AJRI 2003; 50: 48–59



American Journal of Reproductive Immunology
ISSN 8755-8920

# Immunology and Endometriosis

Berkkanoglu M, Arici A. Immunology and endometriosis. AJRI 2003; 50:48–59 © Blackwell Munksgaard 2003

PROBLEM: Accumulating data suggests that aberrant immune responses during retrograde menstruation may be involved in the development of endometriosis.
METHOD OF STUDY: The role of immunology in the etiology of endometriosis is reviewed and summarized from the available literature.
RESULTS: Immunologic factors may affect a woman's susceptibility to implantation of exfoliated endometrial cells. Immune alterations include increased number and activation of peritoneal macrophages, decreased T cell reactivity and natural killer cell cytotoxicity, increased circulating antibodies, and changes in the cytokine network.
CONCLUSION: There is substantial evidence that immunologic factors play a role in the pathogenesis of endometriosis and endometriosis-associated infertility. Decreased natural killer cell cytotoxicity leads to an increased likelihood of implantation of endometriotic tissue. In addition, macrophages and a complex network of locally produced cytokines modulate the growth and inflammatory behavior of ectopic endometrial implants.

**Murat Berkkanoglu and Aydin Arici**

Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Yale University School of Medicine, New Haven, CT, USA

Key words: Autoimmunity, chemokines, cytokines, endometrium, macrophage, natural killer cell

Address reprint requests to Aydin Arici, MD, Division of Reproductive Endocrinology, Department of Obstetrics and Gynecology, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06520-8063, USA.
E-mail: aydin.arici@yale.edu

Submitted July 18, 2002;
revised October 29, 2002;
accepted November 1, 2002.

## INTRODUCTION

Endometriosis is a common gynecologic disorder, affecting 3–10% of reproductive-aged women.[1,2] Prevalence of endometriosis, in fact, changes by diagnosis. In women undergoing tubal ligation, the prevalence of mainly asymptomatic endometriosis was found to vary from 1 to 7%.[3–6] On the other hand, in women with primary infertility the prevalence varied from 9 to 50%.[7,8] There is also racial difference. Two studies have showed that Africans may have a lower risk and East Asians may have higher risk for the disease than Caucasians.[6,9]

Endometriosis is characterized by the growth of endometrial tissue outside the uterine cavity. Nearly more than a century has passed since the first description of endometriosis by von Rokitansky in 1860,[10] but our current understanding of the pathogenesis and pathophysiology of the disease still remains unclear. Various theories have been put forth to explain the mechanisms for the development of this disease. The three main theories accepted presently are implantation of endometrial tissue following retrograde menstruation, coelomic metaplasia and induction theories. Among these theories, Sampson's theory of retrograde menstruation[11] has gained the most supportive evidence. This evidence includes the presence of viable endometrial tissue in the peritoneal fluid that is capable of growth,[12–16] and the anatomical distribution of endometriotic implants.[17]

In fact, no single theory explains all cases adequately. Although retrograde menstruation occurs in at least 76–90% of women undergoing peritoneal dialysis and laparoscopy,[18,19] the much lower prevalence of endometriosis (6.2–8.2%),[20,21] suggests that other factors must determine the susceptibility to developing endometriosis.

The ability of endometrial implants to survive in ectopic locations may be related to an aberrant immune response. The role of the immune system in endometriosis has been studied extensively, and numerous immune anomalies have been identified. Yet, whether immune anomalies are a cause or a result of endometriosis has not been resolved. This article will address the pathogenesis of endometriosis from an immunologic perspective, and characterize immune defects related to this disorder.

## IMMUNOLOGY AND ENDOMETRIOSIS

Many investigators have suggested that there is an association between the presence of endometriosis and

an altered immune system. The theory of an altered immune system and endometriosis suggests that changes in cell-mediated immunity and humoral immunity may contribute to the development of the disease.[22]

*Cell-Mediated Immunity and Endometriosis*

In rhesus monkeys with spontaneous endometriosis cellular immunity to autologous endometrium is suppressed.[23] This observation led to a speculation that endometriosis can develop only in women with altered cellular immunity.[24] A diminished cellular-mediated immune response would make the ectopic implantation of the translocated endometrial cells possible.

*Natural killer cells.* The mechanisms by which the clearance of regurgitated endometrial cells occurs in the peritoneal cavity of the majority of women are poorly understood. However, it has been suggested that natural killer (NK) cells may play a role. There is a decreased cytotoxicity of peripheral and peritoneal fluid NK cells from patients with endometriosis to autologous and heterologous endometrium.[25,26] Furthermore, the decrease in local NK-mediated cytotoxicity in the peritoneal fluid is more pronounced in the moderate and severe stages of endometriosis. It was also shown that the peritoneal fluid from women with endometriosis contained significantly greater NK cell suppressive activity than that of the peritoneal fluid from fertile controls.[27] These findings were confirmed by other researchers in the serum[28] and peritoneal fluid[29] of women with endometriosis.

Thus, it is postulated that the decrease in NK cytotoxicity to retrogradely shed endometrial tissue may allow for the establishment of endometriosis within the peritoneal cavity. The mechanisms that suppress NK cell activity are not clear. Wu et al. reported increased killer inhibitory receptor (KIR) expression on peritoneal NK cells from women with endometriosis, which represents a likely cause of decreased peritoneal NK activity in these patients.[30] In a recent study, it was shown that the proportion of a subclass of killer inhibitory receptors (KIR2DL1) was increased on NK cells in the peritoneal fluid and peripheral blood[31] suggesting a local and systemic decrease in NK activity.

Although it is generally accepted that there is a decrease in NK cytotoxicity, there are discrepancies in the percentage, and the number of these cells in women with endometriosis. Different studies have showed that the number or the percentage of NK cells in women with the disease may be decreased,[32] increased,[33] or unchanged.[25]

It is interesting to note that in women with endometriosis there are progressive increases in both NK cell number[34] and activity[35] following GnRH agonist treatment, which may be because of a direct effect of GnRH agonist or a consequence of decreased estradiol levels.

*Macrophages.* Factors responsible for the suppression of cellular immunity are thought to be products of monocytes or macrophages. It is known that monocyte and macrophage products are modulators of both immune and non-immune cells, and therefore these cells and their products have been investigated intensively in the pathogenesis of endometriosis. Zeller et al. showed that the activational status of peripheral monocytes is increased in women with endometriosis, but they found no difference between the percentage of the peripheral monocytes in women with the disease and that in women without the disease.[36] Furthermore, some other studies showed that peritoneal macrophages are increased in total number, concentration and activational status.[37–41] In association with the activational status of the macrophages there is an increase in the release of their products, such as growth factors and cytokines, which can affect the survival and growth of ectopic endometrial cells.[42]

Scavenger function is one of the vital functions of macrophages in the face of an invading foreign material or when encountering cellular debris and apoptotic cells.[43,44] A variety of surface receptors are involved in this activity.[43–46] A number of factors, including cytokines, glucocorticoids, lipopolysaccharides, interferons, macrophage colony-stimulating factor, and retinoids can regulate these receptors.[47–49] As these cytokines and hormones are present in abnormal levels in the peritoneal fluid of women with endometriosis, they may cause defective scavenger receptor function, which might be another mechanism for abnormally functioning macrophages that could contribute to the growth of ectopic endometrial cells.[50] In addition, it was shown that when macrophages are not adherent and are not attached to the extracellular matrix in tissues, they do not express scavenger receptors of the A-type.[51] Thus, in contrast to tissue macrophages, when the peritoneal macrophages are not attached to extracellular matrix components, they may not be competent scavengers despite their differentiated status. Indeed, the number of non-adherent macrophages is increased in the peritoneal fluid of women with endometriosis.[52] This property may play a causal role in the etiology of this disease through decreased macrophage scavenger activity.

*Lymphocyte.* In endometriosis, it was shown that there is a decrease in the proliferation of peripheral blood lymphocytes in response to recognition of endometrial antigen and cells.[23,24] Similarly, Steele et al. observed that there is a reduced cytotoxic effect of peripheral

blood lymphocytes against autologous endometrial cells, and they suggested that the destruction of endometrial cells is decreased in women with endometriosis.[24] Although the changes in peripheral blood leukocyte profiles were reported in women with endometriosis, data are inconsistent. In one study, the ratio of T helper to T suppressor lymphocytes was found to be increased in peripheral blood of women with endometriosis.[53] But two other studies revealed that peripheral lymphocyte profiles are not markedly affected in endometriosis.[33,54] In addition, data obtained from studies on peripheral lymphocytes must be carefully interpreted because the biologic activities of peripheral lymphocytes may not reflect the biologic activities of lymphocytes from specific tissue sites.[55] Peritoneal T cell and macrophage numbers were increased in baboons with spontaneous endometriosis, but not in those with surgically induced disease.[56] Increase in the T helper to T suppressor ratio and concentration of both T cell types were reported in the peritoneal fluid[33,57] and ectopic endometriotic tissue[58] compared to eutopic endometrium in women with endometriosis. On the other hand, by immunohistochemistry no significant difference in the ratio of T helper to T suppressor lymphocytes could be shown between eutopic endometria of patients with and without endometriosis.[59] A recent study has reported that GnRH agonist treatment in women with endometriosis causes an up-regulation of T cell proliferative activity.[35] In all, there appear to be inconsistencies in the alterations in T cells and their role in endometriosis.

*Immune surveillance*. Defective immune surveillance is another interesting concept in the etiology of endometriosis. It has been postulated that the defective immune surveillance predisposes susceptible women to develop the syndrome. Several mechanisms have been put forth on how endometriotic cells evade leukocyte recognition. One mechanism could be the secretion of proteins that interfere with immunocyte-endometrial implant recognition. One such factor is the soluble form of the intercellular adhesion molecule (ICAM)-1. ICAM-1, a cell surface molecule, acts as a ligand for leukocyte function antigen-1 (LFA-1), and the ICAM-1/LFA-1 system mediates various cell–cell interactions involved in immunity. Soluble ICAM-1 (sICAM-1) is a circulating substance generated by cleavage of the extracellular domain of ICAM-1. It binds with LFA-1 of leukocytes, thus, making leukocytes less available for binding with cell surface ICAM-1 on target cells. As a result, this prevents the activation of these leukocytes. It has been shown that isolated endometriotic stromal cells express more ICAM-1 mRNA and secrete more sICAM-1 than the matched stromal cells from eutopic endometrium.[60]

Another hypothesis involves the Fas–Fas ligand (FasL) system. When a FasL-expressing cell binds a Fas-bearing immune cell, it triggers the Fas-bearing cell's death by apoptosis. Garcia-Velasco et al. have shown that macrophage-conditioned media induces FasL expression by endometrial stromal cells in a concentration-dependent manner, and they have suggested that the proinflammatory nature of the peritoneal fluid of women with endometriosis induces the FasL expression by regurgitated endometrial cells, and signals Fas-mediated cell death of activated immune cells; this could be a mechanism for endometrial cells to escape immune surveillance.[61] Selam et al. have shown that eutopic endometrial stromal cells from women with endometriosis demonstrate higher FasL expression compared with those from women without endometriosis when they attach to extracellular matrix proteins (fibronectin, laminin and collagen IV).[62] These results suggest that the attachment of endometrial stromal cells during retrograde menstruation to a new environment such as peritoneum with increased expression of laminin, fibronectin and collagen IV could lead to an increase in FasL expression. This induction of FasL may take part in the development of a relative immunotolerance by inducing apoptosis of cytotoxic T lymphocytes, which will allow further development of ectopic implants (Fig. 1).

*Humoral Immunity*

*Autoantibodies*. In addition to alterations in cell-mediated immunity, considerable evidence has been gathered indicating that there are alterations in B-cell activity and an increased incidence of autoantibodies in women with endometriosis (Table I). Nearly two decades ago, it was shown that there is an increased B-cell function in women with endometriosis as well as with adenomyosis.[63] Later, Weed et al. demonstrated IgG and complement deposits in the eutopic endometrium and a corresponding reduction in the serum total complement level in women with endometriosis.[64] They hypothesized that ectopic endometrium might act as a foreign trigger to induce an autoimmune response, resulting in infertility. Mathur et al. detected IgG and IgA autoantibodies in sera, cervical and vaginal secretions of women with endometriosis.[65] Later, they found that these autoantibodies recognize endometrial antigens (ranging in molecular weight from 34 to 140 kD) that are candidates for the autoantigens responsible for the immune response.[66] In addition, complement C3 and C4 levels were found to be higher in the serum and the peritoneal fluid of women with endometriosis than those in controls.[67]

The theory that endometriosis could be regarded as an autoimmune disease, since there is an increase in the frequency of autoantibodies, was first introduced by

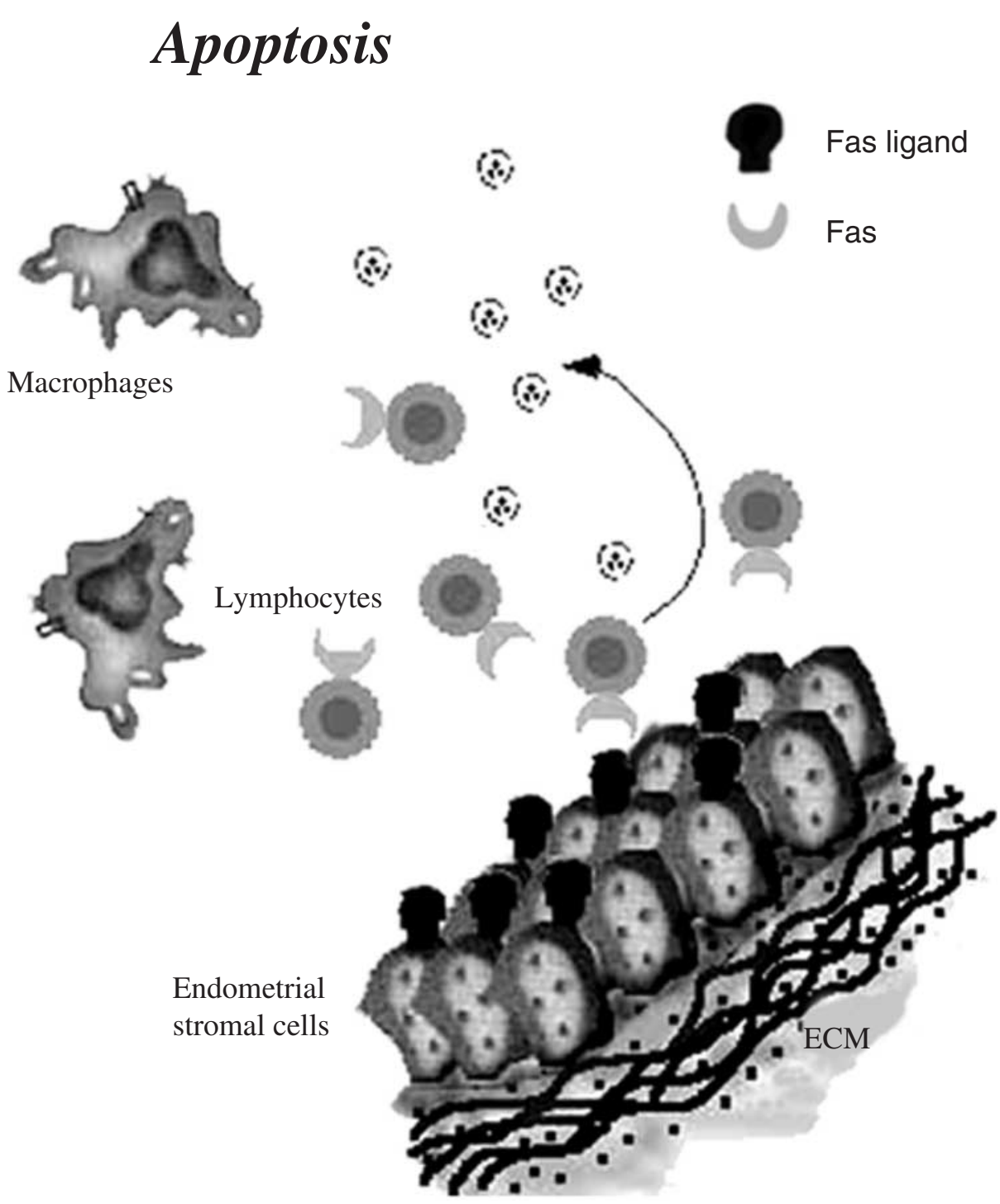


*Fig. 1.* Peritoneal macrophages and extracellular matrix proteins (ECM) can induce Fas ligand expression by endometrial stromal cells, which in turn may result in apoptosis of cytotoxic lymphocytes.

Gleicher et al.[68] These include autoantibodies to a variety of phospholipids, histones, polynucleotides as well as lupus.[68–71] Some of these autoantibodies are organ-specific such as antiendometrial and antiovarian antibodies.[66] Like classical autoimmune diseases, endometriosis has polyclonal B-cell activation, immunological abnormalities in T- and B-cell functions, increased apoptosis, tissue damage, and multiorgan involvement. Moreover, there are familial occurrence, a possible genetic basis, female preponderance, and

TABLE I. Summary of Immunologic Abnormalities Related to Autoantibodies

| | |
|---|---|
| Increased B cell function | Startseva et al.[63] |
| Polyclonal B cell activation | Hang et al.[73] |
| IgG and complement deposits in eutopic endometrium | Weed et al.[64] |
| IgG and IgA autoantibodies against endometrial antigens | Mathur et al.[65,66] |
| Increased C3 and C4 levels in the serum and peritoneal fluid | Badawy et al.[67] |
| Increased autoantibodies of IgG, IgM and IgA against phospholipids, histones, polynucleotides | Gleicher et al.,[68,70] Kennedy et al.,[69] Taylor et al.[71] |
| Antibodies to human chorionic gonadotorpin receptor, isoforms I and II of the enzyme carbonic anhydrase, CA 125, serum transferrin, $\alpha_2$ -Heremans Schmidt glycoprotein | Moncayo et al.,[82] D'Cruz et al.,[83] Barbieri et al.,[84] Mathur et al.,[85] Pilai et al.[86] |
| Autoantibodies against a Thomsen–Friendreich-like carbohydrate antigen | Lang et al.[87] |

increased likelihood of other autoimmune diseases.[72–74] However, there are still some problems in postulating and accepting endometriosis as an autoimmune disease. Autoimmune diseases are associated with certain HLA alleles. Although a strong genetic component exists in endometriosis,[75] no association with specific HLA haplotypes has been demonstrated yet.[76] To define a disease truly autoimmune in nature, it needs to be manifested in normal animals following adaptive transfer of immunoglobulin from the blood or affected tissues of subjects with autoimmune disease, but there is still no such study performed to investigate this phenomenom.[72] There is also no proof that complement activation occurs specifically in the endometrium of women with endometriosis.[77,78] The increased endometrial autoimmunity in endometriosis patients is also thought to be because of enhanced immune reactivity to normal self-antigens because of a genetic predisposition to autoimmunity or an excess of endometrial autoantigens in the peritoneal cavity because of retrograde menstruation. However, there are some investigators who have been able to document these autoantibodies,[79,80] and this subject remains controversial.[81]

Recognized tissue antigens include the human chorionic gonadotropin receptor, isoforms I and II of the enzyme carbonic anhydrase, and CA-125.[82–84] Antibodies to serum transferrin and $\alpha_2$ -Heremans Schmidt glycoprotein have also been described and proposed as diagnostic markers.[85,86] However, none of these tests has provided sufficient evidence concerning the disease and stage specificity to be used for screening. It has recently been shown that a common carbohydrate epitope, the Thomsen–Friedenreich-like (T) antigen, found on many of these antigens is involved in the autoantibody response.[87] The anti-T-like response may be indicative of an underlying genetic defect in glycosylation or in the control of glycosylation by steroid sex hormones.[88]

The association between autoantibody abnormalities and endometriosis could explain endometriosis-related infertility. An increased risk of pregnancy loss has been clearly associated with the presence of abnormal non-organ specific[89] as well as organ-specific autoantibodies.[90] There are some studies showing that treatment with Danazol[91] or GnRH analogues[92] suppresses the levels of autoantibodies associated with endometriosis. Thus, they provide further evidence for a role of autoimmunity or autoantibodies in the infertility associated with endometriosis. Dmowski et al. also showed the effect of autoantibodies on *in vitro* fertilization (IVF) success rates among women with endometriosis. They did a retrospective analysis of IVF cycles of 193 patients and measured autoantibodies in 50 of them who had a history of endometriosis. They found those women with evidence of more than three autoantibodies ('positive') had a significantly lower pregnancy rate per transfer (22.9 versus 45.7%) than women with less than two autoantibodies ('negative'). Among 10 autoantibody-positive patients treated with corticosteroids, eight of them became pregnant, whereas none of the antibody-positive endometriosis patients who were not treated with glucocorticoids conceived.[93] These data can be interpreted to suggest that abnormal autoantibodies may play a role in the infertility associated with endometriosis.

### *Cytokines and Growth Factors*

Cytokines and growth factors are proteins or glycoproteins produced by leukocytes or other cells, and secreted to the extracellular environment.[94] These molecules exert their effects on the same (autocrine activity) or nearby cells (paracrine activity). They are also key mediators of intercellular communication within the immune system. Cytokines may have proliferative, cytostatic, chemoattractant or differentiative effects. In the peritoneal fluid of women with endometriosis, several proinflammatory chemoattractant cytokines for monocytes, macrophages and granulocytes have been identified.

*Interleukin-1*. Interleukin (IL)-1 is a cytokine playing an important role in inflammation and immune response. It is secreted mainly by activated monocytes and macrophages and also by T and B cells, and NK cells. It affects the activation of T cells and the differentiation of B cells. There are two distinct molecular forms of IL-1 (IL-1α and IL-1β) derived from two different genes. An IL-1 receptor antagonist (IL-1ra) has also been defined and acts as an endogenous receptor inhibitor. IL-1 has been isolated from the peritoneal fluid of women with endometriosis. Most researchers found increased levels in such women,[95–97] although others found no difference.[98] It has been suggested that IL-1β plays a role in promoting angiogenesis in endometriotic lesions by inducing the angiogenic factors (vascular endothelial growth factor and IL-6) in endometriotic stromal cells but not in normal endometrial stromal cells.[99] It has also been found that IL-1β increased sICAM-1 shedding from endometrial cells, which may interfere with peritoneal immune surveillance.[60]

*Interleukin-6*. IL-6 is a cytokine mainly produced by T cells, and also secreted by macrophages, fibroblasts and endothelial cells. It has B cell stimulatory activity. It enhances the differentiation of T cells, induces the synthesis of acute phase proteins, modulates the secretion of other cytokines, and also acts as a growth regulator for various human cell lines. It has been shown that IL-6 can inhibit the proliferation of the human endometrial stromal cells.[100] Yoshioka et al. observed that IL-6 has no effect on the growth of endometrial stromal cells from the proliferative phase, but it inhibits proliferation of endometrial stromal cells from the secretory phase.[101] On the other hand, they also found that stromal cells of endometriotic tissues are resistant to IL-6, showing no inhibitory response. Endometriotic cells may behave differently from their eutopic endometrial counterpart in terms of the inhibitory regulation exerted by IL-6. Tabibzadeh et al. reported that IL-6 levels are low in the proliferative phase of the cycle while estrogen concentrations are high, and IL-6 levels are high during the secretory phase of the cycle while estrogenic activity is low. Therefore, they suggested that IL-6 fluctuations during the menstrual cycle reflect an inverse relationship to estrogen action.[102] Peritoneal fluid levels of IL-6 correlate well with the severity of endometriosis, and a high peritoneal fluid level of this cytokine in severe endometriosis is also accompanied by a decrease in IL-6 soluble receptor concentration.[103] For this reason, the endometriotic implants may be resistant to growth inhibition by IL-6. However, other investigators reported an increased concentration of this soluble receptor.[104] The secretion of IL-6 was highest in ectopic lesions, significantly lower in eutopic endometrium from women with endometriosis and lowest in endometrium from women without the disease. Similar results were obtained when cells were stimulated by IL-1β.[105]

*Interleukin-8*. IL-8 is a chemokine that induces chemotaxis of neutrophils and is a potent angiogenic factor. Besides mesothelial cells that form the majority of the peritoneal cells, macrophages and endometrial cells are potential sources of this chemokine.[106,107] We have found that there are menstrual cycle-dependent changes in IL-8 mRNA in the endometrium.[108] IL-8 mRNA levels in the late secretory and early to mid-proliferative phase samples are higher than the level observed in the middle of the cycle. It can be speculated that IL-8 may modulate the timely recruitment of neutrophils and lymphocytes into the endometrium. Moreover, it has been shown that IL-8 significantly stimulates cell proliferation in endometrial and endometriotic stromal cells.[109,110] Iwabe et al. also observed that TNF-α stimulates proliferation of endometriotic stromal cells through induction of IL-8 gene and protein expression.[111] We and other investigators have previously shown that IL-8 is elevated in the peritoneal fluid of women with endometriosis.[107,112,113] We also observed that IL-8 level is correlated with the severity of the disease.[107] We have recently shown that IL-8 stimulates the adhesion of endometrial cells to fibronectin.[114] Thus, IL-8 may be relevant for stimulating the attachment of endometrial implants in the pathogenesis of endometriosis. In addition, the adherence of endometrial cells induces further IL-8 expression by an integrin-dependent mechanism.[115] In summary, IL-8 may act as an autocrine growth factor in the endometrium and may play a role in the pathogenesis of endometriosis by promoting a vicious circle of endometrial cell attachment, cell growth, and further secretion of this cytokine.

*Interleukin-12*. IL-12 is secreted by macrophages and monocytes. It plays an important role in the regulation of NK cell activity. It stimulates secretion of various cytokines, cytotoxicity of NK cells, and also proliferation of T cells and NK cells. Zeyneloglu et al. demonstrated that IL-12 is present in peritoneal fluid of women with endometriosis as well as in women without endometriosis. It was shown that the most likely source of IL-12 in peritoneal fluid is macrophages, and IL-12 does not have value in predicting the inflammatory status of peritoneal fluid.[116] It was found that higher levels of free p40 subunit of IL-12 are present in the peritoneal fluid of women with endometriosis compared to those in women without the

disease. It was also shown that free p40 subunit is a very potent inhibitor of IL-12-induced NK activity and decreases IL-12 receptors on NK cells.[117]

*Monocyte chemotactic protein-1 (MCP-1)*. MCP-1 is a monocyte chemoattractant and activating factor. It is produced by monocytes, T lymphocytes, fibroblasts and endothelial cells. IFN-γ is a potent stimulus for leukocyte release of MCP-1, while IL-13 inhibits its release. Concentration of MCP-1 is elevated in the peritoneal fluid of women with endometriosis compared with women without endometriosis, and the level also correlates with the severity of the disease and tends to decrease with medical treatment.[118,119]

*Rantes*. RANTES (Regulated on Activation, Normal T-cell Expressed and Secreted) is a chemokine and a chemoattractant for monocytes and memory T-cells. It is secreted by T cells, some epithelial cells and mesenchymal cells. It has been shown that the concentration of RANTES is increased in the peritoneal fluid of women with endometriosis and its level correlates with the severity of the disease. It has been postulated that secretion of RANTES may result in the recruitment of peritoneal macrophages and more T lymphocytes after T cell activation in peritoneal fluid.[120] The pattern of RANTES protein distribution, which shows localization primarily in stromal compartment in endometriotic lesions, is similar to that found in normal endometrium.[121] Moreover, there is an important difference between normal endometrial stromal cell cultures and those derived from endometriomas that are under similar conditions. The latter cells show significantly greater secretion of RANTES.[122]

*Transforming growth factor*-β *(TGF*-β*)*. TGF-β is mainly produced by platelets, activated lymphocytes and macrophages. It is a potent chemoattractant for monocytes, which induces angiogenesis, and inhibits T and B lymphocyte and NK cell activity. It was shown that there are increased concentrations of TGF-β in peritoneal fluid in women with endometriosis as compared to both fertile and infertile patients without endometriosis.[123]

*Tumor necrosis factor*-α *(TNF*-α *)*. Neutrophils, activated lymphocytes, