REPRODUCTIVE IMMUNOLOGY

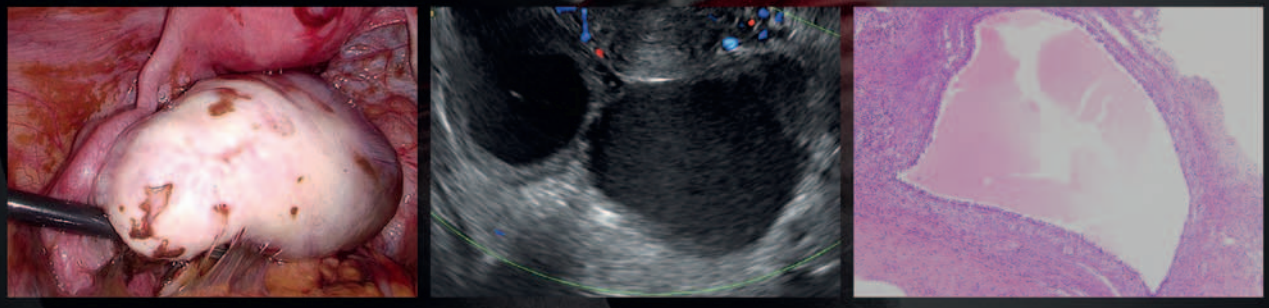
Series Editor GIL MOR

IMMUNOLOGY OF ENDOMETRIOSIS

Pathogenesis and Management

Edited by

KAORI KOGA



VOLUME 2

IMMUNOLOGY OF ENDOMETRIOSIS

About the Series

Reproductive immunology is a growing field that covers multiple aspects of human reproduction: from normal conception, pregnancy, and fetal development, to pathologic conditions such as infertility, pregnancy complications, infections, endometriosis, and cancer.

The series on reproductive immunology will provide a comprehensive source of the up-to-date knowledge on the role of the immune system in normal reproduction and its complications.

Each book discusses a specific clinical complication and its immunological component. A unique objective of the series is to provide a clinical overview of reproductive complications within the scope of the immunological component. The application of immunological markers for detection and immunological approaches for treatment are discussed in each book of the series.

The whole series is intended to reach MDs as well as PhDs. We expect that obstetricians and gynecologists will be interested in many of the subjects covered in this book as well as the following series. Similarly, graduate students may be interested on the area of reproductive immunology.

REPRODUCTIVE IMMUNOLOGY

IMMUNOLOGY OF ENDOMETRIOSIS

PATHOGENESIS AND MANAGEMENT

VOLUME 2

Series Editor

GIL MOR

John M. Malone Jr., MD, Endowed Chair, Scientific Director,
C. S. Mott Center for Human Growth and Development, Wayne State University School of Medicine,
Detroit, MI, United States

Edited by

KAORI KOGA

Associate Professor of the Department of Obstetrics and
Gynecology, School of Medicine at the University of Tokyo, Japan

ELSEVIER

ACADEMIC PRESS

An imprint of Elsevier

Academic Press is an imprint of Elsevier
125 London Wall, London EC2Y 5AS, United Kingdom
525 B Street, Suite 1650, San Diego, CA 92101, United States
50 Hampshire Street, 5th Floor, Cambridge, MA 02139, United States
The Boulevard, Langford Lane, Kidlington, Oxford OX5 1GB, United Kingdom

Copyright © 2022 Elsevier Inc. All rights reserved.

No part of this publication may be reproduced or transmitted in any form or by any means, electronic or mechanical, including photocopying, recording, or any information storage and retrieval system, without permission in writing from the publisher. Details on how to seek permission, further information about the Publisher's permissions policies and our arrangements with organizations such as the Copyright Clearance Center and the Copyright Licensing Agency, can be found at our website: www.elsevier.com/permissions.

This book and the individual contributions contained in it are protected under copyright by the Publisher (other than as may be noted herein).

Notices

Knowledge and best practice in this field are constantly changing. As new research and experience broaden our understanding, changes in research methods, professional practices, or medical treatment may become necessary.

Practitioners and researchers must always rely on their own experience and knowledge in evaluating and using any information, methods, compounds, or experiments described herein. In using such information or methods they should be mindful of their own safety and the safety of others, including parties for whom they have a professional responsibility.

To the fullest extent of the law, neither the Publisher nor the authors, contributors, or editors, assume any liability for any injury and/or damage to persons or property as a matter of products liability, negligence or otherwise, or from any use or operation of any methods, products, instructions, or ideas contained in the material herein.

Library of Congress Cataloging-in-Publication Data
A catalog record for this book is available from the Library of Congress

British Library Cataloguing-in-Publication Data
A catalogue record for this book is available from the British Library

ISBN: 978-0-12-820661-4

For information on all Academic Press publications visit our website at https://www.elsevier.com/books-and-journals

Publisher: Andre Gerhard Wolff
Acquisitions Editor: Linda Versteeg-Buschman
Editorial Project Manager: Billie Jean Fernandez
Production Project Manager: Niranjan Bhaskaran
Cover Designer: Matthew Limbert

Typeset by TNQ Technologies

Reproductive immunology

Series editor

Gil Mor, MD, PhD.

John M. Malone Jr., MD, Endowed Chair, Scientific Director, C. S. Mott Center for Human Growth and Development, Wayne State University School of Medicine, Detroit, MI, USA

This page intentionally left blank

Contents

List of contributors

M.S. Abrão Gynecologic Division, BP - A Beneficencia Portuguesa de Sao Paulo, Sao Paulo, SP, Brazil; Disciplina de Ginecologia, Departamento de Obstetricia e Ginecologia, Faculdade de Medicina FMUSP, Universidade de Sao Paulo, Sao Paulo, SP, Brazil

Jodie Avery Robinson Research Institute, Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia

Fabio Barra Academic Unit of Obstetrics and Gynaecology, IRCCS Ospedale Policlinico San Martino, Genova, Italy; Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DiNOGMI), University of Genova, Genova, Italy

Charles Chapron Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Assistance Publique – Hôpitaux de Paris (AP-HP), Hôpital Universitaire Paris Centre (HUPC), Centre Hospitalier Universitaire (CHU) Cochin, Department of Gynecology Obstetrics II and Reproductive Medicine, Paris, France

Giulio Evangelisti Academic Unit of Obstetrics and Gynaecology, IRCCS Ospedale Policlinico San Martino, Genova, Italy; Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DiNOGMI), University of Genova, Genova, Italy

Simone Ferrero Academic Unit of Obstetrics and Gynaecology, IRCCS Ospedale Policlinico San Martino, Genova, Italy; Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DiNOGMI), University of Genova, Genova, Italy

Akira Fujishita Department of Gynecology, Saiseikai Nagasaki Hospital, Nagasaki, Japan

Linda C. Giudice Obstetrics, Gynecology and Reproductive Sciences, University of California San Francisco, San Francisco, CA, United States

Erin Greaves Division of Biomedical Sciences, Warwick Medical School, University of Warwick, Coventry, United Kingdom

Sun-Wei Guo Shanghai OB/GYN Hospital, Fudan University, Shanghai, China; Shanghai Key Laboratory of Female Reproductive Endocrine-Related Diseases, Fudan University, Shanghai, China

Tasuku Harada Department of Obstetrics and Gynecology, Tottori University School of Medicine, Yonago, Tottori, Japan

Ying He Department of Obstetrics and Gynaecology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China

Takehiro Hiraoka University of Tokyo, Department of Obstetrics and Gynecology, Tokyo, Japan

Mary Louise Hull Robinson Research Institute, Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia; Women's and Children's Hospital, North Adelaide, SA, Australia

Sze Wan Hung Department of Obstetrics and Gynaecology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China

Gentaro Izumi Department of Obstetrics and Gynecology, The University of Tokyo Hospital, Tokyo, Japan

Miaomiao Ji Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, Jinan, Shandong, China

Xue Jiao Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, Jinan, Shandong, China

Yoshimasa Kamei Obstetrics and Gynecology, Saitama Medical University, Morohongo Moroyama-machi, Iruma-gun, Saitama, Japan

Khaleque N. Khan Department of Obstetrics and Gynecology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan

Michio Kitajima Department of Obstetrics and Gynecology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Jo Kitawaki Department of Obstetrics and Gynecology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan

Hiroshi Kobayashi Department of Obstetrics and Gynecology, Nara Medical University, Kashihara, Japan

Kaori Koga Department of Obstetrics and Gynecology, The University of Tokyo Hospital, Tokyo, Japan

Hiroaki Komatsu Department of Obstetrics and Gynecology, Tottori University School of Medicine, Yonago, Tottori, Japan

Mathew Leonardi Robinson Research Institute, Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia; Department of Obstetrics and Gynecology, McMaster University, Hamilton, Canada

Tin-Chiu Li Department of Obstetrics and Gynaecology, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, China

Wan-Ning Li Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan

Harshavardhan Lingegowda Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada

Louis Marcellin Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Assistance Publique – Hôpitaux de Paris (AP-HP), Hôpital Universitaire Paris Centre (HUPC), Centre Hospitalier Universitaire (CHU) Cochin, Department of Gynecology Obstetrics II and Reproductive Medicine, Paris, France

Ryan M. Marks Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada

Alison McCallion Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada

Taisuke Mori Department of Obstetrics and Gynecology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan

Anushka Nair Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada

Yosuke Ono Teine Keijinkai Hospital, Department of Obstetrics and Gynecology, Japan

Yutaka Osuga Department of Obstetrics and Gynecology, The University of Tokyo Hospital, Tokyo, Japan

Kavita Panir Division of Biomedical Sciences, Warwick Medical School, University of Warwick, Coventry, United Kingdom; Robinson Research Institute, Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia

L.G.C. Riccio Gynecologic Division, BP - A Beneficencia Portuguesa de Sao Paulo, Sao Paulo, SP, Brazil; Disciplina de Ginecologia, Departamento de Obstetricia e Ginecologia, Faculdade de Medicina FMUSP, Universidade de Sao Paulo, Sao Paulo, SP, Brazil

Pietro Santulli Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Assistance Publique – Hôpitaux de Paris (AP-HP), Hôpital Universitaire Paris Centre (HUPC), Centre Hospitalier Universitaire (CHU) Cochin, Department of Gynecology Obstetrics II and Reproductive Medicine, Paris, France

Carolina Scala Unit of Obstetrics and Gynecology, Gaslini Institute, Genova, Italy

Masashi Takamura Obstetrics and Gynecology, Saitama Medical University, Morohongo Moroyama-machi, Iruma-gun, Saitama, Japan

Yukiko Tanaka Department of Obstetrics and Gynecology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan

Fuminori Taniguchi Department of Obstetrics and Gynecology, Tottori University School of Medicine, Yonago, Tottori, Japan

Chandrakant Tayade Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada

Shaw-Jenq Tsai Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan; Department of Physiology, College of Medicine, National Cheng Kung University, Tainan, Taiwan

Júlia Vallvé-Juanico Obstetrics, Gynecology and Reproductive Sciences, University of California San Francisco, San Francisco, CA, United States

Chi-Chiu Wang Department of Obstetrics and Gynaecology, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, China; Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, China; School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China; Chinese University of Hong Kong – Sichuan University Joint Laboratory in Reproductive Medicine, The Chinese University of Hong Kong, Hong Kong, China

Guoyun Wang Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, Jinan, Shandong, China

Meng Hsing Wu Department of Physiology, College of Medicine, National Cheng Kung University, Tainan, Taiwan; Department of Obstetrics and Gynecology, College of Medicine, National Cheng Kung University, Tainan, Taiwan

Hui Xu Department of Obstetrics and Gynaecology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China

Osamu Yoshino University of Yamanashi, Department of Obstetrics and Gynecology, Yamanashi, Japan

Tao Zhang Department of Obstetrics and Gynaecology, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, China

This page intentionally left blank

About the series editor

Gil Mor, MD, PhD, is the John M. Malone Jr., MD, Endowed Chair Professor, and Scientific Director of the C. S. Mott Center for Human Growth and Development at Wayne State University. Before moving to Wayne State University, he was a Tenured Professor of Obstetrics and Gynecology and Reproductive Science at Yale University School of Medicine. His research topics were related to the immunology of pregnancy and the role of inflammation in cancer formation and progression. He was the Division Director of the Reproductive Science Division at the Department of Obstetrics and Gynecology Yale, and he directed the Reproductive Immunology Unit and the Translational Research Program "Discovery to Cure." He was the Editor in Chief of the American Journal of Reproductive Immunology from 2009 to 2019 and the President of the American Society for Reproductive Immunology from 2019−2021. He has been funded by grants from the National Institute of Child Health Development (NICHD), National Cancer Institute (NCI), and National Institute of Allergies and Infectious Diseases (NIAID) as well as by several pharmaceutical companies and is widely published in the area of immunology and reproduction with more than 300 publications and is the editor of four books on the immunology of pregnancy and apoptosis and cancer. He is also the Senior Editor of the book series on Reproductive Immunology with Elsevier. He is the recipient of several national and international prizes, including the Pearl River Professor from Jinan University Guangzhou China, the J. Christian Herr Award, and the AJRI Award from the Society for Reproductive Immunology. He is a member of the American Association for Cancer Research, the Society for Gynecologic Investigation, American Association of Immunologists and the American Society of Reproductive Immunology. He is also a Member of the International Advisory Committee for the Sino-American Center of Translational Medicine. Southern Medical University, China, Guest Professor at Tongji Medical College in Wuhan China, and Honorary Member and Professor of several scientific societies in Asia, South America, and Europe.

This page intentionally left blank

About the editor

Kaori Koga, MD, PhD is a physician-scientist and an Associate Professor of the Department of Obstetrics and Gynecology, School of Medicine at the University of Tokyo, Japan. Dr. Koga received her MD degree from Chiba University, Chiba, Japan, and her PhD degree from the University of Tokyo. She undertook postdoctoral fellowships in the Uterine Biology Group (Prof. Lois Salamonsen's laboratory) at Prince Henry's Institute, Melbourne, Australia in 2006, and the Reproductive Immunology Unit (Prof. Gil Mor's laboratory) at the Department of Obstetrics, Gynecology, and Reproductive Sciences in Yale University in 2006−08. Dr. Koga's keen interest lies in Reproductive Immunology, with particular focus on endometriosis and infertility. As a gynecological clinician, Dr. Koga has treated many patients with endometriosis and has performed Minimally Invasive Surgery and Assisted Reproductive Technology. She has been funded by grants from the Japan Society for the Promotion of Science (JSPS) KAKENHI, Japan Agency for Medical Research and Development (AMED), as well as by several pharmaceutical companies. She has published more than 180 peer-reviewed journal articles in the fields of immunology and reproduction. Dr. Koga has received numerous national and international awards, including Kanzawa Award from the Kanzawa Medical Research Foundation in 2018 and Gusdon Award from the American Society for Reproductive Immunology (ASRI) in 2008. She is a member of the ASRI, the International Society for Immunology of Reproduction (ISIR) as well as of the European Society of Human Reproduction and Embryology (ESHRE), and an ambassador of the World Endometriosis Society (WES). Dr. Koga was a member of the Guideline Development Group of the Japan Society of Obstetrics and Gynecology (2016, 2020). She is also an Associate Editor of *Human Reproduction Open* (2021−) and serves on the editorial boards of the *American Journal of Reproductive Immunology*, and the *Journal of Reproductive Immunology*.

This page intentionally left blank

Preface

Endometriosis is called an enigmatic disease, as its causes are unclear, difficult to treat, and affecting the quality of life of millions of women worldwide. The 100th anniversary of Cullen's report on "adenomyomata" (1921)[1] and Sampson's report on "chocolate cyst" (1921)[2] happened in 2021. Since then, many researchers have been struggling to unravel the "enigma." In the last decade we have experienced major progress in understanding of the role of steroid hormones, genetics, the immune system, the nervous systems, and environmental factors in the pathogenesis and management of endometriosis.

In this book, the editors have focused on the role of the immune system, on the pathogenesis and management of this disease. To achieve this goal, we have engaged world's leading researchers to provide an immunological perspective on how the immune system may impact endometriosis-associated conditions, and novel immunologic approaches for the development of better therapeutic strategies.

The editors would like to thank all the authors for their contributions and dedication to this book.

I have selected for the cover of the book pictures from one of my patients with the purpose of emphasize the complexity of the biological changes taking place in the suffering women's body associated with this disease.

On the cover of the book, I have included intraabdominal, ultrasound, and pathological findings of one of my patients to show what is happening inside the suffering women's body.

One hundred years after the reports of Cullen and Sampson, I hope that from the pages of this book, researchers, and clinicians will gain new insights into the biology of endometriosis that will help prevent and halt the suffering of women. I hope that the chapters presented here will stimulate further researchers in order to achieve our final goal to cure endometriosis.

Kaori Koga MD, PhD
The University of Tokyo
Tokyo, Japan

[1] Cullen TS, Arch Surg, 1921.

[2] Sampson JA, Am J Obstet Gynecol, 1921.

This page intentionally left blank

SECTION I

Immune factors in the pathogenesis, and the potential therapeutic target, of endometriosis

This page intentionally left blank

CHAPTER

1

B lymphocytes

L.G.C. Riccio[1,2] *and M.S. Abrão*[1,2]

[1]**Gynecologic Division, BP - A Beneficencia Portuguesa de Sao Paulo, Sao Paulo, SP, Brazil;**
[2]**Disciplina de Ginecologia, Departamento de Obstetricia e Ginecologia, Faculdade de Medicina FMUSP, Universidade de Sao Paulo, Sao Paulo, SP, Brazil**

The pathogenesis of endometriosis is multifactorial, and many studies have explored the role of genetics, environmental factors, and the immune system in contributing to the development of the disease. Several immunological abnormalities have been reported to occur in endometriosis, but the role of the immune system in the pathogenesis of the disease is not completely understood [1,2]. An aberrant immune response associated to a peritoneal environment that allows proliferation of ectopic endometrial cells contribute to the development of the lesions [3].

Many lymphocytes have been identified in endometriotic implants [4] and the immune cells of lymphoid lineage seem to play a key role in the survival and proliferation of ectopic endometrial cells that reach the peritoneal cavity. Reduced cytotoxicity of T lymphocytes, secretion of cytokines by T helper cells and autoantibody production by B lymphocytes have been described in patients with endometriosis [5,6].

B (bone-marrow derived) lymphocytes, or B cells, produce antibodies against antigens and are players of humoral immune response. Its subsets are defined accordingly to their distinct anatomic locations within lymphoid tissues: follicular B cells, marginal zone B cells, and B-1 B cells [7]. The specific markers expressed by B lymphocytes are CD19 [8], CD22, and CD20 [9], and their interaction with antigens leads to B cell activation [10]. Consequently, their activation up-regulates CD40, CD80, CD86, and CD69 in the cell surface [9] and activated B cells differentiate into plasma cells that secrete antibodies [11].

Although peritoneal immunosurveillance is mostly defective in endometriosis, some aspects of the immune system are described to be upregulated, such as the widespread polyclonal activation of B lymphocytes [12]. These cells seem to contribute to the pathogenesis of endometriosis by autoantibodies or antibodies against endometrial epitopes secretion in the lesions, peritoneal fluid and serum [13,14].

https://doi.org/10.1016/B978-0-12-820661-4.00003-0

© 2022 Elsevier Inc. All rights reserved.

The role of estrogens in immunomodulation has been described as a paradox: they can act both as antiinflammatory and proinflammatory substances, and endometriosis is an estrogen-dependent disease. This fact may explain why women have a higher inflammatory response and an increased incidence of autoimmune diseases compared to men. Estrogen can stimulate antibody production by B cells, probably by inhibiting T cell suppression of these cells. In contrast, high concentrations of estrogens may lead to a suppression of B lymphocyte lineage precursors [14].

A recent systematic review [15] evaluated 22 studies concerning the role of B lymphocytes in endometriosis. Most of the authors have reported increased number and/or activation of B cells or higher concentration of antibodies in women with endometriosis [16–29], a few found no difference [4,30–34] and two studies showed decreased number of B cells [35,36]. The results of the studies that evaluated B cells in endometriosis are summarized in Table 1.1 [15].

Increased B lymphocyte number and activation

The studies assessed the direct and indirect role of B cells using different markers and samples, including blood/serum, peritoneal fluid, endometriotic implants, eutopic endometrium, follicular fluid and lymph nodes. In addition, they have used several methods and techniques with different levels of sensibility to evaluate the markers, including ELISA, flow cytometry, immunohistochemistry, immunofluorescence, PCR, avidin–biotin immunoperoxidase (ABC) technique and immunobead rosette technique (IBT). Some of the methods have been improved over time and some of them (e.g., ABC and IBT) are no longer used.

Startseva [38] first reported an increased reactivity of B cells in patients with endometriosis. Increased number and activation of B lymphocytes in the blood and peritoneal fluid of women with endometriosis were also demonstrated by Ref. [17] and later by Refs. [26–28]. They have also described higher concentrations of soluble CD23 in patients with stage I and II endometriosis, suggesting that mild endometriosis may be immunologically more active than severe endometriosis. These findings were consistent with a previous study [22] that identified reduced polyclonal IgG2 production in endometriosis stages III and IV.

B lymphocytes were also shown to be increased in both eutopic and ectopic endometrium and more activated in the lesions of patients with endometriosis [16]. The authors concluded that the development of peritoneal lesions is associated with the activation of local and systemic humoral response due to an increase in the amount of Th2 lymphocytes.

Immunochemical analysis of immune cell infiltrates in endometriotic lesions, myometrium and endometrium of women with endometriosis have shown increased concentration of $CD20^{+}$ B cells [29]. Increased B lymphocytes were also found in pelvic lymph nodes of women with endometriosis during proliferative phase of their menstrual cycle. This observation may support the lymphatic dissemination theory of the pathogenesis of endometriosis [19].

TABLE 1.1 Studies that evaluated the role of B lymphocytes in endometriosis.

Samples	Study design	Population	Methods	Markers	B lymphocytes in EDT	References
Blood/serum	Case-control	EDT × controls	IBT	Monoclonal antibodies	↑ B cells	[17]
	Descriptive	59 EDT	ELISA	FAN; IgG; IgM lupus anticoagulant	Abnormal polyclonal B cells activation	[23]
	Case-control	19 EDT × 26 infertile	IBT; ELISA	B cells; IgA; IgG.	↑ B cells and IgG	[18]
	Case-control	42 EDT × 20 infertile × 22 controls	In vitro stimulation with polyclonal B cell activators	IgG1; IgG2; IgG3	No difference in B cells. ↓ polyclonal IgG2 production in EDT stages III and IV	[22]
	Case-control	21 EDT × 18 controls	ELISA	sCD23	Activation of B cells	[26]
	Case-control	25 EDT and idiopathic infertility	Flow cytometry	CD19	No difference	[31]
	Case-control	57 EDT × 40 controls	ELISA	sCD23; IgG	↑ amount and activation of B cells	[27]
	Case-control	31 EDT × 14 controls	Flow cytometry; IF	CD5; ANA	B Cells are related to ANA production	[20]
	Case-control	175 EDT × 131 controls	Flow cytometry	CD20	↓ B cells	[35]
	Case-control	15 EDT × 20 controls	Flow cytometry	CD20; CD5	No difference	[16]
	Case-control	10 OMA × 10 adenomyosis × 10 leiomyoma	IHC; PCR; ELISA	BLyS; plasma cells	↑ BLyS	[24]
Blood/serum	Case-control	87 EDT × 33 adenomyosis × 205 controls	PCR	BLyS 817C/T polymorphism	Heterozygosity ↓ risk of DIE; BLyS may play a role in the pathogenesis.	[21]
	Case-control	165 infertile EDT × 83 idiopathic infertility × 145 controls	PCR	BLyS −817C/T polymorphism	No difference	[30]
	Case-control	25 EDT × 20 controls	Flow cytometry	PD-1+/PD-L1+ CD19+	↑ PD-1+/PD-L1+ B cells	[37]
Peritoneal fluid	Case-control	EDT × controls	IBT	Monoclonal antibodies	↑ B cells	[17]
		19 EDT × 26 infertile	IBT; ELISA	B cells; IgA; IgG.	↑ B cells, IgA and IgG	[18]

(*Continued*)

TABLE 1.1 Studies that evaluated the role of B lymphocytes in endometriosis.—cont'd

Samples	Study design	Population	Methods	Markers	B lymphocytes in EDT	References
	Case-control					
	Case-control	25 EDT and idiopathic infertility	Flow cytometry	CD 19	No difference	[31]
	Case-control	47 EDT × 35 controls	ELISA	sCD23	↑ B cell activation; higher in stages I and II	[28]
	Case-control	31 EDT × 14 controls	Flow cytometry; IF	CD5; ANA	↑ B-1 cells	[20]
	Case-control	46 EDT × 52 controls	ELISA; PCR	IgG; IgA; Bcl-6; Blimp-1	↓ Bcl-6 and ↑ Blimp-1 No difference in Ig	[34]
Endometrium (eutopic and ectopic)	Descriptive	15 EDT	ABC; IHC	anti-Leu-12	Very few B cells in the lesions	[36]
	Descriptive		IHC	CD22	No difference	[33]
Endometrium (eutopic and ectopic)	Case-control	21 infertile EDT × 18 controls	IHC	CD22	No difference in eutopic endometrium	[4]
	Case-control	30 infertile EDT × 10 controls	IHC	IgG	No difference	[32]
	Case-control	15 EDT × 20 controls	Flow cytometry	CD20; CD5	↑ B cells; ↑ activation in ectopic endometrium	[16]
	Case-control	10 OMA × 10 adenomyosis × 10 leiomyoma	IHC; PCR; ELISA	BLyS; plasma cells	↑ BLyS and plasma cells	[24]
	Case-control	87 EDT × 33 adenomyosis × 205 controls	PCR	BLyS 817C/T polymorphism	Heterozygosity ↓ risk of DIE; BLyS may play a role in the pathogenesis	[21]
	Case-control	48 EDT × 24 adenomyosis × 12 controls	IHC	CD20	↑ B cells in EDT lesions, adenomyosis and endometrium	[29]
Follicular fluid	Case-control	12 infertile EDT × 35 tubal factor × 13 idiopathic	Flow cytometry	CD3; CD4; CD8; CD14; CD20; CD45; CD56	↑ B cells	[25]
Pelvic lymph nodes	Case-control	7 EDT × 9 controls	IHC	CD 20; CD79; plasma cells	↑ B cells during proliferative phase	[19]

ABC, avidin-biotin immunoperoxidase technique; *ANA*, antinuclear antibodies; *Bcl-6*, B cell leukemia lymphoma-6; *Blimp-1*, B lymphocyte inducer of maturation program-1; *BLyS*, B lymphocyte stimulator; *DIE*, deep infiltrating endometriosis; *EDT*, Endometriosis; *ELISA*, enzyme-linked immunosorbent assay; *IBT*, Immunobead rosette technique; *IF*, Immunofluorescence; *IHC*, Immunohistochemistry; *OMA*, ovarian endometrioma; *PD-1*, Programmed cell death 1; *PD-L1*, Programmed cell death 1 ligand; *PCR*, protein chain reaction.
Reproduced from Riccio LGC, Baracat EC, Chapron C, Batteux F, Abrão MS. The role of the B lymphocytes in endometriosis: a systematic review. J Reprod Immunol. 2017;123:29–34.

High concentrations of B lymphocyte stimulator (BLyS) were identified in endometriotic lesions [24]. This molecule is produced by macrophages and induces the development of B cells and its differentiation into plasma cells [39]. Increased BLyS was also described in patients with autoimmune diseases and it could be a target for treating diseases with altered B lymphocytes [40].

While evaluating the BLyS 817C/T polymorphism in women with endometriosis and adenomyosis [21], observed a reduced risk of deep infiltrating endometriosis associated with heterozygosity. The authors concluded that BLyS may play a role in the pathogenesis of the disease. However, these findings were not present in a specific group of women with endometriosis and infertility [30].

Blimp-1 (that regulates plasma cell differentiation) and its antagonist Bcl-6 are transcriptional factors that play an important role in B cell function and were evaluated in endometriosis. Blc-6 mRNA level was significantly lower and Blimp-1 mRNA level was significantly higher in the endometriosis group, with significant correlations among transcriptional factors, immunoglobulins and cytokines [34].

The expression of programmed cell death protein 1 (PD-1) and its ligand (PD-L1) in B lymphocytes were described to be higher in the blood of patients with endometriosis when compared to controls. This protein inhibits peripheral immune tolerance, so its overexpression could lead to continuous B cells activation in women with the disease [37].

Some authors [4,33] analyzed $CD22^{+}$ cells in eutopic and ectopic endometrium of patients with endometriosis compared to controls and found no difference. However [4], concluded that functional differences between these cells could not be excluded and suggested that an analysis of cytokines secreted by B lymphocytes would be helpful to evaluate cell function.

Another study [35] concluded that although some specific B cell clones are activated to secrete autoantibodies in the blood of women with endometriosis, the relative number of total B lymphocytes expressing either HLADR or CD44 was downregulated.

One of the theories to explain infertility in patients with endometriosis includes the effects of B lymphocytes polyclonal activation with B-1-cell proliferation and autoantibody abnormalities [20]. Increased B cells in the follicular fluid of infertile patients with endometriosis also suggest that this factor could impair their fertility [25].

The role of antibodies

The presence of antiendometrial antibodies in the serum of women with endometriosis was described in Ref. [41]. Further immunohistochemical analysis showed that these antiendometrial antibodies could bind to glands of ectopic endometrium [42].

IgG and complement deposits in the endometrium and decreased serum complement were described in endometriosis, suggesting complement consumption by the antigen-antibody complex in an autoimmune response [43].

In addition to antiendometrium, antiovary antibodies, against theca cells and granulosa cells, were also reported in higher concentrations in women with endometriosis compared to controls [44]. Biopsies of endometrial tissue and sera from women with the disease were

analyzed by immunofluorescence and the antibodies identified were mostly IgG and IgA [44]. Higher IgG and IgA concentrations were also described in the peritoneal fluid of patients with endometriosis [34].

Badawy [18] described the presence of increased IgG and IgA in peritoneal cell cultures and also an increased number of B cells and T cells, with increased ratio of $CD4^+/CD8^+$ lymphocytes in the blood and peritoneal fluid of women with endometriosis. The authors concluded that the presence of T helper lymphocytes ($CD4^+$) regulates the production of immunoglobulins by the activated B cells.

A significantly higher concentration of IgG in the serum of women with endometriosis was reported. They were shown to be autoantibodies, as they had increased reactivity against antigens derived from endometrial and ovarian cells [45].

Besides antiendometrium and antiovary antibodies, B lymphocytes seem to contribute to the development of endometriosis by producing antiphospholipid, antideoxyribonucleic acid (anti-DNA) and antinuclear antibodies (ANA), usually identified in autoimmune diseases [6]. These changes may be related to specific genetic variants in autoimmune-related genes [46].

Ref. [20] reported that B cells in the blood of women with endometriosis are related to ANA production and they have also identified increased B-1 cells in the peritoneal fluid of these patients. Although ANA have been detected in 29%–47% of patients with endometriosis [47], their presence seems not to be an aggravating factor to pelvic disease [48].

Endometriosis: an autoimmune disease?

In this context, some authors propose that endometriosis may have an autoimmune etiology [20,49]. Inflammatory reactions and proliferation of ectopic endometrial cells [6] seem to occur due to changes in both humoral and cellular immunity [49], also present in autoimmune diseases. Other common characteristics that have been cited: tissue injury, multiple organ involvement, association with other autoimmune disorders, familial occurrence, possible environmental and genetic factors associated, changes in apoptosis, abnormalities of T and B lymphocytes and polyclonal activation of B cells [49].

However, it is not possible to consider endometriosis an autoimmune disease yet, as a specific association with HLA alleles has not been described [50], neither the specific activation of complement in the endometrium of patients with the disease [51].

A recently described B cell subtype has an immunomodulatory function. These so-called B regulatory cells, or simply Breg, secrete IL-10 and control effector immune responses and even the progression of autoimmune diseases [52]. [53] have compared two strategies in treating endometriosis in a mice model: B cell inactivation with Bruton's tyrosine kinase (Btk) inhibitor Ibrutinib and B cell complete depletion with anti-CD20 antibody. Only the Btk inhibitor was effective, and the authors observed that Breg were depleted by anti-CD20 antibody and preserved by Ibrutinib, suggesting that regulatory B cells might play a role in blocking the development of endometriotic lesions.

Future perspectives in treating endometriosis

There is still no consensus about the exact role of B lymphocytes in endometriosis development and/or progression. The currently available studies in this field use several

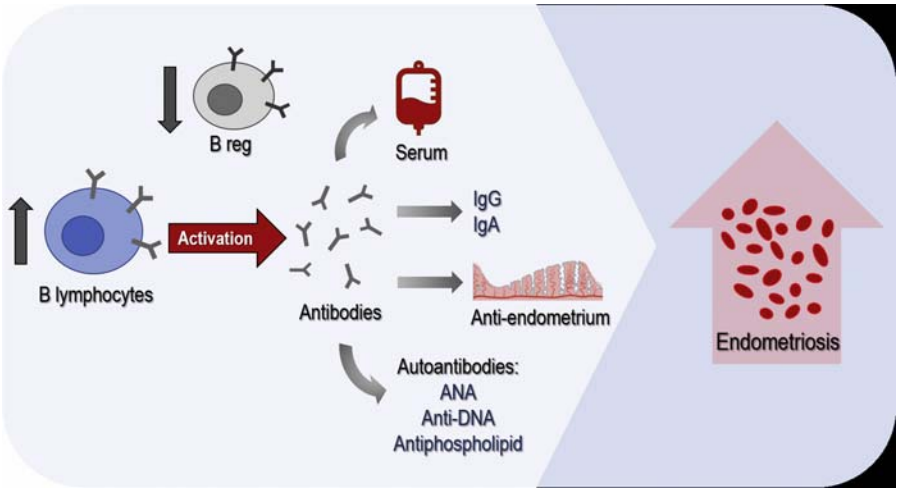


FIGURE 1.1 Effects of B lymphocytes and antibodies on endometriosis pathogenesis and development. *ANA*, antinuclear antibodies; *B reg*, B regulatory cells; *Ig*, immunoglobulin.

approaches and techniques to assess these cells in different types of samples, which makes the comparison of their results very difficult. However, it is possible to consider their findings complementary in describing the various aspects of the disease, and most of authors report an increased B cell activity with antibodies production that contributes to worsening endometriosis. Moreover, the association of these factors with clinical symptoms, location, and severity of the disease has not been investigated.

The treatment of endometriosis is still a challenge. Most of drugs that are effective in controlling disease progression are hormonal and contraceptive, leaving women affected by the disease with the difficult choice between managing the pain and trying to conceive. As the immune system plays a key role in the pathogenesis of the disease, drugs that target immune cells could be an alternative therapeutic strategy. Btk inhibitor Ibrutinib is an FDA approved drug that inactivates B lymphocytes, and it has been recently shown to be effective in controlling endometriosis progression in mice [53].

B lymphocytes and antibody production seem to contribute to endometriosis pathogenesis and development, as summarized in Fig. 1.1. Further studies should evaluate B cells subtypes and drugs that target these cells in order to better understand the pathogenesis and to develop new approaches to treat the disease.

References

[1] Christodoulakos G, Augoulea A, Lambrinoudaki I, Sioulas V, Creatsas G. Pathogenesis of endometriosis: the role of defective immunosurveillance. Eur J Contracept Reprod Health Care 2007;12:194–202.
[2] Riccio LDGC, Santulli P, Marcellin L, Abrão MS, Batteux F, Chapron C. Immunology of endometriosis. Best Pract Res Clin Obstet Gynaecol 2018;50:39–49.
[3] Matarese G, De Placido G, Nikas Y, Alviggi C. Pathogenesis of endometriosis: natural immunity dysfunction or autoimmune disease? Trends Mol Med 2003;9:223–8.

[4] Klentzeris LD, Bulmer JN, Liu DT, Morrison L. Endometrial leukocyte subpopulations in women with endometriosis. Eur J Obstet Gynecol Reprod Biol 1995;63(1):41–7.
[5] Kralickova M, Vetvicka V. Immunological aspects of endometriosis: a review. Ann Transl Med 2015;3(11):153.
[6] Osuga Y, Koga K, Hirota Y, Hirata T, Yoshino O, Taketani Y. Lymphocytes in endometriosis. Am J Reprod Immunol 2011;65(1):1–10.
[7] Abbas A, Lichtman AH, Pillai S. Cellular and molecular immunology. 7th ed. Philadelphia: Elsevier; 2011.
[8] Kurosaki T. Regulation of BCR signaling. Mol Immunol 2011;48:1287–91.
[9] Kaminski DA, Wei C, Qian Y, Rosenberg AF, Sanz I. Advances in human B cell phenotypic profiling. Front Immunol 2012;3:1–15.
[10] Vallvé-Juanico J, Houshdaran S, Giudice LC. The endometrial immune environment of women with endometriosis. Hum Reprod Update 2019;25(5):564–91.
[11] Harwood NE, Batista FD. Early events in B cell activation. Annu Rev Immunol 2010;28:185–210.
[12] Lebovic DI, Mueller MD, Taylor RN. Immunobiology of endometriosis. Fertil Steril 2001;75(1):1–10.
[13] Gajbhiye R, Suryawanshi A, Khan S, Meherji P, Warty N, Raut V, Chehna N. Multiple endometrial antigens are targeted in autoimmune endometriosis. Reprod Biomed Online 2008;16:817–24.
[14] Straub RH. The complex role of estrogens in inflammation. Endocr Rev 2007;28(5):521–74.
[15] Riccio LGC, Baracat EC, Chapron C, Batteux F, Abrão MS. The role of the B lymphocytes in endometriosis: a systematic review. J Reprod Immunol 2017;123:29–34.
[16] Antsiferova YS, Sotnikova NY, Posiseeva LV, Shor AL. Changes in the T helper cytokine profile and in lymphocyte activation at the systemic and local levels in women with endometriosis. Fertil Steril 2005;84(6):1705–11.
[17] Badawy SZ, Cuenca V, Stitzel A, Tice D. Immune rosettes of T and B lymphocytes in infertile women with endometriosis. J Reprod Med 1987;32:194–7.
[18] Badawy SZ, Cuenca V, Kaufman L, Stitzel A, Thompson M. The regulation of immunoglobulin production by B cells in patients with endometriosis. Fertil Steril 1989;51:770–3.
[19] Berbic M, Ng CH, Black K, Markham R, Russell P, Basten A, Fraser IS, Hey-Cunningham AJ. A novel pilot study of endometrial stromal cells and immune cell populations in sentinel uterine-draining lymph nodes during the menstrual cycle and in endometriosis. Reprod Sci 2013;20(1):1339–48.
[20] Chishima F, Hayakawa S, Hirata Y, Nagai N, Kanaeda T, Tsubata K, Satoh K. Peritoneal and peripheral B-1-cell populations in patients with endometriosis. J Obstet Gynaecol Res 2000;26(2):141–9.
[21] de Graaff AA, Dunselman GA, Delvoux B, van Kaam KJ, Smits LJ, Romano A. B lymphocyte stimulator −817C>T promoter polymorphism and the predisposition for the development of deep infiltrating endometriosis. Fertil Steril 2010;94(3):1108–10.
[22] Gebel HM, Braun DP, Rotman C, Rana N, Dmowski WP. Mitogen induced production of polyclonal IgG is decreased in women with severe endometriosis. Am J Reprod Immunol 1993;29(2):124–30.
[23] Gleicher N, el-Roeiy A, Confino E, Friberg J. Is endometriosis an autoimmune disease. Obstet Gynecol 1987;70:115–22.
[24] Hever A, Roth RB, Hevezi P, Marin ME, Acosta JA, Acosta H, Rojas J, Herrera R, Grigoriadis D, White E, Conlon PJ, Maki RA, Zlotnik A. Human endometriosis is associated with plasma cells and overexpression of B lymphocyte stimulator. Proc Natl Acad Sci USA 2007;104(30):12451–6.
[25] Lachapelle MH, Hemmings R, Roy DC, Falcone T, Miron P. Flow cytometric evaluation of leukocyte subpopulations in the follicular fluids of infertile patients. Fertil Steril 1996;65(6):1135–40.
[26] Odukoya OA, Bansal A, Wilson AP, Weetman AP, Cooke ID. Serum-soluble CD23 in patients with endometriosis and the effect of treatment with danazol and leuprolide acetate depot injection. Hum Reprod 1995;10(4):942–6.
[27] Odukoya OA, Bansal A, Cooke I. Serum endometrial IgG antibodies and soluble CD23 concentrations in patients with endometriosis. Acta Obstet Gynecol Scand 1996a;75(10):927–31.
[28] Odukoya OA, Bansal A, Wilson P, Lim K, Weetman AP, Cooke ID. Soluble CD23 protein in the peritoneal fluid of patients with endometriosis. Hum Reprod 1996b;11(9):2018–21.
[29] Scheerer C, Bauer P, Chiantera V, Sehouli J, Kaufmann A, Mechsner S. Characterization of endometriosis-associated immune cell infiltrates (EMaICI). Arch Gynecol Obstet 2016;294(3):657–64.
[30] Christofolini DM, Cavalheiro CM, Teles JS, Lerner TG, Brandes A, Bianco B, Barbosa CP. Promoter −817C>T variant of B lymphocyte stimulator gene (BLyS) and susceptibility to endometriosis-related infertility and idiopathic infertility in Brazilian population. Scand J Immunol 2011;74(6):628–31.

[31] Nava Loya G, Hernández Guerrero C, Pérez Ocharan M, Mojarra Estrada JM, González Morales ME, Bustos López H, Alvarado Durán A, Vadillo Ortega F. Respuesta inmune peritoneal en endometriosis/Peritoneal immune response in endometriosis. Ginecol Obstet Mex 1996;64(4):147–53.
[32] Nomiyama M, Hachisuga T, Sou H, Nakamura K, Matsumoto Y, Iwasaka T, Sugimori H. Local immune response in infertile patients with minimal endometriosis. Gynecol Obstet Invest 1997;44(1):32–7.
[33] Witz CA, Montoya IA, Dey TD, Schenken RS. Characterization of lymphocyte subpopulations and T cell activation in endometriosis. Am J Reprod Immunol 1994;32(3):173–9.
[34] Yeol SG, Won YS, Kim YI, Lee JW, Choi YJ, Park DC. Decreased Bcl-6 and increased Blimp-1 in the peritoneal cavity of patients with endometriosis. Clin Exp Obstet Gynecol 2015;42(2):156–60.
[35] Gagné D, Rivard M, Pagé M, Shazand K, Hugo P, Gosselin D. Blood leukocyte subsets are modulated in patients with endometriosis. Fertil Steril 2003;80(1):43–53.
[36] Oosterlynck DJ, Cornillie FJ, Waer M, Koninckx PR. Immunohistochemical characterization of leucocyte subpopulations in endometriotic lesions. Arch Gynecol Obstet 1993;253(4):197–206.
[37] Walankiewicz M, Grywalska E, Polak G, Korona-Glowniak I, Witt E, Surdacka A, Kotarski J, Rolinski J. The increase of circulating PD-1- and PD-L1-expressing lymphocytes in endometriosis: correlation with clinical and laboratory parameters. Medi Inflamm 2018;25:7041342.
[38] Startseva NV. Clinical immunological aspects of genital endometriosis. Akush Ginekol (Mosk) 1980;3(51):23–6.
[39] Schiemann B, Gommerman JL, Vora K, Cachero TG, Shulga-Morskaya S, Dobles M, Frew E, Scott ML. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. Science 2001;293:2111–4.
[40] Krivosikova M, Dallos T, Maslinski W, Buc M. B cell activating factor, its role in autoimmunity, and targeting in autoimmune diseases. Bratisl Lek Listy 2009;110(3):137–45.
[41] Wild RA, Shivers CA. Antiendometrial antibodies in patients with endometriosis. Am J Reprod Immunol Microbiol 1985;8:84–6.
[42] Fernandez-Shaw S, Hicks BR, Yudkin PL, Kennedy S, Barlow DH, Starkey PM. Anti-endometrial and anti-endothelial auto-antibodies in women with endometriosis. Hum Reprod 1993;8:310–5.
[43] Weed JC, Arquembourg PC. Endometriosis: can it produce an autoimmune response resulting in infertility. Clin Obstet Gynecol 1980;23:885–93.
[44] Mathur S, Peress MR, Williamson HO, Youmans CD, Maney SA, Garvin AJ, Rust PF, Fudenberg HH. Autoimmunity to endometrium and ovary in endometriosis. Clin Exp Immunol 1982;50(2):259–66.
[45] Bohler HC, Gercel-Taylor C, Lessey BA, Taylor DD. Endometriosis markers: immunologic alterations as diagnostic indicators for endometriosis. Reprod Sci 2007;14(6):595–604.
[46] Bianco B, André GM, Vilarino FL, Peluso C, Mafra FA, Christofolini DM, Barbosa CP. The possible role of genetic variants in autoimmune-related genes in the development of endometriosis. Hum Immunol 2012;73(3):306–15.
[47] Iborra A, Palacio JR, Ulcova-Gallova Z, Martinez P. Autoimmune response in women with endometriosis. Am J Reprod Immunol 2000;44:236–41.
[48] Dias JA, de Oliveira RM, Abrão MS. Antinuclear antibodies and endometriosis. Int J Gynaecol Obstet 2006;93(3):262–3.
[49] Nothnick WB. Treating endometriosis as an autoimmune disease. Fertil Steril 2001;76(2):223–31.
[50] Maxwell C, Kilpatrick DC, Haining R, Smith SK. No HLA-DR specificity is associated with endometriosis. Tissue Antigens 1989;34(2):145–7.
[51] Isaacson KB, Galman M, Coutifaris C, Lyttle CR. Endometrial synthesis and secretion of complement component-3 by patients with and without endometriosis. Fertil Steril 1990;53(5):836–41.
[52] Fillatreau S, Sweenie CH, McGeachy MJ, Gray D, Anderton SM. B cells regulate autoimmunity by provision of IL-10. Nat Immunol 2002;3(10):944–50.
[53] Riccio LGC, Jeljeli M, Santulli P, Chouzenoux S, Doridot L, Nicco C, Reis FM, Abrão MS, Chapron C, Batteux F. B lymphocytes inactivation by Ibrutinib limits endometriosis progression in mice. Hum Reprod 2019;34(7):1225–34.

This page intentionally left blank

CHAPTER

2

Macrophages in endometriosis: they came, they saw, they conquered

Kavita Panir[1,2], *Mary Louise Hull*[2] *and Erin Greaves*[1]

[1]Division of Biomedical Sciences, Warwick Medical School, University of Warwick, Coventry, United Kingdom; [2]Robinson Research Institute, Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia

Introduction

Endometriosis is a complex, multifactorial chronic inflammatory disorder defined by the growth of endometrial-like tissue (lesions) usually within the pelvic cavity. It impacts 190 million women worldwide and is associated with debilitating pelvic pain and infertility [1]. The pathophysiology of endometriosis is driven by local peritoneal inflammation associated with aberrant cytokine expression, impaired immune surveillance, and ability for the endometrial tissue to persist within the peritoneal cavity [1–4]. Following the attachment of endometrial-like fragments to the mesothelium of the peritoneal lining, the ectopic tissue manages to thrive in the peritoneal cavity via the development of a vascular network that provides the lesions with nutrients and oxygen [5]. Angiogenesis is frequently accompanied by formation of nerve fibers at the site of implantation, possibly contributing to the chronic pain associated with endometriosis [6].

The most widely recognized theory for the pathogenesis of endometriosis is Sampson's theory of retrograde menstruation, which suggests that endometrial fragments refluxed through the fallopian tubes during menstruation can implant within the peritoneal cavity, giving rise to endometriotic lesions [7]. This theory is supported by the fact that approximately 90% of women experience retrograde menstruation, with viable endometrial epithelial cells and glandular structures being found in the menstrual effluent [8]. The mechanisms underpinning the survival of endometrial tissue in women with endometriosis, in contrast to effective clearance in women *without* endometriosis, are yet to be fully resolved. Several additional theories for the development of endometriosis include the existence of Müllerian remnants and coelomic metaplasia theories based on the assumption that endometriosis develops in situ from local tissue in the peritoneal cavity [1,9]. While the exact etiology of

Immunology of Endometriosis
https://doi.org/10.1016/B978-0-12-820661-4.00019-4

© 2022 Elsevier Inc. All rights reserved.

endometriosis is still under debate, mounting evidence implicates immune dysregulation as a major contributor in the development of endometriosis [10–12].

Complex interactions between ectopic endometrial tissue and leukocytes contribute to the inflammatory cascade associated with endometriosis disease progression. The presence of macrophages is a consistent feature of endometriosis lesions and appears to be a significant driving force in the survival of ectopic endometrial tissue [13]. In 1981, Haney et al. first described an increase in the number of peritoneal macrophages in women with endometriosis [14]. Additional studies have since shown that, activated peritoneal macrophages had a reduced capacity to eliminate refluxed menstrual debris in women with endometriosis [15–17]. Macrophages appeared to facilitate the survival and proliferation of endometrial cells in the peritoneal cavity, through the release of multiple growth and vascular remodeling factors [18]. This chapter summarizes the current understanding of the complex role of macrophages in endometriosis, highlighting the important immunological and, perhaps misplaced, homeostatic functions of these immune cells in the pathogenesis of the disease.

Macrophage origins and phenotype

Macrophages, a group of evolutionary conserved mononuclear phagocytes, are a heterogeneous population of functionally diverse hematopoietic cells that play critical roles in response to inflammatory challenges as well as in maintaining tissue homeostasis and equilibrium. In mammals, macrophage density varies between tissues, constituting high proportions in the liver (Kupffer cells), brain (microglial cells) and skin (Langerhans cells) [19].

Throughout the 20th century, the prevailing view of macrophage ontogeny was that macrophages arose from monocytes derived from hematopoietic stem cells (HSC) in the bone marrow [20]. More recently, however, via the use of parabiotic models and fate mapping tools, multiple studies have confirmed that HSC-derived monocytes are not the only source contributing to tissue-resident macrophages. Macrophages derive from three distinct populations; embryonic progenitors within the yolk sac, fetal liver monocytes and HSC-derived monocytes in adult tissues. Yolk sac-derived macrophages arise from yolk sac blood islands, and are identifiable in mice from the ninth gestational day (E9). These cells are then dispersed throughout the embryo once the circulatory system has been established (day E8.5 to E10) [21–23]. Supporting embryonic microglia development, tissue maturation and clearance of dead cells [24,25], these early erythro-myeloid progenitors are independent of the transcription factor c-Myb and are instead PU.1-dependent [26].

In contrast, fetal monocytes are c-Myb dependent, and arise from erythro-myeloid progenitors seeding the fetal liver between day E11.5 to E12.5 [26–28]. While fetal monocytes are unable to cross the blood-brain barrier, they rapidly infiltrate all other tissues to generate the majority of self-renewing adult tissue-resident macrophages in the peritoneum, skin, spleen, lung, and liver [29–32]. These long-lived tissue-resident cells have important roles in the maintenance of tissue health and integrity (reviewed in detail by Hoffel and Ginhoux [28]). Although both fetal monocytes and their adult counterparts share similar phenotypic traits, fetal monocytes are generated independently of colony-stimulating factor 1 receptor (CSF-1R) and have minimal expression of genes associated with immune activation and pathogen recognition [31,33].

The generation of HSC in bone marrow occurs concurrently with marrow vascularization at day E16.5, giving rise to lineage-specific cells including myeloid and lymphoid progenitors [34]. A proportion of these progenitor cells form the mononuclear phagocyte system, comprising dendritic cells, monocytes and macrophages. Upon maturation, bone marrow-derived monocytes enter circulation and are able to respond to inflammatory challenges (e.g., pathogen exposure, antigenic stimuli, cytokine secretion) by differentiating into effector phagocytes, such as macrophages once they enter peripheral tissues. In the absence of inflammatory stimuli, they perform homeostatic roles (e.g., surveillance of peripheral tissues, tissue repair, maintenance of endothelial integrity) [35].

Macrophages exhibit a huge phenotypic spectrum, which is characterized by the expression of various receptor molecules and via the secretion of a vast range of chemokines and cytokines. Macrophages can be broadly categorized as having primarily either a "proinflammatory" or an "antiinflammatory/prorepair" phenotype. Both proinflammatory and prorepair macrophages play important roles in the initiation and resolution of inflammation. In vitro, undifferentiated macrophages derived from bone marrow progenitors can be induced toward a proinflammatory phenotype following exposure to Interleukin (IL)-1β, tumor necrosis factor (TNF)-α, Interferon (IFN)-γ or lipopolysaccharide (LPS); while prorepair-like macrophages can be generated by treatment with a combination of macrophage colony-stimulating factor (CSF) and IL-10 in mice or the T helper (T_H) two cytokine IL-4 in humans [36–40]. In vivo, macrophage phenotype is infinitely more complex as they respond to local tissue-specific and/or disease-specific local stimuli and modify their transcriptional profile and function accordingly.

Physiological roles of macrophages: same cell, many different occupations

Professional phagocyte and antigen presenter

As effector cells of the innate immune system, macrophages mediate the primary response to pathogens and cellular stress. Recruitment of macrophages to sites of tissue injury or stress is driven by chemokines such as monocyte chemoattractant protein-1 (MCP-1) as part of the "leukocyte adhesion cascade" which involves leukocyte recruitment, extravasation and transendothelial migration [41,42]. Macrophages recognize the presence of either pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs) via pattern recognition receptors including Toll-like receptors (TLRs), which initiates an inflammatory cascade [43,44]. Following identification, these foreign, "nonself" molecules are engulfed by macrophages via phagocytosis [45]. Once encapsulated, these molecules are degraded within lysosomes via exposure to a combination of antimicrobial cationic peptides, superoxide and hydroxyl radicals, and various hydrolytic enzymes [46]. Postdegradation, the additional release of pathogenic peptides and ligands activates macrophages such that they are able to detect viable microbial activity and facilitate effective mechanisms to protect the host. For example, the stimulation of TLRs are able to induce the nuclear factor kappa B (NF-κB) signaling pathway, resulting in the release of inflammatory cytokines including TNF-α and IL-6 [47–49]. Additional procaspase signaling pathways, such as via the activation of NOD-like receptor −1 (NLRP-1) and NLRP-3 inflammasome, result in the

release of IL-1β and IL-18, contributing to inflammation and pathogen clearance [50,51]. Moreover, in tandem with dendritic cells, macrophages also function as antigen presenting cells [52]. Antigenic peptides derived from phagocytosed material are presented on major histocompatibility complex II (MHC II) molecules, initiating the adaptive immune response by activating T_H cells.

The "healer"

While effective proinflammatory activity is beneficial in limiting antigen transmission, extended periods of exposure to inflammatory cytokine activity can result in the exacerbation of inflammation and cellular injury. Following tissue damage, restoration of equilibrium by tissue-resident macrophages and monocyte-derived macrophages recruited from the bone marrow, assist in wound repair, remodeling and renewal of tissue function. To facilitate wound healing, a transition from the predominant proinflammatory macrophage phenotype to a prorepair phenotype occurs. In a mouse model of tissue repair, it was observed that proinflammatory macrophages were present for up to 48 h-post injury and were gradually replaced by increasing numbers of antiinflammatory macrophages [53]. These secondary cells expressed higher levels of transforming growth factor β (TGF-β) and IL-10 compared to the initially recruited macrophages, signifying a subtle change from proinflammatory to prorepair macrophage profile during the transition to tissue repair [53,54]. In addition, specialized proresolving lipid mediators such as lipoxins have been shown to reprogram proinflammatory macrophages to proreparative macrophages by stimulating macrophage efferocytosis and uptake of apoptotic cells, thereby modulating the extent of inflammation and promoting its resolution [55,56].

Coupled with the release of remodeling proteases such as matrix metalloproteinases (MMPs) from macrophages, the recruitment and proliferation of keratinocytes and fibroblasts assist in promoting wound healing or fibrosis. Fibrosis is a pathological endpoint following tissue injury as remodeling and deposition of extracellular matrix components can lead to scar tissue formation and loss of normal organ function [57]. Macrophages contribute to fibrosis via the expression of profibrotic factors including Resistin-like molecule α (RELMα) to support collagen crosslinking in fibroblasts [58] and platelet-derived growth factor (PDGF), which induces fibroblast division and chemotaxis [59]. In addition, macrophages can enhance the survival and proliferation of myofibroblasts, the main cells involved in fibrotic response, via stimulation of NF-κB activity in fibroblasts [60].

Although an inflammatory response is involved in the development of fibrotic tissue, inflammation does not inevitably culminate in fibrosis, and can result in the more preferable outcome of tissue regeneration [61]. The early depletion of macrophages following tissue trauma was found to attenuate the inflammatory response in a mouse model of liver injury, resulting in amelioration of fibrosis [62]. In contrast, macrophage depletion in a mouse model of acute kidney injury resulted in increased histologic injury and delayed regeneration, highlighting the essential role macrophages play in wound healing and repair [63]. Indeed, the complex, multi-faceted role of macrophages in regulating the extent of inflammation, the time to resolution and the initiation of tissue-healing involves the tightly-regulated release of a range of cytokines. The interplay of factors such as IL-10, TGF-β, insulin-like growth factor 1 (IGF-1), PDGF, and vascular endothelial growth factor-α (VEGF-α) act to limit tissue damage and induce proliferation and angiogenesis [64–67]. Importantly, the absence of

effective resolution of inflammation may precede the emergence of chronic or sustained inflammatory disorders. For example, macrophage-driven inflammation and aberrant polarization mechanisms contribute significantly to the pathogenesis of several chronic disorders including cancer, rheumatoid arthritis, type 2 diabetes and periodontitis [68–70] (discussed in more detail below).

The "caretaker"

Macrophages are key to regulation of tissue homeostasis and play diverse roles dependent on their tissue of residence. Microglia, highly specialized macrophages of the central nervous system (CNS), contribute significantly to neuronal growth. These tissue-resident macrophages derive from myeloid progenitors in the yolk sac that migrate to the developing CNS in early embryogenesis [71]. Although the molecular mechanisms controlling microglial function remains elusive, within the brain these effector cells are critical for the regulation of neurogenesis, immune surveillance, synapse regulation, monitoring of cellular turnover and apoptosis [72]. In addition, following CNS injury, microglial cells contribute to wound repair by clearing damaged tissue, restoring CNS integrity and promoting resistance to bacterial infections [73,74]. While complete regeneration of function following central CNS injury is rare, in vivo experiments in paraplegic rats demonstrated marked improved in spinal cord regeneration after the introduction of macrophages, highlighting the important role of these cells in central neuronal repair [75]. In contrast to CNS injuries, strong regenerative capacity is observed following peripheral nerve damage, with macrophages responding to hypoxic conditions and stimulating angiogenesis postinjury [76]. Macrophages also activate the proliferation and differentiation of Schwann cells, which regulate myelin sheath production around axons [77]. Moreover, macrophages modulate the expression of galectin-1, which promotes neural stem cell proliferation, regulates gliosis (glial cell inflammation) and axon growth following nerve damage [78–80].

Macrophages also contribute to the maintenance of homeostasis in other tissues by clearance of apoptotic bodies. As a part of normal tissue turnover, necrosis, and programmed cell death occurs, with the location of these dying cells determining the extent of an elicited immune response [81]. The clearance of apoptotic cells within tolerogenic organs such as the liver and spleen does not commonly elicit an immune response [82]. In contrast, infiltrating macrophages recruited into an inflamed environment may contribute to an exacerbation of inflammation while phagocytosing apoptotic cells [81,83].

In mice, tissue-resident versus monocyte-derived macrophages in the peritoneal cavity are distinguished based on size and differential expression of the macrophage-associated markers cluster of differentiation (CD) 11b, Epidermal growth factor-like module-containing mucin-like hormone receptor-like 1 (F4/80) and MHC II. Self-renewing, embryo-derived, tissue-resident large peritoneal macrophages (LPM) have high F4/80 expression, low MHC II expression and high T-cell immunoglobulin and mucin domain containing 4 (Tim4) expression, with proliferative capacity regulated by the LPM-unique transcription factor GATA-binding factor 6 (GATA6) [84–87]. LPM perform immunosurveillance, have homeostatic functions, regulate intestinal immunity via recruitment of B1 cells using GATA6-TGFβ2 pathways, and phagocytose apoptotic cells during inflammation [84,88,89].

Alternatively, the less abundant monocyte-derived small peritoneal macrophages (SPM) have low F4/80 expression and high MHC II expression [84,87]. Interestingly, in response to inflammatory challenge in the peritoneal cavity, a phenomenon termed the macrophage disappearance reaction (MDR) occurs, in which a significant reduction in LPM numbers coupled with a simultaneous increase in infiltrating SPM and monocytes is observed [90]. When stimulated with LPS in vitro, SPM secrete a range of proinflammatory mediators including nitric oxide, chemokine ligand −3 (CCL-3), CCL-5, and TNF-α, with in vivo studies confirming the release of proinflammatory cytokines IL-1β, IFN-γ and TNF-α [87,91]. In contrast, LPM produce CSF-2 and CSF-3 following LPS stimuli in vitro, and a tissue-healing, regulatory role for any LPM remaining in the peritoneal cavity following MDR has been proposed [87,92]. At the resolution of inflammation, the secretion of antiinflammatory IL-10 by LPM may assist in regulating the number of SPM persisting in the peritoneal cavity [93]. Moreover, remaining SPM can either migrate to lymph nodes, apoptose, or eventually differentiate into LPM-like cells, with a loss of MHC II expression and an increase in F4/80 expression, suggesting that multiple mechanisms exist to restore and maintain steady state conditions in the peritoneal cavity [88,94,95]. Recently, it has been shown that monocyte-derived macrophages (Tim4 negative) gradually replace embryo-derived LPM (Tim4 positive) in a sexually dimorphic manner [32,96]. This phenomenon, which occurs much slower in females, may contribute to differences in homeostasis, susceptibility to inflammation and resolution of diseases between sexes.

The "shaper"

Macrophages play pivotal roles in development. To facilitate the characterization of macrophage function throughout development, mutations in the macrophage lineage differentiation factor CSF-1 and its corresponding receptor CSF-1R have resulted in the generation of mouse models of macrophage ablation. The spontaneous *Csf1*$^{op/op}$ mutation and the genetically engineered Csf-1r knockout (*Csf1r-KO*) results in mice with various osteogenic deficiencies, including impaired bone remodeling, altered calcium deposition and defective tooth growth. These null mutant mice have a systemic reduction in the number of macrophages present compared to age-matched wild-type mice, resulting in delayed hemopoietic development and significantly reduced macrophages in the peritoneal and pleural cavities [97–99]. Although overt pathology appears similar in both strains, macrophage deficiencies are tissue-specific and age-dependent in *Csf1*$^{op/op}$ mice, with higher levels of sustained macrophage depletion seen in *Csf1r-KO* mice. In addition, Langerhans cells and microglia were found to be rare or absent in *Csf1r-KO* mice compared to *Csf1*$^{op/op}$ mice, suggesting CSF-1R has a crucial role in the development of these cells. The generation of transgenic mice carrying a human diphtheria toxin receptor (DTR) inducible system linked with macrophage-associated receptors has allowed for targeted macrophage ablation. Depletion of monocytes and macrophages in CD11b-DTR transgenic mice occurs following the administration of diphtheria toxin, and has been widely used to study the functional roles of macrophages in various physiological conditions, including pregnancy, wherein macrophages promote vascularization to support corpus luteum integrity [100].

Macrophages contribute to ductal branching, as seen during the development of the mammary gland [101], kidney [102], and pancreas [103]. To facilitate tissue growth, the

development of a supporting vascular network is paramount. In particular, vasculogenesis, angiogenesis and the repair of blood vessels, is mediated by Tie2-expressing macrophages (TEMs). Utilizing the angiopoietin-Tie pathway, TEMs influence the angiogenic cascade by regulating blood vessel formation, maturation and remodeling. In an oncogenic mouse model, the expression of Tie2 activates the AKT-dependent signaling pathway to prevent macrophage apoptosis while conditional deletion of Tie2 expression in macrophages significantly impeded angiogenesis [104]. Monocyte-derived macrophages can secrete extracellular remodeling proteins such as MMP-9, which may support basement membrane remodeling and capillary sprouting, further facilitating macrophage trafficking and vascular repair [105].

Factors affecting macrophage activity

Macrophages modify their phenotype and function depending on signals received from their tissue of residence. Macrophage density also alters throughout development, with intrinsic factors such as glycolysis-mediated-ATP generation and DNA methylation contributing to macrophage polarization and efficacy [106,107]. However, a number of extrinsic factors can dictate macrophage activity.

Unsurprisingly, all immune cell populations are affected by exposure to pathogens, which elicit an inflammatory response. However, the extent and duration of the immune reaction in turn depends on multiple factors including antigen load, age, and gender [108]. Critically, sexual dimorphism in macrophage populations have been observed in mice; macrophage cell surface expression of TLR4 and its co-receptor CD14 is significantly higher in male-derived macrophages compared to counterparts [109]. Following exposure to LPS, macrophages from male mice produce higher levels of IL-1β and C-X-C motif chemokine 10 (CXCL-10), and lower levels of prostaglandin E2 (PGE2) compared to female mice, suggesting that disparity in the expression of TLR4 and CD14 may predispose males toward a greater susceptibility to bacterial sepsis and septic shock [109]. Mouse models linking obesity and estrogen have shown inflammatory changes in males, resulting in an expansion of CD11c + adipose tissue macrophages, whereas females exhibited an expansion of CD11c- macrophages [110]. In a mouse model of peritonitis, enhanced phagocytic function was observed in macrophages recovered from female mice, which exhibited reduced inflammation and neutrophil recruitment compared to males [111]. As mentioned previously, the gradual displacement of embryonic-derived macrophages by monocyte-derived macrophages in the murine peritoneal cavity occurs more rapidly in males compared to females, with changes in the local peritoneal microenvironment following sexual maturity contributing to this sexually dimorphic replenishment of LPM [32,96]. The retention of more embryo-derived macrophages in the peritoneal cavity of female mice also appears to protect against pneumococcal peritonitis [96].

Although the exact mechanism governing macrophage sexual dimorphism remains unknown, it appears to occur independently of estrogen signaling and adiposity in mice [96]. Interestingly, while the expression of the lipid transporter and immunoregulator apolipoprotein E (ApoE) is higher in newly differentiated macrophages, the cumulative expression of ApoE is higher in female-derived macrophages compared to males [96,112]. As *ApoE* contains an estrogen response element, it remains possible that estrogen, and the expression of its

receptor on macrophages may subtly influence macrophage differentiation trajectories and function. Indeed, throughout the female menstrual cycle, alteration in macrophage numbers and phenotype in response to changes in ovarian reproductive hormone levels have been observed [113]. Likewise, the presence of iron-laden heme can modulate macrophage inflammatory response via transcription factors BTB Domain And CNC Homolog 1 (BACH1) and Spi-C Transcription Factor (SPIC), impacting hematopoiesis and macrophage polarization [114].

Contact with chemical and environmental toxins also impacts immune activity. For example, exposure to the common immunotoxicant plasticizer bisphenol A (BPA) and phthalate have adverse effects on proinflammatory cytokines and proinflammatory macrophage phagocytic capacity and hormone metabolism [115]. Likewise, various chemical compounds are able to affect macrophage activation through epigenetic mechanisms. MicroRNAs, potent epigenetic regulators of macrophage phenotype programming and plasticity, can be affected by exposure to environmental toxicants [116]. In addition, aberrant expression of histone-modifying enzymes following exposure to plasticizers was found to effect peritoneal macrophages, resulting in a reduced phagocytic capacity [117].

Role of macrophages in disease

As macrophages are intimately involved in the initiation of an inflammatory response, wound healing and maintenance of tissue homeostasis, dysregulation in macrophage activity can contribute significantly to the development and progression of disease. Indeed, a pathogenic role for macrophages has been proposed in multiple chronic illnesses including cancer, metabolic disorders and osteologic diseases [70]. For example, the development of the common autoimmune inflammatory disease, rheumatoid arthritis, is characterized by an infiltration of macrophages and additional lymphocytes [68]. Both proinflammatory and prorepair macrophages contribute to repetitive cycles of synovial fibroblast proliferation, tissue damage and joint destruction [19].

The recruitment of tumor-associated macrophages (TAMs) into the cancer microenvironment is a consistent feature of solid tumors. Although in vitro studies have demonstrated macrophage capability to eliminate tumor cells, in a clinical setting, macrophages are implicated in promoting the survival and invasiveness of tumors (summarized elsewhere [118]). Hypoxic microenvironments within tumors result in the chemoattraction of macrophages expressing the transcription factor hypoxia inducible factor 1α (HIF1α). Expression of HIF1α potentiates the production of VEGF in macrophages. Thus, macrophages assist in the induction of the angiogenic switch, facilitating tumor vascularization to create a network, which supplies oxygen and nutrients to the developing tumor [119]. Macrophages further support cancer metastasis via the expression of MMPs, which enhance remodeling, and epithelial-tumor cell invasion and migration through established vascular systems. For example, following the infiltration of TAMs in the tumor microenvironment, a significant increase in the expression of CXCL8, MMP-9, and VEGF is observed, resulting in accelerated cancer progression [120]. Thus, with a reduced expression of immune-activating transcripts, this prorepair macrophage phenotype observed in cancer promotes malignancy [121].

Macrophage dysfunction has also been implicated in the development of several metabolic disorders. Macrophages are adept at clearing lipoproteins from dying cells, digesting these lipids in the lysosome to generate cholesterol and fatty acids. Ineffective elimination of excess cholesterol from macrophages results in the generation of foam cells, which have been implicated in atherosclerotic cardiovascular disease [122]. Likewise, macrophages are associated with additional diseases including alveolar proteinosis and nonalcoholic fatty liver disease in which lipid turnover is perturbed. Furthermore, the increasing prevalence of nonalcoholic fatty liver disease correlates with increases in obesity and type 2 diabetes, wherein macrophage polarization imbalances contribute to these chronic diseases [123,124]. The accumulation of macrophages is also a contributory factor in the development of several diabetic complications, including neuropathy, nephropathy, and retinopathy [125].

Macrophages in endometriosis

Endometrial macrophages are elevated during menses: seeds for new soil?

Cyclical changes in hormone levels throughout the menstrual cycle regulate endometrial proliferation and differentiation, culminating either in successful embryo implantation with associated vascular modifications or, in the absence of pregnancy, the withdrawal of progesterone results in endometrial breakdown and shedding, prior to repair of the denuded endometrium in preparation for the next cycle. Multiple studies have demonstrated fluctuating immune cell populations throughout the menstrual cycle (reviewed comprehensively by Oertelt-Prigione [126]). Of particular interest is the influx of leukocytes during the secretory and menstrual phases [127,128], with an elevation in the numbers of tissue-resident endometrial macrophages during the secretory phase [129]. Macrophages have a broad spectrum of activation states and function as an important source of both pro- and antiinflammatory mediators in the endometrium [130]. Throughout the menstrual cycle, a steady increase in the proportion of macrophages within the endometrium is observed, with macrophages comprising 6%–15% of all endometrial cells following the withdrawal of progesterone [131]. This increase in macrophage numbers is believed to occur either by in situ proliferation within the endometrium or via chemotaxis of peripheral monocytes into the endometrium [132,133]. Macrophages remain heterogeneous in the endometrium, with three distinct endometrial monocyte-macrophage populations (F4/80- monocytes, F4/80+ monocyte-derived macrophages, and F4/80+ tissue-resident macrophages) identified using flow cytometry in a mouse model of menstruation [134]. Immunohistochemical analysis showed localization of these cells to areas of endometrial breakdown, repair and remodeling respectively [134], suggesting distinct roles for monocytes and macrophages in the initiation of menstruation [129] and in the resolution and subsequent repair of the endometrium [135,136]. Furthermore, it has been postulated that macrophages contribute to the restoration of the functional layer of the endometrium, as studies in an induced menses mouse model have shown increased influx of monocyte-derived macrophages to the endometrium following endometrial shedding [137].

In the context of endometriosis and the widely accepted theory of retrograde menstruation, it is important to consider the contribution of these immune cells and other cellular

components present in the shed menstrual effluent on disease development, as it has been demonstrated that macrophages originating from the endometrium can be detected in endometriosis lesions in mice [138]. To this end, we have recently shown that depletion of donor endometrial macrophages prior to transfer into the peritoneal cavity of recipient mice results in the development of smaller lesions [139]. As the breakdown phase of menstruation is similar to the initial inflammatory phase of tissue injury prior to wound repair, we suggest that the inflammatory tissue forming wounded endometrium that is transferred to the peritoneal cavity causes activation of wound repair processes and establishment of lesions. Depletion of monocytes and macrophages during skin repair results in reduced wound closure [140], thus we postulate that an analogous event could occur in mice receiving endometrium-depleted of monocytes and macrophages, and this may explain our observed reduced lesion size in recipient mice [139]. Interestingly, the repair that takes place in the peritoneal cavity to establish endometriotic lesions is not scar-free, as it is in the eutopic endometrium, as fibrosis is a consistent feature of lesions [141]. Therefore, the environment of the peritoneal cavity must limit scar-free healing of ectopic endometrium, with repeated cycles of tissue breakdown and remodeling facilitating lesion survival through the development of supporting vasculature.

Ectopic endometrium: a new land for colonization

Analysis of eutopic secretory phase endometrium has shown increased proinflammatory macrophage abundance in women with endometriosis compared to women without endometriosis, suggesting that aberrant proinflammatory macrophage activity may contribute to endometriosis [142]. The inherent differences in macrophage phenotype in the eutopic endometrium may affect subsequent recruitment of monocytes following displacement of endometrial tissue in the peritoneal cavity. Moreover, in mice, the monocyte recruitment factor, MCP-1, was significantly higher 4 h following intraperitoneal injection of syngeneic endometrial tissue compared to sham controls [143]. The increased MCP-1 concentration at this time-point demonstrates a key role for displaced endometrial tissue in release of this chemokine, and subsequent monocyte recruitment. The chemoattractant CCL-5, a mediator of leukocyte recruitment in the lesion microenvironment, has also been implicated in the secondary wave of prorepair macrophages to the site of lesion development [144]. The release of CCL-5 from endometriotic stromal cells suggests that the presence of lesions may enhance monocyte recruitment and contribute to peritoneal macrophage accumulation seen in endometriosis. In the peritoneal fluid of women with endometriosis, MCP-1 and CCL-5 (mediators of acute and chronic inflammation) were found to be present at increased levels, providing insight into the chemokine-driven mechanism behind increased macrophage numbers during disease initiation [18,143,144].

Following intraperitoneal injections of endometrial tissue into the peritoneal cavity of baboons, a surge in inflammatory mediators is seen, as well as increased numbers of leukocytes, T-lymphocytes, and TNF-α+ cells, with an associated increase in the levels of the chemokine macrophage inflammatory protein (MIP) [145]. In addition, mouse models of endometriosis have confirmed that the presence of ectopic endometrial tissue triggers an inflammatory response, characterized by the trafficking of neutrophils and monocytes into the peritoneal cavity [146,147]. Within 24 h of disease initiation, numbers of proinflammatory

macrophages in the peritoneal cavity were significantly higher in mice with experimentally induced endometriosis compared to sham controls. In contrast, the levels of prorepair macrophages were not different between experimental and sham mice up to 72 h postdisease initiation, further implicating a central role for proinflammatory macrophages in the initial stages of lesion development [147]. To further evaluate the contribution of macrophages in a mouse model of endometriosis, the transient depletion of macrophages via intraperitoneal injection of chlodronate liposomes was performed [13]. In this model, the loss of macrophages did not impact the adherence capacity of syngeneic endometrium, however, the growth of ectopic endometrial lesions was significantly reduced. Likewise, macrophages were found to predominantly express proinflammatory markers MHCII and inducible nitric oxide synthase (iNOS) in the early stages of lesion development in a heterologous mouse model of endometriosis [148]. Collectively, this suggests that classically activated proinflammatory macrophages are initially recruited to the localized site of inflammation and could impact the survival of attached ectopic endometrial cells in the peritoneal cavity, and contribute to initial disease establishment.

Throughout the reproductive lifespan of women, the endometrium undergoes repeated cycles of hormone-induced proliferation, decidualization, shedding, and remodeling [149]. Endometriosis lesions are dynamic and are likewise influenced by hormonal fluctuations and undergo similar cellular responses, leading to continuous phases of ectopic tissue injury and repair. In women, studying macrophage plasticity during endometriosis is challenging as ectopic lesions are usually surgically removed and evaluated only once the disease is fully established [146]. Although recruited macrophages may differentiate into various phenotypes that assist lesion development, most samples are analyzed at a single menstrual cycle stage, and may not be representative of immunological changes throughout the various stages of disease [150].

Evaluation of endometriotic lesion biopsies has confirmed the presence of HLA-DR, a marker of antigen presenting cell activity [151]. The expression of macrophage markers CD206 and CD163 was upregulated in the peritoneum and lesions from women with endometriosis compared to disease-free peritoneal samples, suggesting upregulation of prorepair activity [146]. In addition, the prorepair macrophage marker peroxisome proliferator-activated receptor-γ (PPAR-γ) was also found in lesions, being expressed by glandular epithelial, and stromal cells, demonstrating that this marker is not exclusively expressed by macrophages, but may be indicative of a predominance of antiinflammatory activity [152]. Collectively, as these studies failed to detect significant presence of proinflammatory macrophage markers within endometriotic tissue samples, this suggests a predominant tissue-healing, prorepair immune profile within lesions, with the caveat that only limited markers were used for evaluation of macrophage phenotype. However, it is important to consider that macrophage phenotype may fluctuate throughout the progression of endometriosis and macrophage heterogeneity within lesions should be investigated. In addition, it remains unknown if different subtypes of lesions (superficial peritoneal, ovarian endometriomas and deep infiltrating) have contrasting resident and infiltrating macrophage phenotypes, which could vastly affect the predisposition for these lesions to persist in the peritoneal cavity and invade underlying tissue.

The evaluation of peritoneal fluid from women with endometriosis has helped to determine how the immune microenvironment affects macrophage plasticity. The proinflammatory macrophage cytokines IL-6 and IL-8 were reported to be higher in women with

endometriosis, compared to disease-free counterparts [153,154]. Expression of MCP-1 by macrophages was also found to be significantly higher in women with severe stage endometriosis, compared to women at a milder stage [155]. IL-4, which suppresses the production of proinflammatory cytokines in human macrophages and polarizes them toward TH_2 response or prorepair phenotype, is elevated in the peritoneal fluid of women with endometriosis and shows a positive correlation with disease stage/severity [156]. Likewise, levels of TGF-β, which promotes macrophage proliferation, cell invasion and angiogenesis, is increased in the serum, peritoneal fluid and ectopic endometrial tissue of women with endometriosis compared to women without this disease [157–160]. While these observations are consistent with the proposed theory that prorepair macrophages are important for lesion growth and maintenance in endometriosis, the characterization of cytokines, chemokines, and growth factors in peritoneal aspirates has shown elevation in both proinflammatory and antiinflammatory cytokine production by endometriosis-associated macrophages [161]. This suggests peritoneal macrophage differentiation, phenotype and composition during lesion progression is likely to be in a constant state of flux, which further influences immune cell recruitment and disease outcomes. However, in contrast to mouse models, human peritoneal macrophages have not yet been extensively studied and characterized, and further work is required to better understand the diversity and complexity of the macrophage populations that constitute this compartment.

Evidence from in vivo mouse and primate studies indicate that proinflammatory macrophages switch phenotype into prorepair macrophages during the progression of endometriosis, but the mechanism behind this change in phenotype remains largely unknown [13]. In a Rhesus model of endometriosis, $CD163^+$ (prorepair scavenger receptor) were significantly higher in endometriotic lesions compared to eutopic endometrium [162]. This data presents the possibility that the peritoneal cavity microenvironment may be responsible for macrophage phenotype skewing in endometriosis. Studies in homologous mouse models of endometriosis have shown a rapid infiltration of macrophages into endometriotic-like lesions within the first few days following disease induction [163]. A shift in macrophage polarization status from a predominantly proinflammatory to a prorepair phenotype has been shown to occur approximately 10 days following disease establishment in mice [148]. Moreover, studies have shown that MCP-1 is secreted at a higher level by murine prorepair macrophages compared to proinflammatory macrophages [164], suggesting a mechanism by which prorepair macrophages recruit additional monocytes, which ultimately become prorepair macrophages within the lesion microenvironment. In a TGF-β1 null mouse model of endometriosis, reduced lesion size with lower numbers of myofibroblasts and macrophages were observed compared to wild-type control mice, indicating a key role for TGF-β1 in facilitating immune cell recruitment in endometriosis [165].

While the classification of proinflammatory and prorepair subsets simplifies the heterogeneity of macrophages in endometriosis, subtle changes in macrophage phenotype due to the cytokine microenvironment or hormone interactions may occur throughout the menstrual cycle [18]. Macrophage activation is dependent on multiple signals and may dynamically alter throughout disease progression [166], and although functional testing of the impact of proinflammatory versus prorepair macrophages in the different stages of endometriosis has been explored in mouse models, activation pathways and markers over time have not been described. Importantly, the sequence of causal pathways linking macrophage

phenotype with the development of endometriosis can only be thoroughly defined in animal models, where sequential changes in lesion establishment and immune profiles can be evaluated effectively. Collectively, these findings necessitate the further characterization of peritoneal macrophages in endometriosis to evaluate the impact of different macrophage subtypes in disease pathogenesis. In addition, the origins of peritoneal and lesion-resident macrophages which may dictate their functional contribution to disease pathogenesis was unknown until recently. We have since demonstrated, using a mouse model of induced endometriosis that lesion-resident macrophages are derived from eutopic endometrial tissue, infiltrating LPM and monocytes that differentiate into macrophages within lesions [139]. Depletion of endometrial macrophages leads to the development of smaller lesions [139], and a previous study depleting F4/80+ LPM with an F4/80 antibody also resulted in smaller lesions [146]. Conversely, when we constitutively depleted monocytes in the mouse model, we saw a significant reduction in the numbers of LPM, SPM and monocytes and a concomitant increase in the number of lesions that developed. This suggests that monocyte-derived macrophages act to protect the peritoneal cavity when challenged with ectopic endometrial tissue. Thus, we have proposed a possible model whereby in endometriosis, macrophages derived from endometrium exhibit proendometriosis functions. Alternatively, monocyte-derived macrophages from the recipient have an antiendometriosis role and are protective against the persistence of ectopic endometrial tissue [139]. The transcriptomic profile of each of these populations and extent of phenotypic heterogeneity is yet to be determined.

Ectopic endometrial lesions as wounds: a perpetual battleground

Following tissue injury, a sequence of four overlapping physiological events occurs (1) an early attempt to restore homeostasis via blood vessel constriction and the formation of blood clots or platelet plugs, (2) a proinflammatory phase with the recruitment of inflammatory immune cells as a defense mechanism and to clear damaged cellular detritus, (3) a proliferative, tissue-healing phase characterized by fibroplasia and angiogenesis, and (4) the resolution of inflammation and remodeling to restore tissue architecture and functionality [167,168]. Macrophages play a critical role in all aspects of wound healing, including phagocytosis of cell debris, collagen degradation, MMP production, neovascularization [168], and are an important source of IGF-1, a potent enhancer of tissue regeneration [169].

In several chronic diseases, aberrant wound healing can give rise to the persistence of an injury, resulting in a continuous recurrence of inflammation as the injury does not progress through the normal sequence toward healing [170]. Likewise, in the peritoneal cavity, displaced endometrial fragments can be considered as injured tissue [171], and endometriotic lesions as wounds undergoing recurrent tissue injury and repair (Re-TIAR) [172], due in part to hormonal changes and cyclic bleeding associated with menses. Remodeling of the endometrium occurs naturally during the human menstrual cycle under the control of estrogen and progesterone, and involves the degradation of the superficial layer of the endometrium and regeneration of a new layer without fibrosis, resulting in scar-free healing, allowing for full functionality of the endometrium to be restored [126,173]. MMPs are the main tissue-remodeling enzyme family involved in this remodeling process, and in endometriosis, the ectopic endometrium has a higher capacity to produce MMP-2 and MMP-9, compared to

eutopic tissues [8,11,126,174]. The levels of MMP-9 were found to increase in correlation with the severity of disease, and the expression of MMP-9 was highest at the proliferative stage [175]. The presence of these enzymes, typically secreted by prorepair macrophages, suggests that the tissue-remodeling process may be a precursor, which promotes attachment and invasion of ectopic endometrial tissue in the peritoneal cavity [13,146,176].

In addition, estrogen contributes to macrophage-mediated tissue regeneration with estrogen/estrogen receptor signaling preferentially polarizing macrophages toward a reparative phenotype [177,178]. In a cutaneous model of wound repair, ablation of estrogen receptor α (ERα) in vivo resulted in fewer prorepair macrophages with corresponding reductions in antiinflammatory cytokine profiles, confirming a role for ERα in the generation of tissue-healing macrophages [177]. In contrast, in a mouse model of endometriosis, macrophages in the lesions predominantly expressed ERβ, with approximately 20% of macrophages expressing ERα [178], suggesting that the impact of hormone signaling on macrophage-assisted wound healing in the peritoneal cavity may be different compared to wound healing elsewhere in the body.

The proposed redefinition of endometriosis as a fibrotic condition [141] supports the assumption that inappropriate wound healing contributes development of fibrotic endometriotic lesions. Prorepair macrophages have been implicated in promoting fibrosis in endometriosis [179]. In mice with induced endometriosis, the number of prorepair $CD163^{+}$ macrophages significantly increased over time, with a simultaneous increase in markers of fibrosis (total collagen, myofibroblast expression, platelet aggregation, epithelial cell abundance), while depletion of macrophages significantly reduces histopathological signs of fibrosis [179]. This suggests that sustained fibrotic, prorepair macrophage activity may impede scar-free repair in endometriosis lesions, and could further contribute to the inability to appropriately resolve inflammation and clear these ectopic sites.

It is particularly interesting that despite repeated cycles of breakdown and repair, eutopic endometrium is able to achieve scar-free healing, whereas the similar cyclical impact on ectopic endometrial tissue within the peritoneal cavity results in fibrosis. While thus far the mechanism differentiating the healing profile in these cells is unknown, it is probable that the constant exposure to peritoneal immune cells prevents appropriate resolution of inflammation leading to fibrotic "healing" to prevent further inflammatory damage to surrounding tissues in the peritoneal cavity. It remains to be determined why ectopic endometrium continues to be recognized as self in women with endometriosis and macrophages activate processes to repair it, while in women without endometriosis ectopic tissue is cleared, relatively silently, from the peritoneal cavity.

We have also demonstrated elevated monocyte numbers and constitutive recruitment of monocytes into the peritoneal cavity in a mouse model of endometriosis [139]. We suggest that newly recruited monocytes initially differentiate into proinflammatory macrophages and may modulate their phenotype to become prorepair. Hence, in endometriosis, continuous recruitment and differentiation may lead to the peritoneal cavity being exposed to both proinflammatory and antiinflammatory cytokines simultaneously [161]. This concurrent action may exacerbate the development of endometriosis, wherein the shift between phases of predominantly proinflammatory to prohealing macrophage phenotype as seen in wound repair is disrupted with continuous waves of proinflammatory cytokine action, healing but then inappropriately resolved inflammation. Therefore, building upon the Re-TIAR model

and incorporating the critical role of macrophages in the pathogenesis of endometriosis, we postulate that the inherent nature of the endometrium to "heal" results in a premature switch in macrophage phenotype to a prorepair phenotype which leads to an inefficient initial inflammatory phase, an early "repair" phase and an aberrant "resolution" of the ectopic tissue such that it forms a fibrotic lesion. With subsequent menstrual cycles, the process of rapid wound healing is re-initiated in the already inadequately resolved ectopic tissue, thus creating fibrotic layers to contain the recurrent microbleeding. Further to this is the assumption that within a single endometriotic lesion, discrete "zones" are established, supported by functionally diverse endometriosis-associated macrophages that contribute to lesion architecture through remodeling, vascularization and neurogenesis.

Macrophages promote urbanization once the new land has been colonized

Once macrophages have infiltrated ectopic endometrial tissue implants, they promote invasion of ectopic endometrial tissue, angiogenesis, neurogenesis and generation of pain. In vitro, coculture systems have been used to evaluate the involvement of macrophages on the invasive potential and growth of endometrial stromal cells. When cocultured with endometriotic stromal cells, isolated autologous peripheral blood-derived monocyte (PBMC)-derived macrophages from women with endometriosis exhibited increased expression of CSF-1 [180]. CSF-1 is known to enhance the attachment, proliferation and invasion of endometriotic stromal cells, and in this coculture system, the clonogenicity and invasiveness of endometriotic stromal cells was increased [180–182]. Similarly, increased survival and growth of ectopic endometrial cells cocultured with PBMC-derived macrophages was observed as well as a reduction in macrophage phagocytic ability compared to macrophages cultured with eutopic endometrial cells [183], indicating that the inherent difference in ectopic endometrial cells may drive a disease-modified macrophage profile. Collectively, these studies suggest that macrophages support the establishment and invasive potential of endometrial stomal cells.

Establishing a blood supply to newly attached ectopic endometrial implants is vital in disease progression. Importantly, peritoneal fluid from women with endometriosis is found to be more proangiogenic than peritoneal fluid from women without endometriosis [151,184]. A combination of macrophage-associated cytokines including VEGF, TNF-α, and IL-8 are elevated in the peritoneal fluid from women with endometriosis compared to those without [176,185,186]. Both TNF-α and IL-8 promote adhesion, proliferation, and angiogenesis of endometrial cells, implying that an overexpression of these cytokines may result in localized vascularization and remodeling of the mesothelium [184]. VEGF, an important angiogenic factor, is abundantly expressed in the glandular compartment of endometriomas [184,187]. Activated peritoneal macrophages have also been implicated as mediators of vascular development, as they are potent sources of VEGF, which results in increased microvascular permeability and release of MMPs, thus facilitating endothelial cell proliferation and migration, and remodeling of vascular networks [13,188]. Moreover, neovascularization is a marker of successful lesion survival, as the development of blood vessels is critical to support lesion growth [189]. In mice, the depletion of macrophages resulted in disruption of the vascularization and growth of endometriosis-like lesions over time [146]. Assessment of microvessel density and endometrial tissue growth in mice highlighted the role of infiltrating VEGF

receptor I (VEGF-R1)-expressing macrophages in enhancing angiogenesis and lesion survival [190]. Collectively, while these studies suggest that disrupting proangiogenic macrophage activity in endometriosis may be beneficial in impeding disease progression, there remains a need to accurately identify, isolate, and target these endometriosis-associated macrophages to ensure specificity of treatment.

Macrophages also have key roles in pain generation as demonstrated through animal models of diabetes [191] and osteoarthritis [192]. Bilateral communication between macrophages and pain-sensing neurons (nociceptors) occurs, and is mediated by a cocktail of proinflammatory cytokines, neurotrophins and microRNAs [193]. Within the endometriotic milieu, complex interactions between newly formed neurons, vasculature, and immune cells occur. Macrophages are an important source of neurotrophins such as nerve growth factor, neurotrophin-3 and brain-derived neurotrophic factor which are essential for neuron sprouting, reorganization and sensitization of peripheral nerve fibers [194]. In endometriotic lesions, macrophages cluster around nerve fibers [195], with the secretion of CSF-1 and CCL-2 from neurons further enhancing macrophage chemotaxis [178]. Additionally, increased expression of cyclooxygenase-2 (COX-2) has been observed in the brain and spinal cord of mice with endometriosis, likely resulting in enhanced pain perception experienced by women with this disease [196]. Importantly, the depletion of peripheral macrophages reduces COX2 and TNF-α expression in the CNS, further supporting a role for macrophages in endometriosis-associated hyperalgesia [197]. In addition, the expression of IGF-1 is increased in endometriosis-associated macrophages, wherein the inhibition of macrophage-derived-IGF-1 in vitro limits neuron growth and selective inhibition of IGF-1 receptor in mice with induced endometriosis reduces endometriosis-associated pain [197].

While the exact mechanisms underpinning neurogenesis in endometriosis are only just being elucidated, the recruitment of macrophages and nerve fibers results in neuroinflammation, possibly contributing to the initiation, maintenance and aggravation of endometriosis-associated pain [198]. As multiple macrophage-associated cytokines can induce neuronal sensitization, therapies targeting macrophage populations, which overexpress neurotrophins, may be an innovative method to overcome the debilitating pain associated with endometriotic lesion persistence. Altered microglial immunoreactivity and astrocyte activation have also been observed in mice with endometriosis, suggesting that the presence of lesions in mice alters spinal glial activity [199] and could contribute to debilitating pain associated with this disorder. The contribution of neurotransmitters and neuropeptides derived from peripheral nerve fibers and neurogenic inflammation can impact macrophage phenotype and differentiation [200,201], and could potentially influence endometriotic stromal cell adhesion, proliferation and apoptosis. Further studies are required in order to determine the role of neurogenic signaling in the context of endometriosis.

Local climate impacts macrophage behavior

Oxygenation plays a key role in dictating macrophage phenotype. Due to an initial lack of supportive vasculature, a hypoxic environment characterizes the early phases of ectopic endometrial tissue survival in the peritoneal cavity. The generation of this hypoxic milieu is primarily mediated by HIF-1, a protein complex derived from an inducible α subunit, comprising three isoforms (HIF-1α, HIF-2α and HIF-3α), and a constitutively expressed β unit [202]. In macrophages, nitric oxide equilibrium is maintained by HIF-1, wherein

HIF-1α regulates inducible nitric oxide synthase (proinflammatory), while HIF-2α controls arginase expression (prorepair) [203]. This suggests that perturbations in oxygen concentrations can influence macrophage polarization, impacting the progression of various pathologies including endometriosis. For example, a defining feature of tumor microenvironments is the presence of hypoxia, which is associated with an accumulation of TAMs with dynamic phenotypes [204]. Under hypoxic conditions, HIF-1$\alpha^{-/-}$ macrophages cocultured with tumor spheroids developed greater antiinflammatory associated TAM markers with reduced tumor toxicity compared to HIF-1$\alpha^{+/+}$ macrophages [205]. In ovarian and breast cancer, chemokines including MCP-1 and CCL5 recruit TAMs, and are further secreted by TAMs during malignant transformation to induce tumor immune tolerance [206,207]. The stabilization of HIF-1 induces a range of proangiogenic mediators including VEGF, angiopoietin-2, and MMPs [208]. Hypoxic conditions in vitro have shown that macrophages with a deletion of HIF-1α (HIF-1$\alpha^{-/-}$) secreted lower quantities of VEGF compared with HIF-1α replete (HIF-1$\alpha^{+/+}$) macrophages [209].

During the menstrual cycle, HIF-1α supports the regeneration of endometrial tissue via regulating VEGF [210,211]. In addition, estrogen induces rapid, transient binding of HIF-1 to the VEGF promoter, thus inducing VEGF transcription [212]. In endometriosis lesions, the expression of HIF-1α was elevated in glandular and stromal compartments, whereas in eutopic endometrium, significantly lower HIF-1α expression was observed and was mainly localized in the glandular compartment [213]. Thus, in the estrogen-dependent environment of endometriosis lesion development, the elevated HIF-1 expression seen in ectopic endometrial tissue [214,215] may stimulate peritoneal macrophage production of VEGF, facilitating disease persistence.

Prolonged periods of oxygen deprivation can initiate cellular epigenetic changes, giving rise to oncogene activation and malignancy [203]. Cancer-associated mutations have been identified in women with endometriosis in the absence of cancer or dysplasia, underscoring the potential for transformation of benign endometriotic lesions into malignant cancers [216,217]. Likewise, several studies have found that endometriosis has the potential to progress to endometriosis-associated ovarian cancer (EAOC) [218–220]. In EAOCs coexisting with endometriosis, the expression of platelet factor 4 (CXCL4) was significantly higher in $CD68^+$ macrophages infiltrating endometriotic lesions compared to $CD68^+$ TAMS in malignant cells [221]. CXCL4 promotes monocyte adhesion and differentiation into macrophages [222]. Interestingly, in vitro stimulation of human peripheral blood monocytes with CXCL4 resulted in macrophages with reduced phagocytic capability and increased expression of MMP-7 and MMP-12 [223,224].

Collectively, these findings suggest that differing patterns of hypoxia in endometriosis may occur throughout lesion growth resulting in the generation of discrete microenvironments within lesions, which in turn could give rise to distinct subpopulations of endometriosis-associated macrophages, however this remains to be evaluated. Thus, endometriotic lesions are histologically heterogenous. Discrete areas of stromal and epithelial cell populations are interspersed with areas of fibrosis, hemorrhage as well as richly vascularized and innervated areas. Macrophage density also differs throughout lesions. Therefore, we propose a model whereby these discrete microenvironments give rise to varied macrophage phenotypes, and that the colonization and role of macrophages in lesions is far more complex than can currently be appreciated (Fig. 2.1).

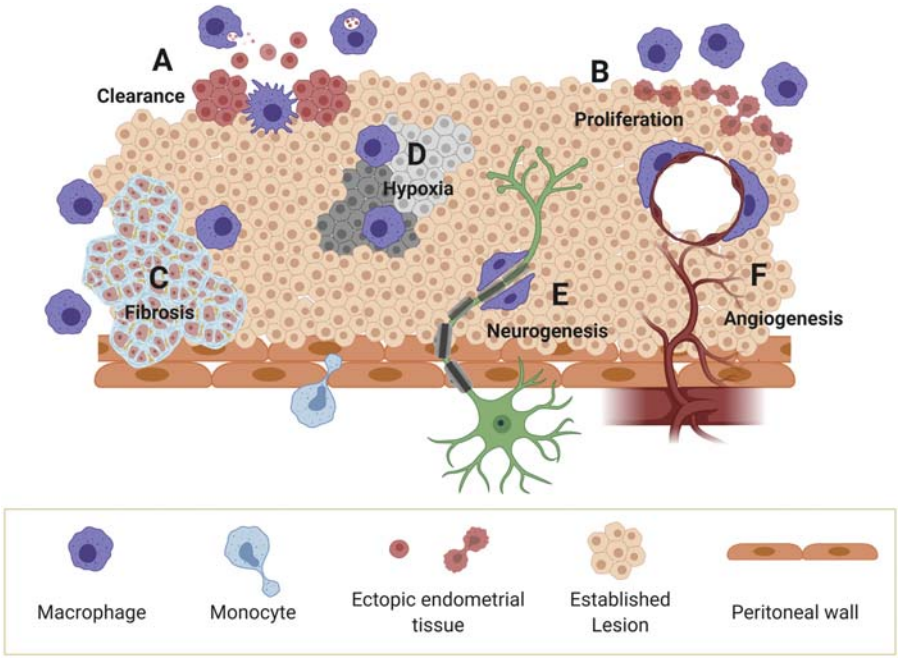


FIGURE 2.1 **Macrophages exist in a dynamic microenvironment within endometriotic lesions.** Here we present a putative model where macrophages exist in discrete microenvironments within lesions that drive phenotype heterogeneity. (A) Proinflammatory macrophages assist in the clearance of ectopic endometrial tissue. (B) Mitogenic macrophages promote ectopic endometrial cell proliferation. (C) Prorepair and profibrotic macrophages drive tissue-healing, scarring and fibrosis. (D) Lack of oxygen results in hypoxic pocket formation within lesions, with macrophages regulating tissue damage and redox status. (E) Macrophages have critical roles in neurogenesis and contribute to pain associated with endometriosis. (F) Proangiogenic macrophages facilitate blood vessel formation in established lesions. Created using Biorender.com.

Macrophages and endometriosis-associated infertility: a hostile habitat

Although chronic pain is the most common presentation of endometriosis, disease symptoms may also include irregular uterine bleeding and infertility [5,126,225,226]. Women with endometriosis have reduced fecundity, increased prevalence of spontaneous abortion, and lower live birth rates [227,228]. While a correlation between endometriosis and subfertility is increasingly apparent, the mechanisms contributing to this phenomenon remains unclear and is likely to be multifactorial. To this extent, immune cells play important roles from the initial peri-conception phase throughout gestation [229]. Macrophages play key roles in the cyclic breakdown and regeneration of the endometrium during the menstrual cycle, and are essential in the establishment and successful maintenance of pregnancy [100]. In mice, immediately following copulation, the presence of seminal fluid facilitates the recruitment of macrophages to the endometrium [230], with subsequent activation and regulation of macrophage proinflammatory/prorepair polarization driving uterine vasculature remodeling to support implantation, placentation, fetal tolerance and growth [231]. In a xenograft model of endometriosis, human endometrial explants exposed to seminal plasma prior to

transfer into mice showed increased proliferation of endometrial epithelial cells resulting in significantly larger lesions compared to mice with control (not exposed to seminal plasma) explants [232]. This suggests that exposure to potential bioactive factors in seminal plasma may contribute to the survival of refluxed endometrium and contribute to endometriosis. Elevated levels of MCP-1 and macrophage migration inhibitory factor (MIF) have been observed in the eutopic endometrium of women with endometriosis compared with controls [233–235]. MIF, an important regulator of the inflammatory cascade, is able to induce MCP-1 expression following a proinflammatory challenge or injury [236]. The tandem action of these cytokines may result in aberrant migration of macrophages into the endometrium, which in turn could negatively impact macrophage availability and function required throughout periconception, potentially contributing to subfertility, embryo implantation failure and preeclampsia.

The expression of macrophage-associated proinflammatory cytokines, including IL-1, IL-6, and IL-8, are elevated in both the eutopic and ectopic endometrium of women with endometriosis [237,238]. This overproduction of proinflammatory mediators generates a chronic inflammatory environment, which is detrimental for trophoblast invasion and generation of immune tolerance [239]. Moreover, macrophages in endometriosis contribute to oxidative stress via increased production of reactive oxygen species, hence further impairing reproductive outcomes [240]. In women, a study evaluating the expression of nitric oxide in endometriosis found significantly higher levels in ectopic tissue compared to paired eutopic endometrial samples [241]. In addition, the same study showed elevated expression of nitric oxide in endometrial samples from women with endometriosis compared to those without. Furthermore, higher levels of iNOS were released from peritoneal macrophages derived from women with endometriosis compared to women without endometriosis, when stimulated with IFN-α in vitro [240]. This finding was linked with the observed subfertility seen in women with endometriosis, as sustained oxidative stress may impact sperm viability postcoitus, as well as affect the quality of oocytes and embryos [242,243]. Additionally, nitric oxide release in the human uterus promotes cervical ripening and uterine contractions, enabling effacement and dilation of the cervix prior to delivery [244]. In early pregnancy however, an increase in cervical nitric oxide levels is associated with intrauterine miscarriages [245], and may be linked with progesterone insufficiency in women with recurrent spontaneous abortions. Therefore, it is possible that progesterone resistance in endometriosis [246] may exacerbate infertility issues associated with the proinflammatory milieu of this disorder.

While a sustained proinflammatory environment contributes considerably toward detrimental fertility outcomes, anomalous tissue-remodeling macrophage activity may also affect the progression of pregnancy. In endometriosis, increased prorepair cytokines in the peritoneal fluid [146] and concomitant elevation of tissue-remodeling factors including MMP-9 [247] and MMP-27 [248], as well as elevated secretion of angiogenic factors such as VEGF [187] is observed. The aberrant expression of angiogenic factors is associated with poor pregnancy outcomes in women with recurrent pregnancy loss [249]. Development of vascular networks to support placentation is critical, and tight regulation of angiogenic cascades is essential at the fetal-maternal interface for successful embryo attachment and development.

Therefore, as macrophages have important roles during gestation, imbalances in proinflammatory/prorepair macrophage activity as seen in endometriosis may be a viable cause for the subfertility seen in women with this disease.

Summary

The complex, heterogeneous manifestation and symptoms of endometriosis contributes to the challenge of understanding the etiology of this disease. Mounting evidence from animal studies implicate multiple roles for macrophages in endometriotic lesion establishment. The evidence presented also underscores the importance of achieving an appropriate balance between proinflammatory and antiinflammatory macrophage responses as well as adequately timed appearance of appropriate macrophage phenotypes in influencing disease outcomes [138,146,148,250].

While acknowledging that the presence of a chronic disease like endometriosis necessitates that the immune system remains in a constant state of flux, strategies, which shift the proinflammatory/prorepair balance, may prove therapeutic by inhibiting the reparative function of prorepair-like macrophages which promotes disease progression. Macrophage-associated therapeutic strategies employed in pathologies with characteristic macrophage-driven inflammatory responses, such as type 2 diabetes, atherosclerosis, and cancer [70], should be appraised to determine suitability for clinical translation in endometriosis. For example, parallels between the heterogeneous tumor microenvironment in cancer and endometriotic lesions comprising similarly diverse cell populations suggests that pharmacological approaches targeting macrophages within the tumor microenvironment (i.e., manipulating macrophage recruitment, macrophage depletion, and macrophage reprograming [251]) may be beneficial in treating endometriosis.

A combination of augmented macrophage function, abnormal immune responses, and epigenetic dysregulation throughout the menstrual cycle may facilitate the growth of endometriotic lesions, and predispose some women to endometriosis, potentially exacerbating this condition over multiple repeated menstrual cycles. It remains critical not to discount the possibility that observed changes in macrophage phenotype in endometriosis could be a consequence of an enhanced inflammatory response toward the presence of ectopic endometrial tissue, rather than the observed peritoneal inflammation driving disease development. Repeated cycles of lesion injury and "repair" may produce discrete microenvironments and heterogeneous lesions, thus influencing macrophage abundance, subtypes, and polarization in endometriosis.

Despite the significant contribution of macrophages in the pathophysiology of endometriosis, it is important to consider the multifactorial nature of this disease. Although studies in animal models have demonstrated correlations between macrophage polarization imbalances and lesion development, it is yet unknown if manipulation of the macrophage polarization balance would be sufficient to reverse established disease, and should be evaluated. Importantly, better identification of macrophage heterogeneity and origin in endometriosis could significantly improve our ability to understand the mechanisms giving rise to this disease and the subsequent persistence of endometriotic lesions. Likewise, it is crucial to define unique markers, expression profiles and functional roles for endometriosis-associated macrophages, with the ultimate goal of developing strategies specifically targeting lesion-associated macrophages without affecting "healthy" macrophage populations. With better understanding of macrophage biology, targeting of epigenetic modulators of macrophage polarization may regulate the balance between proinflammatory and prorepair macrophages, and could prove to be an effective strategy to halt disease progression in women with endometriosis.

References

[1] Zondervan KT, et al. Endometriosis. Nat Rev Dis Primers 2018;4(1):9.
[2] Aznaurova YB, et al. Molecular aspects of development and regulation of endometriosis. Reprod Biol Endocrinol 2014;12(1):1–25.
[3] Benagiano G, Brosens I, Lippi D. The history of endometriosis. Gynecol Obstet Invest 2014;78(1):1–9.
[4] Iwabe T, Harada T. Inflammation and cytokines in endometriosis. In: Harada T, editor. Endometriosis: pathogenesis and treatment. Tokyo: Springer Japan; 2014. p. 87–106.
[5] Giudice LC, Kao LC. Endometriosis. Lancet 2004;364(9447):1789–99.
[6] Hey-Cunningham AJ, et al. Angiogenesis, lymphangiogenesis and neurogenesis in endometriosis. Front Biosci 2013;5:1033–56.
[7] Sampson J. Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol 1927;14.
[8] Halme J, et al. Retrograde menstruation in healthy women and in patients with endometriosis. Obstet Gynecol 1984;64(2):151–4.
[9] Schenken RS. Endometriosis: pathogenesis, clinical features, and diagnosis. Endometriosis November 20, 2018;2018. Available from: http://www.uptodate.com.
[10] Herington JL, et al. Immune interactions in endometriosis. Expet Rev Clin Immunol 2011;7(5):611–26.
[11] Maybin JA, Critchley HO, Jabbour HN. Inflammatory pathways in endometrial disorders. Mol Cell Endocrinol 2011;335(1):42–51.
[12] Riccio L, et al. Immunology of endometriosis. Best Pract Res Clin Obstet Gynaecol 2018;50:39–49.
[13] Capobianco A, Rovere-Querini P. Endometriosis, a disease of the macrophage. Front Immunol 2013;4:9.
[14] Haney AF, Muscato JJ, Weinberg JB. Peritoneal fluid cell populations in infertility patients. Fertil Steril 1981;35(6):696–8.
[15] Kusume T, et al. Human leukocyte antigen expression by peritoneal macrophages from women with pelvic endometriosis is depressed but coordinated with costimulatory molecule expression. Fertil Steril 2005;83(Suppl. 1):1232–40.
[16] Lee K-S, et al. IL-10-dependent down-regulation of MHC class II expression level on monocytes by peritoneal fluid from endometriosis patients. Int Immunopharm 2005;5(12):1699–712.
[17] Yamamoto Y, et al. Decreased human leukocyte antigen-DR expression in the lipid raft by peritoneal macrophages from women with endometriosis. Fertil Steril 2008;89(1):52–9.
[18] Ahmad SF, et al. Macrophages in pathophysiology of endometriosis. In: Harada T, editor. Endometriosis: pathogenesis and treatment. Tokyo: Springer Japan; 2014. p. 61–85.
[19] Pollard JW. Trophic macrophages in development and disease. Nat Rev Immunol 2009;9(4):259–70.
[20] van Furth R, et al. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. Bull World Health Organ 1972;46(6):845–52.
[21] Naito M, et al. Development, differentiation, and maturation of fetal mouse yolk sac macrophages in cultures. J Leukoc Biol 1989;46(1):1–10.
[22] Takahashi K, Yamamura F, Naito M. Differentiation, maturation, and proliferation of macrophages in the mouse yolk sac: a light-microscopic, enzyme-cytochemical, immunohistochemical, and ultrastructural study. J Leukoc Biol 1989;45(2):87–96.
[23] McGrath KE, et al. Circulation is established in a stepwise pattern in the mammalian embryo. Blood 2003;101(5):1669–76.
[24] Squarzoni P, et al. Microglia modulate wiring of the embryonic forebrain. Cell Rep 2014;8(5):1271–9.
[25] Ginhoux F, Guilliams M. Tissue-resident macrophage ontogeny and homeostasis. Immunity 2016;44(3):439–49.
[26] Schulz C, et al. A lineage of myeloid cells independent of Myb and hematopoietic stem cells. Science 2012;336(6077):86–90.
[27] Naito M, Takahashi K, Nishikawa S. Development, differentiation, and maturation of macrophages in the fetal mouse liver. J Leukoc Biol 1990;48(1):27–37.
[28] Hoeffel G, Ginhoux F. Fetal monocytes and the origins of tissue-resident macrophages. Cell Immunol 2018;330:5–15.
[29] Guilliams M, et al. Alveolar macrophages develop from fetal monocytes that differentiate into long-lived cells in the first week of life via GM-CSF. J Exp Med 2013;210(10):1977–92.

[30] Gomez Perdiguero E, et al. Tissue-resident macrophages originate from yolk-sac-derived erythro-myeloid progenitors. Nature 2015;518(7540):547–51.
[31] Hoeffel G, et al. C-Myb(+) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. Immunity 2015;42(4):665–78.
[32] Bain CC, et al. Long-lived self-renewing bone marrow-derived macrophages displace embryo-derived cells to inhabit adult serous cavities. Nat Commun 2016;7(1). ncomms11852.
[33] Ginhoux F, et al. Fate mapping analysis reveals that adult microglia derive from primitive macrophages. Science 2010;330(6005):841–5.
[34] Coskun S, et al. Development of the fetal bone marrow niche and regulation of HSC quiescence and homing ability by emerging osteolineage cells. Cell Rep 2014;9(2):581–90.
[35] Teh YC, et al. Capturing the fantastic voyage of monocytes through time and space. Front Immunol 2019;10:834.
[36] Wynn TA, Barron L. Macrophages: master regulators of inflammation and fibrosis. Semin Liver Dis 2010;30(3):245–57.
[37] Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. Nature 2013;496(7446):445–55.
[38] Italiani P, Boraschi D. From monocytes to M1/M2 macrophages: phenotypical vs. functional differentiation. Front Immunol 2014;5:514.
[39] Jantsch J, et al. Macrophages in homeostatic immune function. Front Physiol 2014;5:146.
[40] Martinez FO, Gordon S. The M1 and M2 paradigm of macrophage activation: time for reassessment. F1000Prime Rep 2014;6:13.
[41] Chavakis E, Choi EY, Chavakis T. Novel aspects in the regulation of the leukocyte adhesion cascade. Thromb Haemostasis 2009;102(2):191–7.
[42] Deshmane SL, et al. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res Off J Int Soc Interferon & Cytokine Res 2009;29(6):313–26.
[43] Zhang X, Goncalves R, Mosser DM. The isolation and characterization of murine macrophages. Curr Protoc Im 2008;83(1):14.1.1–14.1.14. Chapter 14.
[44] Mogensen TH. Pathogen recognition and inflammatory signaling in innate immune defenses. Clin Microbiol Rev 2009;22(2):240–73 [Table of Contents].
[45] Uribe-Querol E, Rosales C. Control of phagocytosis by microbial pathogens. Front Immunol 2017;8(1368).
[46] Odaka C, Mizuochi T. Role of macrophage lysosomal enzymes in the degradation of nucleosomes of apoptotic cells. J Immunol 1999;163(10):5346–52.
[47] Sato S, et al. Essential function for the kinase TAK1 in innate and adaptive immune responses. Nat Immunol 2005;6(11):1087–95.
[48] Wang N, Liang H, Zen K. Molecular mechanisms that influence the macrophage M1–M2 polarization balance. Front Immunol 2014;5(614).
[49] Liu T, et al. NF-κB signaling in inflammation. Signal Transduc & Targ Ther 2017;2(1):17023.
[50] Latz E. The inflammasomes: mechanisms of activation and function. Curr Opin Immunol 2010;22(1):28–33.
[51] Lee C, et al. Inflammasome as a promising therapeutic target for cancer. Life Sci 2019:116593.
[52] Roche PA, Furuta K. The ins and outs of MHC class II-mediated antigen processing and presentation. Nat Rev Immunol 2015;15(4):203–16.
[53] Novak ML, Koh TJ. Macrophage phenotypes during tissue repair. J Leukoc Biol 2013;93(6):875–81.
[54] Sica A, Mantovani A. Macrophage plasticity and polarization: in vivo veritas. J Clin Invest 2012;122(3):787–95.
[55] Mitchell S, et al. Lipoxins, aspirin-triggered epi-lipoxins, lipoxin stable analogues, and the resolution of inflammation: stimulation of macrophage phagocytosis of apoptotic neutrophils in vivo. J Am Soc Nephrol 2002;13(10):2497–507.
[56] Dalli J, Serhan C. Macrophage proresolving mediators-the when and where. Microbiol Spectr 2016;4(3).
[57] Parola M, Pinzani M. Pathophysiology of organ and tissue fibrosis. Mol Aspect Med 2019;65:1.
[58] Knipper JA, et al. Interleukin-4 receptor α signaling in myeloid cells controls collagen fibril assembly in skin repair. Immunity 2015;43(4):803–16.
[59] Alvarez RH, Kantarjian HM, Cortes JE. Biology of platelet-derived growth factor and its involvement in disease. Mayo Clin Proc 2006;81(9):1241–57.

[60] Pradere JP, et al. Hepatic macrophages but not dendritic cells contribute to liver fibrosis by promoting the survival of activated hepatic stellate cells in mice. Hepatology 2013;58(4):1461–73.
[61] Mack M. Inflammation and fibrosis. Matrix Biol 2018;68–69:106–21.
[62] Duffield JS, et al. Selective depletion of macrophages reveals distinct, opposing roles during liver injury and repair. J Clin Invest 2005;115(1):56–65.
[63] Zhang MZ, et al. CSF-1 signaling mediates recovery from acute kidney injury. J Clin Invest 2012;122(12):4519–32.
[64] Rappolee DA, et al. Wound macrophages express TGF-alpha and other growth factors in vivo: analysis by mRNA phenotyping. Science 1988;241(4866):708–12.
[65] Berse B, et al. Vascular permeability factor (vascular endothelial growth factor) gene is expressed differentially in normal tissues, macrophages, and tumors. Mol Biol Cell 1992;3(2):211–20.
[66] Chujo S, et al. Role of connective tissue growth factor and its interaction with basic fibroblast growth factor and macrophage chemoattractant protein-1 in skin fibrosis. J Cell Physiol 2009;220(1):189–95.
[67] Wynn TA, Vannella KM. Macrophages in tissue repair, regeneration, and fibrosis. Immunity 2016;44(3):450–62.
[68] Ma Y, Pope RM. The role of macrophages in rheumatoid arthritis. Curr Pharmaceut Des 2005;11(5):569–80.
[69] Hotamisligil GS. Inflammation and metabolic disorders. Nature 2006;444(7121):860–7.
[70] Parisi L, et al. Macrophage polarization in chronic inflammatory diseases: killers or builders? J Immunol Res 2018;2018:8917804.
[71] Kierdorf K, et al. Microglia emerge from erythromyeloid precursors via Pu.1- and Irf8-dependent pathways. Nat Neurosci 2013;16(3):273–80.
[72] Katsumoto A, et al. Ontogeny and functions of central nervous system macrophages. J Immunol 2014;193(6):2615–21.
[73] Vilhardt F. Microglia: phagocyte and glia cell. Int J Biochem Cell Biol 2005;37(1):17–21.
[74] Jin X, Yamashita T. Microglia in central nervous system repair after injury. J Biochem 2016;159(5):491–6.
[75] Rapalino O, et al. Implantation of stimulated homologous macrophages results in partial recovery of paraplegic rats. Nat Med 1998;4(7):814–21.
[76] Liu P, et al. Role of macrophages in peripheral nerve injury and repair. Neural Regen Res 2019;14(8):1335–42.
[77] Armstrong BD, et al. Lymphocyte regulation of neuropeptide gene expression after neuronal injury. J Neurosci Res 2003;74(2):240–7.
[78] Sakaguchi M, et al. A carbohydrate-binding protein, Galectin-1, promotes proliferation of adult neural stem cells. Proc Natl Acad Sci USA 2006;103(18):7112–7.
[79] Yamane J, et al. Transplantation of galectin-1-expressing human neural stem cells into the injured spinal cord of adult common marmosets. J Neurosci Res 2010;88(7):1394–405.
[80] Gaudet AD, et al. Galectin-1 in injured rat spinal cord: implications for macrophage phagocytosis and neural repair. Mol Cell Neurosci 2015;64:84–94.
[81] Hochreiter-Hufford A, Ravichandran KS. Clearing the dead: apoptotic cell sensing, recognition, engulfment, and digestion. Cold Spring Harb Perspect Biol 2013;5(1):a008748.
[82] Green DR, et al. Immunogenic and tolerogenic cell death. Nat Rev Immunol 2009;9(5):353–63.
[83] Geissmann F, et al. Development of monocytes, macrophages, and dendritic cells. Science 2010;327(5966):656–61.
[84] Ghosn EE, et al. Two physically, functionally, and developmentally distinct peritoneal macrophage subsets. Proc Natl Acad Sci USA 2010;107(6):2568–73.
[85] Wong K, et al. Phosphatidylserine receptor Tim-4 is essential for the maintenance of the homeostatic state of resident peritoneal macrophages. Proc Natl Acad Sci USA 2010;107(19):8712–7.
[86] Rosas M, et al. The transcription factor Gata6 links tissue macrophage phenotype and proliferative renewal. Science 2014;344(6184):645–8.
[87] Cassado Ados A, D'Imperio Lima MR, Bortoluci KR. Revisiting mouse peritoneal macrophages: heterogeneity, development, and function. Front Immunol 2015;6:225.
[88] Gautier EL, et al. Local apoptosis mediates clearance of macrophages from resolving inflammation in mice. Blood 2013;122(15):2714–22.
[89] Okabe Y, Medzhitov R. Tissue-specific signals control reversible program of localization and functional polarization of macrophages. Cell 2014;157(4):832–44.
[90] Barth MW, et al. Review of the macrophage disappearance reaction. J Leukoc Biol 1995;57(3):361–7.

[91] Ruckerl D, et al. Macrophage origin limits functional plasticity in helminth-bacterial co-infection. PLoS Pathog 2017;13(3):e1006233.
[92] Cain DW, et al. Identification of a tissue-specific, C/EBPβ-dependent pathway of differentiation for murine peritoneal macrophages. J Immunol 2013;191(9):4665–75.
[93] Liao CT, et al. IL-10 differentially controls the infiltration of inflammatory macrophages and antigen-presenting cells during inflammation. Eur J Immunol 2016;46(9):2222–32.
[94] Bellingan GJ, et al. In vivo fate of the inflammatory macrophage during the resolution of inflammation: inflammatory macrophages do not die locally, but emigrate to the draining lymph nodes. J Immunol 1996;157(6):2577–85.
[95] Yona S, et al. Fate mapping reveals origins and dynamics of monocytes and tissue macrophages under homeostasis. Immunity 2013;38(1):79–91.
[96] Bain CC, et al. Origin and microenvironment contribute to the sexually dimorphic phenotype and function of peritoneal macrophages. BioRxiv 2019:837336.
[97] Wiktor-Jedrzejczak W, et al. CSF-1 deficiency in the op/op mouse has differential effects on macrophage populations and differentiation stages. Exp Hematol 1992;20(8):1004–10.
[98] Begg SK, et al. Delayed hematopoietic development in osteopetrotic (op/op) mice. J Exp Med 1993;177(1):237–42.
[99] Dai XM, et al. Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects. Blood 2002;99(1):111–20.
[100] Care AS, et al. Macrophages regulate corpus luteum development during embryo implantation in mice. J Clin Invest 2013;123(8):3472–87.
[101] Ingman WV, et al. Null mutation in transforming growth factor beta1 disrupts ovarian function and causes oocyte incompetence and early embryo arrest. Endocrinology 2006:147.
[102] Rae F, et al. Characterisation and trophic functions of murine embryonic macrophages based upon the use of a Csf1r-EGFP transgene reporter. Dev Biol 2007;308(1):232–46.
[103] Banaei-Bouchareb L, et al. A transient microenvironment loaded mainly with macrophages in the early developing human pancreas. J Endocrinol 2006;188(3):467–80.
[104] Chen L, et al. Tie2 expression on macrophages is required for blood vessel reconstruction and tumor relapse after chemotherapy. Cancer Res 2016;76(23):6828–38.
[105] Deryugina EI, Quigley JP. Tumor angiogenesis: MMP-mediated induction of intravasation- and metastasis-sustaining neovasculature. Matrix Biol 2015;44–46:94–112.
[106] Cohen HB, Mosser DM. Extrinsic and intrinsic control of macrophage inflammatory responses. J Leukoc Biol 2013;94(5):913–9.
[107] Chen S, et al. Epigenetic regulation of macrophages: from homeostasis maintenance to host defense. Cell Mol Immunol 2020;17(1):36–49.
[108] Giefing-Kroll C, et al. How sex and age affect immune responses, susceptibility to infections, and response to vaccination. Aging Cell 2015;14(3):309–21.
[109] Marriott I, Bost KL, Huet-Hudson YM. Sexual dimorphism in expression of receptors for bacterial lipopolysaccharides in murine macrophages: a possible mechanism for gender-based differences in endotoxic shock susceptibility. J Reprod Immunol 2006;71(1):12–27.
[110] Griffin C, et al. Sexually dimorphic myeloid inflammatory and metabolic responses to diet-induced obesity. Am J Physiol Regul Integr Comp Physiol 2016;311(2):R211–6.
[111] Scotland RS, et al. Sex differences in resident immune cell phenotype underlie more efficient acute inflammatory responses in female mice. Blood 2011;118(22):5918–27.
[112] Tedla N, et al. Heterogeneous expression of apolipoprotein-E by human macrophages. Immunology 2004;113(3):338–47.
[113] Brown MB, et al. M1/M2 macrophage polarity in normal and complicated pregnancy. Front Immunol 2014;5:606.
[114] Pradhan P, et al. Interplay of heme with macrophages in homeostasis and inflammation. Int J Mol Sci 2020;21(3).
[115] Li Q, et al. Bisphenol A and phthalates modulate peritoneal macrophage function in female mice involving SYMD2-H3K36 dimethylation. Endocrinology 2018;159(5):2216–28.

[116] Shapouri-Moghaddam A, et al. Macrophage plasticity, polarization, and function in health and disease. J Cell Physiol 2018;233(9):6425–40.
[117] Li L, et al. The immunotoxicity of dibutyl phthalate on the macrophages in mice. Immunopharmacol Immunotoxicol 2013;35(2):272–81.
[118] Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. J Pathol 2002;196(3):254–65.
[119] Riabov V, et al. Role of tumor associated macrophages in tumor angiogenesis and lymphangiogenesis. Front Physiol 2014;5:75.
[120] Wu H, et al. Tumour-associated macrophages mediate the invasion and metastasis of bladder cancer cells through CXCL8. Peer J 2020;8:e8721.
[121] Biswas SK, et al. A distinct and unique transcriptional program expressed by tumor-associated macrophages (defective NF-kappaB and enhanced IRF-3/STAT1 activation). Blood 2006;107(5):2112–22.
[122] Remmerie A, Scott CL. Macrophages and lipid metabolism. Cell Immunol 2018;330:27–42.
[123] Angulo P. Obesity and nonalcoholic fatty liver disease. Nutr Rev 2007;65(6 Pt 2):S57–63.
[124] Kraakman MJ, et al. Macrophage polarization in obesity and type 2 diabetes: weighing down our understanding of macrophage function? Front Immunol 2014;5. 470-470.
[125] Tesch GH. Role of macrophages in complications of type 2 diabetes. Clin Exp Pharmacol Physiol 2007;34(10):1016–9.
[126] Oertelt-Prigione S. Immunology and the menstrual cycle. Autoimmun Rev 2012;11(6–7):A486–92.
[127] Kamat BR, Isaacson PG. The immunocytochemical distribution of leukocytic subpopulations in human endometrium. Am J Pathol 1987;127(1):66–73.
[128] Poropatich C, Rojas M, Silverberg SG. Polymorphonuclear leukocytes in the endometrium during the normal menstrual cycle. Int J Gynecol Pathol 1987;6(3):230–4.
[129] Critchley HO, et al. The endocrinology of menstruation–a role for the immune system. Clin Endocrinol 2001;55(6):701–10.
[130] Thiruchelvam U, et al. The importance of the macrophage within the human endometrium. J Leukoc Biol 2013;93(2):217–25.
[131] Salamonsen LA, Zhang J, Brasted M. Leukocyte networks and human endometrial remodelling. J Reprod Immunol 2002;57(1–2):95–108.
[132] Guo Y, et al. Comprehensive analysis of leukocytes, vascularization and matrix metalloproteinases in human menstrual xenograft model. PLoS One 2011;6(2):e16840.
[133] Jenkins SJ, et al. Local macrophage proliferation, rather than recruitment from the blood, is a signature of TH2 inflammation. Science 2011;332(6035):1284–8.
[134] Cousins FL, et al. Evidence from a mouse model that epithelial cell migration and mesenchymal-epithelial transition contribute to rapid restoration of uterine tissue integrity during menstruation. PLoS One 2014;9(1):e86378.
[135] Garry R, et al. Structural changes in endometrial basal glands during menstruation. BJOG An Int J Obstet Gynaecol 2010;117(10):1175–85.
[136] Maybin JA, et al. The presence and regulation of connective tissue growth factor in the human endometrium. Hum Reprod 2012;27(4):1112–21.
[137] Cousins FL, et al. Evidence for a dynamic role for mononuclear phagocytes during endometrial repair and remodelling. Sci Rep 2016;6. 36748-36748.
[138] Greaves E, et al. A novel mouse model of endometriosis mimics human phenotype and reveals insights into the inflammatory contribution of shed endometrium. Am J Pathol 2014;184(7):1930–9.
[139] Hogg C, et al. Macrophages inhibit and enhance endometriosis depending on their origin. Proc Natl Acad Sci 2021;118:e2013776118. USA.
[140] Lucas T, et al. Differential roles of macrophages in diverse phases of skin repair. J Immunol 2010;184(7):3964–77.
[141] Vigano P, et al. Time to redefine endometriosis including its pro-fibrotic nature. Hum Reprod 2017;33(3):347–52.
[142] Vallve-Juanico J, et al. Macrophages display proinflammatory phenotypes in the eutopic endometrium of women with endometriosis with relevance to an infectious etiology of the disease. Fertil Steril 2019;112(6):1118–28.

[143] Cao X, et al. The presence of endometrial cells in the peritoneal cavity enhances monocyte recruitment and induces inflammatory cytokines in mice: implications for endometriosis. Fertil Steril 2004;82(Suppl. 3):999–1007.
[144] Hornung D, et al. Chemokine bioactivity of RANTES in endometriotic and normal endometrial stromal cells and peritoneal fluid. Mol Hum Reprod 2001;7(2):163–8.
[145] D'Hooghe TM, et al. Effect of menstruation and intrapelvic injection of endometrium on inflammatory parameters of peritoneal fluid in the baboon (*Papio anubis* and *Papio cynocephalus*). Am J Obstet Gynecol 2001;184(5):917–25.
[146] Bacci M, et al. Macrophages are alternatively activated in patients with endometriosis and required for growth and vascularization of lesions in a mouse model of disease. Am J Pathol 2009:175.
[147] Burns KA, et al. Early endometriosis in females is directed by immune-mediated estrogen receptor alpha and IL-6 cross-talk. Endocrinology 2018;159(1):103–18.
[148] Johan MZ, et al. Macrophages infiltrating endometriosis-like lesions exhibit progressive phenotype changes in a heterologous mouse model. J Reprod Immunol 2019;132:1–8.
[149] Maruyama T, Yoshimura Y. Molecular and cellular mechanisms for differentiation and regeneration of the uterine endometrium. Endocr J 2008;55(5):795–810.
[150] Halme J, Becker S, Haskill S. Altered maturation and function of peritoneal macrophages: possible role in pathogenesis of endometriosis. Am J Obstet Gynecol 1987;156(4):783–9.
[151] Oosterlynck DJ, et al. Immunohistochemical characterization of leucocyte subpopulations in endometriotic lesions. Arch Gynecol Obstet 1993;253(4):197–206.
[152] McKinnon B, et al. PPAR-gamma expression in peritoneal endometriotic lesions correlates with pain experienced by patients. Fertil Steril 2010;93(1):293–6.
[153] Harada T, Iwabe T, Terakawa N. Role of cytokines in endometriosis. Fertil Steril 2001;76(1):1–10.
[154] Kalu E, et al. Cytokine profiles in serum and peritoneal fluid from infertile women with and without endometriosis. J Obstet Gynaecol Res 2007;33(4):490–5.
[155] Gmyrek GB, et al. Differential flow cytometric detection of intracellular cytokines in peripheral and peritoneal mononuclear cells of women with endometriosis. Eur J Obstet Gynecol Reprod Biol 2008;137(1):67–76.
[156] Fan YY, et al. Expression of inflammatory cytokines in serum and peritoneal fluid from patients with different stages of endometriosis. Gynecol Endocrinol 2018;34(6):507–12.
[157] Oosterlynck DJ, et al. Transforming growth factor-beta activity is increased in peritoneal fluid from women with endometriosis. Obstet Gynecol 1994;83(2):287–92.
[158] Dou Q, Williams RS, Chegini N. Inhibition of transforming growth factor-beta 1 alters the growth, anchor-dependent cell aggregation and integrin mRNA expression in human promonocytes: implications for endometriosis and peritoneal adhesion formation. Mol Hum Reprod 1997;3(5):383–91.
[159] Young VJ, et al. Transforming growth factor-beta induced Warburg-like metabolic reprogramming may underpin the development of peritoneal endometriosis. J Clin Endocrinol Metab 2014;99(9):3450–9.
[160] Young VJ, et al. The peritoneum is both a source and target of TGF-beta in women with endometriosis. PLoS One 2014;9(9):e106773.
[161] Beste MT, et al. Molecular network analysis of endometriosis reveals a role for c-Jun-regulated macrophage activation. Sci Transl Med 2014;6(222):222ra16.
[162] Smith KA, et al. Alternative activation of macrophages in rhesus macaques (*Macaca mulatta*) with endometriosis. Comp Med 2012;62(4):303–10.
[163] Lin YJ, et al. Neutrophils and macrophages promote angiogenesis in the early stage of endometriosis in a mouse model. Endocrinology 2006;147(3):1278–86.
[164] Lolmede K, et al. Inflammatory and alternatively activated human macrophages attract vessel-associated stem cells, relying on separate HMGB1- and MMP-9-dependent pathways. J Leukoc Biol 2009;85(5):779–87.
[165] Hull ML, et al. Host-derived TGFB1 deficiency suppresses lesion development in a mouse model of endometriosis. Am J Pathol 2012;180(3):880–7.
[166] Cassetta L, Cassol E, Poli G. Macrophage polarization in health and disease. Sci World J 2011;11:2391–402.
[167] Guo S, Dipietro LA. Factors affecting wound healing. J Dent Res 2010;89(3):219–29.
[168] Kloc M, et al. Macrophage functions in wound healing. J Tissue Eng Regen Med 2019;13(1):99–109.
[169] Tonkin J, et al. Monocyte/macrophage-derived IGF-1 orchestrates murine skeletal muscle regeneration and modulates autocrine polarization. Mol Ther: J. Amer. Soc. Gene Ther 2015;23(7):1189–200.
[170] Nunan R, Harding KG, Martin P. Clinical challenges of chronic wounds: searching for an optimal animal model to recapitulate their complexity. Dis Model Mech 2014;7(11):1205–13.

[171] Leyendecker G, Wildt L. A new concept of endometriosis and adenomyosis: tissue injury and repair (TIAR). Horm Mol Biol Clin Investig 2011;5(2):125.
[172] Guo S-W. Fibrogenesis resulting from cyclic bleeding: the Holy Grail of the natural history of ectopic endometrium. Hum Reprod 2018;33(3):353–6.
[173] Hong K, Choi Y. Role of estrogen and RAS signaling in repeated implantation failure. BMB Rep 2018;51(5):225–9.
[174] Guo SW, Zilberberg MD, Hummelshoj L. Endometriosis and ovarian cancer. Lancet Oncol 2012;13(5):e189–90. Author reply e190.
[175] Liu H, et al. Correlation between matrix metalloproteinase-9 and endometriosis. Int J Clin Exp Pathol 2015;8(10):13399–404.
[176] McLaren J, et al. Vascular endothelial growth factor is produced by peritoneal fluid macrophages in endometriosis and is regulated by ovarian steroids. J Clin Invest 1996;98(2):482–9.
[177] Campbell L, et al. Estrogen receptor-alpha promotes alternative macrophage activation during cutaneous repair. J Invest Dermatol 2014;134(9):2447–57.
[178] Greaves E, et al. Estradiol is a critical mediator of macrophage-nerve cross talk in peritoneal endometriosis. Am J Pathol 2015;185(8):2286–97.
[179] Duan J, et al. The M2a macrophage subset may be critically involved in the fibrogenesis of endometriosis in mice. Reprod Biomed Online 2018;37(3):254–68.
[180] Chan RWS, et al. Co-culture with macrophages enhances the clonogenic and invasion activity of endometriotic stromal cells. Cell Prolifer 2017;50(3):e12330.
[181] Jensen JR, et al. A potential role for colony-stimulating factor 1 in the genesis of the early endometriotic lesion. Fertil Steril 2010;93(1):251–6.
[182] Aligeti S, et al. Colony-stimulating factor-1 exerts direct effects on the proliferation and invasiveness of endometrial epithelial cells. Fertil Steril 2011;95(8):2464–6.
[183] Mei J, et al. Indoleamine 2,3-dioxygenase-1 (IDO1) in human endometrial stromal cells induces macrophage tolerance through interleukin-33 in the progression of endometriosis. Int J Clin Exp Pathol 2014;7(6):2743–57.
[184] Groothuis PG. Angiogenesis and endometriosis. In: Endometriosis. Wiley-Blackwell; 2012. p. 190–9.
[185] Maas JW, et al. Tumor necrosis factor-alpha but not interleukin-1 beta or interleukin-8 concentrations correlate with angiogenic activity of peritoneal fluid from patients with minimal to mild endometriosis. Fertil Steril 2001;75(1):180–5.
[186] Cho S, et al. Expression of vascular endothelial growth factor (VEGF) and its soluble receptor-1 in endometriosis. Microvasc Res 2012;83(2):237–42.
[187] Krikun G. Endometriosis, angiogenesis and tissue factor, vol. 2012. Scientifica; 2012. p. 306830.
[188] Lohela M, et al. VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. Curr Opin Cell Biol 2009;21(2):154–65.
[189] Hull ML, et al. Antiangiogenic agents are effective inhibitors of endometriosis. J Clin Endocrinol Metab 2003;88.
[190] Sekiguchi K, et al. VEGF receptor 1-expressing macrophages recruited from bone marrow enhances angiogenesis in endometrial tissues. Sci Rep 2019;9(1):7037.
[191] Mert T, et al. Macrophage depletion delays progression of neuropathic pain in diabetic animals. Naunyn-Schmiedeberg's Arch Pharmacol 2009;379(5):445–52.
[192] Miller RE, et al. CCR2 chemokine receptor signaling mediates pain in experimental osteoarthritis. Proc Natl Acad Sci USA 2012;109(50):20602–7.
[193] Chen O, Donnelly CR, Ji RR. Regulation of pain by neuro-immune interactions between macrophages and nociceptor sensory neurons. Curr Opin Neurobiol 2019;62:17–25.
[194] Samah B, Porcheray F, Gras G. Neurotrophins modulate monocyte chemotaxis without affecting macrophage function. Clin Exp Immunol 2008;151(3):476–86.
[195] Tran LV, et al. Macrophages and nerve fibres in peritoneal endometriosis. Hum Reprod 2009;24(4):835–41.
[196] Greaves E, et al. Relevant human tissue resources and laboratory models for use in endometriosis research. Acta Obstet Gynecol Scand 2017;96(6):644–58.
[197] Forster R, et al. Macrophage-derived insulin-like growth factor-1 is a key neurotrophic and nerve-sensitizing factor in pain associated with endometriosis. FASEB J 2019;33(10):11210–22.
[198] Wu J, et al. Macrophage and nerve interaction in endometriosis. J Neuroinflammation 2017;14(1):53.
[199] Dodds KN, et al. Spinal glial adaptations occur in a minimally invasive mouse model of endometriosis: potential implications for lesion etiology and persistent pelvic pain. Reprod Sci 2019. 1933719118773405.

[200] Ahmed AA, Wahbi AH, Nordlin K. Neuropeptides modulate a murine monocyte/macrophage cell line capacity for phagocytosis and killing of Leishmania major parasites. Immunopharmacol Immunotoxicol 2001;23(3):397–409.
[201] Muschter D, et al. Sensory neuropeptides and their receptors participate in mechano-regulation of murine macrophages. Int J Mol Sci 2019;20(3):503.
[202] Serocki M, et al. miRNAs regulate the HIF switch during hypoxia: a novel therapeutic target. Angiogenesis 2018;21(2):183–202.
[203] Kumar V, Gabrilovich DI. Hypoxia-inducible factors in regulation of immune responses in tumour microenvironment. Immunology 2014;143(4):512–9.
[204] Tazzyman S, et al. Macrophage-mediated response to hypoxia in disease. Hypoxia 2014;2:185–96.
[205] Werno C, et al. Knockout of HIF-1alpha in tumor-associated macrophages enhances M2 polarization and attenuates their pro-angiogenic responses. Carcinogenesis 2010;31(10):1863–72.
[206] Soria G, et al. Concomitant expression of the chemokines RANTES and MCP-1 in human breast cancer: a basis for tumor-promoting interactions. Cytokine 2008;44(1):191–200.
[207] Pasquier J, et al. CCL2/CCL5 secreted by the stroma induce IL-6/PYK2 dependent chemoresistance in ovarian cancer. Mol Cancer 2018;17(1):47.
[208] Kelly BD, et al. Cell type-specific regulation of angiogenic growth factor gene expression and induction of angiogenesis in nonischemic tissue by a constitutively active form of hypoxia-inducible factor 1. Circ Res 2003;93(11):1074–81.
[209] Eubank TD, et al. Opposing roles for HIF-1alpha and HIF-2alpha in the regulation of angiogenesis by mononuclear phagocytes. Blood 2011;117(1):323–32.
[210] Sharkey AM, et al. Vascular endothelial growth factor expression in human endometrium is regulated by hypoxia. J Clin Endocrinol Metab 2000;85(1):402–9.
[211] Maybin JA, et al. Hypoxia and hypoxia inducible factor-1alpha are required for normal endometrial repair during menstruation. Nat Commun 2018;9(1):295.
[212] Koos RD, et al. New insight into the transcriptional regulation of vascular endothelial growth factor expression in the endometrium by estrogen and relaxin. Ann N Y Acad Sci 2005;1041:233–47.
[213] Xu TX, et al. Hypoxia responsive miR-210 promotes cell survival and autophagy of endometriotic cells in hypoxia. Eur Rev Med Pharmacol Sci 2016;20(3):399–406.
[214] Wu MH, et al. Estrogen receptor expression affected by hypoxia inducible factor-1alpha in stromal cells from patients with endometriosis. Taiwan J Obstet Gynecol 2012;51(1):50–4.
[215] Wu MH, Hsiao KY, Tsai SJ. Hypoxia: the force of endometriosis. J Obstet Gynaecol Res 2019;45(3):532–41.
[216] Anglesio MS, et al. Cancer-associated mutations in endometriosis without cancer. N Engl J Med 2017;376(19):1835–48.
[217] Suda K, et al. Clonal expansion and diversification of cancer-associated mutations in endometriosis and normal endometrium. Cell Rep 2018;24(7):1777–89.
[218] Okada H, Kohanbash G, Lotze MT. MicroRNAs in immune regulation–opportunities for cancer immunotherapy. Int J Biochem Cell Biol 2010;42(8):1256–61.
[219] Dinulescu D. Molecular mechanisms underlying endometriosis and endometriosis-related cancers. In: Endometriosis. Wiley-Blackwell; 2012. p. 512–8.
[220] Yan GJ, et al. MicroRNA miR-302 inhibits the tumorigenicity of endometrial cancer cells by suppression of Cyclin D1 and CDK1. Cancer Lett 2014;345(1):39–47.
[221] Furuya M, et al. Impaired CXCL4 expression in tumor-associated macrophages (TAMs) of ovarian cancers arising in endometriosis. Cancer Biol Ther 2012;13(8):671–80.
[222] Pervushina O, et al. Platelet factor 4/CXCL4 induces phagocytosis and the generation of reactive oxygen metabolites in mononuclear phagocytes independently of Gi protein activation or intracellular calcium transients. J Immunol 2004;173(3):2060–7.
[223] Gleissner CA, et al. CXC chemokine ligand 4 induces a unique transcriptome in monocyte-derived macrophages. J Immunol 2010;184(9):4810–8.
[224] Erbel C, et al. CXCL4-induced plaque macrophages can be specifically identified by co-expression of MMP7+S100A8+ in vitro and in vivo. Innate Immun 2015;21(3):255–65.
[225] Giudice LC. Clinical practice. Endometriosis. N Engl J Med 2010;362(25):2389–98.
[226] Parazzini F, Vercellini P, Pelucchi C. Endometriosis: epidemiology, and etiological factors. In: Endometriosis. Wiley-Blackwell; 2012. p. 19–26.

[227] Santulli P, et al. Increased rate of spontaneous miscarriages in endometriosis-affected women. Hum Reprod 2016;31(5):1014–23.
[228] Farland LV, et al. Endometriosis and risk of adverse pregnancy outcomes. Obstet Gynecol 2019;134(3):527–36.
[229] Schumacher A, et al. Immune cells at the fetomaternal interface: how the microenvironment modulates immune cells to foster fetal development. J Immunol 2018;201(2):325–34.
[230] Robertson SA, et al. Role of high molecular weight seminal vesicle proteins in eliciting the uterine inflammatory response to semen in mice. J Reprod Fertil 1996;107(2):265–77.
[231] Lash GE, et al. Decidual macrophages: key regulators of vascular remodeling in human pregnancy. J Leukoc Biol 2016;100(2):315–25.
[232] McGuane JT, et al. Seminal plasma promotes lesion development in a xenograft model of endometriosis. Am J Pathol 2015;185(5):1409–22.
[233] Akoum A, et al. Secretion of monocyte chemotactic protein-1 by cytokine-stimulated endometrial cells of women with endometriosis. Le groupe d'investigation en gynecologie. Fertil Steril 1995;63(2):322–8.
[234] Jolicoeur C, et al. Increased expression of monocyte chemotactic protein-1 in the endometrium of women with endometriosis. Am J Pathol 1998;152(1):125–33.
[235] Veillat V, et al. Macrophage migration inhibitory factor is involved in a positive feedback loop increasing aromatase expression in endometriosis. Am J Pathol 2012;181(3):917–27.
[236] Xie J, et al. Macrophage migration inhibitor factor upregulates MCP-1 expression in an autocrine manner in hepatocytes during acute mouse liver injury. Sci Rep 2016;6:27665.
[237] Cao WG, et al. Stimulation of macrophage migration inhibitory factor expression in endometrial stromal cells by interleukin 1, beta involving the nuclear transcription factor NFkappaB. Biol Reprod 2005;73(3):565–70.
[238] Khoufache K, et al. Anomalies in the inflammatory response in endometriosis and possible consequences: a review. Minerva Endocrinol 2012;37(1):75–92.
[239] Anton L, et al. Lipopolysaccharide induces cytokine production and decreases extravillous trophoblast invasion through a mitogen-activated protein kinase-mediated pathway: possible mechanisms of first trimester placental dysfunction. Hum Reprod 2012;27(1):61–72.
[240] Osborn BH, et al. Inducible nitric oxide synthase expression by peritoneal macrophages in endometriosis-associated infertility. Fertil Steril 2002;77(1):46–51.
[241] Wu MY, et al. Nitric oxide synthesis is increased in the endometrial tissue of women with endometriosis. Hum Reprod 2003;18(12):2668–71.
[242] Agarwal A, Gupta S, Sharma R. Oxidative stress and its implications in female infertility - a clinician's perspective. Reprod Biomed Online 2005;11(5):641–50.
[243] Carvalho LF, et al. Minimal and mild endometriosis negatively impact on pregnancy outcome. Rev Assoc Med Bras 2012;58(5):607–14.
[244] Vaisanen-Tommiska MR. Nitric oxide in the human uterine cervix: endogenous ripening factor. Ann Med 2008;40(1):45–55.
[245] Vaisanen-Tommiska M, Mikkola TS, Ylikorkala O. Increased release of cervical nitric oxide in spontaneous abortion before clinical symptoms: a possible mechanism for preabortal cervical ripening. J Clin Endocrinol Metab 2004;89(11):5622–6.
[246] Patel BG, et al. Progesterone resistance in endometriosis: origins, consequences and interventions. Acta Obstet Gynecol Scand 2017;96(6):623–32.
[247] Wang DB, et al. [Study of tissue morphology and vascular endothelial growth factor and matrix metalloproteinase-9 gene expressions in nude mouse endometriosis model]. Zhonghua Fu Chan Ke Za Zhi 2004;39(11):759–62.
[248] Cominelli A, et al. Matrix metalloproteinase-27 is expressed in CD163+/CD206+ M2 macrophages in the cycling human endometrium and in superficial endometriotic lesions. Mol Hum Reprod 2014;20(8):767–75.
[249] Su M-T, et al. Gene-gene interactions and gene polymorphisms of VEGFA and EG-VEGF gene systems in recurrent pregnancy loss. J Assist Reprod Genet 2014;31(6):699–705.
[250] Capobianco A, et al. Proangiogenic Tie2(+) macrophages infiltrate human and murine endometriotic lesions and dictate their growth in a mouse model of the disease. Am J Pathol 2011;179(5):2651–9.
[251] Poh AR, Ernst M. Targeting macrophages in cancer: from bench to bedside. Front Oncol 2018;8:49.

This page intentionally left blank

CHAPTER

3

Dendritic cells

Gentaro Izumi, Kaori Koga and Yutaka Osuga

Department of Obstetrics and Gynecology, The University of Tokyo Hospital, Tokyo, Japan

Introduction

Dendritic cells (DCs) are heterogeneous bone-marrow derived cells and known to be professional antigen-presenting cells. The function of DCs is to recognize antigens, phagocyte them and present them to T lymphocytes. In addition, DCs also stimulate various cytokines and costimulatory molecules to determine immune activation or anergy [1]. Thus, DCs bridge innate and adoptive immune systems and orient immune response.

In 1973, Ralph Steinman and Zan Cohn discovered novel cell types in adherent cells from mouse lymphoid organs [2]. These cells were a part of mononuclear phagocyte, but they were distinguished from monocytes and macrophages on the basis of several features. Thus, they named this population as "dendritic cell" after their unique stellate morphology.

Since DCs controls a variety of immune response, they had been thought heterogeneous. In reality, technical developments such as monoclonal antibodies enable to analyze DC subsets, and numerous studies have been discussed about the classification of DCs. Broadly, DCs are divided into two subsets; conventional/myeloid DCs (cDCs) and plasmacytoid DCs (pDC). cDCs are also called "classical DCs", and pDCs are an additional DC subset, which are characterized by their ability to produce large amount of type I interferon.

DCs and monocytes develops from common myeloid precursor cells (CMP). Pre-DC develops from CMP and they differentiate into pDCs and cDCs. cDCs are divided into two major subsets: cDC1 and cDC2. They are immature DCs at first, and they have higher ability to recognize and phagocyte antigens. After inflammatory stimulation with antigen, they differentiate into mature DCs, which have higher ability of migration, antigen presentation, and T cell differentiation. Thus, the combination of these DC subsets fine-tuned the immune environment in steady-state, and their aberrant function causes inflammatory or immune disease.

As mentioned in other sections, many studies reported aberrant functions of helper T cell or regulatory T cells, which differentiated under the control of DCs. Macrophages, another subset of antigen-presenting cells, were also reported as a key factor in the pathogenesis of endometriosis, however, their ability of T cell differentiation and proliferation is far lower

https://doi.org/10.1016/B978-0-12-820661-4.00014-5

© 2022 Elsevier Inc. All rights reserved.

than that of DCs. Thus, we can speculate that DCs must play an important role in endometriosis development. It is true the number of DC study in endometriosis is relatively lower than that of T cells and macrophages, but several important studies were published to develop new insight into endometriosis pathology and novel therapeutic strategies. In this section, we review these studies and discuss DC contribution in endometriosis and future direction of DC study.

DC population in endometriosis patients

Endometrium and peritoneal cavity were known to be important organ in the development of endometriosis. Many studies reported that aberrant T cell profile or function in these organs [3,4]. Thus, several studies hypothesized that abnormal DC frequency in uterus or peritoneal cavity may contribute endometriosis (Table 3.1).

Focusing on DC populations in endometrium, Schuke et al. reported two interesting DC profile change in the endometriosis patients [5]. First, using CD1a as a marker of immature DC, they reported that fluctuation of immature DC during menstrual cycle were lost in endometriosis patients. Immature DC in basal layers of endometrium were increased during secretory phase than proliferative phase in non-endometriosis patients, however, there are no difference in endometriosis patients. Next, using CD83 as a marker of mature DC, the frequency of mature DC were significantly lower in endometriosis patients during all phase of menstrual cycle.

Focusing on peritoneal DCs, Tariverdian et al. also analyzed peritoneal leukocytes of infertile patients [6]. Their result was not statistically significant, but they revealed moderate decrease of the number of HLA-DR+ CD11c+ CD123+ peritoneal DC in endometriosis patients. To analyze peritoneal DC population in detail, we use BDCA3 and BDCA1 as a

TABLE 3.1 Summary of DC frequency analysis.

Tissue	DC markers	Feature of endometriosis patients	References
Endometrium	CD1a+ immature DC	Loss of fluctuation during menstrual cycle	Schuke et al. [5]
	CD83+ mature DC	Lower frequency	Schuke et al. [5]
Peritoneal cavity	HLA-DR+ CD11c+ CD123+ DC	Moderate decrease	Tariverdian et al. [6]
	BDCA3+ CD14− cDC1	No change	Izumi et al. [7]
	BDCA1+ CD19− cDC2	No change	Izumi et al. [7]
	BDCA2+ pDC	No change	Izumi et al. [7]
	CD83+ BDCA3+ CD14-mature cDC1	No change	Izumi et al. [7]
	CD83+ BDCA1+ CD19-mature cDC2	No change	Izumi et al. [7]

marker of cDC1 (=MDC2) and cDC2 (=MDC1) respectively. In addition, we use CD83 as a marker of their mature subtypes. However, there was no significant difference of the frequency of cDC1 and cDC2 and their mature subtypes [7].

The cause of fluctuation of immature DC number in the endometrium during normal menstrual cycle is still unclear. One hypothesis is DC generation were affected by ovarian steroid. However, we have no strong consensus about their effect on DC generation. Human hematopoietic stem cell express ERs [8]. Mice hematopoietic stem cell, monocyte DC precursors and common DC progenitors also express Esr1, Esr2 and Pgr (The Immunological Genome Project; http://www.immgen.org/). Thus, DC generation were thought to be affected by ovarian steroid. However, both positive effect and negative effect of estradiol on DC generation were reported [9–11]. In contrast to estrogen, few studies showed progesterone effect on DC generation, but Ivanova et al. reported that progesterone induce monocyte derived dendritic cells [12]. Thus, we can speculate that endometrial DC population change during menstrual cycle is induced not by systematic change but local factors, such as growth factors, cytokines, and cell-to-cell interactions. The fact that no study reported the fluctuation of DC frequency in the peritoneal cavity also supports this hypothesis.

Maturation of DC is induced by various factors such as inflammatory cytokines, bacterial, and viral induced antigens. The frequency of mature DCs is lower in the endometrium of endometriosis patients [13], but not changed in the peritoneal cavity [7]. In classical paradigm, mature DCs migrate to lymph nodes to present antigens to naïve T cells. Then, there is a question why mature DC increased in endometrium of endometriosis patients. One possible mechanism is endometriosis-related inflammatory stimuli induce DC maturation and the number of mature DC increased both endometrium and regional lymph nodes. Another is that a part of endometrium DC is tissue resident DC subtype, which is not migrate to lymph node even after maturation, and their number increases in endometriosis patients. To discuss this mechanism, further study with new technology to describe DC heterogeneity will be needed.

Plasmacytoid dendritic cell

PDC is a distinct DC subset from conventional myeloid DCs. It detects pathogen-derived nucleic acid and produce massive type I interferon.

Interferon has been postulated to be a therapeutic target of endometriosis. Ingelmo et al. reported interferon alpha-2b administration to peritoneal cavity or subcutaneous reduced the size of endometriotic lesion of rat model [14]. On the other hand, a clinical trial showed interferon alpha-2b progress endometriosis of human. They administrated interferon alpha-2b into peritoneal cavity during conservative surgery of endometriosis, and showed higher recurrence rate of endometriosis [15]. Thus, the role of interferon on the pathogenesis of endometriosis is still unclear.

Few studies on endometriosis examined pDC, which is an important producer of type I interferon, but one study focused on another feature of plasmacytoid dendritic cell. Suen et al. reported pDC is a major source of antiinflammatory cytokines, Interleukin 10 (IL10) in the endometriosis lesion [16]. They also reported that pDC administration increased endometriotic lesion in mouse model, but pDC from IL10 knockout mouse did not [17].

On the other hand, in the peritoneal cavity, the frequency of pDC is not altered in endometriosis patients compared with control [7], and no study focused on the function of peritoneal pDCs. Thus, we need further study to prove that pDC contribute to the pathogenensis of endometriosis.

Animal study using endometriosis mouse models

To evaluate the development of endometriosis in vivo, endometriosis mouse model is used [18]. Mice do not develop spontaneous endometriosis because they lack a menstruation, and their uterine cavity and fallopian tubes are isolated from peritoneal cavity. Thus, to induce endometriosis-like lesion, we need to transfer endometrial tissue to their peritoneal cavity. However, the details of the protocols are varied by their aim of the studies. Their whole uterine or scraped endometrium is transplanted to the peritoneal cavity by injection or surgical suture. After transplantation, endometrial tissue engrafts on the peritoneum and develops into endometriosis-like lesion. In some study, E2 is administrated to enhance endometriosis-like lesion formation. Ovariectomized mice may be used to avoid internal ovarian steroid during estrus cycle.

In 2008, Fainaru et al. demonstrated that injection of granulocyte macrophage colony-stimulating factor (GM−CSF)−derived DCs into the peritoneal cavity of endometriosis model mice promoted lesion formation. Their result suggested that DCs, by enhancing angiogenesis, contribute to the lesion growth [13]. GM−CSF−derived dendritic cells are artificially induced DC-like cells, which develops from mice bone-marrow cells or human blood monocytes after in vitro culture with GM-CSF and Interleukin-4 [19,20]. It is still discussing that GM−CSF−derived DC is spontaneous DC, however, it is the best method to obtain large amount of human DCs. Thus, GM−CSF−derived DC is widely used in immune therapy against malignant neoplasm. Thus, GM−CSF−derived DC would be potentially used for the treatment of endometriosis, if future study would reveal novel GM−CSF−derived DC subtype to regress endometriosis.

CD11c+ DTR/GFP trangesnic mice were established by Jung et al. in 2002, to place human diphtheria toxin receptor (DTR) under the control of the promoter of Itgax (CD11c), which is DC specific surface marker protein. In these transgenic mice, CD11c+ cells express DTR and temporarily depleted after diphtheria toxin administration. It is true that certain macrophage subtypes also express CD11c and their depletion may cause for the effect of diphtheria toxin administration [21]; however, they were widely used for analysis of DC functions.

Using the transgenic mice, two studies generated contradictory results. Pencovich et al. demonstrated that endometriotic lesion formation was reduced in their DC depleted mice [22]; however, Stanic et al. reported that depletion resulted in greater lesion size [23]. The comparison of protocol of two studies is shown in Table 3.2. Many difference of the study design may account for the outcomes; however the timing of diphtheria toxin injection should be the most important. These results imply that DCs prevent the establishment of endometriotic lesions by activating T cells, but they promote the progression of endometriotic lesions after it formed. Another thing we need to consider when reading these result is a

TABLE 3.2 Comparison of two studies on endometriosis model of DC depleted mouse.

	Stanic et al. [23]	Pencovich et al. [22]
Recipient strain	C57BL/6, B6.FVB-Itagx-hDTR-EGFPtg	
Diphtheria toxin doze	4 ng/g	3 ng/g
Diphtheria toxin administration	**Day −1**	**Day 2, 5, and 9**
Evaluation date	Day 7	Day 10
E2 treatment	Subcuateous implant	–
Ovaluectomy	No	
Transplanted tissue	Minced uterine	Self-uterine hone
Treatment for donor	E2 on day −4	–
Method of transplantation	Peritoneal injection	Punched tissue suture
Main result	Lesion weight	Lesion area
Result (compared with control)	**Higher**	**Lower**
Other result	Lower CD69 MFI on T cell	Reduced endometrial tisuue

part of macrophages also express CD11c and might depleted in these mice model. In fact, a report in reproductive medicine showed that the response after diphtheria toxin injection of this transgenic mouse is attributed to not DC depletion but a subset of macrophage depletion [24].

Molecular target on dendritic cells

As mentioned above, the contribution of DCs on the pathogenesis of endometriosis is still controversial. One possible reason of that is DCs are heterogenous and multifunctional. Since total DCs are discussed, their subpopulations which have different functions are huddled, and may cause these inconsistent results.

Thus, more DC subpopulation specific study is needed to explore therapeutic target on DCs. Since BDCA1+ cDC2 is a major DC subset in peritoneal cavity, we analyzed their surface markers of the endometriosis patients. Several pattern recognition receptors, such as DEC-205, DC-SIGN, on the peritoneal cDC2 are evaluated by flow cytometry, and the frequency of macrophage mannose receptor (MMR) positive cells of cDC2s is higher in endometriosis patients than in controls [7]. Since retrograde menstruation is well known risk factor of endometriosis, we hypothesized MMR+ cDC2 interact with debris of retrograde menstruation to progress endometriosis. In vitro assay indicated that MMR+ DCs had higher ability of phagocytosis of dead endometrial cells and produce inflammatory cytokines to progress endometriosis. Thus we concluded MMR+ cDC2 progress endometriosis by interaction with retrograde menstruation.

Taking this idea one step further, modulating MMR expression and/or activity may enable the regulation of immune responses and potentially help treat endometriosis. However, it is still unclear whether MMR+ cDC2 is distinct subset from MMR-cDC2. Further studies about cDC2 subpopulation are needed to clarify the pathogenesis of endometriosis.

Discussion

Though the relatively small number of studies are published, these studies evidenced that DCs are not bystander but one of the key players in endometriosis pathology. However, DCs functions and their subpopulations are still unclear. More precise knowledge of DC subpopulation is needed to reveal the relationship between endometriosis and DCs.

Recent studies showed that cDC2s are divided into several clusters, which have distinct RNA expression patterns [25]. These clustering will contribute to defining cDC2 subpopulations and their specific functions. In addition, these studies revealed a subgroup of cDC2 showed monocyte markers and monocyte signature genes, and they proposed to call them "cDC3" [25]. If this concept is established, we need to review all studies about DCs and monocyte-macrophages, because we have been categorized these DCs as monocyte linage cells (Fig. 3.1).

It is true that DCs have their tissue specific subtypes, and their surface marker, functions, and development are not common between all organs, but subpopulation of DCs in endometriosis lesion or peritoneal cavity would be revealed in the future, and contribute to establish novel therapeutic approach against endometriosis.

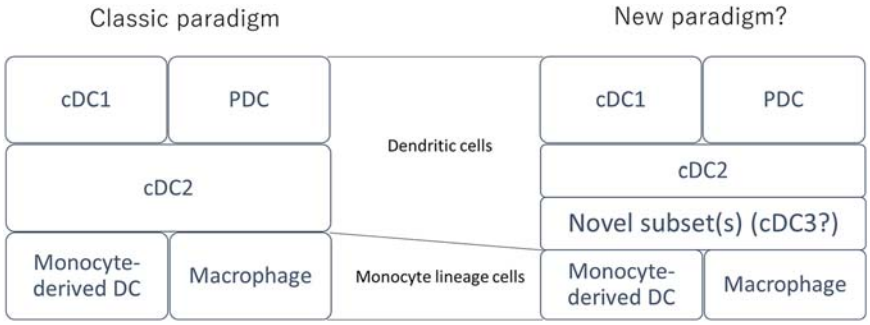


FIGURE 3.1 Paradigm shift of classification of antigen-presenting cells.

References

[1] Steinman RM. Decisions about dendritic cells: past, present, and future. Annu Rev Immunol 2012;30:1–22.
[2] Steinman RM, Cohn ZA. Identification of a novel cell type in peripheral lymphoid organs of mice. I. Morphology, quantitation, tissue distribution. J Exp Med 1973;137(5):1142–62.
[3] Szyllo K, et al. The involvement of T lymphocytes in the pathogenesis of endometriotic tissues overgrowth in women with endometriosis. Mediat Inflamm 2003;12(3):131–8.
[4] Takamura M, et al. Simultaneous detection and evaluation of four subsets of CD4+ T lymphocyte in lesions and peripheral blood in endometriosis. Am J Reprod Immunol 2015;74(6):480–6.
[5] Schulke L, et al. Dendritic cell populations in the eutopic and ectopic endometrium of women with endometriosis. Hum Reprod 2009;24(7):1695–703.

[6] Tariverdian N, et al. Intraperitoneal immune cell status in infertile women with and without endometriosis. J Reprod Immunol 2009;80(1–2):80–90.
[7] Izumi G, et al. Mannose receptor is highly expressed by peritoneal dendritic cells in endometriosis. Fertil Steril 2017;107(1):167–73 e2.
[8] Igarashi H, et al. Age and stage dependency of estrogen receptor expression by lymphocyte precursors. Proc Natl Acad Sci USA 2001;98(26):15131–6.
[9] Paharkova-Vatchkova V, Maldonado R, Kovats S. Estrogen preferentially promotes the differentiation of CD11c+ CD11b(intermediate) dendritic cells from bone marrow precursors. J Immunol 2004;172(3):1426–36.
[10] Seillet C, et al. Estradiol promotes functional responses in inflammatory and steady-state dendritic cells through differential requirement for activation function-1 of estrogen receptor alpha. J Immunol 2013;190(11):5459–70.
[11] Carreras E, et al. Estradiol acts directly on bone marrow myeloid progenitors to differentially regulate GM-CSF or Flt3 ligand-mediated dendritic cell differentiation. J Immunol 2008;180(2):727–38.
[12] Ivanova E, et al. CD83 monocyte-derived dendritic cells are present in human decidua and progesterone induces their differentiation in vitro. Am J Reprod Immunol 2005;53(4):199–205.
[13] Fainaru O, et al. Dendritic cells support angiogenesis and promote lesion growth in a murine model of endometriosis. FASEB J 2008;22(2):522–9.
[14] Ingelmo JM, Quereda F, Acien P. Intraperitoneal and subcutaneous treatment of experimental endometriosis with recombinant human interferon-alpha-2b in a murine model. Fertil Steril 1999;71(5):907–11.
[15] Acien P, et al. Use of intraperitoneal interferon alpha-2b therapy after conservative surgery for endometriosis and postoperative medical treatment with depot gonadotropin-releasing hormone analog: a randomized clinical trial. Fertil Steril 2002;78(4):705–11.
[16] Suen JL, et al. Serum level of IL-10 is increased in patients with endometriosis, and IL-10 promotes the growth of lesions in a murine model. Am J Pathol 2014;184(2):464–71.
[17] Suen JL, et al. IL-10 from plasmacytoid dendritic cells promotes angiogenesis in the early stage of endometriosis. J Pathol 2019;249(4):485–97.
[18] Cummings AM, Metcalf JL. Induction of endometriosis in mice: a new model sensitive to estrogen. Reprod Toxicol 1995;9(3):233–8.
[19] Pickl WF, et al. Molecular and functional characteristics of dendritic cells generated from highly purified CD14+ peripheral blood monocytes. J Immunol 1996;157(9):3850–9.
[20] Bender A, et al. Improved methods for the generation of dendritic cells from nonproliferating progenitors in human blood. J Immunol Methods 1996;196(2):121–35.
[21] Jung S, et al. In vivo depletion of CD11c(+) dendritic cells abrogates priming of CD8(+) T cells by exogenous cell-associated antigens. Immunity 2002;17(2):211–20.
[22] Pencovich N, et al. The development of endometriosis in a murine model is dependent on the presence of dendritic cells. Reprod Biomed Online 2014;28(4):515–21.
[23] Stanic AK, et al. Dendritic cells attenuate the early establishment of endometriosis-like lesions in a murine model. Reprod Sci 2014;21(10):1228–36.
[24] Ono Y, et al. CD11c+ M1-like macrophages (MPhis) but not CD206+ M2-like MPhi are involved in folliculogenesis in mice ovary. Sci Rep 2018;8(1):8171.
[25] Villani AC, et al. Single-cell RNA-seq reveals new types of human blood dendritic cells, monocytes, and progenitors. Science 2017;356(6335).

This page intentionally left blank

CHAPTER

4

Neutrophils

Masashi Takamura[1], *Kaori Koga*[2], *Yoshimasa Kamei*[1] *and Yutaka Osuga*[2]

[1]Obstetrics and Gynecology, Saitama Medical University, Morohongo Moroyama-machi, Iruma-gun, Saitama, Japan; [2]Department of Obstetrics and Gynecology, The University of Tokyo Hospital, Tokyo, Japan

Introduction

Endometriosis is a chronic, inflammatory, estrogen-dependent disease defined as a presence of endometrial tissue outside of the uterine cavity. Although its pathophysiology is not completely understood, an aberrant immune response seems involved in the development of this disease [1]. Leukocytes within the ectopic tissues or peritoneal cavity in patients with endometriosis contribute to the survival and growth of the endometriotic lesions [2]. Peritoneal fluid leukocyte profiles were comprehensively observed for the first by Hill et al. to find difference between groups [3]. The most significant elevations in total leukocytes, macrophages, helper T lymphocytes, and natural-killer cells were observed in women with stage I and II endometriosis. With respect to neutrophils, Milewski et al. [4] reported the accumulation of neutrophils in the peritoneal fluid of patients with endometriosis, and neutrophils accumulation in ovarian endometriosis was also reported later [5].

Neutrophils are derived from the myeloid lineage of hematopoietic stem cells in the bone marrow and the most abundant granulocytes in the blood. They are the first cells recruited to the infected inflammatory site to respond to viral, bacterial, and fungal infections, and constitute an integral component of granulocytes and provide the bacterial phagocytic arm of the innate immune system [6]. Neutrophils fulfill their protective functions through multiple mechanisms, including phagocytosis, release of antimicrobial peptides and proteases, and the formation of neutrophil extracellular traps [7]. Their half-life span was thought very short, namely, 6–8 h in the blood circulation, however in vivo labeling revealed a human neutrophil lifespan to be 5.4 days [8].

https://doi.org/10.1016/B978-0-12-820661-4.00012-1

© 2022 Elsevier Inc. All rights reserved.

With regards to the physiological change and function of neutrophils in endometrium, leukocytes in general are more abundant in endometrium than in other female reproductive tracts. Neutrophils in the endometrium profoundly increase in the premenstrual period, up to 6%–15% of endometrial cells [9] and have been suggested to play a pivotal role in the destruction of endometrial tissue at menstruation and in its concomitant repair [10]. These neutrophils together with the shed endometrium may play a role in the pathogenesis of endometriosis.

The neutrophil effector functions are all well-known for their importance in host defense, but abnormalities in these functions are also associated with development of autoimmune disease. In SLE, neutrophils are more activated, have a lower phagocytic capacity, a decreased production of NOX2 ROS, an increase in mitochondrial ROS, and are more prone to spontaneously release NETs [11]. Similar reports are observed in other chronic disease, RA [12], and asthma [12]. Given that endometriosis has an aspect of chronic inflammatory disease and similar pathological features, it can be easily presumed that neutrophils play an important role in the pathogenesis of endometriosis. In fact, there is emerging evidence indicating that neutrophils play a very important role in the pathogenesis of endometriosis. In this review, we will provide an overview of the involvement of neutrophils and discuss their putative therapeutic target related to neutrophils. The figure later in the chapter illustrates the summary of this text.

Neutrophil abundance and dysregulated function in endometriosis

Numerous studies focused on neutrophil accumulation in either peritoneal fluid, endometriotic lesion, or peripheral blood of patients with endometriosis. As for the function of neutrophils, dysregulated neutrophil-related protein (e.g., cytokines, chemokines, granules) level has been also reported. These findings have indicated deep involvement of neutrophils in the pathophysiology of endometriosis. The representative studies are shown below.

Neutrophil chemokines

More than a quarter century ago, chemotaxis of neutrophils and macrophages by peritoneal fluid of patients with endometriosis were investigated. In this study, a significantly higher chemotactic activity was observed compared to that of patients without endometriosis or with medical suppression. Patients who had received medical treatment had the lowest chemotactic activity [13]. Many studies have focused on the concentration of chemokines in peritoneal fluid later. Some of them are known as chemoattractant of neutrophils. One of the best-known characterized chemokine is interleukin-8 (IL-8). Cell activation in response to IL-8 is mediated via the receptor either CXCR1 or CXCR2 and neutrophils is known to express CXCR1. Monocytes and macrophages represent principal cellular sources of IL-8; however, a wide variety of cell types, including endometrial cells are sources of IL-8 [14]. Besides chemotaxis of neutrophils and other immune cells, the function of IL-8 includes angiogenesis and endometrial cell proliferation [15]. Most researchers pointed to the increased peritoneal and serum IL-8 levels and the peritoneal cytokine level correlates with the severity of the disease, size and number of the active lesions [16]. In women with

moderately severe endometriosis, the plasma level of IL-8 was significantly higher than in the controls [17]. Cystic fluids from ovarian endometriosis also contained significantly higher IL-8 compared to other benign ovarian cysts. Interestingly, the levels of VEGF are also higher in endometriotic cysts and show correlation with that of IL-8 [18], indicating IL-8 derived accumulation of neutrophils risen the level of VEGF. The expression of IL-8 is higher in endometriotic tissue compared to eutopic endometrium [19]. This enhancement is thought through inflammatory stimuli like IL-1b [19], TNFa [20], IL-17A [21], and interferon-gamma [22] confirmed by in vitro study.

Growth-regulated alpha (Gro-alpha) are also known as neutrophil chemotactic factor and acts via the same receptor of IL-8. The levels of Gro-alpha are elevated in the peritoneal fluid of women with moderate and severe endometriosis [23]. Other group also confirmed Gro-alpha elevation in peritoneal fluids though no correlation between concentration of Gro-alpha and stage of endometriosis were found [24]. IL-17A increased the secretion of Gro-alpha from endometriotic stromal cells and localization of IL-17A, Gro-alpha and neutrophils were similar in stroma beneath the epithelium, indicating this cascade play a role in recruiting neutrophils in the endometriotic lesion [5]. Epithelial neutrophil-activating peptide 78 is also known as a chemoattractant of neutrophils. This chemokine is higher in the peritoneal fluids of patients with endometriosis [25,26].

Compared with the plasma and peritoneal fluid of healthy controls, the addition of plasma and peritoneal fluid from patients with endometriosis to an in-vitro culture of neutrophils from healthy subjects reduced the percentage of apoptotic neutrophils. Neutralizing interleukin-8 antibody abrogated the delay of neutrophil apoptosis induced by peritoneal fluid.

Indicates that IL-8 is one of the neutrophil survival factors in the peritoneal fluid of endometriosis patients [27].

Indeed, the accumulation of neutrophils was confirmed in a comprehensive investigation of the frequency and phenotype of leukocytes in peritoneal fluid of infertile women. Neutrophils as well as CD4+ and CD8+ cells were increased in endometriosis patients compared to infertile women without endometriosis while other immune cells were not [28].

Neutrophil in peripheral blood and candidate for disease marker

Patients with endometriosis show increased number of neutrophils in peripheral blood compared to control group [29]. This observation was also confirmed recently [30]. This augmentation can be useful for a less invasive method for diagnosing endometriosis. The most extensively studied marker for endometriosis is CA-125. Although CA-125 is often elevated in advanced endometriosis, the low sensitivity of this assay limits its usefulness for detecting minimal and mild disease. Cho et al. advocated the use of WBC subtypes and the neutrophil-to-lymphocyte ratio (NLR) as simple diagnostic marker for this disease with its sensitivity of 69.3% and specificity of 83.9% with a cutoff value of 55.7 [31]. In addition to this, NLR level was also an independent risk factor of endometriosis with infertility [32].

As for the function of neutrophils in peripheral blood, Lukács et al. reported significantly reduced phagocyte function of monocytes and neutrophil granulocytes in women with endometriosis. Interestingly, this reduction showed significant improvement compared to the

preoperative results of women with endometriosis and this increment reached the values of the healthy women [33]. This alteration in phagocyte function can lead the decreased clearance of endometrial tissue from the abdominal cavity, and accordingly illustrate the pathogenesis of endometriosis. Neutrophils and monocytes are known to play a role in the defending mechanisms against tumor cells or infections, their decreased phagocyte function can explain the increased risk of lower genital tract infections and ovarian cancer in patients with endometriosis. The cause of this alternation was speculated to be circulating factors in peripheral blood produced in connection with the presence of ectopic endometrial cells since the postoperative values revealed significant improvement [33].

Dysregulated function of neutrophils in endometriosis

Aberrant function of neutrophils was observed in numerous studies. Most of them are estimated through the altered concentration of products from neutrophils. Most of them means augmented function in patients with endometriosis.

Neutrophil extracellular traps (NETs): More than a decade ago, a novel extracellular-killing mechanism was described in which activated neutrophils may expel their entire chromatin, which is scattered with intracellular proteins, serving as catch and kill scaffold against microorganisms [34] The procedure was designated as NETosis and the expelled structure as NETs. NETs play a crucial role in fighting against microorganisms, however exaggerated NET production may lead to tissue damage in their vicinity in pathological conditions and has been described to be related to the pathogenesis of inflammatory and autoimmune conditions [35]. A study investigating NETs in peritoneal fluids from patients undergoing laparoscopic surgery, NETs could be detected in 49% of peritoneal fluid from patients with endometriosis, meanwhile in the control group NETs were present in only 18% of patients. The amount of NETs was also significantly higher in endometriosis patients compared to the controls [36]. A case-control study to compare the circulating plasma NETs levels in patients with surgically confirmed endometriosis and those of patients without surgical findings of endometriosis revealed circulating plasma NETs levels were significantly higher in the endometriosis group compared with control group and subanalysis clarified patients with deep infiltrating endometriosis showed more concentrated plasma NETs levels than those without deep infiltrating endometriosis [37]. The exact role of NETs in the pathophysiological mechanisms of endometriosis is necessary for further understanding of these findings.

YKL-40: a new biomarker of inflammation, is secreted by activated macrophages and neutrophils in different tissues with inflammation. Serum YKL-40 levels were statistically higher in the endometriotic group compared to control group. YKL-40 levels were significantly higher in severe endometriosis group (Stage 3−4) compared to mild endometriosis group (Stage 1−2) [38]. Other group reported similar result [39].

Human neutrophil peptides 1, 2, and 3 (HNP 1−3): are α-defensin family proteins, play a crucial role in innate immunity against infections and may exert immunoregulatory effects. The levels of HNP 1−3 were significantly increased in the peritoneal fluid of endometriosis patients, compared with control women, and correlated with severity of the disease and the levels of HNP 1−3 strongly correlated with concentrations of neutrophils [4].

Oxidative stress: Neutrophils as well as other inflammatory cells like eosinophils and macrophages generate reactive oxygen species that contribute to the development of oxidative stress in the peritoneal cavity. Oxidative stress augments immune response in affected sites and can exacerbate the development of endometriosis by inducing chemokines and endometrial cell growth-promoting activity, however the association of the amount of oxidative stress and endometriosis is not consistent [40].

PD-L1: In ovarian endometrioma, correlation between the counts of neutrophils and the severity of ovarian endometrioma was revealed. From the in vitro study using ovarian endometrioma conditional supernatants, ovarian endometrioma microenvironment delayed the onset of apoptosis of neutrophils and induce PD-L1 expression in neutrophils, which inhibit CD8 T cell proliferation and activation and may allow the endometrial cells to growth [41].

VEGF: Neutrophil granulocytes infiltrating the human endometrium express VEGF [42] The peritoneal fluid from endometriosis patients induced the production and release of VEGF by neutrophils while the release from macrophage did not change, suggesting that neutrophils may be a source of peritoneal VEGF [43].

Findings obtained from animal model

To elucidate the disease mechanism of endometriosis, surgically transplanted endometriosis model has been widely used [44–46] and some of the function of neutrophils has been clarified through animal model.

Lin et al. observed the sequential change of neutrophil infiltration in mouse endometriotic lesions and found that neutrophil infiltration peaked early in lesion formation and dramatically dropped in the later stage. Peritoneal neutrophils and macrophages secreted vascular endothelial growth factor and 4–7 days after transplantation angiogenesis was initiated. From this observation, neutrophils and macrophages may promote angiogenesis in the early stage of endometriosis [45]. With neutrophil depleting antibody, neutrophil depletion at the time of endometrial attachment to the peritoneum reduced the number and total weight of the endometriotic lesion whereas depletion of neutrophils 8–12 days after transplantation did not alter the endometriosis formation suggesting the neutrophil function is important mainly in the early stage of endometriosis [46].

Burns et al. found substantial infiltration of neutrophils and macrophages into the peritoneal cavity even in estrogen receptor α knockout mice. Estrogen does not further increase lesion marker gene expression like markers for neutrophils (S100A8), macrophages (F4/80), and granulocytes (G-CSFR). From these findings it is suggest there are two phases of endometriosis—an immune-dependent phase and a hormone-dependent phase, and that targeting the innate immune system could prevent lesion attachment in this susceptible population of women [47].

Another group confirmed rapidly recruited neutrophils remain present in murine lesions long term and In vivo neutrophil depletion altered the systemic and peritoneal immune microenvironment of mice with endometriosis as demonstrated by changes in proinflammatory and angiogenic mediators including VEGF [48].

Putative therapeutic targets

As is well-known, medical strategy for endometriosis is solely on hormonal ones, like OC GnRH agonist, and progestin. They all have inhibitory effect on either ovulation or proliferation of endometrium. Other drugs without hormonal mechanism are definitely necessary. As discussed above, neutrophils are deeply involved in the pathology of endometriosis, therefore it is rational to seek the target of endometriosis in the candidates modifying the function of neutrophils. Below are the lists of putative therapeutic targets.

Cabergoline

Cabergoline is known as a dopamine receptor agonist with a direct inhibitory effect on pituitary lactotroph and used for patients with hyperprolactinemia. Recently, a Ca^{2+} signaling modulator and antioxidant actions of cabergoline has been reported in some cells. It was also effective in reducing oxidative stress caused by activated neutrophils in endometriosis patients via modulating calcium ion signaling [49]. In this study, no information about the effect on the lesion size and symptom were investigated, further investigation was necessary.

Formyl peptide receptor 1 (Fpr1)

Formyl peptide receptors are expressed on macrophages, monocytes and neutrophils and can bind some proinflammatory peptides with chemotactic activity and release reactive oxygen species (ROS) and granule constituents from immune cells [50]. In particular it has been described the up-regulation of the Fpr1 in patients affected by endometriosis and its role in cell differentiation and proliferation [51]. Surgically induced endometriosis in Fpr1 KO mice showed lower duration of uterine pain behaviors, lower size of developed cysts and reduced neutrophils accumulation and nitrosative stress formation. Fpr1 gene has key role in immune cell recruitment including neutrophils, suggesting it as a new target to control the pathologic features of endometriotic lesions [52].

Cyclooxygenase (COX)-2 inhibitor

COX-2 is highly expressed in endometriosis and the application of selective COX-2 inhibitors has been suggested as an option for the management of endometriosis-associated pain [53]. Experimental studies further indicate that the inhibition of COX-2 reduces the vascularization of endometriotic lesions [54]. In COX-2 inhibitor treated mice, impaired early vascularization and stromal tissue growth as well as reduced glandular secretory activity of the lesions were observed. Parecoxib-treated lesions further contained less proliferating and more apoptotic cells and exhibited lower numbers of infiltrating macrophages and neutrophilic granulocytes, suggesting these effects were through reduction of these inflammatory cell recruitment [55]. In this context, prophylactic use may have effect on preventing the onset of endometriosis.

All-transretinoic acid (ATRA)

Retinoids are known to regulate growth and induce differentiation of normal and malignant cells, and have been used to treat cancers in which VEGF is overexpressed [56]. Retinoic acid synthesis appears to be impaired in the endometrium of women with endometriosis [57]. ATRA induced a dose- and time-dependent suppression of VEGF mRNA and protein from neutrophils like cells: HL-60 cells in vitro, suggesting that the up-regulated VEGF and angiogenesis in tissue from women with endometriosis may reflect failure of neutrophil differentiation in these cases. These findings provide a rationale for retinoid therapy in this condition [56].

Other candidate related to neutrophil function

Conventional disease-modifying drugs like TNF-α monoclonal antibody (Adalimumab), AnIL-6R monoclonal antibody (Tocilizumab), anti-RANKL monoclonal antibody (Denosumab) cDMARDs (Leflunomide, Methotrexate, Glucocorticoids) used for chronic inflammatory disease like rheumatoid arthritis (RA) have been shown to extensively modify neutrophil function [58].

Other candidates are inhibitor of cytokines or receptor (anti-IL-8 antibody, anti-IL-17 antibody, CXCR1/2 inhibitor, and anti-GM-CSF antibody) or inhibitor of NETs formation (PAD4-specific inhibitor, and PAD2/4 inhibitor) are putative targets for future treatment (Fig. 4.1).

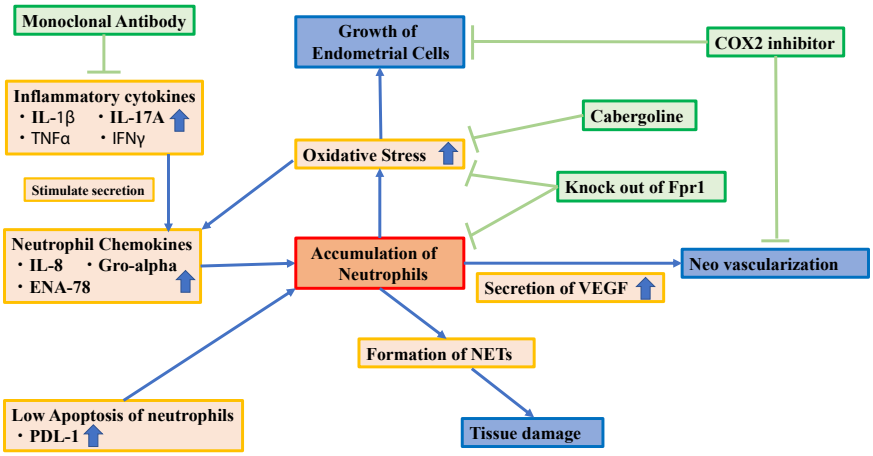


FIGURE 4.1 This figure illustrates the pathology of endometriosis related to neutrophils and their putative therapeutic targets. *Orange box*: Alteration of relevant factors observed in endometriosis lesion. *Blue box*: Histological changes observed in endometriosis tissue. *Green box*: Putative therapeutic agents targeting neutrophil-derived etiology of endometriosis. *Green T arrows* mean the suppression of the targets.

References

[1] Izumi G, Koga K, Takamura M, Makabe T, Satake E, Takeuchi A, Taguchi A, Urata Y, Fujii T, Osuga Y. Involvement of immune cells in the pathogenesis of endometriosis. J Obstet Gynaecol Res 2018;44(2):191–8.

[2] Vallve-Juanico J, Houshdaran S, Giudice LC. The endometrial immune environment of women with endometriosis. Hum Reprod Update 2019;25(5):564–91.

[3] Hill JA, Faris HM, Schiff I, Anderson DJ. Characterization of leukocyte subpopulations in the peritoneal fluid of women with endometriosis. Fertil Steril 1988;50(2):216–22.

[4] Milewski L, Dziunycz P, Barcz E, Radomski D, Roszkowski PI, Korczak-Kowalska G, Kaminski P, Malejczyk J. Increased levels of human neutrophil peptides 1, 2, and 3 in peritoneal fluid of patients with endometriosis: association with neutrophils, T cells and IL-8. J Reprod Immunol 2011;91(1–2):64–70.

[5] Takamura M, Osuga Y, Izumi G, Yoshino O, Koga K, Saito A, Hirata T, Hirota Y, Harada M, Hasegawa A, Taketani Y. Interleukin-17A is present in neutrophils in endometrioma and stimulates the secretion of growth-regulated oncogene-alpha (Gro-alpha) from endometrioma stromal cells. Fertil Steril 2012;98(5):1218–24. e1211-1212.

[6] Mayadas TN, Cullere X, Lowell CA. The multifaceted functions of neutrophils. Annu Rev Pathol 2014;9:181–218.

[7] Chen W, Wang Q, Ke Y, Lin J. Neutrophil function in an inflammatory milieu of rheumatoid arthritis. J Immunol Res 2018;2018:8549329.

[8] Pillay J, den Braber I, Vrisekoop N, Kwast LM, de Boer RJ, Borghans JA, Tesselaar K, Koenderman L. In vivo labeling with $2H_2O$ reveals a human neutrophil lifespan of 5.4 days. Blood 2010;116(4):625–7.

[9] Salamonsen LA, Woolley DE. Menstruation: induction by matrix metalloproteinases and inflammatory cells. J Reprod Immunol 1999;44(1–2):1–27.

[10] Kaitu'u-Lino TJ, Morison NB, Salamonsen LA. Neutrophil depletion retards endometrial repair in a mouse model. Cell Tissue Res 2007;328(1):197–206.

[11] Wirestam L, Arve S, Linge P, Bengtsson AA. Neutrophils-important communicators in systemic lupus erythematosus and antiphospholipid syndrome. Front Immunol 2019;10:2734.

[12] Edilova MI, Akram A, Abdul-Sater AA. Innate immunity drives pathogenesis of rheumatoid arthritis. Biomed J 2021;44(2):172–82.

[13] Leiva MC, Hasty LA, Pfeifer S, Mastroianni Jr L, Lyttle CR. Increased chemotactic activity of peritoneal fluid in patients with endometriosis. Am J Obstet Gynecol 1993;168(2):592–8.

[14] Arici A, Seli E, Senturk LM, Gutierrez LS, Oral E, Taylor HS. Interleukin-8 in the human endometrium. J Clin Endocrinol Metab 1998;83(5):1783–7.

[15] Li MQ, Luo XZ, Meng YH, Mei J, Zhu XY, Jin LP, Li DJ. CXCL8 enhances proliferation and growth and reduces apoptosis in endometrial stromal cells in an autocrine manner via a CXCR1-triggered PTEN/AKT signal pathway. Hum Reprod 2012;27(7):2107–16.

[16] Arici A, Tazuke SI, Attar E, Kliman HJ, Olive DL. Interleukin-8 concentration in peritoneal fluid of patients with endometriosis and modulation of interleukin-8 expression in human mesothelial cells. Mol Hum Reprod 1996;2(1):40–5.

[17] Sikora J, Smycz-Kubanska M, Mielczarek-Palacz A, Kondera-Anasz Z. Abnormal peritoneal regulation of chemokine activation-The role of IL-8 in pathogenesis of endometriosis. Am J Reprod Immunol 2017;77(4).

[18] Fasciani A, D'Ambrogio G, Bocci G, Monti M, Genazzani AR, Artini PG. High concentrations of the vascular endothelial growth factor and interleukin-8 in ovarian endometriomata. Mol Hum Reprod 2000;6(1):50–4.

[19] Akoum A, Lawson C, McColl S, Villeneuve M. Ectopic endometrial cells express high concentrations of interleukin (IL)-8 in vivo regardless of the menstrual cycle phase and respond to oestradiol by up-regulating IL-1-induced IL-8 expression in vitro. Mol Hum Reprod 2001;7(9):859–66.

[20] Sakamoto Y, Harada T, Horie S, Iba Y, Taniguchi F, Yoshida S, Iwabe T, Terakawa N. Tumor necrosis factor-alpha-induced interleukin-8 (IL-8) expression in endometriotic stromal cells, probably through nuclear factor-kappa B activation: gonadotropin-releasing hormone agonist treatment reduced IL-8 expression. J Clin Endocrinol Metab 2003;88(2):730–5.

[21] Hirata T, Osuga Y, Hamasaki K, Yoshino O, Ito M, Hasegawa A, Takemura Y, Hirota Y, Nose E, Morimoto C, Harada M, Koga K, Tajima T, Saito S, Yano T, Taketani Y. Interleukin (IL)-17A stimulates IL-8 secretion, cyclooxygensase-2 expression, and cell proliferation of endometriotic stromal cells. Endocrinology 2008;149(3):1260–7.

[22] Bersinger NA, Frischknecht F, Taylor RN, Mueller MD. Basal and cytokine-stimulated production of epithelial neutrophil activating peptide-78 (ENA-78) and interleukin-8 (IL-8) by cultured human endometrial epithelial and stromal cells. Fertil Steril 2008;89(5 Suppl. l):1530–6.
[23] Oral E, Seli E, Bahtiyar MO, Olive DL, Arici A. Growth-regulated alpha expression in the peritoneal environment with endometriosis. Obstet Gynecol 1996;88(6):1050–6.
[24] Szamatowicz J, Laudanski P, Tomaszewska I, Szamatowicz M. Chemokine growth-regulated-alpha: a possible role in the pathogenesis of endometriosis. Gynecol Endocrinol 2002;16(2):137–41.
[25] Mueller MD, Mazzucchelli L, Buri C, Lebovic DI, Dreher E, Taylor RN. Epithelial neutrophil-activating peptide 78 concentrations are elevated in the peritoneal fluid of women with endometriosis. Fertil Steril 2003;79 (Suppl. 1):815–20.
[26] Suzumori N, Katano K, Suzumori K. Peritoneal fluid concentrations of epithelial neutrophil-activating peptide-78 correlate with the severity of endometriosis. Fertil Steril 2004;81(2):305–8.
[27] Kwak JY, Park SW, Kim KH, Na YJ, Lee KS. Modulation of neutrophil apoptosis by plasma and peritoneal fluid from patients with advanced endometriosis. Hum Reprod 2002;17(3):595–600.
[28] Tariverdian N, Siedentopf F, Rucke M, Blois SM, Klapp BF, Kentenich H, Arck PC. Intraperitoneal immune cell status in infertile women with and without endometriosis. J Reprod Immunol 2009;80(1–2):80–90.
[29] Hrycek A, Kalina Z, Cuzytek A. [Selected immunologic markers for evaluation of peripheral blood in patients with internal endometriosis]. Wiad Lek 1996;49(1–6):10–4.
[30] Ottolina J, Bartiromo L, Dolci C, Salmeri N, Schimberni M, Villanacci R, Vigano P, Candiani M. Assessment of coagulation parameters in women affected by endometriosis: validation study and systematic review of the literature. Diagnostics 2020;10(8).
[31] Cho S, Cho H, Nam A, Kim HY, Choi YS, Park KH, Cho DJ, Lee BS. Neutrophil-to-lymphocyte ratio as an adjunct to CA-125 for the diagnosis of endometriosis. Fertil Steril 2008;90(6):2073–9.
[32] Jing X, Li C, Sun J, Peng J, Dou Y, Xu X, Ma C, Dong Z, Liu Y, Zhang H, Shao Q, Zhang H, Wang L, Zhang Y, Qu X. Systemic inflammatory response markers associated with infertility and endometrioma or uterine leiomyoma in endometriosis. Therapeut Clin Risk Manag 2020;16:403–12.
[33] Lukacs L, Kovacs AR, Pal L, Szucs S, Kover A, Lampe R. Phagocyte function of peripheral neutrophil granulocytes and monocytes in endometriosis before and after surgery. J Gynecol Obstet Hum Reprod 2020:101796.
[34] Brinkmann V, Reichard U, Goosmann C, Fauler B, Uhlemann Y, Weiss DS, Weinrauch Y, Zychlinsky A. Neutrophil extracellular traps kill bacteria. Science 2004;303(5663):1532–5.
[35] Fousert E, Toes R, Desai J. Neutrophil extracellular traps (NETs) take the central stage in driving autoimmune responses. Cells 2020;9(4).
[36] Berkes E, Oehmke F, Tinneberg HR, Preissner KT, Saffarzadeh M. Association of neutrophil extracellular traps with endometriosis-related chronic inflammation. Eur J Obstet Gynecol Reprod Biol 2014;183:193–200.
[37] Munros J, Tassies D, Reverter JC, Martin L, Perez A, Carmona F, Martinez-Zamora MA. Circulating neutrophil extracellular traps are elevated in patients with deep infiltrating endometriosis. Reprod Sci 2019;26(1):70–6.
[38] Tuten A, Kucur M, Imamoglu M, Oncul M, Acikgoz AS, Sofiyeva N, Ozturk Z, Kaya B, Oral E. Serum YKL-40 levels are altered in endometriosis. Gynecol Endocrinol 2014;30(5):381–4.
[39] Ural UM, Tekin YB, Cure M, Sahin FK. Serum YKL-40 levels as a novel marker of inflammation in patients with endometriosis. Clin Exp Obstet Gynecol 2015;42(4):495–7.
[40] Augoulea A, Mastorakos G, Lambrinoudaki I, Christodoulakos G, Creatsas G. The role of the oxidative-stress in the endometriosis-related infertility. Gynecol Endocrinol 2009;25(2):75–81.
[41] Xu H, Zhao J, Lu J, Sun X. Ovarian endometrioma infiltrating neutrophils orchestrate immunosuppressive microenvironment. J Ovarian Res 2020;13(1):44.
[42] Mueller MD, Lebovic DI, Garrett E, Taylor RN. Neutrophils infiltrating the endometrium express vascular endothelial growth factor: potential role in endometrial angiogenesis. Fertil Steril 2000;74(1):107–12.
[43] Na YJ, Yang SH, Baek DW, Lee DH, Kim KH, Choi YM, Oh ST, Hong YS, Kwak JY, Lee KS. Effects of peritoneal fluid from endometriosis patients on the release of vascular endothelial growth factor by neutrophils and monocytes. Hum Reprod 2006;21(7):1846–55.
[44] Hirata T, Osuga Y, Yoshino O, Hirota Y, Harada M, Takemura Y, Morimoto C, Koga K, Yano T, Tsutsumi O, Taketani Y. Development of an experimental model of endometriosis using mice that ubiquitously express green fluorescent protein. Hum Reprod 2005;20(8):2092–6.

[45] Lin YJ, Lai MD, Lei HY, Wing LY. Neutrophils and macrophages promote angiogenesis in the early stage of endometriosis in a mouse model. Endocrinology 2006;147(3):1278–86.
[46] Takamura M, Koga K, Izumi G, Urata Y, Nagai M, Hasegawa A, Harada M, Hirata T, Hirota Y, Wada-Hiraike O, Fujii T, Osuga Y. Neutrophil depletion reduces endometriotic lesion formation in mice. Am J Reprod Immunol 2016;76(3):193–8.
[47] Burns KA, Thomas SY, Hamilton KJ, Young SL, Cook DN, Korach KS. Early endometriosis in females is directed by immune-mediated estrogen receptor alpha and IL-6 cross-talk. Endocrinology 2018;159(1):103–18.
[48] Symons LK, Miller JE, Tyryshkin K, Monsanto SP, Marks RM, Lingegowda H, Vanderbeck K, Childs T, Young SL, Lessey BA, Koti M, Tayade C. Neutrophil recruitment and function in endometriosis patients and a syngeneic murine model. FASEB J 2020;34(1):1558–75.
[49] Ekici EI, Guney M, Naziroglu M. Protective effect of cabergoline on mitochondrial oxidative stress-induced apoptosis is mediated by modulations of TRPM2 in neutrophils of patients with endometriosis. J Bioenerg Biomembr 2020;52(3):131–42.
[50] Marasco WA, Phan SH, Krutzsch H, Showell HJ, Feltner DE, Nairn R, Becker EL, Ward PA. Purification and identification of formyl-methionyl-leucyl-phenylalanine as the major peptide neutrophil chemotactic factor produced by *Escherichia coli*. J Biol Chem 1984;259(9):5430–9.
[51] Paula Jr R, Oliani AH, Vaz-Oliani DC, D'Avila SC, Oliani SM, Gil CD. The intricate role of mast cell proteases and the annexin A1-FPR1 system in abdominal wall endometriosis. J Mol Histol 2015;46(1):33–43.
[52] Fusco R, D'Amico R, Cordaro M, Gugliandolo E, Siracusa R, Peritore AF, Crupi R, Impellizzeri D, Cuzzocrea S, Di Paola R. Absence of formyl peptide receptor 1 causes endometriotic lesion regression in a mouse model of surgically-induced endometriosis. Oncotarget 2018;9(59):31355–66.
[53] Cobellis L, Razzi S, De Simone S, Sartini A, Fava A, Danero S, Gioffre W, Mazzini M, Petraglia F. The treatment with a COX-2 specific inhibitor is effective in the management of pain related to endometriosis. Eur J Obstet Gynecol Reprod Biol 2004;116(1):100–2.
[54] Ozawa Y, Murakami T, Tamura M, Terada Y, Yaegashi N, Okamura K. A selective cyclooxygenase-2 inhibitor suppresses the growth of endometriosis xenografts via antiangiogenic activity in severe combined immunodeficiency mice. Fertil Steril 2006;86(4 Suppl. l):1146–51.
[55] Rudzitis-Auth J, Nickels RM, Menger MD, Laschke MW. Inhibition of cyclooxygenase-2 suppresses the recruitment of endothelial progenitor cells in the microvasculature of endometriotic lesions. Am J Pathol 2018;188(2):450–60.
[56] Tee MK, Vigne JL, Taylor RN. All-trans retinoic acid inhibits vascular endothelial growth factor expression in a cell model of neutrophil activation. Endocrinology 2006;147(3):1264–70.
[57] Osteen KG, Bruner-Tran KL, Ong D, Eisenberg E. Paracrine mediators of endometrial matrix metalloproteinase expression: potential targets for progestin-based treatment of endometriosis. Ann N Y Acad Sci 2002;955:139–46. discussion 157–138, 396–406.
[58] O'Neil LJ, Kaplan MJ. Neutrophils in rheumatoid arthritis: breaking immune tolerance and fueling disease. Trends Mol Med 2019;25(3):215–27.

CHAPTER

5

Role of Th1, Th2, Th17, and regulatory T cells in endometriosis

Khaleque N. Khan[1], *Akira Fujishita*[2], *Yukiko Tanaka*[1] *and Jo Kitawaki*[1]

[1]Department of Obstetrics and Gynecology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan; [2]Department of Gynecology, Saiseikai Nagasaki Hospital, Nagasaki, Japan

Abbreviations

B cells B lymphocytes
Ccl-2 C−C motif chemokine ligand 2 (MCP-1)
COX2 Cyclooxygenase 2
ER Estrogen receptor
ESCs Eutopic/ectopic endometrial stromal cells
GnRHa Gonadotropin-releasing hormone agonist
HBD Human beta defensin
IL Interleukin
LPS Lipopolysaccharide
MIP1 Macrophage inflammatory protein 1
MyD88 Myeloid differentiation factor 88
Mφ Macrophages
NK cells Natural killer cells
NK-kB Nuclear factor-kappa B
PAMP Pathogen-associated molecular pattern
PB Peripheral blood
PF Peritoneal fluid
PRR Pattern recognition receptor
Ptgs-2 Prostaglandin-endoperoxide synthase-2
revised-ASRM Revised classification of the American Society of Reproductive Medicine
RIF Repeated implantation failure
SLPI Secretory leukocyte protease inhibitor
TGFβ Transforming growth factors beta
Th cells T-helper cells
TIRAP TIR domain-containing adapter protein

Immunology of Endometriosis
https://doi.org/10.1016/B978-0-12-820661-4.00009-1

© 2022 Elsevier Inc. All rights reserved.

TLR Toll-like receptor
TNFα Tumor necrosis factor alpha
TRAM TRIF-related adapter molecule
Treg Regulatory T cells
TRIF TIR (Toll/IL-1 receptor)-domain-containing-adaptor inducing IFN-β
VEGF Vascular endothelial growth factor

Introduction

Innate (natural or constitutive) immunity in our body depends on toll-like receptors (TLRs). From flies to mammals, these proteins provide a first line defense and are implicated in infectious and autoimmune diseases. While scientists have been studying the adaptive (acquired) immune response for several decades, the recognition of the importance of innate immunity was established only during the past few years to understand the association between adaptive and innate immune system. Why is innate immunity necessary for our body? There was always a question of how an adaptive immune system could defend us if it were alone because adaptive immunity depends on the multiplication of host cells with a generation time of at least 12 h, whereas microbes can divide every 20 min. To cover this lag, the rapidly reactive innate immune system responds immediately to infectious agents, protecting the host until slower adaptive system kicks in and eventually makes memory cells for long-term response [1,2]. Therefore, innate immune responses are, in many cases, necessary for triggering an adaptive immune response just as adjuvant is necessary for a significant vaccine response.

Functional characterization of TLRs has established that innate immunity is a skillful system that detects invasion of microbial pathogens. Recognition of microbial components by TLRs initiates signal transduction pathways, and triggers expression of genes. These genes control innate immune responses and further instruct development of antigen-specific adaptive immunity. In adaptive immunity, B and T lymphocytes utilize antigen receptors such as receptor for immunoglobulins and T cell receptors to recognize nonself, such as foreign antigens. However, these receptors are present only in vertebrates. In contrast, innate immune system operates in both vertebrates and non-vertebrates. Mammalian innate immune cells such as macrophages and dendritic cells can be activated by microbial components (nonself) such as endotoxin or lipopolysaccharide (LPS) from Gram-negative bacteria.

Analysis of the female reproductive tract indicates that the key cells of the innate and adaptive immune systems are present and functionally responsive to antigens [3,4]. The innate immune system has evolved to recognize foreign structures that are not normally found in the host. It relies on conserved germ-line-encoded receptors that recognize conserved pathogen-associated molecular patterns (PAMP) found in groups of microorganism [5]. The pattern recognition receptors (PRR) of the host that recognize PAMP in female reproductive tract are expressed on the cells of the innate immune system. TLRs are one group of PRRs that are expressed on macrophages (Mφ), dendritic cells, and as more recently shown, on neutrophils, natural killer cells, and on epithelial cells [4–6].

At the end of the 20th century, Toll was shown to be an essential receptor for host defense against fungal infection in *Drosophila* (fly), which has only innate immunity [7]. One year

later, a mammalian homolog of the Toll receptor (now termed TLR4) was shown to induce expression of genes involved in inflammatory responses [8]. In addition, a point mutation in the Tlr4 gene has been identified in a mouse strain that is unresponsive to LPS [9]. These studies have made rapid progress in our understanding that innate immune system senses invasion of microbial pathogens by TLRs. Furthermore, activation of the innate immunity is a critical step to the development of antigen-specific adaptive (acquired) immunity.

Now it is well recognized that innate and adaptive immune system are the two key branches that determine host protection throughout the female reproductive tract and at other mucosal surfaces, including the respiratory, gastrointestinal and urinary tracts. During the last decade, investigations of the innate immune system have shown that microbial pathogens are recognized by TLRs that, in turn, regulate the activation of both innate and adaptive immunity [10,11]. Association of microbial components, their ligands and interaction with Mφ and TLR4 in the growth of endometriosis has been reported recently in a number of literatures [12–15]. Natural Killer (NK) cells are cytotoxic effector lymphocytes and a defect in NK cell function to distort its ability to eliminate endometrial cells in ectopic sites is involved in the development of endometriosis. Alteration of the innate immunity mediated by NK cells may promote impairments of adaptive immunity, can contribute to the development and progression of endometriosis and infertility associated with endometriosis [16–19]. Here we discuss the link between innate and adaptive immunity in the generation of inflammation and disease and systematically elaborate the involvement of different components of both innate immunity and adaptive immunity in human endometriosis and their experimental evidence in animal model.

Cross talk between innate immunity and adaptive immunity

The TRIF/TRAM pathway provides a direct link between TLR4 activation and adaptive (acquired) immunity. Although purified LPS acts like a strong adjuvant, its effects are abolished in the mutant mouse strains. This suggests that both inflammatory and adjuvant effects of LPS flow through TLR4. Combination of MyD88/TIRAP and TRIF/TRAM pathways provides a biochemical basis for how adjuvants work. Activation of adaptive immune system requires antigen-presenting cells such as Mφ and dendritic cells to express costimulatory molecules such as CD40, CD80 and CD86, and to produce proinflammatory cytokines. When TLR4 recognizes LPS on the surface of Mφ or dendritic cells, it leads to the production of cytokines via MyD88/TIRAP pathway and costimulatory molecules via TRIF/TRAM pathway, providing both components to activate T helper lymphocytes of the adaptive immune system [1,3].

Some evidence suggests that the initial innate immune process influences the type of acquired immune response that is generated [1,20]. When naïve T helper (Th0) cells are presented with antigens by antigen-presenting cells, they differentiate into four subsets of $CD4^+$ T cells. Two subsets are classified as proinflammatory, T helper 1 (Th1) cells and Th17 cells, and the remaining two are antiinflammatory, T helper 2 (Th2) cells and regulatory T (Treg) cells based on their functionality and type of cytokine produced (Fig. 5.1) [21]. A balance (Fig. 5.1) between effector T cell subsets such as Th1 or Th17 cells and Th2 or Treg cells dictate a state of either immune tolerance or immune rejection. In other words, a

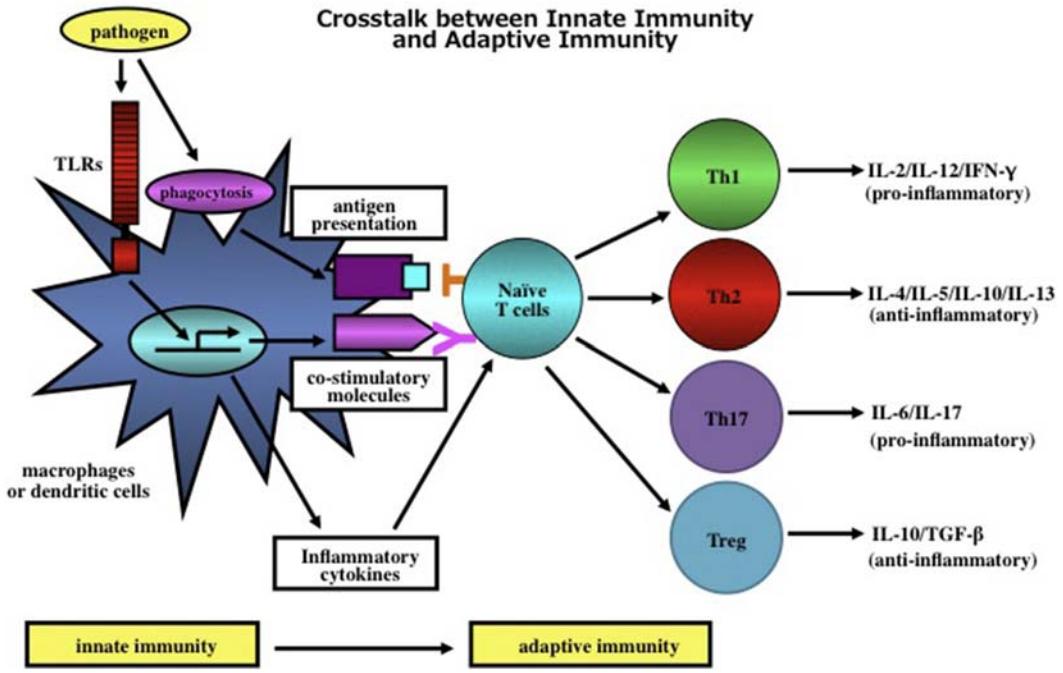


FIGURE 5.1 Shows cross talk between innate and adaptive immunity. Innate immune cells, such as macrophages and dendritic cells, engulf pathogens by phagocytosis, and present pathogen-derived peptide antigens to naïve T cells. In addition, toll-like receptors (TLRs) recognize pathogen-derived components and induce expression of genes, such as costimulatory molecules and inflammatory cytokines. Phagocytosis-mediated antigen presentation, together with TLR-mediated expression of costimulatory molecules and inflammatory cytokines, instruct development of antigen specific adaptive immunity. When naïve T helper cells are presented with antigens by antigen-presenting cells, they differentiate into four subsets of $CD4^+$ T cells. Two subsets are classified as proinflammatory, T helper 1 (Th1) cells and Th17 cells, and the remaining two are antiinflammatory, T helper 2 (Th2) cells and regulatory T (Treg) cells. The production of respective cytokines by each of Th1, Th2, Th17 and Treg cells are shown on the extreme right side of this figure.

fine-tuning between Th1/Th17 cells, Th2/Treg cells or Th17/Treg cells can decide the occurrence or clearance of infection and disease. It has been demonstrated that autoimmune diseases such as Crohn's disease and multiple sclerosis are associated with an abnormally strong Th1 response, whereas allergic diseases seem to involve an abnormally strong Th2 response [22].

Which limb of acquired immunity predominates may be modulated by innate immune response. For instance, MyD88-deficient mice are skewed toward a Th2 response and activation of TLR4 by LPS stimulates Th1 activity. In patients suffering from obesity or type 2 diabetes mellitus, there were alterations in the proliferation of T cells and Mφ and impairment in function of NK cells and B cells representing abnormal innate and adaptive innate immunity [23,24]. Similarly, besides estrogen-dependent pathway, an alteration of different components of innate or adaptive immunity might be involved in the pathogenesis of endometriosis. This integrated knowledge of innate and adaptive immunity may give future therapeutic possibilities for infectious diseases, allergic diseases and autoimmune diseases including endometriosis.

Role of innate immunity in endometriosis

Microbial agents, ligands and TLRs

For most of the reproductive cycle in humans and animals, the uterus is thought to be a sterile or at least clear of pathogenic bacteria, but it is readily contaminated with bacteria during sexual intercourse and around the time of parturition. In fact, upper genital tract is vulnerable to the spread of microorganisms from the lower genital tract, resulting in the development of infectious diseases such as endometritis and salpingitis [25]. An enormous number of Gram-negative and Gram-positive microbes are present in the vaginal cavity. All these microbes reside in the vaginal cavity as normal vaginal flora and may cause genitourinary infections upon ascending migration [26]. Details of normal vaginal flora/microbes associated with genitourinary tract infection and different TLRs and their ligands are described elsewhere [14,22].

A number of widely accepted mechanisms (coelomic metaplasia, retrograde menstruation/transplantation/implantation, and immune defect or tolerance) have been reported to be involved in the development of endometriosis. The production of proinflammatory cytokines and growth of endometriosis in pelvic environment can be regulated by innate immune system. We proposed for the first time a new concept "bacterial contamination hypothesis" in endometriosis and involvement of LPS/TLR4 cascade in the growth regulation of endometriosis. Our results suggest that a substantial amount of endotoxin (LPS) in peritoneal fluid due to reflux of contaminated menstrual blood is involved in pelvic inflammation and may promote TLR4-mediated growth of endometriosis. Use of anti-TLR4 antibody and/or polymyxin B (an LPS antagonist) prior to LPS treatment was able to significantly suppress pelvic inflammation as well as growth of endometriosis [12]. We proposed that targeting bacterial endotoxin, TLR4 or NK-kB could be useful as a therapeutic strategy to suppress growth of endometriosis with consequent improvement in the quality of life and fertility rate of women who suffer from endometriosis. In fact, different reports demonstrated the involvement of ligands from Gram-negative and Gram-positive bacteria in both female and male infertility [27,28].

Subclinical infection as source of initial inflammation

Ligands of different microbial components are a constant source of initial inflammation in pelvis that in turn stimulate different innate immune cells for the secretion of secondary inflammatory mediators [12–14]. In addition to bacteria culture system, we confirmed occurrence of subclinical infection in intrauterine environment and cystic fluid of ovarian endometrioma using second generation sequencing system [29]. 16S metagenome assay indicated that intrauterine microbial colonization was significantly increased in GnRHa-treated women with endometriosis than in GnRHa-untreated women and consequent occurrence chronic endometritis in GnRHa-treated women with and without endometriosis [29,30]. Decreased expression of antimicrobial peptides (HBD and SLPI) in endometria in response to GnRHa-mediated estrogen suppression might be responsible in intrauterine microbial colonization [30].

LPS/TLR4/NF-kB in endometriosis: an experimental evidence

The role of LPS, TLR4 and NF-kB has been confirmed in murine endometriosis model. Intraperitoneal (IP) injection of LPS in BALB/c mice increased the total number and size of endometriosis-like lesions, increased Ki-67-positive cells and F4/80-positive Mφ in endometriosis lesions. In addition, IP injection of LPS increased mRNA expression of *Ptgs-2*, *VEGF*, *Ccl-2* and *Il-6* in endometriosis-like lesions [31]. The same group demonstrated that while LPS injection up-regulates expression of TLR4 and p65 isoform of NF-kB, simultaneous treatment with LPS + parthenolide (an NF-kB inhibitor) and/or LPS + dexamethasone (an antiinflammatory agent) significantly decreased TLR4 and phospho-p65NF-kB expression with consequent decrease of pelvic inflammation and growth of endometriosis in murine model [31]. Based on these findings, LPS enhanced the development of murine endometriosis-like lesions by activating peritoneal inflammatory status via the pleiotropic TLR4/NF-kB pathway and reconfirmed our findings in human study [12].

Association between ovarian steroids and immune cells

Besides a role in reproduction, the ovarian steroid hormones such as 17β-estradiol (E_2) and progesterone (P) have been recognized to influence numerous immune and inflammatory responses. The immunomodulating actions of E_2 are thought to mainly result from their specific effects on the different cellular components of the immune system, because most of them express estrogen receptors [32]. Local biosynthesis of E_2 by endometriotic lesions in concert with pronounced inflammation in pelvis fosters an aberrant immune-endocrine microenvironment that may be ideal for growth and survival of ectopic lesions [33,34].

We previously observed that a variable amount of E_2 and LPS is available in the pelvis of women with and without endometriosis across the phases of the menstrual cycle. Since endometriotic lesions are constantly exposed to a combined milieu of E_2 and LPS in pelvic environment, E_2 and LPS might act together as an additive promoter in the growth of endometriosis [32,33]. Comparing to single treatment, combined treatment with E_2 and LPS additively promoted IL-6 and TNFαsecretion by peritoneal Mφ and growth of eutopic/ectopic ESCs. The additive effects of E_2+LPS on cytokine secretion and growth of ESCs were effectively suppressed after combined blocking of ER and TLR4. These findings confirmed a cross talk between E_2 and LPS in promoting proinflammatory response in pelvis and growth of endometriosis. A recent report provides evidence that the early initiation of endometriosis is predominantly dependent on immune system, whereas E_2/ER-α/IL-6-mediated cross talk plays a partial role [35,36]. These findings suggest that there are two phases of endometriosis, an immune-depended phase and a hormone-depended phase, and that targeting the innate immune system could prevent lesion attachment in this susceptible population of women.

Role of adaptive (acquired) immunity in endometriosis

The cell-mediated and humoral components of adaptive immunity that are regulated by T lymphocytes (T cells) have been implicated in the pathophysiology of endometriosis. Effector

T cells or T helper cells are $CD4^+$ T cells that can be categorized into four subsets: Th1, Th2, Th17 and regulatory T (Treg) cells. Because of the presence of elevated type 2 cytokines in the plasma and PF, endometriosis has been characterized as skewed toward a Th2 immune response [37,38]. However, the typical Th2 response associated with wound healing and fibrosis, has not been fully elucidated in the context of endometriosis [39].

Th1/Th2 cells in endometriosis

Th1 cells are differentiated $CD4^+$ T cells characterized by the production of proinflammatory cytokines. Th2 cells are differentiated from activated $CD4^+$ T cells, which drive the production of key Th2 cell lineage-defining cytokines [40,41]. Th1 cells secrete IL-2, IL-12, interferon-γ, which promote mainly cellular immunity; Th2 cells produce IL-4, IL-5, IL-10, and IL-13, and promote mainly humoral immunity (Fig. 5.1) [1,14]. Studies of Mφ have shown that M2 polarization is associated with the development of endometriosis and Th2 cells have been assumed to cause disease progression [42]. It has been shown that cytokine secretion by peritoneal T cells is characteristic of Th2 in endometriosis tissue [43]. In the endometriosis lesion, the frequency of Th1 cells is lower than in normal endometrium [44]. Furthermore, Th2 cytokines, such as IL-4 and IL-10 was found to be involved in disease progression [45–48]. Studies of peripheral blood, however, have revealed a higher Th1/Th2 cytokine ratio and Th1 cell frequency in endometriosis patients compared with controls [44]. These findings indicated that $CD4^+$ T-lymphocyte profile in lesions and PB is altered in women with endometriosis. This increased proinflammatory condition in the pelvis might be associated with adverse reproductive outcome in women with endometriosis.

There is a general agreement that pregnancy is associated with Th2 dominance and Th1 immune response is associated with embryonic rejection. A recent ART clinical trial demonstrated that women with RIF have increased Th1 immune responses with increased peripheral blood Th1/Th2 cell ratio [49,50]. The same group found that an immunosuppressive treatment using tacrolimus improved pregnancy outcome of RIF patients with elevated Th1/Th2 ratios. These findings indicated that elevation of Th1/Th2 cell ratio in PB/PF can be utilized as a biomarker to select women with RIF or inflammatory status in the pelvis of women with endometriosis. A proper selection of immunosuppressive agent might be useful to suppress both local and systemic immune response in order to achieve successful reproductive outcome in women with endometriosis. Medications with danazol and GnRHa seem to down-regulate cellular and humoral immune responses concomitant with their effect on endometriosis implants [51]. Immunomodulatory effects of danazol and GnRHa are likely to contribute to the observed clinical improvement associated with their use.

Th17/Treg cells in endometriosis

While Th17 cells produce IL-6/IL-17 in local and systemic environment, Treg cells secrete IL-10/TGF-β (Fig. 5.1). When $CD4^+$ T cells are encountered by IL-6 and TGF-β, they tend to differentiate to Th17 cells, which play a pathological role in various inflammatory disorders [52]. Treg cells express CD4, CD25, and forkhead family transcription factor FoxP3. They represent a small subset of T lymphocytes constituting only 5%–20% of the $CD4^+$

compartment, suppressing effector T cell responses, limiting inflammation, and preventing autoimmunity [52–54]. Upon its first molecular characterization as $CD4^+CD25^+$Treg cells in 2001, Treg cells were detected in a diversity of inflammatory pathologies such as allergies, autoimmune diseases, and cancer [55]. Regulatory T cells are potent suppressors of inflammatory immune responses and are essential in preventing destructive immunity in all tissues. Currently, Treg cells are characterized by the expression of $CD25^+CD127^-FoxP3^+$ cells, since CD25 and forkhead box P3 (FoxP3) are constitutive markers to isolate Treg cells [56–58]. Among three subsets of T cells (Tr1/Th3/Treg) involved in the suppression of immune response, $FoxP3^+$Treg cells have been recognized as the main subset in the female reproductive tract [59].

$CD4^+$ T cells tend to polarize to Treg cells in vitro when treated with TGF-β, which appears to be dependent upon fatty acid oxidation and cholesterol metabolism rather than glycolysis [60,61]. Treg cells suppress the levels of effector T cells by various pathways: (1) inhibition of TCR-induced proliferation and IL-2 transcription of conventional T cells, (2) release of antiinflammatory cytokines like IL-10 and TGF-β, (3) expression of the coinhibitory molecule cytotoxic T lymphocyte antigen-4 (CTLA-4), (4) expression of the hallmark transcription factor of suppressed $CD4^+$ T cells, and (5) ability to migrate by activation of glycolysis [21,62,63]. The balance between Treg and Th1 or Th17 cells is important in immune response to different inflammatory diseases including endometriosis.

The production of IL-17 by a subset of T cells was discovered in 1999 using T-cell clones from the joints of patients with rheumatoid arthritis [64,65]. The results were subsequently confirmed in mice and the term T-helper-17 (Th17) subset was introduced in 2005 in the mouse as a T-helper subset distinct from Th1 and Th2 cell [65–67]. Sequence screening identified an IL-17 family comprising six members from IL-17A to IL-17F. Among them, IL-17A and IL-17F are the closest members, with 50% homology, and they share most of their activities, with IL-17A being more potent than IL-17F [65]. Interleukin (IL)-17A is secreted from Th17 cells, a discovery leading to revision of mechanism underlying the role of Th1/Th2 in the immune response.

The frequency of Th17 cells in endometriosis lesions is higher than in that of the normal endometrium [44] and their high frequency in peritoneal fluid is associated with the increased severity of disease [68]. IL-17A was found to enhance IL-8 secretion from ESCs in a dose-depended manner and this effect was abrogated after treatment with anti-IL-17 receptor A antibodies or inhibitors of p38MAPK, p42/44MAPK. IL-17A also enhanced expression of COX2 and proliferation of ESCs [69]. These findings indicate that IL-17A may play a role in the development of endometriosis by stimulating inflammatory responses and proliferation of ESCs [70,71]. Many studies have shown that Treg cells are increased in the peritoneal cavity of endometriosis patients. However, two studies did not find a significant difference [44,72]. In a mouse study, the inhibition of Treg induction reduced the number and weight of endometriotic lesions indicating its role in endometriosis progression [73]. In addition to play a pivotal role in the pathogenesis of endometriosis, IL-17 is also involved in miscarriage, preterm labor, and preeclampsia [74,75]. A recent study demonstrated that the effects of exogenous IL-17 on increased abortion rate, as well as decreased IL-10 and TGF-β expression were reversed by a premating transfusion of Treg cells in a mouse model of pregnancy [76]. Further studies are warranted to discover the mechanism and therapeutic targets of Treg cells in endometriosis and in women suffering from adverse reproductive outcome.

Treg cells in ovarian endometrioma and nonendometrioma

Occurrence of peritoneal lesions in women with ovarian endometrioma and nonendometrioma

Many theories have been proposed to explain the development of endometriosis and one of the most accepted is the retrograde menstruation theory [77]. It is still unclear the phenomenon why endometriosis occurs in only 10% of women while retrograde menstruation occurs in more than 90% women. It has been proposed that a complementary theory may be involved by which defective immune response could determine survival and implantation of ectopic endometrial cells [78–81]. According to this theory, inflammatory reactions send signals to immune systems to scavenge endometrial cells at the ectopic sites, while in women with endometriosis an impairment of this process promotes reduced attack to the ectopic endometrial cells with consequent survival and implantation in pelvis. A number of recent elegant studies demonstrated that Treg cells are in higher concentration in tissues and body fluids (eutopic endometrium, PF and PB) of women with endometriosis when compared to women without endometriosis [82–84]. However, none of these studies could describe the pattern of Treg cells in different peritoneal lesions coexistent with ovarian endometrioma and non-endometrioma and their association with the revised-ASRM staging of endometriosis.

In clinical practice, there is a lower occurrence of peritoneal lesions in women with dermoid cysts, serous cyst adenoma (SCA), and mucinous cyst adenoma (MCA) even these women experience similar cyclic menstruation with retrograde menstrual flow. A thorough survey of the coexistence of peritoneal lesions in women with dermoid cyst/SCA/MCA and how this varies with that of ovarian endometrioma is still unknown. We retrospectively reviewed recorded files of laparoscopic surgery (September 1982–April 2008) including women with ovarian endometrioma, dermoid cysts, SCA and MCA and found 2988 cases had surgery with a variable indication. Among the 2988 cases, 350 cases (11.7%) were found to have ovarian endometrioma, 414 cases (13.9%) had mature cystic teratoma (dermoid cysts), and 101 cases (3.4%) had combined SCA and MCA. Comparing to 269 (76.9%) cases with endometrioma with coexisting peritoneal lesions, 74 (17.8%) and 12 (11.9%) cases with dermoid cyst and combined SCA/MCA, respectively, had coexistent peritoneal lesions and this difference was statistically significant ($P < .001$ for both) [85]. Multiple logistic regression analysis after adjustment for the confounding factors further confirmed that occurrence of peritoneal lesions was significantly higher in women with ovarian endometrioma and that significantly less occurrence of peritoneal lesions was observed in women with dermoid cysts (OR 2.36, 95%CI 1.25–4.39, $P = .007$) [85].

FoxP3$^+$Treg cells in ovarian endometrioma and dermoid cysts

The number of FoxP3$^+$Treg cells was significant higher in the peritoneal lesions coexistent with both dermoid cyst ($F = 22.01$, $P = <.001$) and endometrioma ($F = 21.52$, $P = <.001$) comparing to these two groups of women without any coexistent peritoneal lesions. Although a significant difference in the amount of FoxP3$^+$Treg cells was observed between peritoneal lesion and cyst wall derived from women with endometrioma ($P < .001$), this

difference was not found for women with dermoid cysts. When we distributed the number of FoxP3$^+$Treg cells in the combined peritoneal lesions and cyst walls based on the revised-ASRM staging of endometriosis, one way-ANOVA indicated no significant difference in Treg cells among the stages of endometriosis ($F = 2.42$, $P = .087$) [85]. We learned from these findings a significantly less accumulation of FoxP3$^+$Treg cells in the cyst wall and peritoneum of women with dermoid cysts and endometrioma without any coexisting peritoneal lesions comparing to similar tissues derived from these two groups of women harboring visible peritoneal lesions. These results indicate that a persistent inflammatory reaction in the pelvis of women with peritoneal lesions may induce differentiation, maturation and proliferation of Treg cells in these women with coexisting peritoneal lesions. In contrast, a decreased FoxP3$^+$-Treg cell population in women lacking peritoneal lesions may switch signaling to effector immune cells (T1/Th17 cells, Mφ, and NK cells) to scavenge adherent endometrial cells in the peritoneum.

FoxP3$^+$Treg cells in endometria based on phases of the menstrual cycle

The immunoreactions of FoxP3$^+$Treg cells in the eutopic endometria derived from women with ovarian endometrioma and dermoid cyst had coexistent peritoneal lesions or no lesion was analyzed based on the phases of the menstrual cycle. There was no significant difference in the number of FoxP3$^+$Treg cells in the eutopic endometria between peritoneal lesions (+) and peritoneal lesion (−) groups across the phases of the menstrual cycle. Kruskal–Wallis test indicated a higher tendency in the accumulation of FoxP3$^+$Treg cells in the endometria from the secretory phase to the menstrual phase in groups of women coexistent with peritoneal lesions. These findings are in agreement with the report of Prieto et al. [86], who demonstrated that patterns of FoxP3$^+$Treg cells in the endometria were independent of the phases of the menstrual cycle. However, a differential increased pattern of FoxP3$^+$Treg cells accumulation in the endometria was observed during the proliferative phase [87] or during the secretory phase [88] of the menstrual cycle. The current findings together with the results of Berbic et al. [88] may propose the fact that women with FoxP3$^+$Treg cells in the endometria may decrease the ability of the newly recruited effector immune cells to effectively recognize endometrial antigens during the secretory or the menstrual phase, thereby allowing their survival and consequent implantation in the pelvis.

FoxP3$^+$Treg cells based on the color appearance of peritoneal lesions

We were curious to know the distribution of FoxP3$^+$Treg cells in the coexisting peritoneal lesions derived from women with ovarian endometrioma and dermoid cysts based on the color appearance of these lesions. The color appearance of peritoneal lesions was characterized as nonopaque transparent lesions (clear papule, serous bleb), blood-filled opaque red lesions (blood bleb), and black lesions (blue berry spot) [89]. Blood bleb lesions showed significantly higher number of FoxP3$^+$Treg cells than in serous blebs and blue berry spots ($P < .05$ for each, Mann–Whitney U test). The number of FoxP3$^+$Treg cells in the peritoneum adjacent to serous bleb and blood bleb lesions were significantly higher than that in the peritoneum adjacent to blue berry lesions ($P < .05$ for each, Mann–Whitney U test) [85]. This differential expression of FoxP3$^+$Treg cells can be explained by an increased tissue inflammatory reaction

in the pelvis of women harboring active peritoneal lesions. Different macromolecules in pelvis with variable inflammatory condition may differentially trigger Treg cells in women harboring different types of peritoneal lesions.

TGF-beta/IL-6 in PF of women with or without peritoneal lesions

A tug of war between destructive immune cells (Th1/Th17) and protective or immunotolerant cells (Treg cells) operates in human body in an attempt to clear microbes or unwanted attached cells, thereby protecting our body against infection or development of a lesion [90]. The balance of these two compartments of immune system has been reported to be regulated by interleukin (IL)-6/IL-17 and transforming growth factor beta (TGF-β)/IL-10 in systemic or local pelvic environment [91,92]. The competition between the levels of IL-6 and TGF-β plays a key role in the priming of Th17 cells, one of the effector T cells or Treg cells in pelvic environment. When TGF-β levels in sera or peritoneal fluid (PF) dominates over IL-6/IL-17 levels, an increase in the number/activity of FoxP3$^+$Treg cells induces strong suppression to Th17 cells resulting in a state of immune-tolerance and in less clearance of attached cells to the peritoneum with consequent survival of cells and development of lesions in the pelvis. A delicate balance between Treg cells and Th17 cells may be involved in the occurrence of endometriosis (Fig. 5.2).

With this concept in mind, we measured concentrations of TGF-β (one of the differentiation factors of Treg cells) and IL-6 (one of the potential cytokines released by the effector immune cells) in the PF collected from women with ovarian endometrioma and dermoid cyst had coexistent peritoneal lesions or without peritoneal lesions. It was found that PF levels of TGF-βwas significantly higher in women with ovarian endometrioma and dermoid cyst harboring peritoneal lesions comparing to these two groups of women without any coexistent peritoneal lesion in their pelvis. Interestingly, PF levels of IL-6 was significantly decreased than TGF-β in women with ovarian endometrioma and a modest decrease in PF levels of IL-6 was observed in women with dermoid cyst had coexisting peritoneal lesions [85]. These results indicates that an altered cytokine environment in pelvis and consequent abundant generation of Fox3$^+$Treg cells might be involved in immune escape of destructive immune cells (Th1/Th17 cells), thereby resulting in the development of peritoneal lesions. On the other hand, a switching of this event toward Th17 cells in response to low PF levels of TGF-β may be involved in decreased or no occurrence of peritoneal lesions (Fig. 5.2). Considering significantly less occurrence of peritoneal lesions in women with dermoid cyst, our study may clarify the phenomenon why 10% of women in the general population develop endometriosis even cyclic menstruation with retrograde flow occurs in more than 90% of women.

Role of activated Treg cells in endometriosis: human and animal study

Recent studies suggest the presence of a subpopulation of Fox3$^+$Treg cells may control immune response in human tissues because FoxP3$^+$ cells in human but not in murine, include not only pure suppressive T cells but also effector T cells. Miyara et al. [93] demonstrated that CD4$^+$Fox3$^+$Treg cells could be composed of three phenotypes based on the expression of

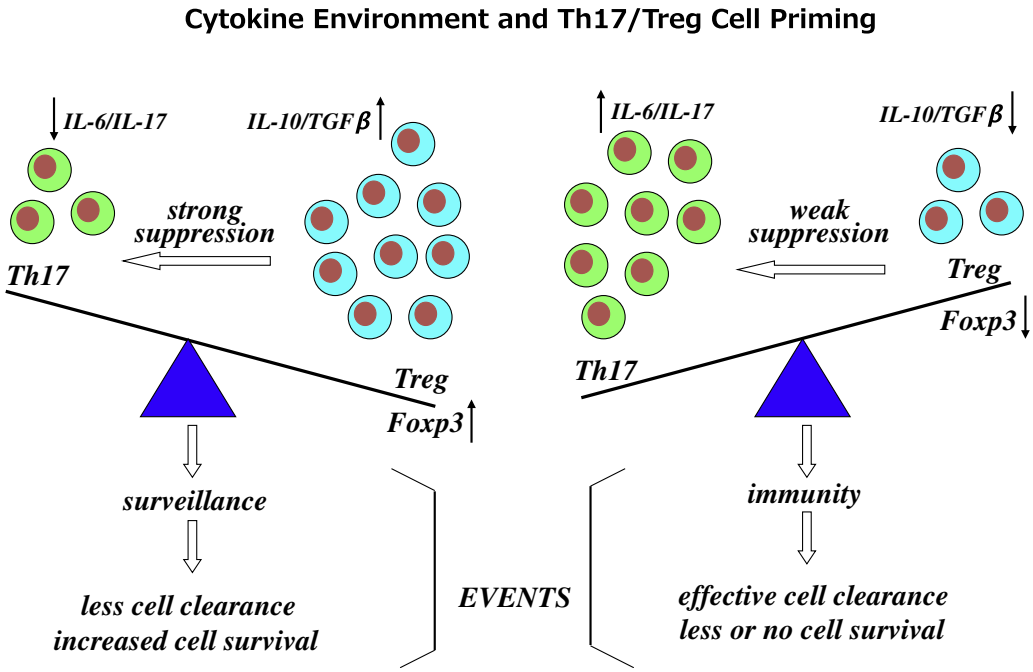


FIGURE 5.2 Shows the biological events between cytokine environment in pelvis and their association with Th17 cells/FoxP3^{+}Treg cells priming. A tug of war between destructive immune cells (Th17 cells) and protective or immuno-tolerant cells (Treg cells) operates in human body in an attempt to clear microbes or unwanted attached cells, thereby protecting our body against infection or development of a lesion. The balance between these two compartments of adaptive immunity has been reported to be regulated by interleukin (IL)-6/IL-17 and transforming growth factor beta (TGF-β)/IL-10 in systemic or local pelvic environment. The competition between the levels of IL-6/IL-17 and IL-10/TGF-β plays a key role in the priming of either Th17 cells or Treg cells in pelvic environment. When IL-10/TGF-β levels in sera or peritoneal fluid dominates over IL-6/IL-17 levels, an increase in the number/activity of FoxP3^{+}Treg cells induces strong suppression to Th17 cells resulting in a state of immune-tolerance or surveillance and in less clearance of attached cells to the peritoneum with consequent survival of cells and development of lesions in the pelvis (left side). A reverse effect of cytokine levels with weak suppression of Treg cells dominates the function of Th17 cells resulting in effective cell clearance and less or no cellular survival and less occurrence of lesion in pelvis (right side).

FoxP3, cell surface phenotype, DNA methylation of FoxP3, DNA microarray profile, proliferation status, T-cell receptor repertoire, and in vitro suppressive activity. The distinct subpopulation consists of (i) CD45RA^{+}FoxP3^{low} resting (naïve) Treg cells, (ii) CD45RA^{-}FoxP3^{high} activated Treg cells, and (iii) CD45RA^{-}FoxP3^{low} non-Treg cells. It has been proposed that activated Treg cells should be considered as possessing the true suppressive characteristics of Treg cells. The functional behavior of activated Treg cells in controlling immune response in endometriosis and how depletion of activated Treg cells in mouse model switch immune modification were investigated [94].

CD45RA^{-}FoxP3^{high} activated Treg cells in human endometriosis

The percentage of activated Treg cells in eutopic (1.5%) and ectopic (2.6%) endometria derived from women with ovarian endometrioma was significantly less than that in

endometria (8.9%) derived from control women without endometriosis. This finding was not consistent with the previous report that FoxP3^{+}Treg cells were more elevated in endometriotic lesions than in normal tissues, resulting in Treg cell-mediated immunosuppression to facilitate the survival of endometrial fragments [88]. The discrepancy between these two studies could be explained by Treg cell definition. No substantial differences were found in the activated Treg cells in PB and PF among patients with and without endometriosis. This contradicts with the findings of Hanada T et al. [95] who found that the proportion of CD45RA^{-}FoxP3^{high} Treg cells was significantly higher in the PF samples of women with endometriosis than in those of control women but this did not differ between the PB samples of patients and controls. A difference in the proportion of activated Treg cells across the phases of menstrual cycle was not observed. Interestingly, the percentage of both total (CD25^{+}FoxP3^{+} cells) Treg cells and activated Treg cells in control endometria was significantly higher than that in PF and PB in patients without endometriosis. In contrast, activated Treg cells in the eutopic/ectopic endometria derived from women with endometrioma were not different from the level in PF and PB, although the percentage of total Treg cells in ectopic endometria was significantly higher than that in PF and PB of women with endometriosis [94].

A model of temporary depletion of Treg cells in mice

A mouse model of endometriosis was made in FoxP3$^{tm3Ayr/J}$ (FoxP3^{DTR}) C57BL/6 Treg cell-depleted mice. Female FoxP3$^{tm3Ayr/J}$ mice which express the diphtheria toxin (DT) receptor (DTR) fused to enhanced green fluorescence protein under the control of FoxP3 promoter [96]. An IP infection of 0.5 μg DT was administered to both FoxP3^{DTR} and WT mice (FoxP3^{DTR}/DT and WT/DT, respectively) on days 25 and 32 to ablate Treg cells temporarily in mice [96]. Time-course analysis of Treg cell-deleted mice showed Treg cell reduction in FoxP3^{DTR} mice after DT injection on days 23 and 30 and slight restoration on day 40. While CD4^{+} T cells were increased, the relative ratio of FoxP3^{+} cells in CD4^{+} cells was significantly decreased. This result indicated that Treg cells were ablated in endometriosis-like lesions.

Changes in endometriosis-like lesions and inflammation in Treg cell ablated mice

The number and weight of endometriosis-like lesions in FoxP3^{DTR}/DT mice was significantly more increased than those in the other control mice. In contrast, no difference was found in number, weight, or size of endometriosis-like lesions between the control mice [WT/DT (+) versus WT/DT (−)]. This was confirmed by the higher immunoreactivity of Ki-67, a cell proliferation marker, in the FoxP3^{DTR}/DT mice than in control mice. As a marker of tissue inflammation, expression of F4/80^{+}CD11b^{+} (M1 macrophages) was higher in endometriosis-like lesions of FoxP3^{DTR}/DT mice than that in the control mice. Analysis of different inflammatory markers showed that gene expression of IL-6, CCL2, MIP1, and VEGF were elevated in endometriosis-like lesions in FoxP3^{DTR}/DT mice. Among these markers, only IL-6 in both PF and PB was significantly increased in FoxP3^{DTR}/DT mice compared with control mice [94]. These results suggests that true suppressive phenotype of Treg cells indeed exist that regulate tissue inflammatory reaction as well as growth of endometriosis.

All these findings in human and animal model demonstrated that decrease of true suppressive activated Treg cells might be involved in the growth, maintenance and progression of endometriosis. Accumulating evidence from both experimental and clinical studies have indicated that the decrease in Treg cells is associated with increased risk of reproductive and prenatal complications such as recurrent spontaneous abortion [97], repeated failure of artificial insemination [98], and preeclampsia [99] by increasing the effector T cell response. Comparing to control women, a significantly less proportion of activated Treg cells in the eutopic endometria derived from women with endometriosis might account not only for the growth of endometriosis but also for the occurrence of endometriosis-associated infertility. Further studies are warranted to confirm this issue and also to clarify the role of activated Treg cells in early and advanced endometriosis.

Treg/Th17 cells in early and advanced endometriosis

The Treg/Th17 balance is critical in maintaining immune homeostasis and persistence of inflammation because of the opposite effect on the immune response [91,100]. Detailed information on Treg and Th17 cell profiles in the peripheral blood (PB) and peritoneal fluid (PF) derived from women with early (revised-ASRM stage I-II) endometriosis and those with advanced (revised-ASRM stage III-IV) endometriosis is limited. The differential levels of $CD25^+FoxP3^+$Treg cells and IL-$17A^+$Th17 cells within the $CD4^+$T-cell population in the PB and PF collected from control women, women with early and advanced endometriosis are described here.

Treg/Th17 cells in PF/PF of early and advanced endometriosis

A comparison of total $CD25^+FoxP3^+$ cells within CD^+ cells was done in the PB and PF of the control group and women with endometriosis. Compared with PB, the percentages of $CD25^+FoxP3^+$ cells were significantly higher in the PF of control subjects and of women with endometriosis. No significant difference was found in the percentage of $CD25^+FoxP3^+$ cells in either PB or PF between control women and women with endometriosis. Although no significant difference was observed in the percentages of $CD25^+FoxP3^+$ cells in the PB between women with early endometriosis and advanced endometriosis, a significantly higher percentage of $CD25^+FoxP3^+$ cells was found in the PF of women with advanced endometriosis (median 10.1%) than in women with early endometriosis (median 6.0%) or in control women (median 6.8%). The differential pattern of PB and PF concentration of Treg cells in both control women and women with endometriosis may reflect their active translocation from the PB to the peritoneal cavity [101]. As a component of effector immune cells, both PB and PF collected from these three groups of women retained one-fifth to one-10th concentration of IL-$17A^+$Th17 cells comparing to $CD25^+FoxP3^+$ cells. There was no difference in the distribution of IL-$17A^+$Th17 cells either in PB or in PF between control subjects and women with endometriosis or between early and advanced endometriosis [101]. We postulate that higher Treg cells in the PF of women with advanced endometriosis may be responsible in the suppression of effector immune cells resulting in the maintenance and progression of higher grades of endometriosis. This was consistent with the persistently lower prevalence of Th17 cells in the PF of both early and advanced endometriosis. Our findings are in agreement with two recent publications [84,85].

Treg/Th17 cells in PB/PF based on the phases of menstrual cycle

A higher trend in the accumulation of Treg cells was found in the proliferative phase and menstrual phase than in the secretory phase. However, a prevalence difference between PB and PF indicated a significantly higher percentage of Treg cells in the PF in any phase of the menstrual cycle than in PB of women with endometriosis. There was so apparent difference in the distribution of Treg cells in the PB and Th17 cells in the PB/PF of women with and without endometriosis. Comparing with $CD25^+FoxP3^+$ cells in PB and PF, a persistently lower percentage of IL-$17A^+$Th17 cells was found in control subjects and in women with endometriosis across the phases of the menstrual cycle [101]. The persistently low levels of proinflammatory IL-$17A^+$Th17 cells in PB and PF across the phases of menstrual cycle may be due to the antagonistic effect of $CD25^+FoxP3^+$ cells on Th17 cells [92,100] or plasticity properties of Th17 cells with a rapid shifting toward the Th1 lineage [102]. This may further confirm a peculiar feature of human Th17 cells that they are very rare in the inflammatory sites in comparison with Th1 cells [103]. These features implicate that the Th17/Treg balance plays a major role in the development of human autoimmune and inflammatory diseases including endometriosis. We found that this balance was disturbed and may promote the initiation, maintenance and progression of endometriosis. There are diverse opinion regarding the interaction between Treg cells and effector immune cells. Recent reports have revealed that the suppressive activities of Treg cells on effector immune cells are related to both cell-contact dependent and cell-contact independent mechanisms of suppression [104,105].

Treg/Th17 cells based on the color appearance of peritoneal lesions

The distribution of $CD25^+FoxP3^+$ cells and IL-$17A^+$Th17 cells within $CD4^+$ T cell population in the PB and PF of women with stage I-II endometriosis based on the color appearance of dominant peritoneal lesions in pelvis was analyzed. It is difficult to collect and recognize color of peritoneal lesions in women with stage III-IV endometriosis due to coexistent adhesions. Peritoneal fluid level of $CD25^+FoxP3^+$ cells was significantly higher in women harboring red lesions than in black lesions. These women with red lesions also showed a statistical significance in the levels of $CD25^+FoxP3^+$ cells between PF and PB. IL-$17A^+$Th17 cells in the PB did not differ among peritoneal lesions. In contrast, PF levels of IL-$17A^+$Th17 cells in women with black lesions in the pelvis were significantly higher than in mixed lesions (coexistence of either black and white or black and red peritoneal lesions) and modestly higher than in red lesions [101]. Different macromolecules in pelvis with variable inflammatory condition may differentially trigger Treg cells and Th17 cells in women harboring different types of peritoneal lesions.

Comparison of Treg cells and effector T cells in PB and PF

$CD25^+FoxP3^-$ effector T (Teff) cells and $CD25^+FoxP3^+$ regulatory T (Treg) cells within $CD4^+$ T cell population in the PB and PF of control group and women with endometriosis were analyzed. In the PB, although there were no difference between Teff cells and Treg cells in the control group, the percentage of Teff cells was significantly higher than that of Treg cells in women with endometriosis. This difference between Teff cells and Treg cells was

lost in the PF of control group and women with endometriosis. When we compared the results of Teff cells and Treg cells on the basis of the revised-ASRM staging of endometriosis, we found a significant increase in the percentage of Teff cells relative to Treg cells in the PB of women with only stage I-II endometriosis. No significant difference between Teff cells and Treg cells was observed in the PF of control group, women with early endometriosis and women with advanced endometriosis [101].

The higher percentages of $CD25^{+}FoxP3^{-}$ T-effector cells in the PB of women with early endometriosis may indicate that the function of effector immune cells may start in the systemic environment but this effect is lost in local environment. On the basis of these findings, we may speculate that an immune defect with less clearance capacity of Th17 cells or other immune cells in the pelvis during retrograde flow of menstruation may facilitate initial survival and implantation of endometrial cells with subsequent maintenance and progression to advanced endometriosis.

TGF-beta/IL-17 levels in PF of early and advanced endometriosis

The balance of Th17/Treg cells, two cellular components of immune system, has been reported to be regulated by interleukin (IL)-6/IL-17 and transforming growth factor beta (TGF-β)/IL-10 in systemic or local pelvic environment [91,92]. The competition between the levels of IL-17 and TGF-β plays a key role in the priming of Th17 cells, one of the effector T cells or Treg cells in pelvic environment (Fig. 5.2). PF levels of both TGF-βand IL-17 were significantly higher in women with endometriosis than in control women. This pattern was also observed in women with early and advanced endometriosis. Interestingly, as a marker of effector immune cells, PF levels of IL-17 was significantly decreased than TGF-β in women advanced endometriosis and a modest decrease in women with early stage endometriosis. A menstrual phase-dependent increasing concentration of both TGF-βand IL-17 was found in the PF of women with endometriosis but this pattern was not observed in control women [101].

A significantly higher PF levels of TGF-β than in IL-17 in women with stage III-IV endometriosis and during the secretory phase coincided with increased prevalence of Treg cells in women with advanced endometriosis. Increased amounts of TGF-βand IL-17 have been reported in the PF of women with endometriosis [106,107]. It is important to mention that in addition to active translocation to the peritoneal cavity, increased numbers of Treg cells may be a result of their local induction in response to some cytokines such as TGF-βand IL-10 [108,109]. Tone et al., demonstrated that TGF-β is necessary for the differentiation and maturation of Treg cells via Smad3 pathway [110]. These results indicate that an altered cytokine environment in pelvis and consequent abundant generation of Treg cells might be involved in immune escape of destructive immune cells (Th1/Th17 cells), thereby resulting in the development of peritoneal lesions and consequent progression to advanced stage of the disease.

Summary and perspective

We demonstrated here the potential role of different components of innate and adaptive immune system either alone or in combination in the pathogenesis of endometriosis. A cross talk between innate and adaptive immune system indeed exists. Delivery of antigen-

presenting cells with expression of costimulatory molecules and production of proinflammatory cytokines by innate immune system activates naïve T-cells to generate different subsets of T helper lymphocytes of the adaptive immune system. Besides ovarian steroid hormones, the initiation, growth and progression of endometriosis can be regulated by the different components of innate and adaptive immune system. When we consider innate immune system, a cascade between effector immune cells and LPS/TLR4/NF-kB may be involved in promoting inflammatory response in pelvis and growth of endometriosis. An imbalance of T cell subsets leads to aberrant cytokine secretion and inflammation that results in further growth and progression of endometriosis. It is still uncertain whether the activity of these immune cells causes endometriosis or whether they act as secondary enhancers to maintain and progress the disease.

Immune studies so far have been observational measuring immune cells and their cytokine products in the systemic and local environment. Moreover, lack of spontaneously menstruating animal models that recapitulate human disease along with the chronic and heterogeneous nature of endometriosis and the confounding influence of concurrent hormonal and pain-related interventions further complicate the cause-effect relationship in immune context in endometriosis. In this critical situation, we have recently progressed our understanding about issues surrounding immune rejection and immune-surveillance or tolerance in endometriosis. Here, we systemically demonstrated the disturbance in the balance between proinflammatory (Th1/Th17 cells) and antiinflammatory (Th2/Treg cells) cellular components in endometriosis.

Considering lower occurrence of peritoneal lesions in women with ovarian nonendometrioma and associated lower or no tissue expression of $FoxP3^{+}$Treg cells may clarify the phenomenon that only 10% of women in the general population develop endometriosis even though cyclic menstruation with retrograde flow occurs in more than 90% of women. Study using mouse model of endometriosis and Treg-depleted mice confirmed that Treg cell deficiency exaggerates local inflammation and growth of endometriosis. Depletion of Treg cells and consequent promotion of local inflammatory response in eutopic endometria of women with endometriosis may also contribute to the occurrence of endometriosis-associated infertility. We postulate that exogenous transfer of Treg cells may resolve this problem. With the support of this proposal, a recent study in animal model demonstrated that abnormally elevated expression of Th17 cells in the fetomaternal interface may result in miscarriage and transfer of antigen-specific Treg cells before mating may have the potential application in preventing abortion rate [76].

In our separate human study we demonstrated that advanced endometriosis may be associated with disturbed compartmentalization of $CD25^{+}FoxP3^{+}$Treg cells within $CD4^{+}$ T cell population but this abrogated immune response was not observed in women with early endometriosis. A persistently lower concentration of $CD4^{+}$Th-$17A^{+}$ cells in peritoneal environment comparing to Treg cells may disturb the balance between proinflammatory and antiinflammatory condition in pelvis and trigger initiation of early endometriosis with time-dependent transition to advanced endometriosis. Our findings reconfirmed the current speculation that endometriosis is related to Treg and Th17 cells alteration causing survival and implantation of ectopic endometrial lesions in the early stage of endometriosis with consequent progression toward the advanced stage of endometriosis. At this moment, we do not know whether increased PF levels of TGF-β and Treg cells precede or follow the

development of endometriosis. A retrograde menstruation, endometrial cells in pelvis, induction of variable degree of inflammation, and most importantly, a competition between Treg cells and effector immune cells such as Th17 cells in peritoneal environment may decide the occurrence, maintenance, and progression of endometriosis. Manipulation of Treg cells to suppress and/or of Th17 cells to increase in the peritoneal environment may be the potential target in the treatment of endometriosis. Our studies on activated Treg cells in animal model and Treg/Th17 cell profiles in human body fluids may shed new light in searching and understanding the exact pathogenesis and open new avenues for the diagnosis and management of endometriosis.

References

[1] Check W. Innate immunity depends on Toll-like receptors. ASM News 2004;70:317–22.
[2] Reichhart JM. TLR5 takes aim at bacterial propeller. Nat Immunol 2003;4:1159–60.
[3] Takeda K, Akira S. Toll-like receptors in innate immunity. Int Immunol 2005;17:1–14.
[4] Wira CR, Fahey JV, Sentman CL, Pioli PA, Shen L. Innate and adaptive immunity in female genital tract: cellular responses and interactions. Immunol Rev 2005;206:306–35.
[5] Medzhitov RM, Janeway CJ. Innate immunity. N Engl J Med 2002;343:338–44.
[6] Yang D, Chertov O, Bykovskaia SN, Chen O, Buffo MJ, Shigan J, Anderson M, Schroder JM, Wang JM, Howard OM, Oppenheim JJ. β-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 1999;286:525–8.
[7] Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffman JA. The dorsoventral regulatory gene cassette spätzle/Toll/cactus controls the potent antifungal response in *Drosophila* adults. Cell 1996;86:973–6.
[8] Medzhitov R, Preston-Hurlburt P, Janeway Jr CA. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. Nature 1997;388:394–6.
[9] Poltorak A, He X, Smirnova I, Liu MI, Van Huffel C, Du X, Birdwell D, Alejos E, Silva M, Galanos C, Freudenberg M, Ricciardi-Castagnoli P, Layton B, Beutier B. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: mutation in *Tlr4* gene. Science 1998;282:2085–8.
[10] Janeway CAJ. Approaching the asymptote? Evolution and revolution in immunology. Cold Spring Harbor Symp Quant Biol 1989;54:1–13.
[11] Janeway CAJ, Medzhitov R. Innate immune recognition. Annu Rev Immunol 2002;20:197–216.
[12] Khan KN, Kitajima M, Hiraki K, Yamaguchi N, Katamine S, Matsuyama T, Fujishita A, Nakashima M, Ishimaru T, Masuzaki H. *Escherichia coli* contamination of menstrual blood and effect of bacterial endotoxin on endometriosis. Fertil Steril December 2010;94(7):2860–3.
[13] Khan KN, Fujishita A, Hiraki K, Kitajima M, Nakashima M, Fushiki S, Kitawaki J. Bacterial contamination hypothesis: a new concept in endometriosis. Reprod Med Biol 2018;17(2):125–33.
[14] Khan KN, Kitajima M, Fujishita A, Nakashima M, Masuzaki H. Toll-like receptor system and endometriosis. J Obstet Gynaecol Res 2013;39(8):1281–92.
[15] Herath S, Fischer FD, Werling D, Williams EJ, Lilly ST, Dobson H, Bryant CE, Sheldon IM. Expression and function of Toll-like receptor 4 in the endometrial cells of the uterus. Endocrinology 2006;147:562–70.
[16] Sikora J, Mielczarek-Palacz A, Kondera-Anasz Z. Role of natural killer activity in the pathogenesis of endometriosis. Curr Med Chem 2011;18(2):200–8.
[17] Oosterlynck DJ, Cornillie FJ, Waer M, Vandeputte M, Koninckx PR. Women with endometriosis show a defect in natural killer activity resulting in a decreased cytotoxicity to autologous endometrium. Fertil Steril 1991;56(1):45–51.
[18] Maeda N, Izumiya C, Taniguchi K, Matsushima S, Fukaya T. Role of NK cells and HLA-G in endometriosis. Front Biosci 2012;4:1568–81. June 1.
[19] Thiruchelvam U, Wingfiled M, O'Farrelly C. Natural killer cells: key players in endometriosis. Am J Reprod Immunol 2015;74(4):291–301.
[20] Takeuchi O, Hoshino K, Kawai T, Sanjo H, Takada H, Ogawa T, Takeda K, Akira S. Differential roles of TLR2 and TLR4 in recognition of gram-negative and gram-positive bacterial cell wall components. Immunity 1999;11:443–51.

[21] Zhou T, Hu Z, Yang S, Sun L, Yu Z, Wang G. Role of adaptive and innate immunity in type 2 diabetes mellitus. J Diabetes Res 2018;2018:7457269. November 8.
[22] Khan KN, Kitajima M, Hiraki H, Fujishita A, Sekine I, Ishimaru T, Masuzaki H. Toll-like receptors in innate immunity: role of bacterial endotoxin and toll-like receptor 4 (TLR4) in endometrium and endometriosis. Gynecol Obstet Invest 2009;68:40–52.
[23] Berrou J, Fougeray S, Venot M, Chardiny V, Gautier JF, Dulphy N, Toubert A, Peraldi MN. Natural killer cell function, an important target for infection and tumor protection, is impaired in type 2 diabetes. PLoS One 2013;8(4):e62418.
[24] Shirakwa K, Yan X, Shinmura K, Endo J, Kataoka M, Matsumata Y, Yamamoto T, Anzai A, Isobe S, Yoshida N, Itoh H, Manabe I, Sekai M, Hamazaki Y, Fukuda K, Minato N, Sano M. Obesity accelerates T cell senescence in murine visceral adipose tissue. J Clin Invest 2016;126(12):4626–39.
[25] Sheldon IM, Noakes DE, Rycroft AN, Pfeiffer DU, Dobson H. Influence of uterine bacterial contamination after parturition on ovarian dominant follicle selection and follicle growth and function in cattle. Reproduction 2002;123:837–45.
[26] Larsen S, Galask RP. Vaginal microbial flora: practical and theoretic relevance. Obstet Gynecol 1980;55:100S–13S.
[27] Kamiyama S, Teruya Y, Nohara M, Kanzawa K. Impact of detection of bacterial endotoxin in menstrual effluent on the pregnancy rate in in vitro fertilization and embryo transfer. Fertil Steril 2004;82:788–92.
[28] Fujita Y, Mihara T, Okazaki T, Shitanaka M, Kushino R, Ikeda C, Negishi H, Liu Z, Richards JS, Shimada M. Toll-like receptors (TLR) 2 and 4 on human sperm recognize bacterial endotoxins and mediate apoptosis. Hum Reprod 2011;26:2799–806.
[29] Khan KN, Fujishita A, Masumoto H, Muto H, Kitajima M, Masuzaki H, Kitawaki J. Molecular detection of intrauterine microbial colonization in women with endometriosis. Eur J Obstet Gynecol Reprod Biol 2016;199:69–75.
[30] Khan KN, Fujishita A, Kitajima M, Hiraki K, Nakashima M, Masuzaki H. Intra-uterine microbial colonization and occurrence of endometritis in women with endometriosis. Hum Reprod 2014;29(11):2446–56.
[31] Azuma Y, Taniguchi F, Nakamura K, Nagira K, Khine YM, Kiyama T, Uegaki T, Izawa M, Harada T. Lipopolysaccharide promotes the development of murine endometriosis-like lesions via the nuclear factor-kapa B pathway. Am J Reprod Immunol 2017;77(4):e12631.
[32] Khan KN, Kitajima M, Inoue T, Fujishita A, Nakashima M, Masuzaki H. 17β-estradiol and lipopolysaccharide additively promote pelvic inflammation and growth of endometriosis. Reprod Sci 2015;22(5):585–94.
[33] Cakmak H, Guzeloglu-Kayisli O, Kayisli UA, Arici A. Immune-endocrine interactions in endometriosis. Front Biosci 2009;1:429–43. June 1.
[34] Giudice LC, Kao LC. Endometriosis. Lancet 2004;364:1789–99.
[35] Burns KA, Rodriguez KF, Hewitt SC, Janardhan KS, Young SL, Korach KS. Role of estrogen receptor signaling required for endometriosis-like lesion establishment in a mouse model. Endocrinology 2012;153:3960–71.
[36] Burns KA, Thomas SY, Hamilton KJ, Young SL, Cook DN, Korach KS. Early endometriosis in females is directed by immune-mediated estrogen receptor α and IL-6 crosstalk. Endocrinology 2018;159:103–18.
[37] Podgaec S, Abrao MS, Dias Jr JA, Rizzo LV, de Oliveira RM, Baracat EC. Endometriosis: an inflammatory disease with a Th2 immune response component. Hum Reprod 2007;22:1373–9.
[38] Antsiferova YS, Sotnikova NY, Posiseeva LV, Shor AL. Changes in the T-helper cell cytokine profile and in lymphocyte activation at the systemic and local levels in women with endometriosis. Fertil Steril 2005;84:1705–11.
[39] Gieseck RL, Wilson MS, Wynn TA. Type 2 immunity in tissue repair and fibrosis. Nat Rev Immunol 2018;18(1):62–76. 117.
[40] Wilson CB, Rowell E, Sekimata M. Epigenetic control of T-helper cell differentiation. Nat Rev Immunol 2009;9(2):91–105.
[41] Ansel KM, Djuretic I, Tanasa B, Rao A. Regulation of Th2 differentiation and *Il4* locus accessibility. Annu Rev Immunol 2006;24(1):607–56.
[42] Osuga Y, Koga K, Hirota Y, Hirata T, Yoshino O, Taketani Y. Lymphocytes in endometriosis. Am J Reprod Immunol 2011;65:1–10.
[43] Szyllo K, Tchorzewski H, Banasik M, Glowacka E, Lewkowicz P, Kamer-Bartosinska A. The involvement of T lymphocytes in the pathogenesis of endometriotic tissues overgrowth in women with endometriosis. Mediat Inflamm 2003;12:131–8.

[44] Takamura M, Koga K, Izumi G, Hirata T, Harada M, Hirota Y, Hiraike O, Fujii T, Osuga Y. Simultaneous detection and evaluation of four subsets of CD^+ T lymphocyte in lesions and peripheral blood in endometriosis. Am J Reprod Immunol 2005;74:480–6.
[45] Ouyang Z, Hirota Y, Osuga Y, Hamasaki K, Hasegawa A, Tajima T, Hirata T, Koga K, Yoshino O, Harada M, Takemura Y, Nose E, Yano T, Taketani Y. Interleukin-4 stimulates proliferation of endometriotic stromal cells. Am J Pathol 2008;173:463–9.
[46] Ouyang Z, Osuga Y, Hirota Y, Hirata T, Yoshino O, Koga K, Yano T, Taketani Y. Interleukin-4 induces expression of eotaxin in endometriotic stromal cells. Fertil Steril 2009;94:58–62.
[47] Urata Y, Osuga Y, Akiyama I, Nagai M, Izumi G, Takamura M, Hasegawa A, Harada M, Hirata T, Hirota Y, Yoshino O, Koga K, Kozuma S. Interleukin-4 and prostaglandin E2 synergistically up-regulate 3beta-hydroxysteroid dehydrogenase type 2 in endometriotic stromal cells. J Clin Endocrinol Metab 2013;98:1583–90.
[48] Suen JL, Chang Y, Chiu PR, Hsieh TH, Hsi E, Chen YC, Chen YF, Tsai EM. Serum level of IL-10 is increased in patients with endometriosis and IL-10 promotes the growth of lesions in a murine model. Am J Pathol 2014;184:464–71.
[49] Nakagawa K, Kwak-Kim J, Ota K, Kuroda K, Hisano M, Sugiyama R, Yamaguchi K. Immunosuppression with tacrolimus improved reproductive outcome of women with repeated implantation failure and elevated peripheral blood TH1/TH2 cell ratio. Am J Reprod Immunol 2015;73:353–61.
[50] Kwak-Kim J, Chung-Bang HS, Ng SC, Ntrivalas EL, Mangubat CP, Beaman KD, Beer AE, Gilman-Sachs A. Increased T cell 1 cytokines responses by circulating T cells are present in women with recurrent pregnancy losses and in infertile women with multiple implantation failure after IVF. Hum Reprod 2003;18:767–73.
[51] Seli E, Arici A. Endometriosis: interaction of immune and endocrine systems. Semin Reprod Med 2003;21(2):135–44.
[52] Zhu J, Yamane H, Paul WE. Differentiation of effector CD4 T cell populations. Annu Rev Immunol 2010;28(1):445–89.
[53] Zheng Y, Rudensky AY. FoxP3 in control of the regulatory T cell lineage. Nat Immunol 2007;8(5):457–62.
[54] Dimeloe S, Burgener AV, Grahlert J, Hess C. T-cell metabolism governing activation, proliferation and differentiation: a modular view. Immunology 2017;150(1):35–44.
[55] Sakaguchi S, Miyara M, Costantino CM, Hafler DA. $Foxp3^+$ regulatory T cells in the human immune system. Nat Rev Immunol 2010;10:490–500.
[56] Liu W, Putnam AI, Xu-Yu Z, Szot GL, Lee MR, Zhu S, Gottlieb PA, Kapranov P, Gingeras TR, Fazekas de St Groth B, Clayberger C, Soper DM, Ziegler SF, Bluestone JA. CD127 expression inversely correlates with foxp3 and suppressive function of human $CD4^+$Treg cells. J Exp Med 2006;203:1701–11.
[57] Josefowicz SZ, Lu LF, Rudensky AY. Regulatory T cells: mechanisms of differentiation and function. Annu Rev Immunol 2012;30:531–64.
[58] Li P, Liu C, Yu Z, Wu M. New insights into regulatory T cell: exosome-and non-coding-mediated regulation of homeostasis and resident Treg cells. Front Immunol 2016;7:574.
[59] Guerin LR, Prins JR, Robertson SA. Regulatory t-cells and immune tolerance in pregnancy: a new target for infertility treatment? Hum Reprod Update 2009;15:517–35.
[60] Michalek RD, Gerriets VA, Jacobs SR, Macintyre AN, Maclver NJ, Mason EF, Sullivan SA, Nichols AG, Rathmell JC. Cutting edge: distinct glycolytic and lipid oxidative metabolic programs are essential for effector and regulatory $CD4^+$T cell subsets. J Immunol 2011;186(6):3299–303.
[61] Berod L, Friedrich C, Nandan A, Freitag J, Hagemann S, Harmrolfs K, Sandouk A, Hesse C, Castro CN, Bahre H, Tschirner SK, Gorinski N, Gohmert M, Mayer CT, Huehn J, Ponimaskin E, Abraham WR, Muller R, LOchner M, Sparwasser T. Erratum: De novo fatty acid synthesis controls the fate between regulatory T and T helper 17 cells. Nat Med 2015;21(4):414.
[62] Guzman-Flores JM, Portales-Perez DP. Mechanisms of suppression by regulatory T cells (Treg). Gac Med Mex 2013;149(6):630–8.
[63] Kishore M, Cheung KCP, Fu H, Bonacina F, Wang G, Coe D, Ward EJ, Colamatteo A, Jangani M, Baragetti A, Matarese G, Smith DM, Haas R, Mauro C, Wraith DC, Okkenhaug K, Catapano AL, De Rosa V, Norata GD, Marelli-Berg FM. Regulatory T cell migration is dependent on glucokinase-mediated glycolysis. Immunity 2018;48(4):831–2.
[64] Aarvak T, Chabaud M, Miossec P, Natvig JB. IL-17 is produced by some proinflammatory Th1/Th0 cells but not by Th2 cells. J Immunol 1999;162(3):1246–51.

[65] Beringer A, Noack M, Miossec P. IL-17 in chronic inflammation: from discovery to targeting. Trends Mol Med 2016;22(3):230–41.
[66] Bettelli E, Korn T, Kuchroo VK. Th17: the third member of the effector T cell trilogy. Curr Opin Immunol 2007;19(6):652–7.
[67] Saito S, Nakashima A, Ito M, Shima T. Clinical implication of recent advances in our understanding of IL-17 and reproductive immunology. Expet Rev Clin Immunol 2011;7(5):649–57.
[68] Gogacz M, Winkler I, Bojarska-Junak A, Tabarkiewicz J, Semczuk A, Rechberger T, Adamiak A. Increased percentage of TH17 cells in peritoneal fluid is associated with severity of endometriosis. J Reprod Immunol 2016;117:39–44.
[69] Hirata T, Osuga Y, Hamasaki K, Yoshino Y, Ito M, Hasegawa A, Takemura Y, Hirota Y, Nose E, Morimoto C, Harada M, Koga K, Tajima T, Saito S, Yano T, Taketani Y. Interleukin(IL)-17A stimulates IL-8 secretion, cyclooxygenase-2 expression, and cell proliferation of endometriotic stromal cells. Endocrinology 2008;149:1260–7.
[70] Ahn SH, Edwards AK, Singh SS, Young SL, Lessey BA, Tayade C. IL-17A contributes to the pathogenesis of endometriosis by triggering proinflamatory cytokines and angiogenic growth factors. J Immunol 2015;195:2591–600.
[71] Hirata T, Osuga Y, Takamura M, Kodama A, Hirota Y, Koga K, Yoshino O, Harada M, Takemura Y, Yano T, Taketani Y. Recruitment of CCR-6 expressing Th17 cells by CCL20 secreted from IL-1beta-, TNF-alpha-, and IL-17A-stimulated endometriotic stromal cells. Endocrinology 2010;151:5468–76.
[72] Gogacz M, Winkler I, Bojarska-Junak A, Tabarkiewicz J, Semczuk A, Rechberger T, Adamiak A. T regulatory lymphocytes in patients with endometriosis. Mol Med Rep 2014;10(2):1072–6.
[73] Wei C, Mei J, Tang L, Liu Y, Li D, Li M, Zhu X. 1-methyl-tryptophan attenuates regulatory T cell differentiation due to the inhibition of estrogen-IDO1-MRC2 axis in endometriosis. Cell Death Dis 2016;7(12):e2489.
[74] Wang WJ, Hao CF, Yin GJ, Bao SH, Qiu LH, Lin QD. Increased prevalence of T helper 17 (Th17) cells in peripheral blood and decidua in unexpected recurrent spontaneous abortion patients. J Reprod Immunol 2010;84:164–70.
[75] Wang WJ, Hao CF, Qu QL, Wang X, Qiu LH, Lin QD. The deregulation of regulatory T cells on interleukin-17 producing T helper cells in patients with unexplained early recurrent miscarriage. Hum Reprod 2010;25:2591–6.
[76] Wang WJ, Liu FJ, Liu X, Hao CF, Bao HC, Qu QL, Liu XM. Adaptive transfer of pregnancy-induced $CD4^+CD25^+$ regulatory T cells reverses the increase in abortion rate caused by interleukin-17 in the CBA/JxBALB/c mouse model. Hum Reprod 2014;29(5):946–52.
[77] Sampson JA. Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol 1927;14:442–69.
[78] Helme J, Hammond MG, Hulka JF, Raj SG, Talbert IM. Retrograde menstruation in healthy women and in patients with endometriosis. Obstet Gynecol 1984;64:151–4.
[79] Berbic M, Fraser IS. Regulatory T cells and other leukocytes in the pathogenesis of endometriosis. J Reprod Immunol 2011;88:149–55.
[80] Olovsson M. Immunological aspects of endometriosis: an update. Am J Reprod Immunol 2011;66(Suppl. 1):101–4.
[81] Kralickova M, Vetvicka V. Immunological aspects of endometriosis: a review. Ann Transl Med 2015;3(11):153.
[82] Chen S, Zhang J, Huang C, Lu W, Liang Y, Wan X. Expression of T regulatory cell transcription factor FoxP3 in peri-implantation phase endometrium in infertile women with endometriosis. Reprod Biol Endocrinol 2012;10:34. https://doi.org/10.1186/1477-7827-10-34.
[83] Podgaec S, Rizzo LV, Fermandes LF, Baracat EC, Abrao MS. $CD4^+CD25highFoxP3^+$ cells increased in the peritoneal fluid of patients with endometriosis. Am J Reprod Immunol 2012;68:301–8.
[84] Olkowska-Truchanowicz J, Boclan K, Maksym RB, Bialoszewska A, Wlodarczyk D, Baranowski W, Zabek J, Korczak-Kowalska G, Malejczyk J. $CD4^+CD25^+Foxp3^+$ regulatory T cells in peripheral blood and peritoneal fluid of women with endometriosis. Hum Reprod 2013;28:119–24.
[85] Khan KN, Yamamoto K, Fujishita A, Koshiba A, Kuroboshi H, Sakabayashi S, Teramukai S, Nakashima M, Kitawaki J. Association between $FOXP3^+$ regulatory T-cells and occurrence of peritoneal lesions in women with ovarian endometrioma and dermoid cysts. Reprod Biomed Online 2019;38(6):857–69.

[86] Prieto GA, Rosentein Y. Oestradiol potentiates the suppressive function of human CD4 CD25 regulatory T cells by promoting their proliferation. Immunology 2006;118(1):58–65.
[87] Arruvito L, Sanz M, Banham AH, Fainboim L. Expansion of $CD4^+CD25^+$ and $FOXP3^+$ regulatory Treg cells during the follicular phase of the menstrual cycle: implications for human reproduction. J Immunol 2007;178(4):2572–8.
[88] Berbic M, Hey-Cunningham AJ, Ng C, Tokushige N, Genewatta S, Markham R, Russel P, Fraser IS. The role of $FoxP3^+$ regulatory T-cells in endometriosis: a potential controlling mechanism for a complex, chronic immunological condition. Hum Reprod 2010;25(4):900–7.
[89] Khan KN, Masuzaki H, Fujishita A, Kitajima M, Sekine I, Ishimaru T. Higher activity by opaque endometriotic lesions than non-opaque lesions in women with endometriosis. Acta Obstet Gynecol Scand 2004;83:375–82.
[90] Prins JR, Zhang B, Schjeken JE, Guerin LR, Barry SC, Robertson SA. Unstable $FoxP3^+$ regulatory T cells and altered dendritic cells are associated with lipopolysaccharide-induced fetal loss in pregnant interleukin10-deficient mice. Biol Reprod 2015;93(4):95. https://doi.org/10.1095/biolreprod.115.128694.
[91] Bettelli E, Carrier Y, Gao W, Kom T, Storm TB, Oukka M, Weiner HL, Kuchroo VK. Reciprocal development pathways for the generation of pathogenic effector Th17 and regulatory T cells. Nature 2006;441(7090):235–8.
[92] Ichiyama K, Yoshida H, Wakabayashi Y, Chinen T, Saeki K, Nakaya M, Takaesu G, Hori S, Yoshimura A, Kobayashhi T. Foxp3 inhibits RORγt-mediated IL-17A mRNA transcription though direct interaction with RORγt. J Biol Chem 2008;283:17003–8.
[93] Miyara M, Yoshioka Y, Kitoh A, Shima T, Wing K, Niwa A, Parizot C, Taflin C, Heike T, Valeyre D, Mathian A, Nakahata T, Yamaguchi T, Nomura T, Ono M, Amoura Z, Gorochov G, Sakaguchi S. Functional delineation and differentiation dynamics of human $CD4^+$ T cells expressing the FoxP3 transcription factor. Immunity 2009;30(6):899–911.
[94] Tanaka Y, Mori T, Ito F, Koshiba A, Takaoka O, Kataoka H, Maeda E, Okimura H, Mori T, Kitawaki J. Exacerbation of endometriosis due to regulatory T-cell dysfunction. J Clin Endocrinol Metab 2017;102:3206–17.
[95] Hanada T, Tsuji S, Nakayama M, Wakinoue S, Kasahara K, Kimura F, Mori T, Ogasawara K, Murakami T. Suppressive regulatory T cells and latent transforming growth factor-β-expressing macrophages are altered in the peritoneal fluid of patients with endometriosis. Reprod Biol Endocrinol 2018;16(9). https://doi.org/10.1186/s12958-0325-2.
[96] Kim JM, Rasmussen JP, Rudensky AY. Regulatory T cells prevent catastrophic autoimmunity throughout the lifespan of mice. Nat Immunol 2007;8(2):191–7.
[97] Sasaki Y, Sasaki M, Miyazaki S, Higuma S, Shiozaki A, Saito S. Decidual and peripheral blood $CD4^+CD25^+$ regulatory T cells in early pregnancy subjects and spontaneous abortion cases. Mol Hum Reprod 2004;10(5):347–53.
[98] Lu Y, Zhang F, Zhang Y, Zeng B, Hu L, Liao A. Quantitative reduction of peripheral blood $CD4^+CD25^+FOXP3^+$ regulatory T cells in reproductive failure after artificial insemination of donor sperm. Am J Reprod Immunol 2013;69(2):188–93.
[99] Toldi G, Svec P, Vasarhelyi B, Meszaros G, Rigo J, Tulassay T, Treszl A. Decreased number of $FoxP3^+$ regulatory T cells in preeclampsia. Acta Obstet Gynecol Scand 2008;87(11):1229–33.
[100] Noack M, Miossec P. Th17 and regulatory T cell balance in autoimmune and inflammatory diseases. Autoimmun Rev 2014;13(6):668–77.
[101] Khan KN, Yamamoto K, Fujishita A, Muto H, Koshiba A, Kuroboshi H, Saito S, Teramukai S, Nakashima M, Kitawaki J. Differential levels of regulatory T-cells and T-helper-17 cells in women with early and advanced endometriosis. J Clin Endocrinol Metab 2019;104:4715–29.
[102] Annunziato F, Cosmi L, Liotta F, Maggi E, Romagnani S. Defining the human T helper 17 cells phenotype. Trends Immunol 2012;33(10):505–12.
[103] Annunziato F, Santarlasci V, Maggi L, Cosmi L, Liotta F, Romagnani S. Reasons for rarity of Th17 cells in inflammatory sites of human disorders. Semin Immunol 2013;25(4):299–304.
[104] Basta P, Koper K, Kazmierczak W, Wisniewski M, Makarewicz A, Dutsch-Wicherek M, Kojs Z, Popiela TJ, Slusarz R, Dubiel M, Wicherek L. The biological role of Treg cells in ectopic endometrium homeostasis. Histol Histopathol 2014;29:1217–33.
[105] Sakaguchi S, Wing K, Onishi Y, Prieto-Martin P, Yamaguchi T. Regulatory T cells: how do they suppress immune responses? Int Immunol 2009;21:1105–11.
[106] Zhang X, Xu H, Lin J, Qian Y, Deng L. Peritoneal fluid concentrations of interleukin-17 correlate with the severity of endometriosis and infertility of this disorder. BJOG 2005;112:1153–5.

[107] Omwandho CO, Konrad L, Halis G, Oehmke F, Tenneberg HR. Role of TGF-betas in normal eutopic endometrium and endometriosis. Hum Reprod 2010;25:101–9.
[108] Okamoto A, Fujio K, Okamura T, Yamamoto K. Regulatory T-cell-associated cytokines in systemic lupus erythematosus. J Biomed Biotechnol 2011;2011:463412.
[109] Lu L, Ma J, Wang X, Zhang F, Yu J, He G, Xu B, Brand DD, Horwitz DA, Shi W, Zheng SG. Synergistic effect of TGF-beta superfamily members on the induction of Foxp3^{+}Treg. Eur J Immunol 2010;40:142–52.
[110] Tone Y, Furuuchi K, Kojima Y, Tykocinski ML, Greene MI, Tone M. Smad3 and NFAT cooperate to induce foxp3 expression through its enhancer. Nat Immunol 2008;9:194–202.

This page intentionally left blank

CHAPTER

6

Auto-immunity and endometriosis: evidence, mechanism and therapeutic potential

Tao Zhang[1], *Tin-Chiu Li*[1] *and Chi-Chiu Wang*[1,2,3,4]

[1]Department of Obstetrics and Gynaecology, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, China; [2]Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, China; [3]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China; [4]Chinese University of Hong Kong – Sichuan University Joint Laboratory in Reproductive Medicine, The Chinese University of Hong Kong, Hong Kong, China

Introduction

Endometriosis , defined by the presence of endometrial tissues outside the uterus, is a common but complex gynecological disorder. The most common locations are the anatomically closed pelvic organs (ovaries, fallopian tubes, and peritoneum) and it seldomly occurs in remote organs, such as lungs and intestines. It affects up to 10% of the females in the general population during their reproductive age, and 20%–90% of women with pelvic pain and infertility [1,2]. Laparoscopy is the gold standard of diagnosis, which is invasive and only indicated in women with symptoms including dyspareunia, pelvic pain, and infertility. Therefore, the exact prevalence remains unknown, especially in asymptomatic women. The current treatments are very limited and often unsatisfactory, resulting in poor life quality in affected women. Although many possible etiologies and theories had been proposed in the literatures, the underlying mechanisms of endometriosis remains unclear.

The most popular and widely accepted etiology is the retrograde menstruation and transplantation theory proposed by Sampson in 1920s [3]. This theory describes how viable endometrial tissue can be spread into the peritoneal cavity through the fallopian tubes during menstruation and found in most common affected sites, including ovaries, uterosacral ligaments and cul-de-sac peritoneum. This was further supported by findings showing women with endometriosis have greater volume of refluxed blood during menstruation than those

https://doi.org/10.1016/B978-0-12-820661-4.00018-2

© 2022 Elsevier Inc. All rights reserved.

TABLE 6.1 Features in endometriosis which are indicative of autoimmune disease.

Population-based association with autoimmune diseases	• increased incidence of autoimmune diseases in women with endometriosis and vice versa. • higher risk for women with endometriosis to develop autoimmune diseases and vice versa.
B-lymphocyte immunological abnormalities (organ-specific and nonspecific autoantibodies)	• anti-endometrial and antiovarian antibodies • Laminin-1, 40% women with endometriosis and infertility were positive, 31% women with endometriosis and recurrent miscarriage were positive • IgA and IgG autoantibodies in the serum, cervical and vaginal secretions of women with endometriosis • IgG and complement deposits in the ectopic endometrium • higher complement C3 and C4 levels in the serum and peritoneal fluid • antinuclear antibodies, positive in 58% of women with endometriosis
T-lymphocyte immunological abnormalities	• alteration of Th cells and effector T cells in peritoneal fluid and ectopic endometrium
Estrogen related	• involvement of estrogen-mediated inflammation • beneficial of low-estrogen state for improving disease activity
Multiorgan involvement	• ovary, peritoneum, intestine, lung
Possible genetic basis	• FOXP-3, FCRL3, NF-κB, and B lymphocyte stimulator
Beneficial potential of immunotherapy	• potential application of TNF-α, pentoxifylline, vitamin D in endometriosis

without endometriosis. However, this may not be the only factor as retrograde menstruation has been observed in approximately 76%–90% of women of reproductive age [4]. To explain this discrepancy, the immunological anomalies in women with endometriosis have been postulated to contribute to the survival and growth of ectopic endometrial cells [5–7]. Owing to the remarkable immunological changes, including the higher incidence of autoantibodies, evidence of chronic inflammatory response and presence of traits similar to other autoimmune diseases, endometriosis has often been debated as an autoimmune disease [8]. If it is indeed the case, immunomodulatory therapy should provide an alternative treatment option to the traditional hormonal and surgical therapy, possibly with reduced side effects. In this chapter, the comparison of, and association between, endometriosis and autoimmune diseases, current evidence, and the link of their underlying mechanisms (Table 6.1) are discussed with a view to identifying potential treatments for endometriosis (Fig. 6.1).

The association between endometriosis and autoimmune diseases

The immune system is delicately educated to develop tolerance to self-antigen. In normal individuals, potentially self-reactive lymphocytes are either deleted or inactivated (clonal deletion and clonal anergy) before encountering self-antigens. The loss or breakdown of

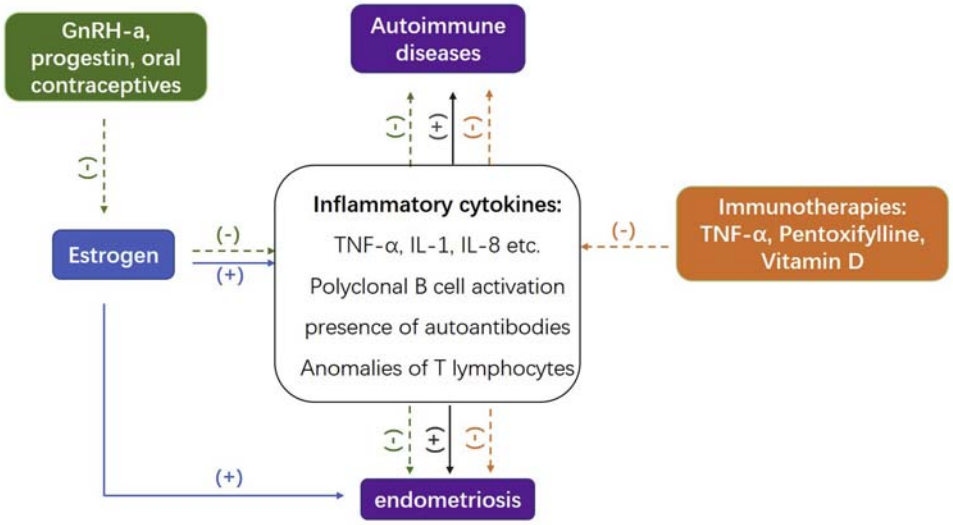


FIGURE 6.1 Potential immunotherapies for endometriosis based on the immunological similarities to autoimmune disease.

this property leads to autoimmune diseases which can affect many parts of body. There are more than 80 autoimmune diseases. The most common ones include systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), Addison's diseases (AS), Celiac diseases (CD), multiple sclerosis (MS), inflammatory bowel diseases (IBD), and SjÖgren's syndrome (SS).

In 1985, Grimes and his colleagues reported that endometriosis was associated with a twofold increased risk of SLE [9]. In later studies, the association between endometriosis and other autoimmune diseases had also been investigated [10]. A more recent metaanalysis study reviewed a total of 26 published population-based, cross-sectional, case-control, and cohort studies to investigate the association between endometriosis and autoimmune diseases. This metaanalysis supported an association between endometriosis and autoimmune diseases [11]. The association remained statistically significant in patients with SLE, SS, RA, CLD, MS, or IBD after excluding poor quality studies [11].

Whether the association between endometriosis and autoimmune diseases is casual or causal remains a matter of debate. The exact relationship between these two diseases cannot be analyzed from cross-sectional and case-control studies which are the majority of currently available association studies. Prospective longitudinal studies in women with endometriosis prior to occurrence of autoimmune diseases could provide better evidence on temporality. Two cohort studies investigated the association between laparoscopically confirmed endometriosis and subsequently diagnosed SLE, but their results were not consistent. Harris's study found that laparoscopically confirmed endometriosis was significantly associated with subsequent SLE or RA diagnosis [12] while this phenomenon was not found in Nete's study [13]. A significantly increased incidence of IBD was also observed in women with endometriosis confirmed during surgery compared with women without endometriosis in a large sized nationwide Danish cohort study [14]. Another study found the absolute risk of endometriosis

in patients with prior CD was increased with the highest risk in the first year after diagnosis [15]. These findings suggest that endometriosis may share some etiological factors with autoimmune diseases. However, several limitations have to be considered. First, there is an estimated average delay of seven years between onset of endometriosis relevant symptoms and its diagnosis by laparoscopy [16]. It is difficult to define the exact time of the start of the disease. Similarly, some cases with autoimmune diseases do not manifest typical symptoms and could remain undetected for years. Secondly, the selection of control subjects might have limitations. For instance, some study recruited women with fibroid confirmed during gynecologic surgery [11] but it can be argued that they were not strictly speaking healthy controls and there is clinical evidence to suggest an association between endometriosis and fibroids as they are both estrogen dependent gynecological pathology [17]. Other studies recruited women who attended obstetrical/gynecological clinics or family planning units and friends of women with endometriosis as endometriosis-free controls, but the absence of endometriosis had not been verified by laparoscopy [11]. Some women with asymptomatic endometriosis could have been inadvertently recruited. Last but not least, almost half of associated studies lack adjustments of confounding factors, such as age, residence location, hormonal factors, sampling year and other potential confounding health conditions [11].

In brief, evidences from the current studies suggest an increased risk of comorbidity of autoimmune diseases in women with endometriosis. Larger follow-up studies in the future would not only help to ascertain the risk, but also understand whether causal relationship exists between endometriosis and autoimmune diseases.

Autoimmune-related mechanisms in endometriosis

The association between endometriosis and autoimmune disease is not only indicated in population-based studies, they also share some serological and pathophysiological characteristics.

Humoral-mediated immunity

Humoral-mediated immunity refers to the antibody-mediated immune responses. Antibodies are produced by plasma cells (activated B cells) and provide immediate protection against infection. Most B cells are tolerized where they are born—in the bone marrow. In some pathological conditions or rare healthy situations, they are activated and produce autoantibodies against self-antigens to destroy self-tissues, resulting in autoimmune diseases or prevent inflammation by binding and removing oxidized lipids and proteins and apoptotic cells [18].

Immunological abnormalities in B cell

The increased B-cell function in women with endometriosis was firstly documented in the 1980's [19]. Around the same year, Weed and coworkers reported that IgG deposited in the endometrium of women with endometriosis is associated with a reduction in the serum total complement level, which suggested an intraendometrial antigen-antibody reaction [20]. Further studies demonstrated higher incidence of auto-antibodies in sera, cervical and

vaginal secretions from women with endometriosis [21]. Moreover, the autoantibody profiles of patients with endometriosis, including autoantibodies of IgG, IgM and IgA isotypes against phospholipids, histones, polynucleotides and even lupus, were very similar to patients with autoimmune diseases [22–24]. Likewise, a higher frequency of antitissue autoantibodies, antiendometrial, and antiendothelial autoantibodies, was also identified in various reports on women with endometriosis [25]. However, the correlation between autoantibodies concentration and the severity of endometriosis remains controversial [26–28]. Hence, the presence of autoantibodies has been considered to be a secondary immunological response which would not play an aggravating role on the pathogenesis and development of endometriosis [29].

On the other hand, the exact reason behind the increased abundance of autoantibodies in women with endometriosis is still uncertain. It has been proposed that excess endometrial proteins outside the uterus during retrograde menstruation may provoke an autoimmune response, immunologic tolerance or rejection of the homograft, resulting in the survival of endometrial implants [30]. Another possible explanation is the overexpression of B lymphocyte activating factor of the tumor necrosis factor family (BAFF) can lead to self-tolerance breakdown [31]. This factor was first identified as a new ligand of the TNF cytokine family and shown to act as a critical regulator during B cell maturation [32]. It has been shown that an increase in plasma concentration of BAFF is found in patients with autoimmune disorders [33]. In a study reported by Aniko et al., it was demonstrated that the B lymphocyte stimulator protein levels in serum were elevated in patients with endometriosis [31]. In addition, the mRNA levels expressed by macrophages and its receptor BCMA expressed by plasma cells has been found to be strongly upregulated in endometriotic lesions, which may play a role in facilitating the survival of plasma cells [31]. Moreover, excessive expression of BAFF might impair B cell tolerance resulting in autoimmune diseases [34]. Thus, future studies on BAFF could provide new insights on the understanding of autoantibodies production in endometriosis and its impact on potential treatment regime.

Autoantibodies associated with infertility

Antiendometrial antibodies are the most well-studied autoantibodies; they have been postulated to be potential diagnostic or prognostic marker of the assessment of treatment and recurrence of endometriosis. The antiendometrial antibodies include transferrin and alpha 2 Heremans Schmidt (α2-HS) glycoprotein. The serum from patients with endometriosis with positive antiendometrial antibody could migrate and react with the luminal epithelium, glandular epithelium, and stroma to alter endometrial receptivity and increase implantation failure [35]. With the concentration of transferrin and α2-HS glycoprotein being found higher in peritoneal fluid from women with endometriosis, it has been shown that transferrin attenuates FSH-induced differentiation of granulosa cells and might consequently lead to a varying degrees of ovarian dysfunction [36]. On the other hand, α2-HS glycoprotein, a negative acute phase protein, suppresses the maturation conversion of mouse embryo zona pellucida protein ZP3 to ZP3f and causes polyspermia [37]. Furthermore, the addition of antibodies specifically against transferrin and α 2-HS glycoprotein to sperm cells in vitro can inhibit motility and survival [30].

Laminin is a major multifunctional basement membrane glycoprotein with at least 15 known isoforms. It plays a critical role during embryo implantation by synthesizing

network-forming complement during early embryo development. Also, it has been found to increase the trophoblast adhesion to the maternal extracellular matrix and decidua in endometrium, which further promotes proliferation and differentiation of trophoblast upon interaction with integrin receptors. In a study conducted by Inagaki and coworkers, they found a significant association between IgG ani-laminin-1 antibodies and infertile patients with endometriosis at stage II or more [38]. The same group also demonstrated that antilaminin-1 antibodies are significantly higher in women with recurrent miscarriage when compared with healthy controls, presumably they interfere with embryogenesis and placental development [39,40]. Moreover, antilaminin-1 in follicular fluid was found to be related to oocyte maturation and adversely affect oocyte quality resulting in reduced fertility [41].

Other autoantibodies have also been found in women with endometriosis undergoing assisted reproduction. A retrospective study examined the impact of circulating IgG, IgM and IgA isotype antibodies to cardiolipin, phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatic acid (PA), histone 2A (H2A) and 2B (H2B) fractions, single-stranded DNA (ssDNA), and double-stranded DNA (dsDNA) on IVF outcome in women with and without endometriosis [42]. The result showed three or more positive autoantibodies were detected in 50% of patients with endometriosis. The pregnancy rates in autoantibody-positive group and autoantibody-negative group was 23% and 46%, respectively [42]. Importantly, administration of corticosteroid for patients with autoantibodies improved the pregnancy rate [42]. A similar study analyzed the presence of several different antinuclear antibodies (ANA), antiphospholipid antibodies (APA), antithyroid antibodies (ATA), and lupus anticoagulant in infertile women with recurrent pregnancy loss [43]. The result showed that rate of positive autoantibodies was similar in women with reproductive failure and endometriosis, which is higher than fertile controls [43]. Based on these evidences, autoantibodies could be a potentially useful marker in infertility and recurrent miscarriage associated with endometriosis.

Immunological abnormalities in T cell function

Two of the most important weapons in adaptive immune system are helper T (Th) cells and killer (cytotoxic) T cells. Th cells can produce many different cytokines by which they communicate with the rest of the immune system. So far, three major subsets have been identified: Th1, Th2 and Th17 based on the secreted cytokines profile. In addition, there is another subset of Th cells, Foxp3+ regulatory T cells (Tregs), which functions to control and suppress a range of immune responses by production of IL-10, TGF-β, and antiinflammatory cytokines that inhibit Th cell activation. Th1/Th2 and Th17/Tregs must be balanced in healthy conditions to eliminate pathogens.

Th cells are central mediators of autoimmune pathology although it is still not yet possible to precisely define their exact functions in specific autoimmune disease. An excessive Th1 or Th2-like response may favor the development of different autoimmune diseases. Th1 cytokines participate in the process of autoimmune disease in which cytotoxic T cells attack organ-specific component. Th1 dominant autoimmune diseases include Hashimoto's thyroiditis and insulin-dependent diabetes mellitus [44]. On the other hand, Th2-driven autoimmune response is involved in Grave's disease and SLE with a strong

humoral component which amplifies the activation of B cells [44]. In addition to the specific cytokines profile, the conventional Th cells (CD4+ T-cells) are characterized by unique expression of chemokine receptors which mediate cells migration to inflamed sites to promote B cell responses and antibody production within pathologically inflamed non-lymphoid tissues in patients with RA [45]. These suggest the chemoattractant from inflamed lesions might be the regulators for modulating T-B-cell interactions. With regards to the balance of Th17/Tregs, the activity of Th17 is usually in excess of that of Tregs and the defect in either the number or the function of Tregs have been found in various autoimmune disorders, which suppresses the self-tolerance of the activation of B cells and promotes the production of auto-antibodies [46,47]. In recent years, high-throughput genomic at a single-cell level discovered the existence of instability and plasticity within the Tregs compartment in different local environments. Hence, modulating Tregs function might be a therapeutic option for patients with autoimmune diseases [48,49]. In brief, the imbalance of Th cells, Th1/Th2, and Th17/Tregs, is involved in autoimmune disease through driving autoantibody response and chronic tissue inflammation [50,51], which could be a target for immunotherapy.

Several studies revealed that total lymphocyte numbers and the ratio of helper/suppressor in peripheral blood and peritoneal fluid were not obviously altered [52–54], while one study reported that helper/suppressor ratio was increased in infertile women with endometriosis [55] and another study showed a decrease in affected women [56]. Nevertheless, the reported balance of Th1/Th2 is consistent in women with endometriosis in published studies. Podgaec et al. found that there was a predominance of IL-4 and IL-10 in peritoneal fluid of women with endometriosis, suggesting endometriosis involves a possible shift toward Th2 immune response component [57] which might stimulate the differentiation and proliferation of B cells through the synthesis of IL-4, IL-5 and IL-10. This observation was reinforced by other studies showing that endometriosis was associated with decreased peritoneal Th1 immune response in peritoneal fluid which may allow the survival, implantation, and proliferation of endometrial cells under conditions that are not yet fully understood [54]. The animal study using a murine model showed that the promotion of a Th1 milieu in the peritoneum reduced the weight and area of the implant [58]. It suggests that the imbalance of Th1/Th2 is involved in the pathogenesis of endometriosis and might be modulated as a therapeutic target. In the local sites, there is no significant difference in total lymphocyte number and helper/suppressor ratio in the eutopic endometrium of women with and without endometriosis [59]. In contrast, the number of T lymphocytes is significantly elevated in the stroma of endometriotic lesions compared with control group during both proliferative and secretory phases [60]. Moreover, the number of activated T cells was increased in ectopic endometrium as compared to eutopic endometrium [60]. This might be caused by the abnormal chemokines such as Interleukin-8 (IL-8) expressed in endometriotic lesions [61], which induces the chemotaxis of T lymphocytes. Unfortunately, previous studies mainly measured the percentage or cell numbers of T cells. Nowadays, with the development of high-throughput sequencing and proteomics at single-cell level, further studies are required to better explore the characteristics and pathological roles of T cells in endometriosis.

In recent years, Th17 cells and Tregs have been investigated in endometriosis. It has been found that the percentage of Th17 cells in peritoneal fluid was significantly higher

in patients with severe endometriosis compared with women with a minimal/mild stage of the disease, namely the increased percentage of peritoneal Th17 cells is associated with the severity of endometriosis [62]. Th17 cells were also found in ectopic endometrium. It has been found that IL-17A, the representative cytokines secreted by Th17, could directly stimulate the production of IL-8 and proliferation of stromal cells [63]. The concentration of IL-8 is significantly increased in peritoneal fluid of women with endometriosis and it is involved in several key steps of endometriosis development through stimulating proliferation, matrix metalloproteinase activity, invasive capacity, angiogenesis, Fas ligand protein expression, and adhesion capability of endometrial stromal cells [64–66]. The same group also found that inflammatory cytokines including IL-17A stimulated the production of CCL-20 in ectopic endometrial stromal cells which regulate the chemotaxis of Th17 cells expressing CCR6 (the receptor of CCL-20) [67]. Th17 cells, IL-17A and ectopic endometrial stromal cells seems to work as a positive feedback loop in the progression of endometriosis. However, there are also debatable findings. In a recent study, Khan et al. reported that the percentage of Th17 cells was persistently lower in both peripheral blood and peritoneal fluid of women with early and advanced endometriosis [68]. The changes and roles of Th17 in the development of endometriosis still need more studies for clarification. In line with a role for a modified immunity in the pathogenesis of endometriosis, CD4+/FoxP3+ Tregs are present in endometriotic lesions [69]. In humans, conflicting results have been obtained regarding whether populations of Tregs in the peritoneal fluid (PF) or peripheral blood (PB) differ significantly between patients with EMS and controls [70–72]. The percentage of Tregs and its cytokines products are significantly higher in peripheral fluid from women with endometriosis, which may contribute to immunotolerance of ectopic endometrium. TECK derived from endometrial stromal cells and macrophages promotes the differentiation and cytokines production of Tregs [73]. According to several studies, a strong association between transforming growth factor-β (TGF-β) levels in PF and EMS has been observed [74], and TGF-β induces FoxP3+ Tregs and inhibits the proliferation of immune cells and cytokine production via FOXP3-dependent and independent mechanisms [75]. On the other side, decreased percentage of Tregs might favor autoimmune activity and inflammatory response. However, there is no study on the correlation of the percentage of Tregs with autoantibodies or autoimmune diseases in women with endometriosis. Alternatively, the changes of Tregs might be caused by the polymorphisms of FOXP3, the key transcription factor of Tregs. In reflecting the immune response, the polymorphisms of FOXP3 gene may change the quantity or function of Tregs. As shown in autoimmune disease, FOXP3 gene was predominantly restricted to the function or cell numbers of Tregs [76]. A remarkable association between polymorphisms of FOXP3 and endometriosis was also observed [128], but the studies exploring the percentage of Tregs in endometriosis did not analyze the polymorphisms of FOXP3. In addition, the differences may be caused by the different recruiting criteria of affected subjects and controls discussed as above. Hence, further studies are warranted to clarify the precise role of Tregs in endometriosis. In future study, it would be of interest to examine the ratio of Th17/Tregs (like the ratio of Th1/Th2) as it is more meaningful than single percentage or absolute number of Th17 and Tregs as immune cells collaborate as a team like “Yin” and “Yang” to maintain the homeostasis.

Estrogen related immune response in autoimmune diseases and endometriosis

Estrogen plays a critical role not only in reproduction, but also in autoimmune diseases by interacting with immune system. That is also one of the reasons why women during fertile age are more often affected by autoimmune diseases than men [77]. Many immune cells have been found to possess estrogen receptors, such as CD8+ T cells, B cells, monocytes and macrophages [78,79]. Estrogens increase humoral immunity while progesterone exerts as a natural immunosuppressor [80–82].

In systemic inflammatory diseases, 17β-estradiol enhances IgG and IgM production by peripheral blood mononuclear cells (PBMCs), leading to increased levels of polyclonal IgG including anti-dsDNA antibody in PBMCs from patients with SLE by enhancing B cell activity via IL-10 [83]. Estrogen is also able to enhance the secretion of proinflammatory cytokines TNF-α, IL-1 and IL-6 by macrophages and CD4+ T cells [84,85]. It has been found that the concentration of estrogen is increased in synovial fluid from patients with RA and it promotes the production of matrix metalloproteinase and IL-1β-induced IL-6 from human fibroblast-like synoviocytes and macrophages [86]. Furthermore, the inflammatory cytokines are able to stimulate the aromatase activity, which mediates the conversion of androgens to estrogens [87,88]. Therefore, the positive feedback loop between estrogens and inflammatory cytokines in synovial fluid might explain the altered balance of androgen (low) and estrogen (high) as well as their pathological roles in RA. The increased aromatase activity and shifted balance of androgens and estrogens is also presented in women with SLE [89]. In all, the interaction between estrogen and immune system is involved in the pathogenesis of autoimmune diseases.

Endometriotic lesion is characterized by the higher biosynthesis and lower inactivation of estradiol compared to healthy women [1], which are caused by aberrant expression of several enzymes metabolizing estrogen (aromatase, 17β-Hydroxysteroid dehydrogenase type 2-17β-HSD2). In addition to directly stimulates the proliferation of ectopic endometrial cells through estrogen receptors, estrogen might be also involved in the pathogenesis of endometriosis by interacting with immune response as above. Estrogen receptor is reported to be expressed in the majority of immunoreactive macrophages isolated from peritoneal cells of women with or without endometriosis [90]. It is also expressed by the macrophages infiltrated into the endometrium. With pretreatment of estrogen at pregnancy level, lipopolysaccharide (LPS) stimulated nitrite production, and TNF-α secretion produced by murine peritoneal macrophages are decreased [91]. This indicates that the increase of estrogen concentration may promote the cytokine production from macrophages in women with endometriosis. The main hormonal treatments for endometriosis including Danazol, GnRH agonist and dienogest are able to suppress the concentration of autoantibodies and peritoneal IL-1β, TNF-α, monocyte chemoattractant protein (MCP)-1 [92–95]. Therefore, estrogen-mediated proinflammatory immune response may favor the development of endometriosis, which further provides the evidence for the potential of developing immunomodulatory therapies for endometriosis.

Autoimmune-related genetics in endometriosis

There is without a doubt that endometriosis is a genetic disease [96,97]. Common genetic polymorphisms related to immune system were both detected in endometriosis and autoimmune disease, including region of the genes, FOXP-3 (discussed as above), FCRL3, NF-κB and B lymphocyte stimulator.

Fc receptor-like 3 (FCRL3) gene encodes a glycoprotein belonging to the immunoglobulin receptor superfamily. It plays a role in B cells differentiation into autoreactive cells through the modulation of signal transduction via activation/inactivation of signaling tyrosine protein kinases [98]. As reported by Bianco et al., FCRL3-169C/T was significantly associated with risk of endometriosis regardless of the stage of the disease [99]. Also, the FCRL3-169C allele was found to be correlated with an increased production of autoantibodies [100].

NF-κB, a major transcription factor of immune response leading to cell apoptosis and growth, was reported to play an important role in mediating antiapoptosis, growth of endometriotic cells, invasion, angiogenesis and cytokine production in endometriosis [101]. Recently, a common insertion/deletion polymorphism (−94 insertion/deletion ATTG) in the NF-κB gene was identified in SLE [102]. The depletion of ATTG results in the loss of binding to nuclear proteins and reduces promoter activity. In endometriosis, the frequency of the ATTG (2)/ATTG (2) genotype and ATTG (2) allele was significantly increased when compared with healthy individuals. This suggested that the polymorphism of NF-κB gene might be a predisposition gene toward endometriosis susceptibility [103].

B lymphocyte stimulator (BLyS), a member of the TNF superfamily implicated in other autoimmune diseases, is necessary for B-cell development and differentiation into plasma cells [32]. When the prevalence of BLyS-817C/T polymorphic variant was assessed in women with endometriosis, it revealed that patients with deep infiltrating endometriosis were less likely to have a BLyS-817C > T genotype as compared with the group of gynecologic patients without symptomatic endometriosis and group of healthy women [104].

Autoimmune diseases are usually associated with certain HLA alleles [105]. Similar phenomenon has also been observed in endometriosis [24]. In the Japanese population, Class I HLA-B54 and HLA-Cw7, class II HLA-DRB1*1403 and HLADQB1*0301 were associated with endometriosis [106,107]. Another study found that endometriosis was associated with class I HLA-B*0702 alleles, in linkage disequilibrium with HLA-A24, HLA-Cw*0702 and class II HLA-DRB1*0101 alleles [108]. While in the Chinese population, class I HLA-B46, class II HLA-DRB1*15, and HLADQA1*0401 were reported to be associated with endometriosis [109−111]. However, this was not replicable in the Korean population [112]. Similarly, the association between HLA alleles and endometriosis was not consistent in Europe. In a study conducted by Sundqvist et al. they found significant association between CCL21, HLA-DRB1 and endometriosis in the Swedish/Belgian population [113], however this association was not found in the Polish population [114]. The disparity between the different populations indicated multiple genetic factors might be involved in variable prevalence of endometriosis among different ethnicities.

Potential immunomodulatory therapy for autoimmunity in endometriosis

Hormonal therapies

Endometriotic lesions are associated with hormonal imbalance, including increased estrogen synthesis and metabolism and progesterone resistance which cause proliferation of endometriotic cells and inflammation [115]. Accordingly, hormonal medication targets on the hormonal imbalance by decreasing systemic and local estrogen synthesis or estrogen activity, counteracting progesterone resistance by progestins or by selective progesterone-receptor modulators [115]. The most commonly used drug acting on estrogen secretion and/or ER activity is GnRH-analogs (GnRH-a) which is highly effective to inhibit the growth of the ectopic endometrial tissue and control pelvic pain [116]. The limitations of GnRH-a include the recurrence rate after therapy discontinuation and side effects associated with low estradiol: bone density loss, worsening of serum lipoprotein cholesterol distribution, hot flashes, genitourinary atrophy, depression, and decreased libido [117]. Danazol (a derivative of 17α-ethynyl testosterone with mild androgenic but strong antiestrogenic activity) is also useful to improve endometriosis-associated symptoms by inhibiting the concentration of estradiol [115]. However, the side effects of androgenic and anabolic properties, including weight gain, acne, oily hair, headache, seborrhea, and liver function are poorly tolerant for the majority [115]. In addition, progestin, combined hormonal contraceptive agents, and selective progesterone-receptor modulators are options to inhibit endogenous ovarian estradiol production and ovulation and create a progestin-dominant hormonal milieu that suppress the growth of endometriotic lesions [118,119], but the incidence of irregular bleeding is high.

Due to the inflammatory effects mediated by estradiol in autoimmune disease, especially in SLE and RA discussed as above, inhibition of estrogen has been proposed to reduce disease activity. An open-labeled and longitudinal study evaluated the use of pregnane progestins in 187 SLE patients over 48 months. It was reported that the disease activity was reduced after progestins treatment although the breakthrough bleeding was reported in 17.7% patients using cyproterone acetate and 12.6% patients using chlormadinone acetate [120]. The recent data from the Norfolk Arthritis Register (NOAR) showed that oral contraceptive use is generally associated with a beneficial functional outcome in women with recent-onset inflammatory polyarthritis [121]. However, another cohort study found the improvement in disease activity and damage was not significant in 54 oral contraceptive users with RA compared to 58 non-users [122]. This inconsistent finding was also reported in earlier small-sized studies [123,124]. The controversial results might be caused by the different assessment of disease activity, follow-up years and confounding factors such as the age of symptom onset, and duration of oral contraceptive use and so on. On the other hand, a large population-based case-control study of 445 women showed reduced risk of developing RA in the following years when oral contraceptive exposure occurred in earlier years [125]. All the studies are mainly assessing the safety of oral contraceptives in women with RA and the results are consistently showing that oral contraceptives can be safely used as contraception in RA. At this point, there is no strong evidence to recommend that oral contraceptives can be used as a preventive or therapeutic regime in RA. It is worthwhile to continue to seek potential applications of hormonal (progestin and androgenic compounds) or antihormonal (estrogen receptor modulators and antagonist metabolites) therapies for autoimmune diseases.

Taken together, hormonal treatment of endometriosis targets on reducing estrogen levels and reversing progesterone resistance, leading to decrease in the growth of both eutopic and ectopic endometrial cells. This regime maybe also have a beneficial effect in treating autoimmune diseases due to the effects of estrogen on immune response, which further sheds light on the potential of developing immunomodulatory therapies or synergistic combination therapies, a strategy which can avoid hypoestrogenic state associated side effects and restoring fertility.

Immunomodulatory therapy

Anti-TNF-α therapies

TNF-α is a cytokine involved in systemic inflammation, which stimulates inflammatory response in the acute phase by activating a cascade of cytokines, such as IL-1,IL-6, etc. and influences the reaction, proliferation and differentiation through binding to either one or both of the two membrane-bound receptors, TNFR1 and TNFR2. It is mainly produced by activated macrophages, lymphocytes and NK cells. Its excessive production is involved in the pathogenesis of some autoimmune inflammatory diseases, such as RA, CD and UC [126]. TNF-α inhibitors are highly effective in the induction and maintenance of clinical remission in about 60%−70% of patients [127]. Different ratio of the two TNF-α receptors on different T cell subsets and the formation of antidrug antibodies induced by TNF-α might decrease the functional drug concentration, leading to a reduced response of treatment [126,128].

TNF-α is involved also in the pathogenesis of endometriosis since higher expression level of TNF-α was found in PF of women with endometriosis [129]. TNF-α expression in endometrial epithelial cells during the secretory phase can be upregulated by IL-1 [130]. Keenan and coworkers demonstrated that peritoneal macrophages might be another potential source to induce elevated TNF-α in PF [131]. Furthermore, TNF-α enhances the adhesion of cultured endometrial stromal cells to mesothelial cells [132]. This may help retrograde endometrial cell attach to peritoneum. In addition to adhesion, TNF-α also stimulates the proliferation of endometrial cells. In a rat model of endometriosis, TNF-α binding protein-1 was found to inhibit the proliferation of endometrial stromal cells [133]. Furthermore, TNF-α induces higher synthesis of IL-8 in endometrial stromal cells, which causes the proliferation of endometrial stromal cells in a dose dependent fashion [134].

Since increased TNF-α has been found in women with endometriosis and plays an essential role in mediating the pathological change of immune response, it is a potential target to suppress endometriosis progression. TNF-α inhibition by administration of TNF binding protein-1 (TBP-1) has been demonstrated to successfully prevent and treat induced or spontaneous endometriosis in rats [133] or baboons model [135] respectively. Infliximab, TNF-α monoclonal antibody, is used to treat RA and inflammatory bowel disease. A small sample randomized controlled trial conducted a decade ago reported that infliximab appeared not to affect the pain associated with deep endometriosis [136]. Etanercept, a recombinant human TNF binding protein-1 binding to TNF-α and inhibiting its action, is one of the treatments for RA and other arthritis types. Barrier et al. found that etanercept effectively reduced the extent of endometriotic lesions and the surface area of red lesions in baboons [135]. Unfortunately,

there has not been any sufficiently powered and well-designed clinical trials to determine the efficacy of TNF-α inhibitors in endometriosis. More trials are required to determine its efficiency as a treatment for endometriosis. Moreover, the ratios of TNF-α receptors and antidrug antibodies should also be considered in the future trials.

Pentoxifylline

Pentoxifylline is a multi-site immunomodulatory agent, which plays an important role in controlling inflammation and apoptosis in different autoimmune diseases [137]. It suppresses phagocytosis and production of toxic oxygen species and proteolytic enzymes by macrophages and neutrophils. Besides, it also inhibits TNF-α generation by macrophages and the proinflammatory effect of TNF-α and IL-1 on granulocytes [137]. In 1991, Steinleitner and colleagues reported that the use of pentoxifylline dramatically abrogated the adverse influence of endometriotic implants on infertility in rodent mice [138]. Later, the same group showed that endometriosis-associated subfertility in mice model were induced by the transfer of hyperactivated macrophages, not basal state macrophages, into peritoneal cavity. The subfertility was reversed with the treatment of periovulatory pentoxifylline [139]. Moreover, pentoxifylline inhibited the growth of endometriotic implants in rat model and did not affect the circulating estrogen and progesterone levels [140]. In 1997, Balasch and coworkers demonstrated that treatment of oral pentoxifylline for 12 months enhanced the pregnancy rate of women with infertility associated with asymptomatic minimal or mild endometriosis [141]. However, a Cochrane review (187) evaluated four clinical trials, and demonstrated a lack of evidence to recommend pentoxifylline for pain relief or to improve the chances of spontaneous pregnancy. So, even if these novel therapeutic agents appear promising in the treatment of endometriosis, further studies from multi-center clinical trials are needed to confirm if it could be recommended as a routine regime for women with endometriosis.

Vitamin D

In recent years, accumulating evidences showed that Vitamin D (VD) supplementation could also be a therapeutic agent for managing endometriosis. VD is a pleiotropic molecule and exerts a broad range of biological activities [142]. It has been well-documented to play a vital role in bone mineralization as well as regulation of plasma calcium levels, and VD has shown immunomodulatory effect [142]. Deficiency in VD is associated with pathogenesis of several autoimmune diseases, including SLE and MS [143]. The active form of VD, 1,25(OH) 2D3, has been reported to inhibit dendritic cell maturation and Th17 cells activation in SLE patients, which decreases autoantibody production [144]. It promotes antiinflammatory cytokine profile and reduces the Th17/Tregs ratio in MS [145] and supresses the migration and function of macrophages to produce cytokines [146,147]. Therefore, VD supplementation has been proposed as an immunomodulator for autoimmune diseases treatment [148,149].

The VD receptor is expressed in multiple reproductive tissues and cells, including granulosa cells [150], decidua [151], placenta [151], endometrium [152], fallopian epithelial cells [152], and pituitary glands [153]. This indicates that VD should be closely associated in the physiologic reproduction process. In a study conducted by Agic et al. they found that the expression of VD receptor at both mRNA and protein levels of the endometrium was significantly higher in women with endometriosis compared with healthy controls [152]. However, there were conflicting reports regarding the serum levels of 1,25(OH)2D3 in women with

endometriosis. The study reported by Hartwell et al. found that 1,25(OH)2D3 levels were higher in women affected by endometriosis [154]. Another study observed a similar trend toward a higher level of 1,25(OH)2D3 in women with endometriosis compared with control women [155]. In contrast, the study of Mariko et al. revealed that serum 1,25(OH)2D3 was significantly lower in women with severe endometriosis compared with controls and women with mild endometriosis, and there was no difference between women with and without endometriosis [156]. A possible explanation for the discrepancy among these studies might be due to the heterogeneity of population studied and the relatively small sample size.

Recently, the therapeutic effect of 1,25(OH)2D3 on endometriosis has been highlighted as a potential therapeutic agent. In brief, rodents supplemented with 1,25(OH)2D3 showed regression of endometriotic lesion by significantly reducing VEGF and MMP-9, and increasing MMP-2 inhibitors [157–159]. The addition of 1,25(OH)2D3 in culture medium of endometriotic stromal cells significantly inhibited IL-1β or TNF-α induced inflammatory responses, such as IL-8 expression and prostaglandin activity [160]. Moreover, 1,25(OH)2D3 can reduce the expressions of MMP-2 and MMP-9 through inhibiting the NF-kB signaling pathway [156]. Importantly, dairy products rich in calcium and VD have been claimed to lower the risk of developing endometriosis in humans [160]. Therefore, VD supplementation may be considered a novel therapeutic approach for the management of endometriosis through its immunomodulatory and antiinflammatory properties. However, the role of VD in cellular and hormonal immune response in endometriosis has not been clearly investigated yet. Randomized clinical trials are required to verify if VD supplementation is indeed beneficial.

Conclusion

Endometriosis shares many characteristics and fulfills some criteria of autoimmune diseases, including immunological abnormalities in T- and B-cell functions, polyclonal B-cell activation, presence of various autoantibodies, aberrant apoptosis, multiorgan involvement, tissue damage, genetic preference, female preponderance, and association with other autoimmune diseases. Although endometriosis is still not currently defined as an autoimmune disease, the link between endometriosis and autoimmune disease provide a new approach to improve and optimize the therapy for patients with endometriosis, with fewer side effects compared with currently used hormonal therapies. Therefore, more research about the mechanisms of chronic local inflammation/autoantibodies in endometriosis and well-designed clinical trials regarding immunomodulatory therapy are needed to improve outcome.

References

[1] Bulun SE. Endometriosis. N Engl J Med 2009;360(3):268–79.
[2] Mathias SD, et al. Chronic pelvic pain: prevalence, health-related quality of life, and economic correlates. Obstet Gynecol 1996;87(3):321–7.
[3] Sampson JA. Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. Am J Pathol 1927;3(2):93–110. 43.
[4] Halme J, Becker S, Wing R. Accentuated cyclic activation of peritoneal macrophages in patients with endometriosis. Am J Obstet Gynecol 1984;148(1):85–90.

[5] Olovsson M. Immunological aspects of endometriosis: an update. Am J Reprod Immunol 2011;66(Suppl. 1):101–4.
[6] Kanzaki H, Nakajima T, Yoshimura T. Immunological aspects of endometriosis. Nihon Rinsho 2001;59(Suppl. 1):44–7.
[7] Sidell N, Han SW, Parthasarathy S. Regulation and modulation of abnormal immune responses in endometriosis. Ann N Y Acad Sci 2002;955:159–73. Discussion 199–200, 396–406.
[8] Matarese G, et al. Pathogenesis of endometriosis: natural immunity dysfunction or autoimmune disease? Trends Mol Med 2003;9(5):223–8.
[9] Grimes DA, et al. Systemic lupus erythematosus and reproductive function: a case-control study. Am J Obstet Gynecol 1985;153(2):179–86.
[10] Greenbaum H, et al. Evidence for an association between endometriosis, fibromyalgia, and autoimmune diseases. Am J Reprod Immunol 2019;81(4):e13095.
[11] Shigesi N, et al. The association between endometriosis and autoimmune diseases: a systematic review and meta-analysis. Hum Reprod Update 2019;25(4):486–503.
[12] Harris HR, et al. Endometriosis and the risks of systemic lupus erythematosus and rheumatoid arthritis in the Nurses' Health Study II. Ann Rheum Dis 2016;75(7):1279–84.
[13] Nielsen NM, et al. The co-occurrence of endometriosis with multiple sclerosis, systemic lupus erythematosus and Sjogren syndrome. Hum Reprod 2011;26(6):1555–9.
[14] Jess T, et al. Increased risk of inflammatory bowel disease in women with endometriosis: a nationwide Danish cohort study. Gut 2012;61(9):1279–83.
[15] Stephansson O, Falconer H, Ludvigsson JF. Risk of endometriosis in 11,000 women with celiac disease. Hum Reprod 2011;26(10):2896–901.
[16] Nnoaham KE, et al. Impact of endometriosis on quality of life and work productivity: a multicenter study across ten countries. Fertil Steril 2011;96(2):366–373 e8.
[17] Uimari O, Jarvela I, Ryynanen M. Do symptomatic endometriosis and uterine fibroids appear together? J Hum Reprod Sci 2011;4(1):34–8.
[18] Lleo A, et al. Definition of human autoimmunity–autoantibodies versus autoimmune disease. Autoimmun Rev 2010;9(5):A259–66.
[19] Startseva NV. [Clinical immunological aspects of genital endometriosis]. Akush Ginekol (Mosk) 1980;(3):23–6.
[20] Weed JC, Arquembourg PC. Endometriosis: can it produce an autoimmune response resulting in infertility? Clin Obstet Gynecol 1980;23(3):885–93.
[21] Mathur S, et al. Autoimmunity to endometrium and ovary in endometriosis. Clin Exp Immunol 1982;50(2):259–66.
[22] Gleicher N, et al. Is endometriosis an autoimmune disease? Obstet Gynecol 1987;70(1):115–22.
[23] Rote NS, Dostal DA. Is endometriosis an autoimmune disease? Obstet Gynecol 1988;71(2):280–2.
[24] Nothnick WB. Treating endometriosis as an autoimmune disease. Fertil Steril 2001;76(2):223–31.
[25] Fernandez-Shaw S, et al. Anti-endometrial and anti-endothelial auto-antibodies in women with endometriosis. Hum Reprod 1993;8(2):310–5.
[26] Taylor PV, et al. Autoreactivity in women with endometriosis. Br J Obstet Gynaecol 1991;98(7):680–4.
[27] Evers JL, Dunselman GA, Van der Linden PJ. Markers for endometriosis. Baillieres Clin Obstet Gynaecol 1993;7(4):715–39.
[28] Badaway SZ, et al. Endometrial antibodies in serum and peritoneal fluid of infertile patients with and without endometriosis. Fertil Steril 1990;53(5):930–2.
[29] Dias Jr JA, de Oliveira RM, Abrao MS. Antinuclear antibodies and endometriosis. Int J Gynaecol Obstet 2006;93(3):262–3.
[30] Mathur SP. Autoimmunity in endometriosis: relevance to infertility. Am J Reprod Immunol 2000;44(2):89–95.
[31] Hever A, et al. Human endometriosis is associated with plasma cells and overexpression of B lymphocyte stimulator. Proc Natl Acad Sci USA 2007;104(30):12451–6.
[32] Schiemann B, et al. An essential role for BAFF in the normal development of B cells through a BCMA-independent pathway. Science 2001;293(5537):2111–4.
[33] Moisini I, Davidson A. BAFF: a local and systemic target in autoimmune diseases. Clin Exp Immunol 2009;158(2):155–63.

[34] Bosello S, et al. BAFF and rheumatic autoimmune disorders: implications for disease management and therapy. Int J Immunopathol Pharmacol 2007;20(1):1–8.
[35] Gajbhiye R, et al. Multiple endometrial antigens are targeted in autoimmune endometriosis. Reprod Biomed Online 2008;16(6):817–24.
[36] Sensky TE, Liu DT. Endometriosis: associations with menorrhagia, infertility and oral contraceptives. Int J Gynaecol Obstet 1980;17(6):573–6.
[37] Kalab P, Schultz RM, Kopf GS. Modifications of the mouse zona pellucida during oocyte maturation: inhibitory effects of follicular fluid, fetuin, and alpha 2HS-glycoprotein. Biol Reprod 1993;49(3):561–7.
[38] Inagaki J, et al. An association of IgG anti-laminin-1 autoantibodies with endometriosis in infertile patients. Hum Reprod 2003;18(3):544–9.
[39] Inagaki J, et al. Pregnancy loss and endometriosis: pathogenic role of anti-laminin-1 autoantibodies. Ann N Y Acad Sci 2005;1051:174–84.
[40] Inagaki J, et al. IgG anti-laminin-1 autoantibody and recurrent miscarriages. Am J Reprod Immunol 2001;45(4):232–8.
[41] Caccavo D, et al. Anti-laminin-1 antibodies in sera and follicular fluid of women with endometriosis undergoing in vitro fertilization. Int J Immunopathol Pharmacol 2011;24(2):481–8.
[42] Dmowski WP, et al. The effect of endometriosis, its stage and activity, and of autoantibodies on in vitro fertilization and embryo transfer success rates. Fertil Steril 1995;63(3):555–62.
[43] Kaider AS, et al. Immunodiagnostic evaluation in women with reproductive failure. Am J Reprod Immunol 1999;42(6):335–46.
[44] Kroemer G, et al. Differential involvement of Th1 and Th2 cytokines in autoimmune diseases. Autoimmunity 1996;24(1):25–33.
[45] Rao DA, et al. Pathologically expanded peripheral T helper cell subset drives B cells in rheumatoid arthritis. Nature 2017;542(7639):110–4.
[46] Petro TM. Regulatory role of resveratrol on Th17 in autoimmune disease. Int Immunopharm 2011;11(3):310–8.
[47] Miyara M, et al. Human FoxP3+ regulatory T cells in systemic autoimmune diseases. Autoimmun Rev 2011;10(12):744–55.
[48] Dominguez-Villar M, Baecher-Allan CM, Hafler DA. Identification of T helper type 1-like, Foxp3+ regulatory T cells in human autoimmune disease. Nat Med 2011;17(6):673–5.
[49] Daniel C, von Boehmer H. Extrathymic generation of regulatory T cells–chances and challenges for prevention of autoimmune disease. Adv Immunol 2011;112:177–213.
[50] Carbajal KS, et al. Th cell diversity in experimental autoimmune encephalomyelitis and multiple sclerosis. J Immunol 2015;195(6):2552–9.
[51] Hoyer KK, et al. Distinct roles of helper T-cell subsets in a systemic autoimmune disease. Blood 2009;113(2):389–95.
[52] Gleicher N, et al. Lymphocyte subsets in endometriosis. Obstet Gynecol 1984;63(4):463–6.
[53] Hill JA, et al. Characterization of leukocyte subpopulations in the peritoneal fluid of women with endometriosis. Fertil Steril 1988;50(2):216–22.
[54] Mier-Cabrera J, et al. Quantitative and qualitative peritoneal immune profiles, T-cell apoptosis and oxidative stress-associated characteristics in women with minimal and mild endometriosis. BJOG 2011;118(1):6–16.
[55] Badawy SZ, et al. Immune rosettes of T and B lymphocytes in infertile women with endometriosis. J Reprod Med 1987;32(3):194–7.
[56] Gallinelli A, et al. Different concentrations of interleukins in the peritoneal fluid of women with endometriosis: relationships with lymphocyte subsets. Gynecol Endocrinol 2004;18(3):144–51.
[57] Podgaec S, et al. Endometriosis: an inflammatory disease with a Th2 immune response component. Hum Reprod 2007;22(5):1373–9.
[58] Mier-Cabrera J, Gonzalez-Gallardo S, Hernandez-Guerrero C. Effect of nitric oxide and TH1/TH2 cytokine supplementation over ectopic endometrial tissue growth in a murine model of endometriosis. Reprod Sci 2013;20(11):1332–8.
[59] Mettler L, et al. Lymphocyte subsets in the endometrium of patients with endometriosis throughout the menstrual cycle. Am J Reprod Immunol 1996;36(6):342–8.
[60] Witz CA, et al. Characterization of lymphocyte subpopulations and T cell activation in endometriosis. Am J Reprod Immunol 1994;32(3):173–9.

[61] Dmowski WP, Gebel HM, Braun DP. The role of cell-mediated immunity in pathogenesis of endometriosis. Acta Obstet Gynecol Scand Suppl 1994;159:7–14.
[62] Gogacz M, et al. Increased percentage of Th17 cells in peritoneal fluid is associated with severity of endometriosis. J Reprod Immunol 2016;117:39–44.
[63] Hirata T, et al. Interleukin (IL)-17A stimulates IL-8 secretion, cyclooxygensase-2 expression, and cell proliferation of endometriotic stromal cells. Endocrinology 2008;149(3):1260–7.
[64] Arici A. Local cytokines in endometrial tissue: the role of interleukin-8 in the pathogenesis of endometriosis. Ann N Y Acad Sci 2002;955:101–9. Discussion 118, 396–406.
[65] Mulayim N, et al. Regulation of endometrial stromal cell matrix metalloproteinase activity and invasiveness by interleukin-8. Fertil Steril 2004;81(Suppl. 1):904–11.
[66] Selam B, et al. Regulation of fas ligand expression by IL-8 in human endometrium. J Clin Endocrinol Metab 2002;87(8):3921–7.
[67] Hirata T, et al. Recruitment of CCR6-expressing Th17 cells by CCL 20 secreted from IL-1 beta-, TNF-alpha-, and IL-17A-stimulated endometriotic stromal cells. Endocrinology 2010;151(11):5468–76.
[68] Khan KN, et al. Differential levels of regulatory T cells and T-helper-17 cells in women with early and advanced endometriosis. J Clin Endocrinol Metab 2019;104(10):4715–29.
[69] Maridas DE, et al. Peripheral and endometrial dendritic cell populations during the normal cycle and in the presence of endometriosis. J Endometr Pelvic Pain Disord 2014;6(2):67–119.
[70] Gogacz M, et al. T regulatory lymphocytes in patients with endometriosis. Mol Med Rep 2014; 10(2):1072–6.
[71] Olkowska-Truchanowicz J, et al. CD4(+) CD25(+) FOXP3(+) regulatory T cells in peripheral blood and peritoneal fluid of patients with endometriosis. Hum Reprod 2013;28(1):119–24.
[72] Takamura M, et al. Simultaneous detection and evaluation of four subsets of CD4+ T lymphocyte in lesions and peripheral blood in endometriosis. Am J Reprod Immunol 2015;74(6):480–6.
[73] Li MQ, et al. CD4+Foxp3+ regulatory T cell differentiation mediated by endometrial stromal cell-derived TECK promotes the growth and invasion of endometriotic lesions. Cell Death Dis 2014;5:e1436.
[74] Harada T, Iwabe T, Terakawa N. Role of cytokines in endometriosis. Fertil Steril 2001;76(1):1–10.
[75] Yoshimura A, Wakabayashi Y, Mori T. Cellular and molecular basis for the regulation of inflammation by TGF-beta. J Biochem 2010;147(6):781–92.
[76] Hori S, Nomura T, Sakaguchi S. Control of regulatory T cell development by the transcription factor Foxp3. Science 2003;299(5609):1057–61.
[77] Kvien TK, et al. Epidemiological aspects of rheumatoid arthritis: the sex ratio. Ann N Y Acad Sci 2006;1069:212–22.
[78] Cutolo M, et al. Presence of estrogen receptors in human myeloid monocytic cells (THP-1 cell line). Eur Cytokine Netw 2001;12(2):368–72.
[79] Cutolo M, et al. Androgen and estrogen receptors are present in primary cultures of human synovial macrophages. J Clin Endocrinol Metab 1996;81(2):820–7.
[80] Bijlsma JW, et al. Neuroendocrine immune system involvement in rheumatology. Ann N Y Acad Sci 2006;1069:xviii–xxiv.
[81] Cutolo M, et al. Nocturnal hormones and clinical rhythms in rheumatoid arthritis. Ann N Y Acad Sci 2005;1051:372–81.
[82] Guery JC. Estrogens and inflammatory autoimmune diseases. Joint Bone Spine 2012;79(6):560–2.
[83] Kanda N, Tsuchida T, Tamaki K. Estrogen enhancement of anti-double-stranded DNA antibody and immunoglobulin G production in peripheral blood mononuclear cells from patients with systemic lupus erythematosus. Arthritis Rheum 1999;42(2):328–37.
[84] Hu SK, Mitcho YL, Rath NC. Effect of estradiol on interleukin 1 synthesis by macrophages. Int J Immunopharm 1988;10(3):247–52.
[85] Correale J, Arias M, Gilmore W. Steroid hormone regulation of cytokine secretion by proteolipid protein-specific CD4+ T cell clones isolated from multiple sclerosis patients and normal control subjects. J Immunol 1998;161(7):3365–74.
[86] Cutolo M, et al. Synovial fluid estrogens in rheumatoid arthritis. Autoimmun Rev 2004;3(3):193–8.
[87] Macdiarmid F, et al. Stimulation of aromatase activity in breast fibroblasts by tumor necrosis factor alpha. Mol Cell Endocrinol 1994;106(1–2):17–21.

[88] Purohit A, et al. Aromatase activity and interleukin-6 production by normal and malignant breast tissues. J Clin Endocrinol Metab 1995;80(10):3052–8.
[89] Folomeev M, et al. Plasma sex hormones and aromatase activity in tissues of patients with systemic lupus erythematosus. Lupus 1992;1(3):191–5.
[90] Khan KN, et al. Estrogen and progesterone receptor expression in macrophages and regulation of hepatocyte growth factor by ovarian steroids in women with endometriosis. Hum Reprod 2005;20(7):2004–13.
[91] Kim JY, Jeong HG. Down-regulation of inducible nitric oxide synthase and tumor necrosis factor-alpha expression by bisphenol A via nuclear factor-kappaB inactivation in macrophages. Cancer Lett 2003;196(1):69–76.
[92] Taketani Y, Kuo TM, Mizuno M. Comparison of cytokine levels and embryo toxicity in peritoneal fluid in infertile women with untreated or treated endometriosis. Am J Obstet Gynecol 1992;167(1):265–70.
[93] Kennedy SH, et al. Antiendometrial antibodies in endometriosis measured by an enzyme-linked immunosorbent assay before and after treatment with danazol and nafarelin. Obstet Gynecol 1990;75(6):914–8.
[94] Ferrero S, et al. Endometriosis: the effects of dienogest. Clin Evid 2015;2015.
[95] Andres Mde P, et al. Dienogest in the treatment of endometriosis: systematic review. Arch Gynecol Obstet 2015;292(3):523–9.
[96] Kennedy S, Mardon H, Barlow D. Familial endometriosis. J Assist Reprod Genet 1995;12(1):32–4.
[97] Hadfield RM, et al. Risk factors for endometriosis in the rhesus monkey (*Macaca mulatta*): a case-control study. Hum Reprod Update 1997;3(2):109–15.
[98] Chistiakov DA, Chistiakov AP. Is FCRL3 a new general autoimmunity gene? Hum Immunol 2007;68(5):375–83.
[99] Bianco B, et al. Association of FCRL3-169T/C polymorphism with endometriosis and identification of a protective haplotype against the development of the disease in Brazilian population. Hum Immunol 2011;72(9):774–8.
[100] Owen CJ, et al. Analysis of the Fc receptor-like-3 (FCRL3) locus in Caucasians with autoimmune disorders suggests a complex pattern of disease association. J Clin Endocrinol Metab 2007;92(3):1106–11.
[101] Gonzalez-Ramos R, et al. Involvement of the nuclear factor-kappaB pathway in the pathogenesis of endometriosis. Fertil Steril 2010;94(6):1985–94.
[102] Gao M, et al. NFKB1 -94 insertion/deletion ATTG polymorphism contributes to risk of systemic lupus erythematosus. DNA Cell Biol 2012;31(4):611–5.
[103] Zhou B, et al. A functional promoter polymorphism in NFKB1 increases susceptibility to endometriosis. DNA Cell Biol 2010;29(5):235–9.
[104] de Graaff AA, et al. B lymphocyte stimulator -817C>T promoter polymorphism and the predisposition for the development of deep infiltrating endometriosis. Fertil Steril 2010;94(3):1108–10.
[105] Kong YC, et al. Application of HLA class II transgenic mice to study autoimmune regulation. Thyroid 2007;17(10):995–1003.
[106] Ishii K, et al. Studies on the human leukocyte antigen class I antigens in Japanese patients with macroscopically diagnosed endometriosis. Gynecol Obstet Invest 2002;54(3):150–3.
[107] Ishii K, et al. Studies on the human leukocyte antigen-DR in patients with endometriosis: genotyping of HLA-DRB1 alleles. Hum Reprod 2002;17(3):560–3.
[108] Kitawaki J, et al. Association of HLA class I and class II alleles with susceptibility to endometriosis. Hum Immunol 2002;63(11):1033–8.
[109] Wang X, et al. [Study on polymorphism of human leukocyte antigen-DRB1 allele in patients with endometriosis]. Zhonghua Fu Chan Ke Za Zhi 2002;37(6):346–8.
[110] Zong L, et al. [Comparative study of HLA-DQA1 and HLA-DRB1 allele in patients with endometriosis and adenomyosis]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2002;19(1):49–51.
[111] Zong L, He Y, Pan D. [Association of human leukocyte antigen-DQA1 with endometriosis of women in southern China]. Zhonghua Fu Chan Ke Za Zhi 2001;36(7):405–7.
[112] Whang DH, et al. No association between HLA-DRB1 alleles and susceptibility to advanced stage endometriosis in a Korean population. Hum Reprod 2006;21(1):129–33.
[113] Sundqvist J, et al. Endometriosis and autoimmune disease: association of susceptibility to moderate/severe endometriosis with CCL21 and HLA-DRB1. Fertil Steril 2011;95(1):437–40.
[114] Roszkowski PI, et al. Susceptibility to ovarian endometriosis in Polish population is not associated with HLA-DRB1 alleles. Hum Reprod 2005;20(4):970–3.

[115] Tosti C, et al. Hormonal therapy for endometriosis: from molecular research to bedside. Eur J Obstet Gynecol Reprod Biol 2017;209:61–6.
[116] Abrao MS, et al. Deep endometriosis infiltrating the recto-sigmoid: critical factors to consider before management. Hum Reprod Update 2015;21(3):329–39.
[117] Streuli I, et al. An update on the pharmacological management of endometriosis. Expet Opin Pharmacother 2013;14(3):291–305.
[118] Angioni S, et al. New trends of progestins treatment of endometriosis. Gynecol Endocrinol 2014;30(11):769–73.
[119] Bergqvist A, Theorell T. Changes in quality of life after hormonal treatment of endometriosis. Acta Obstet Gynecol Scand 2001;80(7):628–37.
[120] Chabbert-Buffet N, et al. Pregnane progestin contraception in systemic lupus erythematosus: a longitudinal study of 187 patients. Contraception 2011;83(3):229–37.
[121] Camacho EM, et al. The relationship between oral contraceptive use and functional outcome in women with recent-onset inflammatory polyarthritis: results from the Norfolk Arthritis Register. Arthritis Rheum 2011;63(8):2183–91.
[122] Drossaers-Bakker KW, et al. Pregnancy and oral contraceptive use do not significantly influence outcome in long term rheumatoid arthritis. Ann Rheum Dis 2002;61(5):405–8.
[123] Hazes JM, et al. Oral contraceptive treatment for rheumatoid arthritis: an open study in 10 female patients. Br J Rheumatol 1989;28(Suppl. 1):28–30.
[124] Gilbert M, et al. Norethynodrel with mestranol in treatment of rheumatoid arthritis. J Am Med Assoc 1964;190:235.
[125] Doran MF, et al. The effect of oral contraceptives and estrogen replacement therapy on the risk of rheumatoid arthritis: a population based study. J Rheumatol 2004;31(2):207–13.
[126] Pecoraro V, et al. The impact of immunogenicity of TNFalpha inhibitors in autoimmune inflammatory disease. A systematic review and meta-analysis. Autoimmun Rev 2017;16(6):564–75.
[127] Ford AC, et al. Efficacy of biological therapies in inflammatory bowel disease: systematic review and meta-analysis. Am J Gastroenterol 2011;106(4):644–59. Quiz 660.
[128] Bystrom J, et al. TNFalpha in the regulation of Treg and Th17 cells in rheumatoid arthritis and other autoimmune inflammatory diseases. Cytokine 2018;101:4–13.
[129] Eisermann J, et al. Tumor necrosis factor in peritoneal fluid of women undergoing laparoscopic surgery. Fertil Steril 1988;50(4):573–9.
[130] Bergqvist A, et al. Production of interleukins 1beta, 6 and 8 and tumor necrosis factor alpha in separated and cultured endometrial and endometriotic stromal and epithelial cells. Gynecol Obstet Invest 2000;50(1):1–6.
[131] Keenan JA, et al. IL-1 beta, TNF-alpha, and IL-2 in peritoneal fluid and macrophage-conditioned media of women with endometriosis. Am J Reprod Immunol 1995;34(6):381–5.
[132] Zhang RJ, Wild RA, Ojago JM. Effect of tumor necrosis factor-alpha on adhesion of human endometrial stromal cells to peritoneal mesothelial cells: an in vitro system. Fertil Steril 1993;59(6):1196–201.
[133] D'Antonio M, et al. Ability of recombinant human TNF binding protein-1 (r-hTBP-1) to inhibit the development of experimentally-induced endometriosis in rats. J Reprod Immunol 2000;48(2):81–98.
[134] Iwabe T, et al. Tumor necrosis factor-alpha promotes proliferation of endometriotic stromal cells by inducing interleukin-8 gene and protein expression. J Clin Endocrinol Metab 2000;85(2):824–9.
[135] Barrier BF, et al. Efficacy of anti-tumor necrosis factor therapy in the treatment of spontaneous endometriosis in baboons. Fertil Steril 2004;81(Suppl. 1):775–9.
[136] Koninckx PR, et al. Anti-TNF-alpha treatment for deep endometriosis-associated pain: a randomized placebo-controlled trial. Hum Reprod 2008;23(9):2017–23.
[137] Hendawy N. Pentoxifylline attenuates cytokine stress and Fas system in syngeneic liver proteins induced experimental autoimmune hepatitis. Biomed Pharmacother 2017;92:316–23.
[138] Steinleitner A, et al. Immunomodulation in the treatment of endometriosis-associated subfertility: use of pentoxifylline to reverse the inhibition of fertilization by surgically induced endometriosis in a rodent model. Fertil Steril 1991;56(5):975–9.
[139] Steinleitner A, Lambert H, Roy S. Immunomodulation with pentoxifylline abrogates macrophage-mediated infertility in an in vivo model: a paradigm for a novel approach to the treatment of endometriosis-associated subfertility. Fertil Steril 1991;55(1):26–31.

[140] Nothnick WB, Curry TE, Vernon MW. Immunomodulation of rat endometriotic implant growth and protein production. Am J Reprod Immunol 1994;31(2–3):151–62.
[141] Balasch J, et al. Pentoxifylline versus placebo in the treatment of infertility associated with minimal or mild endometriosis: a pilot randomized clinical trial. Hum Reprod 1997;12(9):2046–50.
[142] Bouillon R, et al. Vitamin D and human health: lessons from vitamin D receptor null mice. Endocr Rev 2008;29(6):726–76.
[143] Wahono CS, et al. Effects of 1,25(OH)2D3 in immune response regulation of systemic lupus erithematosus (SLE) patient with hypovitamin D. Int J Clin Exp Med 2014;7(1):22–31.
[144] Shahin D, et al. Serum 25-OH vitamin D level in treatment-naive systemic lupus erythematosus patients: relation to disease activity, IL-23 and IL-17. Lupus 2017;26(9):917–26.
[145] Lu M, Taylor BV, Korner H. Genomic effects of the vitamin D receptor: potentially the link between vitamin D, immune cells, and multiple sclerosis. Front Immunol 2018;9:477.
[146] Riek AE, et al. 25(OH) vitamin D suppresses macrophage adhesion and migration by downregulation of ER stress and scavenger receptor A1 in type 2 diabetes. J Steroid Biochem Mol Biol 2014;144 Pt A:172–9.
[147] Zhang Y, et al. Vitamin D inhibits monocyte/macrophage proinflammatory cytokine production by targeting MAPK phosphatase-1. J Immunol 2012;188(5):2127–35.
[148] Marinho A, et al. Vitamin D supplementation effects on FoxP3 expression in T cells and FoxP3(+)/IL-17A ratio and clinical course in systemic lupus erythematosus patients: a study in a Portuguese cohort. Immunol Res 2017;65(1):197–206.
[149] Simpson Jr S, der Mei IV, Taylor B. The role of vitamin D in multiple sclerosis: biology and biochemistry, epidemiology and potential roles in treatment. Med Chem 2018;14(2):129–43.
[150] Thill M, et al. Expression of prostaglandin metabolising enzymes COX-2 and 15-PGDH and VDR in human granulosa cells. Anticancer Res 2009;29(9):3611–8.
[151] Tanamura A, et al. Purification and characterization of 1,25(OH)2D3 receptor from human placenta. J Obstet Gynaecol 1995;21(6):631–9.
[152] Agic A, et al. Relative expression of 1,25-dihydroxyvitamin D3 receptor, vitamin D 1 alpha-hydroxylase, vitamin D 24-hydroxylase, and vitamin D 25-hydroxylase in endometriosis and gynecologic cancers. Reprod Sci 2007;14(5):486–97.
[153] Perez-Fernandez R, et al. Vitamin D receptor gene expression in human pituitary gland. Life Sci 1997;60(1):35–42.
[154] Hartwell D, et al. Vitamin D metabolites–relation to age, menopause and endometriosis. Scand J Clin Lab Invest 1990;50(2):115–21.
[155] Somigliana E, et al. Vitamin D reserve is higher in women with endometriosis. Hum Reprod 2007;22(8):2273–8.
[156] Miyashita M, et al. Effects of 1,25-dihydroxy vitamin D3 on endometriosis. J Clin Endocrinol Metab 2016;101(6):2371–9.
[157] Mariani M, et al. The selective vitamin D receptor agonist, elocalcitol, reduces endometriosis development in a mouse model by inhibiting peritoneal inflammation. Hum Reprod 2012;27(7):2010–9.
[158] Abbas MA, et al. Regression of endometrial implants treated with vitamin D3 in a rat model of endometriosis. Eur J Pharmacol 2013;715(1–3):72–5.
[159] Yildirim B, et al. 1-alpha,25-dihydroxyvitamin D3 regresses endometriotic implants in rats by inhibiting neovascularization and altering regulation of matrix metalloproteinase. Postgrad Med 2014;126(1):104–10.
[160] Jurkiewicz-Przondziono J, et al. Influence of diet on the risk of developing endometriosis. Ginekol Pol 2017;88(2):96–102.

CHAPTER

7

Role of estrogen and estrogen-related factors in endometriosis

Taisuke Mori and Jo Kitawaki

Department of Obstetrics and Gynecology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan

Introduction

Endometriosis is a chronic disease characterized by the presence of endometrial tissue outside the uterus. Its affects approximately 6%–10% of the general female population of reproductive age [1]and it is associated with various clinical symptoms including chronic pelvic pain, dysmenorrhea, dyspareunia, and infertility, seriously affecting women's health and quality of life [2]. Although extensive studies have provided some established theories to understand the etiology of the endometriosis, its exact pathogenesis remains poorly defined.

Scientific evidence has established that endometriosis is foremost an estrogen-dependent disease [3], characterized by estrogen-dependent growth and maintenance of ectopic endometrium and by increased local estrogen production. Clinically, endometriosis-associated pain improves dramatically after menopause in most of cases. Additionally, the lesions usually shrink in a low-estrogen environment after treatment with a gonadotropin releasing hormone (GnRH) agonist [4]. Previous studies have shown that estrogen is elevated in local endometriotic lesions, although serum estrogen levels are not elevated in women with endometriosis [5–8]. The biological action of estrogen is mediated by estrogen receptors (ERs). Estrogen responsiveness depends on the balance of ER expression, distribution, and ER protein function. These features differ between endometriotic tissue and normal endometrium, contributing to the pathological characteristics of endometriosis [9]. Thus, previous evidence suggests the existence of a proliferative signaling mechanism in endometriotic tissues mediated by the estrogen-ER axis [10]. Here we provide current insight into the biological process of estrogen-mediated signaling in endometriosis and the development of therapeutic strategies targeting local estrogen formation.

Immunology of Endometriosis
https://doi.org/10.1016/B978-0-12-820661-4.00022-4

© 2022 Elsevier Inc. All rights reserved.

Estrogen production in endometriosis (Fig. 7.1)

In situ estrogen synthesis and metabolism have been considered to play an important role in the development and progression of an estrogen-dependent disease [11,12]. Estrogen is one of the steroid hormones synthesized from cholesterol. Cholesterol is transferred from the cytoplasm to the mitochondrion by steroidogenic acute regulatory protein (StAR). StAR is expressed in adrenal glands and gonads. The expression is stimulated initially by follicle-stimulating hormone (FSH) and luteinizing hormone (LH) secreted from the pituitary. The function of StAR in the regulation of steroidogenesis involves transport of cholesterol for estrogen production [13]. Previous studies have shown that StAR expression at the protein and

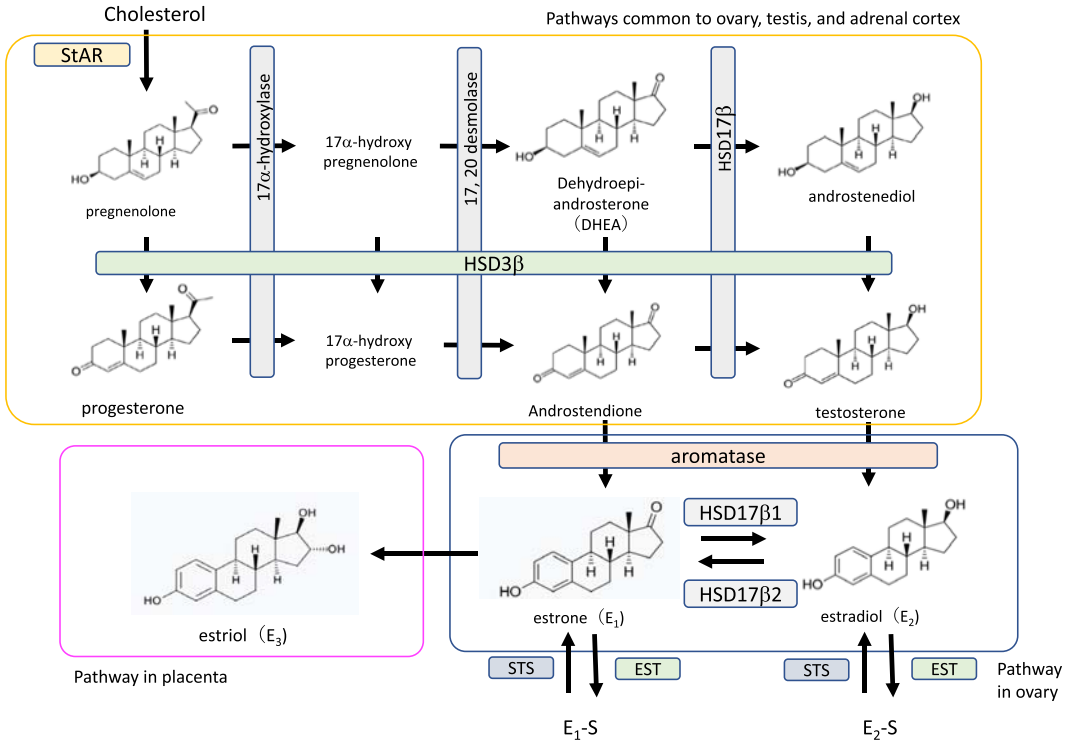


FIGURE 7.1 **Steroid hormone biosynthesis pathway.** Estrogen is one of the steroid hormones synthesized from cholesterol. Cholesterol is transferred from the cytoplasm to the mitochondrion by steroidogenic acute regulatory protein (StAR) which is expressed in adrenal glands and gonads. Pregnenolone is converted to progesterone by 3b-hydroxysteroid dehydrogenase (3b-HSD). 3bHSD is also responsible for converting 17-hydroxyprognenolone to 17-hydroxyprogesterone, dehydroepiandrosterone (DHEA) to androstenedione. Aromatase is the enzyme that converts testosterone and androstenedione to estradiol (E_2) and estrone (E_1), respectively. In situ estrogen synthesis and metabolism have been considered to play an important role in the development and progression of an estrogen-dependent disease. The 17β-hydroxysteroid dehydrogenase type1 (HSD17β1) catalyzes 17β-reduction of biologically inactive E_1 to E_2, while HSD17 type 2 (HSD17β2) preferentially catalyzes the oxidation of E_2 to E_1. The other major source of estrogens is estrone sulfate, an inactive conjugated form abundant in the circulation. Estrone sulfate (S) is desulfated to estrone by steroid sulfatase (STS) and estrone is inactivated by estrogen sulfotransferase (EST).

mRNA level is significantly higher in peritoneal endometriosis and endometriotic stromal cells, when compared with normal endometrium [14,15]. Treatment with prostaglandin E_2 (PGE_2) significantly increased StAR expression in human endometriotic stromal cells. This response could be mediated via phosphorylation of cAMP response element-binding protein (CREB) and binding of CCAAT/enhancer-binding protein (C/EBP) to a cis-element of the StAR promoter [16,17]. Thus, aberrant expression of StAR in endometriotic stromal cells plays a critical role in the development of endometriosis.

3β-hydroxysteroid dehydrogenase (HSD3β2) is responsible for converting progesterone, 17-hydroxyprognenolone to 17-hydroxyprogesterone, dehydroepiandrosterone (DHEA) to androstenedione. HSD3β isozymes are encoded by HSD3β1 and HSD3β2. HSD3β1 is expressed in placenta peripheral tissues including the mammary gland, prostate, and skin; whereas HSD3β2 is exclusively expressed in classic steroidogenic tissues including the adrenal gland, ovary, and testis. In endometriotic tissues, HSD3β2 is highly expressed in comparison with normal endometrium [14,18]. IL-4, a cytokine known to be highly expressed in local endometriotic tissues, in combination with PGE_2, have been shown to enhance the expression of HSD3β2 in endometriosis [18].

Aromatase is the enzyme that converts testosterone and androstenedione to estradiol (E_2) and estrone (E_1), respectively. Aromatase is expressed in a number of human tissues and cells, such as ovarian granulosa cells, adipose tissue, skin fibroblasts, placental trophoblasts, osteoblasts, and brain. In women of reproductive age, aromatase is most potent and periodically secreted by the ovary. Ovarian granulosa cells express high levels of aromatase under the influence of FSH [19]. In contrast, estrogen formation in postmenopausal women takes place in extraglandular sites such as adipose tissue and skin. The main substrate of aromatase in adipose and skin tissues is androstenedione, secreted from adrenal tissues. Interestingly, previous evidence demonstrates that aromatase is highly expressed in endometriosis [12].

Aromatase is detected in endometriotic implants in much larger amounts than in eutopic endometrium, although it is not detected in normal endometrium from disease-free women. Our group showed that local estrogen production by aberrantly elevated aromatase takes place in endometriosis and adenomyosis, but not in normal endometrium tissue using immunochemistry analysis [4]. Conversely, some studies have shown an absence of aromatase activity in endometriotic tissues. This discrepancy may be due to differences in the specificity of the antibodies used or differences between the biopsy specimens investigated. Recently, Huhtinen et al. found that ovarian endometriotic lesions have markedly higher intratissue estrogen concentrations than in those found in normal endometrium, peritoneal, and deep endometriosis [12]. These findings suggest that aromatase plays a critical role in local estrogen production, especially in ovarian endometriosis, and indicates the existence of autocrine and paracrine sources of estrogen in local lesions.

The 17β-hydroxysteroid dehydrogenase (HSD17β) enzyme family is also prominently involved in the local estrogen formation process. These enzymes participate in the formation of biologically active steroid hormones including testosterone, estrone (E_1), and estradiol (E_2). They are responsible for catalyzing the reversible interconversion of E_1 and E_2. Specifically, HSD17β type1 (HSD17β1) catalyzes 17β-reduction of biologically inactive E_1 to E_2, while HSD17β2 preferentially catalyzes the oxidation of E_2 to E_1. In endometriotic tissues, HSD17β1 expression and enzyme activity are increased when compared with those in normal endometrium without endometriosis [20,21]. Our recent study revealed that expression levels

of HSD17β1 at both the mRNA and protein level in endometriotic tissues, including deep infiltrating endometriosis (DIE) lesions, are higher than in normal endometrium. We also showed that progesterone therapy significantly suppressed the catalytic activity of HSD17β1 in ovarian endometrial stromal cells [22]. In contrast, the expression and the enzymatic activity of HSD17β2 in endometriotic tissues is still not well characterized. Some studies have shown that HSD17β2 expression is decreased in endometriotic tissues, including eutopic and ectopic endometrium, resulting in the inactivation of E_2. On the other hand, our group reported that HSD17β2 expression in secretary endometrium was increased with endometriosis, but not in normal endometrium [23]. Other investigators have shown that there are no observable differences in the expression of HSD17β2 between normal endometrium and endometriotic tissue [24]. Our data also showed that progesterone treatment did not alter the expression of HSD17β2 in endometriotic stromal cells [25].

The major source of estrogen is estrone sulfate, an inactive conjugate form abundant in peritoneal tissues, such as circulation serum. Estrogen sulfate is desulfated to estrone, an active form, by steroid sulfatase (STS), and estrone is inactivated by estrogen sulfotransferase (EST) [26]. STS is highly expressed in endometriotic tissues and has been shown to correlate with disease severity [27]. EST is highly expressed in ovarian endometrioma, though its expression is not detectable in normal and eutopic endometrium. Taken together, these findings indicate that aberrant expression of these enzymes in endometriosis contributes to local production and metabolism of estrogens.

Aromatase regulation in endometriosis

The human aromatase gene, mapped to chromosome 15, band q21, is controlled in a tissue-specific manner through the alternative use of at least eight untranslated exons/promoters (I.1, I.2, I.3, I.4, I.5, I.6, I.7, and PII). Various exon I-containing mRNAs are present at different levels in different aromatase-expressing tissues. For example, exon I.1 transcripts, located most distally upstream from the coding region, were found to be elevated in placental tissue [28]. The major exons in breast cancer specimens include I.3 and PII [29]. On the other hand, a very low level of exon/promoters I.3/PII and a low level of exon/promoter I.4 are present in normal breast adipose tissues. These findings suggest that a different regulatory mechanism for aromatase expression could exist between normal breast adipose and cancer tissues. For endometriotic tissues, our group and other investigators have demonstrated that the exon/promoters I.3 and PII are the main regulatory promoters [7,30]. Previous evidence has demonstrated that a positive feedback loop enhances aromatase expression in endometriosis [31]. Prostaglandin E_2 (PGE_2) stimulates aromatase expression in endometriotic stromal cells [32], and PGE_2 is stimulated by the cyclooxygenase type 2 (COX-2) enzyme in endometriotic stromal cells [33]. Furthermore, estrogen-mediated action is directly linked to the promotion of local inflammation, as verified by the induction of various cytokines [34]. Interleukin (IL)-6 and IL-8, one of the representative inflammatory cytokines elevated in local endometriotic tissues, activate a prosurvival signaling pathway as well as COX-2 and PGE_2 [35]. Thus, there is the existence of a feed-forward mechanism controlling the overproduction of estrogen, PGE_2, and various cytokines which promotes the persistence of endometriotic lesions (Fig. 7.2) [36].

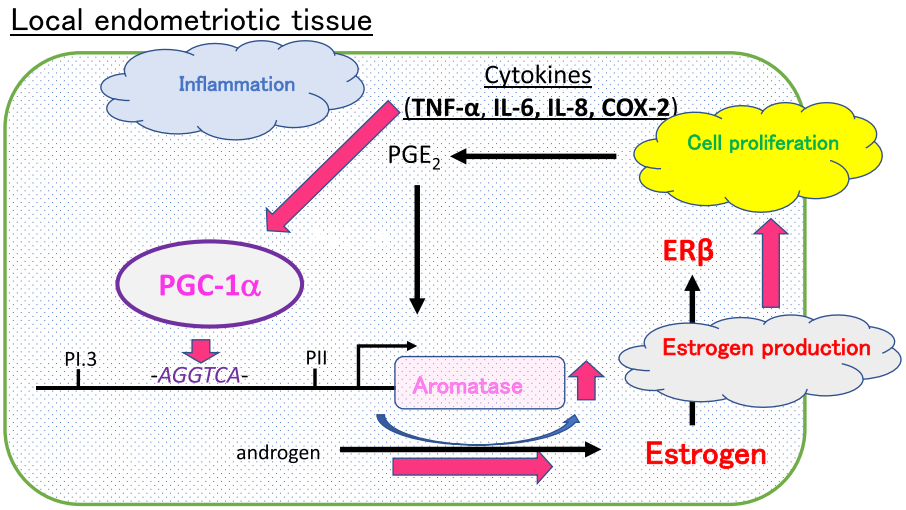


FIGURE 7.2 **The vicious cycle of local estrogen production during endometriosis through PGC-1α and inflammatory cytokines.** The vicious cycle of local estrogen production during endometriosis through PGC-1α and TNF-α. PGC-1α enhanced local estrogen production through aromatase in endometriotic lesions. Estrogen stimulates endometriotic cell proliferation and the activity of macrophages in peritoneal fluid. TNF-α secreted from both endometriotic cells and macrophages upregulates the expression of PGC-1α by a positive feedback mechanism. These findings suggest that this cycle is predominantly involved in the pathogenesis of endometriosis and PGC-1α could be a promising candidate for developing novel therapies for endometriosis.

Transcriptional regulation of aromatase expression in endometriosis

It is important to understand the regulatory mechanism controlling aromatase expression in normal ovarian granulosa cells during the menstrual cycle. Ovarian follicles are the primary source of local and circulating estrogen in humans. Estrogen synthesis is stimulated through the binding of FSH to granulosa cells. Once FSH is secreted from the pituitary gland, a cyclic AMP (cAMP)-dependent signaling cascades is triggered to regulate the transcription of aromatase. Previous studies have shown that steroidogenic factor-1 (SF-1) and cAMP response element-binding protein are recruited to bind to aromatase promoter II to enhance estrogen production at the preovulatory phase [37,38].

In endometriotic tissues, aromatase expression, elevated by cAMP-dependent cascades and PGE_2, is controlled by the binding of SF-1 to the nuclear receptor half-site upstream of aromatase promoter II [7]. In contrast, chicken ovalbumin upstream promoter-transcription factor (COUP-TF) inhibits aromatase expression. SF-1 is expressed in endometriosis but not in normal endometrium, while COUP-TF is expressed in endometriosis in both normal and eutopic endometrium. Importantly, SF-1 can compete with COUP-TF for the same binding site on aromatase promoter. In addition, previous studies have demonstrated that various transcriptional factors could be involved in the regulatory expression of aromatase. Of these, Wilms' tumor-1 acts as a corepressor of SF-1 at the nuclear half-site of aromatase promoter I.3 and II. CCCAAT-enhancer-binding protein (C/EBP)α and C/EBPβ bind to the cAMP response element site, located just upstream of the nuclear half-sites of aromatase PI.3 and

PII. C/EBPα functions as an enhancer of aromatase expression, while C/EBPβ acts as an inhibitor. C/EBPβ is expressed at a lower level in endometriosis, but not in eutopic endometrium. Dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1 (DAX-1) regulates SF-1 transcription in a dominant-negative manner to inhibit SF-1 dependent expression of aromatase in endometriosis [39]. Taken together, these findings indicate that the above described transcription factors play important roles in the regulation of aromatase expression, which differs significantly between endometriotic tissues and normal/eutopic endometrium.

Peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α) is another important factor in the regulation of aromatase expression. PGC-1α is a multifunctional coactivator interacting with a broad range of transcription factors in multiple biological responses including adaptive thermogenesis, mitochondrial biogenesis, oxidative metabolism, and steroidogenesis [40,41]. It is known that PGC-1α is differentially expressed in different tissues and functions as a coactivator interacting with tissue-specific transcription factors. For example, PGC-1α interacts with peroxisome proliferator-activated receptor gamma (PPARγ) to stimulate adipocyte differentiation in brown adipose tissues [42]. On the other hand, PGC-1α plays an important role in progesterone production in ovarian granulosa cells where it acts as a coactivator of SF-1 and LRH-1 [41]. In skeletal muscles, PGC-1α is involved in glucose uptake to downregulate the expression of insulin-sensitive glucose transport type 4 [43]. In endometriosis, our group discovered that PGC-1α is aberrantly elevated in ovarian endometrioma, correlating with the localization of aromatase in endometriotic tissues [30]. We also showed that PGC-1α enhanced aromatase expression at the mRNA level and induced transcriptional activity at the aromatase promoter I.3 and II in ovarian endometriotic stromal cells (OESCs). In general, PGC-1α increases aromatase enzyme activity in the cells. Our chromatin immunoprecipitation assay showed that endogenous PGC-1α can recruit the nuclear receptor half-site 5′-AGGTCA-3′ site between aromatase promoter I.3 and PII. It is also notable that tumor necrosis factor (TNF)-α, produced by peritoneal macrophages and endometriotic lesions, induces PGC-1α expression in OESCs. Despite numerous studies, further investigation to understand the regulation of aromatase in endometriosis is required.

Aromatase targeting as a treatment option for endometriosis

Endocrine agents including oral contraceptives (OC), progestins, and GnRH agonists are current methods of treatment for endometriosis. OC are widely used in women with chronic pelvic pain and clinically suspected endometriosis. OC operate by inhibiting ovulation and substantially reducing the volume of menstrual flow, however, OC might interfere with implantation of refluxed endometrial cells. A recent systematic review of previous clinical studies demonstrated that OC use might reduce the risk of the incidence of endometriosis [44].

Progestins are also used to reduce the symptoms of endometriosis. Progestins inhibit the hypothalamic-pituitary-ovarian axis leading to anovulation, reduction of the serum estrogen level and atrophy of eutopic/ectopic endometrium. Dienogest (DNG) is a fourth-generation progestin with potent oral progesterone activity and no androgenic activity [45]. A double-blind randomized control trial from multicenter institutions demonstrated that DNG exerts

potent efficacy in relieving endometriosis-associated pelvic pain [46]. The efficacy of long term DNG use was verified in other studies [47]. In addition, evidence from basic scientific research supports the hypothesis that DNG directly inhibits progesterone receptor-mediated cell proliferation [48] and the production of inflammatory cytokines including COX-2 and PGE_2 [49,50], toll-like receptor 4 [51], and nerve growth factor [52]. Using a spheroid culture system and endometriotic stromal cells, our group showed that DNG reduced the expression of aromatase and the enzyme activity of HSD17β1, but not of HSD17β2 [25,53]. These findings indicate that DNG not only reduces local estrogen production through aromatase, but also converts the less potent E_1 form to the more potent E_2 form.

Gonadotropin releasing hormone agonist (GnRH-a) is known to cause pharmacological menopause by depleting the production of gonadotropins and suppressing ovulation to reduce ovarian steroidogenesis. Hypogonadism by GnRH-a treatment results in the shrinking of endometriotic lesions and can achieve relief of severe endometriosis-associated pelvic pain [54]. Side effects include hot flashes, vaginal dryness, decreased libido, insomnia, and depression. Therefore, a modification of GnRH-a treatment is to supplement estrogen and progestin using doses typically given to postmenopausal women. Long term use of GnRH-a causes hypoestrogenic side effects and a substantial reduction in bone mineral density [55]. Another issue with GnRH-a treatment is that endometriosis-associated pain often recurs if treatment is interrupted. There are two reasons for the shortfall of GnRH-a treatment of endometriosis. The first is that local estrogen secretion from ovaries resumes after discontinuation of the treatment allowing the development of endometriotic lesions. The second is that GnRH-a treatment affects only the hypothalamo-pituitary-gonadal axis, but does not affect extraglandular lesions, including adipose tissues, skin, and local endometriotic lesions, which can produce estrogen in women.

Aromatase inhibitors (AIs) can also suppress estrogen production by inhibiting the enzymatic activity of aromatase. Recent development of more potent AIs with high specificity, such as anastrozole, letrozole, and exemestane, have led to their use as first line endocrine therapy for postmenopausal women with breast cancer [56]. AIs maintain low-estrogen levels in extraovarian lesions during the course of treatment. However, AIs are known to enhance FSH levels, through positive feedback of the hypothalamo-pituitary-gonadal axis, when used in premenopausal women, occasionally leading to the development of ovarian cysts. Therefore, it is recommended to use AIs with progestins, OC, or GnRH-a for women of reproductive age with endometriosis. A systematic review, evaluating eight clinical studies, showed that AIs decreased pain, reduced the size of extrauterine endometrial lesions, and improved patients quality of life when used in combination with conventional endocrine agents [57]. A more recent meta-analysis of 10 clinical studies including a total of 251 women showed similar results. Of these, letrozole in combination with norethisterone acetate was found to be more effective than norethisterone acetate alone in reducing pain and dyspareunia. In addition, letrozole in combination with GnRH-a for 6 months after surgery, was more effective than GnRH-a alone in patients with endometriosis. Thus, the European Society of Human Reproduction and Embryology (ESHIRE) guidelines recommend concomitant use of AIs and OC, progestins, or GnRH-a in patients suffering from pain associated with drug-resistant and surgery-resistant recto-vaginal endometriosis [58].

We previously demonstrated that PGC-1α contributes to aberrant local estrogen production through aromatase stimulation in ovarian endometrioma, but not in endometrium

with endometriosis or normal endometrium [30]. More recent data revealed that PGC-1α overexpression promoted proliferation of OESC. In addition, PGC-1α knockdown inhibited the proliferation of OESC but not normal endometrial cells. These findings suggest that PGC-1α represents a promising candidate for targeted therapies in the treatment of endometriosis. However, ligands that possess an inhibitory effect on PGC-1α are yet to be found. A chromatin immunoprecipitation assay revealed that PGC-1α was recruited to a region containing the estrogen-related responsive element (ERRE) site. Based on this finding, we established a luciferase assay to screen potential compounds. Of those tested, we found that RXRα antagonist HX531 inhibited PGC-1α-induced ERRE activity in ovarian endometrioma cells [59]. Interestingly, HX531 suppressed PGC-1α-induced cell proliferation and the expression of aromatase, inflammatory cytokines including IL-6 and IL-8, as well as survivin, which is a key molecule in apoptosis resistance in endometriosis. Thus, the PGC-1α-mediated pathway could represent a potential target for molecular therapy of endometriosis, although further studies are required to confirm this issue.

Isoflavones are plant-derived nonsteroidal compounds that exert weak estrogenic activity. Isoflavones also demonstrate estrogen-like activity due to structural similarities allowing isoflavone to bind to ER. Although the estrogenic activity of isoflavones is very weak, i.e., 1/1000 to 1/10,000 that of estradiol, some isoflavones exert antiestrogenic effects in reproductive-aged women with high levels of estrogen. Accumulating evidence has shown that isoflavone intake might reduce the incidence of breast, prostate, and colon cancer, while improving climacteric disorder in peri- or postmenopausal women [60,61]. Previous studies demonstrated that two flavonoids, namely, puerarin and parthenolide, inhibited the proliferation of endometriotic stromal cells [62,63]. Another study revealed that genistein caused regression of an endometriotic implant in a rat model [64]. In addition, our group demonstrated that a dietary supplement, daidzein-rich isoflavone aglycones (DRIAs), suppressed proliferation in OESCs at clinically feasible concentrations [65]. DRIAs decreased IL-6, IL-8, COX-2, and aromatase expression at the mRNA level in OESC. This compound also inhibited aromatase enzymatic activity in these cells. Using a mouse model, DRIAs was found to reduce the extent of endometriotic-like lesions. Therefore, our findings suggest that DRIA might be useful for the management of endometriosis.

Suppression of in situ estrogen production could be a potential therapeutic option for endometriosis. A more detailed understanding of the regulatory mechanisms controlling aromatase expression in endometriosis will provide further insight on aberrant estrogen production in local lesions.

Estrogen receptors in endometriosis

Estrogens promote physiological action after binding to an ER. ER subtypes α and β are proteins with high affinity for estradiol, and are encoded by separate genes. The classical human ERα was first cloned in 1986, and a second receptor, ERβ, was isolated from human testis in 1996 [66,67]. The expression of these receptors is tissue-specific. ERα is highly expressed in bone, kidney, liver, mammary glands, and reproductive organs; while ERβ is expressed in the prostate, ovary, bladder, uterus, and central nervous system [68]. A physiological response to estrogen occurs after the binding of classical estrogen-response elements

(EREs), and is followed by the action of the nuclear activation complex stimulating transcription of a target gene. This represents the classical genomic pathway of estrogen action. Alternatively, estrogen can exert its effect through nongenomic signaling via cell membrane ERs. A seven-pass transmembrane G protein-coupled receptor has been identified as a novel ER with binding capability to E_2 in the cell membranes and the endoplasmic reticulum [69]. The response is regulated by downstream molecules including the phosphatidylinositol-3-kinase (PI3K) and MEK/ERK mitogen-activated protein kinase (MAPK) pathways. Below, we discuss the mechanism responsible for the expression of ERs in endometriosis (Fig. 7.3).

Classical estrogen receptors in endometriosis

Nuclear estrogen receptors have two isoforms, ERα and ERβ. They are encoded by different genes but share significant similarities in their DNA-binding domain. The classic action of estrogen receptors involves estrogen binding to specific response elements, known as ERE, located in target genes that regulate the growth of the tissues. One study revealed that the upregulation of ERs caused the activation of their target genes, c-Myc, cyclin D1, and

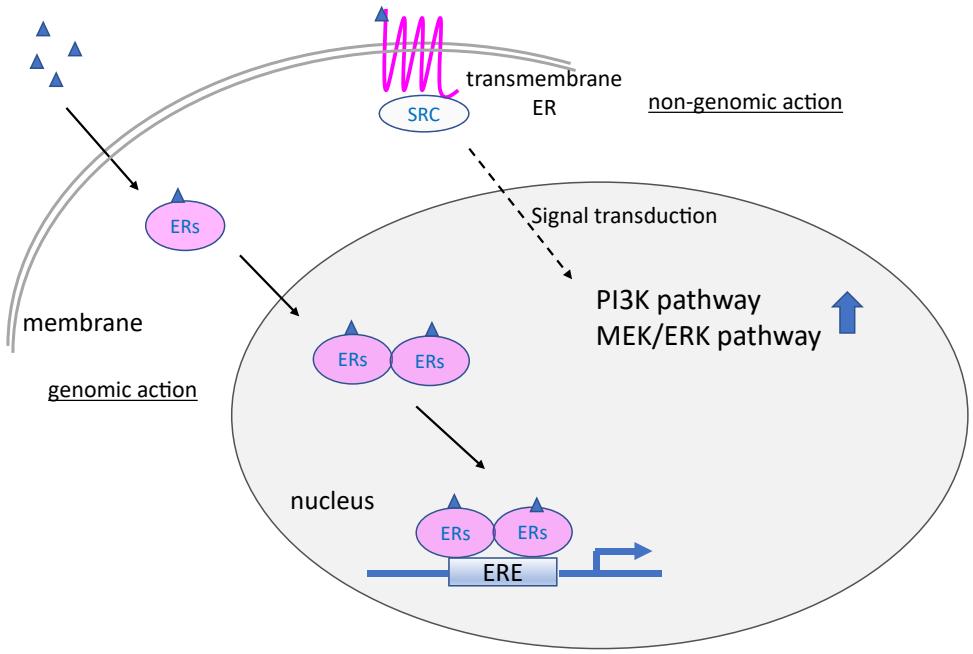


FIGURE 7.3 **Estrogen action through several types of estrogen receptors.** In addition to the classical estrogen receptors (ERα and ERβ), a novel estrogen receptor, a seven-pass transmembrane G protein-coupled receptor (GPER) has been identified. This transmembrane ER mediates the balance between non-genomic rapid cell signaling mechanisms through PI3K and MEK/ERK pathways and slow genomic transcriptional activities in the response to estrogens.

GREB1, to regulate cell mitogenesis in endometriotic tissues. Previously, several studies focused on the functional role, and the expression of classical ERs in endometriosis. In normal endometrium, expression of ERα is significantly higher than that of ERβ, whereas in endometriosis, the expression of ERα is attenuated compared with normal endometrium, and in contrast, ERβ is upregulated [24,70,71]. Although the detailed mechanism involved in the attenuation of ERα and the increase of ERβ remains unclear, previous studies showed that hypomethylation of the ERβ promoter could be associated with the upregulation of this protein in endometriotic tissues [71]. Other investigators have shown that ERβ suppressed ERα expression in stromal cells from ovarian endometrioma, leading to augment ERβ/ERα ratios, which might cause a shift from E_2 stimulation to inhibition [72]. Importantly, a loss of function experiment using siRNA showed that ERβ inhibited the proliferation of endometrial stromal cells. In contrast, treatment with an ERβ agonist reduced endometriotic lesion size in a mouse model, which contradicts the finding that ERβ plays a critical role in the suppression of cell growth and exhibits antiinflammatory action. One recent study clearly demonstrated that ERβ in the cytoplasm suppressed TNF-α induced apoptosis, increased IL-1b-mediated cellular adhesion, and increased epithelial-mesenchymal transition, resulting in enhanced growth of endometriosis. This action, mediated by ERβ, is a nongenomic example of estrogen-mediated action and occurs in the presence of ectopic endometrial tissues. Thus, the complete functional role of ERβ has not been fully elucidated, although evidence for the potential of ERβ as a therapeutic target in endometriosis has been recognized [73].

GPER in endometriosis

G protein-coupled estrogen receptor 1, GPER, mediates the balance between nongenomic rapid cell signaling mechanisms and slow genomic transcriptional activity, in the response to estrogens. GPER is expressed in most tissues, for example heart, brain, placenta, and liver. GPER is also expressed in both within the cell membrane as well as in the surface membrane of intracellular organelles including the endoplasmic reticulum and the Golgi apparatus. It stimulates both the phosphatidylinositol 3-kinase (PI3K) and MEK/ERK mitogen-activated protein kinase (MAPK) pathways. Although there have only been a few reports on GPER in endometriosis, it has been demonstrated that it is expressed at a higher level in endometriotic tissues and in the endometrium of patients with endometriosis when compared with normal endometrium [74] and healthy women, respectively [75]. Furthermore, aberrant expression of GPER in estrogen-dependent diseases suggests its potential involvement in the pathogenesis of endometriosis [76,77]. To further understand the mechanism of GPER activity in endometriosis, we conducted experiments using a GPER agonist called G-1. We found that G-1 inhibited proliferation in a dose-dependent manner and caused G2/M cell cycle arrest in endometriotic stromal cells, leading to the induction of caspase-3-dependent apoptosis [78]. Interestingly, such inhibitory effects might surprisingly occur independently of GPER. Although our preliminary findings suggest that G-1 might represent a therapeutic option for endometriosis treatment, further investigation is required to obtain a more complete understanding of the functional mechanism of GPER.

Estrogen-related receptors in endometriosis

Estrogen-related receptors (ERRs) are members of the nuclear receptor super family that are most closely related to estrogen receptors. ERRs can bind to estrogen receptor elements in DNA-binding domain like ERs and modulate target gene expression, however, endogenous ligands for the ERRs have not been identified. The ERR family consists of three members: ERRα and ERRβ and ERRγ [79]. Of these, ERRβ has been detected in normal endometrium tissue throughout the menstrual cycle [80]. In addition, ERRα and ERRγ have been reported to be expressed in normal endometrium, however, little is known about these receptors in endometriosis. Recently, Cavallini A et al. showed that increased levels of ERβ was associated with a reduction of ERRα, ERRα, and ERRγ in endometriotic lesions, but not in eutopic endometrium with endometriosis or normal endometrium [81]. In addition, ERRβ were found at a similar level in women with and without endometriosis. However, the functional mechanism of ERRs in endometriosis remains unknown. A recent study showed that decidualization of human endometrial stromal cells enhanced the expression of ERRα and its target genes, pyruvate dehydrogenase kinase 4 (PDK4) and medium chain-specific acyl-CoA dehydrogenase (MCAD). These finding indicate that ERRα plays an important role in decidualized stromal cells in preparation for implantation and trophoblast invasion [82]. Further evaluation of the molecular mechanism of ERRs in endometriosis is required.

Summary and perspective

Although the pathogenesis of endometriosis remains unclear, it is apparent from previous basic scientific studies and clinical evidence that it is an estrogen-dependent disease. However, the disease cannot be explained solely by classical proliferative activity, mediated by the ER. In addition to the expression of several ERs, the activities mediated by numerous regulatory factors could play important roles in disease development involving complicated network. Nonetheless, it remains to be determined the important targets for treatment and areas of future research including local estrogen production in endometriotic tissues and estrogen activity via the ERs.

Acknowledgments

We thank Dr. Hisashi Kataoka and Dr. Osamu Takaoka for the contribution to this work.

References

[1] Buck Louis GM, Hediger ML, Peterson CM, Croughan M, Sundaram R, Stanford J, et al. Incidence of endometriosis by study population and diagnostic method: the ENDO study. Fertil Steril 2011;96(2):360–5.
[2] Zondervan KT, Becker CM, Koga K, Missmer SA, Taylor RN, Vigano P. Endometriosis. Nat Rev Dis Primers 2018;4(1):9.
[3] Xiong W, Zhang L, Yu L, Xie W, Man Y, Xiong Y, et al. Estradiol promotes cells invasion by activating beta-catenin signaling pathway in endometriosis. Reproduction 2015;150(6):507–16.

[4] Kitawaki J, Ishihara H, Kiyomizu M, Honjo H. Maintenance therapy involving a tapering dose of danazol or mid/low doses of oral contraceptive after gonadotropin-releasing hormone agonist treatment for endometriosis-associated pelvic pain. Fertil Steril 2008;89(6):1831–5.
[5] Kitawaki J, Noguchi T, Amatsu T, Maeda K, Tsukamoto K, Yamamoto T, et al. Expression of aromatase cytochrome P450 protein and messenger ribonucleic acid in human endometriotic and adenomyotic tissues but not in normal endometrium. Biol Reprod 1997;57(3):514–9.
[6] Yang S, Fang Z, Suzuki T, Sasano H, Zhou J, Gurates B, et al. Regulation of aromatase P450 expression in endometriotic and endometrial stromal cells by CCAAT/enhancer binding proteins (C/EBPs): decreased C/EBPbeta in endometriosis is associated with overexpression of aromatase. J Clin Endocrinol Metab 2002;87(5):2336–45.
[7] Zeitoun KM, Bulun SE. Aromatase: a key molecule in the pathophysiology of endometriosis and a therapeutic target. Fertil Steril 1999;72(6):961–9.
[8] Huhtinen K, Desai R, Stahle M, Salminen A, Handelsman DJ, Perheentupa A, et al. Endometrial and endometriotic concentrations of estrone and estradiol are determined by local metabolism rather than circulating levels. J Clin Endocrinol Metab 2012;97(11):4228–35.
[9] Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. Fertil Steril 2012;98(3):511–9.
[10] Burns KA, Rodriguez KF, Hewitt SC, Janardhan KS, Young SL, Korach KS. Role of estrogen receptor signaling required for endometriosis-like lesion establishment in a mouse model. Endocrinology 2012;153(8):3960–71.
[11] Sasano H, Miki Y, Nagasaki S, Suzuki T. In situ estrogen production and its regulation in human breast carcinoma: from endocrinology to intracrinology. Pathol Int 2009;59(11):777–89.
[12] Huhtinen K, Stahle M, Perheentupa A, Poutanen M. Estrogen biosynthesis and signaling in endometriosis. Mol Cell Endocrinol 2012;358(2):146–54.
[13] Bulun SE, Lin Z, Imir G, Amin S, Demura M, Yilmaz B, et al. Regulation of aromatase expression in estrogen-responsive breast and uterine disease: from bench to treatment. Pharmacol Rev 2005;57(3):359–83.
[14] Attar E, Tokunaga H, Imir G, Yilmaz MB, Redwine D, Putman M, et al. Prostaglandin E2 via steroidogenic factor-1 coordinately regulates transcription of steroidogenic genes necessary for estrogen synthesis in endometriosis. J Clin Endocrinol Metab 2009;94(2):623–31.
[15] Tsai SJ, Wu MH, Lin CC, Sun HS, Chen HM. Regulation of steroidogenic acute regulatory protein expression and progesterone production in endometriotic stromal cells. J Clin Endocrinol Metab 2001;86(12):5765–73.
[16] Sun HS, Hsiao KY, Hsu CC, Wu MH, Tsai SJ. Transactivation of steroidogenic acute regulatory protein in human endometriotic stromal cells is mediated by the prostaglandin EP2 receptor. Endocrinology 2003;144(9):3934–42.
[17] Hsu CC, Lu CW, Huang BM, Wu MH, Tsai SJ. Cyclic adenosine 3′,5′-monophosphate response element-binding protein and CCAAT/enhancer-binding protein mediate prostaglandin E2-induced steroidogenic acute regulatory protein expression in endometriotic stromal cells. Am J Pathol 2008;173(2):433–41.
[18] Urata Y, Osuga Y, Akiyama I, Nagai M, Izumi G, Takamura M, et al. Interleukin-4 and prostaglandin E2 synergistically up-regulate 3beta-hydroxysteroid dehydrogenase type 2 in endometrioma stromal cells. J Clin Endocrinol Metab 2013;98(4):1583–90.
[19] Dewailly D, Robin G, Peigne M, Decanter C, Pigny P, Catteau-Jonard S. Interactions between androgens, FSH, anti-Mullerian hormone and estradiol during folliculogenesis in the human normal and polycystic ovary. Hum Reprod Update 2016;22(6):709–24.
[20] Dassen H, Punyadeera C, Kamps R, Delvoux B, Van Langendonckt A, Donnez J, et al. Estrogen metabolizing enzymes in endometrium and endometriosis. Hum Reprod 2007;22(12):3148–58.
[21] Colette S, Defrere S, Van Kerk O, Van Langendonckt A, Dolmans MM, Donnez J. Differential expression of steroidogenic enzymes according to endometriosis type. Fertil Steril 2013;100(6):1642–9.
[22] Mori T, Ito F, Matsushima H, Takaoka O, Koshiba A, Tanaka Y, et al. Dienogest reduces HSD17beta1 expression and activity in endometriosis. J Endocrinol 2015;225(2):69–76.
[23] Kitawaki J, Koshiba H, Ishihara H, Kusuki I, Tsukamoto K, Honjo H. Progesterone induction of 17beta-hydroxysteroid dehydrogenase type 2 during the secretory phase occurs in the endometrium of estrogen-dependent benign diseases but not in normal endometrium. J Clin Endocrinol Metab 2000;85(9):3292–6.
[24] Smuc T, Pucelj MR, Sinkovec J, Husen B, Thole H, Lanisnik Rizner T. Expression analysis of the genes involved in estradiol and progesterone action in human ovarian endometriosis. Gynecol Endocrinol 2007;23(2):105–11.
[25] Mori T, Ito F, Matsushima H, Takaoka O, Koshiba A, Tanaka Y, Kusuki I, Kitawaki J. Dienogest reduces HSD17b1 expression and activity in endometriosis. J Endocrinol 2015;225(2):69–76.

[26] Suzuki T, Miki Y, Nakamura Y, Ito K, Sasano H. Steroid sulfatase and estrogen sulfotransferase in human carcinomas. Mol Cell Endocrinol 2011;340(2):148–53.
[27] Purohit A, Fusi L, Brosens J, Woo LW, Potter BV, Reed MJ. Inhibition of steroid sulphatase activity in endometriotic implants by 667 COUMATE: a potential new therapy. Hum Reprod 2008;23(2):290–7.
[28] Kumar P, Kamat A, Mendelson CR. Estrogen receptor alpha (ERalpha) mediates stimulatory effects of estrogen on aromatase (CYP19) gene expression in human placenta. Mol Endocrinol 2009;23(6):784–93.
[29] Chen S, Itoh T, Wu K, Zhou D, Yang C. Transcriptional regulation of aromatase expression in human breast tissue. J Steroid Biochem Mol Biol 2002;83(1–5):93–9.
[30] Suganuma I, Mori T, Ito F, Tanaka Y, Sasaki A, Matsuo S, et al. Peroxisome proliferator-activated receptor gamma, coactivator 1alpha enhances local estrogen biosynthesis by stimulating aromatase activity in endometriosis. J Clin Endocrinol Metab 2014;99(7):E1191–8.
[31] Bulun SE. Endometriosis. N Engl J Med 2009;360(3):268–79.
[32] Noble LS, Takayama K, Zeitoun KM, Putman JM, Johns DA, Hinshelwood MM, et al. Prostaglandin E2 stimulates aromatase expression in endometriosis-derived stromal cells. J Clin Endocrinol Metab 1997;82(2):600–6.
[33] Wu MH, Wang CA, Lin CC, Chen LC, Chang WC, Tsai SJ. Distinct regulation of cyclooxygenase-2 by interleukin-1beta in normal and endometriotic stromal cells. J Clin Endocrinol Metab 2005;90(1):286–95.
[34] Reis FM, Petraglia F, Taylor RN. Endometriosis: hormone regulation and clinical consequences of chemotaxis and apoptosis. Hum Reprod Update 2013;19(4):406–18.
[35] Monsivais D, Dyson MT, Yin P, Coon JS, Navarro A, Feng G, et al. ERbeta- and prostaglandin E2-regulated pathways integrate cell proliferation via Ras-like and estrogen-regulated growth inhibitor in endometriosis. Mol Endocrinol 2014;28(8):1304–15.
[36] Bulun SE, Monsivais D, Kakinuma T, Furukawa Y, Bernardi L, Pavone ME, et al. Molecular biology of endometriosis: from aromatase to genomic abnormalities. Semin Reprod Med 2015;33(3):220–4.
[37] Michael MD, Michael LF, Simpson ER. A CRE-like sequence that binds CREB and contributes to cAMP-dependent regulation of the proximal promoter of the human aromatase P450 (CYP19) gene. Mol Cell Endocrinol 1997;134(2):147–56.
[38] Michael MD, Kilgore MW, Morohashi K, Simpson ER. Ad4BP/SF-1 regulates cyclic AMP-induced transcription from the proximal promoter (PII) of the human aromatase P450 (CYP19) gene in the ovary. J Biol Chem 1995;270(22):13561–6.
[39] Gurates B, Amsterdam A, Tamura M, Yang S, Zhou J, Fang Z, et al. WT1 and DAX-1 regulate SF-1-mediated human P450arom gene expression in gonadal cells. Mol Cell Endocrinol 2003;208(1–2):61–75.
[40] Cormio A, Guerra F, Cormio G, Pesce V, Fracasso F, Loizzi V, et al. The PGC-1alpha-dependent pathway of mitochondrial biogenesis is upregulated in type I endometrial cancer. Biochem Biophys Res Commun 2009;390(4):1182–5.
[41] Yazawa T, Inaoka Y, Okada R, Mizutani T, Yamazaki Y, Usami Y, et al. PPAR-gamma coactivator-1alpha regulates progesterone production in ovarian granulosa cells with SF-1 and LRH-1. Mol Endocrinol 2010;24(3):485–96.
[42] Finck BN, Kelly DP. PGC-1 coactivators: inducible regulators of energy metabolism in health and disease. J Clin Invest 2006;116(3):615–22.
[43] Miura S, Kai Y, Ono M, Ezaki O. Overexpression of peroxisome proliferator-activated receptor gamma coactivator-1alpha down-regulates GLUT4 mRNA in skeletal muscles. J Biol Chem 2003;278(33):31385–90.
[44] Vercellini P, Eskenazi B, Consonni D, Somigliana E, Parazzini F, Abbiati A, et al. Oral contraceptives and risk of endometriosis: a systematic review and meta-analysis. Hum Reprod Update 2011;17(2):159–70.
[45] Sasagawa S, Shimizu Y, Kami H, Takeuchi T, Mita S, Imada K, et al. Dienogest is a selective progesterone receptor agonist in transactivation analysis with potent oral endometrial activity due to its efficient pharmacokinetic profile. Steroids 2008;73(2):222–31.
[46] Harada T, Momoeda M, Taketani Y, Aso T, Fukunaga M, Hagino H, et al. Dienogest is as effective as intranasal buserelin acetate for the relief of pain symptoms associated with endometriosis–a randomized, double-blind, multicenter, controlled trial. Fertil Steril 2009;91(3):675–81.
[47] Momoeda M, Harada T, Terakawa N, Aso T, Fukunaga M, Hagino H, et al. Long-term use of dienogest for the treatment of endometriosis. J Obstet Gynaecol Res 2009;35(6):1069–76.

[48] Shimizu Y, Takeuchi T, Mita S, Mizuguchi K, Kiyono T, Inoue M, et al. Dienogest, a synthetic progestin, inhibits the proliferation of immortalized human endometrial epithelial cells with suppression of cyclin D1 gene expression. Mol Hum Reprod 2009;15(10):693–701.
[49] Shimizu Y, Mita S, Takeuchi T, Notsu T, Mizuguchi K, Kyo S. Dienogest, a synthetic progestin, inhibits prostaglandin E2 production and aromatase expression by human endometrial epithelial cells in a spheroid culture system. Steroids 2011;76(1–2):60–7.
[50] Horie S, Harada T, Mitsunari M, Taniguchi F, Iwabe T, Terakawa N. Progesterone and progestational compounds attenuate tumor necrosis factor alpha-induced interleukin-8 production via nuclear factor kappa B inactivation in endometriotic stromal cells. Fertil Steril 2005;83(5):1530–5.
[51] Mita S, Shimizu Y, Notsu T, Imada K, Kyo S. Dienogest inhibits Toll-like receptor 4 expression induced by costimulation of lipopolysaccharide and high-mobility group box 1 in endometrial epithelial cells. Fertil Steril 2011;96(6):1485–1489.e4.
[52] Mita S, Shimizu Y, Sato A, Notsu T, Imada K, Kyo S. Dienogest inhibits nerve growth factor expression induced by tumor necrosis factor-alpha or interleukin-1beta. Fertil Steril 2014;101(2):595–601.
[53] Yamanaka K, Xu B, Suganuma I, Kusuki I, Mita S, Shimizu Y, et al. Dienogest inhibits aromatase and cyclooxygenase-2 expression and prostaglandin E(2) production in human endometriotic stromal cells in spheroid culture. Fertil Steril 2012;97(2):477–82.
[54] Vercellini P, Buggio L, Frattaruolo MP, Borghi A, Dridi D, Somigliana E. Medical treatment of endometriosis-related pain. Best Pract Res Clin Obstet Gynaecol 2018;51:68–91.
[55] Olive DL, Pritts EA. Treatment of endometriosis. N Engl J Med 2001;345(4):266–75.
[56] De Placido S, Gallo C, De Laurentiis M, Bisagni G, Arpino G, Sarobba MG, et al. Adjuvant anastrozole versus exemestane versus letrozole, upfront or after 2 years of tamoxifen, in endocrine-sensitive breast cancer (FATA-GIM3): a randomised, phase 3 trial. Lancet Oncol 2018;19(4):474–85.
[57] Nawathe A, Patwardhan S, Yates D, Harrison GR, Khan KS. Systematic review of the effects of aromatase inhibitors on pain associated with endometriosis. Bjog 2008;115(7):818–22.
[58] Dunselman GA, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, De Bie B, et al. ESHRE guideline: management of women with endometriosis. Hum Reprod 2014;29(3):400–12.
[59] Kataoka H, Mori T, Okimura H, Matsushima H, Ito F, Koshiba A, et al. Peroxisome proliferator-activated receptor-gamma coactivator 1alpha-mediated pathway as a possible therapeutic target in endometriosis. Hum Reprod 2019;34(6):1019–29.
[60] Adlercreutz H. Phyto-oestrogens and cancer. Lancet Oncol 2002;3(6):364–73.
[61] Khaodhiar L, Ricciotti HA, Li L, Pan W, Schickel M, Zhou J, et al. Daidzein-rich isoflavone aglycones are potentially effective in reducing hot flashes in menopausal women. Menopause 2008;15(1):125–32.
[62] Takai E, Taniguchi F, Nakamura K, Uegaki T, Iwabe T, Harada T. Parthenolide reduces cell proliferation and prostaglandin estradiol synthesis in human endometriotic stromal cells and inhibits development of endometriosis in the murine model. Fertil Steril 2013;100(4):1170–8.
[63] Cheng W, Chen L, Yang S, Han J, Zhai D, Ni J, et al. Puerarin suppresses proliferation of endometriotic stromal cells partly via the MAPK signaling pathway induced by 17ss-estradiol-BSA. PLoS One 2012;7(9):e45529.
[64] Yavuz E, Oktem M, Esinler I, Toru SA, Zeyneloglu HB. Genistein causes regression of endometriotic implants in the rat model. Fertil Steril 2007;88(4 Suppl):1129–34.
[65] Takaoka O, Mori T, Ito F, Okimura H, Kataoka H, Tanaka Y, et al. Daidzein-rich isoflavone aglycones inhibit cell growth and inflammation in endometriosis. J Steroid Biochem Mol Biol 2018;181:125–32.
[66] Green S, Walter P, Kumar V, Krust A, Bornert JM, Argos P, et al. Human oestrogen receptor cDNA: sequence, expression and homology to v-erb-A. Nature 1986;320(6058):134–9.
[67] Mosselman S, Polman J, Dijkema R. ER beta: identification and characterization of a novel human estrogen receptor. FEBS Lett 1996;392(1):49–53.
[68] Matthews J, Gustafsson JA. Estrogen signaling: a subtle balance between ER alpha and ER beta. Mol Interv 2003;3(5):281–92.
[69] Revankar CM, Cimino DF, Sklar LA, Arterburn JB, Prossnitz ER. A transmembrane intracellular estrogen receptor mediates rapid cell signaling. Science 2005;307(5715):1625–30.
[70] Matsuzaki S, Murakami T, Uehara S, Canis M, Sasano H, Okamura K. Expression of estrogen receptor alpha and beta in peritoneal and ovarian endometriosis. Fertil Steril 2001;75(6):1198–205.

[71] Xue Q, Lin Z, Cheng YH, Huang CC, Marsh E, Yin P, et al. Promoter methylation regulates estrogen receptor 2 in human endometrium and endometriosis. Biol Reprod 2007;77(4):681–7.
[72] Han SJ, O'Malley BW. The dynamics of nuclear receptors and nuclear receptor coregulators in the pathogenesis of endometriosis. Hum Reprod Update 2014;20(4):467–84.
[73] Han SJ, Jung SY, Wu SP, Hawkins SM, Park MJ, Kyo S, et al. Estrogen receptor beta modulates apoptosis complexes and the inflammasome to drive the pathogenesis of endometriosis. Cell 2015;163(4):960–74.
[74] Samartzis N, Samartzis EP, Noske A, Fedier A, Dedes KJ, Caduff R, et al. Expression of the G protein-coupled estrogen receptor (GPER) in endometriosis: a tissue microarray study. Reprod Biol Endocrinol 2012;10:30.
[75] Plante BJ, Lessey BA, Taylor RN, Wang W, Bagchi MK, Yuan L, et al. G protein-coupled estrogen receptor (GPER) expression in normal and abnormal endometrium. Reprod Sci 2012;19(7):684–93.
[76] Chevalier N, Vega A, Bouskine A, Siddeek B, Michiels JF, Chevallier D, et al. GPR30, the non-classical membrane G protein related estrogen receptor, is overexpressed in human seminoma and promotes seminoma cell proliferation. PLoS One 2012;7(4):e34672.
[77] Tian R, Wang Z, Shi Z, Li D, Wang Y, Zhu Y, et al. Differential expression of G-protein-coupled estrogen receptor-30 in human myometrial and uterine leiomyoma smooth muscle. Fertil Steril 2013;99(1):256–63.
[78] Mori T, Ito F, Matsushima H, Takaoka O, Tanaka Y, Koshiba A, et al. G protein-coupled estrogen receptor 1 agonist G-1 induces cell cycle arrest in the mitotic phase, leading to apoptosis in endometriosis. Fertil Steril 2015;103(5):1228–35.
[79] Vernier M, Dufour CR, McGuirk S, Scholtes C, Li X, Bourmeau G, et al. Estrogen-related receptors are targetable ROS sensors. Genes Dev 2020;34(7–8):544–59.
[80] Bombail V, MacPherson S, Critchley HO, Saunders PT. Estrogen receptor related beta is expressed in human endometrium throughout the normal menstrual cycle. Hum Reprod 2008;23(12):2782–90.
[81] Cavallini A, Resta L, Caringella AM, Dinaro E, Lippolis C, Loverro G. Involvement of estrogen receptor-related receptors in human ovarian endometriosis. Fertil Steril 2011;96(1):102–6.
[82] Bombail V, Gibson DA, Collins F, MacPherson S, Critchley HO, Saunders PT. A Role for the orphan nuclear receptor estrogen-related receptor alpha in endometrial stromal cell decidualization and expression of genes implicated in energy metabolism. J Clin Endocrinol Metab 2010;95(10):E224–8.

This page intentionally left blank

CHAPTER

8

Hypoxia and immune factors

Wan-Ning Li[1], *Meng Hsing Wu*[2,3] *and Shaw-Jenq Tsai*[1,2]

[1]Institute of Basic Medical Sciences, College of Medicine, National Cheng Kung University, Tainan, Taiwan; [2]Department of Physiology, College of Medicine, National Cheng Kung University, Tainan, Taiwan; [3]Department of Obstetrics and Gynecology, College of Medicine, National Cheng Kung University, Tainan, Taiwan

Introduction (immunology in endometriosis)

Endometriosis is a common gynecological disease, which is characterized as aberrant growth of endometriotic lesions outside of the uterine cavity. These endometriotic tissues, without clear mechanism, grow well under the hostile microenvironment and further cause clinical symptoms such as chronic pelvic pain, dysmenorrhea, dyspareunia, and even infertility [1]. Although not being viewed as a malignant disease, endometriosis shows high recurrent rate even after surgical removal in only 1–3 years [2], which severely reduces the quality of life of affected individuals. According to Sampson's theory, lesions are originated from the site of endometrium, reaching the peritoneal cavity by retrograde flow during menstruation [3]. Since over 90% of the women in reproductive age have retrograde menstruation but only 10%–15% suffer from endometriosis [4,5], it is generally believed that there must be other critical factors involved in the pathogenesis of endometriosis.

Endometriosis is a disease of chronic inflammation [6]. It has been hypothesized that immune system first recognizes the abnormally detached cells retrograded to the peritoneal cavity, and initiates the innate immune response to attack and prevent the growth of these foreign invaders [7]. However, due to both strong survival abilities of the endometriotic lesions and the incompetence of immune system, endometriosis develops and the body compromises to a chronic inflammatory state. Elevated levels of proinflammatory cytokines and recruited immune cells are found in ectopic lesions and peritoneal fluid of patients with endometriosis [8–10]. Moreover, it has also been demonstrated these immune factors

https://doi.org/10.1016/B978-0-12-820661-4.00021-2

© 2022 Elsevier Inc. All rights reserved.

consequently serve as effectors for the pathogenesis of endometriosis. Interleukin-1 (IL-1) family cytokines, as one example, are crucial factors in promoting the survival of endometriotic tissues [11]. IL-1β was found highly expressed in endometriosis patients [12], which promotes cyclooxygenase (COX)-2 expression [13] thus enhances angiogenic activity of the endometriotic lesions through increasing the production of prostaglandin (PG) E_2. On the other hand, impaired function of macrophages was also found in patients with endometriosis [14–16]. Wu et al. showed COX-2-derived PGE_2 decreases the phagocytic ability of macrophages through the suppression of matrix metalloproteinase-9 (MMP-9), annexin A2, and CD36 [14–16]. These lines of evidence show that dysfunction and/or imbalance of immune factors may facilitate the survival of the retrograded endometrial cells, and ultimately lead to the development of endometriosis.

The chronically inflamed microenvironment in patients with endometriosis is a hotbed for the growth of endometriotic tissues. However, a great percentage of retrograded endometrial tissues can still grow in this hostile microenvironment. Therefore, it is intriguing to unravel what drives these lesions to survive, implant, and grow in ectopic sites. It has been reported endometriotic lesions are featured with higher adhesive ability, good capability of antiapoptosis, and stronger angiogenesis activity [17–19]. Many studies have indicated that hypoxia plays an important role in promoting lesion growth through mediating the expression of a number of genes [17,19]. Distinct patterns of gene expression regulated by hypoxia in endometriotic cells end up with different characteristics, which may account for different pathological consequences in patients. In this chapter, we summarize the effect of hypoxia on the immunology of endometriosis and discuss the potential therapeutic strategy by targeting hypoxia-mediated gene expression network.

The role of hypoxia in the pathogenesis of endometriosis

The first challenge that retrograded endometriotic tissues encounter in peritoneal cavity is hypoxia. Under hypoxic condition that lacks enough oxygen, most cells die but some survive owing to undergoing epigenome reprogramming so they acquire self-supporting capacity [20]. From the general concepts, hypoxia was originally considered to be hostile for the cells in the microenvironment; however, on the contrary, these endometriotic cells show good adaptabilities to hypoxic stress. The response to hypoxia enables cells to be more resistant to apoptosis and to develop ways of maintaining cellular homeostasis (Fig. 8.1). Moreover, it has been reported hypoxia induces steroidogenesis [17,21], angiogenesis [22,23], cell proliferation [21], and migration/invasion [24] in endometriotic cells, consequently resulting in the sustainable growth of these unwelcome residents.

Hypoxia-inducible factors (HIF), a heterodimeric complex composed of α and β subunits, are the potent regulators in controlling numerous pathophysiological processes during hypoxic response. Both subunits contain basic helix-loop-helix motifs for DNA-binding and dimerization, but only the α subunit possesses oxygen-dependent degradation (ODD) domain (Fig. 8.2). The β subunit, also known as aryl hydrocarbon nuclear translocator, constitutively expresses under both normoxic and hypoxic conditions while the α subunit

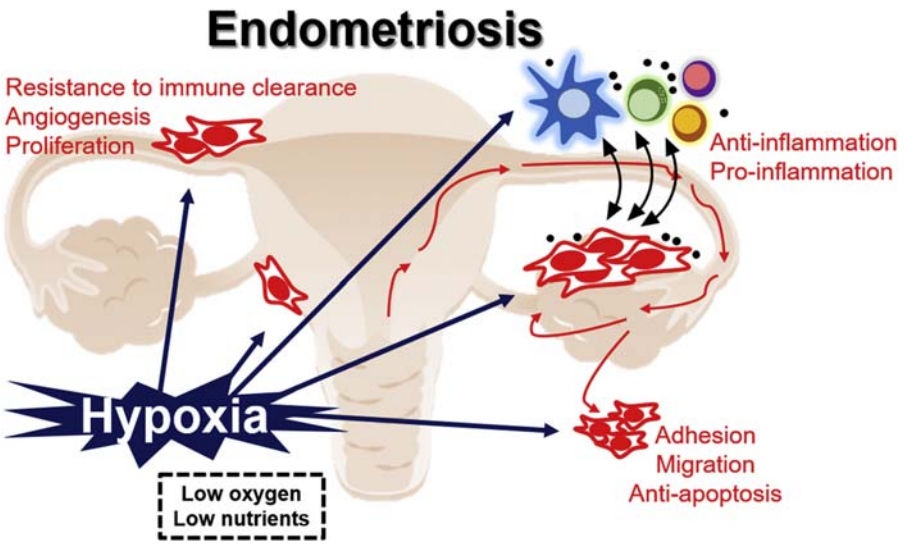


FIGURE 8.1 **Hypoxia promotes endometriotic lesion development and progression.** Hypoxia enables the retrograded tissues to migrate, and be more adhesive to peritoneal wall or organ surface in the peritoneal cavity. It also enhances cell survival by transforming cell phenotype to be more resistant to programmed cell death and immune clearance. Higher angiogenic and proliferative abilities of endometriotic tissues are found largely contributed by the hypoxic effect. Overall, hypoxia shows extensive effect on endometriotic lesions during the disease progression.

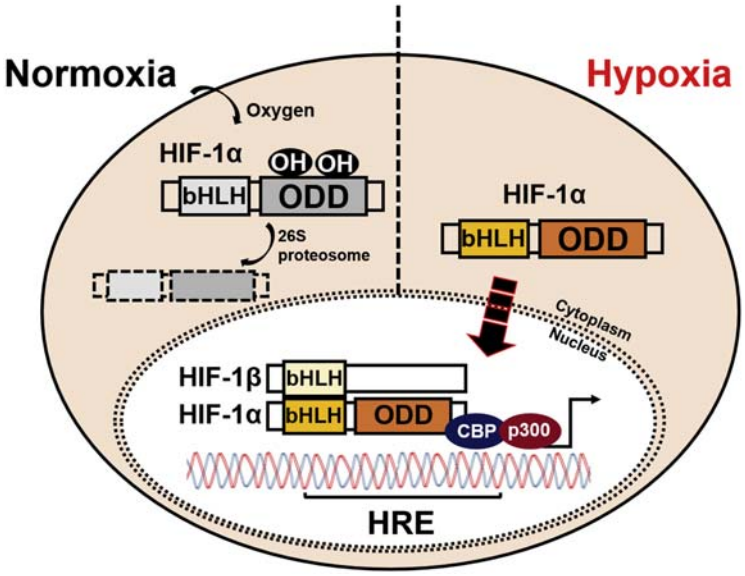


FIGURE 8.2 **Regulation of hypoxia inducible factor-1α (HIF-1α) stability and transcriptional ability under normoxic and hypoxic conditions.** Under normoxia, HIF-1α undergoes 26S proteasome degradation due to the effect of hydroxylation of proline residues in its oxygen-dependent degradation (ODD) domain. Under low oxygen situation (hypoxia), stabilized HIF-1α, together with HIF-1β, forms heterodimer and translocates into the nucleus for gene regulation. p300 and CREB binding protein (CBP) are cofactors that are essential for HIF-1α binding to hypoxia response element (HRE).

undergoes 26S proteasome degradation under ambient oxygen concentration due to the hydroxylation on ODD domain. When cells are exposed to low oxygen, HIF-1α accumulates in the nucleus and regulates the target genes expression through transcriptional modulation by binding to hypoxia response element (HRE) within promoter, 5′, or even 3′ untranslated regions [17,19]. Higher expression levels of HIF-1α mRNA and protein are found in endometriotic tissues compared to the eutopic counterparts [25]. Additionally, there are two coactivators, p300, and CREB binding protein that bind to transactivation domain of HIF-1α, which are essential for HIF-regulated transcription activity [26]. Therefore, upon hypoxia stimulation, stabilized HIF complex alters the gene expression pattern and thus causes functional changes of the cells that enables endometriotic cells to live despite without enough fundamental supplies for survival.

A number of HIF-1α-targeted genes are reported in endometriosis [22,27]. For instance, it has been reported that leptin, a gene that is found highly expressed in endometriotic stroma, is directly regulated by HIF-1α [25]. Wu et al. demonstrated the stabilized HIF-1α under hypoxia promotes the expression of leptin through direct transcriptional binding to the 5′ region of leptin promoter, thus enhances cell proliferation in endometriosis [25]. Hypoxia also upregulates vascular endothelial growth factor A (VEGF-A) in endometriosis. Sharkey et al. provided the evidence that two- to six- fold increase of VEGF-A secretion from both stromal cells and epithelial cells under hypoxia [28], which was thought to be important for angiogenesis in endometriosis [29]. In addition, besides directly controls target genes expression through transcriptional modulation, hypoxia also regulates global gene expression through epigenetic modification [30]. It has been shown that hypoxia causes global hypomethylation in endometriotic stromal cells through destabilizing DNA methyltransferase 1 (DNMT1) mRNA [30]. Hypoxia-mediated DNMT1 downregulation results in upregulation of many genes that had been reported to be critical for and overexpressed in endometriotic cells. These lines of evidence demonstrate that hypoxia plays a critical role in promoting endometriosis development via regulating numerous pathological processes in endometriosis.

Hypoxia modulates immune responses in endometriosis

Hypoxia and inflammation are considered as two main stress factors that regulate the development of endometriosis. Many studies suggest two signaling pathways crosstalk with each other to set up the complicated network [26]. This section will summarize the recent findings regarding the role of hypoxia in immune responses contributing to the development of endometriosis. Herein, we roughly classify immune factors into two parts, proinflammatory cytokines and immune cells, to discuss the regulatory role of hypoxia in regulating the immune responses in endometriosis.

Proinflammatory cytokines

As mentioned in the previous section that IL-1β plays important roles in endometriosis. IL-1β is elevated in the peritoneal fluid of women with endometriosis [12] and contributes to the

upregulation of COX-2 [13,31], VEGF-A [32], and chemoattractant for immune cells [33]. However, why is IL-1β upregulated in the endometriotic lesion was not clearly delineated until recently (Fig. 8.3). Lin and colleagues reported that hypoxia induces Yes-associated protein-1 (YAP1), a key effector in the HIPO pathway, to cause IL-1β upregulation in endometrial stromal cells [22]. Hypoxia transcriptionally suppressed the expression of large tumor suppressor kinase 1 (LATS1), a kinase that directly phosphorylates and inactivates YAP1, in a HIF-1α-dependent manner. Hypoxia-suppressed LATS1 results in activation and nuclear translocation of YAP1, which then turns on numerous genes critical for endometriosis progression, including IL-1β.

IL-6 is another proinflammatory cytokine found highly expressed in endometriosis [34]. Previous studies have shown IL-6 can be secreted from both endometriotic cells and activated macrophages, and are mainly through activation of nuclear factor-κB (NF-κB) and mitogen-activated protein kinase (MAPK) pathway [35]. The study by Hsiao et al. [21] identified a novel pathway by showing that downregulation of dual-specificity phosphatase 2 (DUSP2), a critical threonine/tyrosine phosphatase in extracellular signal-regulated protein kinase (ERK)/MAPK signaling, increases IL-6 expression in ectopic endometriotic stromal cells (Fig. 8.3). Since DUSP2 has been reported as a target gene of HIF-1α, the authors further investigated whether elevated IL-6 is mediated by hypoxia. Indeed, they not only proved that it is hypoxia-inhibited DUSP2 that promotes the expression of IL-6, but also indicated IL-6 promotes cell proliferation and prevents apoptosis through activating signal transducer and activator of transcription (STAT) three signaling in endometriotic stromal cells.

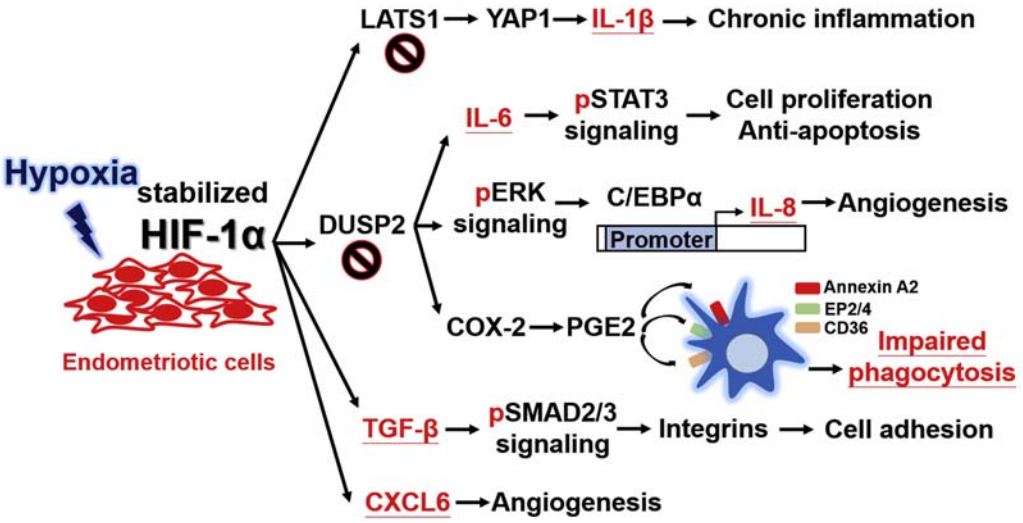


FIGURE 8.3 **Summary of the immune factors modulated by HIF-1α in endometriosis.** Proinflammatory cytokines and chemokines such as interleukin (IL)-1β, IL-6, IL-8, transforming growth factor (TGF)-β1, and CXCL6 are regulated by HIF-1α in different ways. Suppressed phagocytic ability of macrophages is also modulated by HIF-1α. Evidence shows HIF-1α accumulation suppressed the effect of dual specificity protein phosphatase 2 (DUSP2), a phosphatase responsible for ERK inactivation, resulting in the increase of cyclooxygenase-2 (COX-2) and prostaglandin E_2 (PGE_2). High level of PGE_2 further reduces phagocytic function of macrophages in endometriosis.

Apart from IL-6, the expression of IL-8 in endometriosis is also found to be regulated through hypoxia-DUSP2 axis [23]. Hypoxia suppresses DUSP2 leading to prolonged ERK phosphorylation and recruitment of C/EBPα protein onto IL-8 promoter region to increase the transcription of IL-8, which results in subsequent induction of angiogenesis in endometriosis (Fig. 8.3). Using reparixin, an inhibitor for IL-8 receptor, the authors demonstrated that angiogenic effect enhanced by hypoxia can be abolished and the severity of endometriosis is ameliorated [23]. This finding not only reveals underlying mechanism of IL-8 overexpression in endometriotic lesion but also provides answer for the puzzle that blocking VEGF-A signaling is ineffective in treating endometriosis.

Hypoxia also plays an influential role in modulating transforming growth factor (TGF)-β signaling [36]. HIF-1α induces the release of TGF-β1 from endometrial stromal cells, which further promotes the expression of integrin α5, integrin αV, integrin β3, and integrin β5 via autocrine manner [36]. Hypoxia is a strong factor to activate TGF-β1-mediated Smad2/Smad3 phosphorylation in endometriosis, resulting in the highly expressed integrins in endometriotic stromal cells (Fig. 8.3). These data provide the evidence that hypoxia is critically involved in the molecular mechanism of cell adhesion in endometriosis.

Additionally, chemokines are also found to be regulated by hypoxia (Fig. 8.3). It was shown that chemokine ligand 6 (CXCL6) is increased by hypoxia [37], resulting in higher angiogenic activity in endometriotic tissues. Tan et al. indicated hypoxia not only induces angiogenic factors such as CXCL6, but also downregulates migration inhibitor CD26/dipeptidyl peptidase IV; both effects could syngergistically promote the development of endometriosis. These lines of evidence prove that hypoxia modulates cytokines expression may ultimately lead to facilitating ectopic endometriotic lesion growth.

Immune cells

The functions of peritoneal macrophages are closely related to the immune status in peritoneal cavity [38]. The ectopic tissues are enriched with infiltrated leukocytes, such as lymphocytes, neutrophils, and macrophages [39]. Macrophages, served as a master scavenger during innate immune response, are reported to be disabled in endometriosis in many studies [38]. To eliminate cell debris and the invaders, macrophages are regularly recruited to the sites of tissue stress such as hypoxia and inflammation; however, the gathered macrophages in endometriotic lesions show impaired phagocytic capability [8,40] and even being as helpers for lesions growth [41,42]. Hypoxia is reported as a key role in mediating the phagocytic functions of macrophages in endometriosis [38]. Wu et al. showed hypoxia-suppressed DUSP2 enhances COX-2 expression in endometriotic cells, resulting in high level of PGE_2 in peritoneal cavity of patients [43], and the increased PGE_2 has been reported to weaken the phagocytic functions of macrophages. Results from a series of studies indicated PGE_2 suppresses macrophage's phagocytic activity through three distinct but complement mechanisms. First, PGE_2 inhibits macrophage's MMP9 expression and enzymatic activity that weakens the ability of degrading ectopic endometriotic tissues [16]. Second, phagocytosis depends on a group of membrane receptors, called scavenger receptors, to facilitate the recognition and engulfment of foreign entities. CD36, a member of group B scavenger receptor, is especially critical for macrophage's phagocytic ability. Levels of CD36 in peritoneal

macrophages isolated from women with endometriosis are significantly lower than those from normal women [44]. It was later identified that reduced expression of CD36 in peritoneal macrophages in women with endometriosis was due to PGE_2-mediated transcriptional suppression [14]. Third, Annexin A2, a membrane-bound and secreted molecule that functions as an activator to facilitate MMPs-dependent extracellular matrix degradation or serve as scavenger receptor to enhance phagocytosis, is downregulated in peritoneal macrophages isolated from women with endometriosis. Downregulation of Annexin A2 is mediated by PGE_2 via EP2/EP4 signaling, which consequently reduces the scavenger ability of these phagocytes [15].

Previous studies have suggested HIF-1α is able to induce metabolic reprogramming in macrophages, leading to M1/M2 polarization in inflammation [45]. In endometriosis, alternative activation of macrophage has been found to facilitate the growth of endometriotic lesion [41] and may contribute to the development of fibrosis in mouse model of endometriosis [46]. However, whether the M1/M2 polarization of macrophage is modulated by hypoxia or hypoxia-induced proinflammatory cytokines remain to be determined.

Regulation of HIF-1α by immune factors

As we mentioned above, it is reported hypoxia promotes the secretion of TGF-β1 from endometriotic stromal cells, which results in higher adhesive ability via autocrine manner [36]. On the other hand, TGF-β1 directly increases the expression of HIF-1α mRNA and protein in endometriotic cells [26,47–49]. Young et al., showed that alteration of metabolic profiles of endometriotic lesion is a consequence of Warburg effect, and TGF-β1 plays an important role in regulating the expression of some metabolic drivers such as HIF-1α [48]. They also found the expression of inhibitor of DNA-binding (ID), a protein with bHLH but lack of DNA-binding domain, in peritoneal mesothelial cells is suppressed by TGF-β1 [49]. They further hypothesized ID could heterodimerize with other transcription factors with bHLH such as HIF-1α, causing their destabilization to interfere transcription processes. Indeed, they demonstrated TGF-β1-inhibited ID increases the expression of HIF-1α, which may explain the Warburg effect caused by TGF-β1 in endometriosis [49]. Furthermore, some studies suggested the reason why TGF-β1 could lead to HIF-1α stabilization is due to tissue fibrosis [26]. The high level of TGF-β1 during prolonged inflammation increases ECM accumulation which leads to hypoxic microenvironment in endometriosis. Conclusively, the association between hypoxia and TGF-β1 signaling is a bidirectional regulation, and this causes the repeated processes of the tissue damage and reconstruction in endometriotic lesions.

Besides TGF-β1, IL-6 also regulates the accumulation of HIF-1α protein in endometrium of patients with endometriosis [50]. IL-6-activated STAT3 not only phosphorylates and stabilizes HIF-1α protein but also induces *HIF-1α* mRNA expression [50]. The observation that overexpression of estrogen receptor-β in endometriotic cells induces IL-6/Janus kinase (JAK)/STAT3 signaling and HIF-1α expression, further suggesting that endometriosis is a consequence of complicated network composed of hormone dysregulation, chronic inflammation, and hypoxic effect [51].

The activated immune cells also serve as inducers for HIF-1α expression. Recently, it was shown that activated platelets are responsible for local estrogen production in endometriosis, which is through activation of NF-κB and TGF-β1 signaling [52]. In addition, they also found HIF-1α mRNA is upregulated in endometriotic stromal cells upon the stimulation of platelet, which suggests activated platelets may also participate in hypoxic response at early stage of endometriosis.

The potential approaches for therapy

Targeting HIF as a therapeutic strategy has been reported in many cancer articles [53]; however, only handful of articles adapted similar approach to treat endometriosis. Most drugs focus on targeting the synthesis of HIF-1α mRNA and protein, and the inhibition of HIF-1α protein accumulation and dimerization [54,55]. In 2007, Becker et al. released a paper that indicated 2-Methoxyestradiol, a drug that inhibits the synthesis and nuclear translocation activity of HIF-1α, downregulates the expression level of HIF-1α-targeting genes such as VEGF, phosphoglycerate kinase, and glucose transporter-1, which leads to the suppression of lesions growth in endometriosis mice model via decreasing vascular permeability [56]. However, the potential adverse effect on other organs was not accessed. Besides directly targeting HIF proteins, other studies have declared their inhibitory effects on HIF-1α by using different signaling blockers. For instance, Romidepsin, a kind of histone deacetylase (HDAC) inhibitor, showed the successful inhibition on both HIF-1α and VEGF in endometriotic cells, resulting in an antiangiogenesis effect [57]. In addition, Sorafenib, a multiple kinase inhibitor, can decrease the expression level of HIF-1α in ectopic mesenchymal stem cells, finally results in the suppression of endometriotic cell proliferation, migration, and angiogenesis [58]. An in vivo study done by Ren et al. also showed the mammalian target of rapamycin inhibitor, Rapamycin, effectively decreases the serum level of HIF-1α and VEGF in the circulation, and showed good effect on restricting endometriotic lesions growth in mice [59]. Although these animal studies seem promising, since HIF is a master regulator involved in a variety of biological processes and endometriosis is a benign disease, both efficacy and the side effects should be strictly evaluated for clinical treatment of patients with endometriosis.

Conclusion

Hypoxia is a critical factor that mediates many physiological and pathological processes in organisms. A number of studies have reported hypoxia is critically involved in the development of endometriosis through promoting cell survival, adhesion, proliferation, and angiogenesis in endometriotic cells [17,26]. Considering its versatile effects, hypoxia may be a crucial factor to cause the phenotypic and functional remodeling of immune cells in the peritoneal microenvironment. Since immune dysfunction and chronic inflammation had been found in endometriosis, the interaction between hypoxia and immune factors in disease progression is of interesting. Surprisingly, not many studies have been done to investigate the

mutual regulation of hypoxia and immune system. Therefore, further investigation in delineating interplays between hypoxia and immune factors is required in endometriosis. It is undoubtedly that targeting hypoxia and immune system will be a potential therapeutic approach in the future.

References

[1] Agarwal SK, Chapron C, Giudice LC, Laufer MR, Leyland N, Missmer SA, Singh SS, Taylor HS. Clinical diagnosis of endometriosis: a call to action. Am J Obstet Gynecol 2019;220(4). 354 e351–354 e312.

[2] Jerman LF, Hey-Cunningham AJ. The role of the lymphatic system in endometriosis: a comprehensive review of the literature. Biol Reprod 2015;92(3):64.

[3] Sampson JA. Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. Am J Pathol 1927;3(2):93–110. 143.

[4] Halme J, Hammond MG, Hulka JF, Raj SG, Talbert LM. Retrograde menstruation in healthy women and in patients with endometriosis. Obstet Gynecol 1984;64(2):151–4.

[5] Cramer DW, Missmer SA. The epidemiology of endometriosis. Ann N Y Acad Sci 2002;955:11–22.

[6] Garcia-Gomez E, Vazquez-Martinez ER, Reyes-Mayoral C, Cruz-Orozco OP, Camacho-Arroyo I, Cerbon M. Regulation of inflammation pathways and inflammasome by sex steroid hormones in endometriosis. Front Endocrinol 2019;10:935.

[7] Lin YJ, Lai MD, Lei HY, Wing LY. Neutrophils and macrophages promote angiogenesis in the early stage of endometriosis in a mouse model. Endocrinology 2006;147(3):1278–86.

[8] Wu MH, Sun HS, Lin CC, Hsiao KY, Chuang PC, Pan HA, Tsai SJ. Distinct mechanisms regulate cyclooxygenase-1 and -2 in peritoneal macrophages of women with and without endometriosis. Mol Hum Reprod 2002;8(12):1103–10.

[9] Vinatier D, Dufour P, Oosterlynck D. Immunological aspects of endometriosis. Hum Reprod Update 1996;2(5):371–84.

[10] Harada T, Iwabe T, Terakawa N. Role of cytokines in endometriosis. Fertil Steril 2001;76(1):1–10.

[11] Akoum A, Al-Akoum M, Lemay A, Maheux R, Leboeuf M. Imbalance in the peritoneal levels of interleukin 1 and its decoy inhibitory receptor type II in endometriosis women with infertility and pelvic pain. Fertil Steril 2008;89(6):1618–24.

[12] Mori H, Sawairi M, Nakagawa M, Itoh N, Wada K, Tamaya T. Peritoneal fluid interleukin-1 beta and tumor necrosis factor in patients with benign gynecologic disease. Am J Reprod Immunol 1991;26(2):62–7.

[13] Wu MH, Wang CA, Lin CC, Chen LC, Chang WC, Tsai SJ. Distinct regulation of cyclooxygenase-2 by interleukin-1beta in normal and endometriotic stromal cells. J Clin Endocrinol Metab 2005;90(1):286–95.

[14] Chuang PC, Lin YJ, Wu MH, Wing LY, Shoji Y, Tsai SJ. Inhibition of CD36-dependent phagocytosis by prostaglandin E2 contributes to the development of endometriosis. Am J Pathol 2010;176(2):850–60.

[15] Wu MH, Chuang PC, Lin YJ, Tsai SJ. Suppression of annexin A2 by prostaglandin E(2) impairs phagocytic ability of peritoneal macrophages in women with endometriosis. Hum Reprod 2013;28(4):1045–53.

[16] Wu MH, Shoji Y, Wu MC, Chuang PC, Lin CC, Huang MF, Tsai SJ. Suppression of matrix metalloproteinase-9 by prostaglandin E(2) in peritoneal macrophage is associated with severity of endometriosis. Am J Pathol 2005;167(4):1061–9.

[17] Wu MH, Hsiao KY, Tsai SJ. Hypoxia: the force of endometriosis. J Obstet Gynaecol Res 2019;45(3):532–41.

[18] Wu MH, Lu CW, Chuang PC, Tsai SJ. Prostaglandin E2: the master of endometriosis? Exp Biol Med 2010;235(6):668–77.

[19] Hsiao KY, Lin SC, Wu MH, Tsai SJ. Pathological functions of hypoxia in endometriosis. Front Biosci 2015;7:309–21.

[20] Hsiao KY, Wu MH, Tsai SJ. Epigenetic regulation of the pathological process in endometriosis. Reprod Med Biol 2017;16(4):314–9.

[21] Hsiao KY, Chang N, Tsai JL, Lin SC, Tsai SJ, Wu MH. Hypoxia-inhibited DUSP2 expression promotes IL-6/STAT3 signaling in endometriosis. Am J Reprod Immunol 2017;78(4).

[22] Lin SC, Lee HC, Hou PC, Fu JL, Wu MH, Tsai SJ. Targeting hypoxia-mediated YAP1 nuclear translocation ameliorates pathogenesis of endometriosis without compromising maternal fertility. J Pathol 2017;242(4):476–87.

[23] Hsiao KY, Chang N, Lin SC, Li YH, Wu MH. Inhibition of dual specificity phosphatase-2 by hypoxia promotes interleukin-8-mediated angiogenesis in endometriosis. Hum Reprod 2014;29(12):2747–55.
[24] Liu H, Zhang Z, Xiong W, Zhang L, Xiong Y, Li N, He H, Du Y, Liu Y. Hypoxia-inducible factor-1alpha promotes endometrial stromal cells migration and invasion by upregulating autophagy in endometriosis. Reproduction 2017;153(6):809–20.
[25] Wu MH, Chen KF, Lin SC, Lgu CW, Tsai SJ. Aberrant expression of leptin in human endometriotic stromal cells is induced by elevated levels of hypoxia inducible factor-1alpha. Am J Pathol 2007;170(2):590–8.
[26] Wilson RB. Hypoxia, cytokines and stromal recruitment: parallels between pathophysiology of encapsulating peritoneal sclerosis, endometriosis and peritoneal metastasis. Pleura Peritoneum 2018;3(1):20180103.
[27] Lin SC, Wang CC, Wu MH, Yang SH, Li YH, Tsai SJ. Hypoxia-induced microRNA-20a expression increases ERK phosphorylation and angiogenic gene expression in endometriotic stromal cells. J Clin Endocrinol Metab 2012;97(8):E1515–23.
[28] Sharkey AM, Day K, McPherson A, Malik S, Licence D, Smith SK, Charnock-Jones DS. Vascular endothelial growth factor expression in human endometrium is regulated by hypoxia. J Clin Endocrinol Metab 2000;85(1):402–9.
[29] Donnez J, Smoes P, Gillerot S, Casanas-Roux F, Nisolle M. Vascular endothelial growth factor (VEGF) in endometriosis. Hum Reprod 1998;13(6):1686–90.
[30] Hsiao KY, Wu MH, Chang N, Yang SH, Wu CW, Sun HS, Tsai SJ. Coordination of AUF1 and miR-148a destabilizes DNA methyltransferase 1 mRNA under hypoxia in endometriosis. Mol Hum Reprod 2015;21(12):894–904.
[31] Kao AP, Wang KH, Long CY, Chai CY, Tsai CF, Hsieh TH, Hsu CY, Chang CC, Lee JN, Tsai EM. Interleukin-1beta induces cyclooxygenase-2 expression and promotes the invasive ability of human mesenchymal stem cells derived from ovarian endometrioma. Fertil Steril 2011;96(3):678–84. e671.
[32] Lebovic DI, Bentzien F, Chao VA, Garrett EN, Meng YG, Taylor RN. Induction of an angiogenic phenotype in endometriotic stromal cell cultures by interleukin-1beta. Mol Hum Reprod 2000;6(3):269–75.
[33] Lebovic DI, Chao VA, Martini JF, Taylor RN. IL-1beta induction of RANTES (regulated upon activation, normal T cell expressed and secreted) chemokine gene expression in endometriotic stromal cells depends on a nuclear factor-kappaB site in the proximal promoter. J Clin Endocrinol Metab 2001;86(10):4759–64.
[34] Tseng JF, Ryan IP, Milam TD, Murai JT, Schriock ED, Landers DV, Taylor RN. Interleukin-6 secretion in vitro is up-regulated in ectopic and eutopic endometrial stromal cells from women with endometriosis. J Clin Endocrinol Metab 1996;81(3):1118–22.
[35] Yamauchi N, Harada T, Taniguchi F, Yoshida S, Iwabe T, Terakawa N. Tumor necrosis factor-alpha induced the release of interleukin-6 from endometriotic stromal cells by the nuclear factor-kappaB and mitogen-activated protein kinase pathways. Fertil Steril 2004;82(Suppl. 3):1023–8.
[36] Lin X, Dai Y, Xu W, Shi L, Jin X, Li C, Zhou F, Pan Y, Zhang Y, Lin X, Zhang S. Hypoxia promotes ectopic adhesion ability of endometrial stromal cells via TGF-beta1/Smad signaling in endometriosis. Endocrinology 2018;159(4):1630–41.
[37] Tan CW, Lee YH, Tan HH, Lau MS, Choolani M, Griffith L, Chan JK. CD26/DPPIV down-regulation in endometrial stromal cell migration in endometriosis. Fertil Steril 2014;102(1):167–177 e169.
[38] Capobianco A, Rovere-Querini P. Endometriosis, a disease of the macrophage. Front Immunol 2013;4:9.
[39] Osuga Y. Current concepts of the pathogenesis of endometriosis. Reprod Med Biol 2010;9(1):1–7.
[40] Raiter-Tenenbaum A, Baranao RI, Etchepareborda JJ, Meresman GF, Rumi LS. Functional and phenotypic alterations in peritoneal macrophages from patients with early and advanced endometriosis. Arch Gynecol Obstet 1998;261(3):147–57.
[41] Bacci M, Capobianco A, Monno A, Cottone L, Di Puppo F, Camisa B, Mariani M, Brignole C, Ponzoni M, Ferrari S, Panina-Bordignon P, Manfredi AA, Rovere-Querini P. Macrophages are alternatively activated in patients with endometriosis and required for growth and vascularization of lesions in a mouse model of disease. Am J Pathol 2009;175(2):547–56.
[42] Harada T, Enatsu A, Mitsunari M, Nagano Y, Ito M, Tsudo T, Taniguchi F, Iwabe T, Tanikawa M, Terakawa N. Role of cytokines in progression of endometriosis. Gynecol Obstet Invest 1999;47(Suppl. 1):34–9.
[43] Wu MH, Lin SC, Hsiao KY, Tsai SJ. Hypoxia-inhibited dual-specificity phosphatase-2 expression in endometriotic cells regulates cyclooxygenase-2 expression. J Pathol 2011;225(3):390–400.

[44] Chuang PC, Wu MH, Shoji Y, Tsai SJ. Downregulation of CD36 results in reduced phagocytic ability of peritoneal macrophages of women with endometriosis. J Pathol 2009;219(2):232–41.
[45] Corcoran SE, O'Neill LA. HIF1alpha and metabolic reprogramming in inflammation. J Clin Invest 2016;126(10):3699–707.
[46] Duan J, Liu X, Wang H, Guo SW. The M2a macrophage subset may be critically involved in the fibrogenesis of endometriosis in mice. Reprod Biomed Online 2018;37(3):254–68.
[47] Yu YX, Xiu YL, Chen X, Li YL. Transforming growth factor-beta 1 involved in the pathogenesis of endometriosis through regulating expression of vascular endothelial growth factor under hypoxia. Chin Med J 2017;130(8):950–6.
[48] Young VJ, Brown JK, Maybin J, Saunders PT, Duncan WC, Horne AW. Transforming growth factor-beta induced Warburg-like metabolic reprogramming may underpin the development of peritoneal endometriosis. J Clin Endocrinol Metab 2014;99(9):3450–9.
[49] Young VJ, Ahmad SF, Brown JK, Duncan WC, Horne AW. ID2 mediates the transforming growth factor-beta1-induced Warburg-like effect seen in the peritoneum of women with endometriosis. Mol Hum Reprod 2016;22(9):648–54.
[50] Kim BG, Yoo JY, Kim TH, Shin JH, Langenheim JF, Ferguson SD, Fazleabas AT, Young SL, Lessey BA, Jeong JW. Aberrant activation of signal transducer and activator of transcription-3 (STAT3) signaling in endometriosis. Hum Reprod 2015;30(5):1069–78.
[51] Han SJ, Lee JE, Cho YJ, Park MJ, O'Malley BW. Genomic function of estrogen receptor beta in endometriosis. Endocrinology 2019;160(11):2495–516.
[52] Qi Q, Liu X, Zhang Q, Guo SW. Platelets induce increased estrogen production through NF-kappaB and TGF-beta1 signaling pathways in endometriotic stromal cells. Sci Rep 2020;10(1):1281.
[53] Wigerup C, Pahlman S, Bexell D. Therapeutic targeting of hypoxia and hypoxia-inducible factors in cancer. Pharmacol Ther 2016;164:152–69.
[54] Lee SH, Jee JG, Bae JS, Liu KH, Lee YM. A group of novel HIF-1alpha inhibitors, glyceollins, blocks HIF-1alpha synthesis and decreases its stability via inhibition of the PI3K/AKT/mTOR pathway and Hsp90 binding. J Cell Physiol 2015;230(4):853–62.
[55] Lee K, Zhang H, Qian DZ, Rey S, Liu JO, Semenza GL. Acriflavine inhibits HIF-1 dimerization, tumor growth, and vascularization. Proc Natl Acad Sci U S A 2009;106(42):17910–5.
[56] Becker CM, Rohwer N, Funakoshi T, Cramer T, Bernhardt W, Birsner A, Folkman J, D'Amato RJ. 2-methoxyestradiol inhibits hypoxia-inducible factor-1a and suppresses growth of lesions in a mouse model of endometriosis. Am J Pathol 2008;172(2):534–44.
[57] Imesch P, Samartzis EP, Schneider M, Fink D, Fedier A. Inhibition of transcription, expression, and secretion of the vascular epithelial growth factor in human epithelial endometriotic cells by romidepsin. Fertil Steril 2011;95(5):1579–83.
[58] Moggio A, Pittatore G, Cassoni P, Marchino GL, Revelli A, Bussolati B. Sorafenib inhibits growth, migration, and angiogenic potential of ectopic endometrial mesenchymal stem cells derived from patients with endometriosis. Fertil Steril 2012;98(6):1521–30. e1522.
[59] Ren XU, Wang Y, Xu G, Dai L. Effect of rapamycin on endometriosis in mice. Exp Ther Med 2016;12(1):101–6.

This page intentionally left blank

CHAPTER

9

The roles and functions of macrophages in endometriosis

Xue Jiao[1,*], *Miaomiao Ji*[1,*], *Sun-Wei Guo*[2,3] and *Guoyun Wang*[1]

[1]Department of Obstetrics and Gynecology, Qilu Hospital of Shandong University, Jinan, Shandong, China; [2]Shanghai OB/GYN Hospital, Fudan University, Shanghai, China; [3]Shanghai Key Laboratory of Female Reproductive Endocrine-Related Diseases, Fudan University, Shanghai, China

Background

Endometriosis, defined as the presence of endometrial glands and stroma outside the uterine cavity, is a common gynecological disease [1]. It is an estrogen-dependent and chronic inflammatory disease, affecting 10% of women during their reproductive years [2]. It is frequently associated with chronic pelvic pain, dyspareunia, and subfertility, negatively impacting on the afflicted women's quality of life [3]. Although the etiology and pathogenesis of endometriosis are still unclear, Sampson's theory of implantation of endometrial cells and fragments refluxed during menstruation is generally accepted. It holds that endometriosis is caused by retrograde menstruation with intraperitoneal spilling of endometrial cells [4], from the mucosa lining of the uterine cavity, and then implanted into the endoabdominal structures, in particular the peritoneal wall and ovaries [5]. However, nearly all healthy women with patent fallopian tubes undergo retrograde menstruation, yet only 1%–10% of women are diagnosed with endometriosis [6]. Given such a huge gap, it is likely that the genesis of endometriosis is affected by many factors, such as the immune state, inflammation and the changes of hormones levels. It is generally regarded that the pathogenesis of endometriosis is complex, characterized by inflammation of peritoneum, macrophage chemotaxis and polarization, nerve infiltration and increased local estrogen biosynthesis [7].

[*] Xue Jiao and Miaomiao Ji contributed equally to this manuscript.

Immunology of Endometriosis
https://doi.org/10.1016/B978-0-12-820661-4.00007-8

© 2022 Elsevier Inc. All rights reserved.

In recent years, much attention has been paid to the immune aspect of endometriosis, with macrophage being the major focus [8]. The inflammation of peritoneal cavity can be induced by regurgitated endometrial debris during or after menstruation, and the sentinels of the immune system are first recruited to the area harboring the ectopic endometrial cells. Macrophages, as one of the abundant common immune cells in the abdominal cavity, are activated. Consequently, macrophages are likely to be responsible for the elevated proinflammatory and chemotactic cytokines in the pelvic cavity and are involved in the regulation of hypoxia-induced angiogenesis [9]. In addition, the peritoneal macrophages isolated from patients with endometriosis are found to have multiple phenotypes and function [10]. In addition, an imbalance between sympathetic and sensory nerve fibers has been found in peritoneal endometriosis, suggesting that inflammation, neurogenesis and fibrogenesis are important players in driving the pathogenesis of endometriosis [11]. Moreover, estradiol is also implicated as a critical mediator of macrophage-nerve cross talk in peritoneal endometriosis [12]. Therefore, macrophages seem to play a significant role in the genesis and development of endometriosis and its associated dysmenorrhea and chronic pelvic pain. The future therapeutics could pay more attention to the interaction among endometriotic lesions, macrophages, nerve fibers and estrogen.

The aim of this review is to enumerate the roles and functions of macrophage in the development of endometriosis to expose areas in need of further research, and to explore new effective treatment for endometriosis.

The origin, activation, subtype, and biological function of macrophages

Macrophages are classified as part of the mononuclear phagocyte system by Furth and Cohn, and derived mostly from progenitor cells in the bone marrow [13]. It argued that tissue-resident macrophage homeostasis relies on the constant recruitment of blood monocytes [14]. As phagocytic immune cells, they play significant roles in homeostasis and orchestrate multiple responses to pathogens [15]. Interestingly, numerous macrophage subtypes have been identified in different organs, anatomical locations and different pathologies, suggesting that macrophages are heterogeneous, and they acquire specific phenotypes and are activated depending on their respective microenvironment, and perform diverse functions according to their phenotypes, from potent proinflammatory response in eliminating invading pathogens to antiinflammatory response conferring tissue protection, repair and remodeling [16].In this guidance, it is difficult to explain monocytes are the only components of tissue macrophages in the homeostasis. Therefore, recent researches have suggested a new origin for macrophages [17].

Different tissue macrophages may origin either from embryonic progenitors (mainly yolk sac macrophages and fetal monocytes), or from adult blood monocytes [18]. Tissue macrophages derived from embryonic progenitors exist briefly in the yolk sac and the fetal liver, and continue to self-renew throughout adulthood [19]. When infected or inflamed, adult blood macrophages can replace embryonic-derived macrophages and exhibit similar phenotypes and functions [20,21]. Hence, tissue macrophages show functional differences according to their different ontogeny, and the proinflammatory cytokines have various effects on the macrophages [22].

In normal endometrium, macrophages are believed to play important roles during the menstrual cycle, especially in the context of tissue degradation, repair, regeneration, and phagocytic clearance of endometrial tissue debris to reestablish tissue integrity in preparation for implantation and pregnancy [23]. According to the theory of new origin, macrophages in the peritoneal cavity may be also derived from different progenitors, and then be activated to different phenotypes. All these are regulated by estradiol and progesterone [24].

Macrophage recruitment, polarization and their corresponding activating factors

Nearly all women with patent fallopian tubes have endometrial cells reflux, but only a few of them suffer from endometriosis. This seems to indicate that the host immunity and the microenvironment of the peritoneal cavity of the patients with endometriosis are conducive for the growth and infiltration of endometriotic lesions. Indeed, endometriotic lesions exist in a unique microenvironment consisting of epithelial, stromal, endothelial, glandular, and immune cells characterized by increased concentrations of estrogens, and increased degree of inflammation, oxidative stress and iron overload [25]. Furthermore, endometriotic lesions may possess a distinct immune microenvironment reminiscent of a tumorlike inflammatory profile [26].

Specifically, transcriptomic profiling of inflammation-associated alterations within endometriotic lesions compared to matched eutopic and healthy control endometrium revealed elevated expression of genes associated with immune cell recruitment, cytokine−cytokine receptor interactions, cellular adhesion, and apoptosis in ectopic lesions [27]. Endometrial cells refluxed into the peritoneal cavity secrete various chemokines, creating a feed-forward loop that stimulates the infiltration of immune cells especially macrophages [28,29]. In endometriosis patients, macrophage populations are significantly elevated in the peritoneal fluid and eutopic endometrium. In addition, macrophages have been shown to invade endometriotic lesions in greater abundance than healthy peritoneum in both endometriosis patients and control women [30], suggesting that there must be some form of cross talk between endometriotic lesions and macrophages.

Numerous molecular aberrations in the endometriotic lesion have been identified to be responsible for the recruitment of macrophages, including two major classes of chemokines: the CC-chemokine ligands (such as CCL5, CCL2, and CCL11) that target monocytes, T cells and eosinophils and the CXC-chemokine ligands (such as CXCL1, CXCL8, CXCL5, and CXCL12) that recruit monocytes and neutrophils [31].

For instance, an increased secretion of monocyte chemotactic protein-1 (MCP-1, also known as CCL-2) from peritoneal macrophages of women with endometriosis may contribute to paracrine and autocrine activation, leading to increased macrophage accumulation in the peritoneal cavity of patients with endometriosis [32].The ectopic tissue can be stimulated by IL-1β to produce MCP-1, and this response is affected by estradiol [33]. To be more specific, the activation of ERβ in endometriotic stromal cells (ESCs) is involved in macrophage recruitment via NF-κB/CCL2 signaling and subsequently appears to promote the development of endometriosis [34]. These results not only show the important role of MCP-1 in the development of endometriosis, but also reveal the key role of estrogen

in enhancing chemokine induced endometriosis immune-mediated recruitment [35]. This may help to explain the complex interaction between endocrine and immune systems within the lesion. In addition, hypoxia-inducing factor 1α (HIF-1α) mediated vessel remodeling in injured and regenerating tissues may play an important role in macrophage recruitment, as evidenced by the report that ablation of HIF-1α in tumors is associated with decreased CXCL12/SDF1α levels and a less effective recruitment of bone marrow-derived macrophages [36].

According to the function, receptor components, secretion characteristics of macrophages and the response to external stimulation, macrophages have been divided into classically activated macrophage (M1) and alternatively activated macrophage (M2). Many studies [12,37] have shown that M1 macrophages can promote inflammatory response, present antigens, kill invading pathogens and increase the production of reactive oxygen intermediates (ROI). In contrast, M2 macrophages express more antiinflammatory mediators such as IL-10, and are involved in adaptive immune response, angiogenesis, the resolution of inflammation and the coordination of tissue repair [8]. Once stimulated by the signal triggered by the lesions, the macrophages may polarize to the appropriate phenotype. For example, tumor-associated macrophages (TAM) can be polarized to M2 phenotype to promote tumor angiogenesis, growth and metastasis; TAM can also be polarized to M1 phenotype to inhibit tumor growth and progression under opposite stimulation [38,39]. Thus, macrophages can exhibit plasticity and different functional characteristics under different microenvironment induction.

Ectopic endometrium can induce macrophages to polarize to M2, in turn the coculture of macrophages with ESCs increases cellular proliferation and invasiveness of ESCs [40], indicating that lesion growth is likely enhanced in the presence of macrophages. Conversely, Itoh et al. reported no difference in peritoneal M2 macrophages between endometriosis patients and women with other benign gynecologic conditions [41]. Moreover, M1 macrophages have been shown to be predominate in eutopic endometrium from patients with endometriosis as compared with healthy controls [42]. In previous studies, macrophages that coexist in mouse peritoneal cavity (PerC) are defined as two different macrophage subsets. One, provisionally called the large peritoneal macrophages (LPM), contains approximately 90% of the PerC macrophages in unstimulated animals but disappears rapidly from PerC following lipopolysaccharide (LPS) or thioglycolate stimulation. These cells express high levels of the canonical macrophages surface markers, such as CD11b and F4/80. The second subset, referred to as small peritoneal macrophages (SPM), expresses substantially lower levels of CD11b and F4/80 but expresses high levels of MHC-II, which is not expressed on LPM. SPM, which predominates in PerC after LPS or thioglycolate stimulation, does not derive from LPM. Instead, it derives from blood monocytes that rapidly enter the PerC after stimulation and differentiate to mature SPM within 2−4 d. Both subsets show clear phagocytic activity and both produce nitric oxide (NO) in response to LPS stimulation in vivo [43]. The research by Yuan et al. provides a new explanation for the functional status of peritoneal macrophages in endometriosis. Different origins of peritoneal macrophages (SPM and LPM) play different roles in endometriosis in mouse. Specifically, SPMs are polarized to M2 and the LPMs are transformed to M1.Therefore, the entire peritoneal macrophages show a complex phenomenon of mixed, bipolar polarization [44]. Although women with endometriosis have increased production of chemokines and increased local macrophage

recruitment, the potency of the macrophage scavenger function and phagocytic potential seem to be depressed [45], as is shown by reduced expression of the class B scavenger receptor CD36 [46].

Activated macrophages secrete a plethora of adhesion molecules, growth factors and proinflammatory cytokines into the lesional microenvironment and the peritoneal fluid [31] with aberrant activation of the NF-κB pathway. Among these factors, fibronectin, intercellular adhesion molecule 1 (ICAM1), insulin-like growth factor I (IGFI), IL-1, IL-6, IL-8, IL-12, platelet-derived growth factor (PDGF), vascular endothelial growth factor (VEGF), and tumor necrosis factor (TNF) have been widely reported [47]. More importantly, it has been proved that endometriotic lesions develop their own innervation, and macrophages are found to migrate to the nerve. Therefore, the pathophysiology of endometriosis is the end result of complex cross talks among endometriotic lesions, macrophages, nerves and hormones [47].

Thus, macrophages are recruited and polarized in endometriotic lesions, display a continuum of functional status, and vary with disease phenotype, effectively treating endometriotic lesions as a wound and activating accordingly, leading to enhanced cell survival and growth.

Roles and functions of macrophages in endometriosis

Inflammation

During menstruation, the species and contents of various immune cell-derived factors (such as cytokines, chemokines, and growth factors) in eutopic endometrium and peritoneal fluid have been changed. In addition, macrophages are recruited to endometriotic lesions by chemotaxis induced by above factors [34]. The recruited macrophages then release proinflammatory cytokines, which may also accelerate the polarization and recruitment of macrophages, ultimately promoting the development of endometriosis.

Inflammation and macrophages

Cytokines, acting as chemo-attractant mediators, are mainly produced and released by immune cells such as macrophages, including macrophage inflammatory protein (MIP) and MCP-1. Several animal studies have suggested that proinflammatory mediators have the capacity of activating macrophage precursors [48]. Proinflammatory factors such as IL-6 and microbial components especially LPS could induce macrophages to the M1 subtype [8]. The level of IL-6 in the peritoneal fluid and ESCs is higher in patients with endometriosis than controls. It has been reported that the increased IL-6 could promote the secretion of CCL17 in ESCs, and then CCL17 can elevate the expression of CCR4, the receptor on macrophages, which in turn effectively induces the increasing of IL-6 [49]. Similarly, principle source of IL-8 is monocytes and macrophages, and the endometrium has been proven to produce IL-8 [50]. It can not only attract and stimulate the migration of macrophage, but also promote the growth of endometriotic tissue directly. In addition, many other proinflammatory factors are also associated with this process [49]. More importantly, macrophages that are recruited and activated in endometriotic lesions can secrete a variety of cytokines, which in turn promote the development of endometriosis. TNF-α has very proinflammatory,

cytotoxic potential and angiogenesis, which was also found elevating in PF of endometriosis [51]. The most commonly accepted pathways associated with endometriosis include NF-κB and Toll-like receptors (TLR)4-mediated pathway. LPS, an inflammatory medium, is regulated by TLR on the surface and NF-κB at the nucleus. TLRs are mainly expressed in macrophages, dendritic cells, and epithelial cells and macrophages can be activated by LPS, suggesting that LPS/TLR4 and macrophages are involved in endometriosis through NF-κB pathway [51]. MCP-1, secreted by peritoneal macrophages in patients with endometriosis, has significantly increased, and could accelerate the recruitment of macrophages [52].

The interaction between inflammation and macrophages

Macrophages play different roles in regulating the immune response in different microenvironments and different settings, and their functions may be modified by inflammatory mediators in lesions [53]. The prolonged inflammation in the perC results in the reduction of the ability of phagocytosis and removing the endometrial debris. In the recent years, there has been growing evidence suggesting that M2 macrophages are the primary components of macrophages in human endometrium, and are increased in number in the peritoneal cavity and peritoneal fluid from women with endometriosis. As mentioned above, M2 macrophages play a crucial role in tissue remodeling, angiogenesis and tumor progression [42]. MCP-1 as well as RANTES promote the polarization of macrophages from M1 to M2 phenotype, and Arginase 1 is considered as the marker of M2 macrophage [54]. It has been shown recently that endometriosis-exosome is associated with formation of ectopic lesions and infiltration of M2 macrophage in mice, suggesting that M2 macrophages and the factors and pathways that polarize macrophages may be one of the therapeutic targets of endometriosis [55]. In addition, 17β-estradiol has long been found to be at a higher level in ectopic endometrial tissue [34], and that the lesional expression of ERβ may promote endometriosis progression, which provides a new thought about the interaction between estradiol and macrophages [56]. On the other hand, the recruitment and polarization of M2 macrophages are associated with the epithelial−mesenchymal transition (EMT), and it is likely that EMT may be the origin of endometriosis lesion [57]. Furthermore, the level of MIF is consistent with the number of M2 macrophages in the peritoneal cavity, and, as such, MIF may contribute to the incidence of endometriosis by activating macrophages and inducing the polarization [58].Therefore, although endometriosis is a benign disease, it also has several malignant features of hyperplasia, infiltration, recurrence and metastasis, and M2 macrophages play an important role in all these molecular events. However, some researches hold that M1 macrophages are the dominant phenotype, which might be attributable to methodological differences [42]. More studies are needed to further delineate the exact role of macrophages and inflammation in the pathogenesis of endometriosis.

Angiogenesis

Angiogenesis is a prerequisite for successful implantation and long-term survival of ectopic endometrium. However, the vasculature of ectopic lesions remains dependent on the systemic concentration of hormones. As such, these structures undergo a cyclic remodeling, with synchronized tissue destruction and bleeding [8]. Multiple mechanisms contribute to the vascularization of endometriotic lesions, including angiogenesis, vasculogenesis and inosculation. The basis of angiogenesis is the complex regulation of angiogenic factors and hormones,

and the activation of intravascular pathways and related signaling molecules. In addition, circulating endothelial progenitor cells (EPCs) are mobilized from the bone marrow and collected into the endometriosis and incorporated into the new microvascular endothelium, which is called vasculogenesis. Finally, the preformed microvasculature in the endometrial debris combines with the microvascular system of the surrounding host, so as to establish a rapid blood supply system of the ectopic tissue [59]. Common angiogenic factors such as VEGF and matrix metalloproteinase (MMP) whose production may be the result of macrophages action play a key role in promoting endometriosis's angiogenesis. In human endometriotic lesions, VEGF expression has been localized to the stroma, glandular epithelium and infiltrating macrophages [60,61]. A large body of evidence indicates that macrophages are responsible for the angiogenic switch, i.e., the increase in the density of vessels that often characterizes the benign-to-malignant transition in cancer, being involved both in the initial establishment of the vasculature and in the subsequent remodeling of the vessels [62].

In mice, depletion of macrophages using chlorinate liposomes, monoclonal antibodies, or ganciclovir has been shown to reduce lesion weight and vascularity [30,63]. Another mouse model implicated both macrophages and neutrophils in the promotion of angiogenesis in lesions as in vitro culture of isolated peritoneal macrophages and neutrophils in the presence of IL-6, TNF-α, LPS, and estrogen-enhanced VEGF release by these cells [64]. The underlying mechanism may be demonstrated in experimental models in which early phases of lesion establishment are characterized by a transient hypoxia, which results in the up-regulation of HIF-1α, with subsequent overexpression of VEGF [65,66].

Hypoxia and tissue injury within the inflammatory niche are some of the major drivers of CXCL12 production. Indeed, the expression of CXCL12 in a large number of tumors and injured tissues strongly suggests that activation of CXCR4 participates in promoting neoangiogenesis. Then CXCL12 in concert with VEGF aids in recruitment of bone marrow-derived hematopoietic and EPC, their differentiation, and incorporation in the newly formed blood vessels. Under the influence of hypoxia, CXCL12 production is regulated by HIF-1α [67].

Estrogen biosynthesis

Endometriosis is an estrogen-dependent disease, and local production of estrogens promotes endometriotic cell growth. Moreover, estrogen is associated with the immune response, and macrophages in the peritoneal cavity of endometriosis express more estrogen receptors than that of healthy women, suggesting estrogen may be an important mediator for macrophages to promote the pathogenesis of endometriosis [68]. In the women's pelvic, steroid hormone, especially estrogen and its receptors regulate the status and function of the endometrium. There are two subtypes of ERs, Erα, and ERβ. Some studies considered ERβ as more important in the pathogenesis of endometriosis on account of the production of CCL2 via NF-κB signaling, and then recruit macrophages to the ectopic microenvironment [34].

Estrogen is reported to promote the expression of IRF4 in macrophages induced by macrophage colony-stimulating factor (M-CSF) and induce polarization of macrophages to M2 macrophages [69]. In mice with induced endometriosis, the recruitment and activation of macrophages treated with estrogen were increased, and macrophages were stimulated to release neurotrophic factors, thus participating in mediation of pain [12]. Cytokines released

by activated macrophages could promote the adhesion, proliferation, invasion and angiogenesis of endometrial tissue in the peritoneal cavity. Meanwhile, these cytokines could also regulate the macrophages in the endometriosis microenvironment, resulting in changes in gene expression and behavioral function, and high expression of G-protein-coupled estrogen receptor (GPER), thus increasing the sensitivity to estrogen and participating in the development of endometriosis [70]. Hence, estrogen, through its receptors, interacts with macrophages, prompting the development of endometriosis [71].

Endometriosis-associated pain

Pain is one of the most prevalent clinical symptoms of endometriosis, including dysmenorrhea, deep dyspareunia and nonmenstrual chronic pelvic pain [72]. Several studies report that the presence of nerve fibers in the peritoneum is of great significance for endometriosis-associated pelvic pain and dysmenorrhea [73]. There is also evidence that the M2 macrophages polarized in the chronic inflammatory microenvironment may be responsible for the axonal regeneration and the increased density of nerve fibers, which result in the pain symptom with endometriosis [74]. Hence, it is plausible that the interaction between macrophages and nerves may lead to the pathogenesis of endometriosis.

Macrophages and neurogenesis

In recent studies, nerve fiber has been detected with the use of immunohistochemistry with markers, especially protein gene product 9.5 (PGP 9.5) and neurofilament (NF), and found that the nerve fiber density is higher in endometriosis, in line with the amount of macrophages [75]. Neurotrophins play a vital role in the development and differentiation of neurons. It is not only found in endometriosis, but also expressed in macrophages in lesions [76]. One study reports that macrophages are closely related with nerve fibers in lesions of endometriosis, and the neurotrophin-3 (NT-3) and brain-derived neurotrophic factor (BDNF), as members of neurotrophins, are released by macrophages stimulated with colony-stimulating factor 1 (CSF-1) [12]. In addition, nerve growth factor (NGF) has been identified to be responsible for the imbalance of sympathetic and sensory innervation in endometriosis, and it induces the neuropathic pain [11]. Aside from releasing neurotrophins, M2 macrophages could mediate neurogenesis by regulating semaphorins [77]. However, some evidence suggests that the effect of neurotrophins and semaphorins repel each other. It is likely that this may be the reason for the imbalance of sympathetic and sensory innervation, in favor of more sensory innervation with increased pain in endometriosis. Therefore, macrophages mediate the neurogenesis in endometriosis lesions by secreting neurotrophic factors at the expense of sympathetic nerves [78].

Interaction between macrophages and nerve fibers

Not only macrophages could regulate the neurotrophins, nerve fibers can also produce attractants to promote the recruitment and polarization of macrophages. For example, a study demonstrated that CSF-1 produced by nerve fibers play a role on the migration of macrophages [54]. Hence, macrophages and nerve fibers both play important roles in the development of endometriosis, with associated inflammation and hyperinnervation. Sympathetic nerve fibers could secrete norepinephrine (NE), which interacts with the adrenergic β2 receptor (ADRB2) on the macrophages. Then macrophages could generate more TNF-α to regulate

the inflammation, which in turn promotes the recruitment, migration and polarization of macrophages. This also accelerates hyperinnervation in lesions [79,80].The active cross talk between macrophages and nerve fibers impacts on the inflammatory microenvironment in the peritoneal cavity, and exacerbates the painful symptoms of endometriosis. More research is warranted to unravel the underlying pathways and molecules in order to provide better care of women with endometriosis.

Fibrogenesis

Macrophages are well-documented to be a key regulator of tissue repair as they scavenge invading pathogens, remove cellular debris, and express a multitude of cytokines, chemokines, and growth factors—all of which are necessary to mediate tissue repair [81,82]. Since endometriotic lesions are fundamentally wounds undergoing repeated tissue injury and repair (ReTIAR) [83–85], not surprisingly macrophages have long been reported to be involved in the development of endometriosis, as alluded to above.

In fact, one mouse study, published well over a decade ago, showed kinetic changes in the number of lesional immune cell infiltrates. It clearly demonstrates that the number of neutrophils increased dramatically shortly after induction of endometriosis and then declined precipitously, and there was also a significant infiltration of macrophages after the surge of neutrophil infiltration [64]. This pattern is remarkably similar to that of wound healing [81]. Similar to the finding that macrophage depletion impairs tissue repair [86,87], depletion of macrophage also results in attenuated initiation and growth of ectopic implants [30,52].

The analogy/similarity between wound healing and the progression of endometriotic lesions can be exploited further. As alluded to above, macrophages can be classified as M1 and M2 subtypes. Based on their activators, markers of activation, and function, secreted cytokines and chemokines, M2 macrophages can be further classified as four discrete subtypes—M2a, M2b, M2c, and M2d macrophages [88–90]. M2a macrophages promote type 2 immune responses (as opposed to microbial-induced type 1 inflammation), M2b macrophages are involved in immunoregulation, and M2c macrophages are antiinflammatory and initiates tissue remodeling, while M2d macrophages are involved in antiinflammation and angiogenesis [91]. As wound healing typically undergoes four ordered but somewhat overlapping phases, i.e., hemostasis/coagulation, inflammation, proliferation/migration, and remodeling [81], M1 macrophages are found to be involved in the early stages of wound healing while M2 macrophages are involved in middle stages [92]. Not surprisingly, a preemptive depletion of macrophages before induction of endometriosis did not impact much on lesion weight, but depletion after induction resulted in reduced lesional growth and vascularization [30]. Hence, M2 macrophages are involved in the development of endometriosis [30,41].

Unfortunately, tissue repair in endometriotic lesions, as wounds undergoing ReTIAR, is far from normal or well controlled. In fact, the repair simply goes awry because of its repetitive and chronic nature, as seen by the presence of smooth muscle cells and dense fibrotic tissue in and surrounding the lesions [93–96], especially in deep infiltrating endometriosis [93,97–99].

Consistent with the documented M2 macrophages as a key regulator in fibrogenesis [100–102], it is recently reported that lesional infiltration of M2 macrophages increases progressively as lesions progress undisturbed, concomitant with progressive EMT, FMT, SMM and fibrosis [103]. In addition, macrophage depletion after induction of endometriosis substantially reduced lesional infiltration of macrophages, moderately reduced lesional infiltration of M2 macrophages, and significantly reduced lesion weight as well as the lesional fibrotic content [103]. Moreover, adoptive transfer of M2a, but not M1 or M2c, macrophages systemically following macrophage depletion significantly increased the extent of lesional fibrosis [103]. These findings are consistent with the previous report of the roles of M2 macrophages in endometriosis [30,52], and in particular with the finding that the transfer of M1 macrophages reduced the lesion weight [30].

Persistent or prolonged recruitment of the M2a macrophages has been hypothesized to contribute to pathological fibrosis [102]. Macrophages have been demonstrated to promote fibrogenesis through its release of profibrotic TGF-β1 [104], probably enhanced by hypoxia [105], which itself is found to play critical role in the development of endometriosis [106]. Macrophages can also induce NF-κB activation in fibroblasts, promoting their activation [107], and contribute to fibrogenesis.

Of course, macrophages do not act alone. The lesional microenvironment is also conducive for activation of M2a macrophages. M2a macrophages can be induced by.

IL-4/IL-13, and IL-4 greatly stimulates the expression of mannose receptor, which is widely accepted as the canonical marker for M2a macrophages [91]. Despite conflicting reports of IL-13 levels, the IL-4 concentrations in peritoneal fluid from women with endometriosis are reported to be elevated [108–110], and ESC express IL-4 receptor [111]. Once M2a macrophages are recruited and activated, they can induce EMT and FMT in endometriotic lesions through activation of TGF-β1/Smad3 signaling pathway in ESC, with increased cellular contractility and collagen production in vitro [83].

Macrophages undergoes dynamic changes during different stages of tissue repair. Depending on the wound healing phases, M1 macrophages mediate tissue damage and initiate inflammatory responses. This is followed by the involvement of M2 macrophages in mediating tissue repair through synthesizing pro-fibrotic mediators [112] in later phages of tissue repair. Similar to wound healing, in which macrophage depletion suppresses VEGF expression and thus disrupts neovascularization [87,113], macrophage depletion immediately after induction of endometriosis also results in reduced angiogenesis [30]. In agreement with the report that macrophage ablation yielded reduced myofibroblast activation, and decreased expression of TGF-β1, α-SMA, and reduced collagen deposition [87,92,100], similar changes in endometriotic lesions also occur in mouse with induced endometriosis [103].

All these data highlight not only the roles of different macrophage subsets in lesional progression but also the importance of timing of the presence of specific macrophage subsets in the development and fibrogenesis of endometriosis. Viewed from the looking glass of ReTIAR, this is not surprising since in wound healing insufficient accumulation of either M1 macrophages at early stages of tissue repair or of M2 macrophages at later stages have both been associated with disrupted tissue repair [114–117]. Similarly, excessive accumulation of either subtype of macrophages can also be deleterious, since

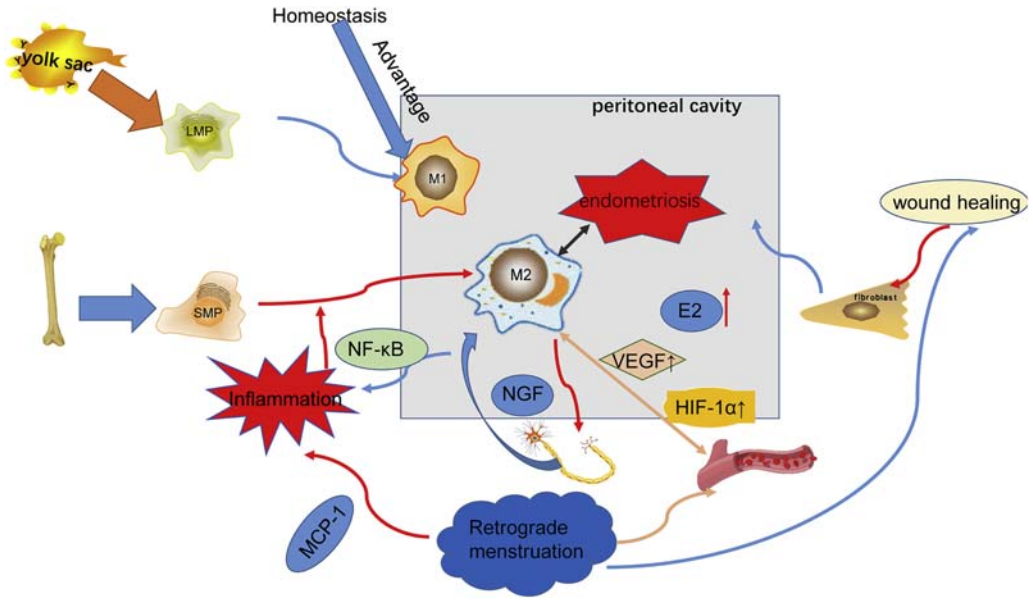


FIGURE 9.1 Endometriotic lesions exist in a unique microenvironment characterized by increased concentrations of estrogens, and increased degree of inflammation, oxidative stress and iron overload which can induce macrophages polarize to M2.Mcrophages that coexist in mouse PerCare defined as two subsets. One, provisionally called the LPM, originating from progenitors from yolk sac, contains approximately 90% of the PerC macrophages. The second subset, referred to as SPM, which derives from blood monocytes. SPM are polarized to M2 and the LPM are transformed to M1. The development of endometriosis may be due to an active crosstalk between macrophages and other multi-factors. **Inflammation**: During menstruation, macrophages are recruited to endometriotic lesions by some chemotaxis, and the recruited macrophages then release pro-inflammatory cytokines, which may also accelerate the polarization and recruitment of macrophages. **Angiogenesis**: Hypoxia drives the production of CXCL12, which regulated by HIF-1α. Then CXCL12 in concert with VEGF aids in the angiogenesis. **Neurogenesis**: Macrophages are closely related with nerve fibers in lesions of endometriosis, and the NGF has been identified to be responsible for the imbalance of sympathetic and sensory innervation in endometriosis. **Fibrogenesis**: Macrophages increased cellular contractility and collagen production through activation of TGF-β1/Smad3 signaling pathway. Moreover, estrogen is also a critical mediator in all of the process. **Abbreviation**: *HIF-1α*, hypoxia-inducing factor 1α; *LPM*, large peritoneal macrophages; *NGF*, nerve growth factor; *PerC*, peritoneal cavity; *SPM*, small peritoneal macrophages; *VEGF*, vascular endothelial growth factor.

excessive M1 macrophages would result in chronic inflammation and tissue destruction [118,119] while excessive M2 macrophages would promote fibrogenesis [104,120].

Fig. 9.1 summarizes various roles and functions of macrophages in the development of endometriosis.

Potential therapeutic targets regarding macrophages

Although a large sum of research on endometriosis has been published in the last decade, the innovation drought in drug research and development has been glaring, especially in nonhormonal drugs [85,121]. Currently, the treatment arsenal includes levonorgestrel-releasing intrauterine system (LNG-IUD), gonadotropin-releasing hormone analogues

(GnRH-a), progestins such as dienogest, and laparoscopic ablation and excision [122]. However, a reappearance of the lesion after surgery is common and repeated surgery increases the risk of premature ovarian failure, adhesion, and organ injury [123–125]. Repeat surgery also diminishes the pregnancy rate in women with infertility [126]. On the other hand, a drug induced hypo-estrogenic state for women who desire a family is simply not conducive to fertility, suggesting that additional approaches are required [25,127].

Since endometriosis and tumors have similar microenvironments and similar biological behaviors, and are closely related to immune disorders, perhaps we can emulate the targeting of tumor-associated macrophages. The potential approaches for treating endometriosis-associated macrophages are described below.

Independent lines of evidence indicate that macrophages undergo activation as a consequence of signals generated within endometriotic lesions, or possibly due to the lack of hormone-regulated anti-inflammatory signals in the ectopic but nonetheless present in the eutopic primary defects, or simply a consequence of endometriosis [8]. Targeting dysregulated immune pathways represents a potential avenue for novel therapeutic development that may not affect fertility negatively. While so far much research has been devoted to interrupt the aberrant interaction of various components in endometriosis or to inhibit various inflammatory pathways, this research has not been translated into better clinical care. This may be due to the fact that a single signal molecule may participate in multiple signal pathways, and suppression one particular pathway may also cause untoward collateral damage to other pathways, resulting unintended side-effects, such as teratogenesis, damage of immune function with increasing infection, etc. In addition, endometriosis is likely to be heterogeneous, with different patients displaying different predominant pathways. For example, NF-κB may represent a potential therapeutic target due to its constitutive activation in peritoneal endometriotic lesions [128]. An overexpression of NF-κB has been confirmed in cultured endometrial stromal cells [129] and peritoneal macrophages [130] isolated from women with endometrioma. But anti-NF-κB treatment has achieved only partial success in treating adenomyosis [131], even though NF-κB has been shown to be activated in adenomyosis and andrographolide is shown to be promising in in vitro and in vivo studies [132,133].

Furthermore, inflammation, oxidative stress and hormones stimulate the immune pathways in endometriotic tissue and therefore they represent a potential target for therapeutic intervention [25]. As described previously, macrophage are an important player in the development chronic pelvic pain. The potential treatment of endometriosis could pay more attention to the interaction of macrophage, nerve fibers and estrogen production, such as the combination therapy of antiinflammatory, antiestrogen, and antinociceptive factor.

It is reported that the anticoagulant treatment using recombinant P-selectin, which is associated with macrophage infiltration, is promising in treating endometriosis [134]. Another study found that dual suppression of estrogenic and inflammatory activities by chloroindazole (CLI) and oxabicycloheptene sulfonate (OBHS) could restrain endometriosis [56]. Similar effect may be achieved by suppressing the recruitment of macrophages, and regulating their function. Bonapace et al. confirm previous findings that when mice with mammary tumors are treated with anti-CCL2 antibodies, the egress of monocytes from the bone marrow is blocked and macrophage infiltration into primary tumors is impaired, leading to reduced formation of lung metastases(136). Unfortunately, the cessation of anti-CCL2 therapy is rapidly followed by

a mobilization of monocytes from the bone marrow to the circulation and their differentiation to macrophages in metastatic tumors, where they promote the formation of tumor blood vessels and tumor growth [135]. Chen's research shows that chloroquine (CQ), a proven antimalarial drug, can function as an antitumor immune modulator that switches TAMs from M2 to tumor-killing M1 subtype in a mice model. They observed that intraperitoneal injection of CQ (75 mg/kg) effectively inhibited melanoma growth and prolonged the survival of the mice [136]. Blood mononuclear cells or CD34+ bone marrow cells could be induced into pluripotent stem cells. These macrophages or induced pluripotent stem cells could then be transfected with the gene or genes of interest, conferring an appropriate regulatory signaling system for therapeutic purpose. In a murine disease model, intrapulmonary transplanted macrophage progenitors displayed selective, long-term pulmonary engraftment and differentiation into functional alveolar macrophages. A single transplantation ameliorated the hereditary pulmonary alveolar proteinosis (herPAP) phenotype for at least nine months, resulting in significantly reduced alveolar proteinosis, normalized lung densities in chest computed tomography, and improved lung function [137]. The efficacy of this intervention would depend on engraftment of these modified cells [138]. Although previous studies provided various novel ideas about these possible intervention modalities, more research is needed to evaluate their safety and efficacy. In future studies, more evidence is needed to demonstrate the potential role of blocking macrophage recruitment, inducing macrophage function changes, and macrophage transplantation in the treatment of endometriosis.

Conclusions and future perspectives

Endometriosis is a common, enigmatic and complex disease. In this review, we have enumerated various the important roles and functions of macrophages recruitment and polarization in the pathophysiology of endometriosis, and briefly touched up the interactions between macrophages and inflammation, angiogenesis, estrogen production and neurogenesis. In conclusion, macrophages are involved in the development of endometriosis via forming the local inflammatory and relatively tolerant immune microenvironment of peritoneal cavity, promoting M2 macrophages polarization, angiogenesis and neurogenesis, and their functions are regulated by estrogen, cytokines and chemicals released by endometriotic cells and other cells in the lesional microenvironment.

However, current treatment such as LNG-IUD, GnRH-a, progestins, and laparoscopic excision only reduce symptoms or stall the lesional development, but still cannot fulfill the pressing need for minimizing or elimination of the risk of recurrence after surgery. At present, the exact regulatory mechanism underlying macrophage recruitment, polarization, and interaction with endometriotic lesions are still largely unclear, and future research is warranted.

In the future, individualized, refined and molecular therapies may be necessary for macrophages-associated interventions to treat endometriosis. On the one hand, precise macrophage markers should be explored to establish a complete and informative signal transduction pathway mechanism. On the other hand, more attention should be paid to the interaction between macrophages and other cells/factors in the lesional microenvironment, such as estrogen levels and angiogenesis. Furthermore, large-scale clinical studies should be carried to verify their safety and effectiveness.

Acknowledgments

This work was supported by the grants (81530040 and 81771553) from the National Science Foundation of China.

References

[1] Olive DL, Schwartz LB. Endometriosis. N Engl J Med 1993;328:1759–69.
[2] Giudice LC. Clinical practice. Endometriosis. N Engl J Med 2010;362:2389–98.
[3] Vercellini P, Vigano P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. Nat Rev Endocrinol 2014;10:261–75.
[4] D'Hooghe TM, Debrock S. Endometriosis, retrograde menstruation and peritoneal inflammation in women and in baboons. Hum Reprod Update 2002;8:84–8.
[5] Sampson JA. Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. Am J Pathol 1927;3:93–110. 43.
[6] Liu DT, Hitchcock A. Endometriosis: its association with retrograde menstruation, dysmenorrhoea and tubal pathology. Br J Obstet Gynaecol 1986;93:859–62.
[7] Laux-Biehlmann A, d'Hooghe T, Zollner TM. Menstruation pulls the trigger for inflammation and pain in endometriosis. Trends Pharmacol Sci 2015;36:270–6.
[8] Capobianco A, Rovere-Querini P. Endometriosis, a disease of the macrophage. Front Immunol 2013;4:9.
[9] Ahn SH, Monsanto SP, Miller C, Singh SS, Thomas R, Tayade C. Pathophysiology and immune dysfunction in endometriosis. BioMed Res Int 2015;2015:795976.
[10] Raiter-Tenenbaum A, Baranao RI, Etchepareborda JJ, Meresman GF, Rumi LS. Functional and phenotypic alterations in peritoneal macrophages from patients with early and advanced endometriosis. Arch Gynecol Obstet 1998;261:147–57.
[11] Arnold J, Barcena de Arellano ML, Ruster C, Vercellino GF, Chiantera V, Schneider A, et al. Imbalance between sympathetic and sensory innervation in peritoneal endometriosis. Brain Behav Immun 2012;26:132–41.
[12] Greaves E, Temp J, Esnal-Zufiurre A, Mechsner S, Horne AW, Saunders PT. Estradiol is a critical mediator of macrophage-nerve cross talk in peritoneal endometriosis. Am J Pathol 2015;185:2286–97.
[13] van Furth R, Cohn ZA. The origin and kinetics of mononuclear phagocytes. J Exp Med 1968;128:415–35.
[14] van Furth R, Cohn ZA, Hirsch JG, Humphrey JH, Spector WG, Langevoort HL. The mononuclear phagocyte system: a new classification of macrophages, monocytes, and their precursor cells. Bull World Health Organ 1972;46:845–52.
[15] Varol C, Mildner A, Jung S. Macrophages: development and tissue specialization. Annu Rev Immunol 2015;33:643–75.
[16] Pineda-Torra I, Gage M, de Juan A, Pello OM. Isolation, culture, and polarization of murine bone marrow-derived and peritoneal macrophages. Methods Mol Biol 2015;1339:101–9.
[17] Ginhoux F, Jung S. Monocytes and macrophages: developmental pathways and tissue homeostasis. Nat Rev Immunol 2014;14:392–404.
[18] Ginhoux F, Schultze JL, Murray PJ, Ochando J, Biswas SK. New insights into the multidimensional concept of macrophage ontogeny, activation and function. Nat Immunol n.d.;17:34–40.
[19] Hoeffel G, Chen J, Lavin Y, Low D, Almeida FF, See P, et al. C-Myb(+) erythro-myeloid progenitor-derived fetal monocytes give rise to adult tissue-resident macrophages. Immunity 2015;42:665–78.
[20] Blériot C, Dupuis T, Jouvion G, Eberl G, Disson O, Lecuit M. Liver-resident macrophage necroptosis orchestrates type 1 microbicidal inflammation and type-2-mediated tissue repair during bacterial infection. Immunity n.d.;42:145–158.
[21] Shemer A, Jung S. Differential roles of resident microglia and infiltrating monocytes in murine CNS autoimmunity. Semin Immunopathol n.d.;37:613–623.
[22] Bilyk N, Holt PG. Cytokine modulation of the immunosuppressive phenotype of pulmonary alveolar macrophage populations. Immunology 1995;86:231–7.
[23] Thiruchelvam U, Dransfield I, Saunders PT, Critchley HO. The importance of the macrophage within the human endometrium. J Leukoc Biol 2013;93:217–25.

[24] Khan KN, Masuzaki H, Fujishita A, Kitajima M, Sekine I, Matsuyama T, et al. Estrogen and progesterone receptor expression in macrophages and regulation of hepatocyte growth factor by ovarian steroids in women with endometriosis. Hum Reprod 2005;20:2004–13.
[25] McKinnon BD, Kocbek V, Nirgianakis K, Bersinger NA, Mueller MD. Kinase signalling pathways in endometriosis: potential targets for non-hormonal therapeutics. Hum Reprod Update 2016;22:382–403.
[26] Suryawanshi S, Huang X, Elishaev E, Budiu RA, Zhang L, Kim S, et al. Complement pathway is frequently altered in endometriosis and endometriosis-associated ovarian cancer. Clin Cancer Res 2014;20:6163–74.
[27] Ahn SH, Khalaj K, Young SL, Lessey BA, Koti M, Tayade C. Immune-inflammation gene signatures in endometriosis patients. Fertil Steril 2016;106. 1420-31.e7.
[28] Lebovic DI, Chao VA, Martini JF, Taylor RN. IL-1beta induction of RANTES (regulated upon activation, normal T cell expressed and secreted) chemokine gene expression in endometriotic stromal cells depends on a nuclear factor-kappaB site in the proximal promoter. J Clin Endocrinol Metab 2001;86:4759–64.
[29] Halme J, Becker S, Hammond MG, Raj MH, Raj S. Increased activation of pelvic macrophages in infertile women with mild endometriosis. Am J Obstet Gynecol 1983;145:333–7.
[30] Bacci M, Capobianco A, Monno A, Cottone L, Di Puppo F, Camisa B, et al. Macrophages are alternatively activated in patients with endometriosis and required for growth and vascularization of lesions in a mouse model of disease. Am J Pathol 2009;175:547–56.
[31] Reis FM, Petraglia F, Taylor RN. Endometriosis: hormone regulation and clinical consequences of chemotaxis and apoptosis. Hum Reprod Update 2013;19:406–18.
[32] Akoum A, Kong J, Metz C, Beaumont MC. Spontaneous and stimulated secretion of monocyte chemotactic protein-1 and macrophage migration inhibitory factor by peritoneal macrophages in women with and without endometriosis. Fertil Steril 2002;77:989–94.
[33] Akoum A, Jolicoeur C, Boucher A. Estradiol amplifies interleukin-1-induced monocyte chemotactic protein-1 expression by ectopic endometrial cells of women with. Endometriosis 2000;85:896–904.
[34] Gou Y, Li X, Li P, Zhang H, Xu T, Wang H, et al. Estrogen receptor β upregulates CCL2 via NF-κB signaling in endometriotic stromal cells and recruits macrophages to promote the pathogenesis of endometriosis. Human Reprod 2019;34:646–58.
[35] Nishida M, Nasu K, Narahara H. Role of chemokines in the pathogenesis of endometriosis. Front Biosci (Schol Ed) 2011;3:1196–204.
[36] Du R, Lu KV, Petritsch C, Liu P, Ganss R, Passegue E, et al. HIF1alpha induces the recruitment of bone marrow-derived vascular modulatory cells to regulate tumor angiogenesis and invasion. Cancer Cell 2008;13:206–20.
[37] Gordon S, Pluddemann A, Martinez Estrada F. Macrophage heterogeneity in tissues: phenotypic diversity and functions. Immunol Rev 2014;262:36–55.
[38] Ruffell B, Coussens LM. Macrophages and therapeutic resistance in cancer. Cancer Cell 2015;27:462–72.
[39] Pyonteck SM, Akkari L, Schuhmacher AJ, Bowman RL, Sevenich L, Quail DF, et al. CSF-1R inhibition alters macrophage polarization and blocks glioma progression. Nat Med 2013;19:1264–72.
[40] Chan RWS, Lee CL, Ng EHY, Yeung WSB. Co-culture with macrophages enhances the clonogenic and invasion activity of endometriotic stromal cells. Cell Prolif 2017;50.
[41] Itoh F, Komohara Y, Takaishi K, Honda R, Tashiro H, Kyo S, et al. Possible involvement of signal transducer and activator of transcription-3 in cell-cell interactions of peritoneal macrophages and endometrial stromal cells in human endometriosis. Fertil Steril 2013;99:1705–13.
[42] Takebayashi A, Kimura F, Kishi Y, Ishida M, Takahashi A, Yamanaka A, et al. Subpopulations of macrophages within eutopic endometrium of endometriosis patients. Am J Reprod Immunol 2015;73:221–31.
[43] Ghosn EEB, Cassado AA, Govoni GR, Fukuhara T, Yang Y, Monack DM, et al. Two physically, functionally, and developmentally distinct peritoneal macrophage subsets. Proc Natl Acad Sci USA n.d.;107:2568–2573.
[44] Yuan M, Li D, An M, Li Q, Zhang L, Wang G. Rediscovering peritoneal macrophages in a murine endometriosis model. Hum Reprod 2017;32:94–102.
[45] Lessey BA, Lebovic DI, Taylor RN. Eutopic endometrium in women with endometriosis: ground zero for the study of implantation defects. Semin Reprod Med 2013;31:109–24.
[46] Chuang PC, Lin YJ, Wu MH, Wing LY, Shoji Y, Tsai SJ. Inhibition of CD36-dependent phagocytosis by prostaglandin E2 contributes to the development of endometriosis. Am J Pathol 2010;176:850–60.

[47] Wu J, Xie H, Yao S, Liang Y. Macrophage and nerve interaction in endometriosis. J Neuroinflammation 2017;14:53.
[48] Greaves E, Cousins FL, Murray A, Esnal-Zufiaurre A, Fassbender A, Horne AW, et al. A novel mouse model of endometriosis mimics human phenotype and reveals insights into the inflammatory contribution of shed endometrium. Am J Pathol 2014;184:1930–9.
[49] Zhou WJ, Hou XX, Wang XQ, Li DJ. The CCL17-CCR4 axis between endometrial stromal cells and macrophages contributes to the high levels of IL-6 in ectopic milieu. Am J Reprod Immunol 2017:78.
[50] Sikora J, Smycz-Kubanska M, Mielczarek-Palacz A, Kondera-Anasz Z. Abnormal peritoneal regulation of chemokine activation-the role of IL-8 in pathogenesis of endometriosis. Am J Reprod Immunol 2017:77.
[51] Zhang Z, Li H, Zhao Z, Gao B, Meng L, Feng X. miR-146b level and variants is associated with endometriosis related macrophages phenotype and plays a pivotal role in the endometriotic pain symptom. Taiwan J Obstet Gynecol 2019;58:401–8.
[52] Haber E, Danenberg HD, Koroukhov N, Ron-El R, Golomb G, Schachter M. Peritoneal macrophage depletion by liposomal bisphosphonate attenuates endometriosis in the rat model. Hum Reprod 2009;24:398–407.
[53] Mei J, Chang KK, Sun HX. Immunosuppressive macrophages induced by Ido1 promote the growth of endometrial stromal cells in endometriosis. Mol Med Rep 2017;15:2255–60.
[54] Kwon MJ, Shin HY, Cui Y, Kim H, Thi AH, Choi JY, et al. CCL2 mediates neuron-macrophage interactions to drive proregenerative macrophage activation following preconditioning injury. J Neurosci Off J Soc Neurosci 2015;35:15934–47.
[55] Sun H, Li D, Yuan M, Li Q, Zhen Q, Li N, et al. Macrophages alternatively activated by endometriosis-exosomes contribute to the development of lesions in mice. Mol Hum Reprod 2019;25:5–16.
[56] Zhao Y, Gong P, Chen Y, Nwachukwu JC, Srinivasan S, Ko C, et al. Dual suppression of estrogenic and inflammatory activities for targeting of endometriosis. Sci Transl Med 2015;7. 271ra9.
[57] An M, Li D, Yuan M, Li Q, Zhang L, Wang G. Interaction of macrophages and endometrial cells induces epithelial-mesenchymal transition-like processes in adenomyosis. Biol Reprod 2017;96:46–57.
[58] Rakhila H, Girard K, Leboeuf M, Lemyre M, Akoum A. Macrophage migration inhibitory factor is involved in ectopic endometrial tissue growth and peritoneal-endometrial tissue interaction in vivo: a plausible link to endometriosis development. PLoS One 2014;9:e110434.
[59] Laschke MW, Menger MD. Basic mechanisms of vascularization in endometriosis and their clinical implications. Hum Reprod Update 2018;24(2):207–24.
[60] Goteri G, Lucarini G, Filosa A, Pierantoni A, Montik N, Biagini G, et al. Immunohistochemical analysis of vascular endothelial growth factor cellular expression in ovarian endometriomata. Fertil Steril 2004;81:1528–33.
[61] McLaren J, Prentice A, Charnock-Jones DS, Millican SA, Muller KH, Sharkey AM, et al. Vascular endothelial growth factor is produced by peritoneal fluid macrophages in endometriosis and is regulated by ovarian steroids. J Clin Invest 1996;98:482–9.
[62] Pollard JW. Macrophages define the invasive microenvironment in breast cancer. J Leukoc Biol 2008;84:623–30.
[63] Capobianco A, Monno A, Cottone L, Venneri MA, Biziato D, Di Puppo F, et al. Proangiogenic Tie2(+) macrophages infiltrate human and murine endometriotic lesions and dictate their growth in a mouse model of the disease. Am J Pathol 2011;179:2651–9.
[64] Lin YJ, Lai MD, Lei HY, Wing LY. Neutrophils and macrophages promote angiogenesis in the early stage of endometriosis in a mouse model. Endocrinology 2006;147:1278–86.
[65] Wu MH, Lin SC, Hsiao KY, Tsai SJ. Hypoxia-inhibited dual-specificity phosphatase-2 expression in endometriotic cells regulates cyclooxygenase-2 expression. J Pathol 2011;225:390–400.
[66] Lin SC, Wang CC, Wu MH, Yang SH, Li YH, Tsai SJ. Hypoxia-induced microRNA-20a expression increases ERK phosphorylation and angiogenic gene expression in endometriotic stromal cells. J Clin Endocrinol Metab 2012;97:E1515–23.
[67] Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, et al. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. Nat Med 2004;10:858–64.
[68] Pepe G, Locati M, Della Torre S, Mornata F, Cignarella A, Maggi A, et al. The estrogen-macrophage interplay in the homeostasis of the female reproductive tract. Hum Reprod Update 2018;24:652–72.
[69] Carreras E, Turner S, Frank MB, Knowlton N, Osban J, Centola M, et al. Estrogen receptor signaling promotes dendritic cell differentiation by increasing expression of the transcription factor IRF4. Blood 2010;115:238–46.

[70] Heublein S, Vrekoussis T, Kuhn C, Friese K, Makrigiannakis A, Mayr D, et al. Inducers of G-protein coupled estrogen receptor (GPER) in endometriosis: potential implications for macrophages and follicle maturation. J Reprod Immunol 2013;97:95–103.
[71] Capellino S, Montagna P, Villaggio B, Sulli A, Soldano S, Ferrero S, et al. Role of estrogens in inflammatory response: expression of estrogen receptors in peritoneal fluid macrophages from endometriosis. Ann N Y Acad Sci 2006;1069:263–7.
[72] Giudice LC, Kao LC. Endometriosis. Lancet 2004;364:1789–99.
[73] Mechsner S, Kaiser A, Kopf A, Gericke C, Ebert A, Bartley J. A pilot study to evaluate the clinical relevance of endometriosis-associated nerve fibers in peritoneal endometriotic lesions. Fertil Steril 2009;92:1856–61.
[74] Chen P, Piao X, Bonaldo P. Role of macrophages in Wallerian degeneration and axonal regeneration after peripheral nerve injury. Acta Neuropathologica 2015;130:605–18.
[75] Cetin C, Serdaroglu H, Tuzlali S. The importance of endometrial nerve fibers and macrophage cell count in the diagnosis of endometriosis. Iran J Reproductive Med 2013;11:405–14.
[76] Artico M, Bronzetti E, Felici LM, Alicino V, Ionta B, Bronzetti B, et al. Neurotrophins and their receptors in human lingual tonsil: an immunohistochemical analysis. Oncol Rep 2008;20:1201–6.
[77] Kao LC, Germeyer A, Tulac S, Lobo S, Yang JP, Taylor RN, et al. Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. Endocrinology 2003;144:2870–81.
[78] Dubovy P. Wallerian degeneration and peripheral nerve conditions for both axonal regeneration and neuropathic pain induction. Annal Anat Anatom Anzeiger Off Organ Anatom Gesellschaf 2011;193:267–75.
[79] Ferrante CJ, Leibovich SJ. Regulation of macrophage polarization and wound healing. Adv Wound Care 2012;1:10–6.
[80] Anaf V, Simon P, El Nakadi I, Fayt I, Buxant F, Simonart T, et al. Relationship between endometriotic foci and nerves in rectovaginal endometriotic nodules. Hum Reprod 2000;15:1744–50.
[81] Shaw TJ, Martin P. Wound repair at a glance. J Cell Sci 2009;122:3209–13.
[82] Brancato SK, Albina JE. Wound macrophages as key regulators of repair: origin, phenotype, and function. Am J Pathol 2011;178:19–25.
[83] Zhang Q, Duan J, Liu X, Guo SW. Platelets drive smooth muscle metaplasia and fibrogenesis in endometriosis through epithelial-mesenchymal transition and fibroblast-to-myofibroblast transdifferentiation. Mol Cell Endocrinol 2016;428:1–16. https://doi.org/10.1016/j.mce.2016.03.015. Epub 2016 Mar 15. PMID: 26992563.
[84] Zhang Q, Duan J, Olson M, Fazleabas A, Guo SW. Cellular changes consistent with epithelial-mesenchymal transition and fibroblast-to-myofibroblast transdifferentiation in the progression of experimental endometriosis in baboons. Reprod Sci 2016 (in press).
[85] Guo SW. Fibrogenesis resulting from cyclic bleeding: the Holy Grail of the natural history of ectopic endometrium. Hum Reprod 2018;33(3):353–6.
[86] Goren I, Allmann N, Yogev N, Schurmann C, Linke A, Holdener M, et al. A transgenic mouse model of inducible macrophage depletion: effects of diphtheria toxin-driven lysozyme M-specific cell lineage ablation on wound inflammatory, angiogenic, and contractive processes. Am J Pathol 2009;175:132–47.
[87] Mirza R, DiPietro LA, Koh TJ. Selective and specific macrophage ablation is detrimental to wound healing in mice. Am J Pathol 2009;175:2454–62.
[88] Wermuth PJ, Jimenez SA. The significance of macrophage polarization subtypes for animal models of tissue fibrosis and human fibrotic diseases. Clin Transl Med 2015;4:2.
[89] Hesketh M, Sahin KB, West ZE, Murray RZ. Macrophage phenotypes regulate scar formation and chronic wound healing. Int J Mol Sci 2017;18.
[90] Novak ML, Koh TJ. Macrophage phenotypes during tissue repair. J Leukoc Biol 2013;93:875–81.
[91] Huang X, Li Y, Fu M, Xin HB. Polarizing macrophages in vitro. Methods Mol Biol 2018;1784:119–26.
[92] Lucas T, Waisman A, Ranjan R, Roes J, Krieg T, Muller W, et al. Differential roles of macrophages in diverse phases of skin repair. J Immunol 2010;184:3964–77.
[93] Bonte H, Chapron C, Vieira M, Fauconnier A, Barakat H, Fritel X, et al. Histologic appearance of endometriosis infiltrating uterosacral ligaments in women with painful symptoms. J Am Assoc Gynecol Laparoscopists 2002;9:519–24.
[94] Guo SW, Ding D, Shen M, Liu X. Dating endometriotic ovarian cysts based on the content of cyst fluid and its potential clinical implications. Reprod Sci 2015;22:873–83.

[95] Itoga T, Matsumoto T, Takeuchi H, Yamasaki S, Sasahara N, Hoshi T, et al. Fibrosis and smooth muscle metaplasia in rectovaginal endometriosis. Pathol Int 2003;53:371–5.
[96] Matsuzaki S, Canis M, Darcha C, Dechelotte P, Pouly JL, Bruhat MA. Fibrogenesis in peritoneal endometriosis. A semi-quantitative analysis of type-I collagen. Gynecol Obstet Invest 1999;47:197–9.
[97] Cornillie FJ, Oosterlynck D, Lauweryns JM, Koninckx PR. Deeply infiltrating pelvic endometriosis: histology and clinical significance. Fertil Steril 1990;53:978–83.
[98] Khare VK, Martin DC, Eltorky M. A comparative study of ovarian and pelvic wall-infiltrating endometriosis. J Am Assoc Gynecol Laparosc 1996;3:235–9.
[99] Nisolle M, Donnez J. Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. Fertil Steril 1997;68:585–96.
[100] Gibbons MA, MacKinnon AC, Ramachandran P, Dhaliwal K, Duffin R, Phythian-Adams AT, et al. Ly6Chi monocytes direct alternatively activated profibrotic macrophage regulation of lung fibrosis. Am J Respir Crit Care Med 2011;184:569–81.
[101] Wynn TA, Barron L. Macrophages: master regulators of inflammation and fibrosis. Semin Liver Dis 2010;30:245–57.
[102] Wynn TA, Ramalingam TR. Mechanisms of fibrosis: therapeutic translation for fibrotic disease. Nat Med 2012;18:1028–40.
[103] Duan J, Liu X, Guo S-W. The M2a macrophage subset may be critically involved in fibrogenesis of endometriosis in mouse. Reprod Biomed Online 2018;37:254–68.
[104] Murray LA, Chen Q, Kramer MS, Hesson DP, Argentieri RL, Peng X, et al. TGF-beta driven lung fibrosis is macrophage dependent and blocked by Serum amyloid P. Int J Biochem Cell Biol 2011;43:154–62.
[105] Ueno M, Maeno T, Nomura M, Aoyagi-Ikeda K, Matsui H, Hara K, et al. Hypoxia-inducible factor-1alpha mediates TGF-beta-induced PAI-1 production in alveolar macrophages in pulmonary fibrosis. Am J Physiol Lung Cell Mol Physiol 2011;300:L740–52.
[106] Hsiao KY, Lin SC, Wu MH, Tsai SJ. Pathological functions of hypoxia in endometriosis. Front Biosci 2015;7:309–21.
[107] Pradere JP, Kluwe J, De Minicis S, Jiao JJ, Gwak GY, Dapito DH, et al. Hepatic macrophages but not dendritic cells contribute to liver fibrosis by promoting the survival of activated hepatic stellate cells in mice. Hepatology 2013;58:1461–73.
[108] Hsu CC, Yang BC, Wu MH, Huang KE. Enhanced interleukin-4 expression in patients with endometriosis. Fertil Steril 1997;67:1059–64.
[109] Gazvani MR, Bates MD, Vince GS, Christmas SE, Lewis-Jones DI, Kingsland CR. Peritoneal fluid concentrations of interleukin-4 in relation to the presence of endometriosis, its stage and the phase of the menstrual cycle. Acta Obstet Gynecol Scand 2001;80:361–3.
[110] Antsiferova YS, Sotnikova NY, Posiseeva LV, Shor AL. Changes in the T-helper cytokine profile and in lymphocyte activation at the systemic and local levels in women with endometriosis. Fertil Steril 2005;84:1705–11.
[111] OuYang Z, Hirota Y, Osuga Y, Hamasaki K, Hasegawa A, Tajima T, et al. Interleukin-4 stimulates proliferation of endometriotic stromal cells. Am J Pathol 2008;173:463–9.
[112] Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. J Pathol 2013;229:176–85.
[113] van Amerongen MJ, Harmsen MC, van Rooijen N, Petersen AH, van Luyn MJ. Macrophage depletion impairs wound healing and increases left ventricular remodeling after myocardial injury in mice. Am J Pathol 2007;170:818–29.
[114] Mirza R, Koh TJ. Dysregulation of monocyte/macrophage phenotype in wounds of diabetic mice. Cytokine 2011;56:256–64.
[115] Wood S, Jayaraman V, Huelsmann EJ, Bonish B, Burgad D, Sivaramakrishnan G, et al. Pro-inflammatory chemokine CCL2 (MCP-1) promotes healing in diabetic wounds by restoring the macrophage response. PLoS One 2014;9:e91574.
[116] Maruyama K, Asai J, Ii M, Thorne T, Losordo DW, D'Amore PA. Decreased macrophage number and activation lead to reduced lymphatic vessel formation and contribute to impaired diabetic wound healing. Am J Pathol 2007;170:1178–91.
[117] Nassiri S, Zakeri I, Weingarten MS, Spiller KL. Relative expression of proinflammatory and antiinflammatory genes reveals differences between healing and nonhealing human chronic diabetic foot ulcers. J Invest Dermatol 2015;135:1700–3.

[118] Mirza RE, Fang MM, Weinheimer-Haus EM, Ennis WJ, Koh TJ. Sustained inflammasome activity in macrophages impairs wound healing in type 2 diabetic humans and mice. Diabetes 2014;63:1103–14.
[119] Sindrilaru A, Peters T, Wieschalka S, Baican C, Baican A, Peter H, et al. An unrestrained proinflammatory M1 macrophage population induced by iron impairs wound healing in humans and mice. J Clin Invest 2011;121:985–97.
[120] Furukawa S, Moriyama M, Tanaka A, Maehara T, Tsuboi H, Iizuka M, et al. Preferential M2 macrophages contribute to fibrosis in IgG4-related dacryoadenitis and sialoadenitis, so-called Mikulicz's disease. Clin Immunol 2015;156:9–18.
[121] Guo SW, Groothuis PG. Is it time for a paradigm shift in drug research and development in endometriosis/adenomyosis? Hum Reprod Update 2018;24:577–98.
[122] Rolla E. Endometriosis: advances and controversies in classification, pathogenesis, diagnosis, and treatment. F1000Research 2019;8.
[123] Garcia-Velasco JA, Somigliana E. Management of endometriomas in women requiring IVF: to touch or not to touch. Hum Reprod 2009;24:496–501.
[124] de Ziegler D, Borghese B, Chapron C. Endometriosis and infertility: pathophysiology and management. Lancet 2010;376:730–8.
[125] Coccia ME, Rizzello F, Mariani G, Bulletti C, Palagiano A, Scarselli G. Ovarian surgery for bilateral endometriomas influences age at menopause. Hum Reprod 2011;26:3000–7.
[126] Vercellini P, Somigliana E, Daguati R, Barbara G, Abbiati A, Fedele L. The second time around: reproductive performance after repetitive versus primary surgery for endometriosis. Fertil Steril 2009;92:1253–5.
[127] Brown J, Farquhar C. An overview of treatments for endometriosis. J Am Med Assoc 2015;313:296–7.
[128] Gonzalez-Ramos R, Donnez J, Defrere S, Leclercq I, Squifflet J, Lousse JC, et al. Nuclear factor-kappa B is constitutively activated in peritoneal endometriosis. Mol Hum Reprod 2007;13:503–9.
[129] Sakamoto Y, Harada T, Horie S, Iba Y, Taniguchi F, Yoshida S, et al. Tumor necrosis factor-alpha-induced interleukin-8 (IL-8) expression in endometriotic stromal cells, probably through nuclear factor-kappa B activation: gonadotropin-releasing hormone agonist treatment reduced IL-8 expression. J Clin Endocrinol Metab 2003;88:730–5.
[130] Lousse JC, Van Langendonckt A, Gonzalez-Ramos R, Defrere S, Renkin E, Donnez J. Increased activation of nuclear factor-kappa B (NF-kappaB) in isolated peritoneal macrophages of patients with endometriosis. Fertil Steril 2008;90:217–20.
[131] Liu X, Yu S, Guo SW. A pilot study on the use of andrographolide to treat symptomatic adenomyosis. Gynecol and Min Inv Ther 2015;3:119–26.
[132] Li B, Chen M, Liu X, Guo SW. Constitutive and tumor necrosis factor-alpha-induced activation of nuclear factor-kappaB in adenomyosis and its inhibition by andrographolide. Fertil Steril 2013;100:568–77.
[133] Mao X, Wang Y, Carter AV, Zhen X, Guo SW. The retardation of myometrial infiltration, reduction of uterine contractility, and alleviation of generalized hyperalgesia in mice with induced adenomyosis by levo-tetrahydropalmatine (l-THP) and andrographolide. Reprod Sci 2011;18:1025–37.
[134] Guo SW, Ding D, Geng J-G, Wang L, Liu X. P-selectin as a potential therapeutic target for endometriosis. Fertil Steril n.d.;103:990–1000.e8.
[135] Keklikoglou I, De Palma M. Cancer: metastasis risk after anti-macrophage therapy. Nature 2014;515:46–7.
[136] Chen D, Xie J, Fiskesund R, Dong W, Liang X, Lv J, et al. Chloroquine modulates antitumor immune response by resetting tumor-associated macrophages toward M1 phenotype. Nat Commun 2018;9:873.
[137] Happle C, Lachmann N, Škuljec J, Wetzke M, Ackermann M, Brennig S, et al. Pulmonary transplantation of macrophage progenitors as effective and long-lasting therapy for hereditary pulmonary alveolar proteinosis. Sci Transl Med n.d.;6:250ra113-250ra113.
[138] Doerschuk CM. Pulmonary alveolar proteinosis and macrophage transplantation. N Engl J Med 2015;372:1762–4.

This page intentionally left blank

SECTION II

The role of immune factors in endometriosis-related conditions

This page intentionally left blank

CHAPTER

10

Pain

Fabio Barra[1,2], *Giulio Evangelisti*[1,2], *Carolina Scala*[3] *and Simone Ferrero*[1,2]

[1]Academic Unit of Obstetrics and Gynaecology, IRCCS Ospedale Policlinico San Martino, Genova, Italy; [2]Department of Neurosciences, Rehabilitation, Ophthalmology, Genetics, Maternal and Child Health (DiNOGMI), University of Genova, Genova, Italy; [3]Unit of Obstetrics and Gynecology, Gaslini Institute, Genova, Italy

Introduction

Endometriosis is an estrogen-dependent chronic inflammatory disease that affects at least 3.6% of women of reproductive age [1]. Pain is a central symptom of this benign chronic disease: in fact, patients may experience a variety of pain symptoms, such as dysmenorrhea, chronic pelvic pain, dyspareunia, and dyschezia. Overall, these symptoms have a negative impact on quality of life, working efficiency and social activities [2]. No direct correlation exists between the extent of endometriosis according to widely accepted classifications (such as that by American Society for Reproductive Medicine [ASRM]) and intensity of pain symptoms [3].

Multiple mechanisms can be involved in the pathogenesis of pain related to endometriosis: in particular, inflammation, nociception, and alterations in peripheral and central neurological pathways may have a critical role in this physio pathological process [4].

It is widely accepted that psychological, physical stress [5] and hormone status [6] may influence pain perception. In particular, chronic pelvic pain is often associated with negative cognitive, behavioral, emotional and sexual impact; these factors can potentially further exacerbate the pain experience [7]. Alterations in both the peripheral and central nervous systems have been reported in women with endometriosis; furthermore, abnormal direct innervation of unmyelinated nociceptive nerve fibers has been reported in endometriotic lesions [8].

Immunology of Endometriosis
https://doi.org/10.1016/B978-0-12-820661-4.00010-8

© 2022 Elsevier Inc. All rights reserved.

Pressure on deep endometriotic nodules during vaginal examination and sexual intercourses causes an exacerbation of pain. This phenomenon of heightened pain arising when a normally nonpainful stimulus is applied is called "hyperalgesia." It is characterized by a displacement to the left of the curve stimulus/response which induces an increase in the pain sensation associated with a lower threshold of pain perception and an increased sensitivity of nociceptors (primary hyperalgesia). In addition, the lower threshold of pain receptors is also observed in surrounding regions (secondary hyperalgesia). In fact, with continuous noxious input into the central nervous system, alterations in gene transcription and expression of neurotransmitters cause a hypersensitive state well beyond the area of the initial noxious input [9].

Overall, the precise mechanisms by which endometriotic lesions cause pain are poorly understood. However, inflammatory mediators and prostaglandins are deeply involved in the pathogenesis of endometriosis-related pain. Moreover, increasing evidence suggests a role of new sensory fibers innervating endometriotic lesions in maintaining the inflammatory phenotype through the release of proinflammatory mediators.

Hormonal and inflammatory microenvironment in endometriosis: a vicious circle

An aberrant hormonal proinflammatory environment is strictly involved in the pathogenesis of endometriotic implants and subsequently in pain related to the disease. Until now, different studies have shown possible mechanisms which may be implicated in the development and progression of endometriosis; however, the precise etiology of this benign chronic disease is still unclear [10,11].

Estrogens and progesterone are key hormones regulating the activity of the endometrium. Similarly to eutopic endometrium, endometriotic lesions are influenced by endocrine environment and subjected to cyclic modifications during the menstrual cycle [12]. In fact, ectopic and eutopic endometrial tissues have apparently similar histological changes in response to estrogens and progesterone [13] and endometriotic implants may cyclically bleed during menstrual period [14,15]. These observations are justified by the fact that both estrogen receptors (ERs) and progesterone receptors (PRs) are present in endometriotic implants [16,17].

It is largely known that estrogens have a pivotal role in the pathogenesis of endometriosis. In particular, estradiol produced in both the ectopic and eutopic endometrium influences the regulation of several immunological mechanisms [18,19]. In endometriotic implants, local production of estradiol can sustain the synthesis of prostaglandins by stimulating COX-2 activity; this constitutes a positive feedback loop augmenting estrogen formation and enhancing inflammation [20]. The synthesis of proinflammatory prostaglandins, such as COX-2-derived PGE_2, can be activated by proinflammatory transcriptional factor nuclear factor (NF)-kB [21]. In general, NF-κB is able to upregulate the expression of proinflammatory genes including cytokines, chemokines, translocating into the nucleus where it binds to specific sequences of DNA (known as response elements) [22].

The development of endometriosis may be favored by the aberrant synthesis of the aromatase enzyme. Whereas there is no detectable aromatase enzyme activity in normal

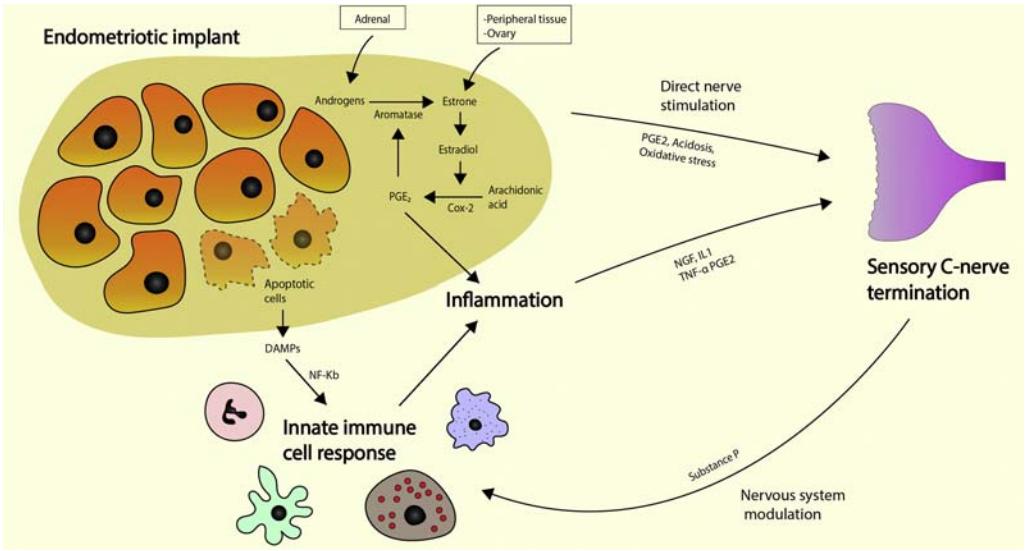


FIGURE 10.1 The bidirectional signaling mechanism constituting neuroinflammation in the pathogenesis of pain related to endometriosis. Under the action of estrogens, produced by aberrant aromatase activity, endometriotic implants can proliferate, promoting the expression and release of pro-inflammatory factors. Moreover, products released from tissue necrosis, such as DAMPs, can activate the innate immune cells through the NF-kB pro-inflammatory pathway. Mediators released during this process by activated mast cells, dendritic cells and macrophages, including cytokines (PGE2) and pro-inflammatory mediators (IL-1β and TNF-α) chemokines can exert a direct stimulation to sensory nerve ending, which generates the nociceptive signal. In response to stimulation, sensory nerves further increase and maintain inflammation by secreting proinflammatory products, such as substance P. NGF is upregulated by inflammatory cytokines and is involved in persistent inflammatory pain by activating further mast cell degranulation and cytokine production.

endometrium, endometriotic tissue has elevate expression of aromatase; its action leads to production of significant quantities of estrogens [23,24], which can further enhance synthesis of prostaglandins by activating COX-2 expression (Fig. 10.1) [20].

At least several growth and proangiogenic factors, such as vascular endothelial growth factor (VEGF), are regulated by estradiol and produced also in response to an inflammatory microenvironment; they promotes proliferation of endothelial cells [12], an essential aspect in the sprouting of blood vessels that vascularize nerve fibers.

Pathogenesis of inflammation in endometriotic implants

Retrograde menstruation of endometrial tissue and intermenstrual bleeding of endometriotic lesions may directly contribute to inflammation with subsequent activation of innate immune cells and mediators within implants of patient with endometriosis [25]. Supporting this concept, higher concentration of innate immune cells have been found in peritoneal fluid of women with endometriosis compared to healthy women; moreover, activated mast cells and macrophages can be detected within peritoneal, ovarian, and deep endometriotic implants

[26–28]. In particular, ERα and ERβ have significantly higher expression on macrophages of women with endometriosis than in controls. The ERα expression is positively correlated with the expression of inflammatory cytokines, such as tumor necrosis factor alpha (TNF-α), interleukin (IL)-1β, and IL-6, only in patients affected; on the other hand, ERβ expression is correlated to the expression of inflammatory cytokines in the both groups [29,30].

Several cellular products released during cell necrosis can induce sterile inflammation by the activation of innate immune cells [31,32]; for this reason, it has been hypothesized that degenerating and necrotic tissue originating from extrauterine menstruation may directly release biological products into the peritoneal cavity, thus leading to a diffuse intraperitoneal sterile inflammation [33].

Damage-associated molecular patterns (DAMPs) are endogenous danger molecules released from damaged or dying cells, activating innate immune system by interacting with pattern recognition receptors (PRRs). The nuclear protein high-mobility-group box 1 (HMGB1), heat shock protein 70 (HSP70), S100, nucleic acids, and soluble extracellular matrix components are only some of the most known DAMPs [34]. Several studies have described the implication of DAMPs in the establishment of the inflammatory microenvironment of endometriotic implants: HMGB1 and HSP70 have been found expressed in endometriotic lesions and present in serum at elevate concentration, respectively, in patients with endometriosis [35,36]. Also subtypes of the S100 family of calcium-modulated proteins (S100A13) have been found to be abnormally expressed in endometriotic implants and in the peritoneal fluid of women affected [37,38]. At least, the presence of extracellular DAMPs, such as fibrinogen, fibronectin, or hyaluronic acid, has been described in endometriotic implants [39].

Overall, the proinflammatory NF-κB pathway is activated in macrophages and mast cells, through the predominant action of the DAMPs on PRRs, which includes, among others, toll-like receptors (TLRs). These are single-pass membrane-spanning proteins usually expressed on immune sentinel cells such as macrophages and dendritic cells, recognizing structurally conserved molecules derived from microbes [40]. Even in endometrium, several TLRs are expressed by immune, epithelial, and stromal cells [41]. In endometriosis, TLR2 and TLR9 are overexpressed in the peritoneal fluid, and TLR4 is upregulated on macrophages and dendritic cells in endometriotic lesions, especially during the secretory phase [42–44]. Interestingly, high concentrations of *Escherichia coli*-derived endotoxin, which is recognized by TLR4, have been found in peritoneal fluid and menstrual blood of patients affected in comparison to healthy women [45]. This suggests that also pathogen-associated molecular patterns (PAMPs) may contribute to generation of inflammatory process in endometriosis.

Iron and oxidative stress also derive from tissue degeneration and cellular injury and for this reason they can be implicated in inflammation within endometriotic tissue. Reactive oxygen species (ROS) produced subsequently to the iron overload enhance activity of mast cells and macrophages through NF-κB action [46]. As proof of this, elevate concentrations of iron have been detected in peritoneal fluid of women affected [47].

The activation of macrophages and mast cells is responsible of stimulating cytokines and chemokines production by lymphocytes. In women with endometriosis, there is substantial evidence of aberrant function of almost all types of acquired immune cells, among which decreased Th1 and Th2 lymphocytes reactivity and polyclonal B cells activation [48,49].

Overall, the secretion of pronociceptive and proinflammatory mediators, which contribute to the generation of nociceptive signals and the feed-forward loop, maintaining and strengthening the inflammation in endometriotic implants [50].

Neurogenesis in endometriotic implants

Neurogenesis is related to both inflammatory response and angiogenesis and, along with an imbalance in sensory and sympathetic innervation, contributes to the growth of nerve fibers, subsequent peripheral neuroinflammation, and generation of chronic pain [12]. The activation of sensory nerve fibers generates nociceptive signals to the central nervous system; this is a critical step in the pathophysiology of pain occurring in endometriosis [50].

Sensory nerve endings are present in endometriotic lesions. Moreover, a positive correlation between the amount of nerve fibers and pain symptoms has been described in the different phenotypes of endometriosis [46]. Persistent nociceptive input originating from endometriotic implants is supposed to cause central sensitization due to the higher responsiveness of spinal cord dorsal horn neurons processing input from the lesions and adjacent viscera [51]. Peripheral sensitization promotes central sensitization, and this is subsequently maintained by persistent input to the central nervous system from sensitized sensory afferent fibers [52].

A greater presence of multiple, small unmyelinated nerve fibers has been reported in peritoneal endometriotic lesions of women with confirmed endometriosis compared with the peritoneum of women without endometriosis. Similarly, there is higher density of nerve fibers in ovarian endometriomas compared with normal ovaries of women with ovarian endometriomas and normal ovaries of healthy women. Deep endometriotic lesions have not only higher fibromuscular content [53], but also higher nerve fiber density than peritoneal and ovarian lesions [54,55]. This phenotype of endometriosis tends to develop in richly innervated anatomical sites, such as uterosacral ligaments or bowel (Fig. 10.2A and B) [8]. These reasons may explain why deep endometriosis is considered the most severe form of endometriosis, causing severe pain in the majority of patients [56]. Whereas a direct correlation between a higher density of nerve fibers in endometriotic lesions and the pain perceived has been reported for peritoneal lesions, this evidence is more controversial for deep rectovaginal nodules [57].

Some authors postulated that a qualitative imbalance of nerve fibers, consisting in an increase of sensory nerve fibers and a concomitant loss of sympathetic nerve, may contribute to the genesis of pain in endometriotic implants. This autonomic dysregulation has been previously reported in peritoneal and deep endometriosis [58,59].

Immune system and neuroangiogenesis: a bidirectional signaling mechanism

One of the consequences of immune cell activation in the endometriosis microenvironment is the production of cytokines, growth factors, and eicosanoids that can simultaneously stimulate lesion innervation and neovascularization through a coordinated mechanism known as neuroangiogenesis [12].

Patients affected by endometriosis have an elevate number of inflammatory cells in proximity to nerve fibers; interestingly, the presence of inflammatory cells near the new peri-endometriotic nerves seems to be more pronounced in women with more severe symptoms [57,60].

