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Review

Emerging bacterial factors for understanding pathogenesis of endometriosis

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SUMMARY

The pathogenesis of endometriosis is a complex process, and recent research has introduced novel hypotheses in this field. This review summarizes recent studies on the pathogenesis of endometriosis. We focused on several classical hypotheses, as well as their interactions with the microenvironment of hormonal dependence and immunosuppression. Furthermore, we highlighted the emergence of bacterial factors associated with endometriosis. Recent advances in next-generation sequencing (NGS) have revealed the presence and detailed distribution of these bacteria as well as the involvement of specific bacteria in pathogenesis. These factors alter the microenvironment in the early stages of endometriosis development, leading to lesion formation. Understanding the mechanisms underlying the early development of endometriosis from a new perspective would be helpful for the development of novel therapeutic agents for endometriosis.

INTRODUCTION

Endometriosis is defined as the presence of endometrial glands and the growth of stromal cells outside the endometrial cavity, in regions such as the peritoneum or ovaries. Endometriosis is prevalent in almost 10% of reproductive-aged women.[1–3] Several hypotheses on the pathogenesis of endometriosis have been proposed; however, none have adequately supported the "10%" prevalence of endometriosis. Current treatment options include surgical excision of endometriotic lesions and administering medicines that suppress ovarian hormone production. Patients who have undergone surgery have reduced ovarian reserves, and various medical treatments have adverse effects.[4–6] Patients who wish to become pregnant have only limited treatment options. Improvements in treatments that are consistent with the pathophysiology of endometriosis, which relieve symptoms and do not limit fertility, are indispensable. In this mini-review, each pathological hypothesis is evaluated in light of the prevalence of endometriosis. In addition, novel treatment strategies based on the pathogenesis are discussed.

CLASSICAL HYPOTHESES

Retrograde menstruation

Retrograde menstruation theory was proposed by Sampson in 1927 and is the most widely accepted theory for the pathogenesis of endometriosis. Sampson was an eminent clinician who discovered that menstrual blood flowed back into the abdominal cavity during menstruation, which is a highly conceivable route for endometrial cells to flow out of the uterus through the fallopian tubes. Many other hypotheses are based on the concept of reflux through the fallopian tubes as a route for endometrial cells, a notion that underlies almost all hypotheses. This theory is supported by the association of short menstrual cycles and excessive menstrual flow with the risk of developing endometriosis. However, retrograde menstruation is a phenomenon observed in almost all women, except those with fallopian tube obstruction. If the endometrium refluxes with each monthly menstruation, the 10% prevalence of endometriosis is too small. In terms of treatment, consistent with this pathological theory, fallopian tubes cannot be removed because they serve a critical function as a pathway for the oocyte and the site of fertilization. Hence, hormonal therapy is used to reduce or eliminate blood reflux.[7]

Coelomic metaplasia

The coelomic metaplasia theory, reported by Robert Meyer in 1924, states that endometriosis is caused by the transformation of the peritoneal mesothelium into endometrial-like cells. This theory is applicable to patients with Mayer–Rokitansky–Küster–Hauser (MRKH) syndrome, who sometimes lack a uterus or endometrium but develop endometriosis. However, the mechanism of metaplasia is unclear. Konrad et al. revealed that patients with MRKH only develop endometriosis if a tiny uterus/endometrium is present and not contradicts the implantation theory of Sampson.[8] Endometriosis sometimes develops in teenagers even before menarche, in girls who have never menstruated,[9,10] and in men who are castrated and treated with estrogen.[11,12] The pathogenesis of endometriosis in these patients could be explained by the

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coelomic metaplasia theory, in which transformation is triggered by estrogen. However, when all reproductive-aged women are considered, it is not possible to explain the 10% prevalence rate using this hypothesis alone; this hypothesis can only explain the incidence under certain conditions.

Stem/progenitor cells

In 2007, Gargett et al. reviewed endometrial stem/progenitor cells and focused on their involvement in eutopic endometrial regeneration and differentiation.[13] This novel theory proposes that endometriosis arises from ectopic endometrial stem/progenitor cells when they are ectopically located.[14] The endometrium is a dynamic and unique structure that displays complete renewal during each monthly menstrual cycle after tissue breakdown. Many studies have identified endometrial stem/progenitor cells that likely reside in the basal layer of the endometrium and regenerate the entire endometrium. Maruyama et al. identified endometrial side population (ESP) cells, even in the functional zone of the endometrium, and bone marrow-derived cells.[15,16] Indeed, bone-marrow-derived cells established endometriotic implants in mice.[17] They insisted on the possibility of endometriosis formation at the single or very few cellular levels within the menstrual blood reflux; however, we should consider the content of ESP cells within the endometrium and their presence within menstrual reflux blood. Masuda et al. have reported that ESP cells account for approximately 2% of the endometrial cell population.[16] This percentage may partially explain the discrepancy between the incidence of endometriosis and the frequency of retrograde menstruation.

MICROENVIRONMENT INTERACTIONS

Hormonal dependence

In the endometrium, estrogen affects the proliferation of endometrial cells, whereas progesterone inhibits the action of estrogen in initiating decidualization. Dysregulation of these two hormones is thought to lead to endometriosis. Endometriosis is an estrogen-dependent benign disease; however, the proliferation of ectopic endometrial cells is clinically problematic. Endometriotic lesions often exhibit abnormal expression of estrogen synthase due to the upregulation of estrogen-producing p450 aromatase expression.[18] This localized increase in estrogen levels is known to induce the proliferation of endometrial cells.[3] In addition, the expression of estrogen receptors (ERα and ERβ) is also the key factor in the development of endometriosis. In endometriosis, overexpression of ERβ is sometimes observed, and ERβ-mediated signaling promotes inflammation.[19] The expression levels of ERα, encoded by the *ESR1* gene, are decreased due to hypermethylation of the promoter lesions. Conversely, the *ESR2* promoter lesions are hypomethylated, resulting in increased expression of ERβ.[19] Upon estrogen binding, ER activates the extracellular-signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) pathways.[20] This signaling pathway triggers the proliferation of endometrial cells, contributing to lesion growth. Furthermore, estrogen/ERβ signaling leads to the activation of the cyclooxygenase (COX-2) and prostaglandin E2 (PGE2), resulting in an increase in the inflammatory response and the pathophysiology of endometriosis.[21,22] Additionally, estrogen/ERβ signaling induces the Nod-like receptor (NLR) family pyrin domain-containing 3 (NLRP3) inflammasome, which plays a role in the maturation of IL-1β from pro-IL-1β.[23,24] The importance of this NLRP3 inflammasome cascade has been demonstrated in endometriotic stromal cells and mast cells of patients with endometriosis.[25–27] Furthermore, α-lipoic acid (ALA) showed potential as a therapeutic agent for endometriosis, reducing the ERβ expression levels and NALP-3 activity.[28] ALA acts as an antioxidant and might contribute to the suppression of inflammatory conditions. Progesterone is another key factor involved in the development of endometriosis. In endometriosis, the expression of the progesterone receptors (PR), including PRA and PRB, are suppressed due to the hypermethylation of their promoter regions.[29] This hypermethylation induces a progesterone-resistant state.[30] In the endometrium, progesterone binds to PRs, leading to the activation of Indian hedgehog (IHH)/chicken ovalbumin upstream promoter-transcription factor II (COUPTF II), and bone morphogenetic protein 2 (BMP2) signaling pathways, which, in turn, induce decidualization.[31] Another PR target is homeobox protein-A10 (HOXA10), which regulates the responsiveness to progesterone.[32] In patients with endometriosis, the expression of progesterone signaling factors is decreased, resulting in an abnormal balance between cell proliferation and decidualization. As PRs inhibit the expression of ERs, the suppression of PRs induces overexpression of ERs and contributes to the development of endometriosis.[33] These endometriosis-related hormones and receptors are critical for the pathogenesis of endometriosis; however, their contributions are largely unknown. Whether the abnormality in hormone receptor description occurs at the time of eutopic endometrium formation or after endometriosis is similar to the egg-first or chicken-first debate. Abnormal expression of these hormonal receptors is an effective therapeutic target in endometriosis treatment. Endometriosis is an estrogen-dependent disorder and estrogen-suppressive medicine is a reasonable treatment option for women of reproductive age. In clinical practice, combined oral contraceptives (OCs) and gonadotrophin-releasing hormone agonists (GnRHa) or antagonists (GnRHant) are the first choice of endometriosis medications to suppress ovarian function. Secondly, because endometriosis is also a progesterone-resistant disorder, dienogest with high progestin activity is now widely used as a hormonal treatment for endometriosis. This treatment works on endometrial regression and ovarian suppression to inhibit the growth of endometriosis, also known as ectopic endometrial tissue.[4,30] The mechanisms of action of these medicines are based on endometriosis pathophysiology and have been proven clinically effective. Unfortunately, they cannot be used in women who wish to become pregnant because these medicines stop the hormone-dependent menstrual cycle.

Immune dysregulation

Endometriosis is also an inflammatory disease in which immune dysregulation induces ectopic endometrial cell adhesion, infiltration, and proliferation. Immune cells involved in the formation and further development of endometriotic lesions are primarily macrophages, natural killer

(NK) cells, and T cells. Although there are several possible causes of this chronic inflammation, we believe that the pathogenesis of chronic inflammation status and immune dysregulation characterize endometriosis.

Macrophages

Macrophages perform phagocytic functions such as clearing apoptotic cells and debris during the menstrual cycle.[34] In particular, M2 macrophages (anti-inflammatory and tissue repair macrophages) are more abundant in endometriotic lesions and peritoneal fluid of women with endometriosis than in those without endometriosis.[35] This anti-inflammatory environment may be permissive to endometriotic lesion formation and promote angiogenesis. In addition, M2 macrophages secrete several cytokines, including transforming growth factor (TGF), which affects the adjacent cells.[36] Moreover, M2 macrophages contribute to the pathogenesis and symptoms of endometriosis by promoting nerve fiber growth, leading to the severe abdominal pain associated with endometriosis.[34] These functions of M2 macrophages may contribute to the development of endometriosis in certain populations by assisting the proliferation of endometrial cells that flow back into the menstrual blood.

Natural killer cells

NK cells are key components of the innate immune system and are present in the eutopic endometrium, peritoneal fluid, and ectopic endometrial lesions. NK cells in the peritoneal fluid of women with endometriosis are less cytotoxic than those in the eutopic endometrium of women without endometriosis.[37] This lower cytotoxicity may allow the proliferation of endometrial fragments in the peritoneal cavity, leading to endometrial implantation in certain populations.

