Trends in Molecular Medicine

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Feature Review

The Immunopathophysiology of Endometriosis

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Endometriosis is a chronic, inflammatory, estrogen-dependent disease characterized by the growth of endometrial tissue outside of the uterine cavity. Although the etiology of endometriosis remains elusive, immunological dysfunction has been proposed as a critical facilitator of ectopic lesion growth following retrograde menstruation of endometrial debris. However, it is not clear whether this immune dysfunction is a cause or consequence of endometriosis. Thus, here we provide in-depth insights into our current understanding of the immunopathophysiology of endometriosis and highlight challenges and opportunities for future research. With the explosion of successful immune-based therapies targeting various chronic inflammatory conditions, it is crucial to determine whether immune dysfunction can be therapeutically targeted in endometriosis.

Highlights

Immunological dysfunction, involving defective immunosurveillance against autologous tissue deposited in the peritoneal cavity, facilitates endometriotic lesion growth in endometriosis patients and ultimately perpetuates disease symptoms.

Conversely, innate and adaptive immune cells from endometriosis patients produce several proinflammatory and blood vessel growth-promoting factors that contribute to hallmarks of disease pathophysiology. Persistent and prolonged endometriosis-associated inflammation further contributes to comorbidities.

Recent studies suggest that endometriotic lesions harbor a unique microenvironment. The interplay between immune cells with stromal and epithelial compartments of lesions is regulated by hormonal pathways.

Targeting dysregulated immune pathways represents a potential avenue for novel therapeutic development that will hopefully not impact fertility.

Endometriosis: A Heterogeneous Inflammatory Disorder

Endometriosis is an enigmatic and often debilitating gynecologic condition that affects an estimated one in ten women of reproductive age worldwide [1]. Commonly associated with symptoms of chronic pelvic pain and infertility, endometriosis can significantly impact the health and quality of life of individuals afflicted by this disease [2,3]. Despite decades of research, non-invasive diagnostic markers for endometriosis are lacking, and no curative treatment is available.

The classical definition of endometriosis refers to the presence of endometrial-like tissue in aberrant locations outside the uterus; however, vast heterogeneity exists among patients regarding phenotypic manifestations of the disease and associated severity of symptoms, if present. Although chronic inflammation and unusually high estrogen concentration are well-established characteristics of endometriosis, the precise etiology of the disease remains largely elusive [4]. This may be attributed to the complex and multifactorial nature of the disease, whereby genetic, endocrine, environmental, and immunological contributions have been previously identified [5]. In particular, the concept of immunological dysfunction has garnered much attention in recent years, as researchers uncover 'innate-adaptive immune pathways' that facilitate the establishment and persistence of endometriotic lesions within the peritoneal cavity.

Thus, we review the literature surrounding the pathophysiology of endometriosis with a focus on immune dysfunction. With the significant associations between immune regulation by endocrine pathways, we further discuss emerging crosstalk between these two axes in the pathogenesis of endometriosis.

Understanding Endometriosis Pathogenesis

The histopathological features of endometriotic lesions show the presence of endometrial, stromal, and glandular tissue. As such, various theories attempt to provide a rationale for

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Box 1. Sampson's Theory of Retrograde Menstruation

This theory postulates that during menstruation, viable endometrial tissue is refluxed into the peritoneal cavity through the fallopian tubes where it subsequently implants into the peritoneal tissue and/or pelvic organs [6]. Evidence for this theory has been demonstrated by the observation of menstrual debris in the peritoneal cavity during menstruation [7,8]. Epidemiological studies have identified that women with primary amenorrhea, other outlet obstructions, and physiological abnormalities in uterine contractility have an increased relative risk for developing endometriosis, perhaps a result of an increased volume of refluxed menstrual debris [9–12]. These observations have been recapitulated in the **iatrogenic, cervical stenosis model** of endometriosis in non-human primates [13]. Taken together, this evidence demonstrates the relevance of viable endometrial effluent to establish as lesions in the peritoneal environment. However, while increased refluxed menstrual effluents may predispose women to endometriosis, one cannot discount the wider prevalence of retrograde menstruation in 76–90% of healthy women [7,8]. Therefore, although this theory provides insight into how endometrial debris is physically disseminated to ectopic regions, particularly the peritoneal cavity, further exploration into the genetic, biochemical, and immunological factors that facilitate survival, invasion, and vascularization of endometriotic lesions is crucial.

Box 2. Immune Dysfunction in Endometriosis

Retrograde menstruation occurs in a majority of women, but endometriosis has been hypothesized to develop in women whose dysregulated innate and adaptive immune mechanisms cannot mount the appropriate response to the refluxed endometrial debris [14]. However, it still remains unclear how or whether immune dysfunction is involved in disease initiation. Previous reports suggest that endometrial fragments from endometriosis patients have increased adhesive capacity [15]. Specifically, the eutopic endometrium of endometriosis patients possesses elevated concentrations of matrix metalloproteinases and plasminogen activator, alluding to the possibility of increased proteolysis of endometrial fragments exiting into the peritoneal cavity via retrograde menstruation and establishing as endometriotic lesions [16,17]. As a natural response of innate and adaptive immune system components to try and clear menstrual debris, immune cell infiltration and resultant tissue repair are initiated. However, the inability to deal with the persistent presence of menstrual debris over time may lead to 'immune system overload' and subsequent 'immune dysfunction'. Nevertheless, it is not clear whether this immune dysfunction is a cause or a consequence of endometriosis.

mechanisms by which uterine tissue is disseminated to ectopic regions, where an environment supporting lesion survival and proliferation is established. The theory of **retrograde menstruation** (see Glossary) proposed by Sampson in 1927 has since become the most widely referenced etiology of endometriosis [6] (Box 1).

Following Sampson's proposition of the retrograde menstruation theory, immune dysfunction has been theorized to facilitate successful lesion development after displacement of endometrial tissue into ectopic locations (Figure 1). Enhanced understanding of immune mechanisms occurring at the site of endometriotic lesion development should therefore provide invaluable insight into disease pathogenesis (Box 2).

The Endometriotic Lesion Immune Microenvironment

Endometriotic lesions establish in a highly complex and dynamic environment that is dominated by inflammatory, angiogenic, and endocrine signals. Consisting of epithelial, stromal, endothelial, glandular, and immune cell components, endometriotic lesions are heterogeneous in nature and display altered immunoinflammatory profiles compared to normal endometrium [18]. Specifically, transcriptomic profiling of inflammation-associated alterations within menstrual-stage-matched endometriotic lesions compared to matched patient 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 compared to eutopic and control endometrial specimens [18]. Furthermore, Suryawanshi *et al.* reported that endometriotic lesions from patients possess a distinct immune

Glossary

Adaptive immunity: arm of the immune system consisting of T and B lymphocytes that is highly specific and responsible for immunological memory.
Alternatively activated macrophage (M2): type of macrophage involved in wound healing and tissue repair that is activated by exposure to certain cytokines such as IL-4, IL-10, or IL-13.
Angiogenesis: the development of new blood vessels from pre-existing vessels through sprouting or intussusception. Angiogenic growth factors include the vascular endothelial growth factor (VEGF) family, angiopoietins (Ang 1/2), fibroblast growth factor (FGF), platelet-derived growth factor-B (PDGF-B), cytokines, and chemokines.
Antibody: also known as an immunoglobulin, is a protein produced by a class of activated B cells called plasma cells of the immune system. These antibodies bind and neutralize antigens to remove them from the body.
B lymphocytes (B cells): cells of the humoral adaptive immune system responsible for mediating the production of antibodies.
Chemokines: class of cytokine proteins that are responsible for immune cell migration.
Classically activated macrophage (M1): type of macrophage that produces high levels of proinflammatory cytokines, mediates resistance to pathogens, and produces reactive nitrogen and oxygen intermediates. Interferon gamma and lipopolysaccharide (LPS) polarize macrophages toward the M1 phenotype.
Cytokines: small proteins involved in signaling between cells and regulation of immune responses.
Damage-associated molecular patterns (DAMPs): host biomolecules that act as endogenous danger signals or alarmins to initiate or perpetuate the inflammatory response.
Dendritic cells (DCs): innate immune cells that function to sample antigens within the surrounding environment and coordinate subsequent immune responses. DCs

microenvironment resembling a tumor-like inflammatory profile [19]. In particular, the complement system was one of the most predominant pathways altered in endometriosis and endometriosis-associated ovarian cancer [19]. While these studies provide insight into major pathways involved in disease pathogenesis, several outstanding knowledge gaps remain: (i) the composition of the endometriotic lesion immune microenvironment and its relationship with different endometriosis stages, phenotypes, and disease symptoms; and (ii) alterations in the functional properties of innate and adaptive immune cells within the lesions and how this may contribute to 'immunosurveillance' defects against autologous tissues.