Activated mast cells and macrophages can release numerous pronociceptive mediators. Cytokines, chemokines, complement components, hydrolytic enzymes, ROS, and growth

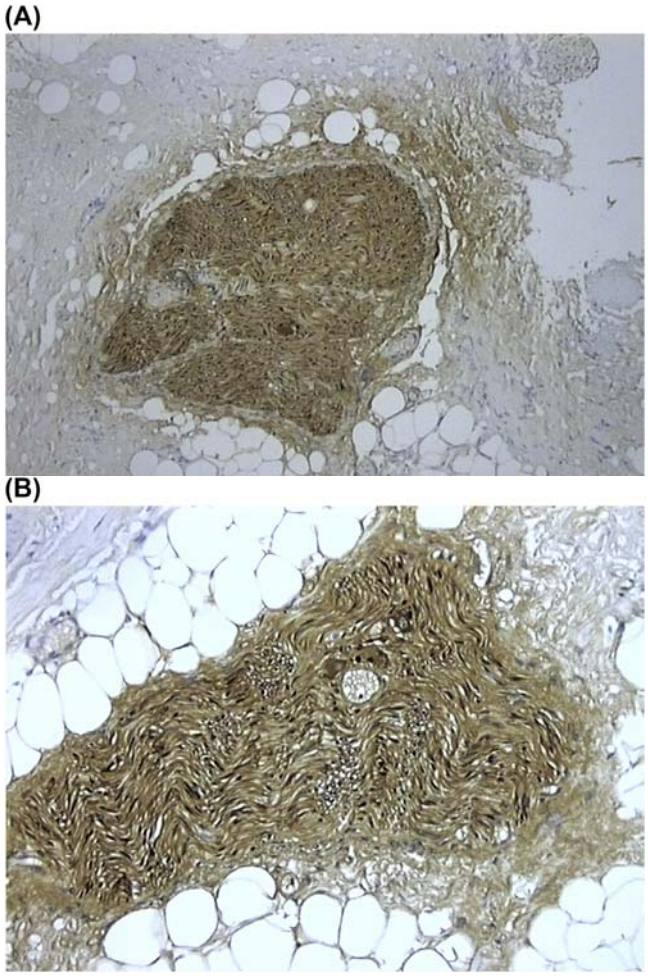


FIGURE 10.2 (A and B) Immunohistochemical staining of ganglionic and nervous cells within a rectal endometriotic nodule. The purified immunoglobulin fraction of rabbit polyclonal anti-serum for cow S-100 protein (Dako, Denmark) is used.

factors, which have higher concentrations in the peritoneal fluid or endometriotic lesions of patients affected [61], are able to directly activate sensory nerve ending [8]. In fact, a pronociceptive effect through the sensitization of sensory nerve fibers by PGE_2, having elevate concentrations in peritoneal fluid of women with endometriosis [62,63], has been largely demonstrated [51].

It is well known that cell necrosis and tissue degeneration can lead to acidification of extracellular microenvironment [52]. This pH decrease is directly detected by several receptors present on sensory nerve fibers, such as transient receptor potential cation channel subfamily V member 1 (TRPV1) [64]. Notably, production of ROS can directly exert a pronociceptive effect by sensitizing sensory nerve fibers through TRPV1 action [65,66]. In

a rat model of endometriosis, sympathetic and sensory C and Aδ fibers expressing TRPV1 protein largely innervated endometriotic lesions. It is presumable that these fibers may contribute to the formation of a proinflammatory microenvironment of dorsal root ganglion neurons from L1-S1, which specifically innervate the pelvis and pelvic organs and, probably, are responsible for pelvic floor hyperalgesia [67]. Furthermore, an increased numbers of TRPV1-positive nerve fibers have been found in endometriotic lesions of women with chronic pelvic pain compared to endometriotic lesions of women without chronic pelvic pain [68].

Nerve fibers themselves also have an active role in the mechanism of inflammatory pain by secreting proinflammatory neuromediators (Fig. 10.1) [69]. In fact, the peritoneal fluid of patients with endometriosis contains high concentrations of nerve growth factor (NGF) and other neuromediators, such as brain-derived neurotrophic factor (BDNF) or neurotrophin (NT) −4 and −5 [70]. Notably, it has been reported that, in an abnormal proinflammatory microenvironment, macrophages can directly stimulate the synthesis of these neurotrophins [71].

NGF is one of most important neurotrophic factor and neuropeptide involved in the regulation of neuronal growth, proliferation, and survival [72]. Produced by innate immune cells under the stimulus of IL-1 and TNF-α [73], this neuropeptide may promote a positive proinflammatory feedback stimulating macrophage chemotaxis, activation of mast cells [74,75] and release of other neuroactive cytokines or inflammatory mediators [76]. Some authors found an elevated immune-intensity of NGF in endometriotic stroma and epithelium from women suffering deep dyspareunia compared to women without deep dyspareunia [77].

Angiogenic and neurogenic growth factors, VEGF and NGF, respectively, are largely coexpressed in both eutopic endometrium and ectopic endometrial tissue of women affected by endometriosis [8]. These molecules can stimulate neuronal migration, axonal growth and blood vessels growth; in fact, the filopodia of both endothelial cells and axons express several receptors, accounting for the different roles of several biological factors in nerve and blood vessel remodeling [8].

Additionally, the presence of substance P has been described within sensory nerve fibers of peritoneal and deep endometriosis lesions [78,79] (Fig. 10.3). This neuropeptide acts as a neurotransmitter and neuromodulator, being able to induce vasodilatation dependent on nitric oxide release and being involved in the axon reflex-mediated vasodilatation to local heating as well as wheal and flare reaction. In endometriotic implants, substance P can enhance activity of mast cells and macrophages, inducing the release of IL-8, IL-6 and TNF-α [57]. Moreover, higher substance P and NGF expressions were found in the superficial dorsal horn of the spinal cord in rats with deep sciatic endometriosis [80]. An aberrant expression of the neurokinin 1 receptor (NK1R) of substance P has been also reported in endometriotic lesions [81]. Notably, the NK1R polymorphism rs811 seems to be associated with a lower risk of disease recurrence; thus, supporting the pathogenic role of neurogenic inflammation in this benign chronic condition [82].

In summary, neurogenic inflammation has a critical role in pain related to endometriosis. Proinflammatory mediators and neuropeptides released by endometriotic cells and sensory nerve fibers, respectively, may constitute a bidirectional signaling mechanism, which activates the nociceptive system response as well as further maintains and increases the inflammatory microenvironment in ectopic implants.

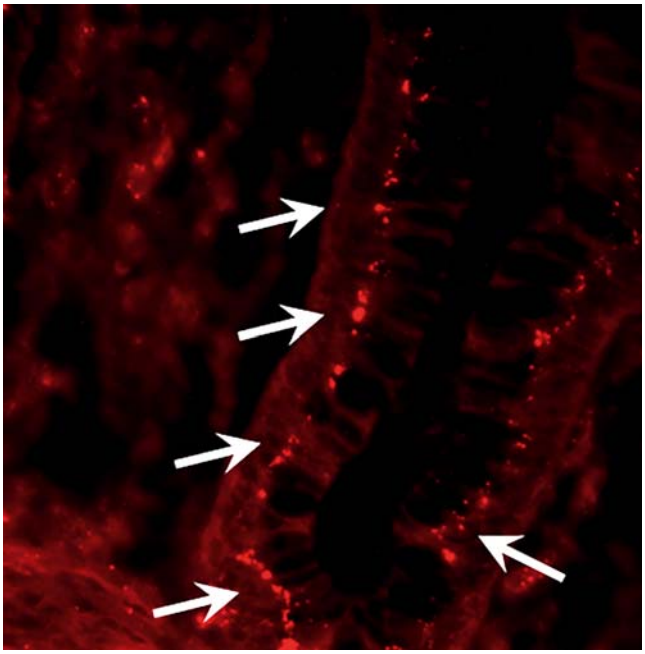


FIGURE 10.3 Immunofluorescence staining of a rectosigmoid endometriotic nodule showing the presence of sensory nerve fibers expressing the neuropeptide substance P. A goat polyclonal antibody against substance P is used (Santa Cruz Biotechnology, Santa Cruz, CA, USA).

Overall, persistent nociceptive input from endometriotic implants may cause central sensitization due to the higher responsiveness of spinal cord dorsal horn neurons processing input from the implants and adjacent viscera. Peripheral sensitization promotes central sensitization, and this can be maintained by persistent input to the central nervous system from sensitized sensory afferent nerve fibers [83]. Thus, in women with a long-standing history of this disease, central neural processing can be altered; therefore, initially peripherally induced central sensitization becomes independent of peripheral sensitization. Clinical manifestations of central sensitization consist in regional allodynia and hyperalgesia, which nevertheless tend to have modest clinical response to current medical options [83,84].

The role of drug therapy in immunomodulation of pain

COX-2 is an essential therapeutic target for antiinflammatory drugs, such as nonsteroidal antiinflammatory drugs (NSAIDs), routinely employed for treating pain related to endometriosis.

The current hormonal medical options for endometriosis comprises combined oral contraceptives, progestins, or short-term treatment with gonadotropin-releasing hormone (GnRH) agonists or antagonists [85–87]. Aromatase inhibitors, such as anastrozole and letrozole, which selectively inhibit the action of the aromatase enzyme, have been investigated as single

regimen or combined with other hormonal drugs. However, their administration is considered off-label in women with endometriosis-related symptoms resistant to other therapies and it should be considered only in the setting of scientific research [88].

All the routinely employed hormonal treatments for endometriosis interfere indirectly with inflammatory pain by suppressing the cyclical impact of steroid hormones on the lesions. Nevertheless, hormonal therapies may have both antinociceptive and immunomodulatory proprieties [89,90]; in fact, a substantially reduction of nerve fiber density in eutopic endometrium and myometrium in women with endometriosis and in peritoneal endometriotic implants has been reported after the use of hormonal drugs [91]. As they can upregulate NGF as well as modulate receptors of neurotrophins [92], estrogens may have a pivotal role in immune system and nerve fiber modulation, in particular, acting on sympathetic nerve sprouting.

Overall, the development, maintenance and progression of endometriotic lesions depend on a variety of altered mechanisms including cell proliferation, immune function, angiogenesis and apoptosis. The growing understanding of the mechanisms that are responsible for the development of pain related to endometriosis is allowing the investigation of new medical opportunities.

Inflammation occurring in endometriosis have been recently targeted by innovative approaches [93]. TNFRSF1A and c5N, two human recombinant TNF-α antagonists, demonstrated an inhibitory action against the growth of endometriotic implants in baboons [94,95]. Etanercept, a dimeric fusion protein consisting of the extracellular ligand-binding portion of TNF receptor linked to the Fc portion of human IgG1, given to rats with endometriotic implants reduced the volume and histopathologic scores and serum levels of VEGF, IL-6, and TNF-α [96–98]. Infliximab, a monoclonal antibody directed against TNF-α, was efficacious in decreasing the size of endometriotic lesions and the plasma levels of nitric oxide in rat model of endometriosis [99]. These results paved the way to a randomized controlled trial including women suffering severe pain caused by rectovaginal endometriosis. Contrary to expectations, infliximab was not able to improve pain related to endometriosis [100]. Several inhibitors of NF-kB, such as urinary preparation human chorionic gonadotropin A, BAY 11−7085 and IkB protease inhibitor (TPCK) have been tested in vitro and on animal models of endometriosis. All these studies showed a reduction of the expression of genes regulating the production of inflammatory mediators, extracellular matrix metalloproteinases and angiogenetic factors [101–103].

A preliminary clinical trial has shown that the administration of palmitoylethanolamide and polydatin, aiming to inhibit degranulation of mast cells, is able to control pain in women affected by deep endometriosis [104]. In a preclinical study on rat model of surgically induced endometriosis, a siRNA blocking β-NGF caused a higher decrease of the spherical volumes of implants in comparison with the control group; the concentrations of NGF-β in the sera and supernatants obtained by peritoneal fluid decreased more in the treatment group compared to control [105].

As reviewed earlier, new innovative targets for the therapy of endometriosis have been identified; however, the majority of new compounds have only been investigated in in vitro/animal studies; in the near future novel experiments should elucidate their efficacy and safety before translating their investigation in humans.

References

[1] Ferrero S, Arena E, Morando A, Remorgida V. Prevalence of newly diagnosed endometriosis in women attending the general practitioner. Int J Gynecol Obstet 2010;110(3):203–7.
[2] La Rosa VL, De Franciscis P, Barra F, Schiattarella A, Torok P, Shah M, et al. Quality of life in women with endometriosis: a narrative overview. Minerva Med 2019;111(1):68–78.
[3] Andres MP, Borrelli GM, Abrao MS. Endometriosis classification according to pain symptoms: can the ASRM classification be improved? Best Pract Res Clin Obstet Gynaecol 2018;51:111–8.
[4] Morotti M, Vincent K, Becker CM. Mechanisms of pain in endometriosis. Eur J Obstet Gynecol Reprod Biol 2017;209:8–13.
[5] Fillingim RB. Individual differences in pain: understanding the mosaic that makes pain personal. Pain 2017;158(Suppl. 1):S11–8.
[6] Maurer AJ, Lissounov A, Knezevic I, Candido KD, Knezevic NN. Pain and sex hormones: a review of current understanding. Pain Manag 2016;6(3):285–96.
[7] Daniels JP, Khan KS. Chronic pelvic pain in women. BMJ 2010;341:c4834.
[8] Morotti M, Vincent K, Brawn J, Zondervan KT, Becker CM. Peripheral changes in endometriosis-associated pain. Hum Reprod Update 2014;20(5):717–36.
[9] Anaf V, Simon P, El Nakadi I, Fayt I, Simonart T, Buxant F, et al. Hyperalgesia, nerve infiltration and nerve growth factor expression in deep adenomyotic nodules, peritoneal and ovarian endometriosis. Hum Reprod 2002;17(7):1895–900.
[10] Augoulea A, Alexandrou A, Creatsa M, Vrachnis N, Lambrinoudaki I. Pathogenesis of endometriosis: the role of genetics, inflammation and oxidative stress. Arch Gynecol Obstet 2012;286(1):99–103.
[11] Lagana AS, Garzon S, Gotte M, Vigano P, Franchi M, Ghezzi F, et al. The pathogenesis of endometriosis: molecular and cell biology insights. Int J Mol Sci 2019;20(22).
[12] Garcia-Gomez E, Vazquez-Martinez ER, Reyes-Mayoral C, Cruz-Orozco OP, Camacho-Arroyo I, Cerbon M. Regulation of inflammation pathways and inflammasome by sex steroid hormones in endometriosis. Front Endocrinol 2019;10:935.
[13] Parente Barbosa C, Bentes De Souza AM, Bianco B, Christofolini DM. The effect of hormones on endometriosis development. Minerva Ginecol 2011;63(4):375–86.
[14] Burney RO, Lathi RB. Menstrual bleeding from an endometriotic lesion. Fertil Steril 2009;91(5):1926–7.
[15] Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. Nat Rev Endocrinol 2014;10(5):261–75.
[16] Noel JC, Chapron C, Bucella D, Buxant F, Peny MO, Fayt I, et al. Estrogen and progesterone receptors in smooth muscle component of deep infiltrating endometriosis. Fertil Steril 2010;93(6):1774–7.
[17] Pellegrini C, Gori I, Achtari C, Hornung D, Chardonnens E, Wunder D, et al. The expression of estrogen receptors as well as GREB1, c-MYC, and cyclin D1, estrogen-regulated genes implicated in proliferation, is increased in peritoneal endometriosis. Fertil Steril 2012;98(5):1200–8.
[18] Bulun SE, Monsavais D, Pavone ME, Dyson M, Xue Q, Attar E, et al. Role of estrogen receptor-beta in endometriosis. Semin Reprod Med 2012;30(1):39–45.
[19] Barra F, Romano A, Grandi G, Facchinetti F, Ferrero S. Future directions in endometriosis treatment: discovery and development of novel inhibitors of estrogen biosynthesis. Expert Opin Invest Drugs 2019;28(6):501–4.
[20] Lai ZZ, Yang HL, Ha SY, Chang KK, Mei J, Zhou WJ, et al. Cyclooxygenase-2 in endometriosis. Int J Biol Sci 2019;15(13):2783–97.
[21] Maia Jr H, Haddad C, Coelho G, Casoy J. Role of inflammation and aromatase expression in the eutopic endometrium and its relationship with the development of endometriosis. Womens Health 2012;8(6):647–58.
[22] Hayden MS, Ghosh S. NF-kappaB in immunobiology. Cell Res 2011;21(2):223–44.
[23] Maia Jr H, Casoy J, Valente Filho J. Is aromatase expression in the endometrium the cause of endometriosis and related infertility? Gynecol Endocrinol 2009;25(4):253–7.
[24] Ferrero S, Remorgida V, Maganza C, Venturini PL, Salvatore S, Papaleo E, et al. Aromatase and endometriosis: estrogens play a role. Annals of the New York Academy of Sciences2014. p. 17-23.
[25] Ferrero S, Vellone VG, Barra F. Pathophysiology of pain in patients with peritoneal endometriosis. Ann Transl Med 2019;7(Suppl. 1):S8.
[26] Anaf V, Chapron C, El Nakadi I, De Moor V, Simonart T, Noël JC. Pain, mast cells, and nerves in peritoneal, ovarian, and deep infiltrating endometriosis. Fertil Steril 2006;86(5):1336–43.

[27] Bedaiwy MA, Falcone T. Peritoneal fluid environment in endometriosis: clinicopathological implications. Minerva Ginecol 2003;55(4):333–45.
[28] Weinberg JB, Haney AF, Xu FJ, Ramakrishnan S. Peritoneal fluid and plasma levels of human macrophage colony-stimulating factor in relation to peritoneal fluid macrophage content. Blood 1991;78(2):513–6.
[29] Montagna P, Capellino S, Villaggio B, Remorgida V, Ragni N, Cutolo M, et al. Peritoneal fluid macrophages in endometriosis: correlation between the expression of estrogen receptors and inflammation. Fertil Steril 2008;90(1):156–64.
[30] Capellino S, Montagna P, Villaggio B, Sulli A, Soldano S, Ferrero S, et al. Role of estrogens in inflammatory response: expression of estrogen receptors in peritoneal fluid macrophages from endometriosis. Ann N Y Acad Sci 2006;1069:263–7.
[31] Kono H, Onda A, Yanagida T. Molecular determinants of sterile inflammation. Curr Opin Immunol 2014;26(1):147–56.
[32] Shen H, Kreisel D, Goldstein DR. Processes of sterile inflammation. J Immunol 2013;191(6):2857–63.
[33] Kajihara H, Yamada Y, Kanayama S, Furukawa N, Noguchi T, Haruta S, et al. New insights into the pathophysiology of endometriosis: from chronic inflammation to danger signal. Gynecol Endocrinol 2011;27(2):73–9.
[34] Patel S. Danger-associated molecular patterns (DAMPs): the derivatives and triggers of inflammation. Curr Allergy Asthma Rep 2018;18(11):63.
[35] Shimizu K, Kamada Y, Sakamoto A, Matsuda M, Nakatsuka M, Hiramatsu Y. High expression of high-mobility group box 1 in menstrual blood: implications for endometriosis. Reprod Sci 2017;24(11):1532–7.
[36] Chehna-Patel N, Warty N, Sachdeva G, Khole V. Proteolytic tailoring of the heat shock protein 70 and its implications in the pathogenesis of endometriosis. Fertil Steril 2011;95(5):1560–7. e3.
[37] Hayrabedyan S, Kyurkchiev S, Kehayov I. Endoglin (cd105) and S100A13 as markers of active angiogenesis in endometriosis. Reprod Biol 2005;5(1):51–67.
[38] Ferrero S, Gillott DJ, Remorgida V, Anserini P, Leung KY, Ragni N, et al. Proteomic analysis of peritoneal fluid in women with endometriosis. J Proteome Res 2007;6(9):3402–11.
[39] Gilabert-Estelles J, Castello R, Gilabert J, Ramon LA, Espana F, Romeu A, et al. Plasminogen activators and plasminogen activator inhibitors in endometriosis. Front Biosci 2005;10:1162–76.
[40] Fitzgerald KA, Kagan JC. Toll-like receptors and the control of immunity. Cell 2020;180(6):1044–66.
[41] Khan KN, Kitajima M, Fujishita A, Nakashima M, Masuzaki H. Toll-like receptor system and endometriosis. J Obstet Gynaecol Res 2013;39(8):1281–92.
[42] Yeo SG, Won YS, Lee HY, Kim YI, Lee JW, Park DC. Increased expression of pattern recognition receptors and nitric oxide synthase in patients with endometriosis. Int J Med Sci 2013;10(9):1199–208.
[43] Hayashi C, Chishima F, Sugitani M, Ichikawa G, Nakazawa-Watanabe T, Sugita K, et al. Relationship between toll-like receptor-4 and mPGES-1 gene expression in local lesions of endometriosis patients. Am J Reprod Immunol 2013;69(3):231–9.
[44] Allhorn S, Böing C, Koch AA, Kimmig R, Gashaw I. TLR3 and TLR4 expression in healthy and diseased human endometrium. Reprod Biol Endocrinol 2008;6.
[45] Khan KN, Kitajima M, Hiraki K, Yamaguchi N, Katamine S, Matsuyama T, et al. *Escherichia coli* contamination of menstrual blood and effect of bacterial endotoxin on endometriosis. Fertil Steril 2010;94(7):2860–3. e3.
[46] Swindle EJ, Metcalfe DD. The role of reactive oxygen species and nitric oxide in mast cell-dependent inflammatory processes. Immunol Rev 2007;217(1):186–205.
[47] Kobayashi H, Yamada Y, Kanayama S, Furukawa N, Noguchi T, Haruta S, et al. The role of iron in the pathogenesis of endometriosis. Gynecol Endocrinol 2009;25(1):39–52.
[48] Riccio L, Santulli P, Marcellin L, Abrao MS, Batteux F, Chapron C. Immunology of endometriosis. Best Pract Res Clin Obstet Gynaecol 2018;50:39–49.
[49] Vallve-Juanico J, Houshdaran S, Giudice LC. The endometrial immune environment of women with endometriosis. Hum Reprod Update 2019;25(5):564–91.
[50] Symons LK, Miller JE, Kay VR, Marks RM, Liblik K, Koti M, et al. The immunopathophysiology of endometriosis. Trends Mol Med 2018;24(9):748–62.
[51] St-Jacques B, Ma W. Peripheral prostaglandin E2 prolongs the sensitization of nociceptive dorsal root ganglion neurons possibly by facilitating the synthesis and anterograde axonal trafficking of EP4 receptors. Exp Neurol 2014;261:354–66.
[52] Reeh PW, Steen KH. Tissue acidosis in nociception and pain. Prog Brain Res 1996;113:143–51.

[53] Barra F, Ferrero S. The use of retinoic acid for the treatment of endometriosis. Archives of Gynecology and Obstetrics; 2018.
[54] Anaf V, Simon P, El Nakadi I, Fayt I, Buxant F, Simonart T, et al. Relationship between endometriotic foci and nerves in rectovaginal endometriotic nodules. Hum Reprod 2000;15(8):1744–50.
[55] Wang G, Tokushige N, Russell P, Dubinovsky S, Markham R, Fraser IS. Hyperinnervation in intestinal deep infiltrating endometriosis. J Minim Invasive Gynecol 2009;16(6):713–9.
[56] Ferrero S, Alessandri F, Racca A, Leone Roberti Maggiore U. Treatment of pain associated with deep endometriosis: alternatives and evidence. Fertil Steril 2015;104(4):771–92.
[57] McKinnon B, Bersinger NA, Wotzkow C, Mueller MD. Endometriosis-associated nerve fibers, peritoneal fluid cytokine concentrations, and pain in endometriotic lesions from different locations. Fertil Steril 2012;97(2):373–80.
[58] Arnold J, Barcena de Arellano ML, Rüster C, Vercellino GF, Chiantera V, Schneider A, et al. Imbalance between sympathetic and sensory innervation in peritoneal endometriosis. Brain Behav Immun 2012;26(1):132–41.
[59] Ferrero S, Haas S, Remorgida V, Camerini G, Fulcheri E, Ragni N, et al. Loss of sympathetic nerve fibers in intestinal endometriosis. Fertil Steril 2010;94(7):2817–9.
[60] Mechsner S, Kaiser A, Kopf A, Gericke C, Ebert A, Bartley J. A pilot study to evaluate the clinical relevance of endometriosis-associated nerve fibers in peritoneal endometriotic lesions. Fertil Steril 2009;92(6):1856–61.
[61] Herington JL, Bruner-Tran KL, Lucas JA, Osteen KG. Immune interactions in endometriosis. Expert Rev Clin Immunol 2011;7(5):611–26.
[62] Karck U, Reister F, Schafer W, Zahradnik HP, Breckwoldt M. PGE2 and PGF2 alpha release by human peritoneal macrophages in endometriosis. Prostaglandins 1996;51(1):49–60.
[63] Dawood MY, Khan-Dawood FS, Wilson Jr L. Peritoneal fluid prostaglandins and prostanoids in women with endometriosis, chronic pelvic inflammatory disease, and pelvic pain. Am J Obstet Gynecol 1984;148(4):391–5.
[64] Holzer P. Acid sensing by visceral afferent neurones. Acta Physiol 2011;201(1):63–75.
[65] Ma F, Zhang L, Westlund KN. Reactive oxygen species mediate TNFR1 increase after TRPV1 activation in mouse DRG neurons. Mol Pain 2009;5.
[66] Wang HJ, Li YL, Zhang LB, Zucker IH, Gao L, Zimmerman MC, et al. Endogenous reactive oxygen species modulates voltage-gated sodium channels in dorsal root ganglia of rats. J Appl Physiol 2011;110(5):1439–47.
[67] Berkley KJ, Dmitrieva N, Curtis KS, Papka RE. Innervation of ectopic endometrium in a rat model of endometriosis. Proc Natl Acad Sci U S A 2004;101(30):11094–8.
[68] Rocha MG, Silva JCRE, Ribeiro Da Silva A, Candido Dos Reis FJ, Nogueira AA, Poli-Neto OB. TRPV1 expression on peritoneal endometriosis foci is associated with chronic pelvic pain. Reprod Sci 2011;18(6):511–5.
[69] McKinnon BD, Bertschi D, Bersinger NA, Mueller MD. Inflammation and nerve fiber interaction in endometriotic pain. Trends Endocrinol Metabol 2015;26(1):1–10.
[70] Barcena de Arellano ML, Arnold J, Lang H, Vercellino GF, Chiantera V, Schneider A, et al. Evidence of neurotrophic events due to peritoneal endometriotic lesions. Cytokine 2013;62(2):253–61.
[71] Asami T, Ito T, Fukumitsu H, Nomoto H, Furukawa Y, Furukawa S. Autocrine activation of cultured macrophages by brain-derived neurotrophic factor. Biochem Biophys Res Commun 2006;344(3):941–7.
[72] Bothwell M. NGF, BDNF, NT3, and NT4. Handb Exp Pharmacol 2014;220:3–15.
[73] Bandtlow CE, Meyer M, Lindholm D, Spranger M, Heumann R, Thoenen H. Regional and cellular codistribution of interleukin 1β and nerve growth factor mRNA in the adult rat brain: possible relationship to the regulation of nerve growth factor synthesis. J Cell Biol 1990;111(4):1701–11.
[74] Kawamoto K, Aoki J, Tanaka A, Itakura A, Hosono H, Arai H, et al. Nerve growth factor activates mast cells through the collaborative interaction with lysophosphatidylserine expressed on the membrane surface of activated platelets. J Immunol 2002;168(12):6412–9.
[75] Horigome K, Bullock ED, Johnson Jr EM. Effects of nerve growth factor on rat peritoneal mast cells. Survival promotion and immediate-early gene induction. J Biol Chem 1994;269(4):2695–702.
[76] Samah B, Porcheray F, Gras G. Neurotrophins modulate monocyte chemotaxis without affecting macrophage function. Clin Exp Immunol 2008;151(3):476–86.
[77] Peng B, Zhan H, Alotaibi F, Alkusayer GM, Bedaiwy MA, Yong PJ. Nerve growth factor is associated with sexual pain in women with endometriosis. Reprod Sci 2018;25(4):540–9.
[78] Wang G, Tokushige N, Russell P, Dubinovsky S, Markham R, Fraser IS. Hyperinnervation in intestinal deep infiltrating endometriosis. J Minim Invasive Gynecol 2009;16(6):713–9.

[79] Tokushige N, Markham R, Russell P, Fraser IS. Nerve fibres in peritoneal endometriosis. Hum Reprod 2006;21(11):3001–7.
[80] Liu Y, Qin X, Lu X, Jiang J. Effects of inhibiting the PI3K/Akt/mTOR signaling pathway on the pain of sciatic endometriosis in a rat model. Can J Physiol Pharmacol 2019;97(10):963–70.
[81] McKinnon BD, Evers J, Bersinger NA, Mueller MD. Induction of the neurokinin 1 receptor by TNFα in endometriotic tissue provides the potential for neurogenic control over endometriotic lesion growth. J Clin Endocrinol Metab 2013;98(6):2469–77.
[82] Renner SP, Ekici AB, Maihöfner C, Oppelt P, Thiel FC, Schrauder M, et al. Neurokinin 1 receptor gene polymorphism might be correlated with recurrence rates in endometriosis. Gynecol Endocrinol 2009;25(11):726–33.
[83] Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain 2011;152(Suppl. 3):S2–15.
[84] Latremoliere A, Woolf CJ. Central sensitization: a generator of pain hypersensitivity by central neural plasticity. J Pain 2009;10(9):895–926.
[85] Grandi G, Barra F, Ferrero S, Sileo FG, Bertucci E, Napolitano A, et al. Hormonal contraception in women with endometriosis: a systematic review. Eur J Contracept Reprod Health Care 2019;24(1):61–70.
[86] Ferrero S, Evangelisti G, Barra F. Current and emerging treatment options for endometriosis. Expert Opin Pharmacother 2018;19(10):1109–25.
[87] Barra F, Scala C, Ferrero S. Elagolix sodium for the treatment of women with moderate to severe endometriosis-associated pain. Drugs Today 2019;55(4):237–46.
[88] Barra F, Grandi G, Tantari M, Scala C, Facchinetti F, Ferrero S. A comprehensive review of hormonal and biological therapies for endometriosis: latest developments. Expert Opin Biol Ther 2019;19(4):343–60.
[89] Barra F, Scala C, Mais V, Guerriero S, Ferrero S. Investigational drugs for the treatment of endometriosis, an update on recent developments. Expert Opin Invest Drugs 2018;27(5):445–58.
[90] Barra F, Scala C, Ferrero S. Current understanding on pharmacokinetics, clinical efficacy and safety of progestins for treating pain associated to endometriosis. Expert Opin Drug Metabol Toxicol 2018;14(4):399–415.
[91] Tokushige N, Markham R, Russell P, Fraser IS. Effects of hormonal treatment on nerve fibers in endometrium and myometrium in women with endometriosis. Fertil Steril 2008;90(5):1589–98.
[92] Richeri A, Bianchimano P, Marmol NM, Viettro L, Cowen T, Brauer MM. Plasticity in rat uterine sympathetic nerves: the role of TrkA and p75 nerve growth factor receptors. J Anat 2005;207(2):125–34.
[93] Barra F, Ferro Desideri L, Leone Roberti Maggiore U, Gaetano Vellone V, Maramai M, Scala C, et al. Endometriosis classification and the role of tumor necrosis factor-alpha polymorphisms as a therapeutic target. Int J Fertil Steril 2020;14(1):76–7.
[94] D'Hooghe TM, Nugent NP, Cuneo S, Chai DC, Deer F, Debrock S, et al. Recombinant human TNFRSF1A (r-hTBP1) inhibits the development of endometriosis in baboons: a prospective, randomized, placebo- and drug-controlled study. Biol Reprod 2006;74(1):131–6.
[95] Falconer H, Mwenda JM, Chai DC, Wagner C, Song XY, Mihalyi A, et al. Treatment with anti-TNF monoclonal antibody (c5N) reduces the extent of induced endometriosis in the baboon. Hum Reprod 2006;21(7):1856–62.
[96] Yildirim G, Attar R, Ficicioglu C, Karateke A, Ozkan F, Yesildaglar N. Etanercept causes regression of endometriotic implants in a rat model. Arch Gynecol Obstet 2011;283(6):1297–302.
[97] Islimye M, Kilic S, Zulfikaroglu E, Topcu O, Zergeroglu S, Batioglu S. Regression of endometrial autografts in a rat model of endometriosis treated with etanercept. Eur J Obstet Gynecol Reprod Biol 2011;159(1):184–9.
[98] Zulfikaroglu E, Kilic S, Islimye M, Aydin M, Zergeroglu S, Batioglu S. Efficacy of anti-tumor necrosis factor therapy on endometriosis in an experimental rat model. Arch Gynecol Obstet 2011;283(4):799–804.
[99] Cayci T, Akgul EO, Kurt YG, Ceyhan TS, Aydin I, Onguru O, et al. The levels of nitric oxide and asymmetric dimethylarginine in the rat endometriosis model. J Obstet Gynaecol Res 2011;37(8):1041–7.
[100] Koninckx PR, Craessaerts M, Timmerman D, Cornillie F, Kennedy S. Anti-TNF-α treatment for deep endometriosis-associated pain: a randomized placebo-controlled trial. Hum Reprod 2008;23(9):2017–23.
[101] Huber AV, Saleh L, Prast J, Haslinger P, Knöfler M. Human chorionic gonadotrophin attenuates NF-κB activation and cytokine expression of endometriotic stromal cells. Mol Hum Reprod 2007;13(8):595–604.
[102] Zhang H, Li M, Wang F, Liu S, Li J, Wen Z, et al. Endometriotic epithelial cells induce MMPs expression in endometrial stromal cells via an NFB-dependent pathway. Gynecol Endocrinol 2010;26(6):456–67.

[103] Nasu K, Nishida M, Ueda T, Yuge A, Takai N, Narahara H. Application of the nuclear factor-κB inhibitor BAY 11-7085 for the treatment of endometriosis: an in vitro study. Am J Physiol Endocrinol Metabol 2007;293(1):E16–23.
[104] Indraccolo U, Barbieri F. Effect of palmitoylethanolamide-polydatin combination on chronic pelvic pain associated with endometriosis: preliminary observations. Eur J Obstet Gynecol Reprod Biol 2010;150(1):76–9.
[105] Chen Y, Li D, Zhang Z, Takushige N, Kong BH, Wang GY. Effect of siRNA against beta-NGF on nerve fibers of a rat model with endometriosis. Reprod Sci 2014;21(3):329–39.

CHAPTER

11

Immune phenotypes and mediators affecting endometrial function in women with endometriosis

Júlia Vallvé-Juanico[a] *and Linda C. Giudice*

Obstetrics, Gynecology and Reproductive Sciences, University of California San Francisco, San Francisco, CA, United States

Introduction

The endometrium is a steroid hormone-dependent tissue that is the anatomic prerequisite for successful pregnancy, and in the absence of pregnancy is sloughed as the menses, self-renewing in subsequent nonpregnancy cycles and postpartum [1]. The tissue is comprised of multiple cell types, including glandular epithelium, luminal epithelium, stromal fibroblasts (eSF), vascular endothelium and smooth muscle cells, resident and transient immune cells, and stem cells. Normal tissue homeostasis and regeneration require programmed cellular proliferation, differentiation, apoptosis, and migration in response to dynamically changing circulating levels of estradiol (E_2) and progesterone (P_4) and in response to paracrine interactions among tissue cellular constituents. Critical are communications among different endometrial immune cells with each other, with other endometrial cells types, and with the invading trophoblast. Abnormalities in any of these cellular responses to steroid hormones or paracrine crosstalk can disrupt the homeostasis and functionality of the tissue. Indeed, several reproductive disorders, including endometriosis, polycystic ovarian syndrome, uterine fibroids, adenomyosis, endometrial hyperplasia, and cancer are associated with abnormal endometrial cellular interactions and hormonal responses that result in tissue dyshomeostasis, infertility, and poor pregnancy outcomes [2].

Endometriosis is a common, E_2-dependent inflammatory disorder causing chronic pelvic pain and infertility [3,4]. Retrograde migration during menses of endometrial tissue into the pelvis results in peritoneal and ovarian disease accompanied by neuroangiogenic and

[a] Current address: Bioliquid Innovative Genetics S.L.U., Barcelona, Spain.

https://doi.org/10.1016/B978-0-12-820661-4.00006-6

© 2022 Elsevier Inc. All rights reserved.

inflammatory responses, scarring and fibrosis [5]. Abnormalities within the eutopic endometrium (within the uterus) are believed to lead to disease formation, with epithelial cells being clonal in endometriotic lesions [6]. There is local inflammation in the ectopic pelvic lesions and within the eutopic endometrium; additionally, women with endometriosis display signs of systemic inflammation [3,4,7]. The inhospitable environment in the pelvis and uterus contribute to infertility in affected women, largely due to adverse inflammatory effects on oocytes, sperm, embryos, and in the endometrium, as well as anatomic distortion due to scarring and fibrosis [8]. Moreover, women with endometriosis who achieve pregnancy have poorer outcomes than those without disease, believed largely due to the proinflammatory environment in the endometrium and effects on the processes of nidation and placentation [2].

We have previously shown that the endometrium of women with endometriosis displays a proinflammatory phenotype [9]. What contributes to this phenotype is an area of active investigation. A recent meta-analysis of mostly our whole genome endometrial transcriptome data reveal activated dendritic cells (DCs), CD4 T effector memory phenotype cells, eosinophils (EN), macrophages (Mφ), and natural killer T cells in endometrium of women with versus without endometriosis [10]. Moreover, other endometrial cells in women with endometriosis contribute to an abnormal endometrial environment and tissue dysfunction [7]. Specifically, eSF display a proinflammatory phenotype acquired within the endometrial niche, and this cell type and its progenitor, the endometrial mesenchymal stem cell (eMSC), from women with versus without endometriosis display abnormal responses to P_4 (*see below*). The proinflammatory cytokine IL-1 and the endometrial niche play roles in these phenotypes [11,12], and IL-11 and seminal plasma can reverse the abnormal P_4 responsiveness of eSF from women with endometriosis [13].

In addition to advances in the field of immunology, technological advances have been made in identifying immune cell activation status and elucidating immune cell differentiation programs and roles of immune cells in the innate and adaptive immune responses, as well cell-cell interactions at the tissue level. Recently, we have described in detail the immune populations and their phenotypes in endometrium of women with versus without endometriosis [7]. Altered immune cells in eutopic endometrium of women with disease can affect other endometrial cell populations, predisposing to lesion establishment, survival, and growth. Several studies have reported changes in eutopic endometrium of women with and without endometriosis in terms of gene expression, angiogenesis, immune components, cytokine production and presence of nerve fibers [9,14–18]. The current chapter focuses on the endometrial immune niche and its role in endometrial dysfunction. We focus on immune and nonimmune cell types in endometrium of women with endometriosis that display a proinflammatory phenotype, their secreted cytokines and chemokines, and how these secreted products affect other immune and nonimmune endometrial cells. Moreover, we discuss the roles of these cells on endometriosis pathogenesis and endometrial dysfunction in women with disease relevant to menstruation, dysmenorrhea, endometrial regeneration and repair, infertility, and poor pregnancy outcomes.

Immune cells in endometrium of women with endometriosis

Leukocytes (CD45+) represent 10%–20% of all endometrial cells [19] and increase in the secretory and menstrual phases versus the proliferative phase of the menstrual cycle

[19–23]. Different immune populations, including those in the innate and adaptive immune systems, are present in this tissue. In women without endometriosis, most endometrial leukocytes in the proliferative phase are T cells (TCs), uterine natural killer (uNK) cells, and Mφ [24]. In contrast, uNK cells predominate (70%–80%) in the secretory phase with Mφ ~30%, and TCs <10% [23]. Not all endometrial immune populations vary equivalently throughout the menstrual cycle in women with and without endometriosis (Figs. 11.1–11.3); herein, we highlight these differences.

Innate immune system

There is a large presence of innate immune populations in the endometrium. Mφ comprise the most abundant immune population in the tissue [19,25–28] and, under normal conditions, they increase in the secretory phase [27,29–33]. However, this increase is not observed in endometrium of women with endometriosis [7]. Mφ are traditionally classified as proinflammatory (Mφ1) and antiinflammatory (Mφ2). Mφ1 are involved in proinflammatory responses and Mφ2 in angiogenesis, antiinflammatory processes, and tissue repair [34,35]. The latter constitute the main Mφ population in healthy endometrium [34,36,37]. However, in endometrium of women with disease, Mφ1 predominate throughout the cycle [37,38], suggesting a persistent, proinflammatory microenvironment therein (Fig. 11.2).

Another antigen-presenting cell found in endometrium is the DC [39]. In normal conditions, immature DCs increase in the secretory phase and menstruation. In contrast, in endometriosis women, this increase is not observed [40]. Densities of immature and mature

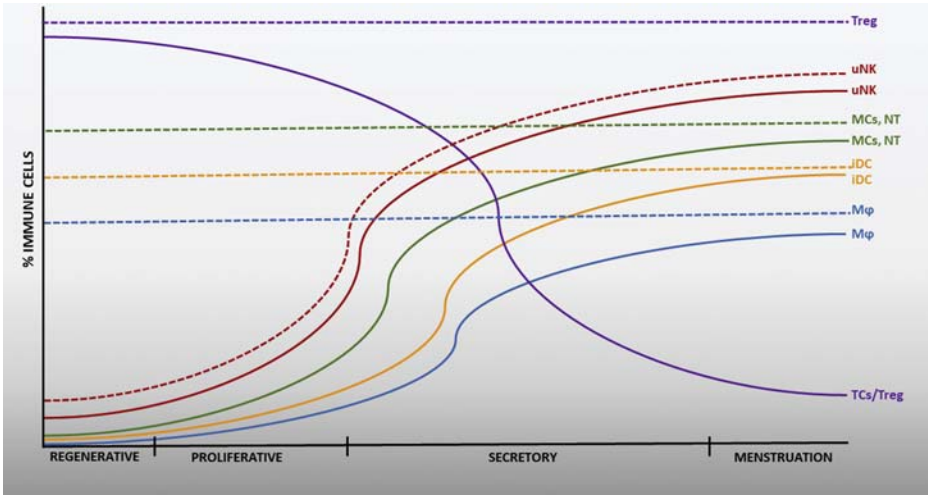


FIGURE 11.1 **Different fluctuations of specific endometrial immune populations across the menstrual cycle in women with and without endometriosis.** *Dashed lines* indicate the distribution of populations in the different cycle phases (regenerative, proliferative, secretory and menstruation) in endometrium of women with disease, and the *solid lines* represent the distribution of the cells in healthy endometrium. Each population is represented with different colors: purple: T cells and regulatory T cells (Treg); red: uterine natural killer cells (uNK); green: mast cells (MCs) and neutrophils (NT); yellow: immature dendritic cells (iDCs); and blue: macrophages (Mφ).

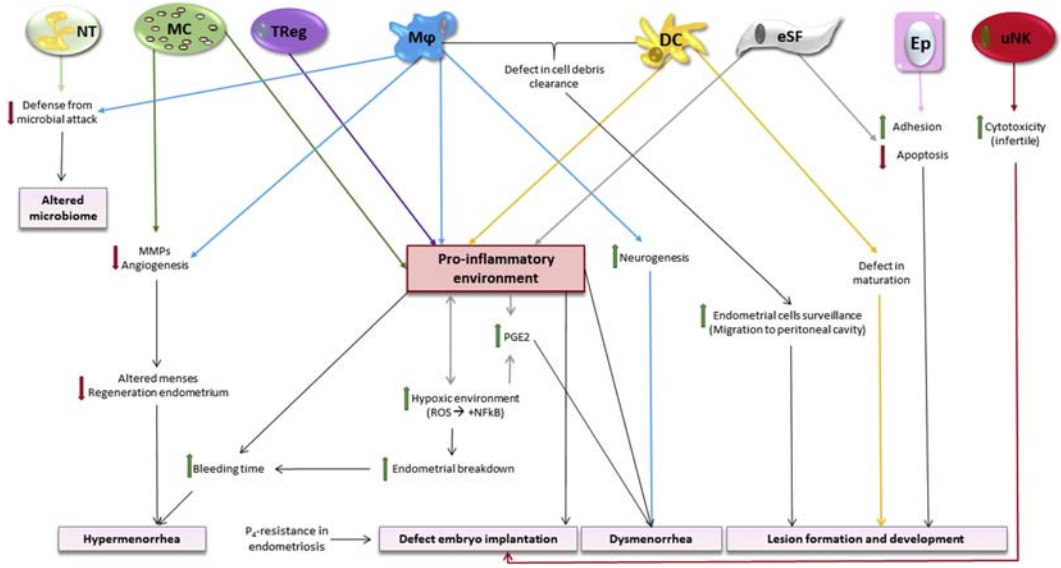


FIGURE 11.2 **Proinflammatory environment in eutopic endometrium of women with endometriosis and its effects.** The main characteristic of eutopic endometrium of women with endometriosis is its proinflammatory phenotype, which is believed to contribute to symptoms of hypermenorrhea and dysmenorrhea and/or aberrancies in embryo implantation. Abnormalities in specific immune cells also may lead to endometriotic lesion formation and development in the peritoneal cavity. The figure shows that defective neutrophils (NT) and macrophages (Mφ) are not able to defend the endometrium from microbial attack during menses, leading teleologically to an altered microbiome in endometrium of women with endometriosis. Moreover, mast cells (MC) in endometriosis, together with Mφ, produce MMPs during menses, and angiogenic factors are decreased, resulting in a decrease in angiogenesis. Thus, the data support abnormalities during menses and during regeneration of the endometrium concomitantly and thereafter. These factors could result in associated hypermenorrhea. Regulatory T cells (Treg) do not decrease during the secretory phase of the cycle in endometriosis, enhancing the proinflammatory environment. This phenomenon can be further enhanced by higher production of proinflammatory factors from MC, Mφ, dendritic cells (DC), and endometrial stromal fibroblasts (eSF). Furthermore, Mφ also enhance neurogenesis and, along with eSF, produce prostaglandins (PGE_2), which contribute to dysmenorrhea. The proinflammatory environment leads to an increase in ROS, producing constitutive activation of the NFkB pathway, leading to transcription of inflammatory genes, including prostaglandin synthase. The hypoxic environment together with the proinflammatory factors lead to an increase of endometrial breakdown and with suboptimal repair can lead to hypermenorrhea. In addition, Mφ and DC do not increase during the secretory and menstrual phases (and endometrial DC exhibit defective maturation in women with disease), potentially resulting in compromised clearance of endometrial cells and cell debris during menses. Thus, endometrial cells (eSF and epithelial cells (Ep)), which have higher adhesion properties and are less apoptotic in women with versus without endometriosis migrate to the peritoneal cavity, adhere, and develop endometriotic lesions. Finally, the proinflammatory endometrium could lead to abnormal embryo implantation, exacerbated by uterine natural killers (uNK), which are more cytotoxic in infertile women with endometriosis. Altogether, the evidence suggests that the proinflammatory environment in endometrium of women with versus without endometriosis, is a result of multiple types of endometrial immune and nonimmune cells and results in disease-associated symptoms, as well as the pathophysiology of endometriosis and reproductive outcomes in affected women. Nonetheless, this diagram should be taken with some reservation, as it is a general summary of multiple factors involved in the endometrial dysfunction in women with endometriosis. More studies are needed to elucidate the specific roles of each endometrial cellular population and their secreted factors in dysfunctionality of the tissue in affected women.

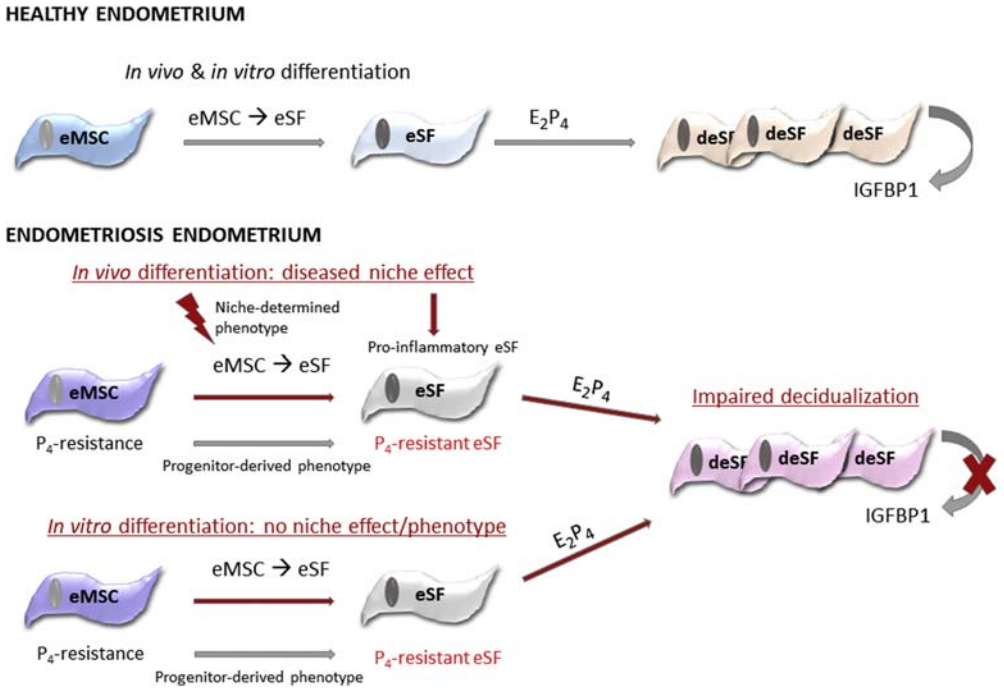


FIGURE 11.3 **Model of disease phenotype of eMSC and eSF in eutopic endometrium of women with endometriosis.** Top panel: normal eMSC progenitors differentiate both in vivo and in vitro to eSF that decidualize in response to P_4. Bottom panel: in endometriosis, in vivo differentiated eSF inherit P_4-resistance from eMSC progenitors, failing to decidualize, and acquire a proinflammatory phenotype within the endometrial niche. The eMSC from endometriosis women differentiate in vitro to eSF that inherit P_4 resistance with impaired decidualization, but eMSC differentiated to eSF outside of the endometrial niche do not acquire the proinflammatory phenotype displayed by their in vivo differentiated counterparts. *eMSC*, endometrial mesenchymal stem cells; *P_4*, progesterone.

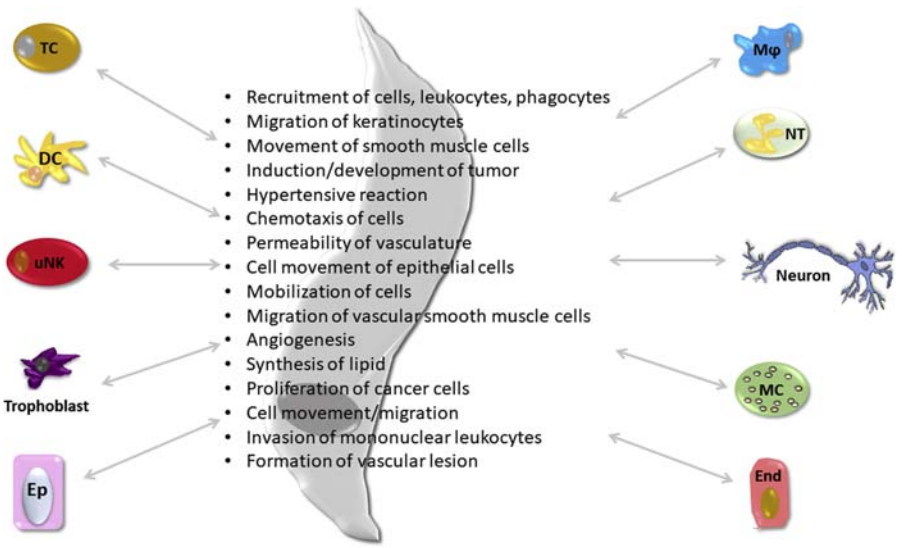


FIGURE 11.4 **eSF as master regulators of eutopic endometrium of women with endometriosis phenotype.** The eSF as a "master regulator" of endometrial function has multiple interactions with immune, epithelial, and endothelial cells and sensorial neurons throughout the menstrual cycle as well as in early pregnancy (along with trophoblasts). As the figure shows, eSF can mediate multiple functions, such as recruitment of cells, migration, angiogenesis, synthesis of lipids, proliferation, and other. *DC*, dendritic cells; *End*, endothelial cells; *Ep*, epithelial cells; *eSF*, endometrial stromal fibroblasts; *MC*, mast cells; *Mφ*, macrophages; *NT*, neutrophils; *TC*, T cells; *uNK*, uterine natural killer cells.

endometrial DCs are higher and lower, respectively, in women with versus without disease [40,41], suggesting blunted DC maturation in women with endometriosis (Fig. 11.2). Recent evidence suggests activation of endometrial DCs in women with disease [10].

Mast cells (MCs), neutrophils (NT), and other innate immune populations do not change in numbers across the cycle [42,43]; however, their activities increase prior to menstruation in normal endometrium [42,44], but not in women with endometriosis [45], indicating a more dormant state in the latter. MCs can be activated by EN, which are granulocytes involved in initiation of inflammatory responses [46]. In general, EN mediators have vasoactive effects, promote histamine release by MCs, and activate Mφ and endothelial cells [44], implicating them in angiogenesis and tissue remodeling. Another key population in endometrium are uNK [47], which increase markedly in the secretory phase and are important in pregnancy establishment [21,23,25,32,48–53]. In women with endometriosis, however, while uNK display the same pattern [54,55], their cytotoxic activity is diminished, suggesting a role in infertility and poor pregnancy outcomes in women with this disorder [56] (Fig. 11.4). Recently, uNK T cells have been suggested to be higher in women with stage I/II disease versus controls [10]. Their roles in the endometriosis proinflammatory endometrial phenotype remains to be determined.

Adaptive immune system

In addition to the innate immune system, the adaptive immune system is a major player in endometrial biology. Regulatory T cells (Tregs) increase prior to ovulation [57], providing an immune-tolerant environment for embryo implantation through secretion of immunosuppressive cytokines such as IL-10 and TGF-β [57–60]. Tregs regulate Mφ, MC degranulation, DCs, NT, EN, B cells, TCs, and uNK cell function and proliferation, all of which having a role in menstruation [22,61]. Conflicting information prevails in the literature about TCs variation across the cycle in women with versus without endometriosis [7]. The most consistent data reveal that TCs are higher in the proliferative phase of women with versus without disease; whereas, in the secretory phase numbers are roughly equivalent and decrease similarly in both groups [62,63]. Some studies have demonstrated higher numbers of Treg cells in the secretory phase compared with the other phases of the cycle (Fig. 11.1), which could have effects in embryo implantation, and others have reported no differences across the cycle. However, it is believed that these cells are abnormal in endometriosis [57,64–67].

CD8+ TC toxicity decreases in normal endometrium during the secretory phase, mediated by progesterone, to minimize an inhospitable environment for an implanting embryo. Their cytotoxicity, however, has been reported to be elevated in endometrium of women with endometriosis [68].

Steroid hormone dependence

Variation of immune cell numbers and activities across the cycle suggests regulation by ovarian-derived steroid hormones. However, there are controversies across studies about steroid hormone receptor expression in endometrial immune populations [7]. Fortunately, technological advances are rapidly developing, providing additional information. Single cell RNA sequencing, e.g., has opened new possibilities to detect expression of these

receptors. In addition, other technologies, such as mass cytometry, will allow detection of specific receptors at the protein level in single cells and activation status of individual cells—something that has been wanting historically in most studies on endometrial immune cells broadly and which can also serve to validate deconvolution analyses. Furthermore, visualization of the combination of RNA and protein at the single cell level is available through in situ hybridization to detect RNA transcripts combined with immunohistochemistry to detect proteins [69]. A major opportunity for future research involves whether steroid hormones directly or indirectly modulate endometrial immune cell functions and why some populations behave differently in endometrium of women with endometriosis.

Proinflammatory endometrial environment in women with endometriosis

Different cell populations and factors are responsible for the characteristic proinflammatory environment in eutopic endometrium of women with endometriosis, and the most common are summarized here.

Immune cells

Macrophages (Mφ)

In healthy endometrium Mφ2 (antiinflammatory responses) are the predominant phenotype [34,36,37]; while in endometrium of women with endometriosis the Mφ1 population (proinflammatory responses) predominates throughout the menstrual cycle [37]. A recent deconvolution study demonstrated that in early stages of the disease (phase I and II), Mφ1 are predominant in endometrium of women with endometriosis and Mφ2 are predominant in advanced stages (III–IV) [10]. We recently demonstrated that Mφ2 in eutopic endometrium of women with endometriosis exhibit proinflammatory properties rather than their normal antiinflammatory phenotype [38]. Our results indicate that Mφ2 could enhance the proinflammatory environment in endometrium of women with endometriosis (Fig. 11.2), as well as have several functional defects, e.g., lack of clearing of endometrial cells and tissue fragments shed during menstruation, as well as promoting a disadvantageous environment for embryo implantation during the secretory phase of the cycle. Also, Mφ promote a proinflammatory gene signature in eSF, including IL-8, CXCL8, MMP3, CYR61, CTGF, among others [70], which are also related to menstruation and cell adhesion.

Dendritic cells (DCs)

DCs produce cytokines and chemokines, such as IL-6, IL-10, IL-12, TNFα, RANTES, and MCP-1 that regulate other lymphocyte populations [33,71], and they have a role in the proinflammatory environment in endometriosis (Fig. 11.2), in the pathophysiology of the disease and they may have a role in embryo implantation (*see below*). The deconvolution study cited above demonstrated that activated DCs are higher in endometrium of women with endometriosis, reinforcing the fact that they are involved in the proinflammatory phenotype of this tissue [10].

Mast cells (MCs)

MCs may also contribute to the proinflammatory phenotype observed in endometrium of women with versus without endometriosis. These cells secrete IL-4, IL-5, IL-6, and TNF-α [44] which stimulate collagenase and prostaglandin E2 (PGE_2) production by eSF [72], which, in turn, activate Mφ. In addition, MCs secrete chemoattractant molecules for EN and NT, including LTC4, LTB4, PGD2, PAF, IL-5 and GM-CSF [44]. The presence of these proinflammatory populations, EN, NT, and Mφ in endometrium of women with endometriosis is enhanced [44], supporting a role for MCs in attracting proinflammatory immune cells in inflammation sites, in this case, the endometrium of women with endometriosis (Fig. 11.2).

Regulatory T cells (Treg)

Another population implicated in the enhanced proinflammatory phenotype in endometrium of women with endometriosis is TCs. Treg cells produce potent antiinflammatory immune responses. They regulate Mφ, MC degranulation, DCs, NT, EO, B cells, TCs and uNK cell function and proliferation [61,73]. Normally, they increase prior to ovulation, creating a hospitable environment for embryo implantation [73]. However, this increase is not consistently observed in endometrium of women with endometriosis [74], suggesting that Tregs cannot trigger the optimal antiinflammatory conditions for embryo implantation in women with disease and contributing to a more proinflammatory phenotype in this tissue (Fig. 11.2).

Nonimmune cells

Microbial environment

Recently, it has been postulated that the microbiota in eutopic endometrium of women with endometriosis may promote a dysfunctional immune response, enhancing the influx of immune populations to the sites of inflammation and thus increasing the proinflammatory phenotype in the endometrial tissue. Different studies have found an increase of *Proteobacteria* in endometrium of endometriosis cohorts [75]. However, more studies are needed to determine the presence and role of the microbiome in endometrium of women with endometriosis [75].

Endometrial epithelial cells

Endometrial epithelial cells can secrete IL-11 by the action of other cytokines and may be involved in the pathophysiology of the disease (*see below*). However, to our knowledge, a clear role of epithelial cells to the proinflammatory phenotype observed in eutopic endometrium of women with endometriosis has not been described.

Endometrial stromal fibroblasts (eSF)

While immune populations predominantly demonstrate a proinflammatory phenotype in endometrium of women with endometriosis, other cell types also do—in particular the eSF. Abnormalities in eSF within the uterus of women with disease (and in ectopic lesions) include aberrant production of E_2 and PGE_2 [76], resistance to P_4 action and a proliferative and invasive phenotype [14,77–79], fibroblast activation marker expression [80], incomplete lineage differentiation and resistance to apoptosis [80]. Thus, there is a defect on eSF differentiation program ("decidualization") during the secretory phase of the menstrual cycle, which is critical for a proper embryo implantation. In addition, eSF in endometriosis exhibit a

proinflammatory phenotype in vivo that is retained in vitro over multiple passages (Fig. 11.3) [79,80]. They exhibit gene expression profiles associated with activation and recruitment of immune cells [14,79,80], and both eutopic and ectopic eSF secrete cytokines, chemokines, and angiogenic and pronociceptive mediators [67,80–82]. The eSF as a "master regulator" of endometrial function has multiple interactions with immune cells, epithelium and endothelial cells throughout the menstrual cycle and in early pregnancy (Fig. 11.4). As chemokines, cytokines and growth factors produced by eSF in response to E_2 and P_4 normally directly affect endometrial leukocyte function [83], aberrancies in the eSF steroid hormone response and paracrine modulators may adversely impact immune cells in endometrium (and in lesions). Therefore, the endometrial immune niche likely contributes significantly to establishing the proinflammatory state of eSF (Fig. 11.2). Recently, we found that eMSC are the progenitors of eSF and that the endometriosis eSF P_4-resistance phenotype, but not its proinflammatory phenotype, is inherited from endometriosis eMSC [80] (Fig. 11.3).

The findings that lineage gene instability and P_4-resistance are inherited from the endometriosis eMSC progenitor, but the proinflammatory phenotype is not, suggest that the endometrial niche may act during the differentiation of eMSC to eSF to confer a persistent proinflammatory phenotype to the progeny eSF in disease [80]. Given the centrality of eSF in endometrial function and of disease-specific fibroblasts in other inflammatory disorders, eSF could be key effector cells in the pathogenesis and pathophysiology of endometriosis with potential as a therapeutic target for disease control and pain relief.

Endometrial mesenchymal stem cells

While eMSC confer on eSF its P_4-resistance and abnormal lineage differentiation, it does not set its inflammatory phenotype [80]. In addition, eutopic eMSC in endometriosis do not display a proinflammatory profile [80]. This leads to the hypothesis that other cells in the endometrial niche establish the inflammatory phenotype of eSF in women with endometriosis. There is growing evidence that eMSC and perhaps other endometrial stem cells contribute to endometriosis lesion formation [84,85], although direct proof is wanting. Stem cells, which express SSEA1 and SOX9, are usually located in the *basalis* layer. Women with endometriosis display an abnormal location of epithelial cells in the *functionalis* layer with this stem cell-like phenotype, compared to controls during the secretory phase [86]. This indicates that they might have an aberrant function in disease and could be involved in the pathogenesis of endometriosis. Finally, in experimental models, lymphocytes can affect MSC differentiation by multiple mechanisms including upregulation of proinflammatory cytokines, suggesting that crosstalk between MSC and resident immune cell niches may play a key role in determining the fate/function of MSC in tissues [87,88]. Whether stem cells are affected by immune cells in endometrium is currently not known and warrants further investigation.

Cytokines and chemokines

Proinflammatory milieu

In endometriosis, there is clear evidence that the immune niche in the endometrium is altered, producing a proinflammatory microenvironment, as described above. Genes encoding for proinflammatory cytokines, chemokines and receptors, including CXCL1, CXCL3, CXCL9, CXCL10, IL-32, CXCR2, and IL-17R are increased in endometrium of women with

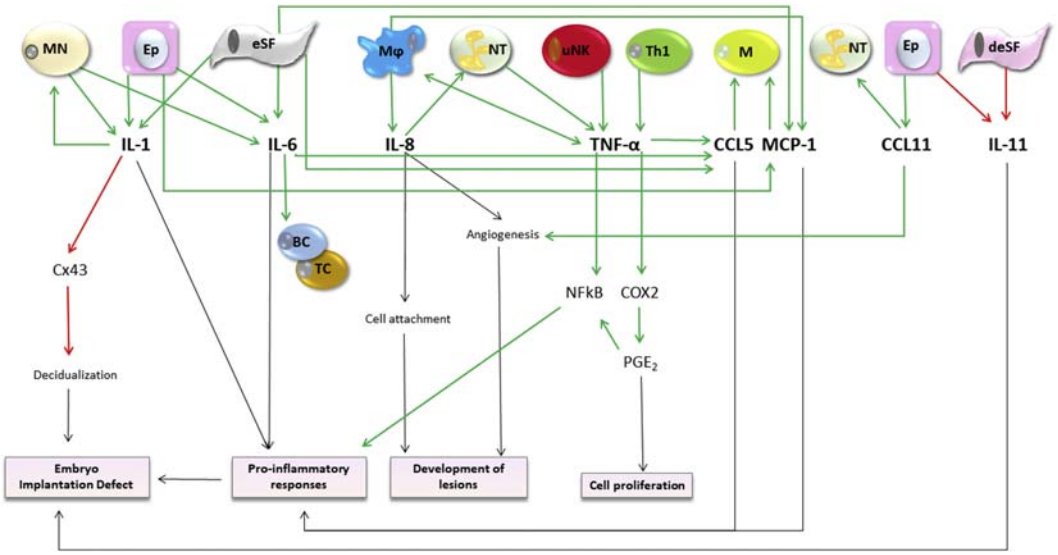


FIGURE 11.5 **De-regulated cytokines in eutopic endometrium of women with endometriosis.** The figure shows the endometrial cells that produce specific cytokines that are deregulated in endometrium of women with endometriosis. *Green arrows* indicate that they activate or promote the expression of the molecules. *Red arrows* indicate inhibition of molecules or processes. The deregulation of these specific cytokines have different consequences (*black arrows*). *BC*, B cells; *deSF*, decidualized endometrial stromal fibroblasts; *Ep*, endometrial epithelial cells; *eSF*, endometrial stromal fibroblasts; *M*, monocytes; *MN*, Mononuclear cells; *Mφ*, macrophages; *NT*, neutrophils; *TC*, T cells; *Th1*, T helpers one; *uNK*, uterine natural killers.

endometriosis [89,90]. Elevated expression of other proinflammatory factors have been reported, including hepatocyte growth factor (HGF) [91], monocyte chemoattractant protein 1 (MCP-1, CCL2) [92], macrophage inhibitory factor (MIF) [93], and eotaxin (CCL11) [94] that can trigger a cascade of proinflammatory reactions in endometrium of women with disease. In addition, some of these cytokines and chemokines, among others, have been related to infertility, including CCL2, CCL5, CXCL1, CXCL8, CXCL13, CXCL14, CX3CL1, CXCL9, CXCL10, IL-32, CXCR2, and IL-7R [90,94]. Herein, we describe the most studied cytokines and chemokines in the context of endometrium in women with endometriosis (Fig. 11.5).

Interleukin-1 (IL-1)

There are 11 members of the IL-1 family that display proinflammatory properties [95]. IL-1α and IL-1β are produced by mononuclear and epithelial cells in response to injury, leading to inflammation [96]. They act on mononuclear cells by positive feedback, increasing further production of IL-1 and IL-6 [97]. IL-1β is increased in eSF of women with endometriosis as well as in ectopic lesions [91,98]. In endometriosis, IL-1β inhibits connexin-43 (Cx43), a critical protein for embryo implantation; moreover, it also inhibits eSF differentiation/decidualization [99], key for pregnancy establishment. Of note, eSF treated with IL-1β in vitro display decreased Cx43, along with inhibited secretion of the decidualization markers prolactin and IGFBP-1 [99]. Mechanisms underlying this process have promise to reveal therapeutic targets to correct the dysregulated decidualization in women with endometriosis.

Interleukin-6 (IL-6)

IL-6 is a cytokine that it is primarily produced in sites of acute and chronic inflammation. Increased production of macrophage-derived IL-6 and IL-8 have been described in patients with endometriosis [8]. IL-6 plays a role in many chronic inflammatory conditions, promotes B cell and TC activation and is secreted by endometrial Mφ and epithelial cells [8]. IL-6 is involved in growth and differentiation of many immunocompetent cell, and is increased in endometrium of women with endometriosis and in ectopic lesions [91,98]. In addition, Tseng et al. found increased basal and IL-1β-stimulated production of IL-6 in an in vitro study by using eSF from endometrium of women with endometriosis compared to controls without the disease [82]. This indicates that endometrium of women with endometriosis displays a chronic inflammatory milieu.

Interleukin-8 (IL-8 or CXCL8)

IL-8 has a role involved in the chemotaxis of NT during inflammation [96] and is a potent angiogenic agent [97]. This molecule, as well as its receptors, is overexpressed in whole tissue endometrium of women with endometriosis and may act as an autocrine growth factor therein [91,98]. It stimulates adhesion of endometrial cells to fibronectin, suggesting a role for it in the pathophysiology of the disease by promoting cell attachment [100]. In addition, IL-8 is elevated in peritoneal fluid of women with endometriosis and its levels correlate with the disease severity [100]. Thiazolidinedione drugs attenuate IL-6 and IL-8 production, resulting in a potent antiinflammatory effect [94] and making these two chemokines candidate targets for endometriosis treatment.

Tumor necrosis factor alpha (TNF-α)

TNF-α is produced by Mφ, uNK, NT, and Th1 cells [101]. It has angiogenic effects, activates the NFkB inflammatory pathway and is increased in endometrium and peritoneal fluid and endometrium of women with early stage endometriosis [96]. TNF-α activates inflammatory leukocytes and stimulates Mφ to produce other proinflammatory cytokines such as IL-1, IL-6, and more TNF-α [97], thereby enhancing the proinflammatory phenotype of endometrium of women with endometriosis. It also induces expression of COX2, an enzyme that regulates the formation of PGE_2, which can attenuate Mφ cytotoxicity and promote local E_2 synthesis, cell proliferation, and angiogenesis, promoting an enhanced tissue growth.

RANTES (CCL5)

RANTES, a chemoattractant for monocytes, secreted by some epithelial and mesenchymal cells [101], T helper cells and EN, is also upregulated in eSF in response to IL-1 and TNF-α [102], suggesting that it is an important mediator in acute and chronic inflammation [101]. In addition, it has been demonstrated that eSF from women with and without endometriosis also produce this cytokine in vitro after stimulation with TNF-α, IFN-γ, E_2 and MPA, the latter mimicking the secretory phase of the menstrual cycle [103]. As with most of the cytokines noted herein, CCL5 is also increased significantly in peritoneal fluid of women with endometriosis compared to controls and correlates with disease stage [104]. There is clear evidence that CCL5 recruits Mφ in ectopic endometriotic lesions [94]. By extrapolation, it is tempting to propose that elevated CCL5 in endometrium of women with disease contributes to the proinflammatory endometrial phenotype detrimental to pregnancy establishment and maintenance in affected women.

Monocyte chemoattractant protein 1 (MCP-1/CCL2)

As its name indicates, monocyte chemoattractant protein 1 (MCP-1), regulates infiltration and migration of monocytes and Mφ to sites of inflammation. In endometrium of women with endometriosis, CCL2 is increased compared to controls. It is produced by epithelial cells and Mφ, consistent with greater infiltration of Mφ in endometrium of women with endometriosis (*see above*). CCL2 is also increased in peritoneal fluid of women with endometriosis, and its levels correlate with the disease severity [94], suggesting that this chemokine contributes to the proinflammatory environment in the endometrium and the pelvic cavity of women with disease.

Eotaxin (CCL11)

Eotaxin, produced by endometrial epithelium, is a chemoattractant for NT and EN and is believed to be involved in the pathogenesis of endometriosis and angiogenesis [105,106]. Epithelial cells in endometrium of women with endometriosis secrete more CCL11, compared to controls [95]. As with CCL2, CCL11 is elevated in peritoneal fluid and is related to disease severity [94].

Interleukin 11 (IL-11)

Interleukin-11 is a member of the IL-6 family of cytokines and is released by decidualized cells and acts on adjacent nondecidualized eSF [22]. In addition, it is released by epithelial cells and eSF in human endometrium, and its release is enhanced by other cytokines, such as TNF-α, IL-1α and TGF-β in vitro, all postulated to have a role in embryo implantation [107]. This interleukin is essential for embryo implantation in mice [108]. In eutopic endometrium of infertile women with endometriosis (regardless of disease stage), IL-11 is decreased during the window of implantation [109]. However, no significant differences between IL-11 levels in uterine flushings of women with minimal stage of disease have been reported [110], suggesting no receptivity defect promoted by endometrial IL-11 levels in infertile women with disease.

Role of endometrial cells in endometriosis pathogenesis and tissue function/dysfunction

Endometriosis pathogenesis

Differences in numbers and, to some extent, activation status of endometrial immune populations in women with and without endometriosis (Fig. 11.1) suggest potential roles for these cells in the pathogenesis of the disease. For example, endometrial Mφ and DCs increase in the secretory and menstrual phases normally, presumably to eliminate cell debris and apoptotic endometrial cells during the endometrial shedding [111]. However, this phenomenon does not occur in women with endometriosis [111], suggesting inefficient clearance of endometrial cells and debris during menses and thereby facilitating establishment of endometriotic lesions (Fig. 11.2). Moreover, coculture of eSF with Mφ significantly increased the eSF clonogenic and invasion properties [112]. These studies overall support the theory that abnormal Mφ can promote *establishment* of endometriosis. Survival of endometriotic lesions is due mainly to their highly angiogenic characteristics and decreased apoptotic potential [113–115].

Another immune population that could be involved in the pathogenesis of the disease is Treg, as in one study, Foxp3, a marker of Treg, was significantly higher in endometrium of patients with advanced endometriosis compared to lesser disease and controls [116].

In addition, BCL2, as well as adhesion molecules, such as ICAM3 and SELL, are increased in endometrium of women endometriosis [89,117]. This indicates that endometrial epithelial cells and eSF in some women have higher survival and greater adhesive properties, increasing their capacity to implant and adhere to the mesothelium favoring, therein, the development of endometriosis (Fig. 11.2). Importantly, cyclic fluctuations of endometrial immune populations and their aberrant functionalities may also affect endometrial function—e.g., embryo implantation (*see below*).

Tissue shedding

Some immune populations are involved in menstruation, the process involving tissue breakdown and bleeding due to multiple factors and involving multiple cell types. An increase of ROS in eSF activates the NFkB pathway, which activates transcription of proinflammatory genes resulting in increased prostaglandins, chemokines and matrix metalloproteinases (MMPs). These activate secretion of degradative enzymes from different leukocytes and other cell types (e.g., eSF) that, together with the hypoxic environment induced by prostaglandins, produce endometrial desquamation. The NFkB pathway is constitutively activated in eSF in women with endometriosis [118], suggesting it may enhance duration of menstruation (Fig. 11.2). Hypermenorrhea is a common symptom of endometriosis, and some immune populations are implicated in heavy menstrual bleeding in some women with disease. In general, the main endometrial immune populations involved in this process are Mφ, MCs, DCs, NT, Treg, and EN [61]. Withdrawal of progesterone during the secretory phase enhances the secretion of specific molecules by endometrial cells, such as IL-8 and MCP-1, which, in turn, increase the influx of these populations to the endometrium.

Importantly, the increase of Mφ during menses suggests an important role for this cell type in defending the host from microbial entry into the degraded endometrium when the epithelial layer is breached. NT have a similar role, as they express defensins. That these two populations behave differently in endometriosis during menstruation suggests that protection against microbial agents may be altered in women with disease (Fig. 11.2). Notably, the endometrial microbiome of women with endometriosis is different from healthy endometrium [75].

Another population involved in the process of menstruation are MCs. MCs trigger a cascade of MMPs necessary to degrade the extracellular matrix resulting in tissue desquamation. As described above, in healthy endometrium, these cells increase their activity during menses; however, this process does not occur in endometriosis [7], indicating that the detachment process of endometrium may be altered in women with disease. Nonetheless, their influx is also increased in menstrual phase [7]. The increase of proinflammatory mediators produced by these cells may contribute to prolonged bleeding during menses in women with endometriosis and can affect recruitment of other endometrial leukocytes necessary for a programmed endometrial breakdown (Fig. 11.2). In addition, MCs also secrete heparin, an anticoagulant, which could also contribute to abnormal uterine bleeding [61].

Dysmenorrhea and pelvic pain

The most common symptoms of endometriosis are dysmenorrhea and chronic pelvic pain. Other symptoms are pain during intercourse (dyspareunia), diarrhea, constipation, fatigue, and nausea [4]. Some symptoms may derive from immune cell aberrations. For example, among other functions, Mφ are also involved in neurogenesis [119]. An imbalance in sensory innervation together with secretion of different cytokines increase neurogenesis in endometrium of women with endometriosis and peripheral neuro-inflammation, which may increase pain perception [119]. Moreover, dysmenorrhea and chronic pelvic pain are mostly derived from the actions of proinflammation cytokines, chemokines and prostaglandins. The latter are key modulators of dysmenorrhea (Fig. 11.2). Under the influence of P_4, prostaglandin dehydrogenase (PGDH) levels are maintained in endometrium, metabolizing prostaglandins [22]. With progesterone withdrawal preceding menses, PGDH is downregulated. As endometrium in women endometriosis is P_4-resistant [11], this mechanism could result in elevated levels of prostaglandins, accompanied by increased uterine peristalsis and cramping during menstruation.

Tissue regeneration and repair

Some immune populations are also involved in tissue regeneration and repair. Mφ and NT can change their phenotypes from pro- to antiinflammatory and contribute to reepithelialization and endometrial repair [22]. Mφ2 are known to have tissue regenerative and angiogenesis properties but, in endometriosis, they show an Mφ1 phenotype. Thus, it is feasible to extrapolate that it could delay regeneration of the endometrium, as they do not express their normal angiogenic phenotype (Fig. 11.2). Since endometrial breakdown and repair occur simultaneously, it is difficult to define the phenotypes associated with each condition and more studies are needed to determine which mechanisms are dysfunctional in this population in endometriosis, e.g., altered activation/inhibition pathways or receptors.

Another population involved in tissue regeneration is the CD8+ TCs, which are cytotoxic in the regenerative and proliferative phases, likely facilitating clearance of tissue debris from the endometrial cavity after endometrial breakdown [61]. However, in endometriosis, and as discussed above, their cytotoxicity is diminished, contributing as well to ineffective cell debris clearance. Endometrial cells that are dysfunctional in women with endometriosis, by either not clearing the cell debris or not properly regenerating the endometrial layer, could have an effect on the duration and volume of menstruation. More studies are needed to elucidate specific mechanisms underlying dysfunctions in endometrial cells from women with endometriosis, as the roles of these populations and mediators in the menstrual phase have been mostly described in women without the disease.

Implications for pregnancy

Immune cells

The immune system is fundamental to human reproduction, since pregnancy success depends on its homeostasis. A network of chemokines and cytokines mediates the dialogue between the maternal immune cells, the trophoblast, and the decidua. The endometrium

takes on great importance, as it plays an indispensable role in embryo implantation and successful pregnancy. Appropriate cytokine balance, sufficient levels of steroid hormones, and correct phenotype of endometrial immune cells and other endometrial cell functionalities are key. Given the dynamic involvement of the endometrial immune niche in endometrial function, pregnancy establishment, tolerance of the fetus, and custodians against infection, abnormalities in this niche can have severe consequences.

Appropriate functions of specific immune populations are crucial for cell-mediated immune tolerance. For example, TCs promote maternal-fetal immune tolerance; uNK cells regulate trophoblast invasion and enhance vascular remodeling by the extravillous trophoblast; and Mφ and DCs and their subtypes protect against infection [48,120,121]. Abnormal functions could result in infertility or have adverse consequences for the fetus. During pregnancy, there is influx of immune cells in the endometrium that are necessary for placental formation and fetal development. However, in abnormal microenvironments, recognizing the embryo as foreign and attacking it, could lead to adverse pregnancy and reproductive outcomes. Notably, women with endometriosis have higher risk of infertility due, in part, to endometrial abnormalities [7,102]. They also have higher risks of ectopic pregnancy and miscarriage, and implantation disorders, e.g., preeclampsia [122].

Specific immune populations may be implicated in embryo implantation abnormalities or complications in pregnancy in women with endometriosis, including mature DCs (mDCs), NT, T helper cells, and uNK cells. For example, mDCs density is significantly reduced in women with the disease [40,123] accompanied by predominance of iDCs in women endometriosis, resulting in a more proinflammatory and inhospitable environment for pregnancy compared to controls. Another example is the augmented NT influx (*see above*) that may also have implications in pregnancy success. NT produce IFN-γ in eutopic endometrium [124]. This cytokine has different roles in endometrium by controlling its growth, differentiation and immune responses accompanying implantation and/or maintenance of pregnancy [125]. If there is dysregulation of NT homeostasis in endometrium, consequences in reproductive outcomes could be significant.

Th2 cells increase during the secretory phase [126] and their numbers are increased in successful early pregnancies [127]. The suppression of a proinflammatory environment in normal eutopic endometrium is a mechanism that facilitates embryo implantation and pregnancy maintenance. Th1 predominate in normal nonpregnant human endometrium, while Th2 are higher in early pregnancy [126]. Cytokines released by Th1 are associated with pregnancy loss and infertility [124], while enhanced Th2 responses are associated with fetal survival [128]. There is lack of information of Th1 in endometrium of women with endometriosis. However, it is known that Th1 responses are increased in peripheral blood of women with endometriosis [74] as well as in endometriotic lesions [59]. Therefore, it is of great interest to study these two populations in endometrium of women with endometriosis. In addition, Treg cells were increased in endometrium of women with primary infertility and endometriosis, compared to controls [116]. Furthermore, one of the most important endometrial immune populations in pregnancy establishment and maintenance is uNK cells [129]. They produce angiogenic factors, such as angiotensin 2 (ANG2), to promote blood vessels maturation, facilitating formation of endometrial spiral arterioles, crucial for a successful implantation and pregnancy [130,131]. They also secrete cytokines such as TNF-α, TGF-β, IL-2, LIF, GM-CSF, CSF-1, CXCL10, and CXCL12 [131] that regulate trophoblast invasion

and promote decidualization of eSF and successful development of the placenta. Hence, uNK are key players in embryo implantation [132]. Dysregulation of their numbers or functionality could produce implantation failure, as has been reported in women with endometriosis [132] (Fig. 11.2). In normal conditions, uNK have decreased cytotoxicity during secretory phase, which allows a suitable environment for the development of the placenta, facilitating embryo implantation [50]. In endometriosis, uNK cells have less cytotoxicity [56]. However, in infertile, but not fertile women with endometriosis, uNK cytotoxicity is increased [133]. uNK may produce cytotoxic factors against trophoblast cells, leading to infertility and/or miscarriage, which are both more prevalent in women with versus without endometriosis [133]. Therefore, uNK have an essential role in infertility in women with disease. A lack of uNK maturation in endometrium of women with endometriosis has been reported [47], suggesting that development of mature uNK cells is dysfunctional in affected women. These defects could lead to an abnormal endometrial environment during pregnancy and increase implantation failure in women with endometriosis.

Nonimmune cells

Cells other than endometrial immune populations are implicated in potential implantation defects in women with endometriosis. As described above, eSF cultured in vitro from women with endometriosis display a P_4-resistance and a proinflammatory phenotype compared to controls [80] and endometrium of women with endometriosis has reduced decidualization capacity [78] due to their resistance to P_4 [80,134]. Teleologically, this may lead to compromised implantation [135]. Interestingly, P_4 has well described antiinflammatory properties [8], suggesting that due to the low response to this hormone by eSF, the endometrial environment may be even more proinflammatory compared with P_4-responsive tissue. During the secretory phase, epithelial cells and eSF differentiate in response to P_4. However, in women with endometriosis P_4-target genes and key receptivity markers are dysregulated during the window of implantation, suggesting aberrant epithelial glandular development in endometrium of women with endometriosis [136]. The poor differentiation of the eSF, due to its P_4-resistance, compromises its paracrine signaling to adjacent epithelial cells and thus differentiation of the latter, important for nidation and pregnancy success (Fig. 11.2). Notably, deficiency of retinoic acid produces deficiency of HDS17B2, an essential enzyme to inactivate E_2 in the epithelium-embryo interface during the implantation window [137]. Thus, an increase of E_2 that enhances estrogen-driven inflammation by recruiting proinflammatory immune cells, may lead to implantation failure in endometrium of women with disease [138].

Altogether, these processes can compromise implantation and lead to poor obstetric outcomes in women with endometriosis. Nonetheless, more studies are needed to elucidate the role of each specific endometrial cell population and the factors involved in these interactions, to drive discovery of practical treatments for implantation failure and poor pregnancy outcomes in women with endometriosis.

Conclusions

Altogether, multiple studies support the concept that the endometrial niche in endometrium of women with endometriosis is more proinflammatory than in women without disease, disrupting endometrial function and contributing to associated symptoms and

compromised pregnancy outcomes. Fig. 11.2 shows a summary of the concepts proposed in this chapter. The impact of the proinflammatory endometrial environment on pregnancy outcomes of women with endometriosis awaits further study. Moreover, deep phenotyping of the immune populations within the endometrium of women with disease is of paramount importance.

Funded by: NIH NICHD National Centers for Translational Research in Reproduction and Infertility P50-HD055764.

References

[1] Hess AP, Hamilton AE, Talbi S, et al. Decidual stromal cell response to paracrine signals from the trophoblast: amplification of immune and angiogenic modulators. Biol Reprod 2007;76(1):102–17. https://doi.org/10.1095/biolreprod.106.054791.

[2] Vannuccini S, Clifton VL, Fraser IS, et al. Infertility and reproductive disorders: impact of hormonal and inflammatory mechanisms on pregnancy outcome. Hum Reprod Update 2016;22(1):104–15. https://doi.org/10.1093/humupd/dmv044.

[3] Giudice LC. Clinical practice: endometriosis. N Engl J Med 2010;362(25):2389–98. https://doi.org/10.1056/NEJMcp1000274.CLINICAL.

[4] Zondervan KT, Becker CM, Koga K, Missmer SA, Taylor RN, Viganò P. Endometriosis. Nat Rev Dis Prim. 2018;4(1):9. https://doi.org/10.1038/s41572-018-0008-5.

[5] Klemmt PA, Starzinski-Powitz A. Molecular and cellular pathogenesis of endometriosis. Curr Women s Heal Rev 2017;13(999):1–11. https://doi.org/10.2174/1573404813666170306163448.

[6] Liu H, Lang JH. Is abnormal eutopic endometrium the cause of endometriosis? The role of eutopic endometrium in pathogenesis of endometriosis. Med Sci Mon Int Med J Exp Clin Res 2011;17(4):RA92–R99. https://doi.org/10.12659/MSM.881707.

[7] Vallvé-Juanico J, Houshdaran S, Giudice LC. The endometrial immune environment of women with endometriosis. Hum Reprod Update 2019;25(5):565–92. https://doi.org/10.1093/humupd/dmz018.

[8] Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. Fertil Steril 2012;98(3):511–9. https://doi.org/10.1016/j.fertnstert.2012.06.029.

[9] Tamaresis JS, Irwin JC, Goldfien GA, et al. Molecular classification of endometriosis and disease stage using high-dimensional genomic data. Endocrinology 2014;155(12):4986–99. https://doi.org/10.1210/en.2014-1490.

[10] Poli-neto OB, Meola J, Rosa-e-silva JC, Tiezzi D. Transcriptome meta-analysis reveals differences of immune profile between eutopic endometrium from stage I-II and III-IV endometriosis independently of hormonal milieu. Sci Rep 2020:1–17. https://doi.org/10.1038/s41598-019-57207-y.

[11] Barragan F, Irwin JC, Balayan S, et al. Human endometrial fibroblasts derived from mesenchymal progenitors inherit progesterone resistance and acquire an inflammatory phenotype in the endometrial niche in endometriosis. Biol Reprod 2016;94(5):1–20. https://doi.org/10.1095/biolreprod.115.136010.

[12] Reis FM, Petraglia F, Taylor RN. Endometriosis: hormone regulation and clinical consequences of chemotaxis and apoptosis. Hum Reprod Update 2013;19(4):406–18. https://doi.org/10.1093/humupd/dmt010.

[13] George AF, Jang KS, Nyegaard M, Neidleman J, Spitzer TB, Xie G, et al. Seminal plasma promotes decidualization of endometrial stromal fibroblasts in vitro from women with and without inflammatory disorders in a manner dependent on interleukin-11 signaling. Hum Reprod 2020;35(3):617–40. https://doi.org/10.1093/humrep/deaa015. PMID: 32219408; PMCID: PMC7105328.

[14] Bulun SE. Endometriosis. N Engl J Med 2009;360(3):268–79. https://doi.org/10.1056/NEJMra0804690.

[15] Ota HI, Shinichi Tanaka T. Expression of γδT cells and adhesion molecules in endometriotic tissue in patients with endometriosis and adenomyosis. Am J Reprod Immunol 1996;35(5):477–82. https://doi.org/10.1111/j.1600-0897.1996.tb00128.x.

[16] Sharpe-Timms KL. Endometrial anomalies in women with endometriosis. Ann N Y Acad Sci 2001;943:131–47. https://doi.org/10.1111/j.1749-6632.2001.tb03797.x.

[17] Tokushige N, Markham R, Russell P, Fraser IS. High density of small nerve fibres in the functional layer of the endometrium in women with endometriosis. Hum Reprod 2006;21(3):782–7. https://doi.org/10.1093/humrep/dei368.

[18] Aghaey Meibody F, Mehdizadeh Kashi A, Zare Mirzaie A, et al. Diagnosis of endometrial nerve fibers in women with endometriosis. Arch Gynecol Obstet 2011;284(5):1157–62. https://doi.org/10.1007/s00404-010-1806-5.
[19] Givan AL, White HD, Stern JE, et al. Flow cytometric analysis of leukocytes in the human female reproductive tract: comparison of fallopian tube, uterus, cervix, and vagina. Am J Reprod Immunol 1997;38(5):350–9.
[20] Yeaman G, Collins J. $CD8^+$ T cells in human uterine endometrial lymphoid aggregates: evidence for accumulation of cells by trafficking. Immunology 2001;2:434–40.
[21] King AE, Critchley HOD. Oestrogen and progesterone regulation of inflammatory processes in the human endometrium. J Steroid Biochem Mol Biol 2010;120(2–3):116–26. https://doi.org/10.1016/j.jsbmb.2010.01.003.
[22] Evans J, Salamonsen LA. Inflammation, leukocytes and menstruation. Rev Endocr Metab Disord 2012;13(4):277–88. https://doi.org/10.1007/s11154-012-9223-7.
[23] Flynn L, Byrne B, Carton J, O'Farrelly C, Kelehan P, O'Herlihy C. Menstrual cycle dependent fluctuations in NK and T-lymphocyte subsets from non-pregnant human endometrium. Am J Reprod Immunol 2000;43(4):209–17. https://doi.org/10.1111/j.8755-8920.2000.430405.x.
[24] Lee SK, Kim CJ, Kim D-J, Kang J-H. Immune cells in the female reproductive tract. Immune Netw 2015;15(1):16–26. https://doi.org/10.4110/in.2015.15.1.16.
[25] Wira CR, Fahey JV, Sentman CL, Pioli PA, Shen L. Innate and adaptive immunity in female genital tract: cellular responses and interactions. Immunol Rev 2005;206:306–35. https://doi.org/10.1111/j.0105-2896.2005.00287.x.
[26] Starkey PM, Clover LM, Rees MC. Variation during the menstrual cycle of immune cell populations in human endometrium. Eur J Obstet Gynecol Reprod Biol 1991;39(3):203–7. https://doi.org/10.1016/0028-2243(91)90058-S.
[27] Salamonsen LA, Zhang J, Brasted M. Leukocyte networks and human endometrial remodelling. J Reprod Immunol 2002;57(1–2):95–108. https://doi.org/10.1016/S0165-0378(02)00011-6.
[28] Ning F, Liu H, Lash GE. The role of decidual macrophages during normal and pathological pregnancy. Am J Reprod Immunol 2016;75(3):298–309. https://doi.org/10.1111/aji.12477.
[29] Deloia JA, Stewart-akers AM, Brekosky J. Effects of exogenous estrogen on uterine leukocytes recruitment. Fertil Steril 2002;77(3):548–54.
[30] De M, Wood GW. Influence of oestrogen and progesterone on distribution in the mouse uterus. J Endocrinol 1990;126:417–24.
[31] Bonatz G, Hansmann M-L, Buchholz F, Mettler L, Radzun HJ, Semm K. Macrophage- and lymphocyte-subtypes in the endometrium during different phases of the ovarian cycle. Int J Gynaecol Obstet 1992;37:29–36.
[32] King A. Uterine leukocytes and decidualization. Hum Reprod Update 2000;6(1):28–36. https://doi.org/10.1093/humupd/6.1.28.
[33] Berbic M, Schulke L, Markham R, Tokushige N, Russell P, Fraser IS. Macrophage expression in endometrium of women with and without endometriosis. Hum Reprod 2009;24(2):325–32. https://doi.org/10.1093/humrep/den393.
[34] Jensen AL, Collins J, Shipman EP, Wira CR, Guyre PM, Pioli PA. A subset of human uterine endometrial macrophages is alternatively activated. Am J Reprod Immunol 2012;68(5):374–86. https://doi.org/10.1111/j.1600-0897.2012.01181.x.
[35] Mantovani A, Sica A, Sozzani S, Allavena P, Vecchi A, Locati M. The chemokine system in diverse forms of macrophage activation and polarization. Trends Immunol 2004;25(12):677–86. https://doi.org/10.1016/j.it.2004.09.015.
[36] Cominelli A, Gaide Chevronnay HP, Lemoine P, Courtoy PJ, Marbaix E, Henriet P. Matrix metalloproteinase-27 is expressed in $CD163^+$/$CD206^+$M2 macrophages in the cycling human endometrium and in superficial endometriotic lesions. Mol Hum Reprod 2014;20(8):767–75. https://doi.org/10.1093/molehr/gau034.
[37] Takebayashi A, Kimura F, Kishi Y, et al. Subpopulations of macrophages within eutopic endometrium of endometriosis patients. Am J Reprod Immunol 2015;73(3):221–31. https://doi.org/10.1111/aji.12331.
[38] Vallvé-Juanico J, Santamaria X, Vo KC, Houshdaran S, Giudice LC. Macrophages display proinflammatory phenotypes in the eutopic endometrium of women with endometriosis with relevance to an infectious etiology of the disease. Fertil Steril 2019;112(6):1118–28. https://doi.org/10.1016/j.fertnstert.2019.08.060.
[39] Schulke L, Manconi F, Markham R, Fraser IS. Endometrial dendritic cell populations during the normal menstrual cycle. Hum Reprod 2008;23(7):1574–80. https://doi.org/10.1093/humrep/den030.
[40] Schulke L, Berbic M, Manconi F, Tokushige N, Markham R, Fraser IS. Dendritic cell populations in the eutopic and ectopic endometrium of women with endometriosis. Hum Reprod 2009;24(7):1695–703. https://doi.org/10.1093/humrep/dep071.

[41] Maridas DE, Hey-Cunningham AJ, Ng CHM, Markham R, Fraser IS, Berbic M. Peripheral and endometrial dendritic cell populations during the normal cycle and in the presence of endometriosis. J Endometr 2014;6(2):92–9. https://doi.org/10.5301/je.5000180.
[42] Sivridis E, Giatromanolaki A, Agnantis N, Anastasiadis P. Mast cell distribution and density in the normal uterus–metachromatic staining using lectins. Eur J Obstet Gynecol Reprod Biol 2001;98(1):109–13. http://www.ncbi.nlm.nih.gov/pubmed/11516809.
[43] Matsuzaki S, Canis M, Darcha C, Fukaya T, Yajima A, Bruhat MA. Increased mast cell density in peritoneal endometriosis compared with eutopic endometrium with endometriosis. Am J Reprod Immunol 1998;40(4):291–4.
[44] Jeziorska M, Salamonsen LA, Woolley DE. Mast cell and eosinophil distribution and activation in human endometrium throughout the menstrual cycle. Biol Reprod 1995;53(2):312–20. https://doi.org/10.1095/biolreprod53.2.312.
[45] Sugamata M, Ihara T, Uchiide I. Increase of activated mast cells in human endometriosis. Am J Reprod Immunol 2005;53(3):120–5. https://doi.org/10.1111/j.1600-0897.2005.00254.x.
[46] Gleich GJ, Adolphson CR. The eosinophilic leukocyte: structure and function. Adv Immunol 1986;39(C):177–253. https://doi.org/10.1016/S0065-2776(08)60351-X.
[47] Thiruchelvam U, Wingfield M, O'Farrelly C. Increased uNK progenitor cells in women with endometriosis and infertility are associated with low levels of endometrial stem cell factor. Am J Reprod Immunol 2016;75(4):493–502. https://doi.org/10.1111/aji.12486.
[48] Lee JY, Lee M, Lee SK. Role of endometrial immune cells in implantation. Clin Exp Reprod Med 2011;38(3):119–25. https://doi.org/10.5653/cerm.2011.38.3.119.
[49] Manaster I, Mizrahi S, Goldman-Wohl D, et al. Endometrial NK cells are special immature cells that await pregnancy. J Immunol 2008;181(3):1869–76. https://doi.org/10.4049/jimmunol.181.3.1869.
[50] Mselle TF, Meadows SK, Eriksson M, et al. Unique characteristics of NK cells throughout the human female reproductive tract. Clin Immunol 2007;124(1):69–76. https://doi.org/10.1016/j.clim.2007.04.008.
[51] Salamonsen LA, Woolley DE. Menstruation: induction by matrix metalloproteinases and inflammatory cells. J Reprod Immunol 1999;44(1–2):1–27. https://doi.org/10.1016/S0165-0378(99)00002-9.
[52] Tang AW, Alfirevic Z, Quenby S. Natural killer cells and pregnancy outcomes in women with recurrent miscarriage and infertility: a systematic review. Hum Reprod 2011;26(8):1971–80. https://doi.org/10.1093/humrep/der164.
[53] Drury JA, Parkin KL, Coyne L, Giuliani E, Fazleabas AT, Hapangama DK. The dynamic changes in the number of uterine natural killer cells are specific to the eutopic but not to the ectopic endometrium in women and in a baboon model of endometriosis. Reprod Biol Endocrinol 2018;16(1):1–11. https://doi.org/10.1186/s12958-018-0385-3.
[54] Jones RK, Bulmer JN, Searle RF. Immunohistochemical characterization of stromal leukocytes in ovarian endometriosis: comparison of eutopic and ectopic endometrium with normal endometrium. Fertil Steril 1996;66(1):81–9. https://doi.org/10.1016/S0015-0282(16)58391-5.
[55] Herington JL, Bruner-Tran KL, Lucas JA, Osteen KG. Immune interactions in endometriosis. Expet Rev Clin Immunol 2011;7(5):611–26. https://doi.org/10.1586/eci.11.53.
[56] Petta CA, Peloggia A, Salamonsen L, De Angelo Andrade L, Zhang J, Bahamondes L. Natural killer cells and telomerase in the endometrium of patients with endometriosis. J Endometr 2010;2(4):182–8.
[57] Arruvito L, Sanz M, Banham AH, Fainboim L. Expansion of $CD4^+CD25^+$ and $FOXP3^+$ regulatory T cells during the follicular phase of the menstrual cycle: implications for human reproduction. J Immunol 2007;178(4):2572–8. https://doi.org/10.4049/jimmunol.178.4.2572.
[58] Sakaguchi S, Miyara M, Costantino CM, Hafler DA. $FOXP3^+$ regulatory T cells in the human immune system. Nat Rev Immunol 2010;10(7):490–500. https://doi.org/10.1038/nri2785.
[59] Takamura M, Koga K, Izumi G, Hirata T, Harada M, Hirota Y. Simultaneous detection and evaluation of four subsets of $CD4^+$ T lymphocyte in lesions and peripheral blood in endometriosis. 2015. https://doi.org/10.1111/aji.12426.
[60] Guerin LR, Prins JR, Robertson SA. Regulatory T-cells and immune tolerance in pregnancy : a new target for infertility treatment. Hum Reprod Update 2009;15(5):517–35. https://doi.org/10.1093/humupd/dmp004.
[61] Berbic M, Ng CHM, Fraser IS. Inflammation and endometrial bleeding. Climacteric 2014;17(Suppl. 2):47–53. https://doi.org/10.3109/13697137.2014.963964.

[62] Bulmer JN, Jones RK, Searle RF. Intraepithelial leukocytes in endometriosis and adenomyosis: comparison of eutopic and ectopic endometrium with normal endometrium. Hum Reprod 1998;13(1O):2910–5. https://doi.org/10.1093/humrep/13.10.2910.
[63] Mettler L, Volkov NI, Kulakov VI, Jurgensen A, Parwaresch MR. Lymphocyte subsets in the endometrium of patients with endometriosis throughout the menstrual cycle. Am J Reprod Immunol 1996;36(6):342–8. https://doi.org/10.1111/j.1600-0897.1996.tb00184.x.
[64] Basta P, Majka M, Jozwicki W, et al. The frequency of $CD25^{+}CD4^{+}$ and $FOXP3^{+}$ regulatory T cells in ectopic endometrium and ectopic decidua. Reprod Biol Endocrinol 2010;8:116. https://doi.org/10.1186/1477-7827-8-116.
[65] Podgaec S, Barbeiro DF, Gueuvoghlanian-Silva BY, Bellelis P, Abrão MS. Foxp3 expression in deep rectosigmoid endometriosis lesions and its association with chronic pelvic pain. J Reprod Immunol 2014;104–105(C):96–9. https://doi.org/10.1016/j.jri.2014.05.002.
[66] Podgaec S, Rizzo LV, Flavio L, Fernandes C, Baracat EC, Abrao MS. $CD4^{+}$ CD25 high $Foxp3^{+}$ cells increased in the peritoneal fluid of patients with endometriosis. Am J Reprod Immunol 2012;3:2–9. https://doi.org/10.1111/j.1600-0897.2012.01173.x.
[67] Berbic M, Hey-Cunningham AJ, Ng C, et al. The role of $Foxp3^{+}$ regulatory T-cells in endometriosis: a potential controlling mechanism for a complex, chronic immunological condition. Hum Reprod 2010;25(4):900–7. https://doi.org/10.1093/humrep/deq020.
[68] White HD, Crassi KM, Givan AL, et al. $CD3^{+}$ $CD8^{+}$ CTL activity within the human female reproductive tract: influence of stage of the menstrual cycle and menopause. J Immunol 1997;158(6):3017–27.
[69] Kersigo J, Pan N, Lederman JD, et al. A RNAscope whole mount approach that can be combined with immunofluorescence to quantify differential distribution of mRNA. Cell Tissue Res 2018;374(2):251–62. https://doi.org/10.1007/s00441-018-2864-4.A.
[70] Eyster KM, Hansen KA, Winterton E, Klinkova O, Drappeau D, Mark-Kappeler CJ. Reciprocal communication between endometrial stromal cells and macrophages. Physiol Behav 2010;17(9):1809–22. https://doi.org/10.1016/j.physbeh.2017.03.040.
[71] Nagorsen D, Marincola FM, Panelli MC. Cytokine and chemokine expression profiles of maturing dendritic cells using multiprotein platform arrays. Cytokine 2004;25(1):31–5. https://doi.org/10.1016/j.cyto.2003.08.012.
[72] Abbas N, Belghazi M, Abdel-mottaleb Y, Tytgat J, Bougis PE. Biochemical and biophysical research communications. Biochem Biophys Res Commun 2008;376(1):525–30. https://doi.org/10.1016/j.bbrc.2008.09.033.
[73] Evans J, Salamonsen LA. Decidualized human endometrial stromal cells are sensors of hormone withdrawal in the menstrual inflammatory cascade. Biol Reprod 2013;90(1):14. https://doi.org/10.1095/biolreprod.113.108175.
[74] de Barros IBL, Malvezzi H, Gueuvoghlanian-Silva BY, Piccinato CA, Rizzo LV, Podgaec S. What do we know about regulatory T cells and endometriosis? A systematic review. J Reprod Immunol 2017;120:48–55. https://doi.org/10.1016/j.jri.2017.04.003.
[75] Leonardi M, Hicks C, El-Assaad F, El-Omar E, Condous G. Endometriosis and the microbiome: a systematic review. BJOG An Int J Obstet Gynaecol 2019. https://doi.org/10.1111/1471-0528.15916.
[76] Noble LS, Takayama K, Zeitoun KM, et al. Prostaglandin E2 stimulates aromatase expression in endometriosis-derived stromal cells. J Clin Endocrinol Metab 1997;82(2):600–6. https://doi.org/10.1210/jc.82.2.600.
[77] Weimar CHE, Macklon NS, Post Uiterweer ED, Brosens JJ, Gellersen B. The motile and invasive capacity of human endometrial stromal cells: implications for normal and impaired reproductive function. Hum Reprod Update 2013;19(5):542–57. https://doi.org/10.1093/humupd/dmt025.
[78] Klemmt PAB, Carver JG, Kennedy SH, Koninckx PR, Mardon HJ. Stromal cells from endometriotic lesions and endometrium from women with endometriosis have reduced decidualization capacity. Fertil Steril 2006;85(3):564–72. https://doi.org/10.1016/j.fertnstert.2005.08.046.
[79] Aghajanova L, Tatsumi K, Horcajadas JA, et al. Unique transcriptome, pathways, and networks in the human endometrial fibroblast response to progesterone in endometriosis. Biol Reprod 2011;84(4):801–15. https://doi.org/10.1095/biolreprod.110.086181.
[80] Barragan F, Irwin JC, Balayan S, et al. Human endometrial fibroblasts derived from mesenchymal progenitors inherit progesterone resistance and acquire an inflammatory phenotype in the endometrial niche in endometriosis. Biol Reprod 2016;94(5):118. https://doi.org/10.1095/biolreprod.115.136010.
[81] Chen JC, Johnson BA, Erikson DW, et al. Seminal plasma induces global transcriptomic changes associated with cell migration, proliferation and viability in endometrial epithelial cells and stromal fibroblasts. Hum Reprod 2014;29(6):1255–70. https://doi.org/10.1093/humrep/deu047.

[82] Tseng JF, Ryan IP, Milam TD, Murai JT, Schiriock ED, Daniel V, Landers RNT. Interleukin-6 secretion in vitro is up-regulated in ectopic and eutopic endometrial stromal cells from women with endometriosis. J Clin Endocrinol Metab 1996;3(81):118–22.
[83] Wira CR, Rodriguez-Garcia M, Patel MV. The role of sex hormones in immune protection of the female reproductive tract. Nat Rev Immunol 2015;15(4):217–30. https://doi.org/10.1038/nri3819.
[84] Gargett CE, Schwab KE, Deane JA. Endometrial stem/progenitor cells: the first 10 years. Hum Reprod Update 2016;22(2):137–63. https://doi.org/10.1093/humupd/dmv051.
[85] Du H, Taylor HS. Contribution of bone marrow-derived stem cells to endometrium and endometriosis. Stem Cell 2007;25(8):2082–6. https://doi.org/10.1634/stemcells.2006-0828.
[86] Hapangama DK, Drury J, Da Silva L, et al. Abnormally located $SSEA1^{+}/SOX9^{+}$ endometrial epithelial cells with a basalis-like phenotype in the eutopic functionalis layer may play a role in the pathogenesis of endometriosis. Hum Reprod 2019;34(1):56–68. https://doi.org/10.1093/humrep/dey336.
[87] Wang Y, Chen X, Cao W, Shi Y. Plasticity of mesenchymal stem cells in immunomodulation: pathological and therapeutic implications. Nat Immunol 2014;15(11):1009–16. https://doi.org/10.1038/ni.3002.
[88] Shi Y, Su J, Roberts AI, Shou P, Rabson AB, Ren G. How mesenchymal stem cells interact with tissue immune responses. Trends Immunol 2012;33(3):136–43. https://doi.org/10.1080/00043249.2000.10792023.
[89] Ahn SH, Khalaj K, Young SL, Lessey BA, Koti M, Tayade C. Immune-inflammation gene signatures in endometriosis patients. Fertil Steril 2016;106(6):1420–31. https://doi.org/10.1016/j.fertnstert.2016.07.005. e7.
[90] Miller JE, Ahn SH, Monsanto SP, Khalaj K, Koti M, Tayade C. Implications of immune dysfunction on endometriosis associated infertility. Oncotarget 2015;8(4):7138–47. https://doi.org/10.18632/oncotarget.12577.
[91] Bondza PK, Maheux R, Akoum A. Insights into endometriosis-associated endometrial dysfunctions: a review. Front Biosci 2009;1:415–28. http://www.ncbi.nlm.nih.gov/pubmed/19482656. [Accessed 29 November 2017].
[92] Jolicoeur C, Boutouil M, Drouin R, Paradis I, Lemay A, Akoum A. Increased expression of monocyte chemotactic protein-1 in the endometrium of women with endometriosis. Am J Pathol 1998;152(1):125–33. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1858106/pdf/amjpathol00013-0122.pdf. [Accessed 12 October 2017].
[93] Akoum A, Metz CN, Al-Akoum M, Kats R. Macrophage migration inhibitory factor expression in the intrauterine endometrium of women with endometriosis varies with disease stage, infertility status, and pelvic pain. Fertil Steril 2006;85(5):1379–85. https://doi.org/10.1016/j.fertnstert.2005.10.073.
[94] Borrelli GM, Carvalho KI, Kallas EG, Mechsner S, Baracat EC, Abrão MS. Chemokines in the pathogenesis of endometriosis and infertility. J Reprod Immunol 2013;98(1–2):1–9. https://doi.org/10.1016/j.jri.2013.03.003.
[95] Dinarello CA. Overview of the IL-1 family in innate inflammation and acquired immunity. Immunol Rev 2018;281(1):8–27. https://doi.org/10.1111/imr.12621.
[96] Ahn SH, Monsanto SP, Miller C, Singh SS, Thomas R, Tayade C. Pathophysiology and immune dysfunction in endometriosis. BioMed Res Int 2015;2015. https://doi.org/10.1155/2015/795976.
[97] Witz CA. Interleukin-6: another piece of the endometriosis-cytokine puzzle. Fertil Steril 2000;73(2):212–4.
[98] Cao W-G, Morin M, Metz C, Maheux R, Akoum A. Stimulation of macrophage migration inhibitory factor expression in endometrial stromal cells by interleukin 1, beta involving the nuclear transcription factor NFκB1. Biol Reprod 2005;73(3):565–70. https://doi.org/10.1095/biolreprod.104.038331.
[99] Yu J, Berga SL, Zou W, et al. IL-1b inhibits connexin 43 and disrupts decidualization of human endometrial stromal cells through ERK1/2 and p38 MAP kinase. Endocrinology 2017;158(12):4270–85. https://doi.org/10.1210/en.2017-00495.
[100] Arici A. Local cytokines in endometrial tissue: the role of interleukin-8 in the pathogenesis of endometriosis. Ann N Y Acad Sci 2002;955:101–9. https://doi.org/10.1111/j.1749-6632.2002.tb02770.x. Discussion 118, 396–406.
[101] Manolova N, Zasheva D, Stamenova M. Immunobiology of endometriosis. Acta Med Bulg 2011;38(1):38–50.
[102] Lessey BA, Lebovic DI, Taylor RN. Eutopic endometrium in women with endometriosis: ground zero for the study of implantation defects. Semin Reprod Med 2013;31(2):109–24. https://doi.org/10.1055/s-0032-1333476.
[103] Hornung D, Dohrn K, Sotlar K, et al. Localization in tissues and secretion of eotaxin by cells from normal endometrium and endometriosis. J Clin Endocrinol Metab 2000;85(7):2604–8. https://doi.org/10.1210/jc.85.7.2604.
[104] Zhang X, Xu H, Lin J, Qian Y, Deng L. Peritoneal fluid concentrations of interleukin-17 correlate with the severity of endometriosis and infertility of this disorder. BJOG An Int J Obstet Gynaecol 2005;112(8):1153–5. https://doi.org/10.1111/j.1471-0528.2005.00639.x.
[105] OuYang Z, Osuga Y, Hirota Y, et al. Interleukin-4 induces expression of eotaxin in endometriotic stromal cells. Fertil Steril 2010;94(1):58–62. https://doi.org/10.1016/j.fertnstert.2009.01.129.

[106] Salcedo R, Young HA, Ponce ML, et al. Eotaxin (CCL11) induces in vivo angiogenic responses by human CCR3$^+$ endothelial cells. J Immunol 2001;166(12):7571–8.
[107] Cork BA, Li TC, Warren MA, Laird SM. Interleukin-11 (IL-11) in human endometrium: expression throughout the menstrual cycle and the effects of cytokines on endometrial IL-11 production in vitro. J Reprod Immunol 2001;50(1):3–17. https://doi.org/10.1016/S0165-0378(00)00089-9.
[108] Menkhorst E, Salamonsen L, Robb L, Dimitriadis E. IL11 antagonist inhibits uterine stromal differentiation, causing pregnancy failure in Mice1. Biol Reprod 2009;80(5):920–7. https://doi.org/10.1095/biolreprod.108.073601.
[109] Dimitriadis E, Stoikos C, Stafford-Bell M, et al. Interleukin-11, IL-11 receptorα and leukemia inhibitory factor are dysregulated in endometrium of infertile women with endometriosis during the implantation window. J Reprod Immunol 2006;69(1):53–64. https://doi.org/10.1016/j.jri.2005.07.004.
[110] Mikolajczyk M, Wirstlein P, Skrzypczak J. Leukaemia inhibitory factor and interleukin 11 levels in uterine flushings of infertile patients with endometriosis. Hum Reprod 2006;21(12):3054–8. https://doi.org/10.1093/humrep/del225.
[111] Capobianco A, Rovere-Querini P. Endometriosis, a disease of the macrophage. Front Immunol January 2013;4:1–14. https://doi.org/10.3389/fimmu.2013.00009.
[112] Chan RWS, Lee CL, Ng EHY, Yeung WSB. Co-culture with macrophages enhances the clonogenic and invasion activity of endometriotic stromal cells. Cell Prolif 2017;50(3):1–9. https://doi.org/10.1111/cpr.12330.
[113] Ding J, Shen J, Rana N, Fernandez BB, Dmowski WP. Relationship between apoptosis and the number of macrophages in eutopic endometrium from women with and without endometriosis. Fertil Steril 2002;78(4):830–5. https://doi.org/10.1016/S0015-0282(02)03334-4.
[114] Gebel MH, Braun DP, Tambur A, Frame D, Rana N, Dmowski WP. Spontaneous apoptosis of endometrial tissue is impaired in women with endometriosis. Fertil Steril 1998;69(6):1042–7. https://doi.org/10.1016/S0015-0282(98)00073-9.
[115] Liu G, Wu C, Wu Y, Zhao Y. Phagocytosis of apoptotic cells and immune regulation. Scand J Immunol 2006;64(1):1–9. https://doi.org/10.1111/j.1365-3083.2006.01771.x.
[116] Chen S, Zhang J, Huang C, Lu W, Liang Y, Wan X. Expression of the T regulatory cell transcription factor FoxP3 in peri-implantation phase endometrium in infertile women with endometriosis. Reprod Biol Endocrinol 2012;10(1):34. https://doi.org/10.1186/1477-7827-10-34.
[117] Cho YJ, Lee SH, Park JW, Han M, Park MJ, Han SJ. Dysfunctional signaling underlying endometriosis: current state of knowledge. J Mol Endocrinol 2018;60(3):R97–133. https://doi.org/10.1530/JME-17-0227.
[118] Kaponis A, Iwabe T, Taniguchi F, et al. The role of NF-kappaB in endometriosis. Front Biosci Sch. 2012;4 S(4):1213–34. https://doi.org/10.2741/s327.
[119] Wu J, Xie H, Yao S, Liang Y. Macrophage and nerve interaction in endometriosis. J Neuroinflammation 2017:1–11. https://doi.org/10.1186/s12974-017-0828-3.
[120] Achache H, Revel A. Endometrial receptivity markers, the journey to successful embryo implantation. Hum Reprod Update 2006;12(6):731–46. https://doi.org/10.1093/humupd/dml004.
[121] Erlebacher A. Immunology of the maternal-fetal interface. Annu Rev Immunol 2013;31(1):387–411. https://doi.org/10.1146/annurev-immunol-032712-100003.
[122] Bulleti C, Coccia M, Battistoni SBA. Endometriosis and infertility. J Assist Reprod Genet 2010;27:441–7. https://doi.org/10.1007/978-1-4471-4953-8_3.
[123] Fang R-L, Chen L-X, Shu W-S, Yao S-Z, Wang S-W, Chen Y-Q. Barcoded sequencing reveals diverse intrauterine microbiomes in patients suffering with endometrial polyps. Am J Transl Res 2016;8(3):1581–92.
[124] Yeaman GR, Collins JE, Currie JK, Guyre PM, Wira CR, Fanger MW. IFN-gamma is produced by polymorphonuclear neutrophils in human uterine endometrium and by cultured peripheral blood polymorphonuclear neutrophils. J Immunol 1998;160(10):5145–53.
[125] Tabibzadeh S. Regulatory roles of IFN-gamma in human endometrium. [Review]. Ann New York Acad Sci 1994;734:1–6.
[126] Saito S, Tsukaguchi N, Hasegawa T, Michimata T, Tsuda H, Narita N. Distribution of Th1, Th2, and Th0 and the Th1/Th2 cell ratios in human peripheral and endometrial T cells. Am J Reprod Immunol 1999;42(4):240–5. https://doi.org/10.1111/j.1600-0897.1999.tb00097.x.
[127] Krasnow JS, Tollerud DJ, Naus G, DeLoia JA. Endometrial Th2 cytokine expression throughout the menstrual cycle and early pregnancy. Hum Reprod 1996;11(8):1747–54. https://doi.org/10.1093/oxfordjournals.humrep.a019480.

[128] Wegmann TG, Lin H, Guilbert L, Mosmann TR. Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a TH2 phenomenon? Immunol Today 1993;14(7):353–6. https://doi.org/10.1016/0167-5699(93)90235-D.
[129] Bulmer JN, Morrison L, Longfellow M, Ritson A, Pace D. Granulated lymphocytes in human endometrium: histochemical and immunohistochemical studies. Hum Reprod 1991;6(6):791–8.
[130] Lysakova-Devine T, O'Farrelly C. Tissue-specific NK cell populations and their origin. J Leukoc Biol 2014;96(6):981–90. https://doi.org/10.1189/jlb.1RU0514-241R.
[131] Li XF, Charnock-jones DS, Zhang EKO, et al. Angiogenic growth factor messenger ribonucleic acids in uterine natural killer cells. J Clin Endocrinol Metab 2015;86(4):1823–34.
[132] Thiruchelvam U, Wingfield M, O'Farrelly C. Natural killer cells: key players in endometriosis. Am J Reprod Immunol 2015;74(4):291–301. https://doi.org/10.1111/aji.12408.
[133] Giuliani E, Parkin KL, Lessey BA, Young SL, Fazleabas AT. Characterization of uterine NK cells in women with infertility or recurrent pregnancy loss and associated endometriosis. Am J Reprod Immunol 2014;72(3):262–9. https://doi.org/10.1111/aji.12259.
[134] Patel BG, Rudnicki M, Yu J, Shu Y, Taylor RN. Progesterone resistance in endometriosis: origins, consequences and interventions. Acta Obstet Gynecol Scand 2017;96(6):623–32. https://doi.org/10.1111/aogs.13156.
[135] McKinnon B, Mueller M, Montgomery G. Progesterone resistance in endometriosis: an acquired property? Trends Endocrinol Metabol 2018;29(8):535–48. https://doi.org/10.1016/j.tem.2018.05.006.
[136] Kao LC, Germeyer A, Tulac S, et al. Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease. Based implantation failure and infertility. Endocrinology 2003;144(7):2870–81. https://doi.org/10.1210/en.2003-0043.
[137] Suzuki T, Moghrabi N, Andersson S, et al. Expression in endometriosis: failure to metabolize. J Clin Endocrinol Metab 1998;83(12):4474–80.
[138] Attar E, Bulun SE. Endometriosis. 2nd ed. Endometrium Mol Cell Clin Perspect; 2008. p. 691–710. https://doi.org/10.1210/er.2018-00242.

This page intentionally left blank

CHAPTER

12

Endometriosis and ovarian dysfunction

Michio Kitajima

Department of Obstetrics and Gynecology, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan

Introduction

Endometriosis is an estrogen dependent chronic inflammatory disease. Pelvic pain and infertility are main symptoms of the disease. Although exact pathogenesis of this enigmatic disease is not fully understood, retrograde menstruation may evoke local inflammation via activating innate immune system in pelvis [1]. Although most of women of reproductive age may show retrograde menstruation, the prevalence of endometriosis is estimated as about 10% of women at reproductive age and they may be found in 50% of women with infertility [2]. Implantation and survival (and/or metaplastic change) of cellular components in menstrual reflux may be affected by specific pelvic environment in women with endometriosis. These observations may indicate that individual immune response in pelvic cavity may contribute to the development of endometriosis.

Endometriotic lesions develop at pelvic peritoneum surrounding uterus and adnexa are designated as pelvic peritoneal endometriosis. These lesions may show varieties of color and shape depending on their activity (red, black, and white lesion). In the ovary, superficial implants may progress into cyst which contain chocolate like fluids (endometrioma or chocolate cyst). At the pouch of Douglas, uterosacral ligament, and recto-vaginal septum, fibrotic nodular lesions may be formed, of which are designated as deep infiltrating endometriosis (DIE). Different phenotype of endometriotic lesions may have different pathogenesis [3]. It may also discernible that immune response and inflammation surrounding the lesions may differ according to the anatomical site and phenotype of endometriosis.

There may be three distinct functions of ovary. (1) Follicular development: development of mature follicle from dormant primordial follicles to ovulation. (2) Secretory functions: maturing follicle and corpus luteum secrete sex steroid hormones that could prepare endometrium for embryo implantation. (3) Reservoir of primordial follicles: in women, the number of

Immunology of Endometriosis
https://doi.org/10.1016/B978-0-12-820661-4.00017-0

© 2022 Elsevier Inc. All rights reserved.

follicles is finite. Ovarian reserve is designated as the amount and quality of remaining follicles at certain time [4]. Protecting the dormancy of primordial follicles is one of the key roles of ovaries. Endometriosis possibly affect these ovarian functions.

In this chapter, the association between endometriosis and ovarian dysfunction are discussed according to the association between ovarian functions and local inflammation and immune reaction evoked by endometriosis. Clinical significances of ovarian dysfunction related to endometriosis also discussed.

Functional structure and cellular components of human ovary and endometriosis

Adult human ovary can be separated into two parts, the cortex and the medulla. The ovarian cortex is the outer part of the human ovary and it is covered by ovarian surface epithelium (OSE), which is a continuum of pelvic peritoneum, common developmental site of endometriosis. The OSE may show monolayer of flat, cuboidal, and columnar appearance in different areas of the same ovary. These ovarian epithelia can be invaginated into the inside of ovarian cortex to form epithelial inclusion glands. They become cystic, resulting in epithelial inclusion cysts, which have been postulated as the origin of ovarian endometrioma based on the metaplasia hypothesis [5]. Beneath the OSE, tunica albuginea, a zone of hypocellular connective tissue, and an area of cortex-specific stroma with early stage follicles are present in a layered structure.

Follicles are functional units which maintain the competence of dormant and growing oocytes via surrounding granulosa cells and theca cells. Folliculogenesis in human ovaries begins in the inner part of the cortex at 14–20 weeks of gestation. At birth, approximately 400,000–800,000 primordial follicles are diffusely packed in the ovarian cortex without gap. Primordial follicle is then progressively disappeared due to atresia. As a result, primordial follicles are found scattered irregularly in clusters (nests) throughout a narrow band of densely accumulated stroma in the superficial cortex in women of reproductive age. Fibroblast like stromal cell and connective matrix with microvasculatures surrounding follicle act as mediators of nutrients and molecular signals as well as a source of somatic cells for growing follicles [6–9]. Extracellular matrix in the ovarian cortex and specific collagen fibers may serve as a rigid frame supporting follicles and stromal cells [10]. Microfibrils, a subset of the collagen fibers that form extracellular matrix may also serve as signal transduction mediators for dormant and early growing follicles that lack vascular channels [11,12]. Spindle-shaped ovarian stromal cells are typically arranged in a storiform pattern. The appearance and density of stromal cells may differ according to the histological sites of the ovarian cortex and medulla [13,14]. The characteristics of these cellular components may be affected by menstrual cycles and the developmental stage (maturation) of follicles that differ in sensitivity to gonadotropin or juxtacrine stimuli.

The mechanism of development of endometriomas is not fully understood. The ovary is one of the most frequent anatomic sites where endometriotic lesion develop. Ovarian

endometriotic lesion can be divided into superficial ovarian lesion and ovarian endometriotic cyst. Similar to peritoneal endometriosis, superficial ovarian endometriosis may be developed by implantation of endometrial tissue derived from retrograde menstrual flows. These superficial lesions may form adhesion with pelvic peritoneum nearby. Then incessant bleeding and secretion from growing endometriotic lesion may form pseudocyst [14]. Although most of the cyst show adhesion stigma and histological structure of pseudocyst, ovarian endometriomas may also develop from the metaplasia of ovarian inclusion cyst or corpus luteum cyst [5,15]. From these points of view, endometriomas can be regarded as heterogenetic cyst mixed with multiple pathogenesis.

Along with the development of ovarian endometriotic lesions, local inflammation may occur at surrounding normal ovarian tissue. Even the microscopic superficial lesions may deteriorate homeostasis of residual normal ovarian tissue. Immune cells, such as macrophages, may accumulate at the site of implantation (Fig. 12.1). These macrophages may secrete varieties of macromolecules that promote growth, metaplasia, migration, and inflammation. Infiltrated immune cells in microscopic endometriotic lesion may affect homeostasis of dormant and growing follicles in ovarian cortical tissue (Fig. 12.1).

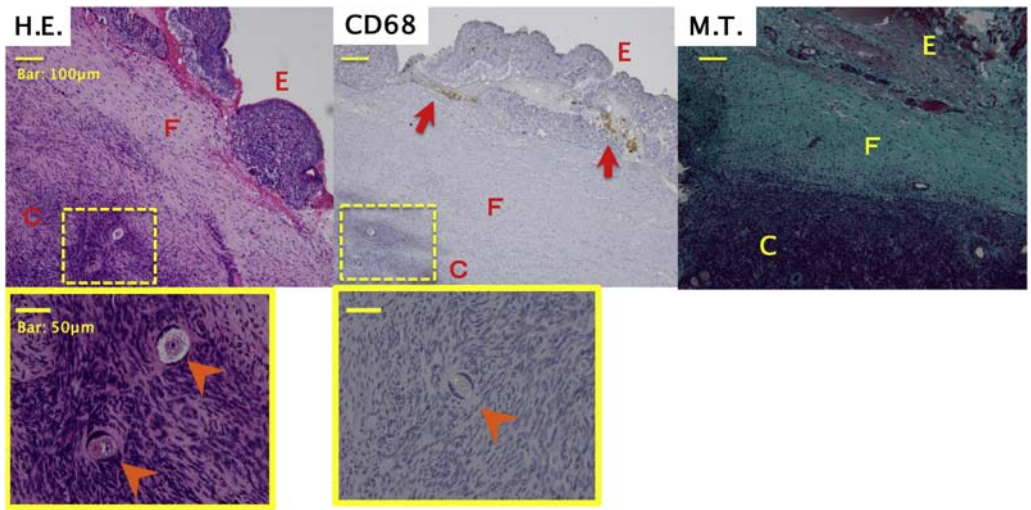


FIGURE 12.1 Representative photomicrograph of hematoxylin-eosin (H.E.) staining, immunohistochemical detection of CD68 positive cells, and Masson's trichrome (M.T.) staining of superficial ovarian cortex with endometriotic implants (E). Fibrotic deposits (F) are present at the beneath of endometriotic implants. These are confirmed by Masson's trichrome staining. Cortical specific stroma with follicles (C) suppressed under the fibrosis. At these cortical areas, morphologically atretic early follicles are found (arrow heads in solid line square, which is the view of high-power field of *dotted line* square). At the border of endometriotic lesion, the accumulation of CD68 positive macrophages are confirmed.

Histological alteration of ovarian cortex in women with endometriosis: burn-out hypothesis

Most of the follicles in the ovaries are dormant (primordial follicle). The growth and maturation of follicles are controlled by several growth factors depending on the different stage of follicular development. Anti-Müllerian hormone (AMH), which is predominantly produced by the granulosa cells of the recruited preantral and early antral follicles from the nest of primordial follicle (AMH negative) until they become sensitive to gonadotropin [16]. AMH may work as inhibitor of activation of primordial follicles [17]. In ovaries affected by endometriotic cyst (endometriomas), enhanced activation of primordial follicles is present [18]. At the same time, increased follicular atresia (apoptosis) simultaneously may occur [19]. Inflammatory response may be evoked at the site of microimplants of superficial endometriosis. Focal inflammation may cause fibrotic deposits on the surface of ovarian cortex, which may alter specific histological structures. Progression of fibrosis may destruct densely packed cortical specific stroma which maintain dormancy of primordial follicles (Fig. 12.1). Once primordial follicles have activated from resting state, these growing follicles may be exposed to inflammatory environment, which may be detrimental. Atresia of early growing follicles may decline local concentration of AMH, which may in turn decrease inhibitory signals to primordial follicles. This vicious circle of local inflammation can be hypothesized as the "burn-out" phenomenon (Fig. 12.2). As a result, the demise of early growing follicles is hole mark of dysregulations of follicular development found in the normal ovarian cortex affected by endometriomas.

Dysregulation of ovarian functions in endometriosis and clinical consequences in infertility therapy

Dysregulations of follicular development may result in lower quality oocytes, lower serum sex steroids levels, and defects in corpus luteum formation. In assisted reproduction techniques (ART), clinical outcomes related to ovarian function, such as ovarian responses to controlled ovarian stimulation (COS), the number of retrieved oocytes, the quality of retrieved oocytes, serum estradiol (E2) levels, the fertilization rate, etc., in infertility women with and without endometriosis had been studied. The results were controversial. These may be due to the difference in study design and heterogeneity of the subjects. In addition, compensatory effects of COS should be considered. From the recent systematic review, in ART treatment in women with endometriosis comparing to women without disease, cycle cancellation due to poor response of ovary is higher, the number of retrieved oocytes is lower, the fertilization rate is comparable, the implantation rate and clinical pregnancy rate is comparable but may be compromised in women with severe disease, miscarriage rate and take-home baby rate are comparable [20]. Although these results may indicate that quantity rather than quality of follicles are mainly deteriorated in women with endometriosis, oocyte quality may be deteriorated in women with endometriomas [21,22].

In women with endometriomas, the surgery, especially cystectomy for bilateral lesions, may cause detrimental effects on ovarian reserve [23]. COS could overcome decreased

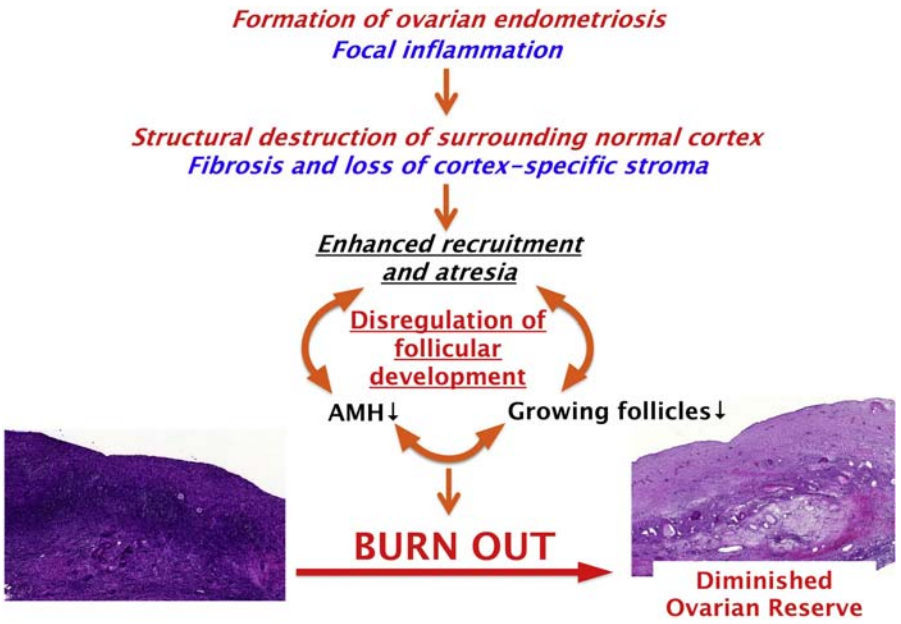


FIGURE 12.2 Altered early follicular development in the ovaries affected by endometriosis may be regarded as "burn-out" by inflammation, the hypothesis which may explain the mechanism partly responsible for the diminished ovarian reserve in women with endometriosis. Formation of ovarian endometriotic lesion may cause focal inflammation in ovarian cortex. This inflammation could result in structural alteration to the ovarian cortex, which manifest as massive fibrosis and loss of cortex-specific stroma that protect the nests of dormant primordial follicles. Focal loss of follicular density may be associated with a vicious circle of dysregulated follicular development that eventually results in burn-out of the stockpile of dormant follicles. *AMH*, anti-Müllerian hormone.

ovarian reserve though the cycles with lower number of growing follicles and low number of retrieved oocytes may be increased and cancellation of the treatment due to poor ovarian response cannot be avoided [24]. The effect of surgery on ovarian reserve may be affected by several confounding variables, such as age, size, the characteristics of the cyst wall, applied surgical technique, and surgeon's skill [25].

The effects of the endometriomas without previous surgical interventions on ART results is controversial. As afore mentioned, endometrioma per se may affect ovarian reserve [14]. However, similar to normal population, ovarian reserve may show large interindividual variance in women with endometriosis. Severity and durations of intrapelvic inflammation in women with endometriomas may be affected by coexisting other phenotypes of endometriosis, such as pelvic peritoneal lesions or DIE. Prior medical treatment and the methods of ovarian stimulation in ART cycle may modify the results. These biases should take into consideration.

There have been a few reports evaluating ovarian reserve in women with endometriosis who do not have ovarian involvement. The direct effects of pelvic subtle lesions on ovarian functions may be restrictive. Although some study indicated diminished ovarian reserve even in women without endometriomas, the data were controversial [27,28]. The efficacy of ART for women without ovarian involvement may not depend on ovarian functions.

The metaanalysis revealed that women with endometriosis but without ovarian lesions may show similar clinical results in ART therapy comparing to women without endometriosis [21,29]. On the other hand, recent reports evaluated the efficacy of endometriosis in ART treatments in women with poor ovarian response with women had been operated for endometriomas, women with unoperated endometriomas and control women with diminished ovarian reserve. The results indicated that the impact of endometriosis on ART is limited in women with already diminished ovarian reserve [30].

Immunocomplexome analysis of follicular fluids of women with endometriosis

As afore mentioned, local inflammatory reactions surrounding endometriotic lesions may affect follicular development and the process of oocyte maturation. On the other hand, several studies have reported a relationship between endometriosis and autoimmune disease [31,32]. In these reports, endometriosis shows symptoms similar to those of autoimmune disease. In addition, various auto-antibodies are found in women with endometriosis [33]. Although the relationship between autoimmune oophoritis and endometriosis had not been clearly demonstrated, a case with mild endometriosis and autoantibodies showed premature ovarian insufficiency and histological autoimmune oophoritis was reported [34].

Immune complexes (ICs) are formed by noncovalent interactions between foreign antigens or autoantigens and antibody molecules [35]. Enhanced formation and defective clearance of ICs occurs in autoimmune diseases [36]. Disease-specific antigens in circulating ICs (CIC-antigens) in serum or cerebrospinal fluid recovered from subjects with autoimmune diseases, infectious diseases, and cancers, as well as those who are liver transplant recipients were reported [37–41]. Formation and deposition of ICs may evoke local inflammatory reactions. In addition, formation of ICs with disease-specific antigens may be involved in disease pathophysiology by loss of functions of specific molecules that related to these antigens.

The contents of follicular fluid are formed by osmotic pressure gradient generated by hyaluronan and versican recruits blood exudate into the follicle, and antibodies can pass through into the fluid [42,43]. Moreover, cytokines, reactive oxygen species, and antioxidants produced by local inflammation may also be accumulated in the follicular fluid of women with endometriosis [44]. As well as defective oocyte maturation, altered follicular milieu may affect the growth of endometriosis. Follicular fluid may spill over the surface of ovary at the time of ovulation, and may affect the activity of ovarian endometriotic lesion [45].

Follicular fluids derived from women with and without endometriosis were examined by immunocomplexome analysis, in which ICs are separated from certain body fluids and then subjected to direct tryptic digestion and nano-liquid chromatography tandem mass spectrometry [38] to comprehensively identify and profile constituent antigens in ICs [46]. These analyses revealed that eight disease-specific antigens were present in women with endometriosis. Among them, antigens related to proteins might be involved in the pathogenesis of endometriosis, such as interleukin-6 receptor subunit beta (also known as gp130), probable ubiquitin carboxyl terminal hydrolase FAF-Y (deubiquitinating enzyme FAF-Y), and fibroblast growth factor receptor 1 (FGFR1) [46]. These proteins are known to regulate local inflammation, inflammasome formation, or the epithelial-to-mesenchymal transition (EMT) [47–50]. Loss of function of these molecules by formation of immunocomplex may be related

to the pathophysiology of endometriosis. Aberrant formation of immune complex in follicular fluids of women with endometriosis may indicate that immune reactions in follicular microenvironment are altered and it can deteriorate oocyte maturation in growing follicles and may affect the growth and progression of superficial ovarian endometriotic lesion or pelvic endometriosis.

Conclusions

Ovarian dysfunction is relevant in infertility associated with endometriosis. Local inflammation may affect follicular growth and primordial follicle dormancy. Diminished ovarian reserve is hole mark of dysregulation of ovarian functions in women with endometriosis. Local immune reaction participate in the development of ovarian endometriotic lesion may also affect homeostasis of follicles. Immunologically altered follicular milieu may disturb oocyte maturation and growth of endometriosis via ovulation.

References

[1] Khan KN, Kitajima M, Fujishita A, Nakashima M, Masuzaki H. Toll-like receptor system and endometriosis. J Obstet Gynaecol Res 2013;39:1281–92.
[2] Burney RO, Giudice LC. Pathogenesis and pathophysiology of endometriosis. Fertil Steril 2012;98:511–9.
[3] Nisolle M, Donnez J. Peritoneal endometriosis, ovarian endometriosis, and adenomyotic nodules of the rectovaginal septum are three different entities. Fertil Steril 1997;68:585–96.
[4] Broekmans FJ, Kwee J, Hendriks DJ, Mol BW, Lambalk CB. A systematic review of tests predicting ovarian reserve and IVF outcome. Hum Reprod Update 2006;12:685–718.
[5] Donnez J, Nisolle M, Gillet N, Smets M, Bassil S, Casanas-Roux F. Large ovarian endometriomas. Hum Reprod 1996;11:641–6.
[6] Van Langendonckt A, Donnez J, Defrère S, Dunselman GA, Groothuis PG. Antiangiogenic and vascular-disrupting agents in endometriosis: pitfalls and promises. Mol Hum Reprod 2008;14:259–68.
[7] Skinner MK. Regulation of primordial follicle assembly and development. Hum Reprod Update 2005;11:461–71.
[8] Oktem O, Urman B. Understanding follicle growth in vivo. Hum Reprod 2010;25:2944–54.
[9] Motta PM, Nottola SA, Familiari G, Makabe S, Stallone T, Macchiarelli G. Morphodynamics of the follicular-luteal complex during early ovarian development and reproductive life. Int Rev Cytol 2003;223:177–288.
[10] Gilks CB, Clement PB. Ovary. In: Mills SE, editor. Histology for pathologist. 4th ed. New York: Lippincott Williams & Wilkins; 2003. p. 1119–48.
[11] Gregory KE, Ono RN, Charbonneau NL, Kuo CL, Keene DR, Bächinger HP, Sakai LY. The prodomain of BMP-7 targets the BMP-7 complex to the extracellular matrix. J Biol Chem 2005;280:27970–80.
[12] Sengle G, Charbonneau NL, Ono RN, Sasaki T, Alvarez J, Keene DR, Bächinger HP, Sakai LY. Targeting of bone morphogenetic protein growth factor complexes to fibrillin. J Biol Chem 2008;283:13874–88.
[13] Reeves G. Specific stroma in the cortex and medulla of the ovary. Cell types and vascular supply in relation to follicular apparatus and ovulation. Obstet Gynecol 1971;37:832–44.
[14] Kitajima M, Defrère S, Dolmans MM, Colette S, Squifflet J, Van Langendonckt A, Donnez J. Endometriomas as a possible cause of reduced ovarian reserve in women with endometriosis. Fertil Steril 2011;96:685–91.
[15] Gordts S, Campo R, Brosens I, Puttemans P. Endometriosis: modern surgical management to improve fertility. Best Pract Res Clin Obstet Gynaecol 2003;17:275–87.
[16] Vercellini P, Somigliana E, Vigano P, Abbiati A, Barbara G, Fedele L. 'Blood on the Tracks' from corpora lutea to endometriomas. BJOG 2009;116:366–71.
[17] Weenen C, Laven JS, Von Bergh AR, Cranfield M, Groome NP, Visser JA, Kramer P, Fauser BC, Themmen AP. Anti-Müllerian hormone expression pattern in the human ovary: potential implications for initial and cyclic follicle recruitment. Mol Hum Reprod 2004;10:77–83.

[18] Visser JA, Themmen AP. Anti-Mullerian hormone and folliculogenesis. Mol Cell Endocrinol 2005;234:81–6.
[19] Takeuchi A, Koga K, Satake E, Makabe T, Taguchi A, Miyashita M, Takamura M, Harada M, Hirata T, Hirota Y, Yoshino O, Wada-Hiraike O, Fujii T, Osuga Y. Endometriosis triggers excessive activation of primordial follicles via PI3K-PTEN-Akt-Foxo3 pathway. J Clin Endocrinol Metab 2019;104:5547–54.
[20] Kitajima M, Dolmans MM, Donnez O, Masuzaki H, Soares M, Donnez J. Enhanced follicular recruitment and atresia in cortex derived from ovaries with endometriomas. Fertil Steril 2014;101:1031–7.
[21] Prefumo F, Rossi AC. Endometriosis, endometrioma, and ART results: current understanding and recommended practices. Best Pract Res Clin Obstet Gynaecol 2018;51:34–40.
[22] Sanchez AM, Vanni VS, Bartiromo L, Papaleo E, Zilberberg E, Candiani M, Orvieto R, Viganò P. Is the oocyte quality affected by endometriosis? A review of the literature. J Ovarian Res 2017;10:43.
[23] Orazov MR, Radzinsky VY, Ivanov II, Khamoshina MB, Shustova VB. Oocyte quality in women with infertility associated endometriosis. Gynecol Endocrinol 2019;35(Suppl. 1):24–6.
[24] Younis JS, Shapso N, Fleming R, Ben-Shlomo I, Izhaki I. Impact of unilateral versus bilateral ovarian endometriotic cystectomy on ovarian reserve: a systematic review and meta-analysis. Hum Reprod Update 2019;25:375–91.
[25] Kitajima M, Khan KN, Harada A, Taniguchi K, Inoue T, Kaneuchi M, Miura K, Masuzaki H. Association between ovarian endometrioma and ovarian reserve. Front Biosci 2018;10:92–102.
[26] Carrillo L, Seidman DS, Cittadini E, Meirow D. The role of fertility preservation in patients with endometriosis. J Assist Reprod Genet 2016;33:317–23.
[27] Lemos NA, Arbo E, Scalco R, Weiler E, Rosa V, Cunha-Filho JS. Decreased anti-Müllerian hormone and altered ovarian follicular cohort in infertile patients with mild/minimal endometriosis. Fertil Steril 2008;89:1064–8.
[28] Seyhan A, Ata B, Uncu G. The impact of endometriosis and its treatment on ovarian reserve. Semin Reprod Med 2015;33:422–8.
[29] Hamdan M, Dunselman G, Li TC, Cheong Y. The impact of endometrioma on IVF/ICSI outcomes: a systematic review and meta-analysis. Hum Reprod Update 2015;21:809–25.
[30] Yang X, Huang R, Cai M, Liang X. Endometriosis has no negative impact on outcomes of in vitro fertilisation in women with poor ovarian response. BJOG 2016;123(Suppl. 3):76–81.
[31] Eisenberg VH, Zolti M, Soriano D. Is there an association between autoimmunity and endometriosis? Autoimmun Rev 2012;11:806–14.
[32] Riccio LDGC, Santulli P, Marcellin L, Abrão MS, Batteux F, Chapron C. Immunology of endometriosis. Best Pract Res Clin Obstet Gynaecol 2018;50:39–49.
[33] Caccavo D, Pellegrino NM, Totaro I, Vacca MP, Selvaggi L, Depalo R. Antilaminin-1 antibodies in sera and follicular fluid of women with endometriosis undergoing in vitro fertilization. Int J Immunopathol Pharmacol 2011;24:481–8.
[34] Bats AS, Barbarino PM, Bene MC, Faure GC, Forges T. Local lymphocytic and epithelial activation in a case of autoimmune oophoritis. Fertil Steril 2008;90:849. e5-8.
[35] Nezlin R. A quantitative approach to the determination of antigen in immune complexes. J Immunol Methods 2000;237:1–17.
[36] Chauhan AK. Editorial: immune complexes in disease pathology. Front Immunol 2017;8:173.
[37] Aibara N, Ichinose K, Baba M, Nakajima H, Satoh K, Atarashi R, Kishikawa N, Nishida N, Kawakami A, Kuroda N, Ohyama K. Proteomic approach to profiling immune complex antigens in cerebrospinal fluid samples from patients with central nervous system autoimmune diseases. Clin Chim Acta 2018;484:26–31.
[38] Ohyama K, Huy NT, Yoshimi H, Kishikawa N, Nishizawa JE, Roca Y, Revollo Guzmán RJ, Velarde FUG, Kuroda N, Hirayama K. Proteomic profile of circulating immune complexes in chronic Chagas disease. Parasite Immunol 2016;38:609–17.
[39] Ohyama K, Yoshimi H, Aibara N, Nakamura Y, Miyata Y, Sakai H, Fujita F, Imaizumi Y, Chauhan AK, Kishikawa N, Kuroda N. Immune complexome analysis reveals the specific and frequent presence of immune complex antigens in lung cancer patients: a pilot study. int j Cancer 2017;140:370–80.
[40] Aibara N, Ohyama K, Hidaka M, Kishikawa N, Miyata Y, Takatsuki M, Eguchi S, Kuroda N. Immune complexome analysis of antigens in circulating immune complexes from patients with acute cellular rejection after living donor liver transplantation. Transpl Immunol 2018;48:60–4.
[41] Haller-Kikkatalo K, Salumets A, Uibo R. Review on autoimmune reactions in female infertility: antibodies to follicle stimulating hormone. Clin Dev Immunol 2012;2012:762541.

[42] Rodgers RJ, Irving-Rodgers HF. Formation of the ovarian follicular antrum and follicular fluid. Biol Reprod 2010;82:1021–9.
[43] Wu G, Bersinger NA, Mueller MD, von Wolff M. Intrafollicular inflammatory cytokines but not steroid hormone concentrations are increased in naturally matured follicles of women with proven endometriosis. J Assist Reprod Genet 2017;34:357–64.
[44] Bahtiyar MO, Seli E, Oral E, Senturk LM, Zreik TG, Arici A. Follicular fluid of women with endometriosis stimulates the proliferation of endometrial stromal cells. Hum Reprod 1998;13:3492–5.
[45] Ohyama K1, Ueki Y, Kawakami A, Kishikawa N, Tamai M, Osaki M, Kamihira S, Nakashima K, Kuroda N. Immune complexome analysis of serum and its application in screening for immune complex antigens in rheumatoid arthritis. Clin Chem 2011;57:905–9.
[46] Murakami N, Kitajima M, Ohyama K, Aibara N, Taniguchi K, Wei M, Kitajima Y, Miura K, Masuzaki H. Comprehensive immune complexome analysis detects disease-specific immune complex antigens in seminal plasma and follicular fluids derived from infertile men and women. Clin Chim Acta 2019;495:545–51.
[47] Rose-John S. IL-6 trans-signaling via the soluble IL-6 receptor: importance for the pro-inflammatory activities of IL-6. Int J Biol Sci 2012;8:1237–47.
[48] Dupont S, Mamidi A, Cordenonsi M, Montagner M, Zacchigna L, Adorno M, Martello G, Stinchfield MJ, Soligo S, Morsut L, Inui M, Moro S, Modena N, Argenton F, Newfeld SJ, Piccolo S. FAM/USP9x, a deubiquitinating enzyme essential for TGFbeta signaling, controls Smad4 monoubiquitination. Cell 2009;136:123–35.
[49] Weng L, Mitoma H, Trichot C, Bao M, Liu Y, Zhang Z, Liu YJ. The E3 ubiquitin ligase tripartite motif 33 is essential for cytosolic RNA-induced NLRP3 inflammasome activation. J Immunol 2014;193:3676–82.
[50] Shimbori C, Bellaye PS, Xia J, Gauldie J, Ask K, Ramos C, Becerril C, Pardo A, Selman M, Kolb M. Fibroblast growth factor-1 attenuates TGF-β1-induced lung fibrosis. J Pathol 2016;240:197–210.

This page intentionally left blank

CHAPTER

13

The role of immune-related redox biology in malignant transformation of endometriosis

Hiroshi Kobayashi

Department of Obstetrics and Gynecology, Nara Medical University, Kashihara, Japan

Introduction

Endometriosis is an estrogen-dependent, chronic inflammatory disease that affects approximately 10% of reproductive age women and accounts for up to 50% of female infertility cases [1,2]. Endometriosis is defined as the presence of endometrial glands and stroma in extrauterine locations, which is commonly found in the pelvic cavity, namely peritoneal, ovarian, and deep infiltrating endometriosis. This disorder often causes dysmenorrhea and pelvic pain that affects patients' quality of life [3,4]. Although the etiology of endometriosis remains elusive, endometrial debris generated by retrograde menstruation can escape immune surveillance and progress to ectopic lesions, suggesting immunological dysfunction as one of the multiple mechanisms [4–6]. Different types of immune cells are involved in the process of endometriosis development: the recruitment of macrophages and their polarization phenotype and impaired function of T cells, B cells, and natural killer (NK) cells [7]. Macrophages are recognized to regulate a wide range of processes, such as removal of tissue debris, inflammation, antiinflammation, and tissue remodeling. Although a cause or a consequence is not easy to distinguish, immune dysfunction may be involved in the etiology of endometriosis.

Furthermore, endometriosis is clearly associated with an increased risk for type 1 epithelial ovarian cancer (EOC), including clear cell carcinoma and endometrioid carcinoma (endometriosis-associated ovarian cancer, EAOC) [8]. Similar risk factors interact to predispose to both endometriosis and type 1 EOC [8]. The proposed etiology and observed pathophysiological factors, including steroid hormone, altered immune system, inflammation, oxidative stress, environmental factors, familial predisposition, genetic alterations, diet, early

Immunology of Endometriosis
https://doi.org/10.1016/B978-0-12-820661-4.00008-X

© 2022 Elsevier Inc. All rights reserved.

menarche, low parity, late menopause, or infertility, may have similar characteristics or be causative factors in carcinogenesis [9]. Among these factors, aberrant immune system and inflammation may mainly stimulate the progression of both endometriosis and its malignant transformation [8]. In this review, we will summarize the current knowledge in the development and maintenance of endometriosis and its malignant transformation, with a focus on recent progress in oxidation-reduction (redox) signaling, regulating immune function, and redox biology.

Immune dysfunction linked to endometriosis development and malignant transformation

Immune cells ingest and process endometrial debris, dead cells, and heme-iron, and then protect the development of endometriosis in normal individuals. Therefore, the occurrence of retrograde menstruation may be insufficient to explain the development of endometriosis in women with normal immune system. These observations suggest that immunomodulatory activities and factors are the main biological and molecular mechanism underlying the development of endometriosis. Endometriosis has been associated with risk of several chronic diseases or autoimmune diseases, including systemic lupus erythematosus, Sjögren's syndrome, rheumatoid arthritis, autoimmune thyroid disorder, celiac disease, multiple sclerosis, or inflammatory bowel disease [10], but the etiology and exact molecular relationship between them remains largely unknown. These observations suggest that there are possible mechanisms, shared risk factors and predisposing factors underlying the link between endometriosis and adverse immune diseases. Like autoimmune diseases, immune suppression or dysfunction of the innate and adaptive immune system may interferes with an effective elimination of endometriosis [11]. The following hypothesis could help to analyze the complex mechanisms of endometriosis development. A functional immunosurveillance process consists of three phases, including elimination, equilibrium and escape in endometriosis suppression and promotion [11]. In the elimination (immunosurveillance) phase, immune cells in women who are likely to be predisposed to endometriosis have limited capability of eliminating eutopic endometrial cells generated by retrograde shedding into the pelvic cavity. The equilibrium phase is the period of immune-mediated endometrial cell dormancy. During this latent phase, endometrial cells acquire biological functions, such as the capacity to proliferate. In the escape phase, the incompetent immune system fails to restrict endometriosis outgrowth; endometriotic cells begin to grow progressively, invade surrounding tissues and promote carcinogenesis. The eutopic endometrium of women with endometriosis or those who appears to predispose to endometriosis later in life may contribute to a variety of functions including endometrial tissue survival, resistance to apoptosis, adhesion, growth, angiogenesis, and invasion within the host tissue and progression to disease [12].

Immune cells, such as macrophages, lymphocytes, mast cells, neutrophils, and NK cells in endometriotic lesions or peritoneal cavity are suggested to play important roles in the progression of the disease [13]. In addition, macrophages particularly provide support to tumor-initiating cells during the transition to malignancy by suppressing immune clearance,

stimulating angiogenesis, promoting proliferation and modulating redox biology that interfere with an antitumor response and tumor elimination, and greatly contributes to carcinogenesis [14]. In the next section, we summarize current knowledge about the potential implication of macrophages contributing to immunomodulatory, antiinflammatory and the antioxidant activities in the pathogenesis of the development of endometriosis and its malignant transformation. In particular, we focus on deciphering the role of redox biology in neoplastic transformation.

The recruitment and phenotype of macrophages in endometriosis and its malignant transformation

The toxic factors, such as menstrual endometrial fragments, apoptotic endometrial tissues, lysed erythrocytes, hemoglobin (Hb), heme and free iron, can induce inflammation and immune response in the peritoneal cavity [15]. Macrophages recruited to this environment release a wide array of proinflammatory chemotactic cytokines and propagate inflammation and tissue damage [16]. Macrophages alter multiple aspects of humoral and cell-mediated immunity [17]. The concentration and proportion of macrophages in the various types of immune cells are increased in the peritoneal fluid of women with endometriosis [18,19]. In addition, the abnormal accumulation and distribution of macrophages are observed within the endometriotic lesion [18,19]. The recruitment, distribution, and changes in macrophage subsets in the peritoneal fluid and within the endometriotic lesion likely compromise immune fitness and facilitate endometriosis initiation and progression. Indeed, an in vivo animal model revealed that macrophage depletion displayed reduced weight, size, vascularization and attenuation of the endometriotic lesion, supporting the notion that macrophages play a direct role in endometriosis pathology [27]. Other immune cells, including neutrophils, mast cells, and dendritic cells, are activated by menstrual debris or in response to these cytokines produced by macrophages [4]. These immune cells also play an important role in the pathogenesis of endometriosis [20–22]. Neutrophils from women with endometriosis entail apoptosis resistance [20]. Dendritic cells are paramount in the activation of adaptive immunity through antigen presentation to naïve T cells. Dendritic cells are found within the endometriotic lesions and promote angiogenesis [21]. Somigliana et al. [22] indicated possible association of diminished cytotoxicity of NK cells with immune dysfunction in endometriosis.

Tissue-resident macrophages are characterized by great functional diversity and plasticity and produce both proinflammatory and antiinflammatory cytokines [23,24]. Macrophage plasticity and polarization are mainly controlled by epigenetic pathways, microRNA (miRNA), the tissue microenvironment (pathogen-associated molecular pattern molecules [PAMPs] and damage-associated molecular pattern molecules [DAMPs]), and cytokines released in inflammation (granulocyte-macrophage colony-stimulating factor [GM-CSF], interferon-beta [IFN-β], IFN-γ and lipopolysaccharide [LPS] vs. IL-4, IL-10 and IL-13) [23,24]. Macrophages are divided into two main phenotypes: the classical macrophages (known as proinflammatory M1 macrophages) and alternatively activated macrophages (antiinflammatory M2 macrophages) [23]. Upon exposure to hemorrhage and lysed

erythrocytes, recruitment of monocytes from the systemic circulation and activation toward M1 macrophages are enhanced at the lesion site, which produces a variety of proinflammatory cytokines, nitric oxide, ROS, and exhibits tissue injury and impaired tissue remodeling and wound healing [25]. Once activated in response to new environmental influences, M2 macrophages retain the ability to continue changing and exhibit phagocytosis of red blood cells and apoptotic cells. The shift between M1 and M2 states of macrophage polarization contributes to maintain tissue homeostasis and diverse immune functions including apoptotic cell removal, inflammatory resolution, myofibroblast proliferation, fibrosis, and tissue healing. Macrophage recruitment and progressive shift in M1 to M2 macrophage polarization are central players in endometriosis development in both human specimens and mouse models [26–29], but the results are inconclusive [30].

In general, M1/kill-type macrophages can stop cancer growth, whereas M2/repair-type macrophages are immunosuppressive cells that can promote cancer growth [31–33]. A high M1/M2 ratio of tumor-associated macrophages is reported to be associated with improved survival in patients with type 2 EOC (i.e., high grade serous ovarian cancer) [34], nonsmall cell lung cancer [35], and other cancers [36]. In contrast, the number of M1 and M2 macrophages were significantly lower in patients with EAOC than in women with endometriosis, although M2 macrophages predominantly infiltrated into the type 1 EOC [28]. We hypothesize that ROS production in endometriotic lesions plays critical roles in the activation and functions of M1 macrophages, which is necessary for the shift in M1 to M2 macrophage polarization to suppress excessive oxidative stress (see the next section).

The role of oxidative stress in endometriosis and its malignant transformation

We highlight emerging concepts of how the redox balance has advanced our understanding in the pathophysiology of endometriosis and discuss molecular mechanisms of redox biology. When erythrocytes are lysed in peritoneal fluid or within endometriotic cysts, Hb, heme and free iron are released from lysed erythrocytes [19]. Once cell-free Hb massively released into the peritoneal cavity or endometriotic cyst fluid space during menstruation, oxyhemoglobin (oxyHb [Hb – Fe^{2+}]) is converted to methemoglobin (metHb [Hb – Fe^{3+}]) by autooxidation [37]. Toxic ROS such as $O_2.^-$ are produced during Hb autoxidation, as follows [37]: $Hb - Fe^{2+} + O_2 \Leftrightarrow Hb - Fe^{2+}O_2 \rightarrow Hb - Fe^{3+} + O_2^-$. In addition, free iron (Fe^{2+}) also stimulates Haber-Weiss-Fenton reaction, contributing to the generation of harmful ROS (•OH) in endometriotic cyst, as follows [37]: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH\text{-} + \bullet OH$ In endometriosis, autoxidation and Fenton reaction of Hb from the ferrous Fe^{2+} (oxyHb) state to the ferric Fe^{3+} (metHb) state lead to production of excess ROS such as O_2^- and •OH, which causes distortion in the homeostatic redox balance [37]. Therefore, metHb and oxyHb are considered to be markers of oxidative stress and antioxidant capacities, respectively. Excessive metHb and free iron induces the ROS-activated signaling pathways, which regulates DNA damage and cell death or is linked to DNA mutations and carcinogenesis, depending on the ROS dose. ROS is involved in redox biology; Redox activates protumorigenic signaling at low ROS levels, while oxidative stress causes damage to DNA, proteins and lipids at high ROS levels [38].

The role of the redox/metallobiology in the malignant transformation of endometriosis is still poorly understood. Iwabuchi et al. [39] for the first time assessed the levels of cyst fluid Hb species and investigated the role of redox status in endometriosis and its malignant transformation. They have investigated a spectrophotometric determination of Hb species such as metHb and oxyHb contents on the basis of wavelength spectrophotometry in the cyst fluids of endometriosis and EAOC. The ratio of each peak wavelength, 620 and 580 nm, in the absorbance spectra (the 620/580 nm ratio) was used as a surrogate indicator of the metHb/(oxyHb + metHb) ratio in each cyst fluid [39]. The 620/580 nm ratio in EAOC patients was significantly lower than that in women with endometriosis (0.39 ± 0.27 vs. 0.67 ± 0.19, $P = .021$), demonstrating that oxyHb is the abundant Hb species in EAOC cyst fluids. EAOC is associated with decreased oxidative stress in cyst fluids, marked by an increase in oxyHb production. These data suggest that metHb shift to oxyHb state plays a role in the process of malignant transformation, which creates a favorable microenvironment for tumor progression by increasing the antioxidant property. Taken together, redox biology is required for cancer initiation, while antioxidant property may be a potent regulator of tumor progression.

The role of antioxidant property in endometriosis and its malignant transformation

Iron deposits, a characteristic of endometriosis, originate from retrograde menstruation or bleeding in the ectopic endometrial lesions [40]. Macrophages phagocyte lysed erythrocytes and also actively participate in iron homeostasis. Macrophages upregulate several antioxidant enzymes including heme oxygenase 1 (HO-1), a stress-inducible protein, through the two main pathways of NF-E2-related factor 2 (NRF2)/BTB domain and CNC homolog 1 (BACH1) system and IL-10/HO-1 axis [37]. HO-1 is induced by various signals such as heme, apoptotic cell supernatants, inflammation and oxidative stress [23,41,42]. HO-1 catalyzes heme and produces carbon monoxide, biliverdin/bilirubin and free iron, which has been shown to have antioxidant, antiinflammatory, antiproliferative, immunomodulatory and neuromodulatory effects in animal and human systems [23,41]. HO-1 induction can promote phenotypic switch toward M2 macrophages [23,41]. How is the role of redox signaling and oxidative stress in endometriosis? Cyst fluid levels of 8-OHdG, a marker of oxidative stress, in women with endometriosis were significantly increased compared with patients with EAOC (median, 2.023 ng/mL [range, 0.16−49.98 ng/mL] vs. 0.820 ng/mL [0.39−3.89 ng/mL], $P = .013$) [43], while the levels of antioxidation TAC (total antioxidation capacity) in women with endometriosis were also significantly higher compared with those in patients with EAOC (median, 0.973 mmol/L [range, 0.230−1.957 mmol/L] vs. 0.616 mmol/L [0.401−0.883 mmol/L], $P < .001$) (unpublished data; personal communication from Yuki Yamada, Nara Medical University). Thus, endometriosis is considered to be characterized by an increase in oxidative stress and also antioxidant capacity, triggering cell death at high ROS levels.

We next focus on the expression of HO-1 protein in both cyst fluids and tissue specimens of endometriosis and EAOC. Fujimoto et al. [43] reported that compared to endometriosis,

EAOC is associated with a decreased antioxidant capacity and also a marked reduction of oxidative stress in cyst fluids. Yamada et al. [28] have quantified the numbers of macrophages polarized as M1 or M2 phenotypes and the expression of HO-1 in tissue sections from patients with endometriosis and its malignant transformation. HO-1 expression in two conditions was primarily detected in $CD163^+$ macrophages, which are known to exhibit the M2 phenotype. As reported previously, CD163 is a member of scavenger receptor family that contributes to the clearance of Hb [44]. The number of the M1 phenotype ($CD11c^+$, $P = .001$) and the M2 phenotype ($CD163^+$, $P = .009$) was significantly lower in EAOC patients than in endometriosis patients [28]. The number of M2 phenotypes expressing HO-1 was also significantly decreased in the EAOC group, compared with the endometriosis group ($P < .001$), demonstrating sustained downregulation of HO-1 in EAOC [28]. Furthermore, the antioxidant-to-oxidant ratio was significantly higher in EAOC group compared with endometriosis group because of an increase in oxyHb levels, possibly activating protumorigenic signaling at low ROS levels [28,43]. This may be a reason why the distortion of the prooxidation (oxyHb) and antioxidation (HO-1) balance has an important role in promoting the carcinogenic process.

In addition, CD44 is another key regulator of antioxidant factors. Expression of CD44 variant isoforms (CD44v8-10) on the cell surface has been identified as a candidate marker for cancer stem cells and metastatic features [45]. CD44v9 modulates intracellular glutathione synthesis and contributes to ROS defense through upregulation of the intracellular antioxidants through the glutamate-cysteine transporter solute carrier family 7 member 11 (SLC7A11, also known as xCT) [46]. In general, CD44v9 expression has been associated with chemoresistance, tumor recurrence, metastasis and poor prognosis of a variety of cancers [47–49]. On the other hand, the clinical impact of CD44v9 on the progression of type 1 EOC showed opposite results [46]. Niiro et al. [46] evaluated the expression of CD44v9 protein in endometriosis, endometriosis adjacent to clear cell carcinomas (benign-adjacent) and clear cell carcinomas. Percentage of CD44v9 positive cells was 68.5% of endometriosis, 25.0% of benign-adjacent and 16.7% of clear cell carcinomas ($P < .001$) [46]. The analysis revealed a remarkable immunoreactivity for CD44v9 in endometriosis and a decreasing gradient of staining in benign-adjacent, and then up to become negative in the majority of clear cell carcinomas. Interestingly, endometriosis adjacent to EAOC showed significantly less CD44v9 staining as compared with distant endometriosis [46]. This is the first study that showed a decreasing gradient of CD44v9 expression in benign, adjacent-benign and cancer tissues in malignant transformation of endometriosis. These data allow us to speculate that loss of CD44v9 is an early event although this loss is not sufficient to drive the development of EAOC. However, the molecular mechanisms regulating loss of CD44v9 protein expression during the process of malignant transformation remain unknown. Considering a clear separation between the overall redox state in endometriosis and its malignant transformation, characteristic alterations in redox balance may be helpful for understanding the pathogenesis of the malignant transformation of endometriosis.

Mechanism underlying malignant transformation of endometriosis

Endometriosis is considered to be a common precursor lesions to ovarian cancer, however, the pathogenesis of EAOC is poorly understood. It was hypothesized that endometriotic

lesions would progress, over time, to potential precursor or premalignant lesions (atypical endometriosis) and then EAOC. This review discusses the fine tuning of the generation of ROS and their antioxidant/detoxification capacity during the process of malignant transformation of endometriosis.

Both genetic mutations and epigenetic silencing of AT-rich interaction domain 1A (ARID1A) [50,51] and phosphatase and tensin homolog (PTEN) [52] genes have been recognized as a fundamental mechanism that promotes EAOC carcinogenesis. Loss of ARID1A and PTEN genes has been demonstrated in several cancers and was found to be independent prognostic factors [53]. Mutation of these genes contributes to carcinogenesis via the PI3K/AKT pathway [54]. Furthermore, innate and adaptive immunity system triggers an inflammatory process in endometriotic foci, which releases various mediators, including cytokines and ROS [55]. ROS can be T cell immunosuppressive [56]. ROS is excessively generated in endometriosis, promoting an oxidative stress in its microenvironment. Since ROS (O_2^- and •OH) is created mainly through metHb ($Hb - Fe^{3+}$) and free iron (Fe^{2+}) in endometriotic cyst fluids, elevated expression of 8-OHdG is observed in the endometriotic epithelial cells [28]. Oxidative stress promote many aspects of tumor development and progression such as aberrant promoter hypermethylation of tumor suppressor genes, ARID1A and PTEN [50–52]. The local and mutagenic concentrations of ROS are important for the cellular functions or gene promoter hyper-methylation via the upregulation of DNA methyltransferase 1 (DNMT1) gene expression [51]. Excess production of ROS causes cell death [37]. On the other hand, reductase systems catalyze the reduction of ferric iron Fe^{3+} to the ferrous Fe^{2+} state in EAOC, demonstrating that metHb shift to oxyHb state plays a critical role in the downregulation of oxidative stress. HO-1 [28], CD44v9 [46], cytochrome P450 family and glutathione transferases are involved in antioxidant defense in EAOC. Thus, EAOC appears to be associated with an increase in the antioxidant-to-oxidative stress ratio, which alleviates cell death by scavenging ROS and allows neoplastic cells to survive and proliferate.

Fig. 13.1 summarizes the mechanism of malignant transformation of endometriosis associated with a fine-tuned balance between oxidants and endogenous antioxidants. The first phase of the carcinogenic process in endometriosis is generally associated with epigenetic alterations and genetic mutations. Prolonged exposure to high ROS concentrations may lead to DNA damage. ROS cause a loss-of-function of tumor suppressor genes, ARID1A [50,51] and PTEN [52]. Genetic and epigenetic events give birth to a subclone that acquired high levels of autonomous proliferation. Despite high-level concentrations of ROS, endometriosis possesses defense mechanisms, including scavenger and enzymatic systems (HO-1, CD44v9, catalase, or superoxide dismutase), against the intrinsic mutagens, such as metHb, free iron, and ROS. An increased antioxidant capacity in endometriosis may reduce further DNA damages and be involved in the elimination process of endometriotic cells with unrepaired or misrepaired DNA (i.e., cancer immunosurveillance). In the second phase, antioxidant capacity may decrease in precancerous lesions due to the reduced accumulation of activated M2 macrophages. Uncontrolled production of ROS as an imbalance in the oxidant/antioxidant mechanisms leads to a state of excessive oxidative stress. The steady accumulation of oxidative stress has adverse consequences. Unrepaired DNA lesions introduce genome instability and is implicated in not only compromising cell viability, but also alterations in gene expression levels, signal transduction pathways, DNA mutations, and

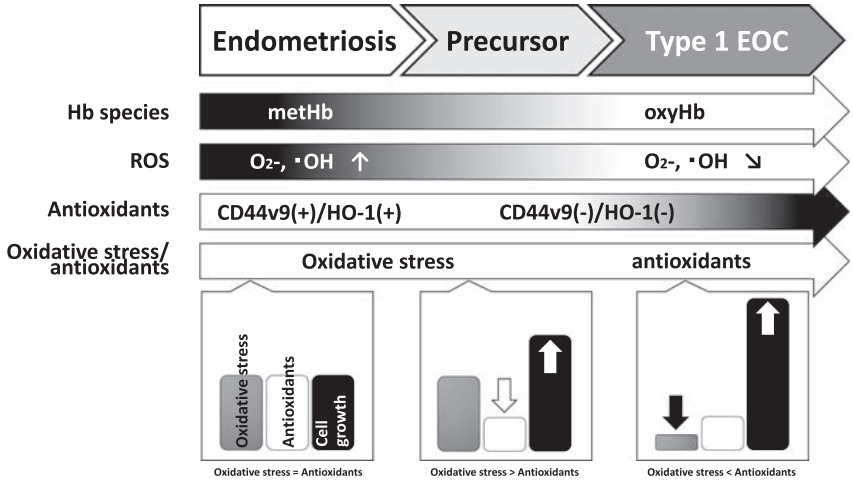


FIGURE 13.1 A fine-tuned balance between oxidants and antioxidants as an underpinning mechanism for malignant transformation of endometriosis.

cancer initiation. In the final phase, reduced oxidative stress may favor the survival of precancerous cells and contribute to tumor progression, clonal amplification, and all phases of tumorigenesis.

Conclusion

We summarize the fine-tuned balance between immune-related oxidative stress, antioxidant capacity, and cellular transformation and discuss the advances in understanding of the mechanisms of malignant transformation of endometriosis. Although it has not yet been experimentally and clinically investigated, the redox imbalance hypothesis might be currently the best explanation for malignant transformation.

Acknowledgments

We extend special thanks to Yuki Yamada, Emiko Niiro and Takuya Iwabuchi for previous work on the experiments.

Author's contribution

HK made contribution to conception of the study, collected data using Web survey method and contributed to the study design and interpretation of included data. The final version of the manuscript has been read and approved by this author.

References

[1] Khan KN, Kitajima M, Fujishita A, Hiraki K, Matsumoto A, Nakashima M, Masuzaki H. Pelvic pain in women with ovarian endometrioma is mostly associated with coexisting peritoneal lesions. Hum Reprod 2013;28(1):109–18.

[2] Liang Y, Yao S. Potential role of estrogen in maintaining the imbalanced sympathetic and sensory innervation in endometriosis. Mol Cell Endocrinol 2016;424:42–9.
[3] D'Hooghe T, Hummelshoj L. Multi-disciplinary centres/networks of excellence for endometriosis management and research: a proposal. Hum Reprod 2006;21(11):2743–8.
[4] Ahn SH, Monsanto SP, Miller C, Singh SS, Thomas R, Tayade C. Pathophysiology and immune dysfunction in endometriosis. BioMed Res Int 2015;2015:795976.
[5] Sourial S, Tempest N, Hapangama DK. Theories on the pathogenesis of endometriosis. Int J Reprod Med 2014;2014:179515.
[6] Králíčková M, Vetvicka V. Immunological aspects of endometriosis: a review. Ann Transl Med 2015;3(11):153.
[7] Riccio LDGC, Santulli P, Marcellin L, Abrão MS, Batteux F, Chapron C. Immunology of endometriosis. Best Pract Res Clin Obstet Gynaecol 2018;50:39–49.
[8] Ness RB. Endometriosis and ovarian cancer: thoughts on shared pathophysiology. Am J Obstet Gynecol 2003;189(1):280–94.
[9] Verit FF, Yucel O. Endometriosis, leiomyoma and adenomyosis: the risk of gynecologic malignancy. Asian Pac J Cancer Prev APJCP 2013;14(10):5589–97.
[10] Shigesi N, Kvaskoff M, Kirtley S, Feng Q, Fang H, Knight JC, Missmer SA, Rahmioglu N, Zondervan KT, Becker CM. The association between endometriosis and autoimmune diseases: a systematic review and meta-analysis. Hum Reprod Update 2019;25(4):486–503.
[11] Sikora J, Mielczarek-Palacz A, Kondera-Anasz Z. Role of natural killer cell activity in the pathogenesis of endometriosis. Curr Med Chem 2011;18(2):200–8.
[12] Kyama CM, Debrock S, Mwenda JM, D'Hooghe TM. Potential involvement of the immune system in the development of endometriosis. Reprod Biol Endocrinol 2003;1:123.
[13] Osuga Y. Current concepts of the pathogenesis of endometriosis. Reprod Med Biol 2009;9(1):1–7.
[14] Guo X, Zhao Y, Yan H, Yang Y, Shen S, Dai X, Ji X, Ji F, Gong XG, Li L, Bai X, Feng XH, Liang T, Ji J, Chen L, Wang H, Zhao B. Single tumor-initiating cells evade immune clearance by recruiting type II macrophages. Genes Dev 2017;31(3):247–59.
[15] Chen GY, Nuñez G. Sterile inflammation: sensing and reacting to damage. Nat Rev Immunol 2010;10(12):826–37.
[16] Capobianco A, Rovere-Querini P. Endometriosis, a disease of the macrophage. Front Immunol 2013;4:9.
[17] Herington JL, Bruner-Tran KL, Lucas JA, Osteen KG. Immune interactions in endometriosis. Expet Rev Clin Immunol 2011;7(5):611–26.
[18] Scheerer C, Bauer P, Chiantera V, Sehouli J, Kaufmann A, Mechsner S. Characterization of endometriosis-associated immune cell infiltrates (EMaICI). Arch Gynecol Obstet 2016;294(3):657–64.
[19] Wickström K, Stavréus-Evers A, Vercauteren O, Olovsson M, Edelstam G. Effect of lignocaine on IL-6, IL-8, and MCP-1 in peritoneal macrophages and endometriotic stromal cells. Reprod Sci 2017;24(3):382–92.
[20] Kwak JY, Park SW, Kim KH, Na YJ, Lee KS. Modulation of neutrophil apoptosis by plasma and peritoneal fluid from patients with advanced endometriosis. Hum Reprod 2002;17(3):595–600.
[21] Schulke L, Berbic M, Manconi F, Tokushige N, Markham R, Fraser IS. Dendritic cell populations in the eutopic and ectopic endometrium of women with endometriosis. Hum Reprod 2009;24(7):1695–703.
[22] Somigliana E, Viganò P, Gaffuri B, Candiani M, Busacca M, Di Blasio AM, Vignali M. Modulation of NK cell lytic function by endometrial secretory factors: potential role in endometriosis. Am J Reprod Immunol 1996;36(5):295–300.
[23] Naito Y, Takagi T, Higashimura Y. Heme oxygenase-1 and anti-inflammatory M2 macrophages. Arch Biochem Biophys 2014;564:83–8.
[24] Murray PJ. Macrophage polarization. Annu Rev Physiol 2017;79:541–66.
[25] Laskin DL, Sunil VR, Gardner CR, Laskin JD. Macrophages and tissue injury: agents of defense or destruction? Annu Rev Pharmacol Toxicol 2011;51:267–88.
[26] Bacci M, Capobianco A, Monno A, Cottone L, Di Puppo F, Camisa B, Mariani M, Brignole C, Ponzoni M, Ferrari S, Panina-Bordignon P, Manfredi AA, Rovere-Querini P. Macrophages are alternatively activated in patients with endometriosis and required for growth and vascularization of lesions in a mouse model of disease. Am J Pathol 2009;175(2):547–56.
[27] Wang Y, Fu Y, Xue S, Ai A, Chen H, Lyu Q, Kuang Y. The M2 polarization of macrophage induced by fractalkine in the endometriotic milieu enhances invasiveness of endometrial stromal cells. Int J Clin Exp Pathol 2014;7(1):194–203.

[28] Yamada Y, Uchiyama T, Ito F, Kawahara N, Ogawa K, Obayashi C, Kobayashi H. Clinical significance of M2 macrophages expressing heme oxygenase-1 in malignant transformation of ovarian endometrioma. Pathol Res Pract 2019;215(4):639–43.
[29] Nie MF, Xie Q, Wu YH, He H, Zou LJ, She XL, Wu XQ. Serum and ectopic endometrium from women with endometriosis modulate macrophage M1/M2 polarization via the Smad2/Smad3 pathway. J Immunol Res 2018;2018:6285813.
[30] Takebayashi A, Kimura F, Kishi Y, Ishida M, Takahashi A, Yamanaka A, Wu D, Zheng L, Takahashi K, Suginami H, Murakami T. Subpopulations of macrophages within eutopic endometrium of endometriosis patients. Am J Reprod Immunol 2015;73(3):221–31.
[31] Chen P, Bonaldo P. Role of macrophage polarization in tumor angiogenesis and vessel normalization: implications for new anticancer therapies. Int Rev Cell Mol Biol 2013;301:1–35.
[32] Ruffell B, Coussens LM. Macrophages and therapeutic resistance in cancer. Cancer Cell 2015;27(4):462–72.
[33] Mills CD, Lenz LL, Harris RA. A breakthrough: macrophage-directed cancer immunotherapy. Cancer Res 2016;76(3):513–6.
[34] Zhang M, He Y, Sun X, Li Q, Wang W, Zhao A, Di W. A high M1/M2 ratio of tumor-associated macrophages is associated with extended survival in ovarian cancer patients. J Ovarian Res 2014;7:19.
[35] Jackute J, Zemaitis M, Pranys D, Sitkauskiene B, Miliauskas S, Vaitkiene S, Sakalauskas R. Distribution of M1 and M2 macrophages in tumor islets and stroma in relation to prognosis of non-small cell lung cancer. BMC Immunol 2018;19(1):3.
[36] Rhee I. Diverse macrophages polarization in tumor microenvironment. Arch Pharm Res 2016;39(11):1588–96.
[37] Iwabuchi T, Yoshimoto C, Shigetomi H, Kobayashi H. Oxidative stress and antioxidant defense in endometriosis and its malignant transformation. Oxid Med Cell Longev 2015;2015:848595.
[38] Schieber M, Chandel NS. ROS function in redox signaling and oxidative stress. Curr Biol 2014;24(10):R453–62.
[39] Iwabuchi T, Yoshimoto C, Shigetomi H, Kobayashi H. Cyst fluid hemoglobin species in endometriosis and its malignant transformation: the role of metallobiology. Oncol Lett 2016;11(5):3384–8.
[40] Donnez J, Binda MM, Donnez O, Dolmans MM. Oxidative stress in the pelvic cavity and its role in the pathogenesis of endometriosis. Fertil Steril 2016;106(5):1011–7.
[41] Lowe DT. Cupping therapy: an analysis of the effects of suction on skin and the possible influence on human health. Compl Ther Clin Pract 2017;29:162–8.
[42] Weis N, Weigert A, von Knethen A, Brüne B. Heme oxygenase-1 contributes to an alternative macrophage activation profile induced by apoptotic cell supernatants. Mol Biol Cell 2009;20(5):1280–8.
[43] Fujimoto Y, Imanaka S, Yamada Y, Ogawa K, Ito F, Kawahara N, Yoshimoto C, Kobayashi H. Comparison of redox parameters in ovarian endometrioma and its malignant transformation. Oncol Lett 2018;16(4):5257–64.
[44] Onofre G, Kolácková M, Jankovicová K, Krejsek J. Scavenger receptor CD163 and its biological functions. Acta Med 2009;52(2):57–61.
[45] Di Stefano C, Grazioli P, Fontanella RA, De Cesaris P, D'Amore A, Regno M, Starace D, Padula F, Fiori ME, Canipari R, Stoppacciaro A, Pesce M, Filippini A, Campese AF, Ziparo E, Riccioli A. Stem-like and highly invasive prostate cancer cells expressing CD44v8-10 marker originate from CD44-negative cells. Oncotarget 2018;9(56):30905–18.
[46] Niiro E, Kawahara N, Yamada Y, Yoshimoto C, Shimada K, Sudo T, et al. Immunohistochemical expression of CD44v9 and 8-OHdG in ovarian endometrioma and the benign endometriotic lesions adjacent to clear cell carcinoma. J Obstet Gynaecol Res 2019;45(11):2260–6.
[47] Yasui W, Kudo Y, Naka K, Fujimoto J, Ue T, Yokozaki H, Tahara E. Expression of CD44 containing variant exon 9 (CD44v9) in gastric adenomas and adenocarcinomas: relation to the proliferation and progression. Int J Oncol 1998;12(6):1253–8.
[48] Zavros Y. Initiation and maintenance of gastric cancer: a focus on CD44 variant isoforms and cancer stem cells. Cell Mol Gastroenterol Hepatol 2017;4(1):55–63.
[49] Taniguchi D, Saeki H, Nakashima Y, Kudou K, Nakanishi R, Kubo N, Ando K, Oki E, Oda Y, Maehara Y. CD44v9 is associated with epithelial-mesenchymal transition and poor outcomes in esophageal squamous cell carcinoma. Cancer Med 2018;7(12):6258–68.
[50] Liou GY, Storz P. Reactive oxygen species in cancer. Free Radic Res 2010;44(5):479–96.
[51] Xie H, Chen P, Huang HW, Liu LP, Zhao F. Reactive oxygen species downregulate ARID1A expression via its promoter methylation during the pathogenesis of endometriosis. Eur Rev Med Pharmacol Sci 2017;21(20):4509–15.

[52] Zhu Y, Hoell P, Ahlemeyer B, Sure U, Bertalanffy H, Krieglstein J. Implication of PTEN in production of reactive oxygen species and neuronal death in in vitro models of stroke and Parkinson's disease. Neurochem Int 2007;50(3):507–16.
[53] Wise HM, Hermida MA, Leslie NR. Prostate cancer, PI3K, PTEN and prognosis. Clin Sci 2017;131(3):197–210.
[54] Takeda T, Banno K, Okawa R, Yanokura M, Iijima M, Irie-Kunitomi H, Nakamura K, Iida M, Adachi M, Umene K, Nogami Y, Masuda K, Kobayashi Y, Tominaga E, Aoki D. ARID1A gene mutation in ovarian and endometrial cancers. Oncol Rep 2016;35(2):607–13.
[55] Georgescu SR, Mitran CI, Mitran MI, Caruntu C, Sarbu MI, Matei C, Nicolae I, Tocut SM, Popa MI, Tampa M. New insights in the pathogenesis of HPV infection and the associated carcinogenic processes: the role of chronic inflammation and oxidative stress. J Immunol Res 2018;2018:5315816.
[56] Fisher RI, Bostick-Bruton F. Depressed T cell proliferative responses in Hodgkin's disease: role of monocyte-mediated suppression via prostaglandins and hydrogen peroxide. J Immunol 1982;129(4):1770–4.

This page intentionally left blank

CHAPTER

14

Pregnancy complications

Louis Marcellin, Pietro Santulli and Charles Chapron

Université Paris Descartes, Sorbonne Paris Cité, Faculté de Médecine, Assistance Publique – Hôpitaux de Paris (AP-HP), Hôpital Universitaire Paris Centre (HUPC), Centre Hospitalier Universitaire (CHU) Cochin, Department of Gynecology Obstetrics II and Reproductive Medicine, Paris, France

The data on the association between pregnancy and endometriosis are limited or have poor-quality available evidence [1–3]. Historically, pregnancy was typically considered to have a positive effect, although temporary, on endometriosis probably due to anovulation, amenorrhea and pregnancy-related hormonal changes, preventing bleeding within ectopic endometriotic tissues [4]. Pregnancy complications have been reported to be increased in women with endometriosis [5]. Growth changes of ectopic endometriotic lesions are variably observed [1]. In addition, rare but severe and unpredictable complications during pregnancy have been reported with puerperal changes in ectopic lesions [6]. The relationship between endometriosis and pregnancy can be considered from two key perspectives: first, the influence of endometriosis on pregnancy outcomes; second, the influence of pregnancy on the endometriosis natural history.

Pathogenesis of the relationship between endometriosis and pregnancy

Decidualization

The menstrual cycle is the natural modification of the female reproductive system that allows implantation of pregnancy: estradiol and progesterone induce cyclical changes in the endometrium in preparation for blastocyst implantation [7]. There are two dominant successive phases including the proliferative phase, which follows menstruation and precedes ovulation, and the secretory phase, which occurs after ovulation. During the secretory phase, the endometrium transforms into a receptive tissue that is suitable for implantation [8].

This endometrial transformation after ovulation, named decidualization, results in the conversion of the endometrium into a specialized uterine tissue [9]. This process prepares the

https://doi.org/10.1016/B978-0-12-820661-4.00013-3

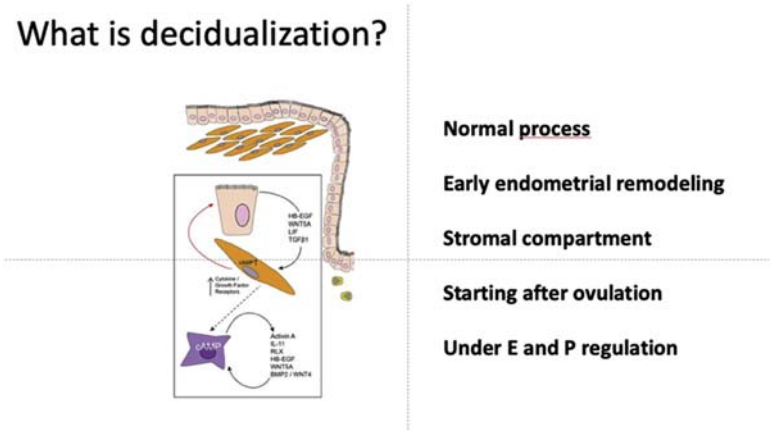
© 2022 Elsevier Inc. All rights reserved.

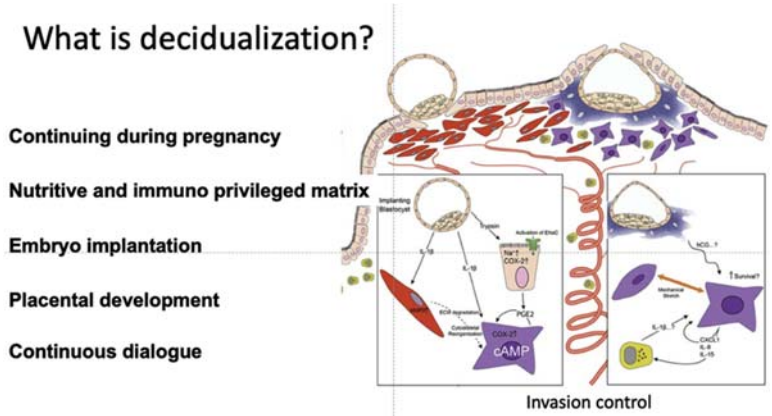
endometrial surface for pregnancy, and is maintained when implantation of the blastocyst occurs [10,11]. The decidualization aims to control trophoblast invasion guaranteeing optimal accommodation of the gestation [12] and protect the embryo from maternal immunological rejection.

The decidualization, mainly induced by estrogen and progesterone, consists of the transformation of endometrial stromal fibroblasts into particular secretary decidual cells [13].

This transformation involves different metabolic, hormonal (estradiol and progesterone), angiogenic (soluble fms-like tyrosine kinase-1 [SflT1]), vascular endothelial growth factor (VEGF), and immune system changes related to steroid exposure [13]. The two main secretory proteins of decidual stromal cells include prolactin and insulin-like growth factor-binding protein-1 (IGFBP -1) that may participate to stimulate trophoblast growth and invasion, to prevent immune rejection, to modulate uterine natural killer cell survival, and to promote angiogenesis. Specific hormonal changes involved in decidualization notably the estradiol interaction with the progesterone receptors expression during secretory phase.

In mice, decidualization is controlling tolerance toward allogenic fetus through several process including [14]: (1) lack of direct allorecognition of paternal major histocompatibility complex expressed by fetal cells, (2) trapping of dendritic cells within the decidua minimizing the immunogenic presentation of conceptus-derived antigens, (3) suppression T cell activation by Tregs in response to conceptus-derived antigens, (4) epigenetic silencing of chemokine genes in decidual stromal cells which prevents activated T cells to decidualization. These local and systemic processes participate to maintain the tolerance of the semiallogenic fetus [14]. Altogether, these molecular processes observed during normal decidualization are the cornerstone of normal placentation that is a prerequisite to normal pregnancy outcomes.





From Gellersen B, Brosens JJ. Cyclic decidualization of the human endometrium in reproductive health and failure. Endocr Rev 2014;35:851–905.

Compromised decidualization in case of endometriosis

Decidualization defect are a major cause of reproductive failures. In a mouse model evaluating the uterus age on pregnancy courses, young mice showed litter pattern that appears normal without defect, whereas aged mice showed altered litter pattern with more heterogeneity and fetal resorptions. These clinical observations were correlated to a clear change in gene expression in placenta with age, reduction of cell proliferation with change in KI-67 expression [15]. Likewise to age, several data have demonstrated the alteration of eutopic endometrium in endometriosis and adenomyosis by numerous cellular and biochemical alterations. Women with endometriosis, alteration of endometrium could contribute to impaired reproduction and decidualization processes [13]. Several structural and molecular features of the endometrium are deregulated including immune factors and adhesion molecules [16], cell proliferation and apoptosis [17] cytokine and inflammatory mediators [18,19], steroid and epigenetic factor [20], oxidative stress and free radical metabolism [18,19]. This participate to a presupposed sequence that profoundly compromise the normal decidual response [21]. More specifically, several genes have been shown aberrantly expressed in endometrium from women with endometriosis including aromatase, 17b hydroxysteroid dehydrogenase, Hoxa-10, Hoxa-11, Lif, MMP progesterone receptors [22,23].

One of the suspected pathogenesis is based on the potential increased risk of defective deep placentation in an altered eutopic endometrium due to endometriosis [24,25]. During decidualization, in case of endometriosis, endometrium showed histologic changes with lower molecular changes and progesterone resistance linked to failure to metabolize estradiol [26]. Other studies showed reduced decidualization capacities with a decrease PRL secretion in cultured stromal cells also a decrease of IGFB-1, molecules that are both main actors of decidualization process [27]. Decreased PRL and IGFBP1 associated with increased aromatase activity [28] and activation of the PI3/AKT signaling pathway participate to the reduced expression of the decidua-specific gene potentially through reduced levels of nuclear factor FOXO1 [29]. All these alterations may participate to affect normal development of fetal

adnexes and in this way fetal membranes have also been reported to show structural (endometriosis-like glands) and molecular changes (gene expression and methylation) in the choriodecidual layer in case of endometriosis [30].

Decidualization of ectopic lesions

Formation of ectopic decidua (deciduosis) during pregnancy is a well-documented phenomenon, usually asymptomatic, that has been attributed to hormonal effects on the ectopic endometrium [31]. Ectopic decidua formation during pregnancy (defined as stromal cell transformation of peritoneum) has been attributed to hormonal effects during pregnancy (mainly progesterone) on the ectopic endometrium [31].

Decidualization also occur in the ectopic endometriotic lesions [32,33]. This pregnancy-associated ectopic decidualization could however lead to suspect image mimicking malignancy [9,34,35]. Historically, ectopic endometriotic lesions were reduced in size at inspection and clinical pelvic examination [36] probably secondary to decidualization of the lesions [32]. It is not clear whether these changes in ectopic lesions persist after delivery. A previous study evaluated the clinical features and the change in size based on US during pregnancy of 25 endometrioma [37], and a three-case series of deep infiltrating endometriosis (DIE) lesions [32], both of which exhibited a change in size. Although there is limited data regarding the growth dynamics of lesions with pregnancy, most investigators have reported regression or cessation of growth during pregnancy [38,39]. In a retrospective study, Ueda et al. reported on the natural progression of 25 ovarian endometriotic lesions observed during pregnancy in 24 women, with a decrease in the volume for 13 lesions (52%), and an increase in the volume for five lesions (20%) [37]. It has been reported that MRI-based DIE lesion size changes before and after pregnancy with a significant decrease in the volume of DIE lesions and endometrioma after pregnancy [40].

Both estrogen and progesterone are key regulators of ectopic endometrial tissues that respond to hormonal stimulation [26]. Hence, the specific hormonal environment during pregnancy may impact the physical appearance of ectopic lesions through several processes: (1) amenorrhea and the absence of cyclic retrograde fallopian bleeding could contribute to a reduction of peritoneal and ectopic implant stimulation [41]; (2) anovulation secondary to the well-known hormonal ovarian blockade occurring during pregnancy may be beneficial to endometriotic lesions [42]; (3) during pregnancy, high placental production of steroid hormones might directly and positively impact endometriotic lesions [43], with a decrease in the intra- and perilesional inflammatory status and a reduced production of prostaglandins and cytokines [6]; and (4) decidualization of endometrium during pregnancy corresponds with the transformation of stromal fibroblasts into epithelioid-like decidual cells and to an adjoined massive influx of immune cells [10,11]. Hence decidualization of ectopic endometrial lesions may participate in the intrinsic changes that can persist long after delivery.

Impact of pregnancy on endometriosis symptoms

Traditionally, pregnancy has been considered to have beneficial effects on endometriosis. In 1949, Beecham considered pregnancy as an efficient prophylactic and curative measure against endometriosis, probably due to exposition of high level of progesterone [4].

Interestingly, pregnancy appears to have a beneficial, albeit transient, effect on endometriosis-related symptoms [44−46]. It appears that the intensity of dysmenorrhea significantly improves over time in multiparous patients, but it is not specific to endometriosis patients [47]. Previously published studies have reported a beneficial role of pregnancy on endometriosis-related symptomatology when comparing symptoms before et after pregnancy notably severe dysmenorrhea (before pregnancy 69% after pregnancy 34%, $P < .001$), severe deep dyspareunia (before pregnancy 40%; after pregnancy 24%; $P = .001$), severe nonmenstrual pelvic pain (before pregnancy 17% after pregnancy 10%, $P < .01$) and severe dyschezia (before pregnancy 27% after pregnancy 13%, $P < .01$) [45,46].

Impact of endometriosis on pregnancy outcomes

Evidence is accumulating of a slightly increased obstetrical risk in endometriosis-affected women [33,48] including miscarriage, premature birth, fetal growth retardation, preeclampsia, placentation disorder, and cesarean section [49−56], irrespective of the use of assisted reproductive technologies [57−59].

Miscarriage

Available studies that have investigated the association between endometriosis and miscarriage are controversial. Some previous uncontrolled studies plead for increased previous miscarriages in endometriosis [60−62], whereas other studies suggest that the spontaneous abortion rate in endometriosis may not be as high as previously reported [63]. Based on an IVF-based population, an increased previous spontaneous abortions rate was found in endometriosis-affected women [64]. In a systematic review and metaanalysis, Barbosa et al. observed a miscarriage relative risk of 1.31 (95% CI, 1.07−1.59) in case of endometrioisis, without any difference in women with different stages of the disease [65]. In a retrospective cohort study comparing exposed women (with histological confirmation of endometriosis) and control (without visible endometriosis at surgery) the previous miscarriage rate was significantly higher in women with endometriosis compared with the controls (139/478 [29] vs. 187/964 [19%], respectively; $P = .001$). After a subgroup analysis, the miscarriage rates of women with endometriosis and the controls were, respectively: 20 versus 12% ($P = .003$) among women without a previous history of infertility and 53 versus 30% ($P = .001$) for women with a previous history of infertility [54]. Recent metanalysis from Zullo et al. found an increased risk of miscarriage (OR 1.75; 95% CI, 1.29−2.37) [48].

Preterm birth

Major studies assessing the relationship between endometriosis and preterm birth are contradictory. The nationwide study found a higher risk of preterm birth in women affected with endometriosis aOR = 1.33; 95% CI, 1.23−1.44 [55], aOR = 1.26; 95% CI, 1.07−1.49 [66]. However, in the Swedish study, adjusted Odd Ratios for preterm birth stratified by assisted reproductive technology were not statistically significant in case of pregnancy with assisted reproductive technology OR = 1.24, 95% CI, 0.99 −1.54 [55]. In a 12 years cohorts study Aris et al. didn't found any statistical difference for preterm birth between woman with

and without endometriosis, with the particularity that all women were issued from a socially deprivated milieu OR = 1.15; 95% CI, 0.91−1.45 [67]. Recent metaanalysis, found that women who had a prior diagnosis of endometriosis had a statistically significantly higher risk of preterm birth <37 weeks (OR 1.63; 95% CI, 1.32−2.01) and <34 weeks (OR 1.58; 95% CI, 1.09−2.67) [48], and preterm birth <37 weeks (OR = 1.70; 95% CI, 1.40−2.06), that remain significant in subgroups of women with and without assisted reproductive technologies [59], or preterm birth <37 weeks OR = 1.38, 95% CI, 1.01−1.89) [5]. Conversely, in a study based on women achieving in vitro fecundation singleton pregnancies, the rate of preterm birth was similar between woman with endometriosis and controls [68]. However, in these studies no precaution was taken about the nature of preterm delivery (spontaneous or induced) and the impact of disease phenotype on preterm birth remain questioning.

Intrauterine growth retardation, low birth weight and small for gestational age

Data regarding association between endometriosis and small for gestational age (SGA), low birth weight (LBW), and intrauterine growth restriction (IUGR) baby risk are still controversial. Due to study designs, nature of control groups and definitions of SGA, LBW and IUGR. Among large nationwide studies, conflicting result are available about IUGR; Significant association between endometriosis and LBW have been previously reported (aOR = 1.7, 95% CI, 1.0−2.6) [69]. An association between endometriosis and LBW (<2500 g) in the group of endometriosis without assisted reproductive technology treatment (adjusted OR 1.46, 95% CI, 1.07−1.99; $P < .05$) but not in the group of endometriosis who underwent assisted reproductive technology (aOR = 0.97; 95% CI, 0.70−1.33) [70]. Conversely, in the Swedish and Scottish cohort, no statistical association was found between IUGR and endometriosis after adjusting for confounding factors (aOR = 1.04, 95% CI, 0.92−1.17) [55], (aOR = 1.12; C95% CI, 0.94−1.32). In a study based on women achieving in vitro fecundation singleton pregnancies, SGA newborns did not differ between woman with endometriosis and control [68]. In a large nationwide Danish study, based on birth weight related to the sex and gestational age−specific average, but without adjusting for assisted reproductive technology procedures, the risk of SGA was higher in the endometriosis cohort (OR 1.5; 95% CI, 1.4−1.6; $P < .05$) [71]. Metaanalysis also found higher risk for SGA <10th% (OR = 1.27; 95% CI, 1.03−1.57 [48], and LBW OR = 1.28; 95% CI, 1.11−1.49 [59].

Hypertensive disease

The potential correlation between endometriosis and hypertensive disease during pregnancy is still matter of debate. Most of studies did not found any association between endometriosis and preeclampsia. Among population based studies, no association between endometriosis and preeclampsia was found in Scottish (aOR: 1.06; 95% CI, 0.91−1.24) [66], or Australian population (OR 0.96; 95% CI, 0.9−1.3) [72]. Conversely, the Swedish study observed an increased risk for preeclampsia among women with endometriosis (adjusted OR = 1.13, 95%; CI, 1.02−1.26; P, 0.05), but no stratification was done for assisted reproductive technology and no systematic surgical confirmation for endometriosis was provided [55]. A recent review and metanalysis regarding specifically the association of endometriosis and preeclampsia in women conceiving spontaneously or through assisted reproductive

technology did not found any association between preeclampsia, eclampsia, and HELLP syndrome risk in women with endometriosis conceiving spontaneously (OR 1.21; 95% CI, 0.94−1.56) or through assisted reproductive technology (OR 0.74; 95% CI, 0.41−1.35) [73]. Metanalysis of Lalani et al. doesn't found association between gestational hypertension and/or preeclampsia and endometriosis in both subgroup of women with known spontaneous conception and with known assisted reproduction [59]. In women achieving in vitro fecundation singleton pregnancies, hypertensive disorders did not differ between woman with endometriosis and controls [68].

Placenta abnormalities

Previous studies on IVF cohort showed an increased risk of placenta previa (aOR = 2.31; 95% CI, 9−2.9) [74]. Available studies are in favor of the association between endometriosis and placenta previa. The metanalysis of Lalani observed an increased risk (OR = 3.31; 95% CI, 2.37−4.63), in both subgroup of women with known spontaneous conception (OR = 6.83; 95% CI, 2.10−22.24) and with known assisted reproduction (OR = 3.33; 95% CI, 1.52−7.30) [59]. In a specific population pf pregnancy obtained by IVF, placenta previa was more common in women with endometriosis than controls (6% vs. 1%, respectively; $P = .006$, aOR = 4.8; 95% CI, 1.4−17.2) [68]. No evident data are available on the association of placenta accreta spectrum and endometriosis.

Cesarean section

Most of available studies demonstrated an increased risk of cesarean section in case of endometriosis (aOR: 1.47; 95% CI, 1.40−1.54) [55], aOR: 1.40; 95% CI, 1.26−1.55) [66]. The available metanalysis are in line with these observation with increased OR = 1.76; 95% CI, 1.51−2.06) [59], OR 1.57; 95% CI, 1.39−1.78 [48], OR = 1.98; 95% CI, 1.64−2.38 [5].

Surgical endometriosis-related complication during pregnancy

Surgical endometriosis-related complication during pregnancy is routinely reported. The risk of such complications during pregnancy is particularly low but severe, potentially life-threatening and unpredictable complications [6]. These complications are mainly due to gestational changes of ectopic lesions, due to likewise in eutopic endometrium, ectopic endometriosis changes due to decidualization processes during pregnancy. However it cannot be excluded either that local inflammation associated with endometriosis may concomitantly weaken the vessel urinary tract and bowel wall. Finally, adhesions associated with presence of endometriosis may lead to unnatural traction that may further facilitate anatomical damages [75].

Spontaneous hemoperitoneum in pregnancy

Spontaneous hemoperitoneum during pregnancy is rare, but a dramatic cause of perinatal mortality and morbidity which occurs predominantly during the third trimester of pregnancy. Emerging evidence suggests that pelvic endometriosis may play an important role in the pathogenesis [10,11]. A case note review reports on 15 events of spontaneous

hemoperitoneum in pregnancy in 11 women and reported absence of maternal-fetal or perinatal mortality, despite a high rate of preterm births (54.5%), an increased value of imaging as a diagnostic tool, notably presence of hemoperitoneum, and recurrence within the same or a subsequent pregnancy. In all women, endometriosis was diagnosed at a certain moment in time before pregnancy, suggesting increasing awareness of this serious complication of pregnancy especially in women diagnosed with history of endometriosis [76].

Uterine rupture

The question of uterine rupture in case of endometriosis is not well addressed, and to date, few cases of uterine rupture related to adenomyosis have been reported. Based on a recent study enrolling 605,362 deliveries, uterine rupture reported rate was 5.6/10,000 deliveries [77]. The global incidence of rupture of an unscarred uterus is one in 8000 to 15,000 deliveries, or 0.006% [78], with various etiopathogenic factors including adenomyosis [79]. Berlac et al. reported 52 cases of uterine rupture before and during labor. In women with endometriosis risk of uterine rupture was higher than without (OR = 2.7; 95% CI, 2.0−3.6). Among those cases, 15/52 (28.8%) occurred in women with history of gynecologic surgery [71]. Spontaneous uterine on primigravid women and unscarred uterus rupture before labor and before term [80] or at term [81] have been previously reported. The transformation of stromal cells in adenomyosis could lead to atrophy and necrosis of the myometrium with reduction of the uterine muscle mass that weak the myometrium during pregnancy [81−83].

Urinary tract complications

Very rare case of urinary tract injuries related to endometriosis have been reported during pregnancy. A case of active hemorrhage arising from right uterine artery and interruption of the ureter in an area of previously documented but not treated endometriotic nodule at 31 weeks of gestation, with fetal death, have been described [84]. Another case of uroperitoneum was observed 6 h after a preterm delivery at 27 weeks of gestation in a patient with clinical history remarkable for intestinal and bladder endometriosis and who undergone transurethral resection of a bladder nodule two years before conception [85].

Bowel complications

There are several reports of bowel perforations during pregnancy and all are not related to endometriosis. In a literature review, Roberti Maggiore et al. [86] reported total of 17 cases of bowel perforations during pregnancy related to the presence of endometriosis. The most frequent location of perforations was the sigmoid colon or rectum. Decidualized endometriosis was demonstrated in most of the cases after histological, and all pregnancy ended in live births [86]. Rare cause of acute appendicitis during pregnancy have been reported, attributed to decidualization of appendiceal locations of endometriosis [87−90].

Conclusion

Women with endometriosis have a higher but low risk of perinatal complications with rare but severe surgical complication during pregnancy. Assisted reproductive therapy does not appear to increase perinatal risk. It is nonetheless not demonstrated that modification of conventional pregnancy monitoring for patients with endometriosis is needed.

References

[1] Leeners B, Damaso F, Ochsenbein-Kolble N, Farquhar C. The effect of pregnancy on endometriosis-facts or fiction? Hum Reprod Update 2018;24:290–9.
[2] Lessey BA, Kim JJ. Endometrial receptivity in the eutopic endometrium of women with endometriosis: it is affected, and let me show you why. Fertil Steril 2017;108:19–27.
[3] Miravet-Valenciano J, Ruiz-Alonso M, Gomez E, Garcia-Velasco JA. Endometrial receptivity in eutopic endometrium in patients with endometriosis: it is not affected, and let me show you why. Fertil Steril 2017;108:28–31.
[4] Beecham CT. Surgical treatment of endometriosis with special reference to conservative surgery in young women. J Am Med Assoc 1949;139:971.
[5] Horton J, Sterrenburg M, Lane S, Maheshwari A, Li TC, Cheong Y. Reproductive, obstetric, and perinatal outcomes of women with adenomyosis and endometriosis: a systematic review and meta-analysis. Hum Reprod Update 2019;25:592–632.
[6] Leone Roberti Maggiore U, Ferrero S, Mangili G, Bergamini A, Inversetti A, Giorgione V, Vigano P, Candiani M. A systematic review on endometriosis during pregnancy: diagnosis, misdiagnosis, complications and outcomes. Hum Reprod Update 2016;22:70–103.
[7] Jabbour HN, Kelly RW, Fraser HM, Critchley HO. Endocrine regulation of menstruation. Endocr Rev 2006;27:17–46.
[8] Okada H, Tsuzuki T, Murata H. Decidualization of the human endometrium. Reprod Med Biol 2018;17:220–7.
[9] Sammour RN, Leibovitz Z, Shapiro I, Degani S, Levitan Z, Aharoni A, Tal J, Lurie M, Ohel G. Decidualization of ovarian endometriosis during pregnancy mimicking malignancy. J Ultrasound Med 2005;24:1289–94.
[10] Brosens IA, Fusi L, Brosens JJ. Endometriosis is a risk factor for spontaneous hemoperitoneum during pregnancy. Fertil Steril 2009;92:1243–5.
[11] Brosens JJ, Parker MG, McIndoe A, Pijnenborg R, Brosens IA. A role for menstruation in preconditioning the uterus for successful pregnancy. Am J Obstet Gynecol 2009;200(615):e611–616.
[12] Schatz F, Guzeloglu-Kayisli O, Arlier S, Kayisli UA, Lockwood CJ. The role of decidual cells in uterine hemostasis, menstruation, inflammation, adverse pregnancy outcomes and abnormal uterine bleeding. Hum Reprod Update 2016;22:497–515.
[13] Gellersen B, Brosens JJ. Cyclic decidualization of the human endometrium in reproductive health and failure. Endocr Rev 2014;35:851–905.
[14] Erlebacher A. Mechanisms of T cell tolerance towards the allogeneic fetus. Nat Rev Immunol 2013;13:23–33.
[15] Woods L, Perez-Garcia V, Kieckbusch J, Wang X, DeMayo F, Colucci F, Hemberger M. Decidualisation and placentation defects are a major cause of age-related reproductive decline. Nat Commun 2017;8:352.
[16] Riccio L, Santulli P, Marcellin L, Abrao MS, Batteux F, Chapron C. Immunology of endometriosis. Best Pract Res Clin Obstet Gynaecol 2018;50:39–49.
[17] Ngo C, Chereau C, Nicco C, Weill B, Chapron C, Batteux F. Reactive oxygen species controls endometriosis progression. Am J Pathol 2009;175:225–34.
[18] Santulli P, Chouzenoux S, Fiorese M, Marcellin L, Lemarechal H, Millischer AE, Batteux F, Borderie D, Chapron C. Protein oxidative stress markers in peritoneal fluids of women with deep infiltrating endometriosis are increased. Hum Reprod 2015;30:49–60.
[19] Santulli P, Marcellin L, Tosti C, Chouzenoux S, Cerles O, Borghese B, Batteux F, Chapron C. MAP kinases and the inflammatory signaling cascade as targets for the treatment of endometriosis? Expert Opin Ther Targets 2015;19:1465–83.
[20] Borghese B, Zondervan KT, Abrao MS, Chapron C, Vaiman D. Recent insights on the genetics and epigenetics of endometriosis. Clin Genet 2017;91:254–64.
[21] Benagiano G, Brosens I, Habiba M. Structural and molecular features of the endomyometrium in endometriosis and adenomyosis. Hum Reprod Update 2014;20:386–402.
[22] Aghajanova L, Tatsumi K, Horcajadas JA, Zamah AM, Esteban FJ, Herndon CN, Conti M, Giudice LC. Unique transcriptome, pathways, and networks in the human endometrial fibroblast response to progesterone in endometriosis. Biol Reprod 2011;84:801–15.
[23] Herndon CN, Aghajanova L, Balayan S, Erikson D, Barragan F, Goldfien G, Vo KC, Hawkins S, Giudice LC. Global transcriptome abnormalities of the eutopic endometrium from women with adenomyosis. Reprod Sci 2016;23:1289–303.
[24] Brosens I, Pijnenborg R, Benagiano G. Defective myometrial spiral artery remodelling as a cause of major obstetrical syndromes in endometriosis and adenomyosis. Placenta 2013;34:100–5.

[25] Brosens I, Pijnenborg R, Vercruysse L, Romero R. The "Great Obstetrical Syndromes" are associated with disorders of deep placentation. Am J Obstet Gynecol 2011;204:193–201.
[26] Bulun SE, Cheng YH, Yin P, Imir G, Utsunomiya H, Attar E, Innes J, Julie Kim J. Progesterone resistance in endometriosis: link to failure to metabolize estradiol. Mol Cell Endocrinol 2006;248:94–103.
[27] Fluhr H, Krenzer S, Deperschmidt M, Zwirner M, Wallwiener D, Licht P. Human chorionic gonadotropin inhibits insulin-like growth factor-binding protein-1 and prolactin in decidualized human endometrial stromal cells. Fertil Steril 2006;86:236–8.
[28] Aghajanova L, Hamilton A, Kwintkiewicz J, Vo KC, Giudice LC. Steroidogenic enzyme and key decidualization marker dysregulation in endometrial stromal cells from women with versus without endometriosis. Biol Reprod 2009;80:105–14.
[29] Yin X, Pavone ME, Lu Z, Wei J, Kim JJ. Increased activation of the PI3K/AKT pathway compromises decidualization of stromal cells from endometriosis. J Clin Endocrinol Metab 2012;97:E35–43.
[30] Marcellin L, Santulli P, Gogusev J, Lesaffre C, Jacques S, Chapron C, Goffinet F, Vaiman D, Mehats C. Endometriosis also affects the decidua in contact with the fetal membranes during pregnancy. Hum Reprod 2015;30:392–405.
[31] Zaytsev P, Taxy JB. Pregnancy-associated ectopic decidua. Am J Surg Pathol 1987;11:526–30.
[32] Coccia ME, Rizzello F, Palagiano A, Scarselli G. The effect of the hormonal milieu of pregnancy on deep infiltrating endometriosis: serial ultrasound assessment of changes in size and pattern of deep endometriotic lesions. Eur J Obstet Gynecol Reprod Biol 2012;160:35–9.
[33] Vannuccini S, Clifton VL, Fraser IS, Taylor HS, Critchley H, Giudice LC, Petraglia F. Infertility and reproductive disorders: impact of hormonal and inflammatory mechanisms on pregnancy outcome. Hum Reprod Update 2016;22:104–15.
[34] Machida S, Matsubara S, Ohwada M, Ogoyama M, Kuwata T, Watanabe T, Izumi A, Suzuki M. Decidualization of ovarian endometriosis during pregnancy mimicking malignancy: report of three cases with a literature review. Gynecol Obstet Invest 2008;66:241–7.
[35] Richard F, Canlorbe G, Bazot M, Darai E. Management of pregnancy in woman with suspected malignant deep infiltrating endometriosis fistulised to the uterine cervix. BMJ Case Rep 2014;2014.
[36] McArthur JW, Ulfelder H. The effect of pregnancy upon endometriosis. Obstet Gynecol Surv 1965;20:709–33.
[37] Ueda Y, Enomoto T, Miyatake T, Fujita M, Yamamoto R, Kanagawa T, Shimizu H, Kimura T. A retrospective analysis of ovarian endometriosis during pregnancy. Fertil Steril 2010;94:78–84.
[38] Bailleux M, Bernard JP, Benachi A, Deffieux X. Ovarian endometriosis during pregnancy: a series of 53 endometriomas. Eur J Obstet Gynecol Reprod Biol 2017;209:100–4.
[39] Benaglia L, Somigliana E, Calzolari L, Busnelli A, Cardellicchio L, Ragni G, Fedele L. The vanishing endometrioma: the intriguing impact of pregnancy on small endometriotic ovarian cysts. Gynecol Endocrinol 2013;29:863–6.
[40] Millischer AE, Marcellin L, Santulli P, Maignien C, Bourdon M, Borghese B, Goffinet F, Chapron C. Magnetic resonance imaging presentation of deep infiltrating endometriosis nodules before and after pregnancy: a case series. PLoS One 2019;14:e0223330.
[41] Sampson JA. Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. Am J Pathol 1927;3:93–110. 143.
[42] Santulli P, Borghese B, Noel JC, Fayt I, Anaf V, de Ziegler D, Batteux F, Vaiman D, Chapron C. Hormonal therapy deregulates prostaglandin-endoperoxidase synthase 2 (PTGS2) expression in endometriotic tissues. J Clin Endocrinol Metab 2014;99:881–90.
[43] Vercellini P, Crosignani PG, Somigliana E, Berlanda N, Barbara G, Fedele L. Medical treatment for rectovaginal endometriosis: what is the evidence? Hum Reprod 2009;24:2504–14.
[44] Alberico D, Somigliana E, Bracco B, Dhouha D, Roberto A, Mosconi P, Facchin F, Vercellini P. Potential benefits of pregnancy on endometriosis symptoms. Eur J Obstet Gynecol Reprod Biol 2018;230:182–7.
[45] Busacca M, Chiaffarino F, Candiani M, Vignali M, Bertulessi C, Oggioni G, Parazzini F. Determinants of long-term clinically detected recurrence rates of deep, ovarian, and pelvic endometriosis. Am J Obstet Gynecol 2006;195:426–32.
[46] Porpora MG, Pallante D, Ferro A, Crisafi B, Bellati F, Benedetti Panici P. Pain and ovarian endometrioma recurrence after laparoscopic treatment of endometriosis: a long-term prospective study. Fertil Steril 2010;93:716–21.

[47] Weissman AM, Hartz AJ, Hansen MD, Johnson SR. The natural history of primary dysmenorrhoea: a longitudinal study. BJOG An Int J Obstet Gynaecol 2004;111:345–52.
[48] Zullo F, Spagnolo E, Saccone G, Acunzo M, Xodo S, Ceccaroni M, Berghella V. Endometriosis and obstetrics complications: a systematic review and meta-analysis. Fertil Steril 2017;108:667–72. e665.
[49] Benaglia L, Bermejo A, Somigliana E, Scarduelli C, Ragni G, Fedele L, Garcia-Velasco JA. Pregnancy outcome in women with endometriomas achieving pregnancy through IVF. Hum Reprod 2012;27:1663–7.
[50] Conti N, Cevenini G, Vannuccini S, Orlandini C, Valensise H, Gervasi MT, Ghezzi F, Di Tommaso M, Severi FM, Petraglia F. Women with endometriosis at first pregnancy have an increased risk of adverse obstetric outcome. J Matern Fetal Neonatal Med 2015;28:1795–8.
[51] Exacoustos C, Lauriola I, Lazzeri L, De Felice G, Zupi E. Complications during pregnancy and delivery in women with untreated rectovaginal deep infiltrating endometriosis. Fertil Steril 2016;106:1129–35. e1121.
[52] Fernando S, Breheny S, Jaques AM, Halliday JL, Baker G, Healy D. Preterm birth, ovarian endometriomata, and assisted reproduction technologies. Fertil Steril 2009;91:325–30.
[53] Kortelahti M, Anttila MA, Hippelainen MI, Heinonen ST. Obstetric outcome in women with endometriosis–a matched case-control study. Gynecol Obstet Invest 2003;56:207–12.
[54] Santulli P, Marcellin L, Menard S, Thubert T, Khoshnood B, Gayet V, Goffinet F, Ancel PY, Chapron C. Increased rate of spontaneous miscarriages in endometriosis-affected women. Hum Reprod 2016;31:1014–23.
[55] Stephansson O, Kieler H, Granath F, Falconer H. Endometriosis, assisted reproduction technology, and risk of adverse pregnancy outcome. Hum Reprod 2009;24:2341–7.
[56] Vercellini P, Parazzini F, Pietropaolo G, Cipriani S, Frattaruolo MP, Fedele L. Pregnancy outcome in women with peritoneal, ovarian and rectovaginal endometriosis: a retrospective cohort study. BJOG An Int J Obstet Gynaecol 2012;119:1538–43.
[57] Glavind MT, Forman A, Arendt LH, Nielsen K, Henriksen TB. Endometriosis and pregnancy complications: a Danish cohort study. Fertil Steril 2017;107:160–6.
[58] Harada T, Taniguchi F, Onishi K, Kurozawa Y, Hayashi K, Harada T, Japan E. Children's study G. Obstetrical complications in women with endometriosis: a cohort study in Japan. PLoS One 2016;11:e0168476.
[59] Lalani S, Choudhry AJ, Firth B, Bacal V, Walker M, Wen SW, Singh S, Amath A, Hodge M, Chen I. Endometriosis and adverse maternal, fetal and neonatal outcomes, a systematic review and meta-analysis. Hum Reprod 2018;33:1854–65.
[60] Groll M. Endometriosis and spontaneous abortion. Fertil Steril 1984;41:933–5.
[61] Naples JD, Batt RE, Sadigh H. Spontaneous abortion rate in patients with endometriosis. Obstet Gynecol 1981;57:509–12.
[62] Wheeler JM, Johnston BM, Malinak LR. The relationship of endometriosis to spontaneous abortion. Fertil Steril 1983;39:656–60.
[63] Metzger DA, Olive DL, Stohs GF, Franklin RR. Association of endometriosis and spontaneous abortion: effect of control group selection. Fertil Steril 1986;45:18–22.
[64] Matalliotakis I, Cakmak H, Dermitzaki D, Zervoudis S, Goumenou A, Fragouli Y. Increased rate of endometriosis and spontaneous abortion in an in vitro fertilization program: no correlation with epidemiological factors. Gynecol Endocrinol 2008;24:194–8.
[65] Barbosa MA, Teixeira DM, Navarro PA, Ferriani RA, Nastri CO, Martins WP. Impact of endometriosis and its staging on assisted reproduction outcome: systematic review and meta-analysis. Ultrasound Obstet Gynecol 2014;44:261–78.
[66] Saraswat L, Ayansina DT, Cooper KG, Bhattacharya S, Miligkos D, Horne AW, Bhattacharya S. Pregnancy outcomes in women with endometriosis: a national record linkage study. BJOG An Int J Obstet Gynaecol 2017;124:444–52.
[67] Aris A. A 12-year cohort study on adverse pregnancy outcomes in Eastern Townships of Canada: impact of endometriosis. Gynecol Endocrinol 2014;30:34–7.
[68] Benaglia L, Candotti G, Papaleo E, Pagliardini L, Leonardi M, Reschini M, Quaranta L, Munaretto M, Vigano P, Candiani M, et al. Pregnancy outcome in women with endometriosis achieving pregnancy with IVF. Hum Reprod 2016;31:2730–6.
[69] Borghese B, Sibiude J, Santulli P, Lafay Pillet MC, Marcellin L, Brosens I, Chapron C. Low birth weight is strongly associated with the risk of deep infiltrating endometriosis: results of a 743 case-control study. PLoS One 2015;10:e0117387.

[70] Stern JE, Luke B, Tobias M, Gopal D, Hornstein MD, Diop H. Adverse pregnancy and birth outcomes associated with underlying diagnosis with and without assisted reproductive technology treatment. Fertil Steril 2015;103:1438–45.
[71] Berlac JF, Hartwell D, Skovlund CW, Langhoff-Roos J, Lidegaard O. Endometriosis increases the risk of obstetrical and neonatal complications. Acta Obstet Gynecol Scand 2017;96:751–60.
[72] Hadfield RM, Lain SJ, Raynes-Greenow CH, Morris JM, Roberts CL. Is there an association between endometriosis and the risk of pre-eclampsia? A population based study. Hum Reprod 2009;24:2348–52.
[73] Perez-Lopez FR, Calvo-Latorre J, Alonso-Ventura V, Bueno-Notivol J, Martinez-Dominguez SJ, Chedraui P, Health Outcomes SAP. Systematic review and meta-analysis regarding the association of endometriosis and pre-eclampsia in women conceiving spontaneously or through assisted reproductive technology. Pregnancy Hypertens 2018;14:213–21.
[74] Healy DL, Breheny S, Halliday J, Jaques A, Rushford D, Garrett C, Talbot JM, Baker HW. Prevalence and risk factors for obstetric haemorrhage in 6730 singleton births after assisted reproductive technology in Victoria Australia. Hum Reprod 2010;25:265–74.
[75] Inoue T, Moriwaki T, Niki I. Endometriosis and spontaneous rupture of utero-ovarian vessels during pregnancy. Lancet 1992;340:240–1.
[76] Lier M, Malik RF, van Waesberghe J, Maas JW, van Rumpt-van de Geest DA, Coppus SF, Berger JP, van Rijn BB, Janssen PF, de Boer MA, et al. Spontaneous haemoperitoneum in pregnancy and endometriosis: a case series. BJOG An Int J Obstet Gynaecol 2017;124:306–12.
[77] Colmorn LB, Petersen KB, Jakobsson M, Lindqvist PG, Klungsoyr K, Kallen K, Bjarnadottir RI, Tapper AM, Bordahl PE, Gottvall K, et al. The Nordic Obstetric Surveillance Study: a study of complete uterine rupture, abnormally invasive placenta, peripartum hysterectomy, and severe blood loss at delivery. Acta Obstet Gynecol Scand 2015;94:734–44.
[78] Posthumus L, Donker ME. Uterine rupture in a primigravid patient, an uncommon but severe obstetrical event: a case report. J Med Case Rep 2017;11:339.
[79] Sun Y, Huang J, Kong HF. Spontaneous rupture of unscarred uterus in the third trimester after in vitro fertilization-embryo transfer because of bilateral salpingectomy: a case report. Medicine 2019;98:e18182.
[80] Indraccolo U, Iannicco A, Micucci G. A novel case of an adenomyosis-related uterine rupture in pregnancy. Clin Exp Obstet Gynecol 2015;42:810–1.
[81] Nohuz E, Albaut M, Bayeh S, Tamburro S, Chene G. Adenomyosis and pregnant uterus: an alliance doomed to rupture? J Gynecol Obstet Hum Reprod 2020;49:101632.
[82] Delepine O, Curinier S, Agar N, Piquier-Perret G, Gallot D, Houlle C, Canis M, Pouly JL. [About a case of uterine per-partum rupture, 37months after resection of a rectovaginal endometriosis nodule]. J Gynecol Obstet Biol Reprod 2016;45:985–9.
[83] Wada S, Kudo M, Minakami H. Spontaneous uterine rupture of a twin pregnancy after a laparoscopic adenomyomectomy: a case report. J Minim Invasive Gynecol 2006;13:166–8.
[84] Chiodo I, Somigliana E, Dousset B, Chapron C. Urohemoperitoneum during pregnancy with consequent fetal death in a patient with deep endometriosis. J Minim Invasive Gynecol 2008;15:202–4.
[85] Leone Roberti Maggiore U, Remorgida V, Sala P, Vellone VG, Biscaldi E, Ferrero S. Spontaneous uroperitoneum and preterm delivery in a patient with bladder endometriosis. J Minim Invasive Gynecol 2015;22:923–4.
[86] Leone Roberti Maggiore U, Inversetti A, Schimberni M, Vigano P, Giorgione V, Candiani M. Obstetrical complications of endometriosis, particularly deep endometriosis. Fertil Steril 2017;108:895–912.
[87] Dimitriadis PA, Makar RR, Kingston G, Farouk R. Appendiceal endometriosis and carcinoid presented as acute appendicitis in pregnancy: a rare case report and review of the literature. Case Rep Obstet Gynecol 2013;2013:360459.
[88] Lam KJ, Tagaloa S. Endometriosis: a rare cause of acute appendicitis in pregnancy. ANZ J Surg 2020;90(5):935–7.
[89] Lebastchi AH, Prieto PA, Chen C, Lui FY. Appendiceal endometriosis in a pregnant woman presenting with acute perforated appendicitis. J Surg Case Rep 2013;2013.
[90] Murphy SJ, Kaur A, Wullschleger ME. Endometrial decidualization: a rare cause of acute appendicitis during pregnancy. J Surg Case Rep 2016;2016.

SECTION III

Immunology and the management of endometriosis

This page intentionally left blank

CHAPTER

15

Prevalent innate and adaptive immune mechanisms in endometriosis

Ryan M. Marks, Harshavardhan Lingegowda, Alison McCallion, Anushka Nair and Chandrakant Tayade

Department of Biomedical and Molecular Sciences, Queen's University, Kingston, ON, Canada

Introduction

Endometriosis is an inflammatory gynecological disease that affects approximately 1 in 10 reproductive age women globally [1]. The disease is characterized by the presence of ectopic endometrial tissue (uterine lining/mucosa), developing outside uterus [1,2]. Endometriotic lesions are typically observed developing superficially on the pelvic peritoneum, ovaries (as endometriomas), and/or invading the rectovaginal septum (deep-infiltrating endometriosis, DIE) and other pelvic structures. The most commonly accepted etiology is Sampson's theory of retrograde menstruation, which suggests that these ectopic lesions originate from within the uterus, whereby during menses, menstrual debris is refluxed via the fallopian tubes into the peritoneal cavity and proliferate [3]. This phenomenon is believed to be accompanied by a variety of immunological responses [4]. Invasion of lesions into these structures is typically accompanied by adhesions and fibrosis causing anatomical distortions, in addition to becoming highly vascularized and innervated during development [2]. Clinically, patients will often present with symptoms including chronic pelvic pain, menstrual irregularities, and infertility, which significantly reduce quality of life for afflicted women [5]. As a remarkably under studied disease, we are only just beginning to understand the complex origin of ectopic endometrial lesion origin and development, the interplay between the local immune microenvironment and the induction of inflammation, and the synergistic role of endocrinological factors, which combine to promote disease pathophysiology. In this chapter, we provide critical insights into the complexities of immune-inflammatory pathways and mediators relevant to the pathophysiology of endometriosis.

Immunology of Endometriosis
https://doi.org/10.1016/B978-0-12-820661-4.00002-9

© 2022 Elsevier Inc. All rights reserved.

The dynamic immune landscape in the eutopic endometrium

Endometrial cyclicality is accompanied by dramatic morphological changes driven by cycling ovarian hormones. Indeed, the proliferation of the endometrium is accompanied by alterations in the local immune cell populations in a menstrual phase dependent manner, and has been reviewed masterfully by Vallvé-Juanico et al. [6]. In summary, leukocytes may comprise up to 10%–20% of cells in the endometrial functionalis layer during the secretory phase, increasing from only 6%–8% during the proliferative phase [6–8]. Most immune cells in the healthy endometrium are presumed to be tissue-resident, while some populations have been speculated to arise from peripherally circulating sources [9]. Immune cells are commonly observed to be dispersed throughout both the stromal compartments and intraepithelial regions in the functionalis layer, in addition to lymphoid aggregates in the basalis layer. These aggregates develop transiently during the proliferative phase and comprise a core of B cells surrounded by a population of CD8+ T lymphocytes and macrophages [6,10]. The complexity of the endometrial immune landscape has likely evolved to satisfy specific demands and immunological challenges associated with human pregnancy. As a result, it is unsurprising that the evolutionary need for such a highly regulated uterine immune environment or disruption of immunological niches, either by alterations in cell function or proportion, have devastating consequences on reproductive capacity and the promotion of gynecological pathologies.

Retrograde menstruation theory commonly proposes that during menstruation, refluxed endometrial tissue may adhere and invade at ectopic sites, where they may ultimately proliferate and sustain lesion growth [1,2]. Many evidences support that the eutopic endometrium from patients who develop disease may harbor predisposing immunomodulatory factors which may ultimately support peritoneal invasion and the stimulation of inflammation from refluxed menstrual debris. Evidence for this hypothesis falls into three broad observations, collectively discussed as (1) retrograde menstruation occurs ubiquitously among women lacking fallopian tube pathology (i.e., maintenance of patency), (2) endometrial cells from menstrual debris are viable and maintain proliferative status should favorable conditions be provided by the aberrant immune response, and (3) alterations in immunological homeostasis which promote tissue remodeling and lesion development [4]. Ultimately, it has been proposed that these changes in immunological condition of the eutopic endometrium are responsible for transforming the reflux cellular debris to a phenotype with aptitude for ectopic growth. This refluxed tissue in endometriosis patient may therefore be capable of avoiding apoptosis and immune clearance, as well as predisposing the patient to chronic pelvic inflammatory sequela.

Prominent roles of macrophages in endometrial-associated inflammation

Macrophages have an intimate relationship with the dynamic physiological changes in the uterus. In the healthy endometrium, macrophages can be observed throughout epithelial and stromal compartments and can accumulate up to 15% of total endometrial cells by the menstrual phase [11]. This increase in population has likely evolved as a product of their

phagocytic properties and ability to clear cellular debris and apoptotic cells, as well as facilitate endometrial repair following menses. As such, it has been suggested that macrophages in the uterus assume varying activation states depending on the menstrual phase. Fluctuations in uterine macrophage populations may be a direct or indirect effect of cycling ovarian hormones; however, little is known about their specific roles in the healthy endometrium or how they interact with other resident immune cells (i.e., uterine natural killer cells) to facilitate normal menstrual regeneration, facilitate implantation/placentation, promote mucosal immunity, or cause adverse inflammation and disease. Nonetheless, evidence suggests that women with endometriosis experience perturbations in both the cyclical turnover of endometrial macrophages throughout the menstrual cycle and activation phenotypes. Varying reports suggest that women with endometriosis have significantly greater abundance of macrophages in the proliferative phases compared to the typical decrease seen in healthy women, while others have observed increases macrophage numbers in all menstrual phases, not adhering to typically cycling dependent behavior [6,12,13]. It has been proposed that increases in macrophage accumulation may be caused by higher local concentration of the potent macrophage chemokines such as monocyte chemoattractant protein-1 (MCP-1) [14,15]. It has been suggested that this may ultimately lead to aberrant cytokine signaling eutopically, stimulating local proinflammatory responses which may induce an antiapoptotic state in endometrial cells, enhancing their aptitude for survival upon reflux and facilitating the ability for these cells to avoid senescence prior to peritoneal invasion and lesion establishment. Specifically, macrophages may secrete tumor necrosis factor (TNF) to induce apoptosis in endometrial cells in the homeostatic clearing of menstrual debris. Han et al. demonstrated that endometrial cells from endometriosis patients conveyed resistance to TNF-mediated apoptosis, in which apoptotic signaling was diverted to induce inflammasome activation and avoid cellular senescence [16]. This accumulates to increased production of interleukin (IL)-1β, which has been documented to predominate the endometriotic milieu, promote peritoneal inflammation and drive the adhesive and invasive phenotypes observed in endometriotic cells [16]. Further investigation into the relevance of the phenomenon of macrophage accumulation and their functions in the eutopic endometrium toward promoting endometriosis are required. In vivo animal models focusing on manipulating local macrophage recruitment and activation status are likely to be beneficial to explore the causative roles of macrophages in predisposing the eutopic endometrium for lesion formation and if these populations can be altered therapeutically to reduce disease incidence and support fertility.

Macrophage activation paradigms

Macrophages were originally classified as bona fide members of the mononuclear phagocyte system, the family of leukocytes comprising bone marrow progenitors, blood monocytes, tissue macrophages, and professional antigen presenting cells including dendritic cells [17]. Macrophages are a major resident leukocyte population in most tissues of the body whose resident population can increase significantly in response to a variety of inflammatory stimuli not limited to infection, tissue regeneration/wound healing, and malignancy. Perhaps the most critical function of macrophage is to serve as master regulators of the innate immune

response, primarily by coordinating the release of key chemokines and cytokines involved in the recruitment and regulation of leukocyte activity, respectively. Thus, macrophages have central functions in the elicitation of inflammatory responses and promotion of homeostatic resolution. In the case of sterile inflammation and wound healing, macrophages are responsible for the clearance of cellular debris, release of matrix metalloproteases to facilitate tissue remodeling, and facilitate the neovascularization of hypoxic tissue by secreting potent angiogenic factors, including vascular endothelial growth factor (VEGF) [18]. Nonetheless, this broad scope of macrophage activity and tissue-specific functions require these cells to be highly plastic and acquire a variety of functional states. Indeed, macrophages adopt distinct activation paradigms in response to local microenvironmental cues and as a result may propagate inflammatory responses or promote resolution, tissue remodeling and neoangiogenesis. The "classical" proinflammatory phenotype (M1) is most commonly seen in the immune response to pathogen associated molecular patterns (i.e., lipopolysaccharides) and endogenous danger signals released during cellular damage [18,19]. These cells secrete inflammatory cytokines, including IL-1β and TNF, and generate molecules involved in the antimicrobial responses, such as reactive oxygen species. Conversely, alternative activated macrophages (M2) are generally involved in wound healing and tissue remodeling and are often observed in the resolution stages of the immune response. This phenotype is responsible for the secretion of IL-10 (an immunosuppressive cytokine), the production of tissue growth factors, and are typically associated with the T-helper 2 response [18]. Importantly, we must appreciate that the acquired macrophage phenotypes are dictated by local microenvironmental signal and are bidirectional and transient in the process of inflammatory homeostasis. Many disease conditions seek advantages in the extremes of macrophage activation status. For example, developing tumors may thrive by promoting in the immunosuppressive and highly vascularized environments induced by M2 macrophages, whereas chronic inflammation in inflammatory bowel disease can be attributed, at least in part, to unregulated M1 macrophage activity [20,21]. Homeostasis between phenotypes ensures the successfully instigation and resolution of inflammation and it is probable that these phenotypes exist on a spectrum of activation states in both health and disease.

Macrophages are major constituents of the endometriotic lesion-immune microenvironment

The endometriotic lesion-immune microenvironment is undoubtably a complex and dynamic landscape that is only recently undergone evaluation. Many studies to date are beginning to characterize the various leukocyte populations which fluctuate in both local peritoneal and systemic compartments and appear to correlate with clinical findings of endometriosis. Of these reports, macrophages are consistently among the most commonly associated leukocyte population accompanying the development of endometriosis in women [22]. Indeed, macrophage populations are significantly elevated in the local peritoneal environment, which we believe is likely in response to inflammatory signals derived from refluxed menstrual debris [23]. As innate immune sentinels, macrophages are likely among the first leukocytes recruited to the developing endometriotic lesions in attempt to phagocytose

cellular debris. Additionally, macrophages have been suggested to possess active roles in the pathophysiology and development of endometriotic lesions and are not merely inert immunological bystanders [4]. Specifically, macrophages in the peritoneal fluid of endometriosis patients have been observed to produce increased peritoneal concentration of both proinflammatory and proangiogenic cytokines, including VEGF, TNF, and IL-1β and IL-8 [4,18]. Specially, TNF has been reported in higher levels in the endometrium and peritoneal fluid of women with endometriosis in mild or early stages of the disease, suggesting that it may have a role in the early stages of disease while lesions are establishing [24,25]. TNF can also stimulate the expression of prostaglandin synthase-2, which in turn increases the production of prostaglandin E2 (PGE2) [26]. In addition to the diverse role of PGE2 in promoting inflammatory effects, it may also up-regulate the expression of aromatase in endometriotic lesions, leading to excessive endogenous steroid synthesis which may further promote lesion development [26]. Although endometriosis is a benign condition, the functionality of macrophages and their interaction with the developing lesions shares many characteristics with tumor-associated macrophages (TAMs). During the development of neoplastic tissues, macrophages have prominent role in inducing inflammation, tissue remodeling, and neovascularization and much of their role in endometriosis has been evaluated thorough the lens of cancer immunology [18].

The infiltration of macrophage is a consistent feature of endometriosis, however the stimuli responsible for the recruitment and pathological activation of macrophages remains elusive. Nonetheless, macrophage activation has been demonstrated to have vital roles in the progression of endometriosis. Experimental evidence has demonstrated that depletion of macrophage from mouse models results in endometriotic lesions which are not only smaller in volume, but accordingly display reduced vascularization compared to their immunological competent counterparts [27]. Importantly, depletion of macrophage did not inhibit the development of endometriosis in this model, but rather attenuated the lesion development. Coculture systems of endometriotic stromal cells with patient macrophages support the role of immune cells in promoting the invasive phenotypes of endometriotic cells observed, indicating they may only function to facilitate lesion development inadvertently, but are not essential to disease progression [28]. Furthermore, Bacci et al. was able to provide evidence that adoptively transferring M2 polarized macrophages were able to significantly enhance lesions growth and vascularization, whereas the adoptive transfer of classically M1 macrophages resulted in smaller, poorly vascularized structures [27]. Although M2 macrophages have been reported to dominate the intralesion environment, the activation of macrophages is likely to comprise of a variety of functional states depending on a variety of microenvironmental queues and the presence of TNF and IL-1β suggesting M1 macrophages are likely to have some role in endometriosis pathophysiology. Ultimately, there are substantial differences in macrophage recruitment and activation phenotypes between healthy women and those who develop endometriosis. However, significant work is required to elucidate the functional and casual roles of subpopulations that comprise the lesion and peritoneal microenvironments. As such, we can predict that the detrimental activities of macrophages in endometriosis are likely the product of disruptions in normal homeostatic responses to endometrial DAMPs and their propensity for lesion development is a consequence of a failure to eliminate these endometriosis-related inflammatory stimuli.

T lymphocytes: adaptive immune roles in endometriosis

Retrograde menstruation is a common phenomenon among women of menstruating age with a prevalence of approximately 9 out of 10 women [29]. However, risk of menstrual debris proliferating in ectopic locations is largely reduced by the process of apoptosis without eliciting extensive inflammation [30,31]. This physiological function of clearing menstrual debris specifically is regulated by immune cells such as macrophages, cytotoxic T lymphocytes, and natural killer (NK) cells, while sparing eutopic endometrium that undergoes reconstruction [32,33].

Lymphocyte subpopulation broadly include CD4 and CD8 T cells and several other subtypes discussed below. CD4+ T cells are instrumental in achieving immune response against pathogens. The cytokine profile in the local environment leads to activation of naïve CD4+ T cells and its subsequent function, discussed in detail elsewhere [34]. On the other hand, pathogens in the system trigger the activation and differentiation of naïve CD8+ T cells into cytotoxic effector cells to clear the pathogens effectively [35]. Extensive studies on endometriosis suggests considerably higher number of CD4+ and CD8+ T cells infiltrating the lesion microenvironment when compared to normal endometrium [36–38]. CD4+ T-helper cells are further segregated into four groups based on the type of immune response. Type 1 (Th1) response activates T cell mediated cytotoxicity via secretion of cytokines such as IL- 2, IL-12, TNF and interferon gamma (IFNγ). Apart from Th2 cells promoting antibody mediated response, they also counteract the Th1 mediated cytotoxicity through secretion of cytokines such as IL-4, -5, -6, -10, and -13 [39,40]. Th1 and Th2 type responses are strictly balanced under normal circumstances [41]. However, elevated levels of Th2 type cytokines such as IL-4 and IL-10 have been reported in the peritoneal fluid and plasma of patients with endometriosis when compared to normal healthy controls [42–44]. Furthermore, IL-4, IL-5 and IL-10 cytokines have been linked with cellular proliferation, adhesion and suppression of cell mediated immunity [45,46]. Due to this imbalance in the regulation of Th1 and Th2 responses, a potential bias of Th2 cytokines (i.e., IL-10) might suppress the cytotoxic T cell activity in the peritoneum of women with endometriosis. CD8+ cytotoxic T cells are subsets of T cells that are imperative in cell mediated immunity [47]. Activated CD8+ TCR-MHC class 1 complex eliminates antigen containing cells by releasing cytokines, cytotoxins or by Fas receptor (FasR) and ligand (FasL) signaling, all of which leads to apoptosis [48]. FasR-FasL signaling in the immune system also regulates the cytotoxic T cell activity which eventually leads to the apoptosis of cells that present foreign antigens. Interestingly, Garcia-Velasco et al. in 2002 found that soluble levels of Fas ligand in the serum and peritoneum fluid of patients with endometriosis were significantly increased when compared to healthy fertile controls [49]. Although speculative, these findings suggest that higher levels of soluble FasL could perhaps induce apoptosis by activating FasR expressed on cytotoxic T lymphocytes which in turn attenuates scavenging activity. Likely, endometriotic lesions develop higher resistance to FasL induced apoptosis in the peritoneum leading to increased rate of survival and proliferation [30].

Additional subsets of T cells include T-helper 17 cells (Th17) and T regulatory lymphocytes (Treg). Th17 cells are found to be the predominant source of IL-17A production. Apart from mediating defense against pathogens, IL-17 has also been linked with the pathogenesis of

various autoimmune diseases, discussed elsewhere [50]. Treg possess a wide variety of immunoregulatory roles controlling proliferation of T lymphocytes [51,52], B lymphocytes, macrophages and dendritic cells, while also mediating cytokine release [53]. Th17 and Treg were found to be increased in numbers in the endometriotic lesions [38,54] and peritoneal fluid [55] of women with endometriosis when compared to women without endometriosis. Similarly, Braundmeier et al. in 2012 showed that by inducing endometriosis in nonhuman primates, they observed increased transcript levels of FOXP3 (regulator of T-reg development) in the ectopic tissues of animals with endometriosis, when compared to the eutopic endometrium. However, they also found that Treg numbers were significantly reduced in the eutopic endometrium of nonhuman primates when compared to animals without endometriosis [56]. Increased Treg populations may have a role in suppressing local immune response that ultimately prevents the elimination of ectopic tissues in the peritoneum cavity [57]. On the contrary, a study conducted by Y. Tanaka et al. demonstrated that Treg that are suppressive in nature ($CD45RA^{-}Foxp3^{hi}$) were found to be significantly lower in the endometriotic lesions when compared to the normal endometrium [58]. Additionally, absence of Treg cells in a mouse model of endometriosis showed increased lesion growth and elevated inflammatory cytokines (IL-6, MIP-1, VEGF and MCP-1), indicating that complete ablation of Treg could worsen endometriosis by facilitating cellular proliferation [58] and angiogenesis via indirect mechanisms such as, suppressing angiostatic cytokines (TNF and IFNγ) producing Th1 effector cells [59]. Likely, IL-17, a predominant functional product of Th17 cells and a known proinflammatory cytokine was found to be significantly elevated in the peritoneal fluid of women with endometriosis [60–62]. Chang et al. in 2017 showed the elevated numbers of IL-10 producing Th17 cells in the peritoneal fluid of women with endometriosis as opposed to women without endometriosis [63]; suggesting a potential interplay that aids in development and progression of endometriotic lesions, as IL10+ Th17 cells are pivotal in limiting autoimmunity and inflammatory responses [64]. Although, these interactions of Th17 cells and cytokines are significant, robust and reliable mechanistic evidences in endometriosis are limited.

The role of B cells and correlative autoimmune features in endometriosis

Endometriosis is generally not considered as an autoimmune disease. However, dysregulation in the immune system has been explored as cause of ectopic lesion development [65,66], and hence, potential association between endometriosis and autoimmune diseases has been proposed [67]. A metaanalysis by Shigesi et al. in 2019 has showed that women with endometriosis have a higher risk of acquiring autoimmune comorbidities including systemic lupus erythematous, rheumatoid arthritis, celiac disease, Sjogren's syndrome, autoimmune thyroid disorder, inflammatory bowel disease, multiple sclerosis and inflammatory bowel disease [68]. However, there are discrepancies in associating endometriosis with autoimmune disease as Nielsen et al. in 2011 reported no significant correlation [69]. Consistent with the established role of CD4+ T cells in activating B cells for antibody production, there are several reports confirming increased CD4+ T cells correlated with increased antibody production in autoimmune-inflammatory conditions [70,71]. Lang et al. in 2001 demonstrated

that patients with endometriosis had higher amounts of auto-antibodies against the endometrial antigens such as a_2-Heremans Schmidt glycoprotein (a2-HSG), transferrin, and carbonic anhydrase in the serum when compared to healthy fertile controls [72]. Similarly, Inagaki et al. in 2003 analyzed IgG antilaminin-1 antibody of endometriosis patients, which were significantly increased in the serum when compared to healthy fertile controls [73]. Likewise, R. Gajbhiye et al. demonstrated that over 60% of the endometriosis patients showed presence of IgG and IgM classes of endometrial antibodies in the serum. Moreover, there are several studies in the past that have confirmed the presence of antiendometrial antigens around 30−45 kd molecular weight [74,75].

B lymphocytes are primary source of antibodies that are produced upon activation by Th2 type cells. The role of B cells in the pathophysiology of endometriosis is not well established. Thus, studies interpreting endometriosis as an autoimmune condition have focused to establish the presence of autoantibodies against refluxed endometrial debris [76]. In concordance, studies have found elevated levels of circulating B cells in blood and peritoneal fluids [77] with higher concentration of autoantibodies among women with endometriosis when compared to healthy fertile controls [72,73,78]. Mathur et al. reported the presence of IgG and IgA antibodies in ectopic tissues and sera of women with endometriosis [79]. Surprisingly, there were no differences observed in mature B cell activity when characterized via CD22 surface marker. Contrary to that, lower levels of IgG antibodies [80] and increased activation of B cells [81] have been identified in blood and peritoneal fluid of women with endometriosis. Additionally, B cell lymphoma 6, a transcription factor responsible for the development and regulation of B cells has been found to be highly expressed in endometrium of women with endometriosis [82]. On the other hand, Yeol et al. show contrary evidences in this regard, where Bcl-6 mRNA has found to be decreased in the endometriosis group [83]. Interestingly, Riccio et al. in their murine model of endometriosis have adopted the idea that B cells might be responsible in developing antiendometrial antibodies and have intervened the mechanism using Bruton's tyrosine kinase (Btk) inhibitor. They have utilized Ibrutinib, a drug that can induce regulatory phenotype of activated B cells. On the other hand, they depleted B cell population in the mice using anti-CD20 antibody as a proof of concept. The authors found that the animals treated with Ibrutinib showed reduced lesion growth and increased regulatory B cells in the spleen and peritoneal fluid, whereas the animals treated with depleting antibody did not show any changes in the progression of endometriosis [84]. Although these evidences are convincing, B cell involvement and its role in the context of endometriosis is arguably most controversial topic due to confounding and unreliable results. Additionally, as mentioned above, potential autoimmune disease comorbidities are associated with endometriosis [68,69] and so far, studies have not completely addressed this. Hence, further research is required to completely understand the involvement of B cells in terms of their activation status, regulatory phenotype and association with specific types of antibodies claimed in the literature associated with endometriosis. This knowledge will potentially help us to determine diagnostic/therapeutic relevance of endometriosis specific autoantibodies in near future.

Recent developments in the field of genome-wide association studies (GWAS) has helped us to better understand the correlation and risk of various diseases [85]. To date there are over eight GWAS involving endometriosis patients among three different ancestry to map heritability of endometriosis influenced by environmental and genetic factors [86]. A metaanalysis

in 2017 has demonstrated the involvement of 14 independent SNP risk loci, while discovering novel secondary risk loci that includes genes such as estrogen receptor 1 (ESR1) and kinase insert domain receptor (KDR) [87]. KDR gene encodes VEGF receptor two and evidences suggest that high levels of estrogen triggers upregulation of KDR, which further contributes to survival and migration of endothelial cells [6]. On the other hand, GWAS have also been instrumental in showing the risk of developing autoimmune comorbidities among women with endometriosis when compared to healthy fertile controls. A metaanalysis of population based cross-sectional and case study in 2019 has showed the significant association between endometriosis and autoimmune diseases such as rheumatoid arthritis, systemic lupus erythematous, Sjogren's syndrome, Celiac disease, inflammatory bowel disease and multiple sclerosis in close to 30% patients when compared to normal female population [68]. Although, GWAS studies provide a potential connection between endometriosis and autoimmune diseases, there are certain limitations that appears to be challenging to address. As mentioned above several studies have identified the autoantibodies in circulation in patients with endometriosis suggesting autoimmune disease phenotypes. However, one of the major limitations is that knowledge is limited regarding the biological pathways shared between endometriosis and autoimmune diseases. Additionally, predicting risk of complex diseases through GWAS studies based on genetic profiles has picked up traction due to increase number of GWAS being analyzed [85]. However, due to low sample size and small number of disease risk loci identified (predicted to be around 2%) of all the risk variants restricts the ability to predict disease risks among endometriosis patients.

The endocrine-immune interface in endometriosis

While immune dysfunction is recognized as a driving factor, endometriosis has long been characterized as endocrinological in nature. Specifically, estrogen dominance is a key characteristic of endometriosis, which occurs largely due to the excessive local production of estrogen by lesion-specific aromatase activity. Consequently, hormone therapy dominates in the clinical management of endometriosis patients. In fact, all of the available pharmacological therapies for endometriosis are endocrine modulating drugs: hormonal contraceptives, GnRH agonists, and synthetic androgens including danazol [84–86]. The underlying objective is to regulate the patient's hormonal imbalance by suppressing endogenous estrogen production. Importantly, these treatment options are counterproductive regarding patients seeking counseling for endometriosis-related infertility who wishes to become pregnant. As well, many patients report persistence of pain symptoms after treatment with hormonal contraceptives, and none of the aforementioned hormonal therapies are curative [87]. The dilemma remains that endometriosis cannot be effectively treated by addressing a patient's endocrine factors alone, as a consequence of the acknowledged multi-faceted nature of the disease.

The interface of immune and endocrine systems is complex and its role in health and disease is poorly understood. While many of the mechanisms remain elusive, aberrant endocrine regulation of immune cells has been implicated in the pathogenesis of endometriosis. Several researchers have considered that hyperphysiological estrogen secretion from lesions acts as

an activating factor for many different leukocytes in the lesion microenvironment [6,87–89]. The functions of estrogen in immune modulation is complex, as it has both anti- and proinflammatory actions identified on different cell types and at different phases of the menstrual cycle [88,90,91]. Different actions of estradiol often depend upon what receptor is expressed by the leukocyte. There are two main estrogen receptor (ER) subtypes: ERα and ERβ. These receptor subtypes are distributed differently in various tissues, with ERα generally dominating the reproductive organs and tissues, notably uterus, theca cells of ovary, testes, mammary glands, bone, and liver [92]. Meanwhile ERβ are found more abundantly in the prostate, bladder, colon, granulosa cells of ovary, and immune system generally [92,93].

Progesterone also has immune modulating roles, and acts on cells via progesterone receptor A (PR-A) and progesterone receptor B (PR-B) [94,95]. ERs and PRs act as nuclear transcription factors, and it is now known they can function via nongenomic membrane receptor activity in certain leukocytes to produce a rapid onset of cellular effects [93,96,97]. A notable endocrine characteristic of endometriosis is progesterone resistance. This refers to the observed lack of responses from endometriotic tissue to progesterone stimulation. Progesterone acts to regulate endometrial apoptosis rate and limit proliferative effects of estradiol. Researchers have found that endometriotic tissue from patients contain significantly low levels of PR-A mRNA and no PR-B mRNA when compared to samples from healthy controls [98]. This observation is particularly relevant because in order to produce the estradiol-metabolizing enzyme 17β-HSD-2, endometrial stromal cells require stimulation by progesterone [99]. The lack of PR expression by stromal cells in endometriotic lesions therefore likely contributes to excess estradiol production and in turn lesion proliferation [99]. These findings may also explain why progestin-based endometriosis therapies often fail to ameliorate disease. Progesterone resistance and its impact on fertility in endometriosis patients has been extensively reviewed elsewhere [1,100,101]. The roles of estrogen and progesterone imbalance in endometriosis pathophysiology have been extensively reviewed in the literature, however the translation of this knowledge to the clinical management of inflammation in patients remains to be demonstrated [1,93,99,100].

Many leukocytes express ERs and PRs, including mast cells, macrophage, T lymphocytes, and NK cells. Activation of these receptors by their respective steroid hormones modulates the leukocyte function relating to proliferation and inflammation. However, there are some indirect ways by which sex hormones influence immune cell activity in the endometrium. For example, estradiol acts on endometrial stromal cells via the β-catenin/Wnt pathway to induce secretion of VEGF, which could then act as a chemokine to monocytes and macrophage and contribute toward angiogensis [102,103]. There are several other chemokines produced by stromal and epithelial cells upon hormonal stimulation that result in chemotaxis of immune cells including RANTES, macrophage-inflammatory protein 1β, CXCL9, and stromal cell-derived factor-1 [104–107].

It is established in the literature that immune cell numbers fluctuate in the menstrual cycle [108–110]. Mast cells have been documented to be present in the endometrium throughout the uterine cycle and high rates of degranulation are observed just before and during menstruation, indicating mast cells have a role in menstruation and are cyclically, hormonally regulated [111]. Expression of both ERs and PRs have been identified in mast cells in humans and mice [112]. In a study by Jensen et al. [113] it was found that both estrogen and progesterone treatment could increase mast cell number at the mouse uterus, and that treatment

with either hormone appeared to induce maturation and degranulation [113]. Progesterone has also been found to decrease secretion of histamine by mast cells in vivo [114]. Estradiol can initiate a Ca^{2+} influx via membrane receptor ERα and kinase activation in mast cells aiding the release of allergic mediators [96]. Aberrant mast cell behavior has been noted in patients with endometriosis. Specifically stem cell factor, which signals through c-Kit and is required for maturation and differentiation of mast cells was present in higher concentrations in the peritoneal fluid from patients with endometriosis as compared with healthy controls [115]. Similarly, endometriotic lesions from endometriosis patients reported to have high numbers of activated mast cells when compared to normal peritoneum, eutopic endometrium, and healthy controls [116,117]. Notably, a positive correlation has been found between number of mast cells and disease stage, with highest mast cell number found in deep-infiltrating endometriosis when compared to peritoneal or ovarian endometriosis [117].

Macrophage number varies over the course of the menstrual cycle, increasing during the proliferative phase, aligning with hormonal regulation [13,118]. In endometriosis patients, macrophage number has been found to be significantly greater in samples from the functional layer of eutopic endometrium when compared to healthy controls [13]. This may be due in part to the endocrine dysregulation characteristic of endometriosis, specific chemokines from the lesion microenvironment, or these in concert together. Macrophage express both ERα and ERβ, and it has been found that ERβ expression in macrophage is positively correlated with the expression of proinflammatory cytokines such as TNF, IL-1α and IL-1β [119,120]. As well, Montagna et al. found that the expression of ERα was positively correlated with production of proinflammatory cytokines such as TNF-a, IL-6, and IL-1β specifically in women with endometriosis [120]. Estradiol was found to classically activate macrophage, polarizing to the M1 phenotype to produce proinflammatory cytokines such as TNF-a, IL-1β, and IL-6; and also stimulates macrophage proliferation in the presence of IFNγ [121]. Multiple other studies have found that ERα activation causes macrophage to polarize toward the M2 "permissive" phenotype, which might allow proliferation of ectopic endometrial lesions in endometriosis[122–124]. The mechanisms of this phenomenon have not yet been delineated, and describing the actions of macrophage has been a challenge in the field due to the tendency of these cells to switch phenotypes [125,126]. At present, evidence is inconclusive as to whether dendritic cells, another predominant antigen presenting cells, are modulated cyclically. Some reports have found no difference in dendritic cell numbers across menstrual cycle while others have noted an increase during the secretory and menstrual phases [127,128].

T lymphocytes are the predominating leukocyte in the endometrium during the proliferative phase and decrease significantly during the secretory phase correlating with estrogen levels [109]. As with most leukocytes, hormonal modulation is indicated. Faas et al. [129] was the first to describe the shift of Th1 (proinflammatory) to Th2 (immune-regulatory) dominance between the follicular (estrogen dominant) and luteal (progesterone dominant) phases [129]. Progesterone appears to influence a shift toward the Th2 type cytokine profile, marked specifically by a significant increase in Th2 cytokine IL-4 in the luteal phase. Polanczyk et al. [130] conducted experiments that elucidated the role of estradiol in T regulatory cell suppression, both through programmed death (PD)-1 -dependent and -independent pathways [130]. Mohammed et al. conducted a study wherein mice with a T cell ERα knockout consistently expressed significantly lower concentrations of proinflammatory cytokines TNF, IFNγ, and

IL-6; severely dampening T cells' pathogenic capacity within the disease model of colitis, representing autoimmune inflammation [131]. This showed that activation of ERα has an important role in T cell activation, survival, and regulation of T cell functions.

Based on the literature, we can conclude that (1) majority of the immune cells express receptors for estrogen and progesterone, (2) both estrogen and progesterone has activating and or inhibitory effects on the immune cell functions depending on the disease or health status, and (3) both estrogen and progesterone modulate chemokine expression in the tissues ultimately regulating leukocyte traffic. However, the major knowledge gap revolves around the crosstalk between endocrine and immune functions and whether the aberrant signaling (endocrine or inflammation) is independently contributing to endometriosis pathology. In other words, does hormonal imbalance contribute to abnormal resolution or triggering inflammation, or it is the inflammation that modulates hormone receptor expression in immune cells and their end response to the environmental cues? Regardless, immune modulating effects of estrogen are well established in the literature. One of the simple ways some of these questions can be addressed is by performing prospective studies in endometriosis patients undergoing hormonal treatment and broadly assessing the impact on systemic immune cell profiles, similar to a CyTOF-based study that documented immunological events in pregnancy [132]. Although descriptive, these studies will shed light on the intricacies of inflammation and endocrine issues. Most of the literature in endometriosis have tackled these two issues independently by either measuring hormones or cytokines in the systemic circulation without any correlations in the disease context. If the consensus is that endometriosis patients experience chronic inflammation combined with hormonal imbalance, future therapeutics should be aimed at targeting both of these processes.

Immunomodulatory therapeutic opportunities and future of endometriosis management

The progression of endometriosis is intimately connected with estrogen-dependence, which has largely predominated our understanding of treatment options for patients. However, the last several decades of research, and even earliest modern documentations of endometriosis, have provided evidence for the critical role of dysregulated immunologic function in the development and progression of disease. Despite being one of the most frequently encountered gynecological diseases, new developments in therapeutic management remain limited, likely as a result of our poor understating of disease pathogenesis in patients. At the time of clinical presentation, most individuals will already have established disease, and it is not always feasible to conduct long-term studies in human populations due to a variety of ethical considerations, primarily those concerning the lack of nonsurgical/invasive imaging modalities [5]. Current therapies are often unsatisfactory as they are primarily concerned with managing symptoms, rather than treating the causes of disease. Many of these therapies focus on targeting the hypothalamic-pituitary axis through the use of gonadotrophin-releasing hormone agonist to maintain a hypoestrogenic state [1]. Although these hormonal therapies do provide benefits to some patients with regards to limiting lesions proliferation and decreasing pain symptoms, patients are often unsatisfied with side

effects, may be unlikely to maintain long-term compliance, and active disease often returns upon cessation of treatment [3]. There is subsequently a strong argument to suggest that hormone therapies for endometriosis are not entirely sufficient for managing disease. Therefore, manipulating the most well understood immunological dysfunctions in endometriosis may be beneficial for addressing the clinical concerns of endometriosis patients by targeting the specific pathobiological mechanisms not currently addressed.

Endometriosis remains a difficult disease to study clinically due to heterogeneity in presentation and difficulty in long-term disease monitoring, as previously mentioned. As a result, it is likely that the future of immunotherapy in endometriosis will rely on adapting the safe and efficacious therapies developed in analogous inflammatory disorders for clinical trial in endometriosis patients. One of the most obvious immunotherapy candidates for endometriosis is etanercept, a fusion protein consisting of human recombinant soluble TNF receptor-2 (p75) conjugated to a human Fc antibody subunit, which can functionally neutralize the activity of TNF and is currently used to reduce signs and symptoms of disease including rheumatoid arthritis and psoriasis [133]. A study from 2004 conducted by Barrier et al. demonstrated that etanercept effectively reduced the amount of spontaneously occurring active endometriosis in the baboon model of endometriosis [134]. However, to date only few case reports have debated the utility of this biologic therapy for endometriosis with limited clinical success [135]. Interestingly, in 2018, Önalan et al. evaluated the therapeutic benefit of etanercept on pregnancy outcomes in women with endometrioma who were pursuing assisted reproductive technologies and demonstrated that antagonizing TNF may be a promising nonhormonal adjunct in the treatment of endometrioma-associated infertility [136]. Nonetheless, our group has published several papers highlighting the pathologic functions of IL-17 in endometriosis [53,137,138]. IL-17 is the major effector cytokine produced from Th17 cells and serves to induce proinflammatory responses and has been associated with promoting allergy and inflammatory disorders, notably psoriasis [139,140]. We recently demonstrated that IL-17 is elevated in the plasma and peritoneal fluid of women with endometriosis compared to controls and that endometriotic lesions produce IL-17 [53]. Additionally, Th17 cells are also elevated in women with endometriosis, increasing in stage dependent manner, compared to healthy controls [45]. We are hopeful that the successes of biological therapy in psoriasis through the development of IL-17 neutralizing antibodies, may provide amenable therapeutic benefits, at least in part, to related Th17-mediated pathologies such as endometriosis [141]. Ultimately, we believe that these novel immunomodulatory therapies will likely serve to augment today's standards of care, rather than replace traditional therapies outright. Endometriosis is a highly complex and broad classification of what is believed to be a variety of different subtypes of disease, which may have different clinical presentations and may respond differently to endocrinological and immunological therapies altogether.

References

[1] Giudice LC, Kao LC. Endometriosis. Lancet 2004;364(9447):1789–99. https://doi.org/10.1016/S0140-6736(04)17403-5.

[2] Zondervan KT, Becker CM, Koga K, Missmer SA, Taylor RN, Viganò P. Endometriosis. Nat Rev Dis Prim 2018. https://doi.org/10.1038/s41572-018-0008-5.

[3] Zondervan KT, Becker CM, Missmer SA. Endometriosis. In: Longo DL, editor. N Engl J Med. vol. 382; 2020. p. 1244–56. https://doi.org/10.1056/NEJMra1810764. 13.

[4] Symons LK, Miller JE, Kay VR, et al. The immunopathophysiology of endometriosis. Trends Mol Med 2018. https://doi.org/10.1016/j.molmed.2018.07.004.
[5] Giudice LC. Clinical practice. Endometriosis. N Engl J Med 2010;362(25):2389–98. https://doi.org/10.1056/NEJMcp1000274.
[6] Vallvé-Juanico J, Houshdaran S, Giudice LC. The endometrial immune environment of women with endometriosis. Hum Reprod Update 2019;25(5):565–92. https://doi.org/10.1093/humupd/dmz018.
[7] Evans J, Salamonsen LA. Inflammation, leukocytes and menstruation. Rev Endocr Metab Disord 2012;13(4):277–88. https://doi.org/10.1007/s11154-012-9223-7.
[8] King AE, Critchley HOD. Oestrogen and progesterone regulation of inflammatory processes in the human endometrium. J Steroid Biochem Mol Biol 2010;120(2–3):116–26. https://doi.org/10.1016/j.jsbmb.2010.01.003.
[9] Lee SK, Kim CJ, Kim D-J, Kang J. Immune cells in the female reproductive tract. Immune Netw 2015;15(1):16. https://doi.org/10.4110/in.2015.15.1.16.
[10] Yeaman GR, Collins JE, Fanger MW, Wira CR. CD8+ T cells in human uterine endometrial lymphoid aggregates: evidence for accumulation of cells by trafficking. Immunology 2001;102(4):434–40. https://doi.org/10.1046/j.1365-2567.2001.01199.x.
[11] Salamonsen LA, Zhang J, Brasted M. Leukocyte networks and human endometrial remodelling. J Reprod Immunol. 57(1–2):95-108. doi:10.1016/s0165-0378(02)00011-6
[12] Takebayashi A, Kimura F, Kishi Y, et al. Subpopulations of macrophages within eutopic endometrium of endometriosis patients. Am J Reprod Immunol 2015;73(3):221–31. https://doi.org/10.1111/aji.12331.
[13] Berbic M, Schulke L, Markham R, Tokushige N, Russell P, Fraser IS. Macrophage expression in endometrium of women with and without endometriosis. Hum Reprod 2009;24(2):325–32. https://doi.org/10.1093/humrep/den393.
[14] Deshmane SL, Kremlev S, Amini S, Sawaya BE. Monocyte chemoattractant protein-1 (MCP-1): an overview. J Interferon Cytokine Res 2009;29(6):313–26. https://doi.org/10.1089/jir.2008.0027.
[15] Borrelli GM, Carvalho KI, Kallas EG, Mechsner S, Baracat EC, Abrão MS. Chemokines in the pathogenesis of endometriosis and infertility. J Reprod Immunol 2013;98(1–2):1–9. https://doi.org/10.1016/j.jri.2013.03.003.
[16] Han SJ, Jung SY, Wu SP, et al. Estrogen receptor β modulates apoptosis complexes and the inflammasome to drive the pathogenesis of endometriosis. Cell 2015. https://doi.org/10.1016/j.cell.2015.10.034.
[17] Geissmann F, Manz MG, Jung S, Sieweke MH, Merad M, Ley K. Development of monocytes, macrophages, and dendritic cells. Science 2010;327(5966):656–61. https://doi.org/10.1126/science.1178331.
[18] Capobianco A, Rovere-Querini P. Endometriosis, a disease of the macrophage. Front Immunol 2013;4:9. https://doi.org/10.3389/fimmu.2013.00009.
[19] Murray PJ. Macrophage polarization. Annu Rev Physiol 2017;79(1):541–66. https://doi.org/10.1146/annurev-physiol-022516-034339.
[20] Mantovani A, Sozzani S, Locati M, Allavena P, Sica A. Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. Trends Immunol 2002;23(11):549–55. https://doi.org/10.1016/S1471-4906(02)02302-5.
[21] Na YR, Stakenborg M, Seok SH, Matteoli G. Macrophages in intestinal inflammation and resolution: a potential therapeutic target in IBD. Nat Rev Gastroenterol Hepatol 2019;16(9):531–43. https://doi.org/10.1038/s41575-019-0172-4.
[22] Hill JA, Faris HMP, Schiff I, Anderson DJ. Characterization of leukocyte subpopulations in the peritoneal fluid of women with endometriosis. Fertil Steril 1988;50(2):216–22. https://doi.org/10.1016/S0015-0282(16)60062-6.
[23] Akoum A, Lemay A, McColl S, Turcot-Lemay L, Maheux R. Elevated concentration and biologic activity of monocyte chemotactic protein-1 in the peritoneal fluid of patients with endometriosis. Fertil Steril 1996;66(1):17–23. http://www.ncbi.nlm.nih.gov/pubmed/8752605.
[24] Cheong YC. IL-1, IL-6 and TNF-alpha concentrations in the peritoneal fluid of women with pelvic adhesions. Hum Reprod 2002;17(1):69–75. https://doi.org/10.1093/humrep/17.1.69.
[25] Pizzo A, Salmeri FM, Ardita FV, Sofo V, Tripepi M, Marsico S. Behaviour of cytokine levels in serum and peritoneal fluid of women with endometriosis. Gynecol Obstet Invest 2002;54(2):82–7. https://doi.org/10.1159/000067717.
[26] Wu MH, Lu CW, Chuang PC, Tsai SJ. Prostaglandin E2: the master of endometriosis? Exp Biol Med 2010;235(6):668–77. https://doi.org/10.1258/ebm.2010.009321.

[27] Bacci M, Capobianco A, Monno A, et al. Macrophages are alternatively activated in patients with endometriosis and required for growth and vascularization of lesions in a mouse model of disease. Am J Pathol 2009;175(2):547–56. https://doi.org/10.2353/ajpath.2009.081011.
[28] Chan RWS, Lee CL, Ng EH, Yeung WS. Co-culture with macrophages enhances the clonogenic and invasion activity of endometriotic stromal cells. Cell Prolif 2017;50(3). https://doi.org/10.1111/cpr.12330.
[29] Halme J, Hammond MG, Hulka JF, Raj SG, Talbert LM. Retrograde menstruation in healthy women and in patients with endometriosis. Obstet Gynecol 1984;64(2):151–4. PMID: 6234483.
[30] Harada T, Kaponis A, Iwabe T, et al. Apoptosis in human endometrium and endometriosis. Hum Reprod Update 2004. https://doi.org/10.1093/humupd/dmh007.
[31] Dmowski WP, Ding J, Shen J, Rana N, Fernandez BB, Braun DP. Apoptosis in endometrial glandular and stromal cells in women with and without endometriosis. Hum Reprod 2001. https://doi.org/10.1093/humrep/16.9.1802.
[32] Braun DP, Muriana A, Gebel H, Rotman C, Rana N, Dmowski WP. Monocyte-mediated enhancement of endometrial cell proliferation in women with endometriosis. Fertil Steril 1994. https://doi.org/10.1016/S0015-0282(16)56456-5.
[33] Tabibzadeh S, Kong QF, Satyaswaroop PG, et al. Distinct regional and menstrual dependent distribution of apoptosis in human endometrium. Potential regulatory role of T cells and TNF-alpha. Endocrine 1994.
[34] Witz CA, Montoya IA, Dey TD, Schenken RS. Characterization of lymphocyte subpopulations and T cell activation in endometriosis. Am J Reprod Immunol 1994.
[35] Scheerer C, Bauer P, Chiantera V, Sehouli J, Kaufmann A, Mechsner S. Characterization of endometriosis-associated immune cell infiltrates (EMaICI). Arch Gynecol Obstet 2016. https://doi.org/10.1007/s00404-016-4142-6.
[36] Szyllo K, Tchorzewski H, Banasik M, Glowacka E, Lewkowicz P, Kamer-Bartosinska A. The involvement of T lymphocytes in the pathogenesis of endometriotic tissues overgrowth in women with endometriosis. Mediat Inflamm 2003. https://doi.org/10.1080/0962935031000134842.
[37] Harada T, Iwabe T, Terakawa N. Role of cytokines in endometriosis. Fertil Steril 2001. https://doi.org/10.1016/S0015-0282(01)01816-7.
[38] Malutan AM, Drugan T, Costin N, et al. Pro-inflammatory cytokines for evaluation of inflammatory status in endometriosis. Cent Eur J Immunol 2015. https://doi.org/10.5114/ceji.2015.50840.
[39] Podgaec S, Abrao MS, Dias JA, Rizzo LV, de Oliveira RM, Baracat EC. Endometriosis: an inflammatory disease with a Th2 immune response component. Hum Reprod 2007. https://doi.org/10.1093/humrep/del516.
[40] Podgaec S, Dias Junior JA, Chapron C, de Oliveira RM, Baracat EC, Abrão MS. Th1 and Th2 immune responses related to pelvic endometriosis. Rev Assoc Med Bras 2010. https://doi.org/10.1590/s0104-42302010000100022.
[41] Jørgensen H, Hill AS, Beste MT, et al. Peritoneal fluid cytokines related to endometriosis in patients evaluated for infertility. Fertil Steril 2017. https://doi.org/10.1016/j.fertnstert.2017.03.013.
[42] Hsu CC, Yang BC, Wu MH, Huang KE. Enhanced interleukin-4 expression in patients with endometriosis. Fertil Steril 1997. https://doi.org/10.1016/S0015-0282(97)81439-2.
[43] OuYang Z, Hirota Y, Osuga Y, et al. Interleukin-4 stimulates proliferation of endometriotic stromal cells. Am J Pathol 2008. https://doi.org/10.2353/ajpath.2008.071044.
[44] Berbic M, Fraser IS. Regulatory T cells and other leukocytes in the pathogenesis of endometriosis. J Reprod Immunol 2011. https://doi.org/10.1016/j.jri.2010.11.004.
[45] Gogacz M, Winkler I, Bojarska-Junak A, et al. Increased percentage of Th17 cells in peritoneal fluid is associated with severity of endometriosis. J Reprod Immunol 2016. https://doi.org/10.1016/j.jri.2016.04.289.
[46] Nandakumar S, Miller CWT, Kumaraguru U. T regulatory cells: an overview and intervention techniques to modulate allergy outcome. Clin Mol Allergy 2009. https://doi.org/10.1186/1476-7961-7-5.
[47] Giatromanolaki A, Bates GJ, Koukourakis MI, et al. The presence of tumor-infiltrating FOXP3+ lymphocytes correlates with intratumoral angiogenesis in endometrial cancer. Gynecol Oncol 2008. https://doi.org/10.1016/j.ygyno.2008.04.021.
[48] Sakaguchi S, Yamaguchi T, Nomura T, Ono M, Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell 2008;133(5):775–87. https://doi.org/10.1016/j.cell.2008.05.009. Cell. 2008.
[49] Braundmeier A, Jackson K, Hastings J, Koehler J, Nowak R, Fazleabas A. Induction of endometriosis alters the peripheral and endometrial regulatory T cell population in the non-human primate. Hum Reprod 2012. https://doi.org/10.1093/humrep/des083.

[50] Tanaka Y, Mori T, Ito F, et al. Exacerbation of endometriosis due to regulatory t-cell dysfunction. J Clin Endocrinol Metab 2017. https://doi.org/10.1210/jc.2017-00052.
[51] Müller-Hermelink N, Braumüller H, Pichler B, et al. TNFR1 signaling and IFN-γ signaling determine whether T cells induce tumor dormancy or promote multistage carcinogenesis. Cancer Cell 2008. https://doi.org/10.1016/j.ccr.2008.04.001.
[52] Hirata T, Osuga Y, Hamasaki K, et al. Interleukin (IL)-17A stimulates IL-8 secretion, cyclooxygensase-2 expression, and cell proliferation of endometriotic stromal cells. Endocrinology 2008. https://doi.org/10.1210/en.2007-0749.
[53] Ahn SH, Edwards AK, Singh SS, Young SL, Lessey BA, Tayade C. IL-17A contributes to the pathogenesis of endometriosis by triggering proinflammatory cytokines and angiogenic growth factors. J Immunol 2015. https://doi.org/10.4049/jimmunol.1501138.
[54] Zhang X, Xu H, Lin J, Qian Y, Deng L. Peritoneal fluid concentrations of interleukin-17 correlate with the severity of endometriosis and infertility of this disorder. BJOG An Int J Obstet Gynaecol 2005. https://doi.org/10.1111/j.1471-0528.2005.00639.x.
[55] Chang KK, Liu LB, Jin LP, et al. IL-27 triggers IL-10 production in Th17 cells via a c-Maf/RORãt/Blimp-1 signal to promote the progression of endometriosis. Cell Death Dis 2017. https://doi.org/10.1038/cddis.2017.95.
[56] Turovskaya O, Kim G, Cheroutre H, Kronenberg M, Madan R. Interleukin 10 acts on regulatory t cells to maintain expression of the transcription factor foxp3 and suppressive function in mice with colitis. Nat Immunol 2009. https://doi.org/10.1038/ni.1791.
[57] Tscharke DC, Croft NP, Doherty PC, La Gruta NL. Sizing up the key determinants of the CD8+ T cell response. Nat Rev Immunol 2015. https://doi.org/10.1038/nri3905.
[58] White HD, Crassi KM, Givan AL, et al. CD3+ CD8+ CTL activity within the human female reproductive tract: influence of stage of the menstrual cycle and menopause. J Immunol 1997.
[59] Garcia-Velasco JA, Mulayim N, Kayisli UA, Arici A. Elevated soluble Fas ligand levels may suggest a role for apoptosis in women with endometriosis. In: Fertility and sterility; 2002. https://doi.org/10.1016/S0015-0282(02)03320-4.
[60] Ahn SH, Monsanto SP, Miller C, Singh SS, Thomas R, Tayade C. Pathophysiology and immune dysfunction in endometriosis. BioMed Res Int 2015. https://doi.org/10.1155/2015/795976.
[61] Izumi G, Koga K, Takamura M, et al. Involvement of immune cells in the pathogenesis of endometriosis. J Obstet Gynaecol Res 2018. https://doi.org/10.1111/jog.13559.
[62] Harris HR, Simard JF, Arkema EV. Endometriosis and systemic lupus erythematosus: a population-based case-control study. Lupus 2016. https://doi.org/10.1177/0961203316631635.
[63] Shigesi N, Kvaskoff M, Kirtley S, et al. The association between endometriosis and autoimmune diseases: a systematic review and meta-analysis. Hum Reprod Update 2019. https://doi.org/10.1093/humupd/dmz014.
[64] Nielsen NM, Jørgensen KT, Pedersen BV, Rostgaard K, Frisch M. The co-occurrence of endometriosis with multiple sclerosis, systemic lupus erythematosus and Sjögren syndrome. Hum Reprod 2011. https://doi.org/10.1093/humrep/der105.
[65] Palmer MT, Weaver CT. Autoimmunity: increasing suspects in the CD4+ T cell lineup. Nat Immunol 2010. https://doi.org/10.1038/ni.1802.
[66] Skapenko A, Leipe J, Lipsky PE, Schulze-Koops H. The role of the T cell in autoimmune inflammation. Arthritis Res Ther 2005. https://doi.org/10.1186/ar1505.
[67] Lang GA, Yeaman GR. Autoantibodies in endometriosis sera recognize a Thomsen-Friedenreich-like carbohydrate antigen. J Autoimmun 2001. https://doi.org/10.1006/jaut.2000.0465.
[68] Wild RA, Shivers CA. Antiendometrial antibodies in patients with endometriosis. Am J Reprod Immunol Microbiol 1985. https://doi.org/10.1111/j.1600-0897.1985.tb00314.x.
[69] Mathur S, Peress MR, Williamson HO, et al. Autoimmunity to endometrium and ovary in endometriosis. Obstet Gynecol Surv 1983. https://doi.org/10.1097/00006254-198309000-00022.
[70] Inagaki J, Sugiura-Ogasawara M, Nomizu M, et al. An association of IgG anti-laminin-1 autoantibodies with endometriosis in infertile patients. Hum Reprod 2003. https://doi.org/10.1093/humrep/deg148.
[71] Mathur S, Chihal HJ, Homm RJ, Garza DE, Rust PF, Williamson HO. Endometrial antigens involved in the autoimmunity of endometriosis. Fertil Steril 1988. https://doi.org/10.1016/S0015-0282(16)60362-X.
[72] Rajkumar K, Malliah V, Simpson CW. Identifying the presence of antibodies against endometrial antigens: a preliminary study. J Reprod Med Obstet Gynecol 1992.

[73] Kyama CM, Debrock S, Mwenda JM, D'Hooghe TM. Potential involvement of the immune system in the development of endometriosis. Reprod Biol Endocrinol 2003. https://doi.org/10.1186/1477-7827-1-123.
[74] Osuga Y, Koga K, Hirota Y, Hirata T, Yoshino O, Taketani Y. Lymphocytes in endometriosis. Am J Reprod Immunol 2011. https://doi.org/10.1111/j.1600-0897.2010.00887.x.
[75] Inagaki J, Hao L, Nakatsuka M, et al. A Possible mechanism of autoimmune-mediated infertility in women with endometriosis. Am J Reprod Immunol 2011. https://doi.org/10.1111/j.1600-0897.2010.00956.x.
[76] Gebel HM, Braun DP, Rotman C, Rana N, Dmowski WP. Mitogen induced production of polyclonal IgG is decreased in women with severe endometriosis. Am J Reprod Immunol 1993. https://doi.org/10.1111/j.1600-0897.1993.tb00576.x.
[77] Odukoya O, Bansal A, Cooke I. Serum endometrial IgG antibodies and soluble CD23 concentrations in patients with endometriosis. Acta Obstet Gynecol Scand 1996. https://doi.org/10.3109/00016349609055030.
[78] Evans-Hoeker E, Lessey BA, Jeong JW, et al. Endometrial BCL6 overexpression in eutopic endometrium of women with endometriosis. Reprod Sci 2016. https://doi.org/10.1177/1933719116649711.
[79] Yeol SG, Won YS, Kim YI, Lee JW, Choi YJ, Park DC. Decreased Bcl-6 and increased Blimp-1 in the peritoneal cavity of patients with endometriosis. Clin Exp Obstet Gynecol 2015.
[80] Rahmioglu N, Nyholt DR, Morris AP, Missmer SA, Montgomery GW, Zondervan KT. Genetic variants underlying risk of endometriosis: insights from meta-analysis of eight genome-wide association and replication datasets. Hum Reprod Update 2014. https://doi.org/10.1093/humupd/dmu015.
[81] Sapkota Y, Steinthorsdottir V, Morris AP, et al. Meta-analysis identifies five novel loci associated with endometriosis highlighting key genes involved in hormone metabolism. Nat Commun 2017. https://doi.org/10.1038/ncomms15539.
[82] Sugino N, Kashida S, Karube-Harada A, Takiguchi S, Kato H. Expression of vascular endothelial growth factor (VEGF) and its receptors in human endometrium throughout the menstrual cycle and in early pregnancy. Reproduction 2002. https://doi.org/10.1530/rep.0.1230379.
[83] Wray NR, Goddard ME, Visscher PM. Prediction of individual genetic risk to disease from genome-wide association studies. Genome Res 2007. https://doi.org/10.1101/gr.6665407.
[84] Buggio L, Somigliana E, Barbara G, Frattaruolo MP, Vercellini P. Oral and depot progestin therapy for endometriosis: towards a personalized medicine. Expert Opin Pharmacother 2017;18(15):1569–81. https://doi.org/10.1080/14656566.2017.1381086.
[85] Selak V, Farquhar CM, Prentice A, Singla AA. Danazol for pelvic pain associated with endometriosis. Cochrane Database Syst Rev 2007;4. https://doi.org/10.1002/14651858.CD000068.
[86] Vercellini P, Viganò P, Somigliana E, Fedele L. Endometriosis: pathogenesis and treatment. Nat Rev Endocrinol 2014;10(5):261–75. https://doi.org/10.1038/nrendo.2013.255.
[87] Chapron C, Marcellin L, Borghese B, Santulli P. Rethinking mechanisms, diagnosis and management of endometriosis. Nat Rev Endocrinol 2019;24(Box 1). https://doi.org/10.1038/s41574-019-0245-z.
[88] Liang Y, Xie H, Wu J, Liu D, Yao S. Villainous role of estrogen in macrophage-nerve interaction in endometriosis. Reprod Biol Endocrinol 2018;16(1):1–11. https://doi.org/10.1186/s12958-018-0441-z.
[89] Patel BG, Lenk EE, Lebovic DI, Shu Y, Yu J, Taylor RN. Pathogenesis of endometriosis: interaction between Endocrine and inflammatory pathways. Best Pract Res Clin Obstet Gynaecol 2018;50:50–60. https://doi.org/10.1016/j.bpobgyn.2018.01.006.
[90] Straub RH. The complex role of estrogens in inflammation. Endocr Rev 2007;28(5):521–74. https://doi.org/10.1210/er.2007-0001.
[91] Kovats S. Estrogen receptors regulate innate immune cells and signaling pathways. Cell Immunol 2015;294(2):63–9. https://doi.org/10.1016/j.cellimm.2015.01.018 [Estrogen].
[92] Paterni I, Granchi C, Katzenellenbogen J, Minutolo F. Estrogen receptors alpha and beta subtype-selective ligands and clinical potential 2011;18(11):1492–501. https://doi.org/10.1016/j.str.2010.08.012.Structure.
[93] Tang Z-R, Zhang R, Lian Z-X, Deng S-L, Yu K. Estrogen-receptor expression and function in female reproductive disease. Cells 2019;8(10):1123. https://doi.org/10.3390/cells8101123.
[94] Heldring N, Pike A, Andersson S, et al. Estrogen receptors: how do they signal and what are their targets. Physiol Rev 2007;87(3):905–31. https://doi.org/10.1152/physrev.00026.2006.
[95] Carey MA, Card JW, Voltz JW, Germolec DR, Korach KS, Zeldin DC. The impact of sex and sex hormones on lung physiology and disease: lessons from animal studies. Am J Physiol Lung Cell Mol Physiol 2007;293(2):272–8. https://doi.org/10.1152/ajplung.00174.2007.

[96] Zaitsu M, Narita SI, Lambert KC, et al. Estradiol activates mast cells via a non-genomic estrogen receptor-α and calcium influx. Mol Immunol 2007;44(8):1977–85. https://doi.org/10.1016/j.molimm.2006.09.030.
[97] Watson CS, Norfleet AM, Pappas TC, Gametchu B. Rapid actions of estrogens in GH3/B6 pituitary tumor cells via a plasma membrane version of estrogen receptor-alpha. Steroids 1999;64(1–2):5–13. https://doi.org/10.1016/S0039-128X(98)00107-X.
[98] Attia GR, Zeitoun K, Edwards D, Johns A, Carr BR, Bulun SE. Progesterone receptor isoform A but not B is expressed in endometriosis. J Clin Endocrinol Metab 2000;85(8).
[99] Bulun SE, Cheng YH, Yin P, et al. Progesterone resistance in endometriosis: link to failure to metabolize estradiol. Mol Cell Endocrinol 2006;248(1–2):94–103. https://doi.org/10.1016/j.mce.2005.11.041.
[100] Lessey BA, Kim JJ. Endometrial receptivity in the eutopic endometrium of women with endometriosis: it is affected, and let me show you why. Fertil Steril 2017. https://doi.org/10.1016/j.fertnstert.2017.05.031.
[101] McKinnon B, Mueller M, Montgomery G. Progesterone resistance in endometriosis: an acquired property? Trends Endocrinol Metabol 2018. https://doi.org/10.1016/j.tem.2018.05.006.
[102] Zhang L, Xiong W, Xiong Y, Liu H, Liu Y. 17 β-Estradiol promotes vascular endothelial growth factor expression via the Wnt/β-catenin pathway during the pathogenesis of endometriosis. Mol Hum Reprod 2016;22(7):526–35. https://doi.org/10.1093/molehr/gaw025.
[103] Wheeler KC, Jena MK, Pradhan BS, et al. VEGF may contribute to macrophage recruitment and M2 polarization in the decidua. PLos One 2018:1–18. https://doi.org/10.1371/journal.pone.0191040.
[104] Srivastava A, Sengupta J, Kriplani A, Roy KK, Ghosh D. Profiles of cytokines secreted by isolated human endometrial cells under the influence of chorionic gonadotropin during the window of embryo implantation. Reprod Biol Endocrinol 2013;11:116. https://doi.org/10.1186/1477-7827-11-116.
[105] Tsutsumi A, Okada H, Nakamoto T, Okamoto R, Yasuda K, Kanzaki H. Estrogen induces stromal cell-derived factor 1 (SDF-1/CXCL12) production in human endometrial stromal cells: a possible role of endometrial epithelial cell growth. Fertil Steril 2011;95(1):444–7. https://doi.org/10.1016/j.fertnstert.2010.08.037.
[106] Park DW, Yang KM. Hormonal regulation of uterine chemokines and immune cells. Clin Exp Reprod Med 2011;38(4):179–85. https://doi.org/10.5653/cerm.2011.38.4.179.
[107] Dimitriadis E, White CA, Jones RL, Salamonsen LA. Cytokines, chemokines and growth factors in endometrium related to implantation. Hum Reprod Update 2005;11(6):613–30. https://doi.org/10.1093/humupd/dmi023.
[108] Oertelt-Prigione S. Immunology and the menstrual cycle. Autoimmun Rev 2012;11(6–7):A486–92. https://doi.org/10.1016/j.autrev.2011.11.023.
[109] Wira CR, Rodriguez-Garcia M, Patel MV. The role of sex hormones in immune protection of the female reproductive tract. Nat Rev Immunol 2015;15(4):217–30. https://doi.org/10.1016/b978-0-323-48253-0.00105-7.
[110] Wira CR, Fahey JV, Rodriguez-Garcia M, Shen Z, Patel MV. Regulation of mucosal immunity in the female reproductive tract: the role of sex hormones in immune protection against sexually transmitted pathogens. Am J Reprod Immunol 2014. https://doi.org/10.1111/aji.12252.
[111] Jeziorska M, Salamonsen LA, Woolley DE. Mast cell and eosinophil distribution and activation in human endometrium throughout the menstrual cycle. Biol Reprod 1995;53(2):312–20. https://doi.org/10.1095/biolreprod53.2.312.
[112] Khan KN, Masuzaki H, Fujishita A, et al. Estrogen and progesterone receptor expression im macrophages and regulation of hepatocyte growth factor by ovarian steroids in women with endometriosis. Hum Reprod 2005;20(7):2004–13. https://doi.org/10.1093/humrep/deh897.
[113] Jensen F, Woudwyk M, Teles A, et al. Estradiol and progesterone regulate the migration of mast cells from the periphery to the uterus and induce their maturation and degranulation. PLoS One 2010;5(12):1–12. https://doi.org/10.1371/journal.pone.0014409.
[114] Vasiadi M, Kempuraj D, Boucher W, Kalogeromitros D, Theoharides TC. Progesterone inhibits mast cell secretion. Int J Immunopathol Pharmacol 2006;19(4):787–94. https://doi.org/10.1177/039463200601900408.
[115] Osuga Y, Koga K, Tsutsumi O, et al. Stem cell factor (SCF) concentrations in peritoneal fluid of women with or without endometriosis. Am J Reprod Immunol 2000;44(4):231–5. https://doi.org/10.1111/j.8755-8920.2000.440407.x.
[116] Sugamata M, Ihara T, Uchiide I. Increase of activated mast cells in human endometriosis. Am J Reprod Immunol 2005;53(3):120–5. https://doi.org/10.1111/j.1600-0897.2005.00254.x.

[117] Anaf V, Chapron C, El Nakadi I, et al. Pain, mast cells, and nerves in peritoneal, ovarian, and deep infiltrating endometriosis. Fertil Steril 2006;86(5):1336–43. https://doi.org/10.1016/j.jri.2010.11.004.
[118] Bonatz G, Hansmann ML, Buchholz F, Mettler L, Radzun HJ, Semm K. Macrophage- and lymphocyte-subtypes in the endometrium during different phases of the ovarian cycle. Int J Gynecol Obstet 1992;37(1):29–36. https://doi.org/10.1016/0020-7292(92)90974-N.
[119] Jing X, Peng J, Dou Y, et al. Macrophage ERα promoted invasion of endometrial cancer cell by mTOR/KIF5B-mediated epithelial to mesenchymal transition. Immunol Cell Biol 2019;97(6):563–76. https://doi.org/10.1111/imcb.12245.
[120] Montagna P, Capellino S, Villaggio B, et al. Peritoneal fluid macrophages in endometriosis: correlation between the expression of estrogen receptors and inflammation. Fertil Steril 2008;90(1):156–64. https://doi.org/10.1016/j.fertnstert.2006.11.200.
[121] Cutolo M, Capellino S, Montagna P, Ghiorzo P, Sulli A, Villaggio B. Sex hormone modulation of cell growth and apoptosis of the human monocytic/macrophage cell line. Arthritis Res Ther 2005;7(5):1124–32. https://doi.org/10.1186/ar1791.
[122] Wang Y, Chen H, Wang NL, et al. Combined 17β-estradiol with TCDD promotes M2 polarization of macrophages in the endometriotic milieu with aid of the interaction between endometrial stromal cells and macrophages. PLoS One 2015;10(5):1–12. https://doi.org/10.1371/journal.pone.0125559.
[123] Campbell L, Emmerson E, Williams H, et al. Estrogen receptor-alpha promotes alternative macrophage activation during cutaneous repair. J Invest Dermatol 2014;134(9):2447–57. https://doi.org/10.1038/jid.2014.175.
[124] Keselman A, Fang X, White PB, Heller NM. Estrogen signaling contributes to sex differences in macrophage polarization during asthma. J Immunol 2017;199(5):1573–83. https://doi.org/10.4049/jimmunol.1601975.
[125] Stout RD, Jiang C, Matta B, Tietzel I, Watkins SK, Suttles J. Macrophages sequentially change their functional phenotype in response to changes in microenvironmental influences. J Immunol 2005;175(1):342–9. https://doi.org/10.4049/jimmunol.175.1.342.
[126] Pepe G, Braga D, Renzi TA, et al. Self-renewal and phenotypic conversion are the main physiological responses of macrophages to the endogenous estrogen surge. Sci Rep 2017;7:1–14. https://doi.org/10.1038/srep44270. July 2016.
[127] Schulke L, Manconi F, Markham R, Fraser IS. Endometrial dendritic cell populations during the normal menstrual cycle. Hum Reprod 2008;23:1574–80. https://doi.org/10.1093/humrep/den030.
[128] Maridas DE, Hey-Cunningham AJ, Ng CHM, Markham R, Fraser IS, Berbic M. Peripheral and endometrial dendritic cell populations during the normal cycle and in the presence of endometriosis. J Endometr Pelvic Pain Disord 2014;6:92–9. https://doi.org/10.5301/je.5000180.
[129] Faas M, Bouman A, Moesa H, Heineman MJ, De Leij L, Schuiling G. The immune response during the luteal phase of the ovarian cycle: a Th2-type response? Fertil Steril 2000;74(5):1008–13. https://doi.org/10.1016/S0015-0282(00)01553-3.
[130] Polanczyk MJ, Hopke C, Vandenbark AA, Offner H. Treg suppressive activity involves estrogen-dependent expression of programmed death-1 (PD-1). Int Immunol 2007;19(3):337–43. https://doi.org/10.1093/intimm/dxl151.
[131] Mohammad I, Starskaia I, Nagy T, et al. Estrogen receptor contributes to T cell–mediated autoimmune inflammation by promoting T cell activation and proliferation. Sci Signal 2018;11(526):1–13. https://doi.org/10.1126/scisignal.aap9415.
[132] Aghaeepour N, Ganio EA, Mcilwain D, et al. An immune clock of human pregnancy. Sci Immunol 2017. https://doi.org/10.1126/sciimmunol.aan2946.
[133] Scott LJ. Etanercept: a review of its use in autoimmune inflammatory diseases. Drugs 2014;74(12):1379–410. https://doi.org/10.1007/s40265-014-0258-9.
[134] Barrier BF, Bates GW, Leland MM, Leach DA, Robinson RD, Propst AM. Efficacy of anti-tumor necrosis factor therapy in the treatment of spontaneous endometriosis in baboons. Fertil Steril 2004;81(Suppl. 1):775–9. https://doi.org/10.1016/j.fertnstert.2003.09.034.
[135] Shakiba K, Falcone T. Tumour necrosis factor-alpha blockers: potential limitations in the management of advanced endometriosis? A case report. Hum Reprod 2006;21(9):2417–20. https://doi.org/10.1093/humrep/del179.
[136] Önalan G, Tohma YA, Zeyneloğlu HB. Effect of etanercept on the success of assisted reproductive technology in patients with endometrioma. Gynecol Obstet Invest 2018;83(4):358–64. https://doi.org/10.1159/000484895.

[137] Ahn SH, Khalaj K, Young SL, Lessey BA, Koti M, Tayade C. Immune-inflammation gene signatures in endometriosis patients. Fertil Steril 2016. https://doi.org/10.1016/j.fertnstert.2016.07.005.
[138] Miller JE, Ahn SH, Marks RM, et al. IL-17A modulates peritoneal macrophage recruitment and M2 polarization in endometriosis. Front Immunol 2020;11:108. https://doi.org/10.3389/fimmu.2020.00108.
[139] Brembilla NC, Senra L, Boehncke WH. The IL-17 family of cytokines in psoriasis: IL-17A and beyond. Front Immunol August 2018;9. https://doi.org/10.3389/fimmu.2018.01682.
[140] Blauvelt A, Chiricozzi A. The immunologic role of IL-17 in psoriasis and psoriatic arthritis pathogenesis. Clin Rev Allergy Immunol 2018;55(3):379–90. https://doi.org/10.1007/s12016-018-8702-3.
[141] Thomas LW, Lee EB, Wu JJ. Systematic review of anti-drug antibodies of IL-17 inhibitors for psoriasis. J Dermatol Treat 2019;30(2):110–6. https://doi.org/10.1080/09546634.2018.1473552.

CHAPTER

16

Novel therapeutic strategy: antiinflammatory reagents

Role of NF-κB in endometriosis

Hiroaki Komatsu, Fuminori Taniguchi and Tasuku Harada

Department of Obstetrics and Gynecology, Tottori University School of Medicine, Yonago, Tottori, Japan

Introduction

Endometriosis was first described by Daniel Shroen in 1690 in a book entitled "Disputatio Inauguralis Medica de Ulceribus Ulceri" [1]. He described the lesions as inflammations with a tendency to form adhesions that linked visceral areas together [2]. The most important characteristic of endometriosis is that it is a local pelvic inflammatory process with altered function of immuno-related cells in the peritoneal environment. Studies have demonstrated that the peritoneal fluid of women with endometriosis contains an increased number of activated macrophages that secrete various local products, such as growth factors and cytokines [3–5]. Thus, the cytokines secreted in chronic pelvic inflammation are crucial in the pathogenesis of endometriosis. These cytokines include interleukin (IL)-1, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12, IL-13, interferon-γ, tumor necrosis factor (TNF)-α, regulated upon activation normal T-cell express sequence (RANTES), monocyte chemotactic protein-1 (MCP-1), macrophage colony stimulating factor, transforming growth factor (TGF)-β, and vascular endothelial growth factor (VEGF). Macrophages are believed to be the primary source of cytokines. However, endometrial cells produce cytokines independent of macrophages, which contributes to their survival in the peritoneal cavity [6]. We concluded that IL-8 significantly enhanced the proliferation of stromal cells derived from ovarian endometriomas, suggesting that IL-8 promotes the progression of endometriosis [7]. We also found that TNF-α promoted the proliferation of endometriotic stromal cells by inducing IL-8 gene and protein expression [8]. Therefore, it is possible that an altered peritoneal microenvironment with increased expression of proinflammatory cytokines and growth factors enhances the capability of endometrial cells to implant and grow in the peritoneal cavity. Thus, Inflammatory responses are new

Immunology of Endometriosis
https://doi.org/10.1016/B978-0-12-820661-4.00016-9

© 2022 Elsevier Inc. All rights reserved.

thought to be mediated by the activation of the transcription factor, nuclear factor-kappaB (NF-κB), which can be activated by various stimuli, including cytokines.

The NF-KB signaling pathway

NF-κB is a complex protein that acts as a transcription factor. Stimulus-induced NF-κB activity, the central mediator of inflammatory responses and immune function, comprises a family of dimeric transcription factors that regulate diverse gene expression programs comprising several genes. NF-κB may allow cells to be protected and proliferate and can simultaneously initiate an inflammatory response through the recruitment and activation of effector cells of the immune system. p50, p52, p65 (RelA), c-Rel, and RelB are five known proteins of the NF-κB family [9–11] that form various homo- and heterodimers, the most common activated forms of which are the p50/RelA or p52/RelA heterodimers. The NF-κB dimers bind to specific inhibitors of NF-κB (I-κB), forming a complex that is inactive because it is unable to bind to DNA. A family of I-κB proteins controls NF-κB DNA-binding activity and nuclear localization. Cellular stimulation results in specific phosphorylation, ubiquitination, and proteasome-mediated proteolysis of the NF-κB-bound I-κB protein, which renders NF-κB capable of binding DNA and being localized to the nucleus. Some I-κB proteins have different affinities for Rel/NF-kB complexes; these proteins are regulated slightly differently and are expressed in a tissue-specific manner. The I-κB proteins include p105, p100, IkBa, IkBb, IkBg, IkBe, IkBz, Bcl-3, and the Drosophila cactus protein [12,13]. Activation of the cytoplasmic NF-κB occurs mainly through I-κB kinase (IKK)-dependent phosphorylation and degradation of I-κB inhibitory proteins. Moreover, IKK activation induces the polyubiquitination and fast proteolysis of I-κB peptides by the 26S proteasome, leading to the liberation and activation of NF-κB. The active NF-κB translocates into the nucleus where it regulates gene expression by binding to κB elements in the enhancer or promoter regions of the genes [11,12,14–17].

Three pathways of NF-κB activation have been identified. First, the canonical pathway depends on the inducible degradation of inhibitory I-κB proteins, which retain most NF-κB dimers (except those that contain RelB) in the cytoplasm [18]. This pathway can be induced by proinflammatory stimuli such as TNF-α, IL-1, and lipopolysaccharide (LPS). Second, the noncanonical pathway is triggered by stimuli activating IKK-α and is mainly associated with the presence of RelB in the cytoplasm. Finally, atypical pathways are induced by diverse stimuli and activate different forms of NF-κB dimers with distinct functions [19]. In the classical pathway, one of the target genes activated by NF-kB is that which encodes I-κBα. Newly synthesized I-κBα can enter the nucleus, remove NF-kB from the DNA, and export the complex back to the cytoplasm to restore the original latent state. Thus, the activation of the NF-κB pathway is generally a transient process that lasts from 30 to 60 min in most cells [16].

NF-κB activation is required for the regulation of many inflammatory and immune response genes, such as genes involved in cellular proliferation, cell adhesion, and apoptosis [20]. The physiologic role of NF-κB is considered to regulate B-cell development, proliferation, and effector functions [16]. Several reports indicated that NF-κB also controlled the expression of some cytokines and various T-cell functions [21–23].

NF-KB expression in normal endometrium

Some functions of the endometrium in humans are associated with inflammatory-like responses, e.g., implantation and menstruation [24]. Human endometrial cells can constitutively express NF-κB proteins [25,26]. NF-κB is involved in the inflammatory events associated with menstruation periods [26,27]. The immune-cyto-chemical analysis in the cultured epithelial endometrial cells reveals that the staining intensity of p65 is low during the proliferative phase, increases during the secretory phase, and reaches it maximum at the time of implantation [25]. During the secretory phase and implantation, there was decreased staining of the I-κB [26,28]. In contrast, these differences in the expression of NF-κB family members were not so remarkable in human endometrial stromal cells [28].

Progesterone withdrawal induces vasoconstriction with associated hypoxia, which in turn induces I-κB-α phosphorylation and ubiquitination and subsequently NF-κB activation [26,29]. Furthermore, NF-κB activation will induce the production of proinflammatory cytokines that will affect lytic enzyme activity and the production of matrix metalloproteinases (MMPs), which are involved in tissue breakdown [30]. In addition, mediators known to activate NF-κB are present in the endometrium during menstruation and likely to contribute to the stimulation of NF-κB at this time [26].

The IKK complex is also expressed in human endometrium. IKK-α is expressed in the endometrium throughout the menstrual cycle with increased expression in the decidua, whereas IKK-β is decreased in the decidua [26]. IKK-α may be involved via NF-κB and COX-2, in the expression of mediators, e.g., prostaglandin E2, which are vital for implantation and successful pregnancy [26,31]. The reduced amount of IKK-β in the decidua may suggest a mechanism to decrease proinflammatory signaling to NF-κB at a time when local immunosuppression is occurred.

NF-KB expression in women with endometriosis

In the eutopic endometrium of patients with endometriosis, the transcriptional dysfunction of the NF-κB is observed during the late secretory phase. IKK is strongly reduced coincidently with the reduction of NF-κB DNA binding compared with the normal endometrium at the same stage of the menstrual cycle [32]. In addition, NF-κB expression was evaluated in the eutopic endometrium of patients with endometriosis [33]. NF-κB alterations in the eutopic endometrium of women with endometriosis may be involved in other processes contributing to the establishment and development of the disease. Higher expression of nuclear p65 and p52 together with lower expression of progesterone receptor-B and cytoplasmic I-κB-α was observed compared with the expression of these molecules in normal endometrium [33]. NF-κB activation has been concerned with the early development of endometriotic lesions in vivo [19,20,34]. In the development of endometriosis, NF-κB is involved in the immune and inflammatory response and modulates cell proliferation, apoptosis, adhesion, invasion, and angiogenesis in various cell types [34]. Furthermore, local deranged immune abnormalities in the peritoneal cavity may enhance the growth of endometriosis.

Constitutive activation of NF-κB has been shown in ectopic endometrial cells and pelvic macrophages in women with peritoneal endometriosis in vivo [34,35]. Gonzalez-Ramos et al. concluded that active endometriotic lesions show a higher degree of activation of the NF-κB pathway than black, inactive lesions. Transcriptional active p50/p65 heterodimer expression was abundant in red-active endometriotic lesions. Both the canonical and atypical NF-κB activation pathways can produce p50/p65 dimers: the canonical pathway in response to inflammatory stimuli (TNF-α, or IL-1β) in endometrial and endometriotic stromal cells and the atypical pathway in response to hypoxia and genotoxic stress [36–40]. Therefore, it suggesting that both pathways are activated in the red endometriotic lesions.

The role of NF-KB to promote inflammation in endometriosis

Various cytokines are produced by many cell types in the peritoneal fluid; they play a diverse role in constructing the peritoneal environment that induces the development and progression of endometriosis. We previously revealed that IL-6, IL-8, and TNF-α are significantly elevated in the peritoneal fluid of women with endometriosis compared with that of women without endometriosis [4,7].

TNF-α, by mediating the NF-κB pathway, can alter cytokine expression in endometriotic cells, contributing to the progression of the disease. The TNF-α-induced activation of NF-κB and other transcription factors can set off a cascade of changes in the expression of their target genes, resulting in increased production of proinflammatory cytokines and chemokines and increased antiapoptotic, angiogenic, and invasive capabilities [20]. In addition, TNF-α enhances mitogenic activity and up-regulates IL-8 expression through NF-κB activation in endometriotic stromal cells [41].

The involvement of NF-κB activation in LPS-inducible TNF-α and IL-8 up-regulation was verified in experiments using an inhibitor for NF-κB N-α-tosyl epsilon-phenylalanyl-chloromethyl ketone (TPCK) [42]. TPCK reduced LPS-induced IL-8 protein production in endometriotic stromal cells [42]. The fact that IL-8 enhances the proliferation of ectopic endometriotic cells and activates angiogenesis and neutrophil migration and differentiation suggests that IL-8 may promote the progression of endometriosis [7]. Progesterone and progestational compounds (dienogest) attenuate the expression of IL-8 by reducing TNF-α-induced NF-κB activation in endometriotic stromal cells [43]. Furthermore, LPS-induced pelvic inflammation status enhanced the development of murine endometriosis-like lesions via the NF-κB pathway [44].

NF-κB and activator protein (AP-1) activation is critical for TNF-α-induced IL-6 expression in endometriotic stromal cells [45]. Increased serum and follicular fluid levels of IL-6 attenuate aromatase activity and estrogen biosynthesis, which affects the fertility of women with endometriosis [46]. TNF-α gene silencing results in the attenuation of the expression of (cIAP2: cellular inhibitor of apoptosis protein-2) and IL-8 genes, which are major marker genes of the NF-κB pathway [47].

When attempting to define the characteristics of the TNF-α-induced signaling pathway and gene expression in endometriotic stromal cells, Ninomiya et al. observed a novel signaling molecule, transforming growth factor β-activated kinase 1 (TAK1) [48]. TAK1 is a member of the MAPK kinase kinase (MAPKKK) family that can be activated by various

cytokines, such as TGFβ, IL-1β, TNF-α, and toll-like receptor ligands [48,49]. MAPKK kinases are involved in the phosphorylation of the IKK complex. In addition, TAK1 activates the MAPK pathway. TNF-α plays a major role in the constitutive activation of NF-κB via the TAK1-IKK pathway, and TNF-α-activated-TAK1 leads to the activation of downstream kinases, including ERK1/2, p38, and IKK [50,51]. We showed that endogenous TAK1 silencing represses TNF-α-enhanced phosphorylation of I-κBα or MAPKs in ESCs and attenuates IL-6 and IL-8 synthesis and cell proliferation in ESCs [52]. Therefore, it is possible that TAK1 possesses the ability to regulate proinflammatory cytokine synthesis and the mitogenic activity of ESCs, and this mechanism depends mainly on the activation of the NF-κB and MAPK pathways.

Some studies have shown that NF-κB mediates macrophage migration inhibitory factor (MIF) gene activation in response to IL-1B or TNF-α in ectopic and eutopic endometrial stromal cells [39,53,54]. MIF is a multifunctional proinflammatory cytokine that activates a number of immunocompetent cells, promotes endothelial cell proliferation, stimulates in vivo angiogenesis, and induces the synthesis and secretion of matrix metalloproteinases [55–58]. The NF-κB-dependent transcriptional activation of MIF in endometriotic stromal cells may provide a mechanism by which ectopic endometrial cells resist apoptosis, proliferate, and exacerbate the local peritoneal endometriosis-associated inflammatory reaction [59,60].

We have shown that IL-10 attenuates TNF-α-induced IL-6 synthesis in cultured endometriotic cells via a mechanism that is definitively dependent on the activation of the NF-κB pathway [61]. Specifically, the administration of exogenous IL-10 reduced the intranuclear concentration of p65 in endometriotic stromal cells, suggesting that IL-10 possesses antiinflammatory effects through the NF-κB pathway [30]. In addition, other authors have proved that IL-10 inhibits TNF-α expression by interfering with NF-κB [59,60]. Another factor with antiinflammatory effects caused by antagonizing the activities of NF-κB is the peroxisome proliferator activated receptor (PPAR)-γ ligand, pioglitazone. We also observed that pioglitazone effectively reduces TNF-α-induced IL-8 expression in endometriotic stromal cells, likely through an NF-κB-dependent pathway [62]. In addition, the intranuclear concentration of the p65 protein is reduced after the addition of pioglitazone in these cells. Ligands of PPAR-γ have been shown to inhibit the expression of many cytokines in macrophages and other cells, principally by preventing the activation of NF-κB [62,63].

These findings suggest that endometriotic cells might have characteristics that cannot be controlled to maintain the balance between pro- and antiinflammatory cytokines. Mechanisms that alter the cytokine profiles in these cells are mainly modulated through the NF-κB pathway.

NF-KB and macrophages

Peritoneal macrophages have been identified as the crucial cells in the regulation and promotion of pelvic inflammatory disease in women with endometriosis. Once they are activated, macrophages can release a wide range of factors (cytokines, growth factors, angiogenic factors) and express cyclooxygenase-2 (COX)-2 and nitric oxide synthase (iNOS), contributing to the maintenance and progression of endometriosis. The increased activation of NF-κB in peritoneal macrophages may alter their physiological functions. A significant

higher proportion of NF-κB nuclear translocation was found in peritoneal macrophages from women with endometriosis compared with women without the disease [35]. Iron is one of the mediators of the endometriosis-associated inflammatory response and oxidative stress and is a known inducer of the NF-κB pathway [64–66]. In fact, iron overload may be one of the factors explaining the increased activation of NF-κB in peritoneal macrophages of women with endometriosis [19].

Macrophages from women with endometriosis have an increased expression of MIF that likely contributes to the increased macrophage infiltration in the peritoneal fluid of these women [51–53]. Activated macrophages can induce the production of proinflammatory cytokines that are able to potentiate the activation of NF-κB, thereby providing a type of positive feedback [67].

NF-KB regulates cox-2 and prostaglandin expression

Several pieces of evidence indicate that prostaglandins (PGs) contribute to the pathophysiology of endometriosis. The concentration of PGE2 in the peritoneal fluid is higher in women suffering from the disease, and COX-2 is more abundantly expressed in ectopic endometriotic tissues compared with eutopic endometrial tissues [68]. LPS, an inducer of the NF-κB pathway, promotes the proliferation and invasion of endometriotic stromal cells via the up-regulation of COX-2 expression and PGE2 synthesis [69]. COX-2 is an NF-κB-target gene and can be up-regulated via NF-κB activation. An inhibitor of NF-κB activation was able to decrease COX-2 mRNA expression and PGE2 levels in endometrial stromal cells [70]. A region of the COX-2 promoter gene contains a variant nuclear factor NF-κB site that, when mutated, completely abolishes COX-2 promoter activation [71]. Binding of NF-κB p65 to this site is, in part, responsible for the COX-2 promoter activation. However, PGE2 could integrate multiple cell survival signaling pathways that promote the survival of endometriotic cells via the action of its receptors, EP2 and EP4 [72]. Banu and coworkers (2009) proposed a mechanism of action of these receptors: PGE2 → EP2/EP4 → Src/B-arrestin 1 complex → epidermal growth factor receptor and/or TNF-α/IL-1B → extracellular signal regulated kinases (ERK) 1/2 and/or protein kinase AKT and/or NF-κB and/or β-catenin [72]. It is possible that the selective inhibition of these receptors could potentially suppress the adverse effects of most of the pathways contributing to the propagation of endometriosis.

NF-KB and angiogenesis

NF-κB regulates the expression of several angiogenic factors. Macrophages and tumor cells have been reported to produce VEGF under the control of NF-κB activation [72]. Macrophages and endometriotic cells show increased VEGF gene and protein expression in women with peritoneal endometriosis [73,74] Therefore, it is possible, indicating that angiogenesis in endometriosis is an NF-κB-dependent process. The deletion of putative NF-κB-binding sites from the VEGF promoter does not affect LPS-induced VEGF promoter activity, suggesting that NF-κB is not solely and directly involved in VEGF transcription [75]. Other angiogenic

factors include NF-κB target genes, such as MCP-1, intercellular adhesion molecule 1 (ICAM-1), IL-8, and MIF [41,42,54,55,76,77]. Recently, in a rat endometriosis model, a significant reduction in microvessel density was achieved by inhibiting the NF-κB pathway [19,78]. These evidences suggest the involvement of NF-κB with the control of angiogenesis in endometriosis. However, additional in vivo experiments are necessary to unravel the exact mechanism of NF-κB-mediated action in these circumstances.

Future perspective focused on the NF-KB pathway for the treatment of endometriosis

Current therapeutic alternatives consist of various treatments aimed to decrease the inflammatory process. Whereas none of the established drugs for treating endometriosis inhibit specifically NF-κB, most of them likely affect the NF-κB pathways. Thus, NF-κB is an excellent potential candidate to target the inflammatory response in endometriotic cells. Efforts have been made for the development of specific drugs targeting the NF-κB pathway. The inhibition of NF-κB could be achieved by: (1) the direct inhibition of NF-κB; (2) the prevention of NF-κB precursors from processing into mature forms by proteasome inhibitors; (3) the prevention of I-κB degradation; (4) the inhibition of IKK; and (5) the inhibition of NF-κB nuclear translocation [78].

The best-known mechanism of the parthenolide is the direct inhibition of NF-κB activity [79]. We previously exhibited that parthenolide repressed the development of endometriosis by suppressing the inflammatory peritoneal environment through the NF-κB pathway [80]. However, a clinically important issue is whether the dose of parthenolide used in this study corresponds to a reasonable dose for human use. We determined the parthenolide dose based on other cell and animal models [30,31]. In addition, we proved LPS-induced pelvic inflammation enhanced the development of murine endometriosis-like lesions via the NF-κB pathway [44]. We also noted the effects of Tokishakuyakusan (TSS), a traditional Japanese medicine (Kampo), on a murine endometriosis model [81]. TSS ameliorated the hyperalgesia and lesion formation on the endometriosis-like model. Thus, TSS represents a possible ideal target of novel therapeutics for endometriosis patients with dysmenorrhea.

Recently, we showed that another NF-κB inhibitor, apigenin, inhibits TNF-α-induced cell proliferation and prostaglandin E2 synthesis in human endometriotic stromal cells [82]. The in vitro application of various other NF-κB inhibitors (e.g., curcumin, IKK-2 inhibitor, PPAR-γ ligand, IL-10) on endometrial and endometriotic cells resulted in decreased proliferation and invasion and increased apoptosis of these cells [19,39,61,62,83,84]. The introduction of these NF-κB modulators in clinical practice will hopefully enable more effective treatment for endometriosis.

Many efforts have been made to manufacture compounds that suppress NF-κB activation in endometriosis and in patients with tumors. However, NF-κB is one of major transcription factors involved in many cellular signaling pathways that are essential for normal cellular function. Currently, several compounds that suppress NF-κB activation have already been available. In clinical practice, these compounds may result in severe adverse effects and cellular toxicity. The strong inhibition of NF-κB might not be practical,

since its absence would result in the severe immunodeficiency. Investigators should be cautious considering the dual role of this transcription factor and existing crosstalk between individual signaling pathways. There are significant challenges for the manufacturing of drugs that (1) normalize and do not abolish the activity of NF-κB, (2) specifically block NF-κB activation in target cells leaving normal cells unaffected, or (3) target the regulation of specific genes whose expression is regulated by the NF-κB transcription factor. Further extensive research is necessary for treatment of endometriosis as the future clinical applications.

References

[1] Shroen D. Disputatio inauguralis medica de ulceribus uteri. Jena: Krebs. 1690. p. 6–17.
[2] Knapp VJ. How old is endometriosis? Late 17th-and 18th-century European descriptions of the disease. Fertil Steril 1999;72:10–4.
[3] Harada T, Iwabe T, Terakawa N. Role of cytokines in endometriosis. Fertil Steril 2001;76:1–10.
[4] Harada T, Yoshioka H, Yoshida S, Iwabe T, Onohara Y, Tanikawa M, Terakawa N. Increased interleukine-6 levels in peritoneal fluid of infertile patients with active endometriosis. Am J Obstet Gynecol 1997;176:593–7. https://doi.org/10.1016/S0002-9378(97)70553-2.
[5] Pizzo A, Salmeri FM, Ardita FV, Sofo V, Tripepi M, Marsico S. Behaviour of cytokine levels in serum and peritoneal fluid of women with endometriosis. Gynecol Obstet Invest 2002;54:82–7.
[6] Tsudo T, Harada T, Iwabe T, Tanikawa M, Nagano Y, Ito M, Taniguchi F, Terakawa N. Altered gene expression and secretion of interleukin-6 in stromal cells derived from endometriotic tissues. Fertil Steril 2000;73:205–11.
[7] Iwabe T, Harada T, Tsudo T, Tanikawa M, Onohara Y, Terakawa N. Pathogenetic significance of increased levels of interleukine-8 in the peritoneal fluid of patients with endometriosis. Fertil Steril 1998;69:924–30.
[8] Iwabe T, Harada T, Tsudo T, Nagano Y, Yoshida S, Tanikawa M, Terakawa N. Tumor necrosis factor-a promotes proliferation of endometriotic stromal cells by inducing interleukine-8 gene and protein expression. J Clin Endocrinol Metab 2000;85:824–9.
[9] Moynagh PN. The NFkB pathway. J Cell Sci 2005;118:4389–92.
[10] Hoffmann A, Natoli G, Ghosh G. Transcriptional regulation via the NFkB signaling module. Oncogene 2006;25:6706–16.
[11] Hayden MS, West AP, Ghosh S. NF-κB and the immune response. Oncogene 2006;25:6758–80.
[12] Karin M, Delhase M. The I-κB kinase (IKK) and NF-κB: key elements of proinflammatory signalling. Semin Immunol 2000;12:85–98.
[13] Schmid JA, Birbach A. IkappaB kinase B (IKAPPAKAPPAB/IKAPPAKAPPA2/IKAPPABKAPPAB)- a key molecule in signaling to the transcription factor NF-κB. Cytokine Growth Factor Rev 2008;19:157–65.
[14] Sen R, Baltimore D. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. Cell 1986;46:705–16.
[15] Ahn KS, Sethi G, Aggarwal BB. Nuclear factor-kappa B: from clone to clinic. Curr Mol Med 2007;7:619–37.
[16] Kumar A, Takada Y, Boriek AM, Aggarwal BB. Nuclear factor kappaB: its role in health and disease. J Mol Med 2004;82:434–48.
[17] Chen F, Castranova V. Nuclear factor-kappaB, an unappreciated tumor suppressor. Cancer Res 2007;67:11093–8.
[18] Ghosh S, Karin M. Missing pieces in the NF-κB puzzle. Cell 2002;109(Suppl. l.):S81–96.
[19] Gonzalez-Ramos R, Van Langendockt A, Defrere S, Lousse JP, Colette S, Devoto L, Donnez J. Involvement of the nuclear factor-kappaB pathway in the pathogenesis of endometriosis. Fertil Steril 2010;94:1985–94.
[20] Guo SW. Nuclear factor-kappaB (NF-kappaB): an unsuspected major culprit in the pathogenesis of endometriosis that is still at large? Gynecol Obstet Invest 2007;63:71–97.
[21] Senftleben U, Li ZW, Baud V, Karin M. IKKB is essential for protecting T cells from TNFα-induced apoptosis. Immunity 2001;14:217–30. https://doi.org/10.1016/S1074-7613(01)00104-2.
[22] Schmidt-Supprian M, Courtois G, Tian J, Coyle AJ, Israel A, Rajewsky K, Pasparakis M. Mature T cells depend on signalling through the IKK complex. Immunity 2003;19:377–89. https://doi.org/10.1016/S1074-7613(03)00237-1.

[23] Yoshimoto T, Nagase H, Ishida T, Inoue J, Nariuchi H. Induction of interleukin-12 p40 transcript by CD40 ligation via activation of nuclear factor-kappaB. Eur J Immunol 1997;27:3461–70.
[24] Finn CA. Implantation, menstruation and inflammation. Biol Rev Camb Phil Soc 1986;61:313–28.
[25] Laird SM, Tuckerman EM, Cork BA, Li TC. Expression of nuclear factor kappaB in human endometrium; role in the control of interleukine 6 and leukaemia inhibitory factor production. Mol Hum Reprod 2000;6:34–40.
[26] King AE, Critchley HOD, Kelly RW. The NF-κB pathway in human endometrium and first trimester deciduas. Mol Hum Reprod 2001;7:175–83.
[27] Kelly RW, King AE, Critchley HOD. Cytokine control in human endometrium. Reproduction 2001;121:3–19.
[28] Page M, Tuckerman EM, Li TC, Laird SM. Expression of nuclear factor kappaB components in human endometrium. J Reprod Immunol 2002;54:1–13.
[29] Royds JA, Dower SK, Qwarnstrom EE, Lewis CE. Response of tumour cells to hypoxia: role of p53 andNF-κB. Mol Pathol 1998;51:55–61.
[30] Luca M, Huang S, Gershenwald JE, Singh RK, Reich R, Bar-Eli M. Expression of interleukine-8 by human melanoma cells up-regulates MMP-2 activity and increases tumor growth and metastases. Am J Pathol 1997;151:1105–13.
[31] Psychoyos A, Nikas G, Gravanis A. The role of prostaglandins in blastocyst implantation. Hum Reprod 1995;10(Suppl. 2):30–42.
[32] Ponce C, Torres M, Galleguillos C, Sovino H, Boric A, Fuentes A, Johnson MC. Nuclear factor kappa B pathway and interleukine-6 are affected in eutopic endometrium of women with endometriosis. Reprod Res 2009;137:727–37.
[33] Nie J, Lu Y, Liu X, Guo SW. Immunoreactivity of progesterone receptor isoform B, nuclear factor kappaB, and I-κBα in adenomyosis. Fertil Steril 2009;92:886–9.
[34] Gonzalez-Ramos R, Donnez J, Defrere S, Leclercq I, Squifflet J, Lousse JC, Van Langendonckt A. Nuclear factor-kappa B is constitutively activated in peritoneal endometriosis. Mol Hum Reprod 2007;13:503–9.
[35] Lousse JC, Van Langendonckt A, Gonzalez-Ramos R, Defrere S, Renkin E, Donnez J. Increased activation of nuclear factor-kappaB (NF-κB) in isolated peritoneal macrophages of patients with endometriosis. Fertil Steril 2008;90:217–20.
[36] Lebovic DI, Chao VA, Martini JF, Taylor RN. IL-1B induction of RANTES (regulated upon activation, normal T cell expressed and secreted) chemokine gene expression in endometriotic stromal cells depends on a nuclear factor-kappaB site in the proximal promoter. J Clin Endocrinol Metabol 2001;86:4759–64.
[37] Sugino N, Karube-Harada A, Sakata A, Takiguchi S, Kato H. Nuclear factor-kappaB is required for tumor necrosis factor-α-induced manganese superoxide dismutase expression in human endometrial stromal cells. J Clin Endocrinol Metabol 2002;87:3845–50.
[38] Karin M, Yamamoto Y, Wang QM. The IKK NF-κB system: a treasure trove for drug development. Nat Rev Drug Discov 2004;3:17–26.
[39] Cao WG, Morin M, Metz C, Maheux R, Akoum A. Stimulation of macrophage migration inhibitory factor expression in endometrial stromal cells by interleukine 1,B involving the nuclear transcription factor NF-κB. Biol Reprod 2005;73:565–70.
[40] Perkins ND. Integrating cell-signalling pathways with NF-κB and IKK function. Nat Rev Mol Cell Biol 2007;8:49–62.
[41] Sakamoto Y, Harada T, Horie S, Iba Y, Taniguchi F, Yoshida S, Iwabe T, Terakawa N. Tumor necrosis factor-α-induced interleukine-8 (IL-8) expression in endometriotic stromal cells, probably through nuclear factor-kappaB activation: gonadotropin-releasing hormone agonist treatment reduced IL-8 expression. J Clin Endocrinol Metabol 2003;88:730–5.
[42] Iba Y, Harada T, Horie S, Deura I, Iwabe T, Terakawa N. Lipopolysaccharide-promoted proliferation of endometriotic stromal cells via induction of tumor necrosis factor α and interleukine-8 expression. Fertil Steril 2004;82(Suppl. 3):1036–42.
[43] Horie S, Harada T, Mitsunari M, Taniguchi F, Iwabe T, Terakawa N. Progesterone and progestational compounds attenuate tumor necrosis factor α-induced interleukin-8 production via nuclear factor kappaB inactivation in endometriotic stromal cells. Fertil Steril 2005;83:1530–5.
[44] Azuma Y, Taniguchi F, Nakamura K, Nagira K, Khine YM, Kiyama T, Uegaki T, Izawa M, Harada T. Lipopolysaccharide promotes the development of murine endometriosis-like lesions via the nuclear factor-kappa B pathway. Am J Reprod Immunol 2017;77.

[45] Yamauchi N, Harada T, Taniguchi F, Yoshida S, Iwabe T, Terakawa N. Tumor necrosis factor-α induced the release of interleukine-6 from endometriotic stromal cells by the nuclear factor-kappaB and mitogen-activated protein kinase pathways. Fertil Steril 2004;82(Suppl. 3):1023–8.
[46] Deura I, Harada T, Taniguchi F, Iwabe T, Izawa M, Terakawa N. Reduction of estrogen production by interleukine-6 in a human granulosa tumor cell line may have implications for endometriosis-associated infertility. Fertil Steril 2005;83(Suppl. 1):1086–92.
[47] Miyamoto A, Taniguchi F, Tagashira Y, Watanabe A, Harada T, Terakawa N. TNF α gene silencing reduced lipopolysaccharide-promoted proliferation of endometriotic stromal cells. Am J Reprod Immunol 2009;61:277–85.
[48] Ninomiya-Tsuji J, Kishimoto K, Hiyama A, Inoue J, Cao Z, Matsumoto K. The kinase TAK1 can activate the NIKI-κB as well as the MAP kinase cascade in the IL-1 signalling pathway. Nature 1999;398:252–6.
[49] Akira S, Takeda K. Toll-like receptor signaling. Nat Rev Immunol 2004;4:499–511.
[50] Jackson-Bernitsas DG, Ichikawa H, Takada Y, Myers JN, Lin XL, Darnai BG, Chaturvedi MM, Aggarwal BB. Evidence that TNF-TNFR1-TRADD-TRAF2-RIP-TAK1-IKK pathway mediates constitutive NF-κB activation and proliferation in human head and neck squamous cell carcinoma. Oncogene 2007;26:1385–97.
[51] Srivastava AK, Qin X, Wedhas N, Arnoush M, Linkhart TA, Chadwick RB, Kumar A. Tumor necrosis factor-α augments matrix metalloproteinase-9 production in skeletal muscle cells through the activation of transforming growth factor-B-activated kinase (TAK1)-dependent signaling pathway. J Biol Chem 2007;282:35113–24.
[52] Taniguchi F, Harada T, Miyakoda H, Iwabe T, Deura I, Tagashira Y, Miyamoto A, Watanabe A, Suou K, Uegaki T, Terakawa N. TAK1 activation for cytokine synthesis and proliferation of endometriotic cells. Mol Cell Endocrinol 2009;307:196–204.
[53] Cao WG, Morin M, Sengers V. Tumor necrosis factor-{α}up-regulates macrophage migration inhibitory factor expression in endometrial stromal cells via the nuclear transcription factor NF-{kappa}B. Hum Reprod 2006;21:421–8.
[54] Veillat V, Lavoie CM, Metz CN, Roger T, Labelle Y, Akum A. Involvement of nuclear factor-kappaB in macrophage migration inhibitory factor gene transcription up-regulation induced by interleukine-1B in ectopic endometrial cells. Fertil Steril 2009;91(Suppl. l):2148–56.
[55] Calandra T, Roger T. Macrophage migration inhibitory factor: a regulator of innate immunity. Nat Rev Immunol 2003;3:791–800.
[56] Bucala R, Donnelly SC. Macrophage migration inhibitory factor: a probable link between inflammation and cancer. Immunity 2007;26:281–5.
[57] Chesney J, Metz C, Bacher M, Peng T, Meinhardt A, Bucala R. An essential role for macrophage migration inhibitory factor (MIF) in angiogenesis and the growth of murine lymphoma. Mol Med 1999;5:181–91.
[58] Meyer-Siegler K. Macrophage migration inhibitory factor increases MMP-2 activity in DU-145 prostate cells. Cytokine 2000;12:914–21.
[59] Kuwata H, Watanabe Y, Miyoshi H, Yamamoto M, Kaisho T, Takeda K, Akira S. IL-10-inducible Bcl-3 negatively regulates LPS-induced TNF-α production in macrophages. Blood 2003;102:4123–9.
[60] Dokter WH, Koopmanms SB, Vellenga E. Effects of IL-10 and IL-4 on LPS-induced transcription factors (AP-1, NF-IL6 and NF-κB) which are involved in IL-6 regulation. Leukemia 1996;10:1308–16.
[61] Tagashira Y, Taniguchi F, Harada T, Ikeda A, Watanabe A, Terakawa N. Interleukine-10 attenuates TNF-α-induced interleukin-6 production in endometriotic stromal cells. Fertil Steril 2009;91:2185–92.
[62] Ohama Y, Harada T, Iwabe T, Taniguchi F, Takenaka Y, Terakawa N. Peroxisome proliferator-activated receptor-γ ligand reduced tumor necrosis factor-α-induced interleukine-8 production and growth in endometriotic stromal cells. Fertil Steril 2008;89:311–7.
[63] Li Q, Verma IM. NF-κB regulation in the immune system. Nat Rev Immunol 2002;2:725–34.
[64] Defrere S, Lousse JC, Gonzalez-Ramos R, Colette S, Donnez J, Van Langendonckt A. Potential involvement of iron in the pathogenesis of peritoneal endometriosis. Mol Hum Reprod 2008;14:377–85.
[65] Kobayashi H, Yamada Y, Kanayama S, Furukawa N, Noguchi T, Haruta S, Yoshida S, Sakata M, Sado T, Oi H. The role of iron in the pathogenesis of endometriosis. Gynecol Endocrinol 2009;25:39–52.
[66] Xiong S, She H, Takeuchi H, Han B, Engelhardt JF, Barton CH, Zandi E, Giulivi C, Tsukamoto H. Signaling role of intracellular iron in NF-κB activation. J Biol Chem 2003;278:17646–54.
[67] Holtmann MH, Neurath MF. Differential TNF-signaling in chronic inflammatory disorders. Curr Mol Med 2004;4:439–44.
[68] Banu SK, Lee J, Speights Jr VO, Starzinski-Powitz A, Arosh JA. Cycloxygenase-2 regulates survival, migration and invasion of human endometriotic cells through multiple mechanisms. Endocrinology 2008;149:1180–9.

[69] Takenaka Y, Taniguchi F, Miyakoda H, Takai E, Terakawa N, Harada T. Lipopolysaccharide promoted proliferation and invasion of endometriotic stromal cells via induction of cyclooxygenase-2 expression. Fertil Steril 2010;93:325–7.
[70] Tamura M, Sebastian S, Yang S, Gurates B, Fang Z, Bulun SE. Interleukin-1B elevates cyclooxygenase-2 protein level and enzyme activity via increasing its mRNA stability in human endometrial stromal cells: an effect mediated by extracellularly regulated kinases 1 and 2. J Clin Endocrinol Metabol 2002;87:3263–73.
[71] Tamura M, Sebastian S, Yang S, Gurates B, Ferrer K, Sasano H, Okamura K, Bulun SE. Up-regulation of cyclooxygenase-2 expression and prostaglandin synthesis in endometrial stromal cells by malignant endometrial epithelial cells. J Biol Chem 2002;277:26208–16.
[72] Kiriakidis S, Andreakos E, Monaco C, Foxwell B, Feldmann M, Paleolog E. VEGF expression in human macrophages is NF-κB-dependent. Studies using adenoviruses expressing the endogenous NF-κB inhibitorI-κBα and a kinase-defective form of the IkappaB kinase 2. J Cell Sci 2003;116:665–74.
[73] Donnez J, Smoes P, Gillerot S, Casanas-Roux F, Nisolle M. Vascular endothelial growth factor (VEGF) in endometriosis. Hum Reprod 1998;13:1686–90.
[74] Kupker W, Schultze-Mosgau A, Diedrich K. Paracrine changes in the peritoneal environment of women with endometriosis. Hum Reprod Update 1998;4:719–23.
[75] Ramanathan M, Pinhal-Enfield G, Hao I, Leibovich SJ. Synergistic up-regulation of vascular endothelial growth factor (VEGF) expression in macrophages by adenosine A2A receptor agonists and endotoxin involves transcriptional regulation via the hypoxia response element in the VEGF promoter. Mol Biol Cell 2007;18:14–23.
[76] Ueda A, Okuda K, Ohno S, Igarashi T, Matsunaga K, Fukushima J, Kawamoto S, Ishigatsubo Y, Okubo T. NF-κB and Sp1 regulate transcription of the human monocyte chemoattractant protein-1 gene. J Imunnol 1994;153:2052–63.
[77] van de Stolpe A, Caldenhoven E, Stade BG, Koenderman L, Raaijmakers JA, Johnson JP, van der Saag PT. 12-O-tetradecanoylphorbol-13-acetate- and tumor necrosis factor-α-mediated induction of intercellular adhesion molecule-1 is inhibited by dexamethasone. Functional analysis of the human intercellular adhesion molecular-1 promoter. J Biol Chem 1994;269:6185–92.
[78] Celik O, Hascalik S, Elter K, Tagluk ME, Gurates B, Aydin NE. Combating endometriosis by blocking proteasome and nuclear factor-kappaB pathways. Hum Reprod 2008;23:2458–65.
[79] Jain NK, Kulkarni SK. Antinociceptive and anti-inflammatory effects of *Tanacetum parthenium* L. extract in mice and rats. J Ethnopharmacol 1999;68:251–9.
[80] Takai E, Taniguchi F, Nakamura K, Uegaki T, Iwabe T, Harada T. Parthenolide reduces cell proliferation and prostaglandin E2 synthesis in human endometriotic stromal cells and inhibits development of endometriosis in the murine model. Fertil Steril 2013;100:1170–8.
[81] Nagira K, Taniguchi F, Nakamura K, Tokita Y, Tsuchiya N, Khine YM, et al. Tokishakuyakusan, a Kampo medicine, attenuates endometriosis-like lesions and hyperalgesia in murine with endometriosis-like symptoms. Am J Reprod Immunol 2019;82.
[82] Suou K, Taniguchi F, Tagashira Y, Kiyama T, Terakawa N, Harada T. Apigenin inhibits tumor necrosis factor α-induced cell proliferation and prostaglandin E2 synthesis by inactivating NF-κB in endometriotic stromal cells. Fertil Steril 2011;95:1518–21.
[83] Sheldrick EL, Derecka K, Marshall E, Chin EC, Hodges L, Wathes DC, Abayasekara DR, Flint AP. Peroxisome proliferator activated receptos and the control of levels of prostaglandin-endoperoxide synthase 2 by arachidonic acid in the bovine uterus. Biochem J 2007;406:175–83.
[84] Hatcher H, Planalp R, Cho J, Torti FM, Torti SV. Curcumin: from ancient medicine to current clinical trials. Cell Mol Life Sci 2008;65:1631–52.

This page intentionally left blank

CHAPTER

17

Gut microbiota and endometriosis

Osamu Yoshino[1], *Yosuke Ono*[2] *and Takehiro Hiraoka*[3]

[1]University of Yamanashi, Department of Obstetrics and Gynecology, Yamanashi, Japan; [2]Teine Keijinkai Hospital, Department of Obstetrics and Gynecology, Japan; [3]University of Tokyo, Department of Obstetrics and Gynecology, Tokyo, Japan

General remarks

Microbiota

Microbiota is "ecological communities of commensal, symbiotic and pathogenic microorganisms" found within and on the host. Microbiota includes bacteria, archaea, protists, fungi, and viruses. The collective genomes of the microorganisms are named the microbiome. The human microbiome involves approximately 150-fold more genes than the human genome [1]. 16S ribosomal-RNA gene sequencing has been established as a standard method to identify microbiota. In addition, new advanced techniques such as Amplicon sequencing, shotgun metagenomic sequencing, and next-generation RNA sequencing (NGS) have made it possible to detect changes in the expression of microbial genes and their functions in the host [2]. There is growing evidence that microbiota plays essential roles in our bodily functions in the induction, training, and enhancement of the host immune system [3,4]. In biological classification, taxonomic ranks of microbiota are classified as domain, kingdom, phylum, class, order, family, genus, and species. The Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia are known to be the dominant bacterial phyla in the gut in the healthy gastrointestinal tract [5]. Moreover, the small intestine is dominated by Lactobacillaceae and Enterobacteriaceae, whereas the colon is characterized by the presence of Bacteroidaceae, Prevotellaceae, Rikenellaceae, and Lachnospiraceae and Ruminococcaceae [5].

Gut microbiota could be inherited and affected by various factors, including diverse environmental conditions, intake of diet, and medication [5–7]. Buffington et al. reported that the maternal high-fat diet led to a shift in microbial ecology that negatively influenced the social behavior of offspring. Social and behavioral deficits associated with gut microbiota imbalance in offspring, a condition of dysbiosis due to maternal high-fat diet, can be prevented by cohousing of offspring and mothers with a balanced diet and transferable to germ-free mice [7]. Moreover, in the microbial communities, their metabolites and components are essential for

https://doi.org/10.1016/B978-0-12-820661-4.00011-X

© 2022 Elsevier Inc. All rights reserved.

immune homeostasis of the host [8]. Therefore, aberrant microbial communities dysregulate immune homeostasis, causing impaired innate immune responses [3,8]. Recently, aberrations in the communication between the gut microbiota and the innate immune system are known to be associated with a variety of diseases, such as obesity, insulin resistance, atherosclerosis, Alzheimer's disease, and inflammatory bowel diseases [9–12].

Dysbiosis

Dysbiosis is a condition of a microbial imbalance or maladaptation on or inside the body [11] Dysbacteriosis is synonymous with dysbiosis. Microbiota, which composes skin flora, gut flora, and vaginal flora can be disturbed as a result of decreasing of typically dominant species and increasing of usually outcompeted or eliminated species to fill the void. Recently, there is growing evidence on the relationship between dysbiosis and various diseases including inflammatory bowel disease, juvenile idiopathic arthritis, and colon cancer, suggesting that gut microbiota play a pivotal role in systemic inflammatory cellular responses [11,13,14].

Diversity of microbiomes: α-diversity, β-diversity, and F/B ratio

Microbial communities can be represented by the use of diversity indices, α and β diversity. α-diversity describes the diversity of a microbial community within a single sample or site. β-diversity, on the other hand, is an index used to compare the diversity of microbial communities across multiple samples or loci [15].

As mentioned above, in humans, healthy adult gut microbiota is mainly dominated by specific phyla such as Bacteroidetes, Firmicutes, Actinobacteria, Proteobacteria, and Verrucomicrobia [5]. A condition of dysbiosis, an imbalance in the microbiota, could induce alterations in the ratio of the microbe communities Firmicutes (F), and Bacteroidetes (B). Therefore, the F/B ratio can be a potential biomarker for pathological conditions [16]. It has been widely considered that the increase of F/B ratio, caused by an expansion of Firmicutes (F) or a contraction of Bacteroidetes (B), was a signature of gut dysbiosis. A high F/B ratio is also reported to be related to obesity [17], hypertension [18], and irritable bowel function [19].

Endometriosis and microbiome

In the development of endometriosis, little is known about the presence and composition of the microbiome and its role. However, recent data revealed that patients with endometriosis tend to be at high risk of chronic diseases, such as cardiovascular diseases [20] and inflammatory bowel diseases [21], which are related to abnormal microbial communities as described above. Additionally, the levels of proinflammatory cytokine such as IL-1β, IL-6, and IL-8 are typically elevated in peritoneal fluid of endometriosis patients. Gut bacteria can modulate systemic inflammatory responses [22], and the release of bacterial products into the peritoneal cavity accelerates auto-immunity [23]. Accordingly, endometriosis is a disease that has a strong relationship with complex immune disorders and may be associated with gut microbiota through the altered immune system [24].

Endometriosis is also regarded as (1) estrogen-dependent disease and (2) a condition of immune disorder. Notably, some reports demonstrated that in endometriosis, helper T cells exhibit characteristics featured by Th2 skewed, Th17 activated and decreased activity of regulatory T cell. The microbiotas related to these events are described below. However, it remains to be elucidated whether these microbiotas are actually involved in the pathogenesis of endometriosis.

Estrogen and microbiome

The gut microbiota influences estrogen metabolism through β-glucuronidase production, and estrogen, on the other hand, also influences the gut microbiota [25,26]. The gut microbiome, including Bacteroides, Bifidobacterium, Escherichia, and Lactobacillus might increase the circulating estrogen levels by encoding for β-glucuronidase production [27]. Considering that endometriosis is an estrogen-dependent disease, gut dysbiosis may lead to the development of endometriosis through the upregulation of serum estrogen levels [25,28].

Helper T cells (Th1, Th2, Th17, and regulatory T cells) and microbiome

Many types of effector cells, including natural killer cells, macrophages, dendritic cells, helper T cells, and cytotoxic T cells, play critical roles in the activation of the immune response. A dysregulated immune response may cause an abnormal environment that enables the growth of escaped ectopic endometrial cells outside the uterus [29]. As for helper T cells, the feature of endometriosis is Th2 skewed, Th17 activated and attenuated function of regulatory T cell.

Th1/Th2 cells

Polysaccharide A (PSA), which is produced by the intestinal commensal Bacteroides fragilis, activates $CD4^+$ T cells, resulting in a Th1 response via toll-like receptor (TLR)-2 activation [30]. N-palmitoyl-S-(2,3-bis(palmitoyloxy)-(2RS)-propyl)-Cys-Ser-Lys_4 (Pam3) of gram-positive bacteria binds to TLR-2 as a ligand and promotes IFN-γ production, which leads to differentiation of Th1 cells [31] As for Th2 cells, a yeast β-1,3-glucan increased the Th2 cell regulator GATA3 [32]. Commensal A4 bacteria, a member of Lachnospiraceae family, which produces an immunodominant microbiota CBir1 antigen, inhibits Th2-cell development through the induction of TGF-β production from dendritic cell [33].

Th17 cells

Th17 cells, also known as inflammatory helper T cells, are derived from natural T cell precursors and produce interleukin-17. Excessive Th17 responses are also involved in a variety of pathogenic conditions, including endometriosis [34]. The retinoid-related orphan receptor-γt (ROR-γt) is a specific transcriptional regulator of Th17 cells [35]. It has been reported that segmented filamentous bacteria (SFB) nesting in the ileum could induce the differentiation of Th17 cells in mice [36]. Also, Candida albicans, *Escherichia coli* O157 and *Citrobacter rodentium* are known to recruit Th17 cells [37–39]. Atarashi et al. reported that adhesion of microbes to intestinal epithelial cells is a critical trigger of Th17 induction. The group identified 20 bacterial strains isolated from fecal samples of a patient with ulcerative colitis in order to cause a robust induction of Th17 cells in mice colon (Table 17.1) [37].

TABLE 17.1 Microbiota to induce Th17 or regulatory T cell (Treg) cells.

Th17	*Candida albicans* [38]
	Escherichia coli O157 [39]
	Citrobacter rodentium Erysipelatoclostridium ramosum[a]
	Flavonifractor plautii[a]
	Clostridium hathewayi[a]
	Clostridium bolteae[a]
	Dielma fastidiosa[a]
	Clostridium symbiosum[a]
	Clostridium innocuum
	Bacteroides dorei
	Clostridiales bacterium
	Anaerostipes caccae
	Ruminococcus gnavus
	Coprobacillus sp.
	Bifidobacterium breve
	Bifidobacterium pseudolongum [37]
Treg	*Lactobacillus murinus*
	Clostridium saccharogumia
	Clostridiales bacterium
	Anaerotruncus colihominis
	Clostridium asparagiforme
	Clostridium scindens
	Erysipelatoclostridium ramos[a]
	Flavonifractor plautii[a]
	Clostridium hathewayi[a]
	Clostridium bolteae[a]
	Dielma fastidiosa[a]
	Clostridium symbiosum[a] [40]

[a]*Common in Th17 and Treg cell induction.*

Treg cells

Regulatory T (Treg) cells are known to be a subpopulation of T cells that maintain immunological self-tolerance and homeostasis and suppress the excessive immune response to the host [41]. The differentiation and function of Treg cells are regulated by the transcription factor Foxp3 [41]. Tanaka et al. reported that in women with endometrioma, the proportion of activated Treg cells in the endometrioma and the endometrium is significantly decreased compared with that in women without endometriosis. Treg cell deficiency enhances local inflammation and angiogenesis, and simultaneously facilitates the attachment and growth of endometrial implants in mice model [42]. It is well established that colon mucosal Tregs require the presence of microbiota for their development, sustenance, and function [43–45]. In humans, inflammatory bowel diseases (IBD) such as Crohn's and ulcerative colitis, are considered to be associated with depletion of commensal bacteria from the phyla Firmicutes and Bacteroidetes [46]. Not only gut microbiota, but also their metabolites, short-chain fatty acids (SCFA), including butyrate, induce the differentiation of Treg cells [47].

Also, the metabolites of gut microbiota control gut function with its integrity. Butylate derived from lactic acid are known to maintain gut integrity by regulating mucin production and the tight junction of epithelial cells [48]. As shown in Fig. 17.1, Lactobacillales or Bifidobacteriales produce lactic acid. Bifidobacterium is a commonly used probiotic to strengthen the intestinal barrier, modulation of the immune response, and pathogen antagonism [47]. And the metabolites of the lactic acid produced by bacteria are dependent on gut microbiota. In the presence of butyrate producers, such as Faecalibacterium, Eubacterium, and Anaerostipes of Firmicutes phylum, lactic acid is metabolized to butylate, which govern healthy gut function. If the microbiome of the gut encourages the production of other SCFA, such as propionate, acetate, and succinate, mucin synthesis and tight junctions is reduced, and gut permeability may be induced [47]. The decreased gut integrity and leakage of bacterial products from the gut might influence other immune cells, including macrophages [49]. It is known that peritoneal macrophages regulate communication between endometriosis and gut microbiota. In a condition of high gut permeability, dysregulated macrophage can permit the survival of endometrial lesions in the peritoneal cavity [50].

Lactobacillus murinus colonization increases Treg cells in the colon [40]. Atarashi et al. proved that the strains affiliated to clusters IV, XIVa, and XVIII of Clostridia, belonging to Firmicutes phylum, promote the expression of Foxp3^{+} in Tregs by providing bacterial antigens and a TGF-β-rich environment. Oral administration of the combination of 17 strains to mice is effective to suppress colitis in allergic diarrhea model (Table 17.1) [40]. Note that there are some strains that overlap to induce Th17 and T-reg cells, suggesting that not only the presence of specific microbiota but other factors might also be critical to induce Th17 or Treg cells [37,40].

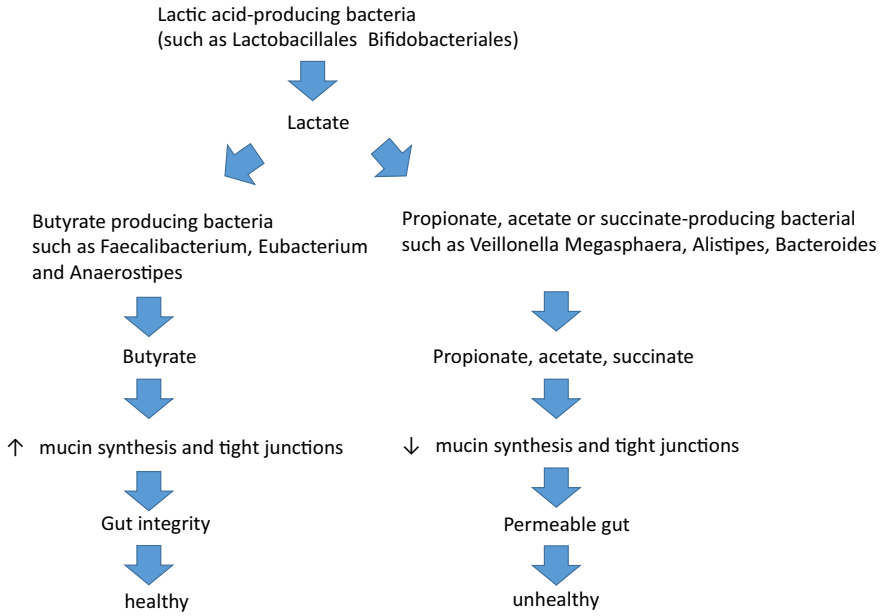


FIGURE 17.1 The concept of healthy and unhealthy gut from the point of lactic acid cascade. *(Modified from Brown C.T., Davis-Richardson A.G., Giongo A., Gano K.A., Crabb D.B., Mukherjee N., Casella G., Drew J.C., Ilonen J., Knip M., Hyöty H., Veijola R., Simell T., Simell O., Neu J., Wasserfall C.H., Schatz D., Atkinson M.A., Triplett E.W., Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes PLoS One 2011;6(10):e25792. doi:10.1371/journal.pone.0025792.)*

Data of gut microbiota in endometriosis

As described above, the gut microbiota is likely to be involved in an etiology of endometriosis, but the numbers of studies using feces are too small in both human and animal models to conclude. Leonardi et al. did great work to summarize the relationship between microbiomes located in various tissues and endometriosis [28].

Human endometriosis

- α- and β-diversity

Ata et al. reported that there was no significant difference of α- and β-diversity in human stool samples from patients with and without endometriosis [51].

- Firmicutes/Bacteroidetes ratio

There is no robust data regarding F/B ration in endometriosis patients.

- Gut microbiota characteristic in endometriosis

At the phylum, class, order, family level.
In the human sample, there is no solid conclusion regarding the difference between endometriosis patients compared to control.
At the genus level.
Genus Sneathia (phylum Fusabacteria), Barnesella (phylum Bacteroidetes) and Gardnerella (phylum Actinobacteria) were significantly decreased in the stool of the endometriosis group [49].

Animal endometriosis model

The phylum-level composition of mice gut microbiota is Firmicutes, Bacteroidetes, and Proteobacteria. Mouse and human share similar gut microbiota composition and function [52]. One of the significant limitations of mice endometriosis models is that mice do not menstruate and therefore require the surgical induction of lesions. Yuan et al. and Chadchan et al. performed a comprehensive study using a murine endometriosis model to determine the changes in gut microbiota [49,53]. Notably, the latter group found that administration of metronidazole, antibiotics, reduced endometriotic lesions in a mouse model of endometriosis, as well as the magnitude of the inflammatory response [53].

- α- and β-diversity

Yuan et al. observed no significant differences in α-diversity, while Chadchan et al. found a difference in the induction of endometriotic lesions. The β-diversity index was significantly higher in the endometriosis mice group compared with controls, suggesting that the gut microbial composition in endometriosis group was altered by the persistent existence of ectopic endometrial tissues in the peritoneal cavity [49].

- Firmicutes/Bacteroidetes ratio

The Firmicutes/Bacteroidetes ratio was elevated in endometriosis mice model [49], indicating that endometriosis may induce dysbiosis.

On the other hand, there is another report that mice with induced endometriotic lesions exhibited lower F/B ratio [53]. Therefore, the studies of the F/B ratio in mice endometriosis model were controversial.

- Gut microbiota characteristic in endometriosis

At the phylum level.

In the mice endometriosis model, the proportions of Actinobacteria and Bacteroidetes were significantly increased, compared to controls [49,53]. Antibiotics treatment decreased endometriotic lesions with a reduction of Bacteroidetes, suggesting that Bacteroidetes are a crucial microbiota to develop endometriotic lesions [53]. As for Firmicutes, data are conflicting.

At the class, order and family level.

The endometriosis mice model exhibited an abundance of Actinobacteria and Betaproteobacteria (the class level), Bifidobacteriales, and Burkholderiales (the order level) and Bifidobacteriaceae and Alcaligenceae (the family level) in the stool compared to control [49].

At the genus level.

Ruminococcaceae-UGG-014, Bifidobacterium, Parasutterella [49], and Bacteroides genera [53] were abundant in the endometriosis mice model.

Conclusion

To date, there are few papers to examine the relationship between the gut microbiomes and endometriosis from the viewpoint of estrogen, helper T cells-related gut microbiome. Endometriosis is a disease that has a strong relationship with complex immune disorders and may be associated with gut microbiota through the altered immune system. Further studies will shed light on the chance of probiotic, prebiotic, and symbiotic strategies in the treatment of endometriosis.

References

[1] Caporaso JG, Lauber CL, Costello EK, Berg-Lyons D, Gonzalez A, Stombaugh J, Knights D, Gajer P, Ravel J, Fierer N, Gordon JI, Knight R. Moving pictures of the human microbiome. Genome Biol 2011;12(5):R50. https://doi.org/10.1186/gb-2011-12-5-r50.

[2] Knight R, Vrbanac A, Taylor BC, Aksenov A, Callewaert C, Debelius J, Gonzalez A, Kosciolek T, McCall L-I, McDonald D, Melnik A v, Morton JT, Navas J, Quinn RA, Sanders JG, Swafford AD, Thompson LR, Tripathi A, Xu ZZ, et al. Best practices for analysing microbiomes. Nat Rev Microbiol 2018;16(7):410–22. https://doi.org/10.1038/s41579-018-0029-9.

[3] Erny D, Hrabě de Angelis AL, Jaitin D, Wieghofer P, Staszewski O, David E, Keren-Shaul H, Mahlakoiv T, Jakobshagen K, Buch T, Schwierzeck V, Utermöhlen O, Chun E, Garrett WS, McCoy KD, Diefenbach A, Staeheli P, Stecher B, Amit I, Prinz M. Host microbiota constantly control maturation and function of microglia in the CNS. Nat Neurosci 2015;18(7):965–77. https://doi.org/10.1038/nn.4030.

[4] Thaiss CA, Zmora N, Levy M, Elinav E. The microbiome and innate immunity. Nature 2016;535(7610):65–74. https://doi.org/10.1038/nature18847.

[5] Donaldson GP, Lee SM, Mazmanian SK. Gut biogeography of the bacterial microbiota. Nat Rev Microbiol 2016;14(1):20–32. https://doi.org/10.1038/nrmicro3552.

[6] Buffington SA, di Prisco GV, Auchtung TA, Ajami NJ, Petrosino JF, Costa-Mattioli M. Microbial reconstitution reverses maternal diet-induced social and synaptic deficits in offspring. Cell 2016;165(7):1762–75. https://doi.org/10.1016/j.cell.2016.06.001.
[7] David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, Ling A v, Devlin AS, Varma Y, Fischbach MA, Biddinger SB, Dutton RJ, Turnbaugh PJ. Diet rapidly and reproducibly alters the human gut microbiome. Nature 2014;505(7484):559–63. https://doi.org/10.1038/nature12820.
[8] Rooks MG, Garrett WS. Gut microbiota, metabolites and host immunity. Nat Rev Immunol 2016;16(6):341–52. https://doi.org/10.1038/nri.2016.42.
[9] Chu H, Khosravi A, Kusumawardhani IP, Kwon AHK, Vasconcelos AC, Cunha LD, Mayer AE, Shen Y, Wu W-L, Kambal A, Targan SR, Xavier RJ, Ernst PB, Green DR, McGovern DPB, Virgin HW, Mazmanian SK. Gene-microbiota interactions contribute to the pathogenesis of inflammatory bowel disease. Science 2016;352(6289):1116–20. https://doi.org/10.1126/science.aad9948.
[10] Shen J, Obin MS, Zhao L. The gut microbiota, obesity and insulin resistance. Mol Aspect Med 2013;34(1):39–58. https://doi.org/10.1016/j.mam.2012.11.001.
[11] Tamboli CP, Neut C, Desreumaux P, Colombel JF. Dysbiosis in inflammatory bowel disease. Gut 2004;53(1):1–4. https://doi.org/10.1136/gut.53.1.1.
[12] Wang Z, Roberts AB, Buffa JA, Levison BS, Zhu W, Org E, Gu X, Huang Y, Zamanian-Daryoush M, Culley MK, DiDonato AJ, Fu X, Hazen JE, Krajcik D, DiDonato JA, Lusis AJ, Hazen SL. Non-lethal inhibition of gut microbial trimethylamine production for the treatment of atherosclerosis. Cell 2015;163(7):1585–95. https://doi.org/10.1016/j.cell.2015.11.055.
[13] Arvonen M, Berntson L, Pokka T, Karttunen TJ, Vähäsalo P, Stoll ML. Gut microbiota-host interactions and juvenile idiopathic arthritis. Pediatr Rheumatol 2016;14(1):44. https://doi.org/10.1186/s12969-016-0104-6.
[14] Huipeng W, Lifeng G, Chuang G, Jiaying Z, Yuankun C. The differences in colonic mucosal microbiota between normal individual and colon cancer patients by polymerase chain reaction-denaturing gradient gel electrophoresis. J Clin Gastroenterol 2014;48(2):138–44. https://doi.org/10.1097/MCG.0b013e3182a26719.
[15] Wagner BD, Grunwald GK, Zerbe GO, Mikulich-Gilbertson SK, Robertson CE, Zemanick ET, Harris JK. On the use of diversity measures in longitudinal sequencing studies of microbial communities. Front Microbiol 2018;9:1037. https://doi.org/10.3389/fmicb.2018.01037.
[16] Mariat D, Firmesse O, Levenez F, Guimarăes V, Sokol H, Doré J, Corthier G, Furet J-P. The Firmicutes/bacteroidetes ratio of the human microbiota changes with age. BMC Microbiol 2009;9(1):123. https://doi.org/10.1186/1471-2180-9-123.
[17] Cox AJ, West NP, Cripps AW. Obesity, inflammation, and the gut microbiota. Lancet Diabetes Endocrinol 2015;3(3):207–15. https://doi.org/10.1016/S2213-8587(14)70134-2. Lancet Publishing Group.
[18] Yang T, Santisteban MM, Rodriguez V, Li E, Ahmari N, Carvajal JM, Zadeh M, Gong M, Qi Y, Zubcevic J, Sahay B, Pepine CJ, Raizada MK, Mohamadzadeh M. Gut dysbiosis is linked to hypertension. Hypertension 2015;65(6):1331–40. https://doi.org/10.1161/HYPERTENSIONAHA.115.05315.
[19] Rajilić-Stojanović M, Biagi E, Heilig HGHJ, Kajander K, Kekkonen RA, Tims S, de Vos WM. Global and deep molecular analysis of microbiota signatures in fecal samples from patients with irritable bowel syndrome. Gastroenterology 2011;141(5):1792–801. https://doi.org/10.1053/j.gastro.2011.07.043.
[20] Mu F, Rich-Edwards J, Rimm EB, Spiegelman D, Missmer SA. Endometriosis and risk of coronary heart disease. Circ Cardiovasc Qual Outcomes 2016;9(3):257–64. https://doi.org/10.1161/CIRCOUTCOMES.115.002224.
[21] Jess T, Frisch M, Jørgensen KT, Pedersen BV, Nielsen NM. Increased risk of inflammatory bowel disease in women with endometriosis: a nationwide Danish cohort study. Gut 2012;61(9):1279–83. https://doi.org/10.1136/gutjnl-2011-301095.
[22] Borody TJ, Khoruts A. Fecal microbiota transplantation and emerging applications. Nat Rev Gastroenterol Hepatol 2012;9(2):88–96. https://doi.org/10.1038/nrgastro.2011.244.
[23] Luckey D, Gomez A, Murray J, White B, Taneja V. Bugs & us: the role of the gut in autoimmunity. Indian J Med Res 2013;138(NOV):732–43.
[24] Laschke MW, Menger MD. The gut microbiota: a puppet master in the pathogenesis of endometriosis? Am J Obstet Gynecol 2016;215(1). https://doi.org/10.1016/j.ajog.2016.02.036. 68.e1-4.
[25] Baker JM, Al-Nakkash L, Herbst-Kralovetz MM. Estrogen-gut microbiome axis: physiological and clinical implications. Maturitas 2017;103:45–53. https://doi.org/10.1016/j.maturitas.2017.06.025.

[26] Plottel CS, Blaser MJ. Microbiome and malignancy. Cell Host Microbe 2011;10(4):324–35. https://doi.org/10.1016/j.chom.2011.10.003.
[27] Kwa M, Plottel CS, Blaser MJ, Adams S. The intestinal microbiome and estrogen receptor-positive female breast cancer. J Natl Cancer Inst 2016;108(Issue 8):19. https://doi.org/10.1093/jnci/djw029. Oxford University Press.
[28] Leonardi M, Hicks C, El-Assaad F, El-Omar E, Condous G. Endometriosis and the microbiome: a systematic review. BJOG Int J Obstet Gynaecol 2020;127(2):239–49. https://doi.org/10.1111/1471-0528.15916.
[29] Riccio L da GC, Santulli P, Marcellin L, Abrão MS, Batteux F, Chapron C. Immunology of endometriosis. Best Pract Res Clin Obstet Gynaecol 2018;50:39–49. https://doi.org/10.1016/j.bpobgyn.2018.01.010.
[30] Wang Q, McLoughlin RM, Cobb BA, Charrel-Dennis M, Zaleski KJ, Golenbock D, Tzianabos AO, Kasper DL. A bacterial carbohydrate links innate and adaptive responses through Toll-like receptor 2. J Exp Med 2006;203(13):2853–63. https://doi.org/10.1084/jem.20062008.
[31] Suzuki N, Akira S, Saito T, Imanishi T, Hara H, Suzuki S. Effector functions cutting edge: TLR2 directly triggers Th1. J Immunol 2007;178(21):6715–9. https://doi.org/10.4049/jimmunol.178.11.6715.
[32] Cao Y, Sun Y, Zou S, Duan B, Sun M, Xu X. Yeast β-glucan suppresses the chronic inflammation and improves the microenvironment in adipose tissues of ob/ob mice. J Agric Food Chem 2018;66(3):621–9. https://doi.org/10.1021/acs.jafc.7b04921.
[33] Wu W, Liu H-P, Chen F, Liu H, Cao AT, Yao S, Sun M, Evans-Marin HL, Zhao Y, Zhao Q, Duck LW, Elson CO, Liu Z, Cong Y. Commensal A4 bacteria inhibit intestinal Th2-cell responses through induction of dendritic cell TGF-β production. Eur J Immunol 2016;46(5):1162–7. https://doi.org/10.1002/eji.201546160.
[34] Hirata T, Osuga Y, Hamasaki K, Yoshino O, Ito M, Hasegawa A, Takemura Y, Hirota Y, Nose E, Morimoto C, Harada M, Koga K, Tajima T, Saito S, Yano T, Taketani Y. Interleukin (IL)-17A stimulates IL-8 secretion, cyclooxygensase-2 expression, and cell proliferation of endometriotic stromal cells. Endocrinology 2008;149(3):1260–7. https://doi.org/10.1210/en.2007-0749.
[35] Jetten AM. Retinoid-related orphan receptors (RORs): critical roles in development, immunity, circadian rhythm, and cellular metabolism. Nucl Recept Signal 2009;7. https://doi.org/10.1621/nrs.07003.
[36] Ivanov II, Atarashi K, Manel N, Brodie EL, Shima T, Karaoz U, Wei D, Goldfarb KC, Santee CA, Lynch Sv, Tanoue T, Imaoka A, Itoh K, Takeda K, Umesaki Y, Honda K, Littman DR. Induction of intestinal Th17 cells by segmented filamentous bacteria. Cell 2009;139(3):485–98. https://doi.org/10.1016/j.cell.2009.09.033.
[37] Atarashi K, Tanoue T, Ando M, Kamada N, Nagano Y, Narushima S, Suda W, Imaoka A, Setoyama H, Nagamori T, Ishikawa E, Shima T, Hara T, Kado S, Jinnohara T, Ohno H, Kondo T, Toyooka K, Watanabe E, et al. Th17 cell induction by adhesion of microbes to intestinal epithelial cells. Cell 2015;163(2):367–80. https://doi.org/10.1016/j.cell.2015.08.058.
[38] Conti HR, Gaffen SL. Host responses to Candida albicans: Th17 cells and mucosal candidiasis. Microb Infect 2010;12(7):518–27. https://doi.org/10.1016/j.micinf.2010.03.013.
[39] Mangan PR, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT. Transforming growth factor-beta induces development of the T(H)17 lineage. Nature 2006;441(7090):231–4. https://doi.org/10.1038/nature04754.
[40] Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, Nishikawa H, Fukuda S, Saito T, Narushima S, Hase K, Kim S, Fritz Jv, Wilmes P, Ueha S, Matsushima K, Ohno H, Olle B, Sakaguchi S, Taniguchi T, et al. Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 2013;500(7461):232–6. https://doi.org/10.1038/nature12331.
[41] Sakaguchi S, Yamaguchi T, Nomura T, Ono M. Regulatory T cells and immune tolerance. Cell 2008;133(5):775–87. https://doi.org/10.1016/j.cell.2008.05.009.
[42] Tanaka Y, Mori T, Ito F, Koshiba A, Takaoka O, Kataoka H, Maeda E, Okimura H, Mori T, Kitawaki J. Exacerbation of endometriosis due to regulatory t-cell dysfunction. J Clin Endocrinol Metab 2017;102(9):3206–17. https://doi.org/10.1210/jc.2017-00052.
[43] Atarashi K, Tanoue T, Shima T, Imaoka A, Kuwahara T, Momose Y, Cheng G, Yamasaki S, Saito T, Ohba Y, Taniguchi T, Takeda K, Hori S, Ivanov II, Umesaki Y, Itoh K, Honda K. Induction of colonic regulatory T cells. Science January 2011;331:337–42. https://doi.org/10.1126/science.1198469.
[44] Bhaskaran N, Cohen S, Zhang Y, Weinberg A, Pandiyan P. TLR-2 signaling promotes IL-17A production in CD4+CD25+Foxp3+ regulatory cells during oropharyngeal candidiasis. Pathogens 2015;4(1):90–110. https://doi.org/10.3390/pathogens4010090.

[45] Geuking MB, Cahenzli J, Lawson MAE, Ng DCK, Slack E, Hapfelmeier S, McCoy KD, Macpherson AJ. Intestinal bacterial colonization induces mutualistic regulatory T cell responses. Immunity 2011;34(5):794–806. https://doi.org/10.1016/j.immuni.2011.03.021.
[46] Frank DN, st Amand AL, Feldman RA, Boedeker EC, Harpaz N, Pace NR. Molecular-phylogenetic characterization of microbial community imbalances in human inflammatory bowel diseases. Proc Natl Acad Sci USA 2007;104(34):13780–5. https://doi.org/10.1073/pnas.0706625104.
[47] Brown CT, Davis-Richardson AG, Giongo A, Gano KA, Crabb DB, Mukherjee N, Casella G, Drew JC, Ilonen J, Knip M, Hyöty H, Veijola R, Simell T, Simell O, Neu J, Wasserfall CH, Schatz D, Atkinson MA, Triplett EW. Gut microbiome metagenomics analysis suggests a functional model for the development of autoimmunity for type 1 diabetes. PLoS One 2011;6(10):e25792. https://doi.org/10.1371/journal.pone.0025792.
[48] McGuckin MA, Lindén SK, Sutton P, Florin TH. Mucin dynamics and enteric pathogens. Nat Rev Microbiol 2011;9(4):265–78. https://doi.org/10.1038/nrmicro2538.
[49] Yuan M, Li D, Zhang Z, Sun H, An M, Wang G. Endometriosis induces gut microbiota alterations in mice. Hum Reprod (Oxf) 2018;33(4):607–16. https://doi.org/10.1093/humrep/dex372.
[50] Li Q, Gao Z, Wang H, Wu H, Liu Y, Yang Y, Han L, Wang X, Zhao L, Tong X. Intestinal immunomodulatory cells (T lymphocytes): a bridge between gut microbiota and diabetes. Mediat Inflamm 2018;2018:9830939. https://doi.org/10.1155/2018/9830939.
[51] Ata B, Yildiz S, Turkgeldi E, Brocal VP, Dinleyici EC, Moya A, Urman B. The endobiota study: comparison of vaginal, cervical and gut microbiota between women with stage 3/4 endometriosis and healthy controls. Sci Rep 2019;9(1):2204. https://doi.org/10.1038/s41598-019-39700-6.
[52] Xiao L, Feng Q, Liang S, Sonne SB, Xia Z, Qiu X, et al. A catalog of the mouse gut metagenome. Nat Biotechnol 2015;33(10):1103–8. https://doi.org/10.1038/nbt.3353. Epub 2015 Sep 28. PMID: 26414350.
[53] Chadchan SB, Cheng M, Parnell LA, Yin Y, Schriefer A, Mysorekar IU, Kommagani R. Antibiotic therapy with metronidazole reduces endometriosis disease progression in mice: a potential role for gut microbiota. Hum Reprod (Oxf) 2019;34(6):1106–16. https://doi.org/10.1093/humrep/dez041.

CHAPTER

18

Immunosuppression and immunotherapy in endometriosis: review of pathophysiology, recent development and future perspectives

Ying He[1], *Sze Wan Hung*[1], *Hui Xu*[1] and *Chi-Chiu Wang*[2,3,4,5]

[1]Department of Obstetrics and Gynaecology, Faculty of Medicine, The Chinese University of Hong Kong, Hong Kong, China; [2]Li Ka Shing Institute of Health Sciences, The Chinese University of Hong Kong, Hong Kong, China; [3]School of Biomedical Sciences, The Chinese University of Hong Kong, Hong Kong, China; [4]Chinese University of Hong Kong – Sichuan University Joint Laboratory in Reproductive Medicine, The Chinese University of Hong Kong, Hong Kong, China; [5]Department of Obstetrics and Gynaecology, Faculty of Medicine, The Chinese University of Hong Kong, Prince of Wales Hospital, Hong Kong, China

Introduction

Endometriosis is a common and complex benign gynecological disorder characterized by the presence of endometrial glands and stroma outside the uterine cavity. Affecting around 10% of women of reproductive age [1] and still under-diagnosed due to the requirement for surgical and pathological diagnosis. Many theories have been proposed to describe the pathogenesis of the disease, including Sampson's retrograde menstruation theory [2] and coelomic metaplastic theory [3], but still cannot explain all the pathogenesis of endometriosis. Endometriosis women have diverse clinical signs and symptoms including pelvic pain, dysmenorrhea, and dyspareunia, which can impact their physical, mental health status, and even social well-being [4]. There is no cure, but treatments are mainly for symptom relief, such as

Immunology of Endometriosis
https://doi.org/10.1016/B978-0-12-820661-4.00001-7

© 2022 Elsevier Inc. All rights reserved.

hormonal therapies or pain relief, while the recurrence rate of endometriosis after the treatment is high [5]. During the past decade, we have seen a considerable amount of research involving alterations of the immune system in endometriosis women and varies of immunotherapies have been tested in clinical trials. In this review, we focus on the underlying mechanisms of immunosuppression and crosstalk of different immune factors in endometriosis. We also summarize current available potential strategies in endometriosis immunotherapy.

Immunosuppressive network in endometriosis (Table 18.1, Fig. 18.1)

Endometriosis establishes a complex microenvironment by immunity, angiogenesis, and endocrine signals. It displays altered immunoinflammatory profiles, which facilitate endometrial tissue to escape immunosurveillance. The immune system has a dual role in endometriosis development, it not only inhibits growth of ectopic endometrial tissue but also promotes the disease progression. Immunosuppressive cells as well as immunosuppressive cytokines form an immunosuppressive network and thereby play a critical role in peritoneal implantation, angiogenesis, and local proliferation of endometrial cells [20,27–30].

Immunosuppressive immune cell in endometriosis

Neutrophils are the most abundant type of granulocytes and one of the first cell types to reach the site of an infection. Only recently it has been accepted that neutrophils have different phenotypes, the N1 antitumor phenotype and N2 protumor phenotype. Neutrophil in early stage endometriosis women may not exhibit a normal inflammatory response in ectopic endometriotic tissue and might have a low respondents to activation signals as in N2 neutrophils. But when endometriosis progresses, and stronger proinflammatory signals are present, the neutrophils become activated [12]. Neutrophils promote angiogenesis of ectopic uterine tissue in the early stage of endometriosis in mouse model [31]. Increased production of vascular endothelial growth factor (VEGF) and matrix metalloproteinase 9 (MMP9) may contribute to the angiogenesis process of neutrophils [32]. Although many studies show women with endometriosis have a higher percentage of peritoneal neutrophils, the potential contribution of neutrophil to disease pathogenesis and subtypes distribution are still unknown [33].

Mast cells Mast cells play an important role in recognition of pathogens or inflammatory disease. Mast cells can regulate immunosuppressive functions in vivo by producing IL-10 which promotes the development of regulatory T cells, inhibits the migration, maturation and activation of DCs, inhibits the production of prostanoids by neutrophils and proinflammatory cytokines by macrophages [34]. Mast cells can also recruit and promote the suppressive activity of MDSCs through CD40: CD40L interaction [35]. Mast cells and degranulating mast cells are significantly more abundant in endometriotic lesions, especially in deep infiltrating lesions than in nonaffected tissues. It indicates that mast cells are associated with pain and hyperalgesia in endometriosis [36]. Numerous mast cells were activated and degranulated in endometriosis lesions which show pathognomonic for a hypersensitivity reaction, presenting a morphological evidence to support the correlation between human endometriosis and allergic disorders [13]. Studies have found that the number of activated mast cells

TABLE 18.1 Abnormal immunological changes in endometriosis.

	Biological role	Finding in endometriosis	References
Cytokines			
IL-4	Antiinflammation	IL-4 increased	[6]
IL-10	Antiinflammation and immunosuppression	IL-10 increased in early stages of endometriosis	[7]
IL-13	Antiinflammation and immunosuppression	IL-13 increased	[8]
TGF-β	Antiinflammation and immunosuppression	Promote the growth of the ectopic endometrial implants, correlate with the severity of the disease	[9]
VEGF	Angiogenesis, inflammation, and immunosuppression	VEGF, VEGFR-2, and VEGF-A increased	[10,11]
Immune cells			
Neutrophils	Protection against infection	Low respondents to activation signals	[12]
Mast cells	Recognition of pathogens or inflammation	Activated and degranulated mast cells increased	[13,14]
Macrophages	Phagocytosis, function both in innate and adaptative immunity	M2 activation	[15]
Dendritic cells	Antigen presentation	Immature DCs increased and mature DCs decreased	[16]
T Cells	Key lymphocytes in cell-mediated immunity	Th17 and Treg increased	[17,18]
B Cells	Key lymphocytes in humoral immunity	Bregs increased	[19]
MDSCs	Suppress T cell responses	MDSCs increased	[20]
Immune checkpoints			
CTLA-4	Negative regulatory molecules on lymphocytes		
PD1/PD-L1	Negative regulatory molecules on lymphocytes	PD-1/PD-L1 expression upregulated	[21]

(*Continued*)

TABLE 18.1 Abnormal immunological changes in endometriosis.—cont'd

	Biological role	Finding in endometriosis	References
Tissue			
MMP	Adhesion	MMP-3 and MMP-9 increased	[22]
NF-kB	Infections	Constitutive activities	[23]
PPARg	Regulator of adipocyte differentiation	Altered expression	[24]
MAPK	Protein kinases	Increased activation	[25]
COX	PGs synthesis	COX-2 increased	[26]
VEGF	Angiogenesis	VEGF increased	[10,11]

in endometriotic lesions are significantly increased, and most of them are concentrated in the vascular and interfiber spaces of ectopic cysts, and are participated in the formation and adhesion of ectopic lesions [14].

Macrophages are derived from monocytes. One of the important functions for macrophages is phagocytosis. In women with endometriosis, scavenger receptor CD36 expression is decreased in the peritoneal macrophages, which contribute to the reduced phagocytic ability of macrophages [37]. Besides phagocytosis, macrophages also play an important role in immunity. Monocyte chemotactic protein-1 (MCP-1) produced by peritoneal mesothelial cells or endometrial cells may attract the accumulation of macrophage [38,39]. Macrophage can in turn produce VEGF and promote angiogenesis of endometriotic lesions [40]. In vitro coculture of endometrial stromal cells (ESCs) from endometriosis women, macrophages and NK cells, cytotoxicity of NK cells was dramatically decreased, and the production of IL-10 and TGF-β were markedly increased [41]. Macrophages are the major immune cells that produce IL-8, which can promote the accumulation of neutrophils [42]. Macrophages have different subsets and functions, classically M1 macrophages are proinflammatory, M2 macrophages are anti-inflammatory that promote tissue repair and fibrosis [43] and could be further divided into M2a, M2b, and M2c [44]. Macrophages from endometriosis women and experimental mice show M2 activation by upregulation of CD163 and CD206 [15]. In endometriosis mouse model, M1 secreted significant amounts of TNF-α while M2 produced higher IL-10, lesions growth was significantly decreased in the presence of M1 macrophages but on the contrary significantly enhanced in the presence of M2 macrophages [15]. Adoptive transfer of M2a macrophages after macrophage depletion

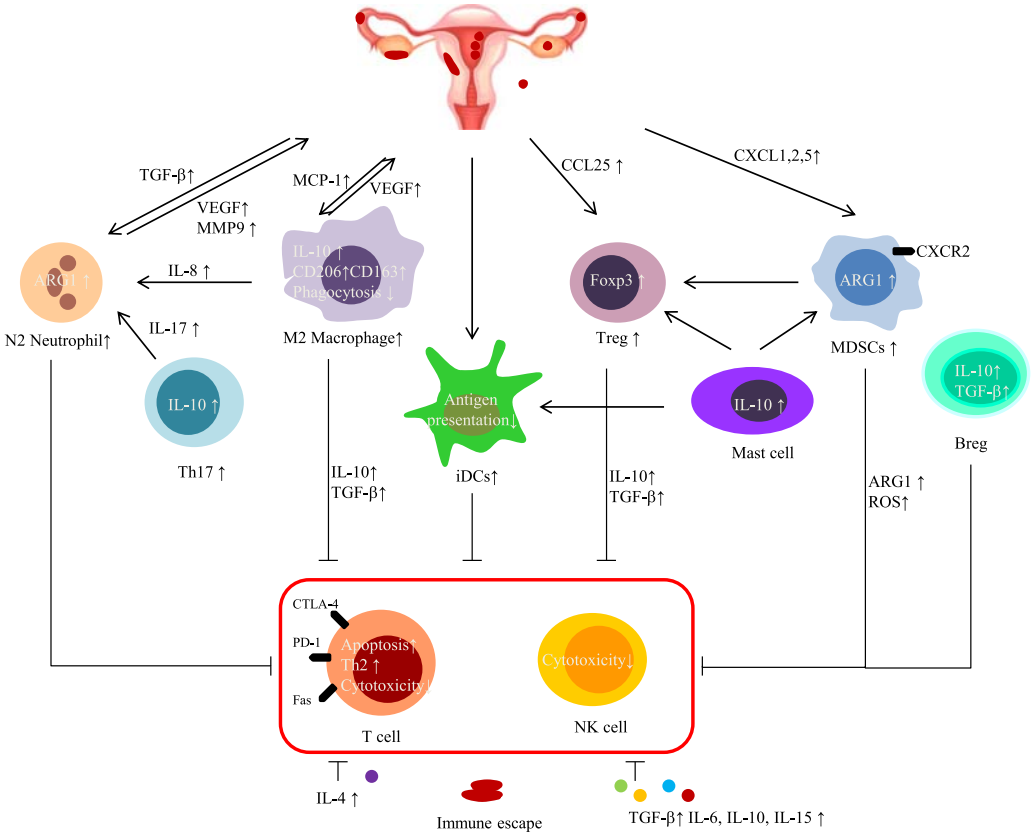


FIGURE 18.1 Immunosuppressive cells and cytokines promote the development of endometriosis. Immunosuppressive cells such as N2 neutrophils, M2 Macrophage, Mast cells, iDC, Tregs, Th17, Breg, MDSCs, and, immunosuppressive cytokines TGF-β, IL-4, IL-10, growth factors VEGF, inhibition factors such as PD-1, CTLA-4, and apoptosis factor Fas suppress the T cell function and NK cell cytotoxicity and promote endometrial cells immune escape and favor their survival.

in endometriosis mouse model significantly increased lesion weight, lesional fibrosis and infiltrated macrophages [27]. Whereas the underlying mechanism of M2c and M2b macrophages in endometriosis is still poorly understood.

Dendritic cells (DCs) are key immune cells for antigen presentation to activate specific T cell response. Under physiological conditions, progenitor cells differentiate into immature DCs (iDCs) then migrate to peripheral tissues to present antigens of diverse origin and initiate their maturation process. The density of endometrial iDCs in peritoneal endometriotic lesions and in the surrounding peritoneum was significantly increased in women with endometriosis compared with controls, while the mDCs was significantly decreased, it indicated

the antigen presentation function of dendritic cells is decreased in endometriosis [16]. Vascular endothelial growth factor receptor 2 (VEGFR2) expressed immature DCs were found to infiltrate peritoneal endometriosis lesions and promote angiogenesis and lesion growth in a murine model [28].

T cells T cells are kind of lymphocyte that play a crucial role in cell-mediated immunity. Once naive CD4+ T helper cells are activated, it can secrete different cytokines and differentiate into several lineages of T helper (Th) cells, including Th1, Th2, Th17, and Treg. In endometriosis women, increased proportion of Th1 and Th17 in endometriosis lesions compared to endometrium [29], higher levels of IL-4 and IL-10 in the peritoneal fluid were found [45], which indicating a shift toward Th2 immune response. In peritoneal fluid of endometriosis women, Th17 cells percentage is increased and even higher in severe stage [17], these cells secreted IL-17A and promote secretion of IL-8 and proliferation of endometriotic stromal cells [46]. IL-27 from ESC and macrophages induces Th17 cells to produce IL-10 and promotes the development of ectopic lesions which may be medicated by c-Maf/RORγt/Blimp-1 signal [47]. In women with ovarian endometriosis, the percentage of Treg cells was significantly increased in the peritoneal fluid [48]. Treg cells are also highly accumulated in eutopic endometrium and ectopic lesions in women with endometriosis along with different stage of the menstrual cycle [18]. The ESC and macrophages derived thymus expressed chemokine (TECK/CCL25) that promotes Tregs differentiation, production of IL-10, TGF-β, CD73, and also suppresses Treg apoptosis, which facilitates immunotolerance of ectopic lesions and ESC proliferation and invasion, then contributes to the development of endometriosis [49].

Regulatory B cells are another type of lymphocytes responsible for humoral immunity by producing antibodies. Many studies demonstrated an increase level of anti-endometrial antibodies in serum, peritoneal fluids, and endometrium in endometriosis women than women unaffected. These studies consider endometriosis as an autoimmune disease [50,51]. A subtype of immunosuppressive B cells, also known as regulatory B (Breg) cells have been associated with immunological tolerance by producing IL-10, TGF-β, or inhibiting T cell functions and other proinflammatory responses [52]. Bruton's tyrosine kinase inhibitor Ibrutinib inhibited mouse endometriotic lesion growth, induced activated B cells toward Bregs in the spleen and peritoneal cavity, and decreased the number of peritoneal M2 macrophages compared to anti-CD20 and control mice [19]. But the role of regulatory B cells in endometriosis patients remains to be elucidated.

Myeloid-derived suppressor cells (**MDSCs**) are a heterogeneous population of myeloid cells that expand under pathological conditions especially in cancer and infections and have immunosuppressive property by suppressing T cell responses [53]. MDSCs have also been reported to induce differentiation of Tregs [54], promote activation of M2 macrophages and impair cytotoxicity of NK cells [55]. It comprises of a mixture of immature myeloid cells and has the morphology of granulocytes (G-MDSC) or monocytes (M-MDSC) [53]. Since G-MDSC shares some phenotype and morphology similarities with neutrophils, some articles hypothesized G-MDSC as a specific subset of neutrophils [56]. Our group previously found that MDSCs significantly increased in peripheral blood of endometriosis women and peripheral blood and peritoneal fluid of endometriosis mouse model. Depletion of MDSCs by anti-Gr-1 antibody significantly inhibited endometrial lesions development in early endometriosis mouse model. These MDSCs can inhibit T cell proliferation by expressing increased level of

ARG1 and ROS [20]. Potential mechanisms of MDSCs in endometriosis and cross-talk between other immune cells in the endometriosis microenvironment remain to be elucidated.

Immunosuppressive cytokines and growth factors

The immune system secrets a group of cytokines, which act as chemical messengers between immune cells, and can recruit numerous cell types to the inflammation sites pleiotropically. Cytokines is also secreted by nonimmune cells such as stromal cells, fibroblasts and endothelium. In addition to immune and inflammatory regulation, their roles are multifunctional. They participate complex network, that facilitate cell growth, hematopoiesis, and tissue repair and cell development [57]. IL-4, IL-10, IL-13, TGF-β, and VEGF are major immunosuppressive cytokines contributing to immune escape.

IL-4 is an anti-inflammatory cytokine induces naïve T cells differentiation into Th2 cells, support immune suppressive function of Treg cells [58], promotes alternative activation of M2 macrophages and suppresses activation of M1 macrophages [59]. In adolescent girls with chronic pelvic pain, concentrations of IL-4 were significantly higher in the serum and peritoneal fluid of adolescents with laparoscopy diagnosed endometriosis than without the disease [60]. IL-4 mRNA expression and intracellular synthesis were significantly higher in peripheral lymphocytes and ectopic endometrial tissue of endometriosis women than health controls [6]. Increased percentage of IL-4 and IL-10 were found in the peritoneal fluid of endometriosis women, also suggest a dominant state of Th2 immune response [45].

IL-10 is an anti-inflammatory and immunosuppressive cytokine. It suppresses the immune response and affects the functions of monocytes and macrophages by inducing release of more anti-inflammatory mediators. The level of IL-10 was significantly elevated in peritoneal fluid of endometriosis women only in early stages of endometriosis but not in later stage, this suppresses activation and leads to a significant reduction of Thl cells [7]. IL-10 down-regulates the cytotoxicity of natural killer cells and trigger the immune escape of ectopic endometrial implants, which are refluxed into the peritoneal cavity and promote the development of endometriosis [30]. An elevated IL-10 level was found in the serum of endometriosis women, depleted the IL-10 activity in an experimental induced endometriosis mouse model lead to the reduction in size of the endometriotic lesions. The lesion size can be promoted by administration IL-10, which attracts the infiltrated plasmacytoid dendritic cells in endometriotic lesions [61].

IL-13 As a cytokine produced by Th2 cells, IL-13 shares some sequence homology and biological functions with IL-4 [62]. IL-13 suppresses inflammatory cytokine secretion, mediates allergic asthma, inhibits tumor immunosurveillance, and promote tissue fibrosis [63]. Elevated levels of IL-13 in ectopic endometrium and peritoneal fluids of endometriosis women compared with normal fertile women were found, it suggested IL-13 may play a role in endometriosis local immune responses [8]. When cultured IL-13 with human ESC, increased levels of IL-6, IL-8, eotaxin, and MCP-1, decreased levels of IL-11, and LIF were found in the culture media, it indicated IL-13 may regulate cytokines and chemokines production of human ESC [64].

TGF-β regulates multifunctional actions of cells, include differentiation, adhesion, invasion, immune functions, and angiogenesis [65]. High levels of TGF-β1 in peritoneal fluid,

serum, and ectopic endometrium were found in endometriosis women and suggest it is importantly involved in the development of endometriosis [9,66]. TGF-β can promote M2 macrophages activation, and is associated with inflammation, apoptotic cells clearance and tissue repairing [67]. TGF-β promotes the growth of the ectopic endometrial implants, correlates with the severity of the disease, and is a chemotactic factor for fibroblasts [9]. Moreover, TGF-β induced an increased proliferation and growth rate of human ESC [68]. In a TGF-β1-null mutant mice model with xenotransplantation of human ectopic endometrial tissues, the deficiency of TGF-β1 suppressed the development of endometriotic lesions associated with decreased macrophages infiltration and myofibroblasts [69].

VEGF is an important angiogenic factor as well as an immunosuppressive factor. VEGF could limit the growth and maturation of immature dendritic cells as well as increase the number of immature Gr-1+ myeloid cell [70]. It was found that the endometriotic lesions in the peritoneal cavity have a high proliferative activity, with a high VEGF-A expression in stromal and epithelium, and a high VEGFR-2 expression in blood vessels [10]. What's more, VEGF and VEGFR-2 have an increased expression in deeply infiltrating endometriosis that affects the rectum [11]. In endometriosis women, the production of IL-1B by activated macrophages resulted in an enhanced expression of VEGF [71], while the secretion of IL-6 and TNF-a by macrophages led to an upregulation of VEGF from macrophages and infiltrating neutrophils [31]. These support that microenvironment of endometriosis acts as a mediator for the secretion of angiogenesis and immunosuppression factor for the development of endometriosis.

Other immunosuppressive cytokines IL-1Ra and IL-37 are anti-inflammatory cytokines belong to the IL-1 family. Elevated serum and peritoneal fluid IL-1Ra levels in endometriosis women were observed, and higher IL-1Ra was found especially in the early stage of endometriosis [72,73]. IL-37 decreases pro-inflammatory cytokines production by macrophages and dendritic cells, it can also inhibit innate and adaptive immune responses in chronic inflammation, autoimmune disorders and cancer [74]. Higher levels of IL-37 in eutopic and ectopic endometrium, serum, and peritoneal fluid of endometriosis women than unaffected women were observed [75,76]. IL-25 (or IL-17E) belongs to the IL-17 cytokine family. IL-25 induces expression of IL-4, IL-5, IL-13, and resulted in activation of Th2 immune response [77]. Increased level of IL-25 was found in the peritoneal fluid of endometriosis women than unaffected women, and its level did not correlate to disease stage [78]. Thymic stromal lymphopoietin (TSLP) promote Th2 immune responses by promoting the functions of Th2 cells and regulatory T cells, while limiting the pro-inflammatory cytokines production by dendritic cells [79]. TSLP concentrations were higher in the serum and peritoneal fluid of endometriosis women compared with unaffected women [80]. IL-1β stimulated the TSLP secretion from human endometrioma stromal cells in vitro [80].

Other immunosuppressive factors

Dysregulation of apoptosis mediator, Fas/Fas-Ligand (FasL), is also involved in endometriosis peritoneal immune privilege of some cell populations. Elevated macrophage products, platelet-derived growth factor (PDGF), and TGF-β1, in the peritoneal fluid of endometriosis women induce an upregulation of FasL expression in ESC and lead to increased Fas-mediated immune cells apoptosis which in turn promote pro-inflammatory immune response

and results in prolonged survival of endometrial cells at the ectopic sites [81]. Negative regulatory molecules expressed on the cell surface of lymphocytes, such as PD-1, could limit T cell immune response and facilitate the development of endometriosis [21]. More and more immunosuppression factors in endometriosis have been identified, indicating that more research is needed to have a complete picture.

Immune microenvironment

Endometrial cells adhesion, angiogenesis, proliferation

Endometriotic lesions establish a very complex microenvironment dominated by altered inflammatory, angiogenesis, and endocrine signals which shows different immunoinflammatory profiles from normal endometrium [82]. Expression of adhesion molecule intercellular adhesion molecule (ICAM-1) is increased in ectopic cells derived from endometriosis and varies during the menstrual cycle [83]. ICAM-1 plays an important role in immune activation and response, while monocyte ICAM-1 expression initiates adherence and activation of T cells and induces ICAM-1 expression on T cells, thereby increases adhesive interaction and additional signaling with macrophages and B cells [84]. Expression of adhesion factors such as endometrial α_V, β3 integrins, and peritoneal vascular cell adhesion molecule 1 (VCAM-1) was increased in women with endometriosis compared with control [85]. In hypoxia, macrophages become active in angiogenesis and produce multiple pro-angiogenic proteinases and growth factors such as VEGF, MMP-9 and urokinase-type PA (uPA) [86]. Higher levels of MMP-3 and uPA in eutopic endometrium and peritoneal fluid which might promote the invasive potential of endometrial cells were found in women with endometriosis when compared with control group [22]. In endometriosis mouse model, elevated levels of TNF-α, IL-6, macrophage inflammatory protein (MIP)-1α and MIP-2 in peritoneal fluids induce neutrophils and macrophages to secrete VEGF and promote angiogenesis in the early stage of endometriosis [31]. Increased concentrations of IL-8, VEGF, MCP-1 and RANTES were found in the peritoneal fluid of women with endometriosis might promote ectopic endometrial tissue adhesion and proliferation [87]. Endometrial cells proliferate after require neovascularization, then invade the extracellular matrix, and further stimulated by various growth factors such as PDGF, insulin-like growth factor (IGF) and macrophage-derived growth factor (MDGF) to establish an endometriotic lesion [88,89].

Epithelial-mesenchymal transition

The epithelial-mesenchymal transition (EMT) occurs in numerous processes, including tissue fibrosis, wound healing, tumor invasion and metastasis [90]. EMT facilities endometrial cells with invasive ability which might also be a prerequisite for the establishment of endometriosis. Several studies examined common markers of EMT in peritoneal, ovarian and rectovaginal endometriosis and endometrium, ectopic have higher expression of Twist, Snail, Slug, MYC, and N-cadherin while eutopic endometrium show increased expression of E-cadherin, this may indicate ectopic and eutopic endometrium have different EMT processes [91–93]. TNF-α, α-enolase and hemoglobin in menstrual effluent induce EMT in mesothelial

cells and cause submesothelial extracellular matrix exposure which facilitated endometrial tissue adhesion to the peritoneum [94]. In endometriosis mouse model, M2a macrophages also play an important role in endometrial tissue repair and fibrogenesis process concomitant with progressive EMT [27].

Therapies in endometriosis

Current therapies in endometriosis

Current therapies for endometriosis have different medical and surgical treatment options. While surgical treatment is sometimes effective but the recurrence risk is high and may cause damages to the ovarian reserve [95]. The hormonal treatments such as oral contraceptives (OC), progestagens, and gonadotrophin-releasing hormone (GnRH) agonists are effective in many women for endometriosis-associated pain but it was not curative, sometimes merely alleviates symptoms and it takes a long time to achieve optimal relief. So there is an urgent need to develop new drugs for endometriosis. The new drugs for endometriosis therapies, including GnRH antagonist, estrogen, and/or progesterone receptors agonist, angiogenesis inhibitor, and immunomodulatory therapies. Here we will focus on the immunomodulatory therapies in endometriosis.

Immunotherapy (Table 18.2)

It is widely accepted that the immune system is critically important to the pathogenesis of endometriosis. The proinflammatory stimulus and pelvic inflammation in peritoneal microenvironment of endometriosis women contribute to their common symptoms, pain and infertility [156]. There are also some particularly cellular and molecular alterations in ectopic endometrium that may act as a potential therapeutic target for endometriosis [157,158]. So immunotherapy and targeted therapy are considered to be the future novel treatment for endometriosis.

Cytokine-mediated therapy

TNF-α is a pro-inflammatory cytokine that has been reported to promote ESC proliferation [159] and adhesion to mesothelial cells [160], then facilitates the development of endometriosis. TNF-α inhibitor Infliximab and Etanercept significantly reduced the volume of endometriotic lesions in rats, decreased the level of plasma nitric oxide (NO), and increased plasma asymmetric dimethylarginine (ADMA) [96], which competes with NO synthase and impairs angiogenesis [161]. Treatment with anti-TNF antibody (c5N) significantly reduced surface area and volume of endometriotic lesions, the number and surface area of red lesions were also decreased in the baboon [104]. However, anti-TNF-α drugs appears to just have a placebo effect on treatment of pelvic pain in women with endometriosis [101,162].

In surgically induced rat endometriosis model, A771726, the active metabolite of Leflunomide, significantly reduced the size and TGF-β1 expression of the endometriotic

TABLE 18.2 Potential immunotherapy in endometriosis.

Strategy	Compound	Used in humans for other indications	Inhibit endometriosis in women/animal	Major outcomes	Any adverse effects	References
Cytokines						
TNF-α inhibitor	Etanercept	Autoimmune diseases such as rheumatoid arthritis, juvenile idiopathic arthritis	Baboon/rat	Inhibit endometriotic lesions in rats and baboons; increase clinical pregnancy rate in patients with endometrioma.	NA	[96–100]
	Infliximab	Autoimmune diseases such as Crohn's disease, rheumatoid arthritis	Rat	Inhibit endometriotic lesions in rats, but no effect observed on human.	NA	[96,101–103]
	c5N	No	Baboon	Inhibit endometriotic lesions in baboons.	Bradycardia	[104]
TGF-β inhibitor	Leflunomide	Rheumatoid arthritis and psoriatic arthritis	Human cell/rat	Inhibit endometriotic lesions in nude mice and rats, and inhibit proliferation of human endometriotic cells.	Teratogenicity	[25,105,106]
	TGFβR1I	No	Mouse	Disease progression was decreased in mice.	NA	[107]
IL-10 inhibitor	F8-IL10	No	Mouse	Inhibit endometriotic lesions in mice.	NA	[108]
MIF inhibitor	ISO-1	No	Mouse	Inhibit endometriotic lesions in mice.	NA	[109]
CSF-1 inhibitor	Imatinib	Chronic myeloid leukemia, gastrointestinal stromal tumors (GIST)	Mouse	Fewer number of endometriotic lesions in a mice.	Increase fetal loss and the rates of certain malformations	[110,111]

(*Continued*)

TABLE 18.2 Potential immunotherapy in endometriosis.—cont'd

Strategy	Compound	Used in humans for other indications	Inhibit endometriosis in women/ animal	Major outcomes	Any adverse effects	References
Immune cells						
Active NK cell	IL-12	No	Mouse	Inhibit endometriotic lesions in mice.	NA	[112]
	BCG	Tuberculosis	Human cell/ rat	Reduce ESC proliferation from endometriosis women in vitro, reduce endometrial implantation to rats.	NA	[113–115]
	Helixor A	Complementary therapy in tumor	Human cell	Enhanced peritoneal NK cell cytotoxicity and relieve pelvic pain of endometriosis women.	NA	[116,117]
Adoptive cell therapy		Tumor immune therapy	Mouse	Reduction in microvessel density (MVD) and volume of endometriotic lesions in mice.	Off-target toxicity and cytokine release syndrome	[118]
Treg inhibitions	CPA	Chemotherapy to treat lymphoma, ovarian cancer	No	NA	NA	[119]
Immune check point blockade						
Anti-CTLA-4	PLGA/anti-CTLA-4	Tumor immune therapy	Mouse	Inhibits ectopic endometrial cells proliferation and invasion in vitro.	NA	[120,121]
Anti-PD1 and anti-PD-L1		Tumor immune therapy	No	Elevated expression od PD-1 and PD-L1in endometriosis women.	NA	[21,122,123]
Tissue targeted therapy						
MMP inhibitors	Metformin	Type 2 diabetes	Rat	Inhibit endometriotic lesions in rats.	NA	[124]

TABLE 18.2 Potential immunotherapy in endometriosis.—cont'd

Strategy	Compound	Used in humans for other indications	Inhibit endometriosis in women/animal	Major outcomes	Any adverse effects	References
	Simvastatin	Decrease elevated lipid levels	Human cell/mouse	Inhibit endometriotic lesions in mice, reduce MMP expression in human ESC.	NA	[125–127]
	Melatonin	Sleep disorders	Human	Relieve chronic pelvic pain in endometriosis women.	NA	[128–130]
	Curcumin	Dietary supplement	Human	Relieve pain in endometriosis women.	NA	[131–133]
	Lipoxin	Eczema	Human cell/mouse	Inhibit endometriotic lesions in mice.	NA	[134,135]
NF-kB inhibitor	Disulfiram	Chronic alcoholism	Rat	Inhibit endometriotic lesions in rats.	NA	[136,137]
	Nobiletin	Neuroprotection	Mouse	Inhibit endometriotic lesions in mice.	NA	[138,139]
	Curcumin	Dietary supplement	Human	Relieve pain in endometriosis women.	NA	[131,140]
PPARg agonist	Ciglitazone	No	Rat	Inhibit endometriotic lesions in rats.	NA	[71]
	Pioglitazone	Type 2 diabetes mellitus	Baboon	Inhibit endometriotic lesions in baboons.	NA	[141,142]
	Rosiglitazone	Type 2 diabetes mellitus	Human	Relief pelvic pain in endometriosis women.	Weight loss	[143,144]

(*Continued*)

TABLE 18.2 Potential immunotherapy in endometriosis.—cont'd

Strategy	Compound	Used in humans for other indications	Inhibit endometriosis in women/animal	Major outcomes	Any adverse effects	References
MAPK inhibitor	Sorafenib	Advanced renal cell carcinoma	Human cell/mouse	Limit endometrial stromal cell proliferation, and inhibit endometriotic lesions in nude mice.	NA	[145,146]
	Leflunomide	Rheumatoid arthritis and psoriatic arthritis	Human cell/rat	Inhibit endometriotic lesions in nude mice and rats, and inhibit proliferation of human endometriotic cells.	Teratogenicity	[25,105]
	Lipoxin	Eczema	Human cell/mouse	Inhibit endometriotic lesions in mice.	NA	[135,147]
COX-2 inhibitor	Rofecoxib	Arthritis, acute pain conditions	Human	Improve pelvic pain and dyspareunia in endometriosis women.	NA	[148,149]
	Curcumin	Dietary supplement	Human	Relieve pain in endometriosis women.	NA	[131]
VEGF inhibitor	EGCG	Dietary supplement	Mouse	Inhibit endometriotic lesions in mice.	NA	[150]
	ATRA	Acute promyelocytic leukemia	Human cell/mouse	Inhibits endometriotic stromal cells proliferation.	NA	[151–153]
	Sunitinib	Renal cell carcinoma and imatinib-resistant GIST	Rat	Inhibit endometriotic lesions in rats.	NA	[154,155]

lesions in rats [105]. In women with endometriosis, treating ectopic endometrial cells with A771726 significantly decreased the phosphorylation of extracellular signal-regulated kinases (ERK) and proliferation of endometrial cells [25].

High levels of TGF-β in peritoneal fluid, serum, and ectopic endometrium were found in endometriosis women [9,66]. Disease progression was decreased when animals were treated with TGF-β type 1 receptor inhibitor (TGFβR1I) in endometriosis mouse model [107]. IL-10 is significantly higher in women with endometriosis than the control group and it also promotes the growth of endometriotic lesions in mouse endometriosis model [61]. Immunocytokines F8-IL10 treatment shows a marked decrease in endometriotic lesion size in endometriosis mouse model [108]. Increased level of macrophage migration inhibitory factor (MIF) was detected in the eutopic and early ectopic endometrium, peritoneal fluid and peripheral blood in endometriosis women [109]. In nude mice implanted with human endometrial tissue, specific antagonist of MIF (ISO-1) treatment caused a significant reduction in number and size of endometriotic lesions [109]. Decreased CSF-1 production by endometrial cells suppress cell proliferation. A CSF-1 inhibitor Imatinib treatment resulted in significantly fewer number of endometriotic lesions in a mouse model [110]. So far there is no human trial yet.

Cell-based therapy

Activate the effector immune cells Some studies examined NK cells have reduced ability to eliminate ectopic endometrial cells which contribute to the development of endometriosis and associated infertility. These indicate upregulation of NK activity may provide a potential immunotherapy for women with severe endometriosis. IL-12 is a heterodimeric cytokine produced by macrophages, dendritic cells [163] which plays an important role in NK cells cytotoxic activity, differentiation and T cells activity, Th1 immune response, and also angiogenesis [164]. In mouse with induced endometriosis, treatment with IL-12 increased splenic NK cells cytotoxicity and reduced the development of the ectopic lesions [112,165], these suggested that IL-12 suppresses endometriotic lesions growth partly through inducing NK cell activation. Depletion NK cell by anti-IL-2Rβ mAb resulted in lower cytotoxicity of NK cells and suppressive effect of IL-12 on eutopic lesions development [112]. Despite encouraging results in mouse models, IL-12 have not been used in endometriosis patients. Mycobacteria BCG could increase NK cells' cytotoxicity to ESC and reduce its proliferation from endometriosis women in vitro [113]. BCG vaccination can reduce the incidence of endometrial implantation to the eye in a rat model, which also indicated its' suppressive effect on endometriosis [114]. While a Mistletoe extract Helixor A could enhanced peritoneal NK cell cytotoxicity through an increased CD107a expression in endometriosis women [116]. Helixor A could also relieve pelvic pain of endometriosis women [166]. Adoptive transfer of healthy women's blood immune cells into nude mice implant with healthy women's endometrium leads to a significantly reduction in MVD and volume of endometriotic lesions [118]. Adoptive cell therapy have a high response rate and caused significant tumor regression in different cancer patients, but before it used in the treatment of human endometriosis, side effects such as off-target toxicity and cytokine release syndrome need to take into consideration.

Suppress the immunosuppressive cells Targeting key immunosuppressive cells such as M2 macrophages, MDSCs, Tregs, Th17, and iDC in endometriosis at different stages can block their recruitment, expansion, activation and immunosuppressive function. Adoptive transfer of M2 macrophages into endometriosis mouse peritoneal cavity dramatically enhanced endometriotic implant growth [15]. Selective Cyclo-oxygenase (COX)-2 inhibitor celecoxib upregulated M1-related cytokine interfron (IFN)-γ, changed the TAM phenotype from M2 back to M1 and reduced the number of endometriotic lesions in mouse [167,168]. Celecoxib also inhibit growth of endometrial epithelial cells from endometriosis women [169]. While COX inhibitors have been widely used in the clinical treatment of endometriosis, whether it also changes immunosuppressive cells in human still need more further studies.

All trans retinoic acid (ATRA) can suppress VEGF expression on neutrophils in eutopic endometrium from women with endometriosis [151]. Retinoic acid could inhibits endometriotic stromal cells proliferation, reduces estradiol production from ovarian endometrial cysts and has the potential to suppress the development of endometriosis [152]. A multi-targeted receptor tyrosine kinase inhibitor Sunitinib reduces the size of endometriotic implants, prevent adhesions, induces luminal epithelium and blood vessels endothelial cells apoptosis, decreased plasma and peritoneal VEGF levels in rat endometriosis model [154,170]. The ATP-binding cassette transporter B1 (ABCB1) can extrude compounds from cells. ABCB1-substrate, cyclophosphamide (CPA), was especially cytotoxic for human Treg cells that lack expression of ABCB1 [119], as higher level of Treg were detected in endometriosis, using CPA to enhance the anti-endometriosis immunity by selectively depleting Treg, which may provide a new strategies for immunotherapy. So far there is no human endometriosis trial yet.

Immune checkpoint blockade is a major mechanism for immune resistance, many immune cells express inhibition molecules, particularly T cells for antigens recognition. Anti-CTLA-4 antibody PLGA reduces Treg cells level, decreases Treg cells IL-10 and TGF-β secretion in peritoneal fluid of endometriosis mouse model, it also inhibits ectopic endometrial cells proliferation and invasion in vitro [120]. Women with advanced endometriosis have higher frequencies of PD-1 positive T and B cells in peripheral blood than healthy individuals, it indicated increased inhibitory signals in lymphocytes may contribute to the development of endometriosis [122]. PD-1 and PD-L1 have elevated expression in endometrial tissues and blood T cells in endometriosis women, estrogen treatment can change their expression in endometrial cells [21]. These data suggest that antibody against immune checkpoints pathway may have a potential effect on endometriosis women. There is no human endometriosis trial yet.

Tissue targeted therapy MMPs is critically important to the adhesion and formation of pathological conditions like endometriosis [171]. In a rat endometriosis model, Metformin which often used in type 2 diabetes significantly decreased volume and weight of endometriotic lesions, reduced VEGF and MMP-9 expression, increased activities of superoxide dismutase (SOD) and tissue inhibitor of metalloproteinase (TIMP)-2 in lesions [124]. A lipid-lowering medication Simvastatin decreased the number and size of endometrial implants and inhibit MMP-3 in mouse endometriosis model [125], it also reduced the expression of MMP2, MMP3 and CD44, but increased TIMP2 in human ESC [126]. In an endometriosis rat model, antioxidant medication Melatonin treatment down-regulated proMMP-9 activity followed by an inverse trend in TIMP-1 expression [128]. And in a phase II placebo-controlled trials, Melatonin relieve chronic pelvic pain and improved sleep quality in endometriosis women [129].

A metabolites of arachidonic acid called Lipoxin inhibited the release of inflammatory factors and MAPK activities in endometriotic stromal cells [147], decreased MMP-9 and VEGF expression in the endometriotic lesions in mouse model [134].

NFκB pathway plays an important role in regulating the immune response to infections. NFκB is constitutive activities in endometriotic lesions may be due to the stimulation such as IL-1β, MIF and TNF-α in peritoneal microenvironment [23]. An NF-κB and proteasome inhibitor Disulfiram decreased NF-κB expression, angiogenesis and proliferation on ectopic endometrium thus reduced the development of endometriosis in rats [136]. Nobiletin can limits inflammation and inhibits the NF-κB activation, significantly altered the VEGF, PCNA expression and proinflammatory mediators in ectopic endometrium, and also impaired the activation of NF-κB in promoting endometriotic cells, thus lead to a reduced ectopic lesion size in mouse endometriosis model [138].

Peroxisome proliferator-activated receptors (PPARs) are a group of nuclear receptor proteins that regulate transcription factors, α (alpha), γ (gamma), and β (delta) are major types of PPARs [172]. A PPAR-γ agonist Rosigliotazone, significantly decreased the size and weight of endometrial implants in a rat model of endometriosis [173]. Rosiglitazone also relief pelvic pain in endometriosis women in a phase 2a clinical trial, although only limited participants in this trial [143]. While PPAR-γ ligand ciglitazone and pioglitazone effectively reduced the endometriosis implants size in rat and baboon models [71,141]. There are increased activation of ERK, a MAPK subtype, in human endometriotic epithelial and stromal cells [25]. Sorafenib is a multi-kinase inhibitor act on MAPK pathway and VEGFR, can significantly limit endometrial stromal cell proliferation, and inhibit the growth of endometriotic lesions in a heterologous nude mouse model [145]. Cyclo-oxygenase (COX) participate in prostaglandins (PGs) synthesis, increased expression of COX-2 has been detected in eutopic and ectopic endometrium from endometriosis women [26]. Treatment with a COX-2 specific inhibitor Rofecoxib significantly improve pelvic pain and dyspareunia in endometriosis women [148].

Alternative medicines Epigallocatechin gallate (EGCG), a potent anti-oxidative and anti-angiogenic catechin extract from green tea, could inhibit endometriotic lesion angiogenesis and growth by selectively suppressed VEGFC and VEGFR2 expression in an experimental mouse model [150]. While the clinical trials to evaluate the efficacy and safety of EGCG in endometriosis women still in progress. Curcumin, a herbal medicine extract from Turmeric, markedly suppresses TNF-α induced ICAM-1 and VCAM-1 expression, promotes IL-6, IL-8 and MCP-1 secretion, inhibits NF-κB activation in human endometriotic stromal cells [140]. Curcumin have a significant effect to relieve pain and decrease serum PGE2 and CA125 levels in endometriosis women [131]. Curcumin also decreased serum MMP-2 and MMP-9 [132], suppress MMP-3 expression and NF-κB translocation in endometriotic lesions in a mouse model [133]. Guizhi Fuling Capsule (GZFLC) significantly reduced implants volume, increased the percentage of CD4+ T cells and enhanced NK cell cytotoxicity in endometriosis rat model [174]. Xiaochaihu decoction treatment remarkly decrease IL-8, TNF-α and VEGF levels in peritoneal fluid and serum and reduced endometriotic lesion size by inducing Fas/FasL expression to promote apoptosis in rat endometriosis model [175,176]. An increasing understanding of the functional molecular mechanisms of natural product of traditional Chinese medicine (TCM) derived herbal extracts provide an attractive strategy for the research and development of novel therapeutics in endometriosis.

Future perspectives: personalized and combination therapy

Different endometriosis women display various immune responses, cellular and molecular changes due to unique characteristic of individual genetic polymorphism, disease progression and environmental interference. Therefore, personalized therapies that target individual immunosuppressive cell profile, hormonal changes and endometriosis tissue should be one of the future directions. Women with endometriosis receiving GnRH agonist show increasing number of natural killer cells and upregulation of T cell proliferative activity accompanied by depressed estradiol level, which indicates hormonal changes will influence the immune response in endometriosis women [177]. In endometriosis mouse model, combination therapy with anti-hypertensive drug telmisartan and selective COX-2 inhibitor parecoxib significantly reduced lesion volume than they use separately. As telmisartan that block receptor angiotensin 1 (AT1) receptor and activate PPAR-γ can also up-regulate COX-2 which promote endometriosis progression, so combination with COX-2 inhibitor enhanced the anti-endometriotic effect [178]. Combine immunotherapy with hormonal or other treatments to target multiple sites of action, reduce side effects and create a personalized therapy for women with endometriosis maybe a promising direction.

Conclusions

A better understanding of immune changes, immune-endocrine communication, immunosuppressive mechanisms in endometriosis will provide potential scientific strategies for endometriosis immunotherapy. One of the important challenges is to identify the pivotal effector immune cells, immunosuppressive cells and immune checkpoint, explore the cellular and molecular mechanisms and key regulatory pathways of these cells in endometriosis microenvironment, which may improve the efficiency of immune therapy. Endometriosis immunotherapy is now emerging as a new approach in addition to conventional therapies. With further understanding of endometriosis pathogenesis and pathology, personalized or combination treatment for each individual may come into the clinical management of endometriosis in the near future. A further challenge is to make suitable plan including optimize dosage and timing of therapies to maximize treatment effect and minimize side effects in order to improve clinical outcomes. Another challenge is to establish meaningful criteria for the evaluation of immune therapies, not only relieve symptoms and/or improve infertility, but also reduce endometriosis implants volume. More well designed clinical trials and animal studies are essential for drug research and development for endometriosis.

References

[1] Eskenazi B, Warner ML. Epidemiology of endometriosis. Obstet Gynecol Clin N Am 1997;24(2):235–58.
[2] Sampson JA. Metastatic or embolic endometriosis, due to the menstrual dissemination of endometrial tissue into the venous circulation. Am J Pathol 1927;3(2):93.
[3] El-Mahgoub S, Yaseen S. A positive proof for the theory of coelomic metaplasia. Am J Obstet Gynecol 1980;137(1):137–40.
[4] Kennedy S, et al. ESHRE guideline for the diagnosis and treatment of endometriosis. Hum Reprod 2005;20(10):2698–704.

[5] Holoch KJ, Lessey BA. Endometriosis and infertility. Clin Obstet Gynecol 2010;53(2):429–38.
[6] Antsiferova YS, et al. Changes in the T-helper cytokine profile and in lymphocyte activation at the systemic and local levels in women with endometriosis. Fertil Steril 2005;84(6):1705–11.
[7] Ho H-N, et al. Peritoneal interleukin-10 increases with decrease in activated CD4+ T lymphocytes in women with endometriosis. Hum Reprod 1997;12(11):2528–33.
[8] Chegini N, Roberts M, Ripps B. Differential expression of interleukins (IL)-13 and IL-15 in ectopic and eutopic endometrium of women with endometriosis and normal fertile women. Am J Reprod Immunol 2003;49(2):75–83.
[9] Pizzo A, et al. Behaviour of cytokine levels in serum and peritoneal fluid of women with endometriosis. Gynecol Obstet Invest 2002;54(2):82–7.
[10] Bourlev V, et al. The relationship between microvessel density, proliferative activity and expression of vascular endothelial growth factor-A and its receptors in eutopic endometrium and endometriotic lesions. Reproduction 2006;132(3):501–9.
[11] Machado DE, et al. Vascular density and distribution of vascular endothelial growth factor (VEGF) and its receptor VEGFR-2 (Flk-1) are significantly higher in patients with deeply infiltrating endometriosis affecting the rectum. Fertil Steril 2008;90(1):148–55.
[12] Finne Riley C, Haase Moen M, Videm V. Inflammatory markers in endometriosis: reduced peritoneal neutrophil response in minimal endometriosis. Acta Obstet Gynecol Scand 2007;86(7):877–81.
[13] Sugamata M, Ihara T, Uchiide I. Increase of activated mast cells in human endometriosis. Am J Reprod Immunol 2005;53(3):120–5.
[14] Kirchhoff D, et al. Mast cells in endometriosis: guilty or innocent bystanders? Expert Opin Ther Targets 2012;16(3):237–41.
[15] Bacci M, et al. Macrophages are alternatively activated in patients with endometriosis and required for growth and vascularization of lesions in a mouse model of disease. Am J Pathol 2009;175(2):547–56.
[16] Schulke L, et al. Dendritic cell populations in the eutopic and ectopic endometrium of women with endometriosis. Hum Reprod 2009;24(7):1695–703.
[17] Gogacz M, et al. Increased percentage of Th17 cells in peritoneal fluid is associated with severity of endometriosis. J Reprod Immunol 2016;117:39–44.
[18] Berbic M, et al. The role of Foxp3+ regulatory T-cells in endometriosis: a potential controlling mechanism for a complex, chronic immunological condition. Hum Reprod 2010;25(4):900–7.
[19] Riccio L, et al. B lymphocytes inactivation by Ibrutinib limits endometriosis progression in mice. Hum Reprod 2019;34(7):1225–34.
[20] Zhang T, et al. MDSCs drive the process of endometriosis by enhancing angiogenesis and are a new potential therapeutic target. Eur J Immunol 2018;48(6):1059–73.
[21] Wu L, et al. Expression of programed death-1 (PD-1) and its ligand PD-L1 is upregulated in endometriosis and promoted by 17beta-estradiol. Gynecol Endocrinol 2018:1–6.
[22] Gilabert-Estelles J, et al. Expression of several components of the plasminogen activator and matrix metalloproteinase systems in endometriosis. Hum Reprod 2003;18(7):1516–22.
[23] McKinnon BD, et al. Kinase signalling pathways in endometriosis: potential targets for non-hormonal therapeutics. Hum Reprod Update 2016;22(3):382–403.
[24] Masoumeh, et al. Adipocyte alterations in endometriosis: reduced numbers of stem cells and microRNA induced alterations in adipocyte metabolic gene expression. Reprod Biol Endocrinol 2019;17(1).
[25] Ngô C, et al. Protein kinase inhibitors can control the progression of endometriosis in vitro and in vivo. J Pathol 2010;222(2):148–57.
[26] Ota H, et al. Distribution of cyclooxygenase-2 in eutopic and ectopic endometrium in endometriosis and adenomyosis. Hum Reprod 2001;16(3):561–6.
[27] Duan J, et al. The M2a macrophage subset may be critically involved in the fibrogenesis of endometriosis in mice. Reprod Biomed Online 2018;37(3):254–68.
[28] Fainaru O, et al. Dendritic cells support angiogenesis and promote lesion growth in a murine model of endometriosis. Faseb J 2008;22(2):522–9.
[29] Takamura M, et al. Simultaneous detection and evaluation of four subsets of CD4+ T lymphocyte in lesions and peripheral blood in endometriosis. Am J Reprod Immunol 2015;74(6):480–6.
[30] Yang H-L, et al. The crosstalk between ESC and Mφ impairs cytotoxicity of NK cell by secreting IL-10 and TGF-β in EMS. Reproduction 2017;154(6).

[31] Lin Y-J, et al. Neutrophils and macrophages promote angiogenesis in the early stage of endometriosis in a mouse model. Endocrinology 2006;147(3):1278–86.
[32] Mantovani A, et al. Neutrophils in the activation and regulation of innate and adaptive immunity. Nat Rev Immunol 2011;11(8):519.
[33] Tariverdian N, et al. Intraperitoneal immune cell status in infertile women with and without endometriosis. J Reprod Immunol 2009;80(1–2):80–90.
[34] Galli SJ, Grimbaldeston M, Tsai M. Immunomodulatory mast cells: negative, as well as positive, regulators of immunity. Nat Rev Immunol 2008;8(6):478.
[35] Danelli L, et al. Mast cells boost myeloid-derived suppressor cell activity and contribute to the development of tumor-favoring microenvironment. Cancer Immunol Res 2015;3(1):85–95.
[36] Anaf V, et al. Pain, mast cells, and nerves in peritoneal, ovarian, and deep infiltrating endometriosis. Fertil Steril 2006;86(5):1336–43.
[37] Chuang PC, et al. Downregulation of CD36 results in reduced phagocytic ability of peritoneal macrophages of women with endometriosis. J Pathol 2009;219(2):232–41.
[38] Arici A, et al. Monocyte chemotactic protein-1 concentration in peritoneal fluid of women with endometriosis and its modulation of expression in mesothelial cells. Fertil Steril 1997;67(6):1065–72.
[39] Akoum A, et al. Secretion of monocyte chemotactic protein-1 by cytokine-stimulated endometrial cells of women with endometriosis. Fertil Steril 1995;63(2):322–8.
[40] McLaren J, et al. Vascular endothelial growth factor is produced by peritoneal fluid macrophages in endometriosis and is regulated by ovarian steroids. J Clin Invest 1996;98(2):482–9.
[41] Yang H-L, et al. The crosstalk between endometrial stromal cells and macrophages impairs cytotoxicity of NK cells in endometriosis by secreting IL-10 and TGF-β. Reproduction 2017;154(6):815–25.
[42] Sikora J, et al. Abnormal peritoneal regulation of chemokine activation—the role of IL-8 in pathogenesis of endometriosis. Am J Reprod Immunol 2017;77(4):e12622.
[43] Murray LA, et al. TGF-beta driven lung fibrosis is macrophage dependent and blocked by Serum amyloid P. Int J Biochem Cell Biol 2011;43(1):154–62.
[44] Novak ML, Koh TJ. Macrophage phenotypes during tissue repair. J Leukoc Biol 2013;93(6):875–81.
[45] Podgaec S, et al. Endometriosis: an inflammatory disease with a Th2 immune response component. Hum Reprod 2007;22(5):1373–9.
[46] Hirata T, et al. Interleukin (IL)-17A stimulates IL-8 secretion, cyclooxygensase-2 expression, and cell proliferation of endometriotic stromal cells. Endocrinology 2007;149(3):1260–7.
[47] Chang K-K, et al. IL-27 triggers IL-10 production in Th17 cells via a c-Maf/RORγt/Blimp-1 signal to promote the progression of endometriosis. Cell Death Dis 2017;8(3):e2666.
[48] Olkowska-Truchanowicz J, et al. CD4+ CD25+ FOXP3+ regulatory T cells in peripheral blood and peritoneal fluid of patients with endometriosis. Hum Reprod 2012;28(1):119–24.
[49] Li M-Q, et al. CD4+ Foxp3+ regulatory T cell differentiation mediated by endometrial stromal cell-derived TECK promotes the growth and invasion of endometriotic lesions. Cell Death Dis 2014;5(10):e1436.
[50] Badaway S, et al. Endometrial antibodies in serum and peritoneal fluid of infertile patients with and without endometriosis. Fertil Steril 1990;53(5):930–2.
[51] Mathur S, Garza DE, Smith LF. Endometrial autoantigens eliciting immunoglobulin (Ig) G, IgA, and IgM responses in endometriosis. Fertil Steril 1990;54(1):56–63.
[52] Rosser EC, Mauri C. Regulatory B cells: origin, phenotype, and function. Immunity 2015;42(4):607–12.
[53] Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. Nat Rev Immunol 2009;9(3):162.
[54] Huang B, et al. Gr-1+ CD115+ immature myeloid suppressor cells mediate the development of tumor-induced T regulatory cells and T-cell anergy in tumor-bearing host. Cancer Res 2006;66(2):1123–31.
[55] Ostrand-Rosenberg S, et al. Cross-talk between myeloid-derived suppressor cells (MDSC), macrophages, and dendritic cells enhances tumor-induced immune suppression. Sem Cancer Biol 2012;22(4):275–81.
[56] Pillay J, et al. Immune suppression by neutrophils and granulocytic myeloid-derived suppressor cells: similarities and differences. Cell Mol Life Sci 2013;70(20):3813–27.
[57] Turner MD, et al. Cytokines and chemokines: at the crossroads of cell signalling and inflammatory disease. Biochim Biophys Acta Mol Cell Res 2014;1843(11):2563–82.

[58] Yang W-C, et al. Interleukin-4 supports the suppressive immune responses elicited by regulatory T cells. Front Immunol 2017;8:1508.
[59] Ponomarev ED, et al. CNS-derived interleukin-4 is essential for the regulation of autoimmune inflammation and induces a state of alternative activation in microglial cells. J Neurosci 2007;27(40):10714–21.
[60] Drosdzol-Cop A, Skrzypulec-Plinta V, Stojko R. Serum and peritoneal fluid immunological markers in adolescent girls with chronic pelvic pain. Obstet Gynecol Surv 2012;67(6):374–81.
[61] Suen J-L, et al. Serum level of IL-10 is increased in patients with endometriosis, and IL-10 promotes the growth of lesions in a murine model. Am J Pathol 2014;184(2):464–71.
[62] Rael EL, Lockey RF. Interleukin-13 signaling and its role in asthma. World Allergy Organ J 2011;4(3):54.
[63] Wynn TA. IL-13 effector functions. Annu Rev Immunol 2003;21(1):425–56.
[64] Nasu K, et al. Interleukin-13 and tumor necrosis factor-β differentially regulate the production of cytokines by cultured human endometrial stromal cells. Fertil Steril 2003;79:821–7.
[65] Young VJ, et al. The role of TGF-β in the pathophysiology of peritoneal endometriosis. Hum Reprod Update 2017;23(5):548–59.
[66] Young VJ, et al. Transforming growth factor-β induced Warburg-like metabolic reprogramming may underpin the development of peritoneal endometriosis. J Clin Endocrinol Metab 2014;99(9):3450–9.
[67] Gong D, et al. TGFβ signaling plays a critical role in promoting alternative macrophage activation. BMC Immunol 2012;13(1):31.
[68] Hammond MG, et al. The effect of growth factors on the proliferation of human endometrial stromal cells in culture. Am J Obstet Gynecol 1993;168(4):1131–6.
[69] Hull ML, et al. Host-derived TGFB1 deficiency suppresses lesion development in a mouse model of endometriosis. Am J Pathol 2012;180(3):880–7.
[70] Gabrilovich D, et al. Vascular endothelial growth factor inhibits the development of dendritic cells and dramatically affects the differentiation of multiple hematopoietic lineages in vivo: presented in part at the Keystone Symposium "cellular and molecular biology of dendritic cells," Santa Fe, NM, March 3-9, 1998, and at the annual meeting of the American association for cancer research, March 28-April 1, 1998. Blood 1998;92(11):4150–66.
[71] Lebovic DI, Kir M, Casey CL. Peroxisome proliferator–activated receptor-gamma induces regression of endometrial explants in a rat model of endometriosis. Fertil Steril 2004;82:1008–13.
[72] Kondera-Anasz Z, et al. Concentrations of interleukin (IL)-1α, IL-1 soluble receptor type II (IL-1 sRII) and IL-1 receptor antagonist (IL-1 Ra) in the peritoneal fluid and serum of infertile women with endometriosis. Eur J Obstet Gynecol Reprod Biol 2005;123(2):198–203.
[73] Mălutan AM, et al. Serum anti-inflammatory cytokines for the evaluation of inflammatory status in endometriosis. J Res Med Sci 2015;20(7):668.
[74] Wang L, et al. Interleukin-37: a crucial cytokine with multiple roles in disease and potentially clinical therapy. Oncol Lett 2018;15(4):4711–9.
[75] Jiang J-F, et al. Increased expression of interleukin 37 in the eutopic and ectopic endometrium of patients with ovarian endometriosis. Reprod Sci 2016;23(2):244–8.
[76] Jiang J, Jiang Z, Xue M. Serum and peritoneal fluid levels of interleukin-6 and interleukin-37 as biomarkers for endometriosis. Gynecol Endocrinol 2019:1–5.
[77] Fort MM, et al. IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. Immunity 2001;15(6):985–95.
[78] Bungum HF, et al. Increased IL-25 levels in the peritoneal fluid of patients with endometriosis. J Reprod Immunol 2016;114:6–9.
[79] Ziegler SF, Artis D. Sensing the outside world: TSLP regulates barrier immunity. Nat Immunol 2010;11(4):289.
[80] Urata Y, et al. Interleukin-1β stimulates the secretion of thymic stromal lymphopoietin (TSLP) from endometrioma stromal cells: possible involvement of TSLP in endometriosis. Hum Reprod 2012;27(10):3028–35.
[81] Garcia-Velasco JA, et al. Macrophage derived growth factors modulate Fas ligand expression in cultured endometrial stromal cells: a role in endometriosis. Mol Hum Reprod 1999;5(7):642–50.
[82] Symons LK, et al. The immunopathophysiology of endometriosis. Trends Mol Med 2018;24(9):748–62.
[83] Vigano P, et al. Expression of intercellular adhesion molecule (ICAM)-1 mRNA and protein is enhanced in endometriosis versus endometrial stromal cells in culture. Mol Hum Reprod 1998;4(12):1150–6.
[84] Dougherty GJ, Murdoch S, Hogg N. The function of human intercellular adhesion molecule-1 (ICAM-1) in the generation of an immune response. Eur J Immunol 1988;18(1):35–9.

[85] Kyama CM, et al. Endometrial and peritoneal expression of aromatase, cytokines, and adhesion factors in women with endometriosis. Fertil Steril 2008;89(2):301–10.
[86] Lin EY, Pollard JW. Tumor-associated macrophages press the angiogenic switch in breast cancer. Cancer Res 2007;67(11):5064–6.
[87] Mier-Cabrera J, et al. Quantitative and qualitative peritoneal immune profiles, T-cell apoptosis and oxidative stress-associated characteristics in women with minimal and mild endometriosis. BJOG An Int J Obstet Gynaecol 2011;118(1):6–16.
[88] Kim JG, et al. Insulin-like growth factors (IGFs), IGF-binding proteins (IGFBPs), and IGFBP-3 protease activity in the peritoneal fluid of patients with and without endometriosis. Fertil Steril 2000;73(5):996–1000.
[89] Halme J, et al. Peritoneal macrophages from patients with endometriosis release growth factor activity in vitro. J Clin Endocrinol Metab 1988;66(5):1044–9.
[90] Kalluri R, Weinberg RA. The basics of epithelial-mesenchymal transition. J Clin Invest 2009;119(6):1420–8.
[91] Matsuzaki S, Darcha C. Epithelial to mesenchymal transition-like and mesenchymal to epithelial transition-like processes might be involved in the pathogenesis of pelvic endometriosis. Hum Reprod 2012;27(3):712–21.
[92] Bartley J, et al. Epithelial to mesenchymal transition (EMT) seems to be regulated differently in endometriosis and the endometrium. Arch Gynecol Obstet 2014;289(4):871–81.
[93] Proestling K, et al. Enhanced epithelial to mesenchymal transition (EMT) and upregulated MYC in ectopic lesions contribute independently to endometriosis. Reprod Biol Endocrinol 2015;13(1):75.
[94] Demir AY, et al. Proteome analysis of human mesothelial cells during epithelial to mesenchymal transitions induced by shed menstrual effluent. Proteomics 2004;4(9):2608–23.
[95] Muzii L, et al. The effect of surgery for endometrioma on ovarian reserve evaluated by antral follicle count: a systematic review and meta-analysis. Hum Reprod 2014;29(10):2190–8.
[96] Cayci T, et al. The levels of nitric oxide and asymmetric dimethylarginine in the rat endometriosis model. J Obstet Gynaecol Res 2011;37(8):1041–7.
[97] Yildirim G, et al. Etanercept causes regression of endometriotic implants in a rat model. Arch Gynecol Obstet 2011;283(6):1297–302.
[98] Barrier BF, et al. Efficacy of anti-tumor necrosis factor therapy in the treatment of spontaneous endometriosis in baboons. Fertil Steril 2004;81:775–9.
[99] Onalan, et al. Effect of etanercept on the success of assisted reproductive technology in patients with endometrioma. Gynecol Obstet Invest 2018;83(4):358–64.
[100] Horneff G, et al. The German etanercept registry for treatment of juvenile idiopathic arthritis. Ann Rheum Dis 2004;63(12):1638–44.
[101] Koninckx P, et al. Anti-TNF-α treatment for deep endometriosis-associated pain: a randomized placebo-controlled trial. Hum Reprod 2008;23(9):2017–23.
[102] Colombel JF, et al. Infliximab, azathioprine, or combination therapy for Crohn's disease. N Engl J Med 2010;362(15):1383–95.
[103] Smolen JS, et al. Efficacy and safety of leflunomide compared with placebo and sulphasalazine in active rheumatoid arthritis: a double-blind, randomised, multicentre trial. Lancet 1999;353(9149):259–66.
[104] Falconer H, et al. Treatment with anti-TNF monoclonal antibody (c5N) reduces the extent of induced endometriosis in the baboon. Hum Reprod 2006;21(7):1856–62.
[105] Yang J, et al. The effect of leflunomide on the transplanted endometriosis lesions in SD rats. Clin Exp Obstet Gynecol 2017;44(1):129–32.
[106] Aytan H, et al. Effect of the immunomodulator leflunomide on the induction of endometriosis in an experimental rat model. Fertil Steril 2007;87(3):698–701.
[107] Correa LF, et al. TGF-β induces endometriotic progression via a noncanonical, KLF11-mediated mechanism. Endocrinology 2016;157(9):3332–43.
[108] Schwager K, et al. The antibody-mediated targeted delivery of interleukin-10 inhibits endometriosis in a syngeneic mouse model. Hum Reprod 2011;26(9):2344–52.
[109] Khoufache K, et al. Macrophage migration inhibitory factor antagonist blocks the development of endometriosis in vivo. PLoS One 2012;7(5):e37264.
[110] Jensen JR, et al. A potential role for colony-stimulating factor 1 in the genesis of the early endometriotic lesion. Fertil Steril 2010;93(1):251–6.
[111] Peng B, Lloyd P, Schran H. Clinical pharmacokinetics of imatinib. Clin Pharmacokinet 2005;44(9):879–94.

[112] Itoh H, et al. Interleukin-12 inhibits development of ectopic endometriotic tissues in peritoneal cavity via activation of NK cells in a murine endometriosis model. Cytotechnology 2011;63(2):133–41.
[113] Clayton R, et al. Increase in peripheral blood mononuclear cell (PBMC)-and CD56+ cell-mediated killing of endometrial stromal cells by mycobacteria; a possible role in endometriosis immunotherapy? Hum Reprod 2004;19(8):1886–93.
[114] Gül A, Yasar T, Uğras S. BCG vaccination to prevent implantation of endometriosis: an experimental study in rats. Eur J Obstet Gynecol Reprod Biol 2001;98(2):209–12.
[115] Organization WH. BCG vaccine: WHO position paper, February 2018–recommendations. Vaccine 2018;36(24):3408–10.
[116] Jeung I-C, et al. Effect of helixor A on natural killer cell activity in endometriosis. Int J Med Sci 2015;12(1):42.
[117] Piao B, et al. Impact of complementary Mistletoe extract treatment on quality of life in breast, ovarian and non-small cell lung cancer patients A prospective randomized controlled clinical trial. Anticancer Res 2004;24(1):303–10.
[118] Bruner-Tran KL, et al. Experimental endometriosis in immunocompromised mice after adoptive transfer of human leukocytes. Fertil Steril 2010;93(8):2519–24.
[119] Dimeloe S, et al. Human regulatory T cells lack the cyclophosphamide-extruding transporter ABCB 1 and are more susceptible to cyclophosphamide-induced apoptosis. Eur J Immunol 2014;44(12):3614–20.
[120] Liu Q, et al. Evaluation of PLGA containing anti-CTLA4 inhibited endometriosis progression by regulating CD4+ CD25+ Treg cells in peritoneal fluid of mouse endometriosis model. Eur J Pharmaceut Sci 2017;96:542–50.
[121] Callahan MK, Wolchok JD, Allison JP. Anti–CTLA-4 antibody therapy: immune monitoring during clinical development of a novel immunotherapy. Semin Oncol 2010;37(5):473–84.
[122] Walankiewicz M, et al. The increase of circulating PD-1-and PD-L1-expressing lymphocytes in endometriosis: correlation with clinical and laboratory parameters. Mediat Inflamm 2018;2018.
[123] Zitvogel L, Kroemer G. Targeting PD-1/PD-L1 interactions for cancer immunotherapy. Oncoimmunology 2012.
[124] Yilmaz B, et al. Metformin regresses endometriotic implants in rats by improving implant levels of superoxide dismutase, vascular endothelial growth factor, tissue inhibitor of metalloproteinase-2, and matrix metalloproteinase-9. Am J Obstet Gynecol 2010;202(4):368. e1–8.
[125] Bruner-Tran KL, Osteen KG, Duleba AJ. Simvastatin protects against the development of endometriosis in a nude mouse model. J Clin Endocrinol Metab 2009;94(7):2489–94.
[126] Sokalska A, et al. Simvastatin decreases invasiveness of human endometrial stromal cells. Biol Reprod 2012;87(1). p. 2, 1-6.
[127] Vaughan CJ, Murphy MB, Buckley BM. Statins do more than just lower cholesterol. Lancet 1996;348(9034):1079–82.
[128] Paul S, et al. Role of melatonin in regulating matrix metalloproteinase-9 via tissue inhibitors of metalloproteinase-1 during protection against endometriosis. J Pineal Res 2008;44(4):439–49.
[129] Schwertner A, et al. Efficacy of melatonin in the treatment of endometriosis: a phase II, randomized, double-blind, placebo-controlled trial. PAIN® 2013;154(6):874–81.
[130] Hardeland R, et al. Melatonin—a pleiotropic, orchestrating regulator molecule. Prog Neurobiol 2011;93(3):350–84.
[131] Signorile PG, Viceconte R, Baldi A. Novel dietary supplement association reduces symptoms in endometriosis patients. J Cell Physiol 2018;233(8):5920–5.
[132] Jana SK, Chakravarty B, Chaudhury K. Letrozole and curcumin loaded-PLGA nanoparticles: a therapeutic strategy for endometriosis. J Nanomed Biother Discov 2014;4(1):1.
[133] Jana S, Paul S, Swarnakar S. Curcumin as anti-endometriotic agent: implication of MMP-3 and intrinsic apoptotic pathway. Biochem Pharmacol 2012;83(6):797–804.
[134] Xu Z, et al. Lipoxin A 4 inhibits the development of endometriosis in mice: the role of anti-inflammation and anti-angiogenesis. Am J Reprod Immunol 2012;67(6):491–7.
[135] Wu SH, et al. Efficacy and safety of 15 (R/S)-methyl-lipoxin A4 in topical treatment of infantile eczema. Br J Dermatol 2013;168(1):172–8.
[136] Celik O, et al. Disulfiram, as a candidate NF-κB and proteasome inhibitor, prevents endometriotic implant growing in a rat model of endometriosis. Eur Rev Med Pharmacol Sci 2016;20(20):4380–9.
[137] Suh JJ, et al. The status of disulfiram: a half of a century later. J Clin Psychopharmacol 2006;26(3):290–302.

[138] Wei X, Shao X. Nobiletin alleviates endometriosis via down-regulating NF-κB activity in endometriosis mouse model. Biosci Rep 2018;38(3). BSR20180470.
[139] Huang H, et al. The multifunctional effects of nobiletin and its metabolites in vivo and in vitro. Evid base Compl Alternative Med 2016;2016.
[140] Kim KH, et al. Curcumin attenuates TNF-α-induced expression of intercellular adhesion molecule-1, vascular cell adhesion molecule-1 and proinflammatory cytokines in human endometriotic stromal cells. Phytother Res 2012;26(7):1037–47.
[141] Lebovic DI, et al. Peroxisome proliferator-activated receptor-γ receptor ligand partially prevents the development of endometrial explants in baboons: a prospective, randomized, placebo-controlled study. Endocrinology 2010;151(4):1846–52.
[142] Derosa G. Efficacy and tolerability of pioglitazone in patients with type 2 diabetes mellitus. Drugs 2010;70(15):1945–61.
[143] Moravek MB, Ward EA, Lebovic DI. Thiazolidinediones as therapy for endometriosis: a case series. Gynecol Obstet Invest 2009;68(3):167–70.
[144] Richter B, et al. Rosiglitazone for type 2 diabetes mellitus. Cochrane Database Syst Rev 2007;3.
[145] Leconte M, et al. Inhibition of MAPK and VEGFR by Sorafenib controls the progression of endometriosis. Reprod Sci 2015;22(9):1171–80.
[146] Escudier B, et al. Sorafenib in advanced clear-cell renal-cell carcinoma. N Engl J Med 2007;356(2):125–34.
[147] Wu R, et al. Lipoxin A 4 suppresses the development of endometriosis in an ALX receptor-dependent manner via the p38 MAPK pathway. Br J Pharmacol 2014;171(21):4927–40.
[148] Cobellis L, et al. The treatment with a COX-2 specific inhibitor is effective in the management of pain related to endometriosis. Eur J Obstet Gynecol Reprod Biol 2004;116(1):100–2.
[149] Matheson AJ, Figgitt DP. Rofecoxib. Drugs 2001;61(6):833–65.
[150] Xu H, et al. Green tea epigallocatechin-3-gallate inhibits angiogenesis and suppresses vascular endothelial growth factor C/vascular endothelial growth factor receptor 2 expression and signaling in experimental endometriosis in vivo. Fertil Steril 2011;96(4):1021–8. e1.
[151] Tee MK, Vigne J-L, Taylor RN. All-trans retinoic acid inhibits vascular endothelial growth factor expression in a cell model of neutrophil activation. Endocrinology 2006;147(3):1264–70.
[152] Yamagata Y, et al. Retinoic acid has the potential to suppress endometriosis development. J Ovarian Res 2015;8(1):49.
[153] Degos L, Wang ZY. All trans retinoic acid in acute promyelocytic leukemia. Oncogene 2001;20(49):7140.
[154] Pala H, et al. The effects of sunitinib on endometriosis. J Obstet Gynaecol 2015;35(2):183–7.
[155] Adams VR, Leggas M. Sunitinib malate for the treatment of metastatic renal cell carcinoma and gastrointestinal stromal tumors. Clin Therapeut 2007;29(7):1338–53.
[156] de Ziegler D, Borghese B, Chapron C. Endometriosis and infertility: pathophysiology and management. Lancet 2010;376(9742):730–8.
[157] Wu Y, et al. Aberrant methylation at HOXA10 may be responsible for its aberrant expression in the endometrium of patients with endometriosis. Am J Obstet Gynecol 2005;193(2):371–80.
[158] Tang L, et al. The DNA methylation status of genes encoding Matrix metalloproteinases and tissue inhibitors of Matrix metalloproteinases in endometriosis. Mol Reprod Dev 2018;85(1):17–25.
[159] Iwabe T, et al. Tumor necrosis factor-α promotes proliferation of endometriotic stromal cells by inducing interleukin-8 gene and protein expression. J Clin Endocrinol Metab 2000;85(2):824–9.
[160] Zhang R-j, Wild RA, Ojago JM. Effect of tumor necrosis factor-α on adhesion of human endometrial stromal cells to peritoneal mesothelial cells: an in vitro system. Fertil Steril 1993;59(6):1196–201.
[161] Jang JJ, et al. Angiogenesis is impaired by hypercholesterolemia: role of asymmetric dimethylarginine. Circulation 2000;102(12):1414–9.
[162] Lu D, Song H, Shi G. Anti-TNF-α treatment for pelvic pain associated with endometriosis. Cochrane Database Syst Rev 2010;3.
[163] Kaliński P, et al. IL-12-deficient dendritic cells, generated in the presence of prostaglandin E2, promote type 2 cytokine production in maturing human naive T helper cells. J Immunol 1997;159(1):28–35.
[164] Manetti R, et al. Natural killer cell stimulatory factor (interleukin 12 [IL-12]) induces T helper type 1 (Th1)-specific immune responses and inhibits the development of IL-4-producing Th cells. J Exp Med 1993;177(4):1199–204.

[165] Somigliana E, et al. Endometrial ability to implant in ectopic sites can be prevented by interleukin-12 in a murine model of endometriosis. Hum Reprod 1999;14(12):2944–50.
[166] Rim S-Y, Oh S-T. The Effect of intraperitoneal instillation of Mistletoe extract during the diagnostic laparoscopy for pain of endometriosis. Obstet Gynecol Sci 2005;48(4):1004–8.
[167] Nakanishi Y, et al. COX-2 inhibition alters the phenotype of tumor-associated macrophages from M2 to M1 in Apc Min/+ mouse polyps. Carcinogenesis 2011;32(9):1333–9.
[168] Olivares C, et al. The inhibitory effect of celecoxib and rosiglitazone on experimental endometriosis. Fertil Steril 2011;96(2):428–33.
[169] Olivares C, et al. Effects of a selective cyclooxygenase-2 inhibitor on endometrial epithelial cells from patients with endometriosis. Hum Reprod 2008;23(12):2701–8.
[170] Abbas MA, Disi AM, Taha MO. Sunitinib as an anti-endometriotic agent. Eur J Pharmaceut Sci 2013;49(4):732–6.
[171] Sillem M, et al. Extracellular matrix remodelling in the endometrium and its possible relevance to the pathogenesis of endometriosis. Hum Reprod Update 1998;4(5):730–5.
[172] Michalik L, et al. International union of pharmacology. LXI. Peroxisome proliferator-activated receptors. Pharmacol Rev 2006;58(4):726–41.
[173] Demirturk F, et al. Effect of peroxisome proliferator—activated receptor-γ agonist rosiglitazone on the induction of endometriosis in an experimental rat model. J Soc Gynecol Invest 2006;13(1):58–62.
[174] Ji X, et al. Immunological regulation of Chinese herb Guizhi Fuling Capsule on rat endometriosis model. J Ethnopharmacol 2011;134(3):624–9.
[175] MA W-g, et al. Empirical study of effect of Yiwei Ning on expression of Fas/FasL in rat with endometriosis. China J Tradit Chin Med 2007;12:021.
[176] Wang Z, et al. Effects of Xiaochaihutang, a Chinese medicine, and danazol on angiogenesis in a rat endometriosis model. Chin J Pathophysiol 1989;05.
[177] Hsu C-C, et al. Immunomodulation in women with endometriosis receiving GnRH agonist. Obstet Gynecol 1997;89(6):993–8.
[178] Nenicu A, et al. Combination therapy with telmisartan and parecoxib induces regression of endometriotic lesions. Br J Pharmacol 2017;174(16):2623–35.

This page intentionally left blank

CHAPTER

19

Novel diagnostic strategies for endometriosis

Mathew Leonardi[1,2], *Jodie Avery*[1] *and Mary Louise Hull*[1,3]

[1]Robinson Research Institute, Adelaide Medical School, University of Adelaide, Adelaide, SA, Australia; [2]Department of Obstetrics and Gynecology, McMaster University, Hamilton, Canada; [3]Women's and Children's Hospital, North Adelaide, SA, Australia

Introduction

Dysmenorrhea is the hallmark of endometriosis, and for 38% of those with endometriosis, it is experienced from menarche [1,2]. It is commonly normalized by health providers as part of the menstrual process. This narrative can be supported by other female family members who, because of the polygenic inheritance of endometriosis, may themselves experience undiagnosed period pain. People with endometriosis face an average delay of six to 12 years from becoming symptomatic to surgical diagnosis [3]. As diagnostic surgery is often the gatekeeper to initiating medical care, many will face major socioeconomic and geographical barriers to endometriosis treatment and be at risk of developing chronic pain sequelae (somatic visceral and central sensitization) [4] and infertility [5]. Pain symptoms correlate poorly with identification of endometriotic lesions at surgery, and for one in three women the risks of surgery will be undertaken without the benefit of obtaining a diagnosis regarding the cause of pain [6].

Because surgery carries risk and is expensive, researchers have sought to find and improve noninvasive ways to diagnose endometriosis, and these have been summarized in several Cochrane reviews. Investigative tests have included pelvic imaging (transvaginal ultrasound [TV-US] and magnetic resonance imaging [MRI]) [7], blood markers [8], eutopic endometrium characteristics [9], urinary markers [10], and peritoneal fluid markers [11].

In line with international guidelines, current investigative pathways for women presenting with symptoms of endometriosis including dysmenorrhea, dyspareunia, chronic pelvic pain, or difficulty conceiving, recommend initial a pelvic ultrasound scan to assess for endometriosis and other pathologies [3,12,13]. In some cases, diagnostic laparoscopy can be undertaken to achieve a diagnosis through direct visualization or histopathological confirmation of

https://doi.org/10.1016/B978-0-12-820661-4.00024-8

© 2022 Elsevier Inc. All rights reserved.

endometriosis. This is generally reserved for those without a diagnosis based on imaging or those who have failed medical treatments. Diagnostic laparoscopy is often done in conjunction with surgical treatment rather than as standalone surgery. Some suggest that MRI may be an alternative to ultrasound, yet it is used more conservatively because of its cost and accessibility. Several blood-based tests have shown promise as diagnostics for endometriosis, but currently, none are specific or sensitive enough to replace invasive surgery or to be used in selecting women for surgery [14].

Beyond imaging, tissue and blood-based biomarkers hold potential for the noninvasive diagnosis of endometriosis and several immunological markers have been evaluated as diagnostic tools for endometriosis. Although many biomarkers are elevated in blood or tissues from women with endometriosis, none have yet been demonstrated to be sensitive or specific enough to reliably detect or exclude endometriosis and their use is restricted to research settings at present [15]. Studies combining biomarkers and imaging are needed, as are studies using big data and machine learning. This chapter summarizes evidence-based research undertaken to identify noninvasive diagnostic tests for endometriosis.

What is a noninvasive diagnostic test for endometriosis?

Although some imaging tests are associated with an intracavitary approach (e.g., transvaginal, transrectal), these tests are generally considered to be noninvasive or minimally invasive when compared with diagnostic surgery for endometriosis. Here, we have defined all tests that do not involve anesthesia and surgery as noninvasive.

A recent series of Cochrane diagnostic test accuracy [4,7,10,15] for endometriosis defined the functions noninvasive diagnostic tests can have in providing clinically useful information that facilitate patient care. Diagnostic tests for endometriosis can fulfill one of three roles:

- **Replacement test:** used to replace an existing test by providing greater or similar accuracy, and other advantages.
- **Triage test:** an initial step in a diagnostic pathway used to identify women who need to undergo further testing with an existing test. Ideally, a triage test has high sensitivity and specificity, however, it may have lower sensitivity but higher specificity than the current test used, or vice versa. A triage test does not aim to improve the diagnostic accuracy of the existing test but rather to reduce the number of individuals undergoing an unnecessary diagnostic test; or
- **Add-on test:** used in addition to an existing test to improve diagnostic performance [16].

Triage tests can also have two roles by either having a high degree of certainty of the diagnosis or by excluding a diagnosis. This review refers often to the terms SpPin and SnNout, and to understand these, we also need to understand the notions of sensitivity and specificity.

- **SpPin:** When a sign, test, or symptom has an extremely high specificity (say, over 95%), a positive result provides near certainty in (rules in) the diagnosis.
- **SnNout:** When a sign, test, or symptom has a high sensitivity, a negative result rules out the diagnosis.

A few other definitions, were defined in the series of Cochrane diagnostic test accuracy reviews for endometriosis and provide context:

- **Replacement test:** when the imaging test has a sensitivity ≥94% and specificity ≥79%. These numbers are based on diagnostic performance of surgery.
- **SnNout triage test:** when the imaging test has a sensitivity ≥95% and specificity ≥50%. This is the threshold at which it is safe to "rule out" endometriosis.
- **SpPin triage test:** when the imaging test has a sensitivity ≥50% and specificity ≥95%. This is the threshold at which it is safe to "rule in" endometriosis.

Which noninvasive tests show the most promise?

Women who present with symptoms suggestive of endometriosis commonly have a full history and examination. They may be provided a presumptive clinical diagnosis of endometriosis, (although not encouraged by some) to initiate early treatment [4]. While this is a noninvasive diagnosis, it is not always preferred by patients because uncertainty in the etiology of symptoms remains. Indeed, a reliable diagnosis based solely on presenting symptoms is difficult to obtain due to the heterogeneity in clinical presentation, the high prevalence of asymptomatic endometriosis (2%–50%), and the poor association between presenting symptoms and severity of the disease [17–19]. While there is a wide differential diagnosis for most positive physical findings, in 70%–90% of cases, an abnormal pelvic examination correlates with the presence of endometriosis on laparoscopy [20]. Normal clinical findings do not exclude endometriosis, as laparoscopically proven disease has been diagnosed in more than 50% of women with a clinically normal pelvic examination [21].

There are many potential advantages of using imaging tests for the diagnosis of endometriosis including their ready availability and acceptability; they do not require anesthetic or incisions; they provide rapid results; and are more cost-effective when compared to surgery [22]. Their diagnostic potential is, however, dependent on the skills of the operator, as only specialized scans, conducted specifically to detect endometriosis will provide reasonable diagnostic accuracy. The most widely reported diagnostic modalities for endometriosis include ultrasound (transabdominal [TA-US], TV-US, and transrectal [TR-US]) and MRI. The International Deep Endometriosis Analysis (IDEA) group has published a consensus opinion on the ultrasound approach to patients with signs and symptoms of endometriosis [23]. In addition to the direct visualization of endometriosis, markers of adhesions including ovarian fixation and obliteration of the pouch of Douglas (POD) are incorporated into the IDEA consensus opinion. Overall pelvic endometriosis (including superficial endometriosis) remains difficult to diagnose or rule out using imaging, though this is an active area of research [24].

While ultrasound is a routine investigation and may provide radiological confirmation of ovarian endometriosis, large limitations exist regarding the implementation of specialist, IDEA-based, endometriosis scans, as experienced sonographers and longer timeframes are required to perform this type of scan well. This is despite evidence that the IDEA-based ultrasound approach will confirm deep endometriosis with high diagnostic accuracy, over and above the diagnostic information from clinical examination. On a broader level, ultrasound has the capacity to predict the severity of endometriosis when compared to the revised

American Society of Reproductive Medicine (rASRM) [25]. In most ways, MRI has the same capacity as ultrasound [26,27], but lacks the dynamic nature of ultrasound and the ability to interact directly with the patient during the assessment. In addition to the operator-dependent nature (i.e., the reliance on the performance and interpretation), which MRI shares with ultrasound, there may also be issues with access and cost. Conversely, MRI may have a higher sensitivity, at the expense of a higher false positive rate for deep endometriosis [7].

Biomarkers in blood, urine and endometrium have been the focus of many small studies to differentiate between women with and without endometriosis. However, once external validation is sought in larger studies by different research groups, many lose their convincing potential as a diagnostic test. The diagnostic value of these tests has been fully systematically evaluated and summarized using Cochrane methods [7,10,15], but also evaluated systematically in other published reviews [28–30]. A simple, noninvasive test for the diagnosis of endometriosis has not been developed that is routinely implemented in clinical practice.

Surgical diagnosis of endometriosis

There are some procedures available for the surgical diagnosis of endometriosis. These include laparoscopy (minimal access, or keyhole surgery) or laparotomy (open surgery via an abdominal incision). Laparoscopy has largely replaced traditional open surgery [31] for several reasons including shorter recovery times, fewer complications, better peritoneal cavity visualization with a magnified view and because laparoscopy is the preferred technique for surgical treatment of endometriosis [32]. Surgery remains the most accepted method to determine the extent and severity of endometriosis, despite improvements in noninvasive diagnostic tools [25].

Although there are several different classification systems for endometriosis [34–37], the rASRM classification is the most commonly used, as it is internationally accepted and respected as a tool for the objective assessment of the disease [33] (Table 19.1). The lack of correlation between laparoscopic staging, the severity of symptoms, and response to treatment, has attributed little value to this classification system in clinical practice [38–40]. A consensus around the optimal classification for endometriosis is very much needed and the World Endometriosis Society has recently endeavored to attain this [41].

In their diagnostic and treatment guidelines, the European Society of Human Reproduction and Embryology (ESHRE) Special Interest Group for Endometriosis has stated women presenting with symptoms of endometriosis, cannot obtain a definitive diagnosis without a visual inspection of the pelvis at laparoscopy, as the gold standard investigation for endometriosis [42]. These authors disagree with the certitude of this statement as noninvasive imaging can identify endometriosis reliably. Although, for those where endometriosis cannot be identified using imaging or examination, visual inspection and histopathology is necessary for definitive diagnosis. In some circumstances, imaging modalities may more accurately detect endometriosis, as endometriosis can exist deep to the peritoneum [43] or under dense adhesions [44] and may not be visualized adequately at laparoscopy [45,46].

TABLE 19.1 Staging of endometriosis, rASRM classification.

Location of endometriosis	Extent	Depth		
		< 1 cm	1–3 cm	> 3 cm
Peritoneum	Superficial	1	2	4
	Deep	2	4	6
Ovary	R superficial	1	2	4
	Deep	4	16	20
	L superficial	1	2	4
	Deep	4	16	20
Posterior Cul de sac obliteration		**Partial**	**Complete**	
		4	40	
Adhesions		**< 1/3 Enclosure**	**1/3–2/3 Enclosure**	**> 2/3 Enclosure**
Ovary	R Filmy	1	2	4
	Dense	4	8	16
	L Filmy	1	2	4
	Dense	4	8	16
Tube	R Filmy	1	2	4
	Dense	4[a]	8[a]	16
	L Filmy	1	2	4
	Dense	4[a]	8[a]	16

[a]*If the fimbriated end of the fallopian tube is completely enclosed, change the point assignment to 16.*
American Society for Reproductive Medicine 1997 [33].

There are disadvantages of laparoscopic surgery, and these include (but are not limited to) high cost, need for general anesthesia, and potential for adhesion formation postprocedure. A 2% risk of injury to pelvic organs, a 0.001% risk of damaging a major blood vessel, and a mortality rate of 0.0001% have been associated with laparoscopy [47]. Major complications of laparoscopy are rare; however, it is difficult to determine the exact incidence of complications, and delayed recognition adds to surgical morbidity and mortality. One-third of women will receive a diagnosis of endometriosis after a laparoscopic procedure; many disease-free women are thus unnecessarily exposed to surgical risk [48]. The validity of laparoscopy is highly dependent on surgical skill. A sole systematic review comparing the diagnostic accuracy of laparoscopic visualization with histological confirmation estimated sensitivity of 0.94 and specificity of 0.79 [49]. Indeed, a recent meta-analysis has demonstrated that conventional white-light laparoscopy may not be the best laparoscopic modality in the detection of endometriosis, yet it is the standard laparoscopic approach [45].

Incorporating histological verification in the diagnosis of endometriosis has been suggested to improve diagnostic accuracy [50–52]. The clinical significance of histological

verification is debatable, and as long as trained and experienced surgeons perform an appropriate pelvic cavity inspection; a generally reliable diagnosis can be based on visual findings [53]. Histological verification also is reliant on the surgeon obtaining an appropriate biopsy. If no biopsy is taken, histology cannot be used as the true confirmation. Unfortunately, even histology is exposed to the operator-dependent limitation: pathologists can often miss small lesions in mild disease, as excised potential endometriotic tissues are rarely serially sectioned in clinical practice [54]. The accuracy of histological reporting is thus often influenced by sampling inconsistencies.

Imaging modalities for the noninvasive diagnosis of endometriosis

The most widely reported diagnostic modalities for endometriosis include ultrasound (TV-US, TA-US, TR-US) and MRI. One of the earliest systematic reviews summarizing the diagnostic performance of ultrasound for endometriosis-associated ovarian masses (endometriomas) concluded that TV-US has clinical utility in differentiating endometriomas from other types of ovarian cysts [55]. Since then, many ultrasound and MRI studies have resulted in various adaptations of these foundational techniques [23,32,46,56–64]. Other techniques including computed tomography (CT)-based imaging [65] and barium enema [66] have been suggested as diagnostic tests for endometriosis. The diagnostic ability of imaging tests to detect endometriosis has also been positively affected by improvements in imaging technology over time. Reevaluation of diagnostic test accuracy since the 2016 Cochrane systematic reviews for a variety of imaging modalities is currently being undertaken [7,10,15].

The most recent Cochrane systematic review examined data from 4807 reproductive-aged women with symptoms of endometriosis, who undertook a noninvasive imaging test followed by diagnostic surgery for endometriosis, in 49 articles published from 1993 through 2015. This is the first diagnostic test review to use Cochrane methods and the most comprehensive review to date [7].

For overall pelvic endometriosis, no imaging method met the criteria for a SpPin or SnNout triage test. This is because superficial endometriosis remains the most difficult subtype of endometriosis to diagnose using imaging and yet, it is the most common subtype. For endometriomas, TV-US met the criteria for a SpPin triage test and approached the criteria for a replacement test and a SnNout triage test, whereas MRI met the criteria for a replacement and SnNout triage test and approached the criteria for a SpPin test. In practical terms, this means that an endometrioma identified on a TV-US can be reliably labeled as such, but the lack of an endometrioma does not necessarily mean there is not one present. Conversely, MRI is better at identifying endometriomas but has a greater chance of mislabeling a structure as an endometrioma when it is not. In routine clinical practice, for cost reasons, TV-US usually precedes MRI as an imaging investigative tool, but it is not usually for the purpose of identifying or clarifying ovarian endometriomas as they are diagnosed reliably by both imaging modalities.

Certainly, MRI is more known as a tool to identify deep endometriosis. For posterior compartment disease, MRI approached the criteria for a replacement test and a SnNout triage

test, and TV-US approached the criteria for a SpPin triage test. In short, this means that neither TV-US nor MRI on their own, have a high enough accuracy to replace, rule in, or rule out overall deep endometriosis when compared to surgery.

For the most frequently evaluated anatomical site of deep endometriosis, rectosigmoid endometriosis, TV-US, TR-US, and MRI reached the criteria for a SpPin triage test and approached the criteria for a SnNout triage test. This has been verified in an updated meta-analysis, where TR-US had the highest pooled sensitivity and specificity, followed by TV-US and finally MRI [67]. For other anatomical locations, TV-US met the criteria for a SpPin triage test for mapping deep endometriosis to uterosacral ligaments, rectovaginal septum, vaginal wall, and identification of POD obliteration, and MRI could qualify as a SpPin triage test only for POD obliteration and vaginal wall endometriosis. Data on TR-US for the other sites was scant, so it could not be adequately assessed in a meta-analysis. However, it would be unusual for a patient to undergo a TR-US when TV-US is possible as TR-US is usually reserved for virginal patients when MRI is less accessible. Expertise in performing TR-US may be less available, which may influence a clinical decision to use TR-US or MRI in these circumstances.

Modified ultrasound methods (TV-US with bowel preparation or rectal water contrast TV-US) and specific MRI modalities (3.0 TMRI and MRI jelly method with introduction of ultrasonographic gel into both the rectum and the vagina) showed the highest diagnostic accuracy for evaluated posterior compartment locations of endometriosis, however, data were insufficient for formal comparative analyses with TV-US and MRI methods. These modified methods generally less available and require increase logistical coordination and expertise but are valuable to advance the technology and techniques in non-invasive imaging of endometriosis.

In the Cochrane review, there were too few studies diagnosing anterior deep endometriosis for a prudent evaluation. However, Gerges et al. recently published a meta-analysis on the imaging diagnosis of bladder endometriosis, which identified pooled sensitivities of 55% and 53% for TV-US and MRI, respectively, and pooled specificities of 99% and 99% for TV-US and MRI, respectively [68]. This translates to both imaging modalities meeting criteria for a SpPin triage test but not a SnNout triage test or replacement test.

Since the publication of the 2016 Cochrane review [7], several authors have attempted to improve the non-invasive imaging diagnosis of superficial endometriosis. Some have attempted to use soft markers (ovarian immobility [69], uterosacral ligament thickening) [70], though this has not been reliable. Using a modified TV-US protocol, Leonardi et al. have proposed a method to directly visualize superficial lesions called saline-infusion sonoPODgraphy (SPG) [25]. Subsequently, in a pilot prospective diagnostic test accuracy study, they demonstrated SPG has a sensitivity of 78% and specificity of 100% when other forms of endometriosis (deep and ovarian) are excluded [24]. It is still necessary for these optimistic findings to be studied in a larger population and to be externally validated including in unique populations such as those with infertility without pain.

While there may not yet be a noninvasive imaging *replacement test* for endometriosis, there is hope that ongoing research in this realm will achieve this outcome. More realistic is that a combined imaging and biomarker test will produce the most accurate noninvasive diagnosis of endometriosis.

Blood biomarkers for the noninvasive diagnosis of endometriosis

Endometriotic lesions and peritoneal fluid have been characterized by specific cellular and molecular processes identified in human and animal models [71–73]. If a blood test, utilizing markers that reflect this pathophysiology, could impart an accurate diagnosis of endometriosis, it would have several potential advantages when compared to surgery including being minimally invasive, readily available, acceptable to women, cost-effective and providing a rapid result. The reliability of laboratory techniques and quality control protocols, however, influence the effectiveness of blood testing. Furthermore, blood biomarker levels can vary during the menstrual cycle, or if reproductive steroids like the combined oral contraceptive pill are used, requiring careful evaluation at different stages in the cycle in diagnostic blood test development.

Over 100 putative biomarkers were identified in a large systematic review of all proposed biomarkers for endometriosis in serum, plasma, and urine. However, the authors were unable to identify any biomarker (single or in a panel) ready for use in clinical practice [28]. In the most recent Cochrane review addressing this topic, diagnostic performance was evaluated for 47 of 122 blood biomarkers [8].

Blood biomarkers have been evaluated as a single test or in combination, in numerous trials. The categories of studied blood markers include: angiogenic and growth factors; markers of apoptosis; cell adhesion molecules and other matrix related proteins; cytoskeleton molecules; DNA-repair/telomere maintenance molecules; hormonal markers; high-throughput molecular markers; hormonal markers; immune system and inflammatory markers; nerve growth markers; oxidative stress markers; posttranscriptional regulators of gene expression (circulating nuclear DNAs, microRNAs); tumor markers; and other peptides/proteins which have been shown to influence key events implicated in endometriosis [8]. A limited number of small studies have evaluated these blood-based tests using varying methods, laboratory techniques, and types of assays.

Cancer antigen-125 (CA-125), a glycoprotein expressed on coelomic epithelial tissues including the peritoneum is the most extensively studied blood maker for endometriosis. A limitation of studies that assessed the utility of CA-125 as a diagnostic test is they analyzed multiple cut-off values within the following groups: $>$ 10.0–14.7 U/mL, $>$16.0–17.6 U/mL, $>$ 20.0 U/mL, $>$ 25.0–26.0 U/mL, $>$ 30.0–33.0 U/mL, $>$ 35.0–36.0 U/mL, and $>$ 42.0–43.0 U/mL. Regardless of the cut-off, none of these tests were found to be sensitive or specific enough to be considered as a replacement or triage test [8]. This is likely due to the indirect nature of the comparisons and heterogeneous study groups from different populations. The summary estimates of the mean sensitivity and the mean specificity of CA-125 did not all show the expected pattern (higher sensitivity and lower specificity with lower thresholds). The cut-off $>$ 16.0–17.6 U/mL performed best of all the CA-125 thresholds subjected to meta-analysis, but it only approached the criteria for a SpPin triage test and showed substantial heterogeneity.

Hirsh et al. found that when looking at all comers with endometriosis, CA-125 may approach a SpPin triage test with a pooled specificity of 93% (95% CI 89%–95%) and sensitivity 52% (95% CI 38%–66%) in a systematic review [74]. However, CA 125 was significantly less useful at detecting minimal to mild endometriosis than moderate or severe endometriosis

($P = .001$). Considering noninvasive imaging has higher accuracy in detecting ovarian and deep endometriosis (usually equivalent to moderate to severe endometriosis), CA-125 does not fill the gap of identifying harder to visualize superficial endometriosis. As further studies evaluating CA-125 have been published and diagnostic test review methodologies continue to improve, an updated review of CA-125 is warranted.

Meta-analysis was feasible for only three other biomarkers: CA-19.9, IL-6, and antiendometrial antibodies. For the detection of endometriosis, the sensitivity of CA-19.9 with a cut-off value of >37 U/mL was too low to meet the criteria for a replacement or triage test and other thresholds did not show promising results. Similarly, antiendometrial antibodies and IL-6 with a cut-off threshold of >1.90–2.00 pg/mL displayed unsatisfactory diagnostic estimates to qualify for either a replacement or triage test. Too few studies existed to perform a meaningful evaluation for other cut-off values of IL-6. IL-6 with a high cut-off of >12.20 pg/mL, did have a sufficiently high sensitivity and specificity to satisfy the criteria for a replacement test, in only one study and thus requires further validation. The findings of the meta-analyses from this review should be interpreted with caution. The results do not seem to be reliable enough to inform clinical practice, considering both the level of heterogeneity and the high/unclear risk of bias of the included studies.

Several tests were assessed and found to have little value in identifying endometriosis. Further research into these biomarkers is unlikely to yield a diagnostic tool. As a guide to researchers, the remaining biomarkers were classified as follows:

- Tests *needing to be validated for their diagnostic potential* (Table 19.2), including:
  - Tests with adequate diagnostic performance, but insufficient data to confidently comment on their diagnostic role and
  - Tests with diagnostic estimates that approached the criteria for replacement or triage tests in a small number of studies
- Tests *of limited diagnostic value* (i.e., studies with low diagnostic estimates).
- Tests *that appear to have limited diagnostic value, but there is insufficient data to confidently comment on their diagnostic role*.

Several groups have identified endometriosis-associated microRNAs in serum or plasma and speculated on their promising diagnostic potential for endometriosis [75–77]. High diagnostic accuracy has been demonstrated in some case-control cohorts [78,79] and in small retrospectively selected validation cohorts [80]. However, heterogeneity between studies in patient selection, microRNA discovery methods, normalization, and control comparators has meant that very few microRNAs have been identified in more than one study [81–83]. Two groups have validated their findings in prospective validation cohorts and did not detect microRNAs with enough sensitivity and specificity to accurately classify women with and without endometriosis at surgery [82,83]. As microRNA detection methods improve and become more consistent, it may be that these markers will be useful as standalone tests or, more likely, as add on markers in a combination test. Currently, blood-borne microRNAs do not display a high enough diagnostic accuracy to meet the criteria for a replacement or triage test for endometriosis [75].

Even though tens of thousands of papers have explored blood biomarkers, a blood-based diagnostic tool remains elusive [8]. This is likely to reflect pathological mechanisms such as inflammation and wound healing which, although upregulated in endometriosis, are not

TABLE 19.2 Blood biomarkers to be validated for their diagnostic potential in endometriosis.

Blood biomarkers[a]	Replacement test	SnOUT triage test	SpIN triage test
1. Angiogenesis and growth markers			
VEGF >680 pg/mL	±	±	+
VEGF >236 pg/mL	±	±	
2. High-throughput markers			
Metabolome by ESI-MS/MS (SMOH C16:1 + PCaa C36:2/PCae C34:2)		±	
Proteome by SELDI-TOF-MS (6 peaks with molecular weights of 1.63, 3.05, 3.53, 3.77, 5.05 and 5.07 Da)			+
3. Immune system and inflammatory markers			
IL-6 > 12.2 pg/mL	+	+	
4. Oxidative stress markers			
PON-1 < 141.5 U/L	+	+	
Carbonyls < 14.9 μM		±	
5. Posttranscriptional regulators of gene expression (microRNAs)			
miR-9*			+
miR-141*			+
miR-145*			+
miR-20a < 0.69			±
miR-22 < 0.56	±	±	
miR-532-3p			±
6. Tumor markers			
CA-125 (cut-o% value > 43 U/mL)	+	+	
7. Combined blood tests			
IL-6 > 12.2 pg/mL + TNF-M > 12.45 pg/mL			+
IL-6 > 12.2 pg/mL + CRP > 438 μg/mL			+
TNF-M > 12.45 pg/mL + CRP > 438 μg/mL			+
miR-199a + miR-542-3p	+	+	
CA-125 + STX-5 + LN-1	±	+	
IL-6 > 12.2 pg/mL + TNF-M > 12.45 pg/mL + CRP > 438 μg/mL			+
miR-199a + miR-122 + miR-145* + miR-542-3p	±	±	
CA-125 > 17.6 IU/mL + VEGF >236 pg/mL _			±

TABLE 19.2 Blood biomarkers to be validated for their diagnostic potential in endometriosis.—cont'd

Blood biomarkers[a]	Replacement test	SnOUT triage test	SpIN triage test
CA-125 + CA-19-9 + survivin _			±
CA-125 > 50 IU/mL +/or CCR1 > 1.16 +/or MCP-1 > 140 pg/mL	±	±	
CA-125 > 20 IU/mL + MCP-1 > 152.744 pg/mL + leptin > 3.14 ng/mL			±
CA-125 + IL-8 + TNF-M		±	
CA-125 + CA-19.9 + IL-6 + IL-8 + TNF-M + hs-CRP (in menstrual phase of the cycle)	±	±	
8. Tests that specifically differentiate endometrioma from other benign ovarian cysts in women of reproductive age			
Urocortin > 29 pg/mL	+	+	
Urocortin > 33 pg/mL			±
Follistatin > 1433 pg/mL	±	±	±
CA-125 > 30 U/mL and > 36 U/mL _			+
CA-125 O 25 U/mL + CA-19.9 O 22 U/mL			±

[a]*This groupincluded: tests with an adequate diagnostic performance, but insufficient data to confidently comment on their diagnostic role (less than three studies with the diagnostic estimates meeting the criteria for either a replacement or triage test); and tests where the diagnostic estimates were approaching the criteria for replacement or triage tests in a small number of studies, and it is possible that they would reach this criteria if further studies were performed (less than three studies with the diagnostic estimates within 5% of the criteria for either replacement or triage tests).*

Notes: +, meets the criteria, Replacement test: sensitivity ≥ 94 and specificity ≥ 79, SnOUT triage test: sensitivity ≥ 95 and specificity ≥ 50, SpIN triage test: sensitivity ≥ 50 and specificity ≥ 95, ± approaches the criteria (within 5% of the pre-defined criteria).

specific to this disease. Biomarkers representing pathological events are elevated in populations of women with endometriosis when compared to controls, but upregulation of these markers in an individual cannot determine causation, as blood levels overlap considerably between the groups. It may be that future studies on blood biomarkers which focus on specific phases of the menstrual cycle, specific types of endometriosis, different cut-off values, or different laboratory methods will reveal a unique diagnostic biomarker for endometriosis, but more likely blood-based "add-ons" in combination with more specific diagnostic tools will be developed in this field of research.

Endometrial biomarkers for the noninvasive diagnosis of endometriosis

An endometrial biopsy can be obtained with a plastic pipette device in an outpatient setting, without general or local anesthetic. The potential advantages of using endometrial tissue samples for the diagnosis of endometriosis include their non- or minimally invasive nature, lower cost, and increased availability when compared to surgery. It is likely most patients would find these methods acceptable if they were able to obtain an accurate

diagnosis of endometriosis. However, testing is likely dependent on the skills of the gynecologist performing the biopsy, the timing of the biopsy in the menstrual cycle, the time taken to process the sample, the reliability of laboratory techniques, and the quality control protocols in place.

Researchers have identified cellular and molecular processes that distinguish endometriosis-associated eutopic endometrium in human and animal models [71–73], suggesting that endometriosis may induce characteristic changes in eutopic endometrial tissues [84–86] or that the eutopic endometrium of women who develop endometriosis is different from the endometrium of women who are endometriosis-free [87]. A growing body of literature has identified aberrant gene expression in the endometrium of women with endometriosis which may have diagnostic potential [88]. Proteolytic enzymes and immune cell populations have displayed a differential expression in eutopic endometrium of women with and without endometriosis [89–91] Additional studies have evaluated and eliminated glycodelin A, CYR61, annexin 1, osteopontin, and aromatase P450 as potential endometrial biomarkers [73,92–95].

The Gupta et al. Cochrane review presents data from 2729 reproductive-aged women who had presented with symptoms of endometriosis and underwent eutopic endometrial biopsies during or prior to diagnostic [9]. The diagnostic performance for 22 endometrial biomarkers was evaluated and another 77 markers were explored but excluded as potential biomarkers. Most of the biomarkers were assessed in a limited number of studies, and only two biomarkers (protein gene product 9.5(PGP 9.5) and aromatase cytochrome P450 (CYP19)) had sufficient data for a meta-analysis.

PGP 9.5, the marker of small unmyelinated sensory C nerve fibers, was the most studied biomarker [96–100]. The meta-analysis revealed that evaluation of nerve fiber density with PGP 9.5 staining could qualify as a replacement test (mean sensitivity of 0.96, 95% CI 0.91 to 1.00; specificity 0.86, 95% CI 0.70 to 1.00) in studies performed by an Australian group that emphasized meticulous sampling and processing techniques [97]. Most independent research groups were not able to confirm these findings [101] and current evidence suggests that the presence of PGP 9.5 nerve fibers may be more closely associated with pain from any gynecological cause (including adenomyosis and fibroids), than with endometriosis [98]. Commercialization of this biomarker has not occurred, and further development and validation would be required for it to become a clinically useful tool.

Estimates of CYP19, the key enzyme in the conversion of C19 steroids into estrogen, did not meet the diagnostic criteria to qualify for either a replacement or triage test (mean sensitivity 0.77, 95% CI 0.70 to 0.85; specificity 0.74, 95% CI0.65 to 0.84). TIMP-1 (tissue inhibitor of metalloproteinases-1) protein ormRNA [102–106] and vascular endothelial growth factor (VEGF) protein or mRNA [106–108] also demonstrated limited diagnostic value with further research into the endometrial presence of these factors unlikely to produce diagnostic benefits. Other tests showed some diagnostic potential but insufficient evidence to comment on their reliability as diagnostic markers.

The findings of the meta-analyses presented by Gupta et al. need to be carefully interpreted and are currently being updated to incorporate new evidence from the literature [9]. Considering the level of heterogeneity and the high or unclear risk of bias of the included studies, the results do not seem to have sufficient reliability to direct clinical practice at present.

Urinary biomarkers for the noninvasive diagnosis of endometriosis

Urine is the most readily available bodily fluid and a urine endometriosis biomarker test would be acceptable and easily self-collected and the results rapid and much more cost-effective than surgery. However, reliable laboratory techniques and quality control protocols are required, and urinary biomarkers are susceptible to confounding from menstrual cycle variation and urinary tract pathologies. Markers of pathophysiological processes in endometriosis have been evaluated in urine, which is increasingly favored as a fluid for biological testing. A limited number of endometriosis urinary biomarkers have been evaluated to date and most were assessed in small individual studies.

Similar to the categories of markers in other body specimen sites, evaluated urinary biomarkers include:

1. Angiogenesis and growth factors;
2. Cell adhesion molecules and other matrix related proteins;
3. Cytokines;
4. Cytoskeleton molecules;
5. High-throughput molecular markers;
6. Oxidative stress markers; and
7. Other peptides/proteins shown to influence key events implicated in endometriosis.

A large systematic review using Cochrane methodologies on diagnostic test accuracy of urine tests for endometriosis showed that only a few urinary biomarkers have been assessed in small numbers of individual studies providing insufficient data to perform a meta-analysis. No urinary test met the criteria of either replacement or triage test for detecting endometriosis.

Wang et al. used a proteomic technique to discover protein and peptide biomarkers of endometriosis [109]. Three algorithms were developed for the selection of peptide peak clusters; the algorithm called genetic algorithm (GA) was a combination of five urinary peptides of 1433.9 Da, 1599.4 Da, 2085.6 Da, 6798.0 Da, and 3217.2Da. The GA demonstrated the highest diagnostic estimates for detecting endometriosis, which approached but did not meet the criteria for the replacement of both the SnNout and SpPin triage tests [109]. The algorithm was validated in an independent test group but, as this test was only reported in one study, it is hard to draw meaningful conclusions regarding its value in clinical practice. Certain urinary peptides identified through the high-throughput MALDI-TOF-MS method showed potential in detecting endometriosis. However, urinary proteome studies showed considerable heterogeneity with respect to the population studied, the way the samples were processed, and the data analysis. Establishing standardized analytical processes, consistent sets of markers, and defined cut-off thresholds would improve the assessment of urinary peptides as a diagnostic tool for endometriosis and further large-scale studies are required.

Enolase 1 (NNE), vitamin-D-binding protein (VDBP), cytokeratin 19 (CK 19), tumor necrosis factor alpha (TNF-a), and VEGF testing did not achieve sufficient performance to meet the criteria for a replacement test or a triage test [10]. All of these biomarkers were assessed in small individual studies and could not be statistically evaluated in a meaningful way. Given the paucity of data, further large studies are still needed to support this statement.

Combination of the noninvasive tests for the diagnosis of endometriosis

In general, it is thought that a combination of noninvasive tests will achieve a superior diagnostic performance than any individual noninvasive test. The potential advantages of combined tests reflect the same advantages outlined above in each individual section. The concept of combined noninvasive tests must be looked at adjacent to both clinical and surgical diagnostic approaches.

Nisenblat et al. performed another systematic review and meta-analysis using Cochrane methodologies that aimed to assess the diagnostic performance of combination noninvasive tests [15]. Part of the series of reviews on noninvasive diagnostic tests for endometriosis, this review assesses combinations of tests, including blood, urine, and endometrial biomarkers and imaging modalities that have been proposed as noninvasive tests for the diagnosis of endometriosis (Table 19.3) [15].

Clinical parameters (history and examination) have low reliability in the diagnosis of endometriosis; however, they may improve the diagnostic performance of other noninvasive tests when incorporated in a diagnostic model. So far, combinations of noninvasive tests have only been assessed in a limited number of small studies, which vary in the type of methodology and tests used and type of endometriosis evaluated. Fifteen combinations of

TABLE 19.3 Combination of the noninvasive tests for endometriosis evaluated in this review.

N	Test
1	1 IL-6 (>15.4 pg/mL) [serum] + PGP 9.5 [endometrium]
2	CA-125 [serum] (>35 U/mL) + P450 aromatase [endometrium]
3	VDBP-Cr [urine] x CA-125 [serum] (>2755)
4	NNE Cr [urine] + CA-125 [serum] (>27.23)
5	History (dysmenorrhoea, dyspareunia) + PV examination + TV-US (fixed ovary)
6	History (length of menses) + CA-125 [serum] (>35 U/mL) + leukocytes [endometrium]
7	History (parity, past IUD, past endometriosis, alcohol intake, dyspareunia) + CA-125 [serum]
8	PV examination (menstrual nodularities) + CA125 (>35 U/mL) [serum]
9	PV examination (menstrual nodularities) OR CA125 (>35 U/mL) [serum]
10	TV-US + CA-125 [serum] (25 U/mL) + CA-19.9 [serum] (12 U/mL)
11	TV-US + (CA-125 [serum] (25 U/mL) OR CA-19.9 [serum] (12 U/mL))
12	TV-US + CA-19.9 [serum] (12 U/mL))
13	TV-US OR CA-19.9 [serum] (12 U/mL))
14	TV-US + CA-125 [serum] (20 U/mL; 25 U/mL; 35 U/mL)
15	PV examination + TV-US

CA-125, cancer antigen; *IL*, interleukin; *IUD*, intrauterine device; *NNE*, nonneuronal enolase; *PV*, per vaginam; *TV-US*, transvaginal ultrasound; *VDBP*, vitamin-D-binding protein.

noninvasive methods for the diagnosis of endometriosis were evaluated in 11 studies comprising 1339 participants (Table 19.3). The composite tests were assessed in small individual studies, providing insufficient data to perform a meta-analysis.

None of the included studies were of high methodological quality. There were too few studies to perform a meaningful evaluation for any of the combination tests. Although some tests were sensitive and specific enough to qualify as a replacement or triage test for detecting endometriosis, each was explored in only one study and warrant further validation.

Combinations of biomarkers had higher diagnostic estimates than those reported for each component of the combined test in all studies. TV-US was a component in seven of 10 combinations of tests that reached replacement or triage status. This confirms the addition of CA-125 made only a small contribution to the diagnostic performance of the test combinations. We also observed that combinations of biomarkers with TV-US for detecting ovarian endometrioma had lower sensitivity and largely comparable specificity than that presented for TV-US alone in the review on imaging tests [7]. Considering the results of the meta-analysis from the imaging tests review, the addition of pelvic examination to TV-US improved diagnostic performance to detect vaginal and rectal endometriosis but did not seem to be superior to TV-US alone for detecting POD obliteration and RVS endometriosis.

The combinations of testing methods that met the criteria for a replacement test (from Table 19.3) included:

1. IL-6 >15.4 pg/mL [serum] + PGP 9.5 [endometrium]—for overall pelvic endometriosis
2. Pelvic examination + TV-US—for rectal DE

The combinations of several testing methods that met the criteria for a SpPin triage test (from Table 19.3) included:

1. VDBP-Cr [urine] x CA-125 [serum] >2755 - for overall pelvic endometriosis
2. History (length of menses) + CA-125 [serum] >35 U/mL + leukocytes [endometrium] - for overall pelvic endometriosis
3. TV-US + CA-125 [serum] $\geq$ 25 U/mL OR CA-19.9 [serum] $\geq$ 12 U/mL) - for ovarian endometrioma
4. TV-US + CA-19.9 [serum] $\geq$ 12 U/mL - for ovarian endometrioma
5. TV-US + CA-125 [serum] $\geq$ 20 U/mL - for ovarian endometrioma
6. TV-US + CA-125 [serum] $\geq$ 25 U/mL - for ovarian endometrioma
7. TV-US + CA-125 [serum] $\geq$ 35 U/mL - for ovarian endometrioma
8. Pelvic examination + TV-US - for the following anatomic locations: (1) POD obliteration; (2) vaginal wall; and (3) RVS.

The findings of this "combination of the tests" review need to be interpreted in a tempered way. Considering both the level of heterogeneity and the high/unclear risk of bias of included studies, the results are not sufficiently reliable to inform clinical practice. Furthermore, TV-US conferred the largest influence in assigning diagnostic potential in combination tests and the diagnostic benefit of additional tests needs to be well evaluated to ensure there is added value and not just added cost.

Conclusion

Accurate noninvasive diagnostics would represent a major breakthrough in care for the 10% of reproductive-aged women across the world who are estimated to have endometriosis. Surgery is a significant barrier to diagnosis, as the resources required for all women with endometriosis to have surgery is too high for health care systems to bear. Instead, diagnosis is delayed, symptoms are normalized, and women have the anxiety and uncertainty as to the cause of their symptoms.

Many thousands of papers are dedicated to identifying an accurate noninvasive diagnostic, and the clinical utility of noninvasive imaging diagnostic techniques is being increasingly recognized. With improvements in radiological equipment and sonographic techniques, TV-US and MRI imaging of lesions produce promising results approaching the criteria for a replacement test and meeting the criteria for triage tests in ovarian and deep endometriosis.

Surgery and imaging tests visualize lesions either directly or indirectly, which is the most differentiating biological feature when women with and without endometriosis are compared. Although immune, inflammatory, metabolic, and genomic markers can be dysregulated when large populations are compared, individual measures cannot distinguish women with and without endometriosis accurately, as there is significant overlap between groups. The biomarkers differentially expressed in endometriosis, also contribute to other pathological states and thus are not specific enough to characterize the endometriotic disease process in the way the presence of a visualized endometriotic lesion can. For these reasons, tissue and blood markers have not been identified with enough diagnostic accuracy for use in clinical practice.

Combinations of imaging, biomarkers, symptoms, and clinical examination may contribute to best practice in diagnosing endometriosis in the future, and complex methodologies using machine learning and artificial intelligence may be required to implement these tools. Endometriosis is an underresearched and underresourced area of clinical need, which is pressing for better diagnostic options, and we are starting to deliver them.

References

[1] Kontoravdis A, Hassan E, Hassiakos D, Botsis D, Kontoravdis N, Creatsas G. Laparoscopic evaluation and management of chronic pelvic pain during adolescence. Clin Exp Obstet Gynecol 1999;26(2):76–7.
[2] Ballweg ML. Impact of endometriosis on women's health: comparative historical data show that the earlier the onset, the more severe the disease. Best Pract Res Clin Obstet Gynaecol 2004;18(2):201–18.
[3] Dunselman GA, Vermeulen N, Becker C, Calhaz-Jorge C, D'Hooghe T, De Bie B, Heikinheimo O, Horne AW, Kiesel L, Nap A, Prentice A, Saridogan E, Soriano D, Nelen W. ESHRE guideline: management of women with endometriosis. Hum Reprod 2014;29(3):400–12.
[4] Agarwal SK, Chapron C, Giudice LC, Laufer MR, Leyland N, Missmer SA, Singh SS, Taylor HS. Clinical diagnosis of endometriosis: a call to action. Am J Obstet Gynecol 2019;220(4):354. e1–e12.
[5] Meuleman C, Vandenabeele B, Fieuws S, Spiessens C, Timmerman D, D'Hooghe T. High prevalence of endometriosis in infertile women with normal ovulation and normospermic partners. Fertil Steril 2009;92(1):68–74.
[6] Taylor HS, Adamson GD, Diamond MP, Goldstein SR, Horne AW, Missmer SA, Snabes MC, Surrey E, Taylor RN. An evidence-based approach to assessing surgical versus clinical diagnosis of symptomatic endometriosis. Int J Gynaecol Obstet 2018;142(2):131–42.
[7] Nisenblat V, Bossuyt PM, Farquhar C, Johnson N, Hull ML. Imaging modalities for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev 2016;2:Cd009591.

[8] Nisenblat V, Bossuyt PM, Shaikh R, Farquhar C, Jordan V, Scheffers CS, Mol BW, Johnson N, Hull ML. Blood biomarkers for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev 2016;2016(5):Cd012179.
[9] Gupta D, Hull ML, Fraser I, Miller L, Bossuyt PM, Johnson N, Nisenblat V. Endometrial biomarkers for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev 2016;4(4):Cd012165.
[10] Liu E, Nisenblat V, Farquhar C, Fraser I, Bossuyt PM, Johnson N, Hull ML. Urinary biomarkers for the non-invasive diagnosis of endometriosis. Cochrane Database Syst Rev 2015;2015(12):Cd012019.
[11] Bedaiwy MA, Falcone T, Sharma RK, Goldberg JM, Attaran M, Nelson DR, Agarwal A. Prediction of endometriosis with serum and peritoneal fluid markers: a prospective controlled trial. Hum Reprod 2002;17(2):426–31.
[12] Society of Obstetricians Gynaecologists of Canada. Endometriosis: diagnosis and management. SOGC clinical practice guideline no. 244. J Obstet Gynaecol Can 2010;32.
[13] The American College of Obstetricians and Gynecologists. Practice bulletin No. 114: management of endometriosis. Obstet Gynecol 2010;116(1):223–36.
[14] Coutinho J A, Bittencourt LK, Pires C, Junqueira F, de Oliveira Lima C, Coutinho E, Domingues M, Domingues RC, Marchiori E. MR imaging in deep pelvic endometriosis: a pictorial essay. Radiographics.31(2) 549–567.
[15] Nisenblat V, Prentice L, Bossuyt PM, Farquhar C, Hull ML, Johnson N. Combination of the non-invasive tests for the diagnosis of endometriosis. Cochrane Database Syst Rev 2016;7:Cd012281.
[16] Bossuyt P, Reitsma J, Bruns D, Gatsonis C, Glasziou P, Irwig L, Lijmer J, Moher D, Rennie D, de Vet H, Kressel H, Rifai N, Golub R, Altman D, Hooft L, Korevaar D, Cohen J, for the STARD Group. Stard 2015: an updated list of essential items for reporting diagnostic accuracy studies. Clin Chem 2015;61(12):1446–52.
[17] Ballard KD, Seaman HE, de Vries CS, Wright JT. Can symptomatology help in the diagnosis of endometriosis? Findings from a national case-control study–Part 1. BJOG 2008;115(11):1382–91.
[18] Fauconnier A, Chapron C. Endometriosis and pelvic pain: epidemiological evidence of the relationship and implications. Hum Reprod Update 2005;11(6):595–606.
[19] Spaczynski RZ, Duleba AJ. Diagnosis of endometriosis. Semin Reprod Med 2003;21(2):193–208.
[20] Ling FW. Randomized controlled trial of depot leuprolide in patients with chronic pelvic pain and clinically suspected endometriosis. Pelvic Pain Study Group. Obstet Gynecol 1999;93(1):51–8.
[21] Eskenazi B, Warner M, Bonsignore L, Olive D, Samuels S, Vercellini P. Validation study of nonsurgical diagnosis of endometriosis. Fertil Steril 2001;76(5):929–35.
[22] Leonardi M, Martin E, Reid S, Blanchette G, Condous G. Deep endometriosis transvaginal ultrasound in the workup of patients with signs and symptoms of endometriosis: a cost analysis. BJOG 2019;126(12):1499–506.
[23] Guerriero S, Condous G, van den Bosch T, Valentin L, Leone FP, Van Schoubroeck D, Exacoustos C, Installe AJ, Martins WP, Abrao MS, Hudelist G, Bazot M, Alcazar JL, Goncalves MO, Pascual MA, Ajossa S, Savelli L, Dunham R, Reid S, Menakaya U, Bourne T, Ferrero S, Leon M, Bignardi T, Holland T, Jurkovic D, Benacerraf B, Osuga Y, Somigliana E, Timmerman D. Systematic approach to sonographic evaluation of the pelvis in women with suspected endometriosis, including terms, definitions and measurements: a consensus opinion from the International Deep Endometriosis Analysis (IDEA) group. Ultrasound Obstet Gynecol 2016;48(3):318–32.
[24] Leonardi M, Robledo KP, Espada M, Vanza K, Condous G. SonoPODography: a new diagnostic technique for visualizing superficial endometriosis. Eur J Obstet Gynecol Reprod Biol 2020;254:124–31.
[25] Leonardi M, Espada M, Choi S, Chou D, Chang T, Smith C, Rowan K, Condous G. Transvaginal ultrasound can accurately predict the American society of reproductive medicine stage of endometriosis assigned at laparoscopy. J Minim Invasive Gynecol 2020;27(7):1581–7.
[26] Indrielle-Kelly T, Frühauf F, Fanta M, Burgetova A, Lavu D, Dundr P, Cibula D, Fischerova D. Diagnostic accuracy of ultrasound and MRI in the mapping of deep pelvic endometriosis using the international deep endometriosis analysis (IDEA) consensus. BioMed Res Int 2020;2020:3583989.
[27] Thalluri AL, Knox S, Nguyen T. MRI findings in deep infiltrating endometriosis: a pictorial essay. J Med Imaging Radiat Oncol 2017;61(6):767–73.
[28] May KE, Conduit-Hulbert SA, Villar J, Kirtley S, Kennedy SH, Becker CM. Peripheral biomarkers of endometriosis: a systematic review. Hum Reprod Update 2010;16(6):651–74.
[29] May KE, Villar J, Kirtley S, Kennedy SH, Becker CM. Endometrial alterations in endometriosis: a systematic review of putative biomarkers. Hum Reprod Update 2011;17(5):637–53.
[30] Fassbender A, Vodolazkaia A, Saunders P, Lebovic D, Waelkens E, De Moor B, D'Hooghe T. Biomarkers of endometriosis. Fertil Steril 2013;99(4):1135–45.

[31] Yeung Jr PP, Shwayder J, Pasic RP. Laparoscopic management of endometriosis: comprehensive review of best evidence. J Minim Invasive Gynecol 2009;16(3):269–81.
[32] Medeiros LR, Rosa MI, Silva BR, Reis ME, Simon CS, Dondossola ER, da Cunha Filho JS. Accuracy of magnetic resonance in deeply infiltrating endometriosis: a systematic review and meta-analysis. Arch Gynecol Obstet 2015;291(3):611–21.
[33] American Society for Reproductive Medicine. Revised American society for reproductive medicine classification of endometriosis: 1996. Fertil Steril 1997;67:817–21.
[34] Endometriosis Fertility Index (EFI): the new validated endometriosis staging system. Art and science of endometriosis. In: Adamson G, editor. Proceedings of the tenth world congress on endometriosis 2008; March 2008. p. 11–4 [Melbourne (Vic)].
[35] Batt RE, Mitwally MF. Endometriosis from thelarche to midteens: pathogenesis and prognosis, prevention and pedagogy. J Pediatr Adolesc Gynecol 2003;16(6):337–47.
[36] Chapron C, Fauconnier A, Vieira M, Barakat H, Dousset B, Pansini V, Vacher-Lavenu MC, Dubuisson JB. Anatomical distribution of deeply infiltrating endometriosis: surgical implications and proposition for a classification. Hum Reprod 2003;18(1):157–61.
[37] Martin D. Applying STARD criteria to the laparoscopic identification of endometriosis. Fertil Steril 2006;86(270).
[38] Chapron C, Fauconnier A, Dubuisson JB, Barakat H, Vieira M, Bréart G. Deep infiltrating endometriosis: relation between severity of dysmenorrhea and extent of disease. Hum Reprod 2003;18(4):760–6.
[39] Guzick DS, Silliman NP, Adamson GD, Buttram Jr VC, Canis M, Malinak LR, Schenken RS. Prediction of pregnancy in infertile women based on the American Society for Reproductive Medicine's revised classification of endometriosis. Fertil Steril 1997;67(5):822–9.
[40] Vercellini P, Trespidi L, De Giorgi O, Cortesi I, Parazzini F, Crosignani PG. Endometriosis and pelvic pain: relation to disease stage and localization. Fertil Steril 1996;65(2):299–304.
[41] Johnson NP, Hummelshoj L, Adamson GD, Keckstein J, Taylor HS, Abrao MS, Bush D, Kiesel L, Tamimi R, Sharpe-Timms KL, Rombauts L, Giudice LC. World Endometriosis Society consensus on the classification of endometriosis. Hum Reprod 2017;32(2):315–24.
[42] Kennedy S, Bergqvist A, Chapron C, D'Hooghe T, Dunselman G, Greb R, Hummelshoj L, Prentice A, Saridogan E. ESHRE guideline for the diagnosis and treatment of endometriosis. Hum Reprod 2005;20(10):2698–704.
[43] Dinh T, Leonardi M, Espada M, Vanza K, Condous G. The use of ultrasound in detecting endometriosis: endometriotic nodule detected on ultrasound but not visualized on laparoscopy. J Obstet Gynaecol Can 2020;42(8):1016.
[44] Leonardi M, Singh SS, Murji A, Satkunaratnam A, Atri M, Reid S, Condous G. Deep endometriosis: a diagnostic dilemma with significant surgical consequences. J Obstet Gynaecol Can 2018;40(9):1198–203.
[45] Maheux-Lacroix S, Belanger M, Pinard L, Lemyre M, Laberge P, Boutin A. Diagnostic accuracy of intraoperative tools for detecting endometriosis: a systematic review and meta-analysis. J Minim Invasive Gynecol 2020;27(2):433–440.e1.
[46] Goncalves MO, Podgaec S, Dias Jr JA, Gonzalez M, Abrao MS. Transvaginal ultrasonography with bowel preparation is able to predict the number of lesions and rectosigmoid layers affected in cases of deep endometriosis, defining surgical strategy. Hum Reprod 2010;25(3):665–71.
[47] Chapron C, Cravello L, Chopin N, Kreiker G, Blanc B, Dubuisson JB. Complications during set-up procedures for laparoscopy in gynecology: open laparoscopy does not reduce the risk of major complications. Acta Obstet Gynecol Scand 2003;82(12):1125–9.
[48] Frishman GN, Salak JR. Conservative surgical management of endometriosis in women with pelvic pain. J Minim Invasive Gynecol 2006;13(6):546–58.
[49] Wykes CB, Clark TJ, Khan KS. Accuracy of laparoscopy in the diagnosis of endometriosis: a systematic quantitative review. BJOG 2004;111(11):1204–12.
[50] Almeida Filho DP, Oliveira LJ, Amaral VF. Accuracy of laparoscopy for assessing patients with endometriosis. Sao Paulo Med J 2008;126(6):305–8.
[51] Marchino GL, Gennarelli G, Enria R, Bongioanni F, Lipari G, Massobrio M. Diagnosis of pelvic endometriosis with use of macroscopic versus histologic findings. Fertil Steril 2005;84(1):12–5.
[52] Stegmann BJ, Sinaii N, Liu S, Segars J, Merino M, Nieman LK, Stratton P. Using location, color, size, and depth to characterize and identify endometriosis lesions in a cohort of 133 women. Fertil Steril 2008;89(6):1632–6.

[53] Redwine DB. 'Invisible' microscopic endometriosis: a review. Gynecol Obstet Invest 2003;55(2):63–7.
[54] Ross WT, Newell JM, Zaino R, Kunselman AR, Harkins GJ, Benton AS. Appendiceal endometriosis: is diagnosis dependent on pathology evaluation? A prospective cohort study. J Minim Invasive Gynecol 2020;27(7):1531–7.
[55] Moore J, Copley S, Morris J, Lindsell D, Golding S, Kennedy S. A systematic review of the accuracy of ultrasound in the diagnosis of endometriosis. Ultrasound Obstet Gynecol 2002;20(6):630–4.
[56] Kinkel K, Frei KA, Balleyguier C, Chapron C. Diagnosis of endometriosis with imaging: a review. Eur Radiol 2006;16(2):285–98.
[57] Saba L, Sulcis R, Melis GB, de Cecco CN, Laghi A, Piga M, Guerriero S. Endometriosis: the role of magnetic resonance imaging. Acta Radiol 2015;56(3):355–67.
[58] Hudelist G, English J, Thomas AE, Tinelli A, Singer CF, Keckstein J. Diagnostic accuracy of transvaginal ultrasound for non-invasive diagnosis of bowel endometriosis: systematic review and meta-analysis. Ultrasound Obstet Gynecol 2011;37(3):257–63.
[59] Valenzano Menada M, Remorgida V, Abbamonte LH, Nicoletti A, Ragni N, Ferrero S. Does transvaginal ultrasonography combined with water-contrast in the rectum aid in the diagnosis of rectovaginal endometriosis infiltrating the bowel? Hum Reprod 2008;23(5):1069–75.
[60] Dessole S, Farina M, Rubattu G, Cosmi E, Ambrosini G, Nardelli GB. Sonovaginography is a new technique for assessing rectovaginal endometriosis. Fertil Steril 2003;79(4):1023–7.
[61] Grasso RF, Di Giacomo V, Sedati P, Sizzi O, Florio G, Faiella E, Rossetti A, Del Vescovo R, Zobel BB. Diagnosis of deep infiltrating endometriosis: accuracy of magnetic resonance imaging and transvaginal 3D ultrasonography. Abdom Imag 2010;35(6):716–25.
[62] Hottat N, Larrousse C, Anaf V, Noël JC, Matos C, Absil J, Metens T. Endometriosis: contribution of 3.0-T pelvic MR imaging in preoperative assessment–initial results. Radiology 2009;253(1):126–34.
[63] Manganaro L, Vinci V, Bernardo S, Storelli P, Fuggetta E, Sollazzo P, Ticino A, Sergi M, Brunelli R, Benedetti Panici P, Porpora MG. The role of 3.0T MRI in the assessment of deep endometriosis located on the uterosacral ligaments. J Endometr 2013;5:10–6.
[64] Thomeer MG, Steensma AB, van Santbrink EJ, Willemssen FE, Wielopolski PA, Hunink MG, Spronk S, Laven JS, Krestin GP. Can magnetic resonance imaging at 3.0-Tesla reliably detect patients with endometriosis? Initial results. J Obstet Gynaecol Res 2014;40(4):1051–8.
[65] Biscaldi E, Ferrero S, Fulcheri E, Ragni N, Remorgida V, Rollandi GA. Multislice CT enteroclysis in the diagnosis of bowel endometriosis. Eur Radiol 2007;17(1):211–9.
[66] Ribeiro HS, Ribeiro PA, Rossini L, Rodrigues FC, Donadio N, Aoki T. Double-contrast barium enema and transrectal endoscopic ultrasonography in the diagnosis of intestinal deeply infiltrating endometriosis. J Minim Invasive Gynecol 2008;15(3):315–20.
[67] Gerges B, Li W, Leonardi M, Mol BW, Condous G. Optimal imaging modality for detection of rectosigmoid deep endometriosis: systematic review and meta-analysis. Ultrasound Obstet Gynecol 2021;58(2):190–200.
[68] Gerges B, Li W, Leonardi M, Mol BW, Condous G. Meta-analysis and systematic review to determine the optimal imaging modality for the detection of bladder deep endometriosis. Eur J Obstet Gynecol Reprod Biol 2021;261:124–33.
[69] Reid S, Leonardi M, Lu C, Condous G. The association between ultrasound-based 'soft markers' and endometriosis type/location: a prospective observational study. Eur J Obstet Gynecol Reprod Biol 2019;234:171–8.
[70] Robinson AJ, Rombauts L, Ades A, Leong K, Paul E, Piessens S. Poor sensitivity of transvaginal ultrasound markers in diagnosis of superficial endometriosis of the uterosacral ligaments. J Endometr Pelvic Pain Disord 2018;10(1):10–7.
[71] D'Hooghe TM, Bambra CS, Xiao L, Peixe K, Hill JA. Effect of menstruation and intrapelvic injection of endometrium on inflammatory parameters of peritoneal fluid in the baboon (*Papio anubis* and *Papio cynocephalus*). Am J Obstet Gynecol 2001;184(5):917–25.
[72] Hull ML, Escareno CR, Godsland JM, Doig JR, Johnson CM, Phillips SC, Smith SK, Tavaré S, Print CG, Charnock-Jones DS. Endometrial-peritoneal interactions during endometriotic lesion establishment. Am J Pathol 2008;173(3):700–15.
[73] Kao LC, Germeyer A, Tulac S, Lobo S, Yang JP, Taylor RN, Osteen K, Lessey BA, Giudice LC. Expression profiling of endometrium from women with endometriosis reveals candidate genes for disease-based implantation failure and infertility. Endocrinology 2003;144(7):2870–81.

[74] Hirsch M, Duffy J, Davis CJ, Nieves Plana M, Khan KS. Diagnostic accuracy of cancer antigen 125 for endometriosis: a systematic review and meta-analysis. BJOG 2016;123(11):1761–8.
[75] Leonova A, Turpin VE, Agarwal SK, Leonardi M, Foster WG. A critical appraisal of the circulating levels of differentially expressed microRNA in endometriosis. Biol Reprod 2021;(ioab134). https://doi.org/10.1093/biolre/ioab134. Online ahead of print.
[76] Bjorkman S, Taylor HS. MicroRNAs in endometriosis: biological function and emerging biomarker candidates. Biol Reprod 2019;100(5):1135–46.
[77] Panir K, Schjenken JE, Robertson SA, Hull ML. Non-coding RNAs in endometriosis: a narrative review. Hum Reprod Update 2018;24(4):497–515.
[78] Papari E, Noruzinia M, Kashani L, Foster WG. Identification of candidate microRNA markers of endometriosis with the use of next-generation sequencing and quantitative real-time polymerase chain reaction. Fertil Steril 2020;113(6):1232–41.
[79] Kimber-Trojnar Ż, Pilszyk A, Niebrzydowska M, Pilszyk Z, Ruszała M, Leszczyńska-Gorzelak B. The potential of non-invasive biomarkers for early diagnosis of asymptomatic patients with endometriosis. J Clin Med 2021;10(13).
[80] Moustafa S, Burn M, Mamillapalli R, Nematian S, Flores V, Taylor HS. Accurate diagnosis of endometriosis using serum microRNAs. Am J Obstet Gynecol 2020;223(4):557. e1–e11.
[81] Haikalis ME, Wessels JM, Leyland NA, Agarwal SK, Foster WG. MicroRNA expression pattern differs depending on endometriosis lesion type. Biol Reprod 2018;98(5):623–33.
[82] Nisenblat V, Sharkey DJ, Wang Z, Evans SF, Healey M, Ohlsson Teague EMC, Print CG, Robertson SA, Hull ML. Plasma miRNAs display limited potential as diagnostic tools for endometriosis. J Clin Endocrinol Metab 2019;104(6):1999–2022.
[83] Vanhie A, D.O., Peterse D, Beckers A, Cuéllar A, Fassbender A, Meuleman C, Mestdagh P, D'Hooghe T. Plasma miRNAs as biomarkers for endometriosis. Hum Reprod 2019;34(9):1650–60.
[84] Akoum A, Lemay A, Brunet C, Hébert J. Secretion of monocyte chemotactic protein-1 by cytokine-stimulated endometrial cells of women with endometriosis. Le groupe d'investigation en gynécologie. Fertil Steril 1995;63(2):322–8.
[85] Fedele L, Marchini M, Bianchi S, Dorta M, Arcaini L, Fontana PE. Structural and ultrastructural defects in preovulatory endometrium of normo-ovulating infertile women with minimal or mild endometriosis. Fertil Steril 1990;53(6):989–93.
[86] Jones CJ, Denton J, Fazleabas AT. Morphological and glycosylation changes associated with the endometrium and ectopic lesions in a baboon model of endometriosis. Hum Reprod 2006;21(12):3068–80.
[87] Al-Jefout M, Tokushige N, Hey-Cunningham AJ, Manconi F, Ng C, Schulke L, Berbic M, Markham R, Fraser IS. Microanatomy and function of the eutopic endometrium in women with endometriosis. Expet Rev Obstet Gynecol 2009;4(1):61–79.
[88] Brosens J, Timmerman D, Starzinski-Powitz A, Brosens I. Noninvasive diagnosis of endometriosis: the role of imaging and markers. Obstet Gynecol Clin N Am 2003;30(1):95–114 [viii-ix].
[89] Chung HW, Wen Y, Chun SH, Nezhat C, Woo BH, Lake Polan M. Matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-3 mRNA expression in ectopic and eutopic endometrium in women with endometriosis: a rationale for endometriotic invasiveness. Fertil Steril 2001;75(1):152–9.
[90] Cox KE, Piva M, Sharpe-Timms KL. Differential regulation of matrix metalloproteinase-3 gene expression in endometriotic lesions compared with endometrium. Biol Reprod 2001;65(4):1297–303.
[91] Klentzeris LD, Bulmer JN, Liu DT, Morrison L. Endometrial leukocyte subpopulations in women with endometriosis. Eur J Obstet Gynecol Reprod Biol 1995;63(1):41–7.
[92] Absenger Y, Hess-Stumpp H, Kreft B, Krätzschmar J, Haendler B, Schütze N, Regidor PA, Winterhager E. Cyr61, a deregulated gene in endometriosis. Mol Hum Reprod 2004;10(6):399–407.
[93] Dheenadayalu K, Mak I, Gordts S, Campo R, Higham J, Puttemans P, White J, Christian M, Fusi L, Brosens J. Aromatase P450 messenger RNA expression in eutopic endometrium is not a specific marker for pelvic endometriosis. Fertil Steril 2002;78(4):825–9.
[94] Kitawaki J, Kusuki I, Koshiba H, Tsukamoto K, Honjo H. Expression of aromatase cytochrome P450 in eutopic endometrium and its application as a diagnostic test for endometriosis. Gynecol Obstet Invest 1999;48(Suppl. 1):21–8.

[95] Wei Q, St Clair JB, Fu T, Stratton P, Nieman LK. Reduced expression of biomarkers associated with the implantation window in women with endometriosis. Fertil Steril 2009;91(5):1686–91.
[96] Leslie C, Ma T, McElhinney B, Leake R, Stewart CJ. Is the detection of endometrial nerve fibers useful in the diagnosis of endometriosis? Int J Gynecol Pathol 2013;32(2):149–55.
[97] Al-Jefout M, Dezarnaulds G, Cooper M, Tokushige N, Luscombe GM, Markham R, Fraser IS. Diagnosis of endometriosis by detection of nerve fibres in an endometrial biopsy: a double blind study. Hum Reprod 2009;24(12):3019–24.
[98] Zhang X, Lu B, Huang X, Xu H, Zhou C, Lin J. Endometrial nerve fibers in women with endometriosis, adenomyosis, and uterine fibroids. Fertil Steril 2009;92(5):1799–801.
[99] Bektas YT, Sakin Ö, Pirimoğlu ZM, Yılmaz AO, Basak K, Büyükbayrak EE, Özkan S, Simsek EE. Diagnostic value of endometrial nerves in the endometrial tissue of patients with endometriosis. South Clin Ist Euras 2017;28(4):261–5.
[100] Zevallos HB, McKinnon B, Tokushige N, Mueller MD, Fraser IS, Bersinger NA. Detection of the pan neuronal marker PGP9.5 by immuno-histochemistry and quantitative PCR in eutopic endometrium from women with and without endometriosis. Arch Gynecol Obstet 2015;291(1):85–91.
[101] Meibody FA, Mehdizadeh Kashi A, Zare Mirzaie A, Ghajarie Bani Amam M, Behbahani AS, Zolali B, Najafi L. Diagnosis of endometrial nerve fibers in women with endometriosis. Arch Gynecol Obstet 2011;284(5): 1157–62.
[102] Chen QH, Qu JY, Xu YY, Qiu NX, Zhuang YZ, Zhong S, Fang QQ. Expressions of matrix metalloproteinase-9 and tissue inhibitor of metalloproteinase-1 in ectopic and eutopic endometrium. Zhonghua Fu Chan Ke Za Zhi 2004;39(12):809–12.
[103] Gilabert-Estellés J, Estellés A, Gilabert J, Castelló R, España F, Falcó C, Romeu A, Chirivella M, Zorio E, Aznar J. Expression of several components of the plasminogen activator and matrix metalloproteinase systems in endometriosis. Hum Reprod 2003;18(7):1516–22.
[104] Gilabert-Estellés J, Ramón LA, España F, Gilabert J, Vila V, Réganon E, Castelló R, Chirivella M, Estellés A. Expression of angiogenic factors in endometriosis: relationship to fibrinolytic and metalloproteinase systems. Hum Reprod 2007;22(8):2120–7.
[105] Laudanski P, Charkiewicz R, Kuzmicki M, Szamatowicz J, Świątecka J, Mroczko B, Niklinski J. Profiling of selected angiogenesis-related genes in proliferative eutopic endometrium of women with endometriosis. Eur J Obstet Gynecol Reprod Biol 2014;172:85–92.
[106] Lee KF, Lee YL, Chan RW, Cheong AW, Ng EH, Ho PC, Yeung WS. Up-regulation of endocrine gland-derived vascular endothelial growth factor but not vascular endothelial growth factor in human ectopic endometriotic tissue. Fertil Steril 2010;93(4):1052–60.
[107] Cho S, Choi YS, Yim SY, Yang HI, Jeon YE, Lee KE, Kim H, Seo SK, Lee BS. Urinary vitamin D-binding protein is elevated in patients with endometriosis. Hum Reprod 2012;27(2):515–22.
[108] da Silva CM, Belo AV, Passos Andrade S, Peixoto Campos P, Cristina França Ferreira M, Lopes da Silva-Filho A, Carneiro MM. Identification of local angiogenic and inflammatory markers in the menstrual blood of women with endometriosis. Biomed Pharmacother 2014;68(7):899–904.
[109] Wang L, Liu HY, Shi HH, Lang JH, Sun W. Urine peptide patterns for non-invasive diagnosis of endometriosis: a preliminary prospective study. Eur J Obstet Gynecol Reprod Biol 2014;177:23–8.

This page intentionally left blank

Index

Note: 'Page numbers followed by "f" indicate figures and "t" indicate tables.'

D

E

F

G

H

I

This page intentionally left blank

REPRODUCTIVE IMMUNOLOGY

VOLUME 2

IMMUNOLOGY OF ENDOMETRIOSIS

Pathogenesis and Management

Series Editor GIL MOR

John M. Malone Jr., MD, Endowed Chair, Scientific Director, C.S. Mott Center for Human Growth and Development, Wayne State University School of Medicine, Detroit MI, USA

Edited by KAORI KOGA

Faculty of Medicine, Department of Obstetrics and Gynecology, the University of Tokyo, Tokyo, Japan

Immunology of Endometriosis covers all the basic science concepts and associated clinical applications related to the immunology of endometriosis. Endometriosis is one of the most common gynecological diseases and causes pain, infertility, cancer, and other negative impacts on the quality of life of affected women. Various immunological factors are thought to play roles in the pathogenesis of endometriosis, and these factors are potential targets for this disease. Endometriosis is considered a disease of both endocrine and immune dysregulation because it is often associated with autoantibodies, other autoimmune diseases, and possibly, recurrent immune-mediated pregnancy loss. The endocrine–immunologic axis underlines the complexity of this gynecologic disorder. However, the recognition of the direct involvement of two major physiological mechanisms has brought about a change in focus that may represent an interesting advance in understanding this disease as well as provide a new focus for further research. Vast changes in the activities of many cells involved in immune reactions might offer new therapeutic targets. This book covers all the basic science concepts and their clinical applications related to the immunology of endometriosis.

Key Features:

- Provides a detailed immunological background for understanding the etiology and management of endometriosis
- Evaluates various immunological factors involved in the pathogenesis of endometriosis
- Supplies a detailed evaluation of current knowledge about each immune cell type in endometriosis

About the Editor

Kaori Koga, MD, PhD, is a physician-scientist and an Associate Professor of the Department of Obstetrics and Gynecology, School of Medicine at the University of Tokyo, Tokyo, Japan. Dr. Koga received her MD from Chiba University, Chiba, Japan, and her PhD from the University of Tokyo. She undertook postdoctoral fellowships in the Uterine Biology Group (Prof. Lois Salamonsen's laboratory) at Prince Henry's Institute, Melbourne, Australia, in 2006, and in the Reproductive Immunology Unit (Prof. Gil Mor's laboratory) at the Department of Obstetrics, Gynecology & Reproductive Sciences at Yale University in 2006–2008. Dr. Koga's keen interest lies in reproductive immunology, with a particular focus on endometriosis and infertility. As a gynecological clinician, Dr. Koga has treated many patients with endometriosis, has performed minimally invasive surgery, and has employed assisted reproductive technology. Dr. Koga has received several national and international awards, including the Kanazawa Award from the Kanazawa Medical Research Foundation in 2018 and the Gusdon Award from the American Society for Reproductive Immunology (ASRI) in 2008. She is a member of ASRI, the International Society for Immunology of Reproduction, and the European Society of Human Reproduction and Embryology as well as an ambassador of the World Endometriosis Society. Dr. Koga is an Associate Editor of Human Reproduction Open (2021–) and serves on the editorial boards of the American Journal of Reproductive Immunology and the Journal of Reproductive Immunology.

ISBN 978-0-12-820661-4

9 780128 206614

ELSEVIER

ACADEMIC PRESS

An imprint of Elsevier

elsevier.com/books-and-journals