Review
The Role of Peritoneal Immunity in Peritoneal Endometriosis and Related Infertility

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Abstract
Endometriosis is defined as a disorder in which the glands and stroma of the endometrium grow and shed periodically outside the uterine cavity. Highly prevalent in women of reproductive age, the most common clinical manifestations are chronic pelvic pain and infertility. The pathogenesis of endometriosis may be multifactorial, including factors of anatomy, immunity, inflammation, hormones (estrogen), oxidative stress, genetics, epigenetics, and environment. There are generally three types of endometriotic disease, namely peritoneal, ovarian, and deep infiltration. For the same patient, there may be a single or multiple types concurrently. The different manifestations of these types suggests that they each have their own etiology. Numerous studies have shown that the evasion of endometrial cells from endometriosis affect more than 10% of women of reproductive age, whereas it occurs in 25–50% of infertile women. In turn, it has been reported that 35–50% of women with endometriosis suffer from infertility [2]. There are generally three types of endometriotic disease, namely, peritoneal, ovarian, and deep infiltration, each with a different etiology. Of these, peritoneal endometriosis is the most common [3], hence our focus herein. The pathogenesis of endometriosis may be multifactorial, including factors of anatomy, immunity, inflammation, hormones (estrogen), oxidative stress, genetics, epigenetics and environment [4]. The two major theories about the etiology of peritoneal endometriosis are those of menstrual reflux and coelomic metaplasia [5]. The peritoneum is an important immune barrier, and the peritoneal cavity composed of it forms an immune microenvironment. Under the action of cytokines and chemokines, the peritoneum continuously recruits leukocytes from blood, of which monocytes and macrophages (MΦs) are the majority, accounting for more than 50% [6]. The peritoneum itself contains plentiful immune cells, including MΦs, mast cells (MCs), B1 cells, T cells, etc. [7,8].

1. Introduction
Endometriosis is defined as a disorder in which the glands and stroma of the endometrium periodically grow and shed outside the uterine cavity, resulting in severe chronic pelvic pain and infertility [1]. Generally, endometrial lesions contain the endometrial epithelium as well as stromal cells, blood vessels, and lymphocytes. Endometriosis affects more than 10% of women of reproductive age, whereas it occurs in 25–50% of infertile women. In turn, it has been reported that 35–50% of women with endometriosis suffer from infertility [2]. There are generally three types of endometriotic disease, namely, peritoneal, ovarian, and deep infiltration, each with a different etiology. Of these, peritoneal endometriosis is the most common [3], hence our focus herein. The pathogenesis of endometriosis may be multifactorial, including factors of anatomy, immunity, inflammation, hormones (estrogen), oxidative stress, genetics, epigenetics and environment [4]. The two major theories about the etiology of peritoneal endometriosis are those of menstrual reflux and coelomic metaplasia [5]. The peritoneum is an important immune barrier, and the peritoneal cavity composed of it forms an immune microenvironment. Under the action of cytokines and chemokines, the peritoneum continuously recruits leukocytes from blood, of which monocytes and macrophages (MΦs) are the majority, accounting for more than 50% [6]. The peritoneum itself contains plentiful immune cells, including MΦs, mast cells (MCs), B1 cells, T cells, etc. [7,8].

Under normal physiological conditions, peritoneal immunity tends to be in a balanced state. When it is stimulated by external stimuli, pro-inflammatory factors first initiate and promote inflammatory response. For body protection, anti-inflammatory factors then play a role to calm inflammation and begin tissue reconstruction to maintain body homeostasis. In patients with endometriosis, immune imbalance is manifested by reduced cytotoxicity of T lymphocytes, cytokines secreting by T helper (Th) cells, and autoantibody produced by B lymphocytes [9,10]. While menstrual reflex occurs in almost all women of reproductive age, only 10% suffer from endometriosis. Many studies have confirmed that the number and activity of various immune cells in the peritoneal fluid of women with endometriosis, as well as the expression levels of secreted cytokines and inflammatory mediators, have changed. As the first site of broken endometrial fragments (also viewed as an exogenous stimulus) into the peritoneal cavity, what is the role of peritoneum in the occurrence and development of pelvic peritoneal endometriosis, and how does the local immune microenvironment of peritoneal cavity alter? What is the relationship between these alterations and endometriosis-related infertility? This review attempts to identify the role of peritoneal immunity in peritoneal endometriosis and related infertility, especially in the aspects of molecular mechanisms.

Keywords: peritoneum; immune; endometriosis; lymphocyte; macrophage
tissue, known as stroma, contains few immune cells [11]. It is made up of three distinct layers: closest to the peritoneal cavity is a single layer of mesothelial cells, followed by the basement membrane and connective tissue [12]. Connective tissue, known as stroma, contains few immune cells [11]. In men, the peritoneum is a closed sac. In women, however, the peritoneum is an incompletely closed sac as the tubal fimbria is not covered. This also creates conditions for the menstrual reflux into the peritoneal cavity [11]. The peritoneum was thought to not only facilitate intra-coelomic movement by reducing friction on the surface of organs, but also have other functions such as intra-abdominal homeostasis regulation, fluid exchange, inflammation suppression, antigen presentation, and tissue repair [13–16]. When the equilibrium homeostasis of peritoneum is broken under certain pathological conditions, certain disorders such as endometriosis occur [11].