Innate Immune Mechanisms in Endometriosis

Cell populations of the **innate immune system** that are predominantly implicated in endometriosis pathophysiology include **neutrophils**, macrophages, **natural killer (NK) cells**, and **dendritic cells (DCs)**.

Neutrophil Granulocytes

In the context of endometriosis, increased neutrophil infiltration has been observed in the peritoneal fluid of endometriosis patients compared to that of disease-free women [20,21]. This is likely a result of elevated concentrations of potent neutrophil chemo-attractants such as **IL**-8 present in endometriosis patient plasma and peritoneal fluid [22]. In a mouse model of endometriosis, profound neutrophil infiltration in ectopic uterine tissue peaked early in lesion formation (days 1–5) and subsequently declined (day 6 or 7), suggesting an important role for neutrophils in early stages of lesion development [23]. In support of this claim, early depletion of neutrophils (days 1–3) in BALB/c mice by using the anti-granulocyte receptor-1 (Gr-1) **antibody** resulted in reduced endometriotic lesion formation [24]. It should be noted, however, that the same anti-Gr-1 antibody was reported to reduce both blood neutrophils and Gr-1$^+$ monocytes in mice [25]. As such, the observed effect of reduced lesion formation may not be attributable solely to neutrophils.

At sites of inflammation, macrophages and mast cells have been shown to play a key role in neutrophil recruitment through release of **chemokines**. These two cell populations are elevated in endometriotic lesions and may potentially influence neutrophil recruitment and function [26,27]. Recent studies in mammary involution also suggest that estrogen functions to support neutrophil infiltration and neutrophil-mediated establishment of a pro-tumor microenvironment [28]. It is of interest to consider whether a similar estrogen-induced modification of neutrophil function may be relevant to the pathophysiology of endometriosis. In accordance with the emerging concept of neutrophil heterogeneity, attempts should also be made to establish whether neutrophil phenotype and function are altered by the local microenvironment.

Macrophages

In endometriosis patients, **macrophage** populations are significantly elevated in the peritoneal fluid and eutopic endometrium [29,30]. In addition, macrophages have been shown to invade endometriotic lesions in greater abundance than healthy peritoneum from both endometriosis patients and control women [26].

Functionally, macrophages play a critical role in endometriotic lesion development and inflammation. In particular, peritoneal macrophages from endometriosis patients possess reduced phagocytic capacity, which is associated with decreased expression of the class B scavenger receptor CD36 compared to macrophages from control women [31,32]. Peritoneal macrophages isolated from endometriosis patients also display increased activation of the proinflammatory transcription factor nuclear factor-κB, along with increased protein

process and present antigens to naïve T cells, thereby providing an essential link between the innate and adaptive arms of the immune system.

Iatrogenic, cervical stenosis model: model of endometriosis in non-human primates in which an experimentally induced narrowing or obstruction of the cervix hinders the passage of menstrual debris between the uterus and vaginal canal. This results in increased retrograde menstruation and subsequent development of endometriotic lesions within the peritoneal cavity.

IL: a group of cytokines produced and secreted by a variety of lymphoid and non-lymphoid cells that are involved in inflammation and immune system regulation.

Inflammasome: critical sensor of the innate immune system that when activated, promotes caspase-1-mediated cleavage of the precursor cytokines pro-IL-1β and pro-IL-18 into their bioactive and proinflammatory forms.

Innate immune system: comprised of cells and pathways that provide a first line of defense against exogenous or endogenous danger signals. Innate immune responses are rapid and nonspecific in nature.

Macrophage: a specialized innate phagocytic cell that plays an essential role in the clearance of cellular debris as well as in the release of both inflammatory and chemotactic immune effector molecules.

Natural killer (NK) cells: lymphocytes with both innate and adaptive immune features. These cells possess constitutive cytotoxic and cytokine-producing abilities and can participate in rapid response to viral-infected cells and tumor immunosurveillance.

Neutrophils: short-lived innate immune cells that contain cytoplasmic granules and are rapidly recruited to sites of infection. Recent evidence has revealed emerging roles for neutrophils in cancer and chronic inflammatory conditions.

Regulatory T cells (Tregs): CD4$^+$ immune cells that express the IL-2 receptor alpha chain (CD25) and the transcription factor forkhead box protein P3 (FOXP3). Tregs function

expression of the proinflammatory **cytokines** tumor necrosis factor (TNF)-α, IL-6, and IL-1β [33,34]. Indeed, endometriotic lesion growth is likely enhanced in the presence of macrophages, as the co-culture of macrophages with endometrial stromal cells (ESCs) increases cell proliferation and invasiveness of ESCs [35–37]. In addition, within peritoneal endometriotic lesions, it has been documented that increased macrophage numbers colocalize with nerve fibers [38]. As a result, it has been proposed that these macrophages and nerve fibers interact to subsequently promote endometriosis-associated pain symptoms. Notably, Greaves *et al.* recently identified that estradiol is a key mediator of macrophage–nerve interaction in peritoneal endometriosis by using both *in vitro* cultures and a mouse model [39].

Another aspect of macrophage biology that must be recognized is the plastic nature of these cells and their ability to adopt a **classically activated macrophage(M1)** or **alternatively activated macrophage (M2)** phenotype in response to environmental stimuli. The status of M1-M2 macrophage polarization in endometriosis remains highly debated. This is likely attributable to differences in the stratification of endometriosis patients within a study and the variability in the use of 'true' healthy controls versus women with other benign gynecologic or inflammatory conditions. According to Bacci *et al.*, M2 alternatively activated macrophages are more abundant in the peritoneal fluid of endometriosis patients compared to control women undergoing surgery for uterine leiomyomas [26]. In addition, adoptive intraperitoneal transfer of M2 macrophages in a mouse model facilitated lesion growth and neovascularization, suggesting that these alternatively activated cells play a role in lesion establishment and development [26]. Conversely, Itoh *et al.* reported no difference in peritoneal M2 macrophages between endometriosis patients and women with other benign gynecologic conditions [36]. Moreover, M1 macrophages have been shown to predominate in the eutopic endometrium of endometriosis patients compared to healthy controls [40].

to regulate or suppress other cells of the immune system to control the immune response to self and foreign antigens.

Retrograde menstruation: the flow of menstrual fluid backwards through the fallopian tubes into the peritoneal cavity instead of out of the body.

T lymphocytes (T cells): adaptive immune cells responsible for cell-mediated immunity. These cells can recognize foreign antigens by specific cell surface receptors and release cytokines. T cells are further divided into subsets according to their function.

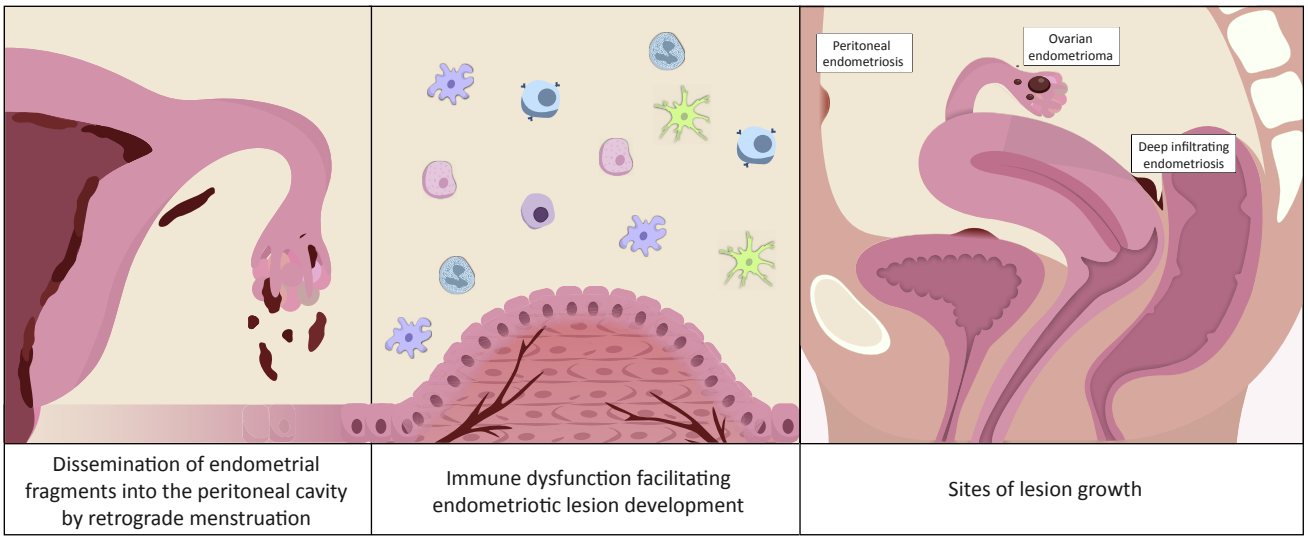

Figure 1. Endometriosis Pathogenesis: Retrograde Menstruation and Immune Dysfunction. Based on Sampson's theory of retrograde menstruation, viable endometrial fragments are first refluxed into the peritoneal cavity during menstruation. The subsequent ability of endometrial debris to implant, proliferate, establish vascular supply, and persist in characteristic locations such as the peritoneum, ovary, and recto-uterine pouch is theorized to be facilitated by immune dysfunction, involving defects in immunosurveillance against autologous cells or immune system overload from the persistent presence of endometrial debris within the peritoneal cavity.

Based on these findings, it is apparent that the M1-M2 polarization paradigm is indeed altered in endometriosis. However, the challenge remains in identifying the primary mediators of these activation states in endometriosis as well as the functional contribution of these M1-M2 populations to disease pathophysiology. Given the complex nature of the lesion microenvironment, it is likely that macrophage polarization in endometriosis lies on a continuum of functional states and varies with disease phenotype.

NK Cells

Populations of NK cells exist in both the peripheral circulation and uterus and are predominantly characterized as $CD56^{dim}CD16^{+}$ and $CD56^{bright}CD16^{-}$, respectively [41]. In endometriosis, it has been well established that the local peritoneal environment suppresses NK cell activity and that NK cells from endometriosis patients exhibit aberrant expression of various activating and inhibitory receptors on their cell surface compared to healthy women [42–45]. Furthermore, elevated peritoneal fluid concentrations of MHC class I chain-related proteins A and B (MICA, MICB), soluble ligands for NKG2D, an activating C-type lectin-like NK cell receptor, have been reported in endometriosis patients compared to disease-free women [46]. Notably, increased MICA and MICB shedding has been proposed as a strategy for tumors to evade immunosurveillance and inhibit NK cell action [47,48]. As such, it has been speculated that NK cell dysfunction in endometriosis contributes to immune escape of ectopic endometrial fragments within the peritoneal cavity.

In recent years, progress has been made in identifying factors that may impair NK cell functionality in endometriosis, as researchers aim to elucidate the roles of specific cytokines and chemokines in disease pathophysiology. Both IL-6 and transforming growth factor (TGF)-β1 in the peritoneal fluid of women with endometriosis have been shown to reduce NK cell cytolytic activity [49,50]. Specifically, IL-6 downregulated NK cell cytolytic granule components by modulating Src homology region 2-containing protein tyrosine phosphatase-2 [50]. In addition, Yu *et al.* demonstrated that IL-15, which is highly expressed by ESCs in ectopic endometrium, also inhibits the killing activity of human NK cells *in vitro* [51]. To imitate the local microenvironment in endometriosis, Yang *et al.* co-cultured ESCs from endometriosis patients with both monocyte-derived macrophages and NK cells and reported that ESC–macrophage interaction downregulated cytotoxicity of NK cells, possibly through increased release of IL-10 and TGF-β [52].

While decreased NK cell cytotoxicity in endometriosis potentially contributes to the defective immunosurveillance against autologous cells, comprehensive analyses of activating and inhibitory receptors on NK cells and their potential modulation with immune checkpoint inhibitors are critically required. Attempts should also be made to establish whether tissue-resident NK cells in the endometriotic lesion microenvironment are of endometrial or peripheral blood origin, as these cells populations likely have distinct roles in endometriosis pathophysiology.

DCs

Currently, the literature pertaining to DC function in endometriosis is lacking. While it has been reported that DCs are present within endometriosis patient peritoneal fluid, their absolute numbers are not significantly altered compared to disease-free women [21]. Conversely, it was recently documented that endometriosis patient peritoneal fluid contains elevated $BDCA1^{+}$ myeloid DCs expressing the mannose receptor [53]. These mannose receptor-expressing DCs are capable of phagocytosing dead ESC debris, but in doing so they may contribute to the inflammatory profile observed in patients through secretion of IL-6 and IL-1β [53]. In addition,

Schulke *et al.* demonstrated that the immunohistological densities of $CD1a^+$ (immature) and $CD83^+$ (mature) DC populations are indeed altered in eutopic and ectopic endometrial tissues from endometriosis patients [54].

In vivo evidence of DC function in endometriosis presents with contradictory findings. According to Stanic *et al.*, conditional DC depletion using the diphtheria toxin in a mouse model of endometriosis resulted in increased lesion size and reduced expression of the T cell activation marker CD69 [55]. These findings suggest that DCs attenuate lesion development through activation of T lymphocytes [55]. Similarly, findings from Fainaru *et al.* suggested that DCs infiltrate surgically induced endometriotic lesions in mice and predominate around the vasculature of the lesion [56]. Further adoptive transfer of bone marrow-derived DCs in mice resulted in enhanced lesion growth and vascularization [56]. By contrast, by ablating DCs using the diphtheria toxin approach, Pencovich *et al.* demonstrated that lesions were approximately fivefold smaller, thereby suggesting that the development of endometriotic lesions is dependent on the presence of endogenous DCs [57]. Besides the observed differences in the role of DCs in endometriosis, it should be noted that these studies did not account for the influence of hormones on lesion development as well as on the activation state and functional maturity of DCs as expected in endometriosis patients. Thus, further research is required to fully establish whether DCs promote or repress lesion formation, as well as the specific mechanisms through which they do so.

Other Mediators of Innate Immunity: Alarmins

Alarmins, also known as **damage-associated molecular patterns (DAMPs)**, are immunostimulatory endogenous molecules that are released in response to tissue injury or inflammation. An example of an alarmin present in menstrual fluid is high-mobility group box 1 (HMGB1) [58]. HMGB1 can act as a proangiogenic factor and inflammatory mediator through the activation of receptor for advanced glycation end products (RAGE) and toll-like receptor-4 pathways. It is logical to assume that the presence of HMGB1 in menstrual fluid may contribute to the pathophysiology of endometriosis following retrograde menstruation, as the RAGE receptor is expressed in both eutopic endometrium and peritoneal endometriotic lesions [58]. In addition to the implications of HMGB1 on increased ESC proliferation, treatment of endometriotic stromal cells with recombinant HMGB1 has been shown to stimulate vascular endothelial growth factor (VEGF) production [58,59]. Given the emerging role of HMGB1 in the development of cancer and chronic inflammatory diseases including rheumatoid arthritis and systemic lupus erythematosus, further attempts should be made to elucidate the functional implications of HMGB1 in endometriosis [60]. Similarly, circulating cell-free DNA in the plasma and serum of endometriosis patients has garnered attention in recent years as a potential non-invasive biomarker for endometriosis [61,62]. However, common to other biomarkers, the clinical cutoffs, sensitivity, and specificity of cell-free DNA as a biomarker have yet to be established.

IL-33 is another alarmin that has been associated with endometriosis [63,64]. IL-33 exerts signaling by binding to its receptor, suppressor of tumorgenicity 2 (ST2), which is expressed by lymphoid and myeloid immune cells as well as non-immune cells including endothelial cells, fibroblasts, and epithelial cells [65]. In recent years, IL-33 has emerged as a critical regulator of many chronic inflammatory and fibrotic diseases [65,66]. In the context of endometriosis, elevated concentrations of IL-33 were observed in the serum and peritoneal fluid of women with deep infiltrating endometriosis patients compared to healthy controls [64]. Our group recently demonstrated that endometriotic lesions from advanced stage endometriosis patients express significantly higher levels of IL-33 protein compared to healthy, fertile controls and that the

endometriotic lesions express the ST2 receptor [63]. This suggests that the endometriotic lesion not only contributes to the production of IL-33 but also responds to IL-33 present in the microenvironment. In a mouse model of endometriosis, we demonstrated that intraperitoneal injections of IL-33 stimulated systemic inflammation and contributed to the growth and vascularization of the lesion [63]. Recently, IL-33 neutralizing monoclonal antibodies have been explored as a potential therapeutic strategy for several chronic inflammatory conditions including asthma and atopic dermatitis [67]. Considering recent evidence suggesting an active role of IL-33 in the progression of endometriosis, neutralization of IL-33 may also serve as a potential avenue for therapeutic intervention.

Despite the increasing body of evidence that portends to the involvement of alarmins in endometriosis pathophysiology, the functional and mechanistic implications of these molecules within the endometriotic lesion microenvironment have yet to be fully described. As modulators of immune cells, it will be prudent to delineate the primary sources of these alarmins in endometriosis.

Adaptive Immune Mechanisms in Endometriosis

The cell-mediated and humoral components of **adaptive immunity** that are regulated by **T lymphocytes (T cells)** and antibodies produced by **B lymphocytes (B cells)**, respectively, have also been implicated in endometriosis pathophysiology.

Cell-Mediated Immunity: Effector T Lymphocytes

Effector T cells or T helper (T_H) cells are $CD4^+$ cells that can be categorized into four subsets: T_H1, T_H2, T_H17, and **regulatory T cells (Tregs)**. Because of the presence of elevated type 2 cytokines in the plasma and peritoneal fluid of endometriosis patients, endometriosis has been characterized as skewed toward a T_H2 immune response [68,69]. However, the typical T_H2 response, associated with wound healing and fibrosis, has not been fully elucidated in the context of endometriosis [70].

The percentage of T_H17 cells in the peritoneal fluid of stage III/IV endometriosis patients has been shown to be significantly higher than in the peritoneal fluid of stage I/II patients [71]. Similarly, another study demonstrated that IL-10^+T_H17 cells are increased in the peritoneal fluid in endometriosis and that the percentage of these IL-10^+T_H17 cells increased with disease severity [72]. Our group and others have shown that IL-17, the major cytokine product of T_H17 cells, is elevated in endometriosis patient plasma and peritoneal fluid and that IL-17 could be contributing to the progression of endometriosis by stimulating the production of cytokines that induce **angiogenesis** and inflammation [73,74]. Taken together, the presence of increased T_H17 cells and IL-17 in the peritoneal fluid of endometriosis patients provides evidence that IL-17 likely contributes to endometriosis pathophysiology by promoting chronic inflammation. In recognition of its established role in chronic inflammatory conditions, the T_H17 pathway has been an attractive therapeutic target [75]. Several Phase I, II, and III clinical trials are being conducted targeting IL-17 or the IL-17 receptor in psoriasis, rheumatoid arthritis, Crohn's disease, asthma, and multiple sclerosis [76].

Currently, the role of Tregs remains largely unclear and controversial in the endometriosis literature [77]. General associations between endometriosis and Tregs have been made such as the association between FOXP3 polymorphisms and endometriosis [78] and the presence of cytokines and chemokines known to be involved in Treg processes [79,80]. Despite proposed

correlations, mechanistic evidence demonstrating their role in the disease pathology is lacking. It has also been suggested that Tregs likely play a role in the two main symptoms of endometriosis: subfertility and pelvic pain [81,82].

Elevated numbers of $CD4^+CD25^+Foxp3^+$ Tregs have been observed in the peritoneal fluid of endometriosis patients compared to healthy, fertile controls, whereas peripheral blood Tregs were reduced [83]. This observation was substantiated by a study demonstrating that suppressive Tregs were elevated in the peritoneal fluid of endometriosis patients compared to control women, with no alterations to peripheral blood Treg populations [84]. Tregs have also been shown to be present in ectopic lesions; however, their numbers have not been shown to be consistently elevated but rather, highly variable [85]. In the baboon model of endometriosis, ectopic lesions had an increased Treg population, while peripheral blood and eutopic endometrial Treg populations were decreased [81]. This evidence, from both human samples and the baboon model, is suggestive of the localized suppressive function of Tregs at the site of lesion development. However, there is no evidence to suggest stage-specific differences in the Treg population as no differences in the levels of Tregs in the peritoneal fluid or plasma have been shown between stage I/II and stage III/IV patients [86]. Based on the presence of Tregs, it is likely that they could be contributing to endometriosis pathophysiology, but without experimental evidence demonstrating cause and effect, it would be purely speculative to establish how and whether Tregs may be involved.

The effect of aberrant local or systemic Treg populations in endometriosis patients has not been fully elucidated; however, Tregs likely play a role in inflammation, proliferation, angiogenesis, and lesion invasion. CCL25 has been shown to be upregulated in endometriosis patients and produced by endometriotic stromal cells and macrophages [87]. CCL25 stimulates Treg differentiation and the production of IL-10 and TGF-β while suppressing peripheral Treg apoptosis [80]. Tregs can also enhance ESC proliferation and invasion *in vitro*; however, this process is dependent on the production of IL-10 and TGF-β [80]. Notably, co-culturing ESCs and monocytes increased Treg chemotaxis and production of TGF-β by Tregs [88]. In mouse models that ablated Tregs, increased macrophage activation and effector T cell numbers as well as increased lesion growth were observed [84,89]. Therefore, it is becoming increasingly apparent that a synergistic effect of many cell types including macrophages, endometrial cells, endothelial cells, and Tregs along with soluble factors such as cytokines and chemokines are facilitating this invasive, pro-angiogenic, proliferative state, where the lesion can adhere and grow.

Humoral Immunity: B Lymphocytes

Since the first characterization of endometriosis as an autoimmune disorder, various reports have confirmed the presence of anti-endometrial antibodies in the serum and peritoneal fluid of endometriosis patients [90,91]. In general, it is understood that autoantibodies likely contribute to endometriosis by stimulating the immune system and perpetuating inflammation; however, the mechanistic evidence is lacking and is mostly speculative. The presence of autoantibodies in endometriosis patients is further complicated by various comorbidities. Specifically, there is a clear association between endometriosis and the presence of other immunological and autoimmune-mediated diseases such as rheumatoid arthritis, psoriasis, and allergies [5,92]. As such, these comorbidities should be taken into account when attempting to isolate specific immune-mediated components of endometriosis pathophysiology. Because of the presence of autoantibodies, B cell populations have been investigated in the context of endometriosis. In a recent meta-analysis examining 22 studies related to the role of B cells in endometriosis, most

of the studies reported the increased presence or activation of B cells in endometriosis patients [93]. Alternatively, seven of 22 studies observed no differences in B cell numbers between endometriosis patients and controls [93].

In addition to producing antibodies, B lymphocytes function to produce cytokines such as IL-6, granulocyte-macrophage colony–stimulating factor, and IL-17, which have been shown to modulate immune cells such as $CD4^+$ T cells and perpetuate chronic inflammatory diseases [94]. These cytokines have also been associated with endometriosis [22,73,95]; therefore, B cells may be involved in the systemic and local production of cytokines in endometriosis and may be contributing to the inflammatory microenvironment. Furthermore, B cell lymphoma 6 (BCL6), a protein required for B cell development, has been shown to be increased in the eutopic endometrium of endometriosis patients and associated with progesterone resistance and endometriosis-associated infertility [96,97]. In discordance, others have shown that BCL6 mRNA expression is decreased in the peritoneal fluid of endometriosis patients [98]. In addition, B lymphocyte stimulator is a protein that has been shown to be upregulated in ectopic endometriotic lesions compared to matched eutopic tissue and has been implicated in differentiating B cells toward a B1 response, suggesting that peritoneal B cells may act independently of T cells [99]. Notably, through the use of knockout mouse models, it has been suggested that simultaneous B cell and T cell deficiency does not directly affect growth or angiogenesis to the endometriotic lesion [100]. Further studies are therefore warranted to fully understand the role of B cells and their interaction with other immune cells within the endometriotic lesion microenvironment (Figure 2).

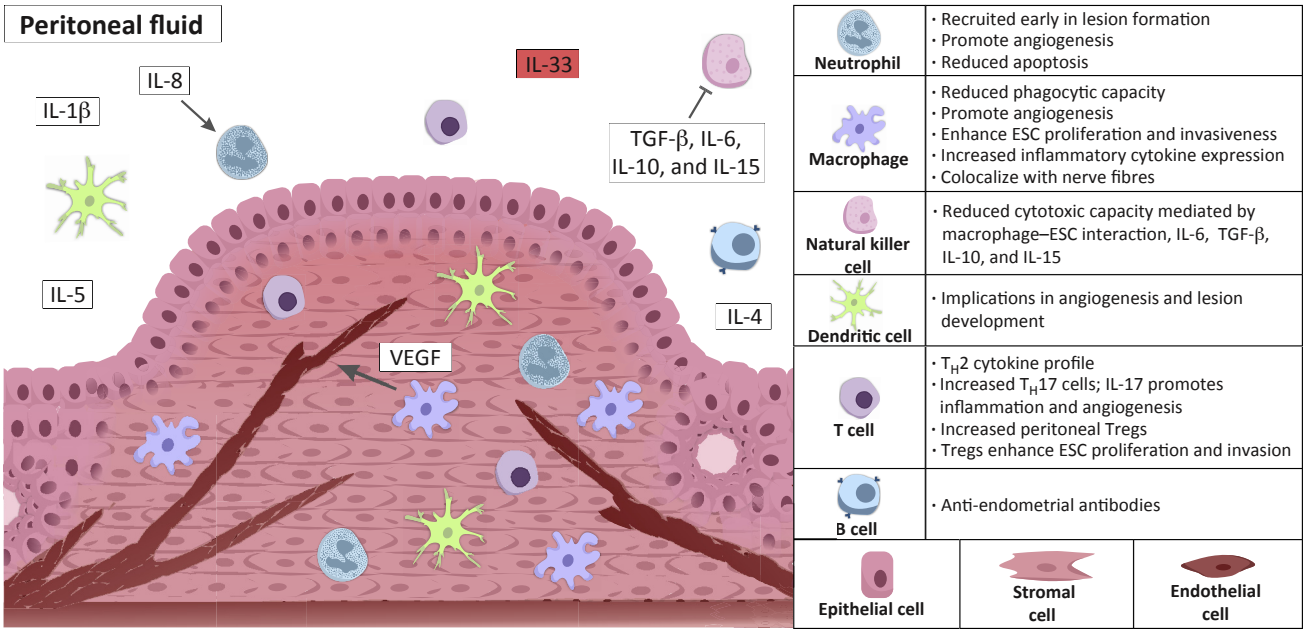

Figure 2. Schematic Representation of the Endometriotic Lesion Immune Microenvironment. Endometriotic lesions consist of epithelial, stromal, and endothelial cell components that interact with various infiltrating immune cell populations. Within the surrounding peritoneal fluid, immune cell types including macrophages, neutrophils, natural killer cells, dendritic cells, and B and T lymphocytes are present along with numerous cytokines (e.g., TNF-α, IL-10, and TGF-β), chemokines (e.g., IL-8), angiogenic growth factors (e.g., VEGF), and alarmins (e.g., IL-33). Emerging roles for each immune cell type in endometriosis pathophysiology are outlined. ESC, endometrial stromal cell; TGF, transforming growth factor; T_H, T helper; T_H2, T helper type 2 immune response; TNF, tumor necrosis factor; Treg, regulatory T cell; VEGF, vascular endothelial growth factor.

