Immunopathogenesis of endometriosis – a novel look at an old problem

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Abstract

This review aims to cast a look at endometriosis as a chronic and progressive gynecological disease. Endometriosis-affected tissues show a variety of pathologic features: alterations in cell growth, apoptosis, activation, angiogenesis, cell adhesion, and cytokine production. Fresh endometriotic lesions are associated with induction of an inflammatory reaction represented by overproduction of prostaglandins (PGE2), metalloproteinases (MMP-2, -3, -9), cytokines (IL-1β, IL-8, IFN-γ, TNF-α, MCP-1 and MIF) and adhesive molecules (ICAM-1, VCAM-1) and activation of synthesis of reactive oxygen and nitrogen species. The inflammatory process may lead to defective folliculogenesis by an altered follicular milieu. An increase in the number and change in function of macrophages, T- and B-lymphocytes and reduction of NK cells have been reported. Treg lymphocytes are known to play an extremely important role in controlling and modulating changes in the aberrant immune response in endometriosis. Dysregulation of the immune system results in both increased progression of endometriosis and its severity. In inflammatory conditions the immune cells provide immune defense at the local level – in peritoneal fluid – and could further cause: 1) a decrease of the number of NK CD16+ cells with expression of KIRs and an increase of NK CD57+; 2) increased numbers of CD8+ cells and CD11b⁻ immature dendritic cells; 3) an increase of FoxP3 expression in the regulatory T cell (Treg) population; 4) an increase of macrophages activating T- and B-lymphocytes leading to elevated synthesis of cytokines and/or autoantibodies. We may conclude that endometriosis resembles an immunodependent disease with the autoimmune background and breakdown of immunosuppressive mechanisms. Further immunological investigations may open a new avenue to discover innovative immunomodulatory treatments of endometriosis.

Key words: *endometriosis, inflammatory response, autoimmunity, immunodependent disease.*

Introduction

Endometriosis is a chronic and often progressive gynecological disease defined by the presence of endometriotic tissue outside the uterine cavity that is sensitive to cyclic steroid hormone regulation. With a prevalence of 6-10% of the female population, it is one of the most frequent gynecologic diseases in women of reproductive age. Endometriosis is associated with dysmenorrhea, dyspareunia, or chronic pelvic pain, and infertility [1-3]. Histologically, lesions are characterized by ectopic endometrial glands and stroma located outside of the uterus, commonly to other organs in the pelvis and beyond. Endometriosis is a disease of inflammatory origin, probably polygenic and multifactorial. Generally, endometriosis can be considered as a disease of both endocrine and immune dysregulation together [4, 5]. However, recognition of the direct involvement of two major physiological mechanisms might represent an interesting advance in the understanding of this disease and it can become a new focus for further research [6].

Although the pathogenesis of endometriosis is still debated, evidence indicates that both the onset and the progression of this disease are supported by: 1) an imbalance of invasive, proliferative and adhesive properties of endometrial cells; 2) increased production of inflammatory molecules [1]. Currently, the most accepted theory seems to be Sampson's retrograde menstruation hypothesis, which suggests that during menstruation, endometrial fragments migrate through the Fallopian tubes to the peritoneal cavity and reach the peritoneum, as they are able to attach, survive, and implant in different locations (Fallopian tubes, ovaries, and pouch of Douglas) [2, 7-9].

The endometrioid tissue in the peritoneal cavity, present on ovaries and in the rectovaginal part, is functional [10].

Endometriosis is known to reduce female fertility and has an impact on the obstetric outcome of affected women. In women with endometriosis multiple factors result in reduction of fertility by: reduced tubal motility and passage, triggering of inflammatory factors derived from the peritoneal fluid, and diminished quality of the oocytes, which compromise the chances of successful implantation in course of natural conception as well as after assisted reproductive technology (ART) [2, 11].

Endometriosis-affected ectopic tissues show different pathologic features, as follows: 1) delayed maturation; 2) altered glycosylation; 3) molecular abnormalities, such as alterations in local steroid biosynthesis, cell growth, apoptosis, immune cell function, angiogenesis, cell adhesion, and cytokine production. All of these might reduce the chances of a successful conception and pregnancy outcome. Pathophysiologic mechanisms might be responsible for increased miscarriage rates observed in women with mild endometriosis; early stages of the disease with more active lesions are known to lead to a more active inflammatory milieu, compared with the more scarring lesions of higher disease stages. Fresh endometriotic lesions are associated with an inflammatory response represented by overproduction of prostaglandins, metalloproteinases and cytokines. Finally this results in an inflammatory process, and may lead to defective folliculogenesis due to an altered follicular milieu. We could speculate that the impact of the mild stage of endometriosis on fertility might be due to nongenetic, e.g. metabolic intracellular processes, leading to insufficient oocyte maturation and/or embryonal development. Additionally, altered endometrial receptivity and therefore reduced "supply" of intracellular embryonal metabolism could possibly impact early trophoblast development [2, 12].

Etiology and pathogenesis of endometriosis

The risk factors of endometriosis may involve: 1) women's age; 2) heavy but short menses [7]; 3) low body mass index [13]; 4) psychological and emotional factors; 5) stress and/a pain response in pelvis; 6) nulliparity; 7) high education and social level [3]. Patients with endometriosis have a high risk for systemic comorbidities including autoimmune, cardiovascular and atopic diseases. It is still unknown whether these comorbidities are a consequence of endometriosis or arise from the same background of risk factors [3, 14]. The pathogenesis of endometriosis still remains controversial: immune, hormonal, genetic, and environmental factors seem to be involved [7].

Immune factors

An aberrant immune system seems to play a key role in the pathogenesis of endometriosis. In these patients, immune alterations occur both locally in the peritoneal fluid and systemically in peripheral blood. It is reported that there is an increase in the number but not in the dysfunction of macrophages, abnormalities in the function and number of T- and B- lymphocytes and reduction in the number and activity of natural killer cells, which in consequence triggers apoptosis dysregulation, change in cytokine pattern and other soluble products secreted in the peritoneal microenvironment [9]. Defective immune surveillance might be responsible for the development of endometriosis. Normally, the clearance of menstrual debris is considered to be the result of immune cells' action. Among the causes of endometriosis are the changes in functions of immune cells that might allow the invasion of endometrial cells to the peritoneal cavity. Direct fault to the immune system results in both increased prevalence of endometriosis and its increased severity [6].

In inflammatory conditions the immune cells supply immune defense proteins at the local level – in peritoneal fluid (PF) [8]. The following changes were identified in PF of patients with endometriosis: 1) decrease of CD16+ NK cell count with expression of killer immunoglobulin like receptors (KIRs), increase of highly cytotoxic NK CD57+ cells [6]; 2) an increase in the number of T-cytotoxic CD8+ lymphocytes and immature dendritic cells, CD11b⁻; 3) an increase of expression of FoxP3 on T-regulatory lymphocytes; 4) increase in macrophages which activate T- and B-lymphocytes for enhanced synthesis of cytokines and autoantibodies [7]. Various changes of innate and acquired immunity occur systemically and locally.

Cellular factors of innate immunity

The peritoneal microenvironment of the endometrium is rich in macrophages [15-17], which may be driven towards a reversible polarization. The "classically activated" macrophages are named M1; they play endocytic functions via the production of cytokines such as interleukin (IL)-1α, IL-6, IL-12, tumor necrosis factor α (TNF-α) and reactive oxygen (ROS) and nitric oxide (NO) species. "Alternatively activated" M2 macrophages are involved in resolution of inflammation and promotion of tissue repair, by secreting anti-inflammatory immunosuppressive cytokines IL-10 and transforming growth factor β (TGF-β), and pro-angiogenic factors (coagulation factor XIII, vascular endothelial growth factor, VEGF) [8, 16, 18, 19]. Infiltrating macrophages in the endometriotic lesions are activated by signals generated within the same lesions or possibly by lack of hormone-dependent antiinflammatory signals in the ectopic but not in the eutopic endometrium. In endometriosis, the cyclic death of endometrial cells leads to the release of cell debris, erythrocytes, and heme-bound iron in the peritoneal cavity. Recruited macrophages perceive ongoing cell death and tissue damage; in endometriotic patients they activate a reparative/regenerative/angiogenic program that is required for lesion maintenance, growth, and spreading. An apparent impairment in the macrophage ability to phagocytose dying cells has been reported. This defect can be the cause or consequence of persistent in-

flammation taking place in the peritoneal cavity associated with endometriosis. The pathogenesis of endometriosis may therefore originate by combination of inappropriate polarization of macrophages, leading to tissue damage (increased M1 response) and immune dysfunction (increased compensatory M2 response). It was demonstrated that recruited macrophages largely develop an immunosuppressive phenotype M2, thereby supporting endometriotic cell survival, attachment, and invasion through matrix remodeling, angiogenesis, and lesion maintenance [7].

Macrophage migration inhibitory factor (MIF) activates peritoneal macrophages and may play a role in retaining macrophages to the inflammatory sites. Macrophages react via phagocytosis, which is regulated through activation of matrix metalloproteinases and expression of the CD36+ receptor. The expression of both these components is reduced in endometriosis. The molecules responsible for this suppression are: prostaglandin E2, TGF-β and intercellular adhesion molecule-1 (ICAM-1) [6]. MIF stimulates cyclooxygenase-2 (COX2) and production of prostaglandin E2 in ectopic endometrial cells [20] and elicits the proangiogenic and proinflammatory phenotype of macrophages, thereby potentiating their capability to stimulate the host angiogenic response and exacerbate the immunoinflammatory reaction in the implantation site [7, 21].