T cells

An imbalance in T cells in the peritoneal fluid and endometriotic lesions may promote and maintain the development of endometriosis. $CD4^+$ T cells (also known as helper-inducer cells) are increased compared with $CD8^+$ T cells (also known as cytotoxic cells) in endometriotic lesions and peritoneal fluid of women with endometriosis.[38] This induces an inflammatory environment that contributes to the establishment, survival, and proliferation of endometriotic lesions. Other types of T cells, such as regulatory T cells (Tregs), are known to modulate the immune system and are related to the progression of endometriosis. Tregs suppress inflammation and prevent autoimmunity.[39] Tregs increase in the peritoneal fluid of women with endometriosis, and the development of autoimmune reactions and suppression of local immune responses are thought to lead to endometriosis.[40]

Immune therapy

Based on the differential infiltration of several immune cells in patients with endometriosis, targeted immune cell depletion opens new opportunities for endometriosis treatments that account for inflammation.[41] However, in practice, immune cells are distributed systemically, which may make it difficult for medicines to exert a localized effect on the endometrium or endometriotic lesions. The following chapters discuss in detail, the bacterial factors that may be closely associated with immune dysregulation.

EMERGENCE OF BACTERIAL FACTORS

Background on genital microbiota

Previously, the only way to confirm bacterial identification was by the culture method; however, setting up a suitable culture environment was complicated, and depending on the number of bacteria that could be collected, it was often impossible to culture disease-related bacteria *in vitro*.[42–44] The association between diseases and microbiota has recently received much attention, owing to advances in next-generation sequencing (NGS), which has revealed detailed bacterial distribution even with only a small number of samples collected.[45] Not only several studies have been reported on the so-called "infectious" state but also the dysbiosis status affects the surrounding microenvironment and results in disease development.[46,47] Dysbiosis has been implicated in various human diseases, including intestinal diseases and cancer.[48–50] Emerging evidence suggests that genital microbiota crosstalk is involved in endometriosis pathogenesis. The majority of clinical studies investigating the microbiota associated with endometriosis are listed in Table 1.[51–62] Advances in NGS have led to the bacterial analysis of female genital organs since 2016. The genital microbiota, especially in the endometrium, which was previously thought to be sterile, is increasingly being studied in the field of endometrial receptivity, implantation, and placentation[63,64] and is also being examined to reveal the cause of endometriosis, recently. Moreover, the analysis of the microbiota in the vagina and cervix, where samples can be easily collected, and the microbiota in the intestinal tract, which might be linked to endometriosis, is also a developing field in terms of biomarkers for endometriosis.

Bacterial contamination theory and bacterial detection methods

This innovative novel theory was presented by Dr. Khan.[65] He examined the endotoxin concentration in menstrual blood and peritoneal fluid and revealed by the bacterial culture method that the menstrual blood of women with endometriosis was highly contaminated with *Escherichia coli* (*E. coli*).[66] This bacterial contamination in menstrual blood could be a constant source of bacterial endotoxins in the peritoneal fluid via retrograde menstruation and can cause endometriosis. Bacterial LPS activates macrophages in the peritoneal fluid, triggering Toll-like receptor 4 (TLR4)-mediated inflammation and promoting endometriosis. LPS/TLR4 signaling stimulates the cascade that results in the production of

Table 1. Clinical studies reporting the microbiota related to endometriosis

Published year	Author	Patient characteristics	Objects	Patient population	Country (Race)	Sample type	Detection of bacteria	Main outcomes in Endometriosis (increased)	Main outcomes in endometriosis (decreased)
2016	Khan et al.[51]	Women without endometriosis (n = 32) vs. with endometriosis (n = 32). Both groups were further divided into GnRHa-treated or untreated groups (n = 16, respectively)	Endometrium	Average age 33.6 years [without endometriosis, GnRHa (−)], 42.1 years [without endometriosis, GnRHa (+)], 35.7 years [with endometriosis, GnRHa (−)], 37.5 years [with endometriosis, GnRHa (+)]	Japan	Endometrial swab, cystic fluid	16S rDNA sequencing (Illumina), restricted to selected 5 genus	Streptococcaceae, Staphylococaceae, Enterobacteriaceae	Lactobacillacae
2019	Ata et al.[52]	Women without endometriosis (n = 14) vs. with endometriosis (n = 14)	Vaginal sample, cervical mucus, feces	Median age 27.5 years (without endometriosis) vs. 28.5 years (with endometriosis)	Turkey	Vaginal swab, cervical swab, and fecal sample	16S rRNA sequencing (Illumina, Miseq)	Alloprevotella (cervical), Escherichia, Shigella (feces)	Atopobium, Gemella (vaginal), Atopobium, Sneathia (cervical), Barnesella, Gardnerrella, Sneathia (feces)
2019	Akiyama et al.[53]	Women without endometriosis (n = 39) vs. with endometriosis (n = 30)	Cervical mucus	Average age 32.5 years without endometriosis vs. 33.9 years with endometriosis	Japan	Cervical mucus aspirated by 1-mL syringe	16S rRNA sequencing (Illumina, Miseq)	Corynebacterium, Enterobactericaea, Flavobacterium, Streptococcus, Pseudomonas	n.a
2020	Wei et al.[54]	Women without endometriosis (n = 14) vs. with endometriosis (n = 36)	Lower third of vagina (CL), posterior vaginal fornix (CU) and cervical mucus (CV), endometrium (ET) and peritoneal fluid (PF)	Average age 31.47 years in total	China	CL, CU, CV: swab, ET: absorption of uterine lavage fluid, PF: aspirated from Douglas pouch	16S rRNA sequencing	Caulobacteraceae, Erysipelotrichaceae, Pseudomonadaceae (CV), Sphingobium, Pseudomonas (ET, PF)	n.a
2020	Hernandes et al.[55]	Women without endometriosis (n = 11) vs. with deep endometriosis (n = 10)	Endometrium, vaginal fluid, deep endometriosis lesion	Age 18 years to 50 years in total	Brazil	Vaginal swab, endometrial curettage, deep endometriosis resection	16S rRNA sequencing (Illumina, Miseq)	Alishewanella, Enterococcus, Pseudomonas (deep endometriosis lesion)	Gardnerellla, Prevotella (vaginal fluid, endometrium)