Immune-Angiogenesis Axis in Endometriosis

Angiogenesis, a process regulated by immune cells and related mechanisms, is integral to the pathogenesis of endometriosis. Similar to tumors, endometriotic lesions must establish a new blood supply to support survival and growth. Indeed, dense vascularization is characteristic of endometriotic lesions, which has led to the idea that suppression of blood vessel growth (antiangiogenic therapy) may be a successful therapeutic approach for endometriosis. The potential effectiveness of antiangiogenic therapies has been assessed in limited animal studies, but there are no reported human clinical trials for testing these therapies in endometriosis.

Surgical removal of endometriotic lesions has been shown to decrease serum VEGF in patients, suggesting that lesion presence in the peritoneum has a profound effect on the microenvironment [101]. Lesions could directly contribute VEGF secretion to the peritoneal fluid, or their presence could drive an immune reaction that promotes an inflammatory and pro-angiogenic environment. In human endometriotic lesions, VEGF expression has been localized to the stroma, glandular epithelium [102,103], and infiltrating macrophages [103,104]. Furthermore, analogous immune cell infiltration to endometriotic lesions and angiogenic activity have been described in animal models. In mice, depletion of macrophages using chlodronate liposomes, monoclonal antibodies, or ganciclovir has been shown to decrease lesion weight and vascularity [26,105]. Similarly, cabergoline treatment to reduce infiltration of macrophages and mast cells in a mouse model decreased the number of immature vessels in lesions [106]. 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, lipopolysaccharide (LPS), and estrogen enhanced VEGF release by these cells [23]. These findings clearly suggest that immune cells create a pro-angiogenic peritoneal microenvironment in endometriosis.

In addition to their effects on immune cell recruitment, various chemokines including CXCL12 [stromal cell-derived factor-1 (SDF-1)] can also have pro-migratory effects on endothelial cells. CXCL12 is a constitutively expressed and inducible chemokine that regulates multiple physiological processes, including embryonic development and organ homeostasis, and has also been implicated in endometriosis pathophysiology [107,108] (Box 3).

Box 3. The CXCL12/CXCR4 Axis and Angiogenesis

Until recently, a single cognate receptor for CXCL12 (SDF-1), CXCR4, was known. CXCR4 is widely and constitutively expressed by numerous cells and tissues, including hematopoietic and endothelial cells [109]. The crucial roles of CXCL12 and CXCR4 in embryonic vasculogenesis were demonstrated by the blood vessel abnormalities manifested in $CXCL12^{-/-}$ and $CXCR4^{-/-}$ mice [110]. Indeed, the expression of CXCL12 in a large number of tumors and injured tissues strongly suggests that activation of CXCR4 participates in promoting neo-angiogenesis. An intensely debated issue regarding the role of the CXCL12/CXCR4 axis in neo-angiogenesis is the 'decoy' (scavenger) function of a recently identified CXCL12 receptor, known as CXCR7 [111]. Despite its phylogenetic and ligand binding similarities with CXCR4, CXCR7 does not mediate typical chemokine receptor responses such as leukocyte trafficking. Only one report describes CXCR7 as minimally expressed in the endometrium across the menstrual cycle and upregulated in decidual tissue as well as in isolated ESCs treated with estrogen or progesterone [112]. Recently, Isaacson *et al.* demonstrated that uropathogenic *Escherichia coli* stimulated production of CXCL12 by bladder epithelial cells, which induced recruitment of NK cells, T cells, and neutrophils to the infected bladder in mice [113]. Blocking of CXCL12 led to reduction in the immune cells and exacerbation of infection, clearly pointing toward an important role of CXCL12 in modulating immune cell recruitment. Hypoxia and tissue injury within the inflammatory niche are some of the major drivers of CXCL12 production. CXCL12 in concert with VEGF aids in recruitment of bone marrow-derived hematopoietic and endothelial progenitor cells, their differentiation, and incorporation in the newly formed blood vessels. Under the influence of hypoxia, CXCL12 production is regulated by hypoxia-inducible factor (HIF)-1α [114]. Blocking of the CXCL12/CXCR4 axis has been shown to reduce EPCs in the ischemic sites [115].

Our group and others have demonstrated increased expression of hypoxia-inducible factor (HIF)-1α [116] and CXCL12 [107] in endometriotic lesions as well as increased CXCL12 in the systemic circulation of endometriosis patients [108]. Blocking of the CXCL12/CXCR4 axis by using AMD3100 led to significant reduction of endothelial progenitor cells in mouse endometriotic lesions and reduced lesion size and vascularization in mice treated with CXCL12 neutralizing antibody compared to control mice receiving isotype antibody [107,108]. Thus, these studies highlight a therapeutic strategy that potentially interferes with early endometriotic lesion establishment by targeting hematopoietic and endothelial progenitor cells in the angiogenic context as well as immune cells in the inflammatory context.

Immune-Endocrine Interactions in Endometriosis

The estrogen-dependent nature of endometriosis is well established. Local biosynthesis of estradiol by endometriotic lesions in concert with pronounced inflammation within the peritoneal cavity fosters an aberrant immune-endocrine microenvironment that is ideal for growth and survival of ectopic lesions [4].

Estradiol exerts its downstream actions through the nuclear estrogen receptors (ERs) ERα and ERβ. Not only is the expression of these receptors altered in endometriotic tissue but also ER signaling has been shown to be necessary for lesion establishment in a syngeneic mouse model [117–119]. More specifically, Burns *et al.* demonstrated that ERα activity drives proliferation, adhesion, and angiogenesis of ectopic lesions [119]. An E_2/ERα/IL-6 immune-mediated signaling axis has also been recently implicated in early endometriosis development in a mouse model [120]. In addition, Han *et al.* revealed the presence of a truncated 70-kDa isoform of steroid receptor co-activator-1 (SRC-1) in endometriotic tissues, which was later shown to interact with ERβ [121,122]. Notably, they demonstrated that, TNF-α, a prominent proinflammatory cytokine in endometriosis, activated matrix metalloproteinase 9-mediated generation of the truncated SRC-1 isoform to promote resistance to apoptosis and ectopic lesion growth [121].

Recently, Han *et al.* sought to elucidate the specific mechanisms through which enhanced ERβ expression in endometriotic tissues contributes to disease progression [122]. Using an ERβ-selective antagonist as well as ERβ knockout and overexpression mouse models, they demonstrated that ERβ promotes ectopic lesion growth through enhanced proliferative activity and reduced apoptotic signaling. Intriguingly, they also revealed a critical role for ERβ in activation of the innate NOD-like receptor pyrin domain-containing 3 **inflammasome**, which perpetuates IL-1β production and lesion growth [122]. In attempt to simultaneously target estrogenic and inflammatory activities in endometriosis, Zhao *et al.* developed the ER antagonists chloroindazole and oxabicycloheptene sulfonate that primarily mediate their actions through ERβ and ERα, respectively [123]. Both ligands were individually able to suppress lesion growth in a mouse model through a reduction in proliferation, vascularization, inflammatory cytokine production, and macrophage infiltration. It is also important to note that neither ligand impacted fertility or estrous cycling at doses effective at suppressing endometriosis [123].

These findings provide invaluable mechanistic insight into how immune and endocrine pathways can converge to promote disease progression in endometriosis. Ultimately, given that excessive estrogen stimulation, immune dysfunction, and angiogenesis are characteristic features of endometriosis, the simultaneous targeting of these pathways represents a challenging, yet exciting opportunity for novel therapeutic development.

Concluding Remarks

In recent years, substantial progress has been made in understanding the cellular and molecular mediators underlying dysfunctional immune responses in endometriosis. However, based on the current literature, we are still not in a position to challenge the central dogma of whether the phenotypic and functional immune adaptations in endometriosis patients facilitate endometriotic lesion establishment and progression, or whether it is merely a bystander effect. To date, most of the immune-based studies have been observational, measuring immune cells or their cytokine products in isolation or combination in the systemic circulation and peritoneal fluid. Moreover, the lack of spontaneously menstruating animal models that recapitulate human disease (except for Old World primates) along with the chronic and heterogeneous nature of the disease, lengthy diagnostic delays of 7–12 years, and the confounding influence of concurrent hormonal and pain-related interventions further complicate the cause-and-effect relationship in the immune context. There is also currently no consensus as to how endometriosis is acquired, or whether genetic, endocrine, immunological, and lifestyle-related factors influence disease pathophysiology separately or in combination. Resultantly, designing mechanistic studies to pinpoint the most relevant immune components of disease pathogenesis remains a paramount challenge. An opportunity to establish a cause-and-effect relationship of certain immune factors lies in conducting a longitudinal study focused specifically on immune transcriptomic alterations and cellular phenotyping in matched ectopic and eutopic endometrium of well stratified patients for disease stage, lesion type, and menstrual cycle phase in comparison with healthy women. It would also be prudent to establish whether hormonal or non-hormonal interventions impact the immunoregulatory and inflammatory pathways in the same longitudinal studies.

Now that we have enhanced understanding about issues surrounding immunosurveillance, 'immune evasion,' and innate-adaptive immune pathways in endometriosis, it is an exciting time to study the endometriotic lesion immune microenvironment more closely than ever in attempt to identify novel therapeutic targets (Box 4). There is a desperate unmet need to find new avenues for diagnosis and therapeutic targeting of endometriosis specifically without compromising patient's reproductive capacity, if desired. The challenges are daunting given that multiple immune-inflammatory pathways, immune-endocrine-angiogenesis interactions, and crosstalk between stromal, epithelial, and immune compartments create a complex puzzle. A comprehensive understanding of endometriosis immunopathophysiology may potentially be attained once we fully integrate all of these underlying components and elucidate the mechanisms through which they interact (see Outstanding Questions). Ultimately, with the

Outstanding Questions

How does the endometriotic lesion microenvironment contribute to the plasticity of immune cells and their functional adaptations? How is the localized and systemic inflammatory milieu in endometriosis patients influenced by this lesion microenvironment?

How do immune-endocrine interactions shape the establishment and subsequent survival of endometriotic lesions? What influence do estrogen and progesterone have on the recruitment and function of relevant innate and adaptive immune cell populations in endometriosis?

How do we establish molecular and phenotypic characteristics of endometriosis lesion types?

How do we establish chronic inflammation clinically in endometriosis patients? What are the specific markers of chronic inflammation?

Does the immune microenvironment dictate the progression from stage I–IV, and/or does this environment/immune profile change throughout the natural history of disease?

Can immune dysfunction be therapeutically targeted using specific inflammatory pathways without affecting fertility?

Box 4. Clinician's Corner

- Given the multi-factorial etiology of endometriosis, it remains a difficult disease to diagnose and treat. The majority of current treatment options solely target symptoms and have significant side effects. Prevention of the disease is currently not feasible.
- The location, stage, and heterogeneous nature of lesions and associated symptoms do not always correlate with disease severity. Modeling endometriosis in animal models also remains a challenge as only Old World primate species spontaneously develop endometriosis. This has led to major knowledge gaps in endometriosis pathogenesis.
- There is a real need to establish sensitivity, specificity, and clinical utility of several biomarkers that have been identified in recent years [92]. This will substantially reduce diagnostic delays and invasive surgical approaches for confirmatory diagnosis.
- Given the recent success of immune-modulatory therapeutics targeting chronic inflammation in other inflammatory diseases, it may be possible to target immune dysfunction in endometriosis in the future. This approach will hopefully not have fertility-related side effects as experienced with some of the widely prevalent hormonal interventions.

application of high-throughput next-generation sequencing platforms, single-cell genomics, and proteomics approaches, endometriosis patient management can be significantly improved via the identification of therapeutically targetable biological pathways.

Acknowledgments

The authors acknowledge funding from Canadian Institutes of Health Research operating grants (M.K., C.T.).

References

1. Giudice, L.C. (2010) Clinical practice: endometriosis. *N. Engl. J. Med.* 362, 2389–2398
2. Dunselman, G.A.J. *et al.* (2014) ESHRE guideline: management of women with endometriosis. *Hum. Reprod.* 29, 400–412
3. Nnoaham, K.E. *et al.* (2011) Impact of endometriosis on quality of life and work productivity: a multicenter study across ten countries. *Fertil. Steril.* 96, 366–373.e8
4. Giudice, L.C. and Kao, L.C. (2004) Endometriosis. *Lancet* 364, 1789–1799
5. Parazzini, F. *et al.* (2017) Epidemiology of endometriosis and its comorbidities. *Eur. J. Obstet. Gynecol. Reprod. Biol.* 209, 3–7
6. Sampson, J.A. (1927) Peritoneal endometriosis due to the menstrual dissemination of endometrial tissue into the peritoneal cavity. *Am. J. Obstet. Gynecol.* 14, 422–469
7. Halme, J. *et al.* (1984) Retrograde menstruation in healthy women and in patients with endometriosis. *Obstet. Gynecol.* 64, 151–154
8. Liu, D.T. and Hitchcock, A. (1986) Endometriosis: its association with retrograde menstruation, dysmenorrhoea and tubal pathology. *Br. J. Obstet. Gynaecol.* 93, 859–862
9. Barbieri, R.L. (1998) Stenosis of the external cervical os: an association with endometriosis in women with chronic pelvic pain. *Fertil. Steril.* 70, 571–573
10. Nawroth, F. *et al.* (2006) Is there an association between septate uterus and endometriosis? *Hum. Reprod.* 21, 542–544
11. Salamanca, A. and Beltrán, E. (1995) Subendometrial contractility in menstrual phase visualized by transvaginal sonography in patients with endometriosis. *Fertil. Steril.* 64, 193–195
12. Sanfilippo, J.S. *et al.* (1986) Endometriosis in association with uterine anomaly. *Am. J. Obstet. Gynecol.* 154, 39–43
13. D'Hooghe, T.M. *et al.* (1994) Development of a model of retrograde menstruation in baboons (*Papio anubis*). *Fertil. Steril.* 62, 635–638
14. Ahn, S.H. *et al.* (2015) Pathophysiology and immune dysfunction in endometriosis. *BioMed Res. Int.* 2015, 795976
15. Klemmt, P.A.B. *et al.* (2007) Endometrial cells from women with endometriosis have increased adhesion and proliferative capacity in response to extracellular matrix components: towards a mechanistic model for endometriosis progression. *Hum. Reprod.* 22, 3139–3147
16. Gilabert-Estellés, J. *et al.* (2003) Expression of several components of the plasminogen activator and matrix metalloproteinase systems in endometriosis. *Hum. Reprod.* 18, 1516–1522
17. Sillem, M. *et al.* (1997) Soluble urokinase-type plasminogen activator receptor is over-expressed in uterine endometrium from women with endometriosis. *Mol. Hum. Reprod.* 3, 1101–1105
18. Ahn, S.H. *et al.* (2016) Immune-inflammation gene signatures in endometriosis patients. *Fertil. Steril.* 106, 1420–1431.e7
19. Suryawanshi, S. *et al.* (2014) Complement pathway is frequently altered in endometriosis and endometriosis-associated ovarian cancer. *Clin. Cancer Res.* 20, 6163–6174
20. Milewski, Ł. *et al.* (2011) 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.* 91, 64–70
21. Tariverdian, N. *et al.* (2009) Intraperitoneal immune cell status in infertile women with and without endometriosis. *J. Reprod. Immunol.* 80, 80–90
22. Monsanto, S.P. *et al.* (2016) Surgical removal of endometriotic lesions alters local and systemic proinflammatory cytokines in endometriosis patients. *Fertil. Steril.* 105, 968–977.e5
23. Lin, Y.-J. *et al.* (2006) Neutrophils and macrophages promote angiogenesis in the early stage of endometriosis in a mouse model. *Endocrinology* 147, 1278–1286
24. Takamura, M. *et al.* (2016) Neutrophil depletion reduces endometriotic lesion formation in mice. *Am. J. Reprod. Immunol.* 76, 193–198
25. Daley, J.M. *et al.* (2008) Use of Ly6G-specific monoclonal antibody to deplete neutrophils in mice. *J. Leukoc. Biol.* 83, 64–70
26. Bacci, M. *et al.* (2009) 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.* 175, 547–556
27. Sugamata, M. *et al.* (2005) Increase of activated mast cells in human endometriosis. *Am. J. Reprod. Immunol.* 53, 120–125
28. Chung, H.H. *et al.* (2017) Estrogen reprograms the activity of neutrophils to foster protumoral microenvironment during mammary involution. *Sci. Rep.* 7, 46485
29. Berbic, M. *et al.* (2009) Macrophage expression in endometrium of women with and without endometriosis. *Hum. Reprod.* 24, 325–332
30. Hill, J.A. *et al.* (1988) Characterization of leukocyte subpopulations in the peritoneal fluid of women with endometriosis. *Fertil. Steril.* 50, 216–222
31. Chuang, P.-C. *et al.* (2009) Downregulation of CD36 results in reduced phagocytic ability of peritoneal macrophages of women with endometriosis. *J. Pathol.* 219, 232–241
32. Chuang, P.-C. *et al.* (2010) Inhibition of CD36-dependent phagocytosis by prostaglandin E2 contributes to the development of endometriosis. *Am. J. Pathol.* 176, 850–860
33. Lousse, J.-C. *et al.* (2008) Increased activation of nuclear factor-kappa B (NF-kappaB) in isolated peritoneal macrophages of patients with endometriosis. *Fertil. Steril.* 90, 217–220
34. Montagna, P. *et al.* (2008) Peritoneal fluid macrophages in endometriosis: correlation between the expression of estrogen receptors and inflammation. *Fertil. Steril.* 90, 156–164
35. Chan, R.W.S. *et al.* (2017) Co-culture with macrophages enhances the clonogenic and invasion activity of endometriotic stromal cells. *Cell Prolif.* 50, e12330
36. Itoh, F. *et al.* (2013) 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.* 99, 1705–1713
37. Shao, J. *et al.* (2016) Macrophages promote the growth and invasion of endometrial stromal cells by downregulating IL-24 in endometriosis. *Reproduction* 152, 673–682
38. Tran, L.V.P. *et al.* (2009) Macrophages and nerve fibres in peritoneal endometriosis. *Hum. Reprod.* 24, 835–841
39. Greaves, E. *et al.* (2015) Estradiol is a critical mediator of macrophage-nerve cross talk in peritoneal endometriosis. *Am. J. Pathol.* 185, 2286–2297

40. Takebayashi, A. *et al.* (2015) Subpopulations of macrophages within eutopic endometrium of endometriosis patients. *Am. J. Reprod. Immunol.* 73, 221–231
41. Shi, F.-D. *et al.* (2011) Organ-specific features of natural killer cells. *Nat. Rev. Immunol.* 11, 658–671
42. Oosterlynck, D.J. *et al.* (1993) Immunosuppressive activity of peritoneal fluid in women with endometriosis. *Obstet. Gynecol.* 82, 206–212
43. Maeda, N. *et al.* (2002) Aberrant expression of intercellular adhesion molecule-1 and killer inhibitory receptors induces immune tolerance in women with pelvic endometriosis. *Fertil. Steril.* 77, 679–683
44. Matsuoka, S. *et al.* (2005) Expression of inhibitory-motif killer immunoglobulin-like receptor, KIR2DL1, is increased in natural killer cells from women with pelvic endometriosis. *Am. J. Reprod. Immunol.* 53, 249–254
45. Wu, M.Y. *et al.* (2000) Increase in the expression of killer cell inhibitory receptors on peritoneal natural killer cells in women with endometriosis. *Fertil. Steril.* 74, 1187–1191
46. González-Foruria, I. *et al.* (2015) Soluble ligands for the NKG2D receptor are released during endometriosis and correlate with disease severity. *PLoS One* 10, e0119961
47. Salih, H.R. *et al.* (2006) Release of MICB molecules by tumor cells: mechanism and soluble MICB in sera of cancer patients. *Hum. Immunol.* 67, 188–195
48. Salih, H.R. *et al.* (2002) Cutting edge: down-regulation of MICA on human tumors by proteolytic shedding. *J. Immunol.* 169, 4098–4102
49. Guo, S.-W. *et al.* (2016) Platelet-derived TGF-β1 mediates the down-modulation of NKG2D expression and may be responsible for impaired natural killer (NK) cytotoxicity in women with endometriosis. *Hum. Reprod.* 31, 1462–1474
50. Kang, Y.-J. *et al.* (2014) An increased level of IL-6 suppresses NK cell activity in peritoneal fluid of patients with endometriosis via regulation of SHP-2 expression. *Hum. Reprod.* 29, 2176–2189
51. Yu, J.-J. *et al.* (2016) IL15 promotes growth and invasion of endometrial stromal cells and inhibits killing activity of NK cells in endometriosis. *Reproduction* 152, 151–160
52. Yang, H.-L. *et al.* (2017) The crosstalk between endometrial stromal cells and macrophages impairs cytotoxicity of NK cells in endometriosis by secreting IL-10 and TGF-β. *Reproduction* 154, 815–825
53. Izumi, G. *et al.* (2017) Mannose receptor is highly expressed by peritoneal dendritic cells in endometriosis. *Fertil. Steril.* 107, 167–173.e2
54. Schulke, L. *et al.* (2009) Dendritic cell populations in the eutopic and ectopic endometrium of women with endometriosis. *Hum. Reprod.* 24, 1695–1703
55. Stanic, A.K. *et al.* (2014) Dendritic cells attenuate the early establishment of endometriosis-like lesions in a murine model. *Reprod. Sci.* 21, 1228–1236
56. Fainaru, O. *et al.* (2008) Dendritic cells support angiogenesis and promote lesion growth in a murine model of endometriosis. *FASEB J.* 22, 522–529
57. Pencovich, N. *et al.* (2014) The development of endometriosis in a murine model is dependent on the presence of dendritic cells. *Reprod. Biomed. Online* 28, 515–521
58. Shimizu, K. *et al.* (2017) High expression of high-mobility group box 1 in menstrual blood: implications for endometriosis. *Reprod. Sci.* 24, 1532–1537
59. Yun, B.H. *et al.* (2016) Pathophysiology of endometriosis: role of high mobility group box-1 and toll-like receptor 4 developing inflammation in wndometrium. *PLoS One* 11, e0148165
60. Bertheloot, D. and Latz, E. (2017) HMGB1, IL-1α, IL-33 and S100 proteins: dual-function alarmins. *Cell. Mol. Immunol.* 14, 43–64
61. Zachariah, R. *et al.* (2009) Circulating cell-free DNA as a potential biomarker for minimal and mild endometriosis. *Reprod. Biomed. Online* 18, 407–411
62. Traver, S. *et al.* (2014) Cell-free nucleic acids as non-invasive biomarkers of gynecological cancers, ovarian, endometrial and obstetric disorders and fetal aneuploidy. *Hum. Reprod. Update* 20, 905–923
63. Miller, J.E. *et al.* (2017) Interleukin-33 modulates inflammation in endometriosis. *Sci. Rep.* 7, 17903
64. Santulli, P. *et al.* (2012) Serum and peritoneal interleukin-33 levels are elevated in deeply infiltrating endometriosis. *Hum. Reprod.* 27, 2001–2009
65. Griesenauer, B. and Paczesny, S. (2017) The ST2/IL-33 axis in immune cells during inflammatory diseases. *Front. Immunol.* 8, 475
66. Li, D. *et al.* (2014) IL-33 promotes ST2-dependent lung fibrosis by the induction of alternatively activated macrophages and innate lymphoid cells in mice. *J. Allergy Clin. Immunol.* 134, 1422–1432.e11
67. Londei, M. *et al.* (2017) A phase 1 study of ANB020, an anti-IL-33 monoclonal antibody in healthy volunteers. *J. Allergy Clin. Immunol.* 139, AB73
68. Podgaec, S. *et al.* (2007) Endometriosis: an inflammatory disease with a Th2 immune response component. *Hum. Reprod.* 22, 1373–1379
69. Antsiferova, Y.S. *et al.* (2005) Changes in the T-helper cytokine profile and in lymphocyte activation at the systemic and local levels in women with endometriosis. *Fertil. Steril.* 84, 1705–1711
70. Gieseck, R.L. *et al.* (2018) Type 2 immunity in tissue repair and fibrosis. *Nat. Rev. Immunol.* 18, 62–76
71. Gogacz, M. *et al.* (2016) Increased percentage of Th17 cells in peritoneal fluid is associated with severity of endometriosis. *J. Reprod. Immunol.* 117, 39–44
72. Chang, K.-K. *et al.* (2017) 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.* 8, e2666–e2666
73. Ahn, S.H. *et al.* (2015) IL-17A contributes to the pathogenesis of endometriosis by triggering proinflammatory cytokines and angiogenic growth factors. *J. Immunol.* 195, 2591–2600
74. Hirata, T. *et al.* (2011) Interleukin-17F increases the secretion of interleukin-8 and the expression of cyclooxygenase 2 in endometriosis. *Fertil. Steril.* 96, 113–117
75. Veldhoen, M. (2017) Interleukin 17 is a chief orchestrator of immunity. *Nat. Immunol.* 18, 612–621
76. Miossec, P. and Kolls, J.K. (2012) Targeting IL-17 and TH17 cells in chronic inflammation. *Nat. Rev. Drug Discov.* 11, 763–776
77. de Barros, I.B.L. *et al.* (2017) What do we know about regulatory T cells and endometriosis? A systematic review. *J. Reprod. Immunol.* 120, 48–55
78. André, G.M. *et al.* (2011) Analysis of FOXP3 polymorphisms in infertile women with and without endometriosis. *Fertil. Steril.* 95, 2223–2227
79. Gueuvoghlanian-Silva, B.Y. *et al.* (2018) Treg and NK cells related cytokines are associated with deep rectosigmoid endometriosis and clinical symptoms related to the disease. *J. Reprod. Immunol.* 126, 32–38
80. Li, M.-Q. *et al.* (2014) CD4+Foxp3+regulatory T cell differentiation mediated by endometrial stromal cell-derived TECK promotes the growth and invasion of endometriotic lesions. *Cell Death Dis.* 5, e1436–e1436
81. Braundmeier, A. *et al.* (2012) Induction of endometriosis alters the peripheral and endometrial regulatory T cell population in the non-human primate. *Hum. Reprod.* 27, 1712–1722
82. Podgaec, S. *et al.* (2014) Foxp3 expression in deep rectosigmoid endometriosis lesions and its association with chronic pelvic pain. *J. Reprod. Immunol.* 104–105, 96–99

83. Olkowska-Truchanowicz, J. *et al.* (2013) CD4+ CD25+ FOXP3+ regulatory T cells in peripheral blood and peritoneal fluid of patients with endometriosis. *Hum. Reprod.* 28, 119–124
84. Hanada, T. *et al.* (2018) 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.* 16, 9
85. Berbic, M. *et al.* (2010) The role of Foxp3+ regulatory T-cells in endometriosis: a potential controlling mechanism for a complex, chronic immunological condition. *Hum. Reprod.* 25, 900–907
86. Gogacz, M. *et al.* (2014) T regulatory lymphocytes in patients with endometriosis. *Mol. Med. Rep.* 10, 1072–1076
87. Wang, Y. *et al.* (2010) Abnormal regulation of chemokine TECK and its receptor CCR9 in the endometriotic milieu is involved in pathogenesis of endometriosis by way of enhancing invasiveness of endometrial stromal cells. *Cell. Mol. Immunol.* 7, 51–60
88. Wang, X.-Q. *et al.* (2017) Synergistic effect of regulatory T cells and proinflammatory cytokines in angiogenesis in the endometriotic milieu. *Hum. Reprod.* 32, 1304–1317
89. Tanaka, Y. *et al.* (2017) Exacerbation of endometriosis due to regulatory T-cell dysfunction. *J. Clin. Endocrinol. Metab.* 102, 3206–3217
90. Wild, R.A. and Shivers, C.A. (1985) Antiendometrial antibodies in patients with endometriosis. *Am. J. Reprod. Immunol. Microbiol.* 8, 84–86
91. Randall, G.W. *et al.* (2007) Serum antiendometrial antibodies and diagnosis of endometriosis. *Am. J. Reprod. Immunol.* 58, 374–382
92. Ahn, S.H. *et al.* (2017) Biomarkers in endometriosis: challenges and opportunities. *Fertil. Steril.* 107, 523–532
93. Riccio, L.G.C. *et al.* (2017) The role of the B lymphocytes in endometriosis: a systematic review. *J. Reprod. Immunol.* 123, 29–34
94. Shen, P. and Fillatreau, S. (2015) Antibody-independent functions of B cells: a focus on cytokines. *Nat. Rev. Immunol.* 15, 441–451
95. Hirata, T. *et al.* (2008) Interleukin (IL)-17A stimulates IL-8 secretion, cyclooxygensase-2 expression, and cell proliferation of endometriotic stromal cells. *Endocrinology* 149, 1260–1267
96. Evans-Hoeker, E. *et al.* (2016) Endometrial BCL6 overexpression in eutopic endometrium of women with endometriosis. *Reprod. Sci.* 23, 1234–1241
97. Yoo, J.-Y. *et al.* (2017) KRAS Activation and over-expression of SIRT1/BCL6 contributes to the pathogenesis of endometriosis and progesterone resistance. *Sci. Rep.* 7, 6765
98. Yeol, S.G. *et al.* (2015) Decreased Bcl-6 and increased Blimp-1 in the peritoneal cavity of patients with endometriosis. *Clin. Exp. Obstet. Gynecol.* 42, 156–160
99. Hever, A. *et al.* (2007) Human endometriosis is associated with plasma cells and overexpression of B lymphocyte stimulator. *Proc. Natl. Acad. Sci. U. S. A.* 104, 12451–12456
100. Söhngen, L. *et al.* (2014) Additional B-cell deficiency does not affect growth and angiogenesis of ectopic human endometrium in T-cell-deficient endometriosis mouse models during long-term culture. *J. Reprod. Immunol.* 106, 50–57
101. Bourlev, V. *et al.* (2010) Signs of reduced angiogenic activity after surgical removal of deeply infiltrating endometriosis. *Fertil. Steril.* 94, 52–57
102. Ceyhan, S.T. *et al.* (2008) Expression of cyclooxygenase-2 and vascular endothelial growth factor in ovarian endometriotic cysts and their relationship with angiogenesis. *Fertil. Steril.* 90, 988–993
103. Goteri, G. *et al.* (2004) Immunohistochemical analysis of vascular endothelial growth factor cellular expression in ovarian endometriomata. *Fertil. Steril.* 81, 1528–1533
104. McLaren, J. *et al.* (1996) Vascular endothelial growth factor is produced by peritoneal fluid macrophages in endometriosis and is regulated by ovarian steroids. *J. Clin. Invest.* 98, 482–489
105. Capobianco, A. *et al.* (2011) Proangiogenic Tie2(+) macrophages infiltrate human and murine endometriotic lesions and dictate their growth in a mouse model of the disease. *Am. J. Pathol.* 179, 2651–2659
106. Novella-Maestre, E. *et al.* (2012) Effect of antiangiogenic treatment on peritoneal endometriosis-associated nerve fibers. *Fertil. Steril.* 98, 1209–1217
107. Laschke, M.W. *et al.* (2011) Endothelial progenitor cells contribute to the vascularization of endometriotic lesions. *Am. J. Pathol.* 178, 442–450
108. Virani, S. *et al.* (2013) Blocking of stromal cell-derived factor-1 reduces neoangiogenesis in human endometriosis lesions in a mouse model. *Am. J. Reprod. Immunol.* 70, 386–397
109. Wang, J. *et al.* (2008) The role of CXCR7/RDC1 as a chemokine receptor for CXCL12/SDF-1 in prostate cancer. *J. Biol. Chem.* 283, 4283–4294
110. Nagasawa, T. *et al.* (1996) Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature* 382, 635–638
111. Boldajipour, B. *et al.* (2008) Control of chemokine-guided cell migration by ligand sequestration. *Cell* 132, 463–473
112. Zhou, W.-H. *et al.* (2015) Co-expression of CXCR4 and CXCR7 in human endometrial stromal cells is modulated by steroid hormones. *Int. J. Clin. Exp. Pathol.* 8, 2449–2460
113. Isaacson, B. *et al.* (2017) Stromal cell-derived factor 1 mediates immune cell attraction upon urinary tract infection. *Cell Rep.* 20, 40–47
114. Ceradini, D.J. *et al.* (2004) Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat. Med.* 10, 858–864
115. Ceradini, D.J. and Gurtner, G.C. (2005) Homing to hypoxia: HIF-1 as a mediator of progenitor cell recruitment to injured tissue. *Trends Cardiovasc. Med.* 15, 57–63
116. Becker, C.M. *et al.* (2008) 2-Methoxyestradiol inhibits hypoxia-inducible factor-1α and suppresses growth of lesions in a mouse model of endometriosis. *Am. J. Pathol.* 172, 534–544
117. Bukulmez, O. *et al.* (2008) Inflammatory status influences aromatase and steroid receptor expression in endometriosis. *Endocrinology* 149, 1190–1204
118. Bulun, S. *et al.* (2012) Role of estrogen receptor-β in endometriosis. *Semin. Reprod. Med.* 30, 39–45
119. Burns, K.A. *et al.* (2012) Role of estrogen receptor signaling required for endometriosis-like lesion establishment in a mouse model. *Endocrinology* 153, 3960–3971
120. Burns, K.A. *et al.* (2018) Early endometriosis in females is directed by immune-mediated estrogen receptor α and IL-6 cross-talk. *Endocrinology* 159, 103–118
121. Han, S.J. *et al.* (2012) A new isoform of steroid receptor coactivator-1 is crucial for pathogenic progression of endometriosis. *Nat. Med.* 18, 1102–1111
122. Han, S.J. *et al.* (2015) Estrogen receptor β modulates apoptosis complexes and the inflammasome to drive the pathogenesis of endometriosis. *Cell* 163, 960–974
123. Zhao, Y. *et al.* (2015) Dual suppression of estrogenic and inflammatory activities for targeting of endometriosis. *Sci. Transl. Med.* 7, 271ra9