Natural killer cells (NK) usually demonstrate an ability to destroy endometrial cells. NK cell activity and cytotoxicity towards autologous endometrial cells have been observed to be decreased in women with endometriosis and correlate with the severity of the disease. The decreased cytotoxicity to endometrial cells in women with endometriosis occurs mainly due to a defect in NK activity but is also partially due to resistance of the endometrium to NK cytotoxicity. It has been suggested that endometriosis may be related to a defect of NK cell cytotoxicity function and the cells' ability to eliminate endometrial cells in ectopic sites. Impaired NK cell cytotoxic activity may be another prominent cause of endometriotic development and endometrial cell escape from targeted destruction [7].

Apoptosis impairment has been observed during endometriosis. It has been reported that the Fas/FasL system is progressively dysregulated throughout the course of the disease, the outcome of which is the situation where endometriotic cells do not undergo Fas/FasL-mediated apoptosis because they do not receive a death signal from peritoneal fluid mononuclear cells (PFMCs), thus implanting themselves and surviving outside of the uterus. Paradoxically, endometriotic cells themselves become capable of killing PFMCs, and this additional factor may allow their establishment in the peritoneum, which in turn becomes an immune semi-privileged environment [7].

Humoral factors of innate immunity

A large number of humoral factors of innate immunity: 1) cell adhesion molecules – intercellular adhesion mole-

cule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1); 2) proinflammatory cytokines – TNF-α, IL-1, IL-6, and IL-8 [22]; 3) chemokines MCP-1, MIF, etc., play key roles in the pathogenesis of endometriosis. These factors are present in the PF as well as in endometriotic implants [7].

In patients with endometriosis eutopic endometrial stromal cells high levels of ICAM-1 [8, 23] are strongly expressed. These data suggest that there may be a cross-talk between endometrial stromal cells and leukocytes in normal as well as endometriotic endometrium and peritoneal fluid via ICAM-1 and its receptors. ICAM-1 expression significantly increases in ectopic endometrial stromal cells from endometriomas by stimulation with proinflammatory cytokines IL-1β and interferon γ (IFN-γ). In addition to ICAM-1, VCAM-1 is also expressed in human endometrial stromal cells. Tumor necrosis factor α has also been shown to be elevated not only in the peritoneal fluid but also in the serum of women with endometriosis. There is a positive correlation between peritoneal levels of TNF-α and the size and number of active endometriotic lesions. In addition to its proinflammatory functions, TNF-α also stimulates the expression of matrix metalloproteinases in endometrial tissue. Matrix metalloproteinases are known to play a role in tissue remodeling and invasion of endometriotic lesions. These data suggest that TNF-α may influence the establishment and progression of disease. Data from the literature documented an impairment of the secretion of the IL-1 cytokine family in endometriosis. For example, a marked imbalance was reported between IL-1 and expression of its decoy receptor (IL1R2). The soluble natural inhibitor IL-1 receptor type 2 (sIL1R2) significantly downregulated the expression of major cell adhesion receptors (αv and β3 integrins), matrix metalloproteinases (MMP-2 and -9), and VEGF [7].

Concentrations of the cytokines IL-6 and IL-8 have been reported to be elevated in the PF of endometriotic patients. The typical course of the disease inflammatory process may start with high concentrations of IL-6. However, a significantly higher IL-6 level was found in moderate-to-severe but not in minimal-to-mild endometriosis. Moreover, serum levels of both IL-6 and IL-8 are significantly higher in patients with ovarian endometrioma, but not in the presence of deep infiltrating endometriosis. The increase in IL-8 production leads to intensification of peritoneal inflammation and activation of synthesis of ROS [16]. The levels of ROS are elevated in patients with endometriosis, but the concentration of catalase is usually decreased. Serum and peritoneal fluid levels of MCP-1 and MIF chemokines in endometriosis are high at early stages of disease and decrease with its severity. The levels of MIF generally were high in women with endometriosis and seem to depend on the stage of disease and its major clinical symptoms (pain and infertility) [7].

Angiogenesis may play an important role in the pathogenesis of endometriosis. Endometriotic implants require

neovascularization to proliferate, invade the extracellular matrix, and establish an endometriotic lesions. In endometriosis, increased levels of VEGF-A, an angiogenic factor, play a major role in the progression of the disease. Moreover, the authors observed an increase of other proteolytic factors, such as urokinase plasminogen activator (uPA) and metalloproteinase-3 (MMP-3), in peritoneal fluid of patients with endometriosis. These factors may enhance the angiogenic and proteolytic capability of ectopic tissue to facilitate the implantation process. The increased levels of VEGF-A may be associated with a decreased rate of pelvic adhesion formation in the course of endometriosis [7].

Metalloproteinases (MMPs) regulate multiple cell functions [19, 23]. The expression levels of MMP-2, MMP-3 and MMP-9 are higher in women with endometriosis than in healthy controls. These enzymes play an important role in the ectopic adhesion, invasion and implantation, and neovascularisation of the endometrium. MMP-2 and MMP-9, by degrading extracellular cellular matrix and promoting the release of key secretion factors, play a critical role in the pathogenesis of endometriosis [7, 24].