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Table 1. *Continued*

Published year	Author	Patient characteristics	Objects	Patient population	Country (Race)	Sample type	Detection of bacteria	Main outcomes in Endometriosis (increased)	Main outcomes in endometriosis (decreased)
2021	Le et al.[56]	Women without endometriosis (n = 9) vs. with endometriosis (n = 20). Both groups included the patients using Ocs; without endometriosis (n = 5) vs. with endometriosis (n = 11)	Urine, feces, vaginal samples	Average age 32.6 years without endometriosis vs. 32.5 years with endometriosis	USA (Caucasian, Hispanic, other)	Urine, fecal swab, vaginal swab	16S rRNA sequencing (Illumina, Miseq)	Lactobacillus (urine), Firmicutes, Bacteroidetes (feces), Firmicutes, Actinobacteria (vaginal)	n.a
2021	Wessels et al.[57]	Women without endometriosis (n = 9) vs. with endometriosis (n = 12)	Endometrium	Average age 35.1 years without endometriosis vs. 33.8 years with endometriosis	Canada	Endometrial biopsy by suction curette	16S rRNA sequencing (Illumina, Miseq)	Actinobacteria phylum, Oxalobacteraceae, Streptococcaceae, Tepidimonas	Brkholderiaceae, Ralstonia
2021	Huang et al.[58]	Women without endometriosis (n = 20) vs. with endometriosis (n = 21)	Feces, cervical mucus, peritoneal fluid	Average age 34.0 years without endometriosis vs. 38.3 years with endometriosis	China	Self-collected feces, cervical swab, aspirated peritoneal fluid	16S rRNA sequencing (Illumina, Miseq)	Eggerthella lenta, Eubacterium dolichum (feces), Pseudomonadaceae Pseudomonas, Prevotellaceae Prevotella, Xanthomonadaceae Luteimonas (peritoneal fluid)	Clostridia Clostridiales, Lachnospiraceae Ruminococcus, Clostridiales Lachnospiraceae, Ruminococcaceae Ruminococcus (feces), Actinomycetales Microbacteriaceae, Lactobacillus iners, Microbacteriaceae Cryocola (peritoneal fluid)
2021	Lee et al.[59]	Women without endometriosis (n = 45) vs. with endometriosis (n = 45)	Extracellular vesicles in peritoneal fluid	Average age 39.4 years without endometriosis vs. 36.2 years with endometriosis	Korea	Aspirated peritoneal fluid from cul-de-sac or uterovesical pouch, extracellular vesicles were collected by ultracentrifugation method	16S rDNA sequencing (Illumina, Miseq)	Acinetobacter, Enhydrobacter, Pseudomonas, Streptococcus	Actinomyces, Propionibacterium, Rothia

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Table 1. *Continued*

Published year	Author	Patient characteristics	Objects	Patient population	Country (Race)	Sample type	Detection of bacteria	Main outcomes in Endometriosis (increased)	Main outcomes in endometriosis (decreased)
2022	Chang et al.[60]	Women without endometriosis (n = 10) vs. with endometriosis (n = 23)	Cervical mucus	Average age 35.4 years with endometriosis	Taiwan	Cervical swab	16S rRNA sequencing (Illumina, Miseq)	Firmicutes	Actinobacteria, Bacteroidete
2022	Oishi et al.[61]	Women without endometriosis (n = 18) vs. with endometriosis (n = 18)	Vaginal fluid, endometrial fluid, peritoneal fluid, ovarian cyst fluid	Average age 35.2 years without endometriosis vs. 37.9 years with endometriosis	Japan	Vaginal swab, endometrial brush, suction of peritoneal fluid, puncturing the ovarian tumor directly in a bag after resection and aspirated	16S rRNA sequencing (Illumina, Miseq	n.a	n.a
2022	Yuan et al.[62]	Women without endometriosis (n = 25) vs. with endometriosis (n = 36)	Peritoneal fluid	Average age 33.3 years without endometriosis vs. 35.2 years with endometriosis	China	Aspirated from Douglas pouch	16S rRNA sequencing (Illumina, Hiseq)	Acidovorax, Devosia, Methylobacterium, Phascolarctobacterium, Streptococcoccus	Brevundimonas, Stenotrophomonas

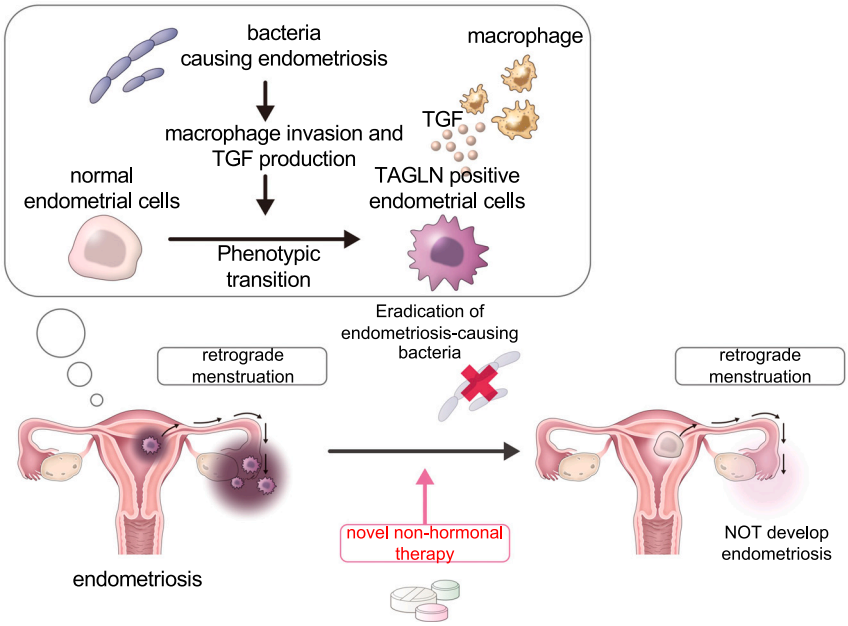

Figure 1. Relationship between bacteria and endometrial cells in the microenvironment of the endometrium

In the endometrium, infected bacteria attract macrophages to the surrounding microenvironment, and transformed M2 macrophages produce TGF. Subsequently, endometrial fibroblasts transform into myofibroblasts in a TGF-rich microenvironment. These phenotypic transformations induce adhesion, migration, and proliferation at the ectopic site to develop endometriosis lesions. The presence of bacteria in the endometrium causes the transformation of fibroblasts that develop endometriosis.

inflammatory cytokines via the activation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) activation.[65,67–69] This critical hypothesis has recently been revised and is being studied at an accelerated pace with the addition of more detailed methods to demonstrate the presence of the microbiota. Traditional methods for microbiota detection involve culturing samples on agar medium. However, culture-based techniques have limitations, as they are labor-intensive and time-consuming. Moreover, most microbes cannot be cultivated under standard laboratory conditions, requiring specialized agar mediums and culture environments. Alternative detection methods for microbiota involve DNA amplification techniques, such as polymerase chain reaction (PCR), and immunological assays, such as enzyme-linked immunosorbent assay (ELISA). These methods require the design of specific primers for each bacterium and do not provide information about bacterial spatial distributions. High-throughput sequencing allows for comprehensive microbiota detection from small samples, revealing the presence and quantity of bacteria. Recent research explores novel approaches to detect microbiota using optical methods, such as fluorescence spectroscopy and optical coherence tomography.[70,71] These optical methods enable the visualization of bacterial distribution within tissue, facilitating the rapid detection of bacteria from patient fluids and even *in vivo*. The advancement and development of several detection methods are valuable for revealing interactions between bacteria and living organisms throughout the body, including in regions previously believed to be sterile, such as the uterus. These detection methods have distinct advantages and disadvantages, depending on factors such as the viability of bacteria, their precise location within the organism, the proportion of target bacteria in the total microbiota, the speed of detection, the comprehensiveness of the microbiota profile obtained, and the ability to identify specific bacteria in samples. A combination of these tests may reveal crucial insights into whether certain bacteria are implicated in specific diseases, their locations within the body, their viability, and their potential roles in disease development, even if they are non-viable, shedding light on the complex interplay among microbiota.

Microbiota in the uterus

In 2017, Chen et al. revealed the existence of certain microbial communities inside the uterus using highly sensitive 16S ribosomal RNA gene amplicon sequencing.[72] This innovative technological advancement has enabled the detection of small amounts of bacteria in the uterus, proving that its interior is not sterile. In addition, the concept of chronic endometritis has recently been established, which is defined as chronic inflammation caused by a persistent bacterial infection in the uterus.[73] Several epidemiological studies have revealed an association between endometritis and endometriosis,[74,75] suggesting infection inside the uterus and the development of endometriosis. We discovered that *Fusobacterium* infection of the endometrium was responsible for the development of endometriosis[76] (Figure 1). These bacteria were included in a dataset published in a comprehensive study by Chen et al. Species of the *Fusobacterium* are common members of the oral and gastrointestinal tract microbiota.[77] We have discovered that this bacterium can infect the uterus by ascending infection from the vagina and induce phenotypic transition in endometrial fibroblasts that cause endometriosis. More interestingly, 64% of patients with endometriosis are positive for *Fusobacterium* in the endometrium, suggesting that this bacterial infection induces a phenotypic transition of endometrial

cells and that transformed endometrial cells that reach the abdominal cavity or ovarian surface during retrograde menstruation develop endometrial lesions. This is potentially close to the 10% prevalence of endometriosis. In the female reproductive organs, *Lactobacillus* is typically recognized for its role in producing lactic acid, which helps maintain a healthy balance by preventing the growth of harmful bacteria inside the uterus.[78,79] Some research has suggested that a decrease in *Lactobacillus* in the cervical microbiome may be associated with endometriosis.[54,55] However, contrary to conventional beliefs, *Lactobacillus* accounted for 46.1% of all bacterial species detected in the endometrium of patients with endometriosis. This percentage was higher than the 36.2% *Lactobacillus* presence found in women without endometriosis. Additionally, we compared the prevalence of bacteria known to cause endometritis[80] in the endometrium of patients with endometriosis and women without endometriosis. Notably, not all bacterial strains were more prevalent in patients with endometriosis. To illustrate, the percentages varied between these two groups (*Gardnerella*; 0.62% [women without endometriosis], 0.16% [women with endometriosis], *Streptococcus*; 0.32%, 3.5%, *Escherichia coli*; 0.93%, 0.27%, *Enterococcus*; 0.78%, 0.44%, *Staphylococcus*; 0.86%, 0.20%, *Mycoplasma*; 0.075%, 0%, *Ureaplasma*; 0.034%, 0.017%, *Proteus*; 0.13%, 0.24%, *Klebsiella*; 0.084%, 1.9%, *Pseudomonas*; 7.9%, 12%, *Corynebacterium*; 0.82%, 0.22%). These percentages are based on our re-analysis of previously published sequencing data from Chen et al. Notably, *Streptococcus*, *Proteus*, *Klebsiella*, and *Pseudomonas* were highly detected in endometrial samples from women with endometriosis, whereas *Lactobacillus* levels were not found to be decreased. The difference in results may be attributed to variations in the sampling location. The previous report collected samples from the cervix, whereas the current re-analysis used samples from the endometrium, the primary source of endometriosis. This suggests that the composition and interaction of bacteria in the uterus are not solely determined by a single organism but rather a balanced ecosystem of various bacteria in the endometrial microenvironment plays a vital role. A more detailed omics analysis focusing on the interaction between healthy and harmful bacteria is urgently needed in the future to better understand the overall association between these bacteria. Detecting bacteria that may be involved in the pathogenesis of endometriosis is challenging, primarily because the total amount of bacteria in the endometrium is considerably lower compared with the vaginal and cervical microenvironments. Selecting organisms from the endometrium that are most likely to contribute to the development of endometriosis might offer a more efficient path to uncovering the condition's pathogenesis. Endometriosis pathophysiology is based on the retrograde menstruation of endometrial cells, and it is highly suggestive that the microbiota present in the endometrium could be a direct trigger for the development of endometriosis.

Microbiota in cervical lesion

If ascending microbial transmission is the cause of endometrial infection, the analysis of microbial colonization in the cervical mucus is important. Akiyama et al. revealed that *Enterobacteriaceae* and *Streptococcus* were identified as more significant candidates in the endometriosis group than in controls.[53] As previously mentioned, the cervical mucus plug is a critical "gatekeeper" that prevents ascending infection from the lower genital tract into the uterine cavity. However, this infection-preventive effect depends on the menstrual cycle, and bacteria in the cervix may invade the uterine cavity during menstruation. The cervical microbiota is of significant value for screening and diagnosing endometriosis due to its ease of sampling. However, the mechanisms through which cervical bacteria influence the endometrial microbiota and contribute to endometriosis remain poorly understood. Although the microbiota in cervical lesions is important, a functional analysis of how these organisms enter the uterus and act on endometrial cells or the cells responsible for endometriosis is necessary in the future. More specifically, the cervical epithelium exhibits distinct histological characteristics from the endometrial epithelium. Therefore, further investigation is required to determine whether bacteria present in the cervix can indeed infect the endometrial epithelium.

Microbiota in vagina

The vagina contains approximately 10^{10}–10^{11} bacteria, mainly *Lactobacillus* species, that maintain a low vaginal pH with lactic acid and maintain a relatively balanced microbial ecosystem.[81] Wang et al. reported microbial translocation from the vagina to the uterus and revealed its role in modulating uterine health.[82] They concluded that the transmission of symbiotic bacteria from other sites to the uterine cavity is presumed to occur from the lower genital tract and that only under special conditions, such as during pregnancy, the bacterium could be transferred from the oral cavity or intestine via the bloodstream. Further studies on how bacteria migrate to the vagina, cervix, and uterus are desirable to elucidate upper and lower genital tract diseases, including endometriosis, endometritis, and endometrial cancer, and the development of functional mechanisms and preventive methods is indispensable. Bacterial vaginosis (BV) is a clinical diagnosis related to the vaginal microbiota and is characterized by a low abundance of *Lactobacillus* and an overgrowth of anaerobes.[83] Human studies have shown an association between endometriosis and *Lactobacillus* depletion, as well as the presence of BV-associated bacteria in the cervicovaginal microbiome.[84] Dysbiosis in the vagina may lead to an increase in harmful bacteria in the vaginal microenvironment, some of which could potentially cause ascending infections in the endometrium. The vagina is the area most exposed to the external ecosystem within the female reproductive organs, and enhancing its barrier function may help reduce the development of endometriosis. Probiotics, live microorganisms that offer health benefits, are commonly used for the prevention or treatment of vaginal disorders.[85] These probiotics often contain *Lactobacillus* species, but there is no consensus on which *Lactobacillus* species are most effective for treating BV. A phase 2 trial was conducted to evaluate the effectiveness of *Lactobacillus crispatus* CTV-05 after antibiotic treatment. This probiotic was found to suppress BV recurrence compared with a placebo, suggesting that re-establishing vaginal eubiosis in combination with antibiotic treatment can be useful.[86] Further research is needed to investigate the relationship between BV and endometriosis, determine whether normalizing vaginal bacteria can prevent the development of endometriosis, and assess whether reestablishing a normal bacteria balance after antibiotic treatment is effective in preventing endometriosis recurrence.

Gut microbiota

The gut microbiota is one of the primary microbiomes in the human body, dominated by five bacterial phyla: Firmicutes, Bacteroidetes, Proteobacteria, Actinobacteria, and Verrucomicrobia.[87] The gut microbiota influences the intestinal microenvironment and can impact other organs through the bloodstream. In women, the gut microbiome plays a role in metabolizing estrogen through β-glucuronidase. Deconjugated estrogens, resulting from this process, circulate in the bloodstream and enhance the estrogen/ER signaling in target organs.[88] The term "estrobolome" refers to the gut microbiota genes that encode β-glucuronidase, which is involved in estrogen metabolism.[89] Specific bacterial strains, such as *Lactobacillus* (Phylum: Firmicutes), *Bifidobacterium* (Phylum: Actinobacteria), and *Enterococcus* (Phylum: Firmicutes), can produce β-glucuronidase, which allows estrogen to be reabsorbed in the gut and reenter circulation. This means that the balance of the gut microbiome can directly impact a woman's lifetime estrogen levels and potentially influence estrogen-related diseases, including endometriosis and estrogen-responsive cancers.[90] An increased Firmicutes/Bacteroidetes ratio, which elevates levels of freely circulating estrogen by inducing β-glucuronidase, might be a factor in the development of endometriosis.[91] Additionally, an animal study using a mouse endometriosis model by intraperitoneal injection of endometrial tissue revealed a significant increase in the Firmicutes/Bacteroidetes ratio compared with control mice.[92] Viewed through the lens of the estrobolome, the gut microbiome is an important factor that can influence endometriosis, and this area has been the focus of recent research.[93] Excess estrogen in the bloodstream has systemic effects that go beyond endometriosis, including estrogen-responsive cancers. As a result, a comprehensive assessment is needed, taking into account factors such as gut microbial balance and the body's response. For instance, systemic antibiotic therapy may temporarily reduce the estrobolome in the gut microbiota, but its effects are not permanent and may disrupt the intestinal microbiota's homeostasis, leading to other diseases. Therefore, such treatments must be approached with caution. Another significant aspect of the gut microbiota is that short-chain fatty acids (SCFAs) are derived from the gut microbiota, which are associated with endometriosis.[94,95] Chadchan et al. revealed the influence of SCFAs on endometriosis using a mouse model and mentioned it as a future treatment option for endometriosis. SCFAs are by-products of bacterial fermentation in digestion and are used as an energy source.[96] Among the SCFAs, n-butyrate inhibited endometriotic lesion growth by activating G-protein-coupled receptor 43 (GPR43) and GPR109A, inhibiting histone deacetylase activity, and activating expression of RAP1GAP to inactivate the RAP1 signaling pathway. Endometriosis alters the gut microbiota, thus resulting in reduced production of n-butyrate. They suggested that several suppletive therapies would represent novel treatments for endometriosis. Moreover, they revealed the usefulness of mixed metabolites, namely quinic acid (QA) (including cytosine, 1-methylhistidine, Ng, NG-dimethyl-L-arginine, 2-aminoheptanoic acid, and N-acetyl aspartic acid) to treat endometriotic lesion in the mouse model.[95] Gut microbiota and their derived metabolites are associated with endometriosis pathological conditions; hence, it is also critical to target these factors in new treatments.

Potential therapeutic approaches

In a mouse endometriosis model, broad-spectrum antibiotics reduced the growth of endometriotic lesions by altering intestinal microbiota.[97] Based on this study, the intestinal microbiota influenced peritoneal endometriosis growth, and antibiotics may be a novel non-hormonal therapy for endometriosis. Several other studies have also revealed the potential of antibiotics in rodent endometriosis models.[98,99] Lu et al. observed differences in vaginal microbiota between women with and without endometriosis, noting a decrease in beneficial bacteria, such as *Lactobacillus*, and an increase in harmful bacteria, such as *Gardnerella* and *Atopobium*, in patients with endometriosis.[98] Subsequently, they investigated the effects of broad-spectrum antibiotics in a mouse endometriosis model, focusing on the NF-kB signaling pathway. Their findings suggested that vaginal dysbiosis could promote the progression of endometriosis. However, it is worth noting that there are differences between the vaginal microbiota of female mice and women, necessitating further detailed studies. In another study, Umezawa et al. explored the effectiveness of clarithromycin and telithromycin in a rat endometriosis model.[99] Although they did not specifically identify target bacteria, they proposed that the reduction in the inflammatory response induced by these antibiotics could suppress endometriosis lesions. Our group also investigated the efficacy of antibiotics in a mouse endometriosis model.[76] Our data revealed a novel pathogenic mechanism of endometriosis involving *Fusobacterium* infection in the endometrium, and its eradication by specific antibiotics against *Fusobacterium* is an attractive option for the treatment of endometriosis. This bacterium expresses the adhesion molecule FadA on its surface, allowing it to bind to E-cadherin on host cells.[100] Additionally, *Fusobacterium* possesses another binding lectin called Fap2, which is recognized for its binding to host polysaccharide Gal-GalNAc in colorectal cancer cells.[101] Notably, Gal-GalNAc, also known as Mucin-1, has been reported to be expressed on the surface of human endometrial luminal epithelial cells during the receptive phase.[102] Furthermore, *Fusobacterium* has the capability to bind to pattern recognition receptors, initiating the TLR4/NF-kB signaling pathway in colorectal cancer cells.[103] Considering these facts, it is plausible that *Fusobacterium* could bind to endometrial epithelial cells through interactions such as FadA/E-cadherin binding or Fap2/Gal-GalNAc binding. Once it penetrates the endometrial epithelial cell barrier, *Fusobacterium* may infiltrate stromal lesions within the endometrium and endometriotic lesions, potentially activating the LPS/TRL4/NF-kB signaling pathway. Moreover, these bacteria could persist within the stromal region, influencing the surrounding cells. Given these potential routes of infection within the uterus, the utilization of antibiotics targeting *Fusobacterium* present inside the endometrium holds promise as an effective and logical treatment approach for addressing the pathogenesis of endometriosis. However, the overuse of antibiotics increases the risk of antimicrobial resistance, and its use changes the profile of the microbial community, thereby affecting healthy microbial communities. Clinical trials are warranted to examine the effectiveness of antibiotics for the treatment of endometriosis. Furthermore, it is essential to acknowledge that the use of antibiotics may carry the potential side effect of disrupting the overall microbiota. Therefore, it becomes imperative to develop more targeted and selective antibiotics that specifically address the bacteria associated with endometriosis. Alternatively, the focus should

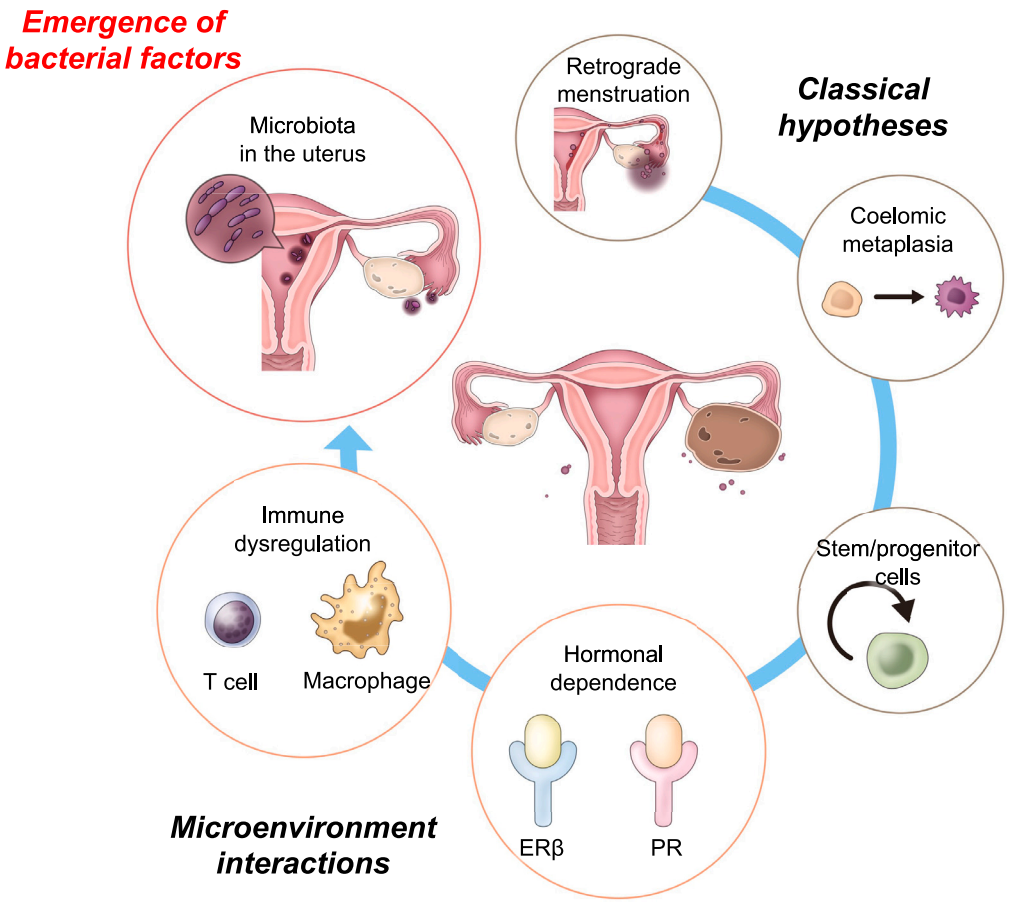

Figure 2. Various hypotheses of endometriosis pathogenesis

Classical hypotheses for endometriosis include retrograde menstruation, coelomic metaplasia, and stem/progenitor cells. Based on these theories, microenvironmental interactions between hormonal dependence and immune dysregulations are important aspects of the pathogenesis of endometriosis. Recently, the presence of bacteria has been highlighted, and various factors have been shown to be involved in the development of endometriosis. ER, estrogen receptor; PR, progesterone receptor.

shift toward creating treatments that center around the intercellular communication and biological signaling pathways influenced by these endometriosis-related bacteria.

The dynamic interplay between bacteria and endometriosis is an area of research that is rapidly expanding, intricately linked with the previously recognized estrogen-dependent and inflammatory aspects of endometriosis. This research is anticipated to yield further insights into the pathological mechanisms underpinning the condition and may pave the way for the development of novel therapeutic strategies.

Conclusion

In this mini-review, we examine the pathogenesis of endometriosis, focusing on its morbidity (Figure 2). The pathogenesis of endometriosis is complex involving many factors and various processes that occur simultaneously. There are interactions between these factors, all of which influence the development and progression of endometriosis. Although many pathological theories have been studied in recent years, no single theory can explain all aspects of endometriosis. Recently, new concepts such as genital microbiota have been proposed, and it is likely that the future perception of endometriosis will incorporate elements of all these etiologic theories. Further research on novel therapeutic targets in the context of endometriosis pathogenesis is warranted.

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DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

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