2.2 Immunological Basis of Peritoneum

The fluid in the peritoneal cavity is called peritoneal fluid. There are different types of immune cells in peritoneal fluid [17]. This lays the foundation for the peritoneum to participate in immune regulation of the body. Studies have shown that there are ample stomata on peritoneal surfaces [13], that are directly connected with the lymphatic system [18–20] and that facilitate communication between the peritoneum and the lymphatic system by helping the cells in the peritoneal cavity to absorb and migrate to the lymphatic system [20,21]. These lymphatic portals located between the mesothelial cells, are usually arranged around milky spots. Milky spots, on the other hand, are considered as secondary lymphoid organs: an aggregation of immune cells, mainly composed of MΦs, B cells, and T lymphocytes. Milky spots can respond to intraperitoneal infection and play an antigen recognition role by amplifying the recruitment of B cells and CD4⁺/CD8⁺ T cells [11]. One of the peritoneal structures, connective tissue, is rich in lymphatic vessels and contains a small number of immune cells. Typically, immune cells in the stoma are scant and inactivated, but under certain pathological or physiological conditions, they are enabled to promote angiogenesis [11].

2.3 Immune Balance and Imbalance

Normally, the peritoneum has an anti-inflammatory effect to prevent infectious peritonitis. The peritoneal inflammatory response is characterized by increased vascular perfusion, aggregated macrophages, and the release of pro- and anti-inflammatory factors [22]. Not only are MΦs abundant in the peritoneal fluid, but the stroma of the peritoneum also contains a certain number of MΦs that recognize and digest foreign material and subsequently recruit more inflammatory leukocytes such as monocytes, lymphocytes and neutrophils from blood. Influenced by these cells, mesothelial cells secrete inflammatory mediators [23–26]. Moreover, adhesion molecules expressed on the surface of mesothelial cells interact with recruited leukocytes to reduce inflammation [27]. Interestingly, mesothelial cells of the peritoneum also act as antigen-presenting cells (APCs), displaying antigen fragments on their cell surface. Valle et al. [28] pointed out that mesothelial cells express major histocompatibility complex (MHC)-II molecules and present antigens to T cells, thus participating in T-cell activation together with resident MΦs, resulting in a cell-mediated immune response to pathogens. This complex peritoneal defense system works well against inflammation while an excessive immune response may lead to angiogenesis, fibrosis and injury of the peritoneum. When the inflammatory triggers remain, peritoneal inflammation does not stop, resulting in peritoneal scarring, impaired tissue function and eventual organ failure. Examples include autoimmune serositis caused by reactions to self-antigens, or peritoneal carcinomatosis and endometriosis caused by reactions to neoplastic or ectopic cells.

3. Peritoneal Endometriosis and Peritoneal Immunity

Pelvic peritoneal endometriosis refers to endometriosis located in the peritoneum around the uterus, fallopian tubes, and ovaries. Retrograde menstruation may cause this disease by activating the innate immune system in the pelvis, which induces local inflammation [29,30]. Although menstrual reflux is common, peritoneal endometriosis does not exist in all women of reproductive ages [2]. In patients with endometriosis, the ability of endometrial cells and glands that flow into the peritoneal cavity with menstrual reflux to grow and survive depends on the specific pelvic environment. The decisive factor seems to be the large number of lymphocytes found in endometriotic lesions [31]. There is increasing evidence that immune disturbance is a major factor in the occurrence and evolution of endometriosis [32–34]. Individual peritoneal immune response promotes evolution of endometriosis [30]. This theory of immune system alterations and endometriosis suggests that changes in cellular and humoral immunity may promote development of the disease [35].

3.1 Innate Immunity

3.1.1 Macrophages (MΦs)

The most common immune cells in peritoneal cavity are MΦs [36]. Peritoneal MΦs (PMΦs) are divided into resident MΦs and monocyte-derived MΦs. The former are representative of MΦs: they represent a strong phagocytic capacity, displaying longevity and self-renewal ability. Activated MΦs can not only remove damaged tis-
nificantly in women with endometriosis, but also regulate the peritoneal microenvironment with the help of their secreted cytokines, prostaglandins, enzymes and complement components [10]. In turn, those mediators secreted by MΦs promote themselves to induce inflammatory responses, tissue remodeling, angiogenesis, and possibly recruitment of endothelial cells [37,38]. Due to their phenotypic plasticity, activated MΦs can be divided into two types with different functions according to the altered microenvironments [39]. M1 type performs pro-inflammatory functions by secreting numerous inflammatory cytokines to specifically eliminate microorganisms and defective cells. M2 type plays the opposite role by secreting anti-inflammatory factor, participating in regulating adaptive immune responses, facilitating angiogenesis, tissue repair and the removal cell debris [40–43].

Normally, the M1/M2 MΦs are in balance to keep the body in homeostasis. This balance is skewed in favor of M1 type in the eutopic endometrium in women with endometriosis [44], whereas it tends to be M2 type polarized in the peritoneum and ectopic lesions [45]. Some scholars have proposed that the resident MΦs in the ectopic lesions are M1 type, while the monocytes recruited from the peritoneum are M2 type [46]. Although M2 type has long been considered to be dominant in endometriotic lesions, few studies have considered the complexity of MΦs phenotype in response to the local tissue microenvironment, where pro-inflammatory and anti-inflammatory factors may co-exist [47]. Recently, another in vitro study confirmed that the decrease of M1 type migration rate of peritoneal macrophages led to the retention of M1 type macrophages in the peritoneal cavity, which caused the continuation of inflammatory response and promoted the development of endometriosis, and it was considered that inducing macrophage differentiation into M2 type might be a method for the treatment of endometriosis [48]. These findings further complicate the role of MΦs in the development of endometriosis.