The data from women with endometriosis showed that expression of annexin A2 in peritoneal macrophages is inhibited by prostaglandin E2 (PGE2), and this impairs the phagocytic ability of macrophages. The level of annexin A2 mRNA in the macrophages is reduced by PGE2 via the EP2/EP4 receptor dependent signaling pathway. Production of prostaglandins PGE2 and PGF2α, spiral arteriole vasoconstriction, and local hypoxia in turn regulate the production of chemokines, such as IL-8 (CXCL8) and CXC chemokine ligand 12 (CXCL12), and stromal cell-derived factor (SDF-1). Increased PG concentrations in the PF of infertile women with endometriosis have been reported. COX-2 expression was upregulated in endometriotic tissue. Well known as a potent vasodilator, PGE2 may play a role in endometriosis-associated angiogenesis and further contribute to ectopic endometrial cell growth. Moreover, PGE2 appears to stimulate the expression of aromatase, an essential enzyme in estrogen synthesis in ectopic endometrium, which may favor ectopic implantation and growth of endometrial tissue. Aromatase, the rate-limiting enzyme for the synthesis of estrogen, is aberrantly expressed in endometriotic implants. Aberrant expression of COX-2 and PGE2 secretion by ectopic endometriotic implants have been reported, although the underlying mechanism is not clearly understood. Local estrogen production hastens prostaglandin synthesis by stimulating COX-2 activity, thus creating a self-perpetuating sequence of estrogen formation and enhanced inflammation [7].

Cellular factors of adaptive immunity

Interactions between T lymphocytes and the extracellular matrix in women with endometriosis are well established. There have been identified two components (fibronectin and collagen IV) with ability for activation of T cells to proliferate, but the lymphocytic proliferative response to autoantigens of autologous endometrial cells is rather decreased in women with endometriosis. Specific T cell-mediated cytotoxicity to autologous endometrial cells was strongly inhibited, suggesting a possible immunological basis. The reduction of T cell-mediated cytotoxicity is based on induction of apoptosis in cytotoxic lymphocytes via the Fas-FasL pathway. Similar to cytotoxic T lymphocytes CD8+, the activity of CD4+ helper T cells in the peritoneal fluid has also been decreased [6].

Beside CD8+ T cells, CD4+ T cells are further diminished in their activity in the PF of patients with endometriosis, probably because PF homeostasis breakdown suppresses activation of T cell helpers. Probably, IL-10 plays a major role in this mechanism. Moreover IL-4 and IL-10 were shown to be upregulated in peripheral lymphocytes in women with endometriosis. Increased IL-4 expression has also been observed in lymphocytes in endometriotic tissues and locally in the PF. On the other hand, production of IFN-γ was reduced in peripheral lymphocytes in endometriosis. PFMCs, as well as endometriotic cells, secrete different patterns of cytokines which may drive the differentiation program of CD4+ T cells towards Th1, Th2, and Th17 and/or Tregs. Th1 cells are characterized by Tbet and STAT-1 and STAT-6 expression, and the production of IL-2, IFN-γ, and TNF-α. Th2 cells are in turn characterized by GATA-3 and STAT-5 and STAT-6 expression, and the production of IL-4, IL-5, and IL-13. However, the Th1/Th2 dogma has been challenged by the introduction of two other subsets of T cells, Th17 cells and regulatory T (Treg) cells, fairly recently. Tregs are characterized by FOXP3 expression and produce IL-10 and TGF-β, suppressing activation of the immune system. A study of an animal experimental model of endometriosis reported a switch towards Th2 and Treg cell profiles, with overrecruitment of Foxp3+ CD4+ Tregs in the draining lymph nodes in the late stage of the disease. This is congruent with our previously reported results [7]. It was proposed that the preserved Treg cells seen in women with endometriosis decrease the ability of newly recruited immune cell populations to effectively recognize and target endometrial antigens during menstruation, allowing survival and implantation of shed endometrial cells. Probably, other immune cells (macrophages, dendritic cells, NK, CD4+, and CD8+ lymphocytes) have their activity suppressed just by Treg cells. A decreased apoptosis rate may cause perpetuation of the disease growth [7].

Generally, a common population of the regulatory T cells (Tregs) includes different subsets: central Tregs from peripheral lymphatic organs, effector Tregs from peripheral lymphatic organs and nonlymphatic tissues, and memory Tregs. Apart from Tregs, the suppressive function is shown by the following immune cells: 1) Treg1 lymphocytes, which are capable of synthesis of IL-10, TGF-β and expression of FoxP3 on their own membrane; 2) Th2

lymphocytes, which are capable of synthesis of IL-4 and inhibition of Th1 lymphocyte functions; 3) two populations of myeloid-derived suppressor cells: granulocyte-like (G-MDSC) and monocyte-like (M-MDSC); the immunosuppressive mechanism that MDSCs use is the production of arginase, reactive oxygen and nitrogen species, COX-2 and induction of T-reg activities [19].

Humoral factors of adaptive immunity

An increased count of activated CD20+ B-lymphocytes was found in ectopic endometrial tissue. The structural components of endometrial cells were degraded upon the influence of the metaplastic processes, which might have resulted in formation of new autoantigens. The expression of peritoneal haptoglobin glycoprotein (PHP), endometrial protein-1 and glycans – novel autoantigens – starts on the surface of modified endometrioid cells, and activates the immune system to produce autoantibodies [2]. These autoantibodies may be synthesized locally (in cervical mucus) and at a central level (in peripheral blood).

Hormonal factors

Immunity and hormonal responses in the reproductive tissues are key points in the scientific literature of the subject [5]. The development of endometriosis is sensitive to cyclic steroid hormone regulation [9]. Physiologically, progesterone (P) induces the decidualization of stromal cells of the endometrium to become receptive for the embryo. As a consequence of P resistance in endometriosis, this process may become dysregulated and lead to suboptimal implantation [2]. Endometriosis is indicative of a resistance to progesterone reaction, related to an overall reduction in the levels of progesterone receptors (PRs) and the lack of the PR isoform named progesterone receptor B (PR-B). In normal endometrium, progesterone causes stromal cells to induce secretion of paracrine factors. These unknown factors influence neighboring epithelial cells to induce expression of the 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD-2) enzyme, which metabolizes the biologically active estrogen E2 to estrogen E1. In endometriotic tissue, progesterone does not induce epithelial 17β-HSD-2 expression due to a defect in stromal cells. The inability of endometriotic stromal cells to produce progesterone-induced paracrine factors that stimulate 17β-HSD-2 may be due to the lack of PR-B [7].

A very low level of progesterone receptor A expression (PR-A) is observed *in vivo* in endometriotic tissue. The observed end point is deficient metabolism of E2 in endometriosis giving rise to high local concentrations of this hormone. Changes in estradiol homeostasis have been observed in endometriosis. A balance observed between local 2-methoxyestradiol production and angiogenesis could promote the development of endometriotic lesions by 7β-estradiol (E2), which is known to play important

roles in the processes that control cell division, differentiation, and proliferation and is considered a major risk factor in the development and progression of endometriosis. The biologically active estrogen estradiol (E2) is the best activator for the growth and inflammation processes in the ectopic endometriotic tissue [7].

A local increase in estrogen levels is characteristic of patients with ovarian endometrioma, and this condition could promote endometriotic cell proliferation. The changes of expression of Krüppel-like factor 9 (KLF9), a progesterone receptor interacting protein, in eutopic endometrium of women with endometriosis may account for progesterone resistance in endometriosis and may influence the development of the peritoneal surface metaplasia or of Müllerian residues [7].

In endometriosis there is a collaboration between immune and endocrine factors. Toll-like receptors (TLRs) are observed to be sensitive to cyclic hormonal regulation. They may be associated with aberrant stimulation of the immune response, possibly contributing to the chronic inflammation. TLRs are a subgroup of pattern recognition receptors (PRRs), which are responsible for innate immunity. Generally, most of these TLRs have been split into two sub-groups. TLR1, -2, -4, -5, -6, and TLR11 comprise the first group and are primarily expressed on the cell surface; the function of this group is to recognize the components of microbial membranes. The second sub-group is composed of TLR3, -7, -8, and TLR9. The endometrial tissue is susceptible to pathogens and microbial antigens. TLR2 and TLR4 are regarded as the major TLRs responsible for sustaining the inflammatory responses in both endometriosis and early miscarriages. This altering of the immunoreactivity and expression of TLRs may result in an impaired immune response, which is marked by abnormal inflammation. The data concerning expression of TLRs and endometriosis are still debated [23, 25].

Treg lymphocytes are known to play an extremely important role in controlling and modulating numerous changes in the immune response in endometriosis. An abundance of Tregs might reflect reduced progesterone responsive endometrial phenotype connected with endometriosis. A premenstrual rise of Tregs in endometriosis is an interesting observation [6].

Genetic and immunogenetic factors

Numerous candidate genes are involved initially in the pathogenesis of endometriosis, including genes governing inflammation, cell cycle regulation, growth factors, hormone receptors and adhesive molecules. However, the exact genetic basis of endometriosis is not yet clear and the exact etiopathogenic mechanism of endometriosis remains to be determined [9, 26].

Gogusev and colleagues reported different copy number variants (CNVs) in three chromosomal regions (1p36, 7p22.1, and 22q12) in patients with endometriosis. Analysis

conducted in Australia and the UK also identified a significant linkage with chromosome 10q26, but no causal gene was found. A genomewide association study (GWAS) in Australia implicated loci 7p15.2 (near the *HOXA10* and *NFE2L3* genes) and 1p36 (containing the *WNT4* gene). A Japanese GWAS also identified the locus 1p36. More recently, retinoid deficiency has been suggested to have a causative role in the etiology of endometriosis. Abnormal methylation of the promoters of genes such as *GATA6*, *ESR2*, and *NR5A1* in endometrial implants leads to an increase in local estrogen and prostaglandin levels, causing inhibition of progesterone receptors. This in turn results in reduced synthesis and absorption of retinoids. These molecular abnormalities have detrimental effects upon cell differentiation, increased cell survival, and enhanced inflammation, and may result in the development of endometriosis [4, 5].