Despite the number of activated PMΦs increasing significantly in women with endometriosis [10], their phagocytic capacity is considerably decreased, so that endometrial cells arriving in the pelvic cavity via retrograde menstruation cannot be cleared. Wu et al. [49] found that the content and activity of matrix metalloproteinases (MMPs) expressed by PMΦs in patients with endometriosis were inhibited, through which MΦs bind to extracellular matrix and play a phagocytic role. The downregulation of MMPs may be mediated by the overexpression of prostaglandin E2 (PEG2) in the peritoneal fluid. Other studies have found an increased proportion of free MΦs in the peritoneal cavity of women with endometriosis [50], suggesting that the decrease of conjugated MΦs implies a decrease in phagocytosis, thus sparing ectopic endometrial cells from phagocytosis and allowing them to plant and develop in the peritoneum. In conclusion, PMΦs may (1) regulate the dynamic changes of secreted proinflammatory factors and anti-inflammatory factors through the change of active phenotype; (2) reduce phagocytosis of the endometrium and stroma contained in reflex menstruation to promote the occurrence and development of peritoneal endometriosis.

3.1.2 Natural Killer (NK) Cells

NK cells, named for their cytotoxic properties, recognize and kill target cells, such as certain tumor cells, virus-infected cells, self-tissue cells (damaged cells), and parasites. They can also synthesize and secrete cytokines to play immune regulation. The regulation of NK cell activity depends on the interaction of two types of receptors expressed on the cell surface, namely activated receptor (KAR) and inhibitory receptor (KIR). KIR protects autologous cells by recognizing MHC-I molecules on their surface to generate inhibitory signals and block the activation of KAR. When the target cells lose MHC-I molecules, the inhibitory effect is relieved and KAR receptor activates NK cells to produce killing effect. KAR receptors, which mainly contain natural killer group 2 member D (NKG2D) and CD16, can bind to target cells coated with immunoglobulin G (IgG) and destroy target cells through an antibody-dependent cell-mediated cytotoxic mechanism. Thus, the increase of NKG2D ligand can trigger cytotoxic reactions of activated NK cells.

Oosterlynck et al. [51] first found reduced cytotoxicity of NK cells in both peritoneal cavity and peripheral blood of patients with endometriosis, confirmed by subsequent studies [52]. González-Foruria et al. [53] found that soluble NKG2D ligands were significantly increased in the peritoneal fluid of patients with endometriosis, implying that fewer NKG2D ligands were expressed on the surface of ectopic endometrial cells. This would make it easier for NK cells to evade recognition due to these soluble NKG2D ligands acting as bait receptors. Wu et al. [54] have found that the excessive expression of KIR in peritoneal NK cells of patients with endometriosis may also explain the decreased activity of NK cells. A recent study found that the chemotaxis (i.e., the ability to migrate to the site of immune response) of peritoneal NK cells decreases in patients with endometriosis, which may lead to the defect of NK cells’ cytotoxic function [55]. It is not clear whether this phenomenon is caused by differences in NK cell function per se or by differences in the peritoneal environment caused by retrograde menstruation, which warrants further exploration. Decreased cytotoxicity and chemotaxis of NK-cells in the peritoneal cavity reduce their ability to clear endometriotic cells and may be associated with the evolution of endometriosis.

3.1.3 Mast Cells (MCs)

Like MΦs, MCs usually reside in the mucosal epithelium of the lungs, digestive tract, and reproductive tract, representing the first line of defense: in humans, they are
also positioned at mesothelium-covered cavities including the peritoneal cavity [56]. MCs are degranulated when stimulated by allergens to produce histamine and protease to participate in immunity [57]. MCs can be divided into at least two populations depending on the protease they contain. MCs containing only tryptase are called mucosal mast cells (MMCs) in mice and MC_T in humans, while MCs containing tryptase, chymase, carboxypeptidase A, and cathepsin G are called connective tissue mass cells (CTMCs) in mice and MC_TC in humans. The latter are thought to be innate MCs, a unique source of heparin, while the former is smaller, often containing fewer granules, and are thought to be adaptive MCs [58]. Their functional differences mainly depend on the response to different stimuli. Peritoneal MCs are the mature MC_TC subgroup. Tryptase is a specific marker of mature mast cells [59]. Previous studies have suggested that MCs may be associated with the progression of endometriosis, but they are limited to certain phenomena, such as the increased number of degranulated MCs in endometriotic lesions and the significantly increased tryptase level in the peritoneal fluid of patients with endometriosis [60,61]. A recent animal study found that estrogen can significantly increase the MCs’ density in endometrial lesions, suggesting that ectopic lesions provide a necessary microenvironment for MCs recruitment and differentiation, while MCs may promote the disease by releasing proinflammatory factors [62]. Tryptase may affect sperm motility and cause low fertility rate of endometriosis, but the results have not been supported [60].

3.1.4 Eosinophils

The main function of eosinophils is to regulate allergic reactions and to activate and attack pathogens during parasitic infections. Early studies found an indirect association between eosinophils and endometriosis. Hornung et al. [63] found that in both eutopic endometrium and ectopic endometrium in women with endometriosis, the expression of extaxin, a highly specific eosinophil chemotactic factor, is significantly elevated versus that in normal controls. Blumenthal et al. [64] found that the level of Eosinophil peroxidase (EPO), an enzyme released by degranulated eosinophils, was increased in ectopic lesions of patients with endometriosis. By establishing a mouse model of endometriosis, Uchiide et al. [65] found that the adherent peritoneal stroma showed the proliferation and infiltration of eosinophils related, which was thought to be the peritoneal manifestation in the early stage of endometriosis. A subsequent study found elevated eosinophil counts in the peritoneal fluid of patients with early endometriosis [66]. These studies suggest that eosinophils may be involved in the pathogenesis of endometriosis.

3.1.5 Neutrophils

Neutrophils play a crucial role in the innate immune system as the second line of defense against microbial invasion, with strong chemotaxis and phagocytosis. The structure and metabolism of peripheral blood neutrophils in patients with endometriosis are changed [67], and the phagocytic function is decreased [68]. After surgical resection of endometriotic lesions, the phagocytic activity of peripheral blood neutrophils can be restored to normal temporarily, but only for a short time [68]. Conversely, the number of neutrophils is increased both in the peritoneal fluid and ectopic lesions of women with endometriosis, potentially secreting cytokines that promote angiogenesis [69,70]. Co-culture with peritoneal fluid from patients with endometriosis induces neutrophils to release more vascular endothelial growth factor (VEGF), a factor that increases vascular permeability and promotes angiogenesis [71]. A recent study showed that granulocyte colony-stimulating factor (G-CSF) and interleukin 6 (IL-6) regulate neutrophils through signal transducer and activator of transcription 3 (STAT3) pathway, altering the expression of angiogenesis-related genes, such as Mmp9, Bombina variegata 8 kDa protein (Bv8), and tumor necrosis factor-related apoptosis-inducing ligand (Trail) to promote the establishment of early endometriosis [72].

3.1.6 Dendritic Cells (DCs)

DCs, the most powerful specialized antigen-presenting cells discovered, can recognize and phagocytose antigens and present them to T lymphocytes. Generally, DCs are divided into two main groups: conventional DCs (cDCs) and plasmacytoid DCs (pDCs). cDCs contain two subpopulations, namely cDC1 and cDC2, which can recognize and phagocytose antigens before maturing. After differentiation and maturation stimulated by inflammatory factors, mature DCs acquire the ability of migration, antigen presentation and T-cell differentiation. For example, cDC1 subgroup is also commonly referred to as Th1-inducing cells [73]. DCs control the differentiation of Th cells or regulatory T (Treg) cells; accordingly, researchers speculate that DCs could cause their functional abnormalities. Several studies have indicated that abnormal DC frequency in the uterus or peritoneal fluid may lead to endometriosis [74–76]. Unlike cDCs, pDCs detect the nucleic acid of pathogens and produce an abundance of interferons (IFNs), currently considered as a therapeutic target in endometriosis. According to Suen et al. [77], in the mouse model, IL-10 in endometriosis is mainly derived from pDC secretion; pDC was found to increase endometriotic lesions, but this phenomenon was not observed in IL-10 knockout mice [78]. However, other researchers found that the frequency of pDCs in the peritoneal fluid of patients with endometriosis did not change [75]. Further studies on the function of peritoneal pDCs are needed to confirm whether they are associated with the establishment and evolution of endometriosis.
3.7 Complement System

The complement system, a part of the innate immune system, consists of a series of proteins that undergo complex cascades upon activation and are highly effective in labeling the non-self (such as pathogens), altered self (such as apoptotic/necrotic cells and protein complex), and transformed self (such as tumor cells) [79,80]. This is followed by lysis of target cells/pathogens, opsonization and subsequent enhancement of their uptake by immune phagocytes via complement receptors, and production of inflammatory mediators. Two anaphylatoxins, C3a and C5a, produced by activated complement, act as activators to stimulate peritoneal MCs and macrophages to produce mediators or cytokines; furthermore, increased endometrial vascular permeability causes inflammation and pain symptoms [81]. Numerous studies have found that the levels of various components of complement, such as C1q, C3a, C3c, C4, and SC5b-9 are significantly increased in the peritoneal fluid of patients with endometriosis. Accordingly, the complement system may also be a vital part of the pathogenesis of endometriosis [82–84].

3.2 Adaptive Immunity

3.2.1 Cellular Immunity

Adaptive immunity refers to the process of activation, proliferation and differentiation of antigen-specific T/B lymphocytes into effector cells and the production of biological effects after being stimulated by antigens in vivo. According to the different cell types and mechanisms involved in immune response, adaptive immunity can be divided into T-cell-mediated cellular immunity and B-cell-mediated humoral immunity. T lymphocytes, derived from bone marrow and embryonic liver, migrate to thymus and mature into immunocompetent T lymphocytes. According to the differences in their expressed glycoproteins CD4 and CD8, which can bind to MHC-II and MHC-I molecules, respectively, T cells are divided into two major categories [85,86], namely, CD4+ T cells and CD8+ T cells. CD4+ T cells can be divided into four subsets according to their cytokine secretion profile: Th1, Th17, Th2 and Treg cells. Th1/Th17 cells are classified as pro-inflammatory cells, whereas Th2/Treg cells have anti-inflammatory effects [87]. Th1 cells synthesize pro-inflammatory cytokines such as IFN-γ, tumor necrosis factor (TNF-α), TNF-β, IL-2, IL-3, IL-10 and IL-12; they are able to promote CD8+ T cell differentiation and cellular immunity via activation of monocytes and MΦs. Th17 cells produce IL-6/IL-17. Th2 cells produce IL-4, IL-5, IL-6, IL-10 and IL-13, and can promote the differentiation of B cells into plasma cells to promote humoral immune response [17]. Treg cells account for a small proportion of T lymphocytes, only 5–20% of CD4+ cells; they secrete IL-10/transforming growth factor (TGF)-β and are able to inhibit effector cell responses, limit inflammatory responses, thereby preventing tissue damage and autoimmunity [88,89]. These four subsets of CD4+ T cells maintain dynamic balance through mutual influence and restriction of cytokines, among which Th1/Th2 and Treg/Th17 are two pairs of balance systems that restrict each other and play an important role in maintaining the immune stability of the body. Disruption of this balance leads to certain diseases including endometriosis.

The concentration of Th2-type cytokines in peripheral blood and peritoneal fluid was found to be elevated in women with endometriosis [90,91]. Studies have found that compared with normal endometrium, Th17 cells are more commonly present in endometriotic lesions [92], and their content in peritoneal fluid is positively correlated with disease grade [93]. IL-17A secreted by Th17 can stimulate inflammatory response and promote the occurrence of endometriosis [94,95]. Authors almost unanimously agree that increased Treg cells in the peritoneal fluid are associated with local immune suppression, allowing ectopic endometrial cells to escape clearance [96,97]. CD8+ T cells have the function of activating macrophages, enabling them to phagocyte cells infected by viruses or intracellular pathogens [85,86]. It was found that the levels of all subset of T lymphocytes in the peritoneal fluid of women with endometriosis were elevated; a higher ratio of CD4/CD8 was also discovered [98]. There are few independent studies on CD8+ T cells in endometriosis.

3.2.2 Humoral Immunity

B lymphocytes are derived from bone marrow and are released into peripheral lymphoid tissues after they develop into mature B cells, which are activated into plasma cells and begin to secrete antibodies, participating in humoral immunity [99,100]. Excessive polyclonal activation of B cells has been found in patients with endometriosis via secretion of autoantibodies in endometriotic lesions, peritoneal fluid and blood [101–103]. Badawy et al. [104] found that the number of B cells was elevated in both the peripheral blood and peritoneal fluid of patients with endometriosis. Another study found that B1 lymphocytes (a subset of B lymphocytes, which do not have memory function but can produce antibodies to attack antigens) in the peritoneal fluid of patients with endometriosis. Levels of anti-endometrial antibodies and anti-ovarian antibodies have been found to be significantly increased in patients with endometriosis, and elevated levels of IgA and IgG-type antibodies in peritoneal fluid also have been discovered [107,108]. In addition to anti-tissue and anti-organ antibodies, B lymphocytes also produce antibodies to cellular components, such as antiphospholipid, anti-DNA and
ANA, which are commonly seen in autoimmune diseases [9]. An immunoregulatory B cell subtype, called B-reg cells, secretes IL-10, which not only controls effector immune responses, but even controls the progression of autoimmune diseases [97]. Animal experiments [98] suggest that B-reg cells may help prevent the progression of endometrial lesions. An immunoregulatory B-cell subtype, known as B reg cells, controls the progression of autoimmune diseases by secreting IL-10 and TGF-β, inhibiting the expansion of pro-inflammatory lymphocytes [109]. An animal drug experiment [110] found that promoting the conversion of activated B cells into B-reg cells prevented the progression of endometriosis, suggesting that B-reg cells play a negative role in the progression of endometriotic lesions. In addition to antibodies, activated B cells can also secrete certain cytokines that regulate immune cells and maintain chronic inflammation, which may be related to endometriosis [111–114].

In conclusion, B cells may participate in the pathogenesis of endometriosis through their own activation and the production of antibodies and a small number of cytokines. However, their role in the microenvironment of endometriosis and their interaction with other immune cells need to be further explored.

### 3.3 Inflammatory Mediators

Many studies have found that the increase in the number of various immune cells in the peritoneal fluid of women with endometriosis directly leads to elevated levels of soluble proteins, including certain cytokines, growth factors, enzymes and antibodies in the peritoneal fluid, lesions and peripheral blood [38,115–119]. All the inflammatory factors and cytokines interact to regulate cellular and humoral immunity [17]. Their roles are shown in Table 1.

#### 3.3.1 Interleukin 1 (IL-1)

IL-1 family are pro-inflammatory and secreted into the peritoneal fluid by activated macrophages [120]. They regulate immune and inflammatory responses by controlling the expression of integrins in leukocytes and endothelial cells [33]; they further mediate the maturity and differentiation of various cells, participating in angiogenesis together with other substances [120]. In this family, IL-1α and IL-1β are two important members, and their concentrations are increased in the peritoneal fluid of patients with endometriosis. IL-1β affects the proliferation of ectopic endometrial cells by regulating the expression of intercellular cell adhesion molecule (ICAM)-1 on cell surface (which can be recognized by NK cells after binding with lymphocyte function-associated antigen (LFA)-1 expressing lymphocytes) [120]. The decrease of soluble IL-1R2 receptor (a receptor with high affinity and inhibitory effect on IL-1β) in the peritoneal fluid of patients with endometriosis may cause the increase of IL-1β concentration in the peritoneal environment [121]. IL-1β not only regulates the proliferation of ectopic endometrial cells, but also promotes the vascularization of ectopic lesions by stimulating the release of VEGF and IL-6 [122]. In addition, IL-1 also has adverse effects on fertility, not only in its toxic effect on embryos [123], but also causes ovulation dysfunction in patients with endometriosis [124].

#### 3.3.2 Interleukin 2 (IL-2)

IL-2, a member of the chemokine family, is mainly produced by activated T lymphocytes and participates in cytotoxic cellular responses. Other functions include stimulating the proliferation and differentiation of lymphocytes, stimulating the proliferation and enhancing the killing activity of NK cells, inducing the production of lymphokine activated killers (LAKs), and activating monocytes and macrophages [125]. Different investigators have different views on the role of IL-2 in the development of endometriosis, as some studies have shown that the concentration of IL-2 in the peritoneal fluid of patients is reduced [126–128], while others have found the converse [93,129]. A recent study showed that IL-2 is an independent protective factor against endometriosis [130]. In other words, the role of IL-2 in the pathogenesis of endometriosis is still unclear and more exploration is warranted.

#### 3.3.3 Interleukin 4 (IL-4)

IL-4 is a pleiotropic cytokine produced by Th2 cells [131]. Its biological effects mainly include stimulating the proliferation of B and T lymphocytes and promoting the differentiation of CD4+ T cells into Th2 cells [132,133]. Increased IL-4 levels in peritoneal fluid of patients with mild endometriosis have been confirmed [134]. However, a contrary report suggests that the level of IL-4 in peritoneal fluid is not related to the presence of endometriosis [135]. IL-4 mRNA and protein expression were increased in both peripheral monocytes and peritoneal fluid cells isolated from patients with endometriosis [136]. IL-4 produced in ectopic endometrial tissue can induce the proliferation of its stroma cells [137] probably through promoting the expression of Eotaxin mRNA in ectopic endometrial stroma in a dose-dependent manner, which promotes angiogenesis and subsequent development of endometriosis [137].

#### 3.3.4 Interleukin 6 (IL-6)

IL-6 is a multifunctional cytokine that is primarily produced in response to acute infection and tissue damage. It can regulate the growth and differentiation of a variety of cells, such as the induction of T lymphocyte activation and B lymphocyte differentiation [85]. While under normal circumstances, IL-6 expression is tightly regulated under pathological conditions, it may be continuously synthesized, affecting chronic inflammation and autoimmunity [138,139]. It is well documented that the proliferation of MΦs in the peritoneal fluid of patients with endometrio-
Table 1. The role of various inflammatory factors and cytokines in endometriosis.

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<th>Inflammatory factors and cytokines</th>
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| IL-1                              | 1. affects the proliferation of ectopic endometrial cells  
|                                   | 2. regulates the expression of ICAM-1 on cell surface  
|                                   | 3. stimulates the release of VEGF and IL-6  
|                                   | 4. has a negative effect on fertility |
| IL-2                              | unclear; more exploration is needed |
| IL-4                              | 1. stimulates the proliferation of B and T lymphocytes  
|                                   | 2. promotes the differentiation of CD4+ T cells into Th2 cells  
|                                   | 3. induces the proliferation of its stroma cells |
| IL-6                              | 1. increases haptoglobin  
|                                   | 2. inhibits NK cell activity  
|                                   | 3. regulates the growth of endometrial stromal cells |
| IL-8                              | 1. chemotaxis to neutrophils  
|                                   | 2. participates in neutrophil activation  
|                                   | 3. proangiogenic effect  
|                                   | 4. contributes to cell adhesion  
|                                   | 5. promotes the proliferation of ovarian endometrioma-derived stromal cells |
| IL-10                             | decreases NK cell cytotoxicity |
| IL-13                             | paradoxical; more exploration is needed |
| TNF-α                             | 1. MΦs recruitment  
|                                   | 2. neutrophils recruitment  
|                                   | 3. cell adhesion (endometrial cells and peritoneum)  
|                                   | 4. stimulates the proliferation of stroma cells |
| IFN-γ                             | 1. activates MΦs  
|                                   | 2. promotes development of T lymphocytes  
|                                   | 3. interacts with IL-2 to shift the Th1/Th2 balance in favor of Th2 |
| TGF-β                             | 1. decreases NK cell cytotoxicity  
|                                   | 2. (TGF-β1) promotes Th cells’ differentiation to produce more IL-10 and IL-17  
|                                   | 3. induces epithelial to mesenchymal transition (EMT)  
|                                   | 4. increases VEGF-A secretion from the peritoneal mesothelium  
|                                   | 5. promoting the vascularization of endometriosis lesions  
|                                   | 6. enhances the migration, invasion and colonization potential of the endometriotic cells |
| VEGF                              | 1. increases vascular permeability  
|                                   | 2. promotes the deformation of extracellular matrix  
|                                   | 3. makes vascular endothelial cells migrate and proliferate, and promotes angiogenesis  
|                                   | 4. formats new blood vessels around endometriotic lesions |

Abbreviations: IL, interleukin; ICAM, intercellular cell adhesion molecule; VEGF, vascular endothelial growth factor; NK, natural killer; TNF, tumor necrosis factor; IFN, interferon; TGF, transforming growth factor.

sis significantly increases IL-6 levels [140–144]. Elevated IL-6, by increasing haptoglobin, allows endometrial implants to evade peritoneal immune surveillance by reducing phagocytosis; NK cell activity can also be inhibited by IL-6 [140]. Other studies have found that endometrial stromal cells are resistant to IL-6 by reducing the expression of IL-6R [145,146]. Elevated IL-6 in the peritoneal cavity not only facilitates the immune evasion of ectopic endometrial cells, but also regulates the growth of endometrial stromal cells.

3.3.5 Interleukin 8 (IL-8)

IL-8, secreted by macrophages and monocytes, regulates inflammatory response by chemotaxis to neutrophils. It can also participate in neutrophil activation and have certain effects on eosinophils, basophils and lymphocytes [147]. It also has a strong proangiogenic effect and can be regarded as a potent angiogenic factor [85], contributing to cell adhesion [118]. IL-8 levels are increased in patients with endometriosis and may be positively correlated with disease severity [148,149]. IL-8 can promote the proliferation of ovarian endometrioma-derived stromal cells, which may be enhanced by TNF-α [122].

3.3.6 Interleukin 10 (IL-10)

IL-10 is a Th2-type cytokine, also secreted by other cells, such as MΦs, keratinocytes and tumor cells [150]. IL-10 regulates cell growth and differentiation, participates in inflammation and immune response, and mainly plays an inhibitory role. Studies found elevated levels of IL-10
in the peritoneal fluid of patients with endometriosis [98], which may be related to the decrease of NK cytotoxicity [151]. Locally elevated IL10 is more conducive to the entry of endometrial debris into the abdominal cavity, facilitating the early stage of pelvic endometriosis [152].

3.3.7 Interleukin 13 (IL-13)

IL-13, secreted by Th2 cells and MCs, can act on Th2 cells, B lymphocytes and MФs, thereby stimulating B cell differentiation and inhibiting the production of inflammatory factors from MФs. Researchers found significantly lower levels of IL-13 in the peritoneal fluid of patients with endometriosis [153]. Further study found that the decrease in concentration of IL-13 was not related to the severity of the disease [154]. On the contrary, other studies suggest that the expression of IL-13 in the peritoneal fluid and endometriosis lesions of patients with endometriosis is increased [155], possibly related to its impaired fertility [156]. Therefore, the role of IL-13 in promoting or inhibiting the pathogenesis of endometriosis remains to be determined.

3.3.8 Tumor Necrosis Factor-α (TNF-α)

TNF-α, mainly derived from the secretion of activated MФs and T lymphocytes, plays a role in various necrosis or apoptosis-signaling pathways [157]. Studies have found that the concentration of TNF-α increased in peritoneal fluid of patients with endometriosis in stage I–II, but did not increase consistently with the progression of the disease [158]. TNF-α can lead to MФs recruitment to the lesion site and differentiate into M1 type, triggering the recruitment of neutrophils to the peritoneal fluid [159], resulting cell adhesion between endometrial cells and peritoneum, stimulating the proliferation of stroma cells [160,161], thereby promoting disease progression [162].

3.3.9 Interferon γ (IFN-γ)

IFN-γ, a highly species-specific glycoprotein, is derived from activated T lymphocytes and NK cells. Its regulatory role on immune effects is mainly reflected in the activation of MФs and development of T lymphocytes [163]. Whether the outcome of the response mediated by IFN is survival or apoptosis depends on the state of the cell at the time and the complex regulatory mechanisms. Maintaining Th1/Th2 balance also requires the participation of IFN-γ and IL-2 interaction. Studies have found that the level of IFN-γ in the peritoneal fluid of women with endometriosis was elevated, with the Th1/Th2 balance skewed toward Th2 [90].

3.3.10 Transforming Growth Factor-β (TGF-β)

TGF-β, secreted by T lymphocytes, promotes the differentiation of Treg cells and Th17 cells to regulate immunity, and can also promote angiogenesis. Young et al. [164] pointed out that peritoneal mesothelial cells are also a source of TGF-β1 in patients with endometriosis through immunohistochemistry of peritoneal biopsy and in vitro study of peritoneal mesothelial cells. Studies have found that the activity of TGF-β in peritoneal fluid of patients with endometriosis is increased, potentially related to the decreased activity of NK cells [113]. Further study found that peritoneal platelet-derived TGF-β1 downregulated NKG2D expression and thus suppressed NK cell activity [165]. These changes reduce the peritoneal immune surveillance function and prevent ectopic endometrial cells from phagocytosis. In vitro experiments showed that human endometrial cells were more likely to adhere to mouse peritoneum after TGF-β1 pretreatment. It was speculated that TGF-β1 may change the expression of cell surface adhesion molecules in peritoneal mesothelial cells or endometrial cells to promote their adhesion [166]. As a well-known epithelial to mesenchymal transition (EMT) inducer, TGF-β1 can increase the migration and invasion ability of endometrial epithelial cells by promoting EMT, thus realizing the implantation of endometrial cells [167]. TGF-β1 may activate the proliferation of ectopic endometrial stromal cells and promote disease progression by upregulating protease activated receptor 2 (PAR2) expression [168]. Vascularization is a necessary condition for the continued growth of the lesion. Young et al. [169] confirmed that TGF-β1 may increase the secretion of VEGF-A in the peritoneal mesothelium through the inhibitor of DNA-binding protein 1 (ID1) pathway, thus promoting the vascularization of endometriosis lesions. TGF-β signaling has been shown to promote the activation of M2 type MФs, a state conducive to the development of endometriosis [170]. In addition, the decrease of NK cell activity induced by TGF-β may affect mouse embryonic development, resulting in infertility [171].

3.3.11 Vascular Endothelial Growth Factor (VEGF)

VEGF can not only increase vascular permeability and promote the deformation of extracellular matrix, but also make vascular endothelial cells migrate and proliferate, and promote angiogenesis. Endometriosis is characterized by the presence of numerous vascular proliferations in and around endometriotic lesions. It is generally believed that the formation of numerous new blood vessels is related to the etiology of endometriosis [162]. This is also confirmed by the increased VEGF levels found in the peritoneal fluid of patients with endometriosis [101], but it has yet to be verified whether these high concentrations of VEGF originate from the ectopic lesions themselves [172,173] or from activated MФs [37]. Thus, researchers speculate that the elevated level of VEGF in peritoneal fluid has notable clinical significance in the progression of the disease.

4. Immunity and Endometriosis Associated Infertility

Endometriosis-related infertility may be attributed to both macro and micro aspects. The former includes me-
mechanical deformation of fallopian tube caused by pelvic adhesion, and closure of fallopian tube and mechanical ovulation disorder. The latter is primarily related to follicle development, oocyte quality, sperm delivery, fertilization, embryo development, embryo delivery and embryo implantation. Studies have found that in donor IVF cycles, the pregnancy rate decreases if the donor is complicated with endometriosis, which may affect the quality of oocytes [174, 175]. High levels of inflammatory factors in peritoneal fluid of patients with endometriosis may lead to reproductive dysfunction through toxic effects on oocyte collection, fertilization, embryo development and implantation [85, 176]. Lachapelle et al. [106] found that the proportions of NK cells, B lymphocytes and monocytes in follicular fluid of patients with endometriosis-related infertility were higher than those with unexplained infertility and tubal infertility. However, it is still controversial whether immunological changes in the eutopic endometrium of patients with endometriosis affect embryo implantation [178, 179].

The concentrations of various cytokines in the peritoneal fluid of women with endometriosis are altered, and the levels of proinflammatory factors such as TNF-α, IL-1, IL-6, IL-10, TGF-β1 and IL-8 are increased [120, 180]. TNF-α can participate in the growth, proliferation and tissue invasion of endometriosis by increasing the transcriptional activity of estrogen-regulated genes in endometrial epithelial cells [181]. It has also been found that the level of TNF-α in follicular fluid of women with endometriosis-related infertility is also increased [30]. IL-1 can affect endometrial decidualization, thus affecting embryo implantation [182]. In addition, IL-1 also has adverse effects on fertility, not only in its toxic effect on embryos [123], but also in ovulation disorders in patients with endometriosis [124]. Increased IL-6 level in peritoneal fluid of patients with endometriosis [30] may induce the differentiation of B cells and cytotoxic T cells, reduce the activity of NK cells, and promote the inflammatory response [140]. Although IL-6 is required for blastocyst development, excessive IL-6 not only significantly reduces sperm motility [183], but also inhibits the proliferation of endometrial stroma [145] and limits the interaction between blastocyst and matrix substrates stroma [184], thus negatively affecting fertilization and embryo implantation. A literature review found that the result of elevated levels of pro-inflammatory cytokines in the peritoneal fluid may lead to decreased oocyte quality, and hence embryo quality, but the exact mechanism has not been determined [185, 186].

Autoantibodies are often associated with autoimmune diseases, but a variety of autoantibodies, including organ-specific and non-organ-specific antibodies, have been found in the peritoneal fluid and blood of women with endometriosis-related infertility, even if the patients do not have clinical manifestations of autoimmune disease [187]. Studies have found that non-organ-specific antibodies, including ANAs and antiphospholipid antibodies, are more common in patients with endometriosis [188]. Further studies have shown that the levels of these antibodies are significantly higher in patients with endometriosis-related infertility than in infertility patients without endometriosis [189]. However, organ-specific antibodies, such as anti-ovary, anti-theca, anti-granulosa cell, anti-zona pellucida, anti-sperm and anti-endometrial antibodies, have been detected in patients with endometriosis-related infertility, but their significance is not clear [107]. These autoantibodies may affect the fertility potential of endometriosis patients by interfering with oogenesis, sperm motility, embryo implantation and other processes [190, 191].

5. Conclusions and Perspectives

Endometriosis is defined as a disorder in which the glands and stroma of the endometrium periodically grow and shed outside the uterine cavity. It mostly appears on the surface of peritoneum and causes chronic pelvic pain and decreased fertility. The pathogenesis of endometriosis may be multifactorial, while the dysfunction of the immune system is a key factor in its occurrence and progression, including local immune regulation in the peritoneal cavity. Currently, Sampson’s theory of menstrual reflux is considered to be an accurate assessment of the basis of peritoneal endometriosis. It points out that the intimal debris entering the abdominal cavity interacts with the peritoneum, followed by a series of events such as adhesion, implantation, proliferation, vascularization and fibrosis. Endometrial cells adhere to the peritoneal mesothelium and acquire the ability to invade the matrix, which is a critical step in disease development. These endometrial cells escape peritoneal clearance and achieve adhesion through a mechanism called “immune evasion”. Whether the subsequent complex immune dysregulation in the peritoneal cavity is the cause of endometriosis or a consequence of the disease itself has yet to be determined. These immune dysregulations include innate and adaptive immune-involved cells, inflammatory factors, and the complement system. They interact with each other to form a complex immune network that promotes disease progression. Peritoneal immune cells and the cytokines they produce are critical in this process. In fact, the local immune changes in the peritoneal cavity of endometriosis are only a part of the body’s immunity, and such changes are difficult to study independently. Further research on the pathogenesis of endometriosis is required for the prevention and early detection of the disease.

Author Contributions

JS and QH together conceptualized of the work. QH, YY, and WX screened and analyzed reliant literatures. QH


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