GWAS and metaanalyses have led to the identification of diseaserisk loci that influence a woman's risk of developing the disorder and provide new insights into potential pathways leading to endometriosis. At present, 19 independent single nucleotide polymorphisms (SNPs) have been robustly associated with endometriosis, explaining 5.19% of the disease variance. The number of these loci is increasing as the proportion of cases analyzed is limited to more severe disease stages (stage III/IV endometriosis) rather than stage I/II, thus indicating that moderate to severe endometriosis cases have a greater genetic burden relative to minimal or mild disease. These results were confirmed by a recent metaanalysis and additionally identified rs11031006 of the *FSHB* gene as a novel endometriosisassociated SNP [9]. Among all SNPs, Falconer *et al.* proposed that genetic polymorphism of hormone receptors, growth factor, and human leukocyte antigen system components showed a stronger correlation than the others [27]. Considering that endometriosis is clearly an estrogen-dependent disease, there are many reports of positive associations with numerous polymorphisms involving sex steroid production and metabolism. There is evidence for a significant correlation between polymorphism of the progesterone receptor gene (PROGINS) and endometriosis. Regarding endometriosis-associated infertility, it has been reported that FoxP3 polymorphisms can be associated with risk of idiopathic infertility (rs2280883 and rs2232368) and endometriosis (rs3761549), and this is in line with other reports indicating the importance of FoxP3+CD4+ Tregs in the pathogenesis of the disease. A more recent study by the same group raised the hypothesis that FoxP3 polymorphism may have a cumulative effect in increasing the risk of developing endometriosis [7].

A hypothesis about the role of the HLA-G antigen (normally involved in maternal tolerance of the semiallogeneic fetus) in suppressed NK and cytotoxic T cell responses was studied in healthy pregnant women. Fetal HLA-G on the trophoblast can interact with uterine NK cells and macrophages through KIR2DL4 [28]. The findings of highly elevated levels of type KIR2DL4 of killer cell inhibitory receptors (KIRs) on NK cells of patients with advanced-stage endometriosis might explain the low activity of NK cells. Some authors suggested a double role – first, the low activity of NK cells might be a primary cause of endometriosis, and further development of this disease lowers their activity even further [6]. The expression of KIR2DL4 leads to inhibition of NK cytotoxicity and results in disturbance of apoptosis of degenerated endometriotic cells [10].

Environmental and metabolic factors

Some authors suggest that exposure to environmental toxicants (dioxin and dioxin-like) plays a role in the pathogenesis of endometriosis. Regardless of the correct etiopathogenetic theory, implanting and proliferation of endometriotic cells seem to depend strictly on the local immune aberrations contained in the PF and inside of endometriotic cysts [7].

In ectopic endometrial tissue the process of glycosylation is affected. Glycosylation is strongly hormonally regulated and is very important for good embryo implantation. At the severe stage of endometriosis the process of glycosylation is abnormal (the number of links between lectins and residues of N-acetylglucosamine is increased) and may be a cause of defective implantation and early miscarriage [2, 6].

Vitamin D is involved in many processes of the reproductive system, and its synthesis may also occur in ectopic endometrium. Vitamin D shows antiproliferative, anti-inflammatory and immunomodulatory effects. The vitamin D receptor (VDR) is ubiquitous and also expressed in cells of reproductive tissues, including the endometrium. The possible link between endometriosis and vitamin D has been recently investigated because endometriosis often shows several characteristics of an autoimmune disease [1].

The development of endometriosis can also be associated with concentration of leptin in PF. Leptin is an adipocytokine, and its concentration is inversely proportional to mass of fat tissue and levels of proinflammatory cytokines, TNF-α and IL-6. Often a low concentration of leptin is associated with development of autoimmune diseases (rheumatoid arthritis, etc.). In endometriosis, production of leptin is altered. The authors hypothesized that impaired fertility in endometriosis also has a metabolic origin. As a consequence of modification of receptiveness of endometrial cells the changes into surrounding tissues begin, resulting in incomplete development and maturation of the oocyte, and formation of a defective zygote and embryo [2].

Endometriosis and autoimmunity

The idea that endometriosis might be a specific type of autoimmune disease is still considered by many authors,

despite the possibility that the increased levels of antibodies might result from inflammatory responses of stimulated macrophages responding to the ectopic endometrial tissue [6]. Reports indicate that the chronic subclinical inflammatory status observed during endometriosis could also increase the incidence of gestational diabetes mellitus (GDM) [13].

In the pathogenesis of endometriosis B lymphocytes have been suggested to play primary roles by secreting autoantibodies. Focusing on the role of B lymphocytes in the pathogenesis of endometriosis, particularly the autoimmune response, the following could be elicited via two major types of autoantibody: 1) antibodies of classes IgG and IgA that specifically respond to the endometrium; 2) antibodies that are commonly observed in various autoimmune disorders (antinuclear antibodies, anti-DNA antibodies, and antiphospholipid antibodies). This suggests that endometriosis is associated with abnormal polyclonal B-cell activation, a classic characteristic of autoimmune disease. The association between autoantibody and endometriosis may also explain endometriosis-related infertility, as these antibodies might bind endometrium, embryos and sperm [7].

In patients with endometriosis showing deficient cellular immunity, this indicates that endometrial cells can implant only in women with altered cell-mediated immune responses. In women with endometriosis the number of Th2 cells and the concentration of cytokine IL-4 (produced by Th2) are increased. We should be able to observe a high incidence of allergic and autoimmune diseases in women with endometriosis [6]. Progression of endometriosis is supported by an imbalance of pro-inflammatory (IL-1, IL-6, IL-8, TNF-α) and anti-inflammatory (IL-10, TGF-β) cytokines, and decreased functions of NK cells and Treg CD4$^+$CD25$^+$Foxp3$^+$. There is additional evidence for the autoimmunological origin of endometriosis [1, 29].

The hypotheses about a link between endometriosis and autoimmune diseases have been based on similarities such as high frequencies of myalgia and arthralgia among patients with endometriosis as well as systemic lupus erythematosus. It is known that there are population differences for endometriosis in terms of disease manifestation, especially ethnic heterogeneity of genetic risk factors for various complex disorders such as systemic lupus erythematosus, juvenile idiopathic arthritis, and rheumatoid arthritis [6, 9]. Although the pathogenesis of endometriosis is still debated, evidence suggests that women with endometriosis may have systemic comorbidities [3].

Another possibility was the finding of high levels of autoantibodies in endometriosis patients. Some studies found even higher levels of antibodies against endometrium. Most of these autoantibodies were directed against endometrial antigens, so it might be the result and not the cause of the disease. A detailed study showed that most of these autoantibodies are directed against carbohydrate epitopes (such as Thomsen-Friedenreich antigen), which led to several hypotheses about the involvement of autoantibodies in endometriosis, mostly by aberrant matrix metalloproteinase function or genetic defects in glycosylation. The question remains why the immune system would attack ectopic endometrial tissue, as it should be recognized as self tissue. An abnormal antibody-antigen reaction resulting in high deposits of complement and antibodies in the endometrium has been described [6].

Moreover, probably endometriosis pathogenesis may depend, at least partially, on a mutation in autoimmunity genes. For example, Ammendola *et al.* investigated *PTPN22*, one of the few known shared-autoimmunity genes, and found that carriers of the PTPN22(∗)T variant were significantly more susceptible to endometriosis than controls [30].

Key questions in research on endometriosis are as follows: 1) Could peritoneal inflammation develop on the basis of pathogenesis of endometriosis? 2) Is endometriosis an autoimmune disease? 3) Or are the observed autoimmune reactions secondary phenomena? Gynecologists and clinical immunologists must address these problems together, in order to optimize novel ways of treatment of infertility in women with endometriosis [31, 32].

Conclusions

Endometriosis is an immunodependent disease with a possible autoimmune background; there are observed changes in immunosuppressive mechanisms which could be mediated via the Treg1 subpopulation of lymphocytes, M2 subpopulation of macrophages and monocyte-like suppressor cells (M-MDSC).

For the prognosis of infertility in women with endometriosis it seems to be necessary to study factors of innate immunity – TLRs, approximately oxygen and nitrogen reactive species secretion, pro- and anti-inflammatory cytokines as well as genetic and immunogenetic factors.

The data from the immunological investigations may be subsequently used to develop novel immunomodulatory treatment of women with endometriosis.

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References

1. Anastasi E, Fuggetta E, De Vito C, et al. (2017): Low levels of 25-OH vitamin D in women with endometriosis and associated pelvic pain. Clin Chem Lab Med 56: 1-3.
2. Kohl-Schwartz AS, Weolfler MM, Mitter V, et al. (2017): Endometriosis, especially mild disease: a risk factor for miscarriages. Fertil Steril 108: 806-814.

3. Vannuccini S, Lazzeri L, Orlandini C, et al. (2018): Mental health, pain symptoms and systemic comorbidities in women with endometriosis: a cross-sectional study. J Psychosom Obstet Gynaecol 39: 315-320.

4. Bulun SE, Monsivais D, Kakinuma T, et al. (2015): Molecular biology of endometriosis: from aromatase to genomic abnormalities. Semin Reprod Med 33: 220-224.

5. Trofimova T, Lizneva D, Suturina L, et al. (2017): Genetic basis of eugonadal and hypogonadal female reproductive disorders. Best Pract Res Clin Obstet Gynaecol 44: 3-14.

6. Miller JE, Ahn SH, Monsanto SP, et al. (2017): Implications of immune dysfunction on endometriosis associated infertility: a review. Oncotarget 8: 7138-7147.

7. Korg Y, Shao Y, Rer C, Yarg G (2021): Endometrial stem/progenitor and their roles in immunity, clinical application, and endometriosis. Stem Cell Res Ther 12: 474.

8. Laganá AS, Garzon S, Gőtte S, et al. (2019): The pathogenesis of endometriosis: molecular and cell biology insight. Int J Mol Sci 20: 5615.

9. Agostinis C, Balsuit A, Mangogna A, et al. (2021): Immunological basis of the endometriosis: the complement system as a potential therapeutic target. Front Immunol 11: 1-17.

10. Matalliotakis M, Zervou MI, Matalliotakis C, et al. (2017): The role of gene polymorphisms in endometriosis. Mol Med Rep 16: 5881-5886.

11. Wilczynska K, Bylinska A, Malejczyk J, et al. (2018): The impact of HLA-G, LILRB1 and LILRB2 gene polymorphisms on susceptibility to and severity of endometriosis. Mol Genet Genomics 293: 601-613.

12. Boujenaha J, Cedrin-Durnerina I, Herbemonta C, et al. (2017): Use of the endometriosis fertility index in daily practice: A prospective evaluation. Eur J Obstet Gynecol Reprod Biol 219: 28-34.

13. Daniilidis A, Pados, G (2017): Comments on the ESHRE recommendations for the treatment of minimal endometriosis in infertile women. Reprod Biomed Online 34: 100-103.

14. Pérez-López, FR, Martínez-Domínguez, SJ, Vińas, A, et al. (2018): Endometriosis and gestational diabetes mellitus risk: a systematic review and meta-analysis. Gynecol Endocrinol 34: 363-369.

15. Ashrafi M, Sadatmahalleh SJ, Akhoond MR, Taleb M (2016): Evaluation of risk factors associated with endometriosis in infertile women. Int J Fertil Steril 10: 11-21.

16. Garcia-Gómez E, Vázquez-Martinez ER, Royes-Mayoral C, et al. (2020): Regulation of inflammation pathways and inflammasome by sex steroid hormones in endometriosis. Front Endocrinol 10: 935.

17. Monsanto SP, Edwards AK, Zhou J, et al. (2016): Surgical removal of endometriotic lesions alters local and systemic proinflammatory cytokines in endometriosis patients. Fertil Steril 105: 968-977.

18. Alderman LF, Tal A, Mamillapalli R, et al. (2018): Hematogenous dissemination of mesenchymal stem cells from endometriosis. Stem Cells 36: 881-890.

19. Golab J, Jakobisiak M, Lasek W, Stoklosa T. (2017): Immunologia (nowe wydanie). PWN SA, Warszawa. Retrieved from https://www.pwn.pl

20. Zhang T, De Carolis C, Man GCW, Wang CC (2018): The link between immunity, autoimmunity and endometriosis: a literature update. Autoimmun Rev 10: 945-955.

21. Bruner-Tran KL, Mokshagundam S, Herington JL, et al. (2018) Rodent models of experimental endometriosis: identifying mechanisms of disease and therapeutic targets. Curr Womens Health Rev 14: 173-188.

22. Fraczek M, Kurpisz M (2015): Cytokines in the male reproductive tract and their role in infertility disorders. J Reprod Immunol 108: 98-104.

23. Fox C, Morin S, Jeong JW, et al. (2016): Local and systemic factors and implantation: what is the evidence? Fertil Steril 105: 873-884.

24. Klemmt PAB, Starzinski-Powitz A (2018): Molecular and cellular pathogenesis of endometriosis. Curr Womens Health Rev 14: 106-116.

25. Ahn SH, Khalaj K, Young SL, et al. (2016): Immune-inflammation gene signatures in endometriosis patients. Fertil Steril 32: 220-227.

26. Koninckx PR, Ussia A, Adamyan L, et al. (2019): Pathogenesis of endometriosis: the genetic/epigenetic theory. Fertil Steril 111: 327-340.

27. Falconer H, Mwenda JM, Chai DC, et al. (2008): Effects of anti-TNF-mAb treatment on pregnancy in baboons with induced endometriosis. Fertil Steril 89 (5 Suppl): 1537-1545.

28. Wisniewski A, Wilczynska K, Krasinski R, et al. (2017): Recurrent implantation failure and soluble HLA-G plasma levels. J Reprod Immunol 122: 46-47.

29. Walankiewicz M, Grywalska E, Polak G, et al. (2018): The increase of circulating PD-1- and PD-L1-expressing lymphocytes in endometriosis: correlation with clinical and laboratory parameters. Mediators Inflamm 2018: 7041342.

30. Ammendola M, Bottini N, Pietropolli A, et al. (2008): Association between PTPN22 and endometriosis. Fertil Steril 89: 993-994.

31. Lessey BA, Kim JJ (2017): Endometrial receptivity in the eutopic endometrium of women with endometriosis: it is affected, and let me show you why. Fertil Steril 108: 19-27.

32. Geoffron S, Legendre G, Darai E, et al. (2017): Medical treatment of endometriosis: hormonal treatment of pain, impact on evolution and future perspectives. Presse Med 46: 1199-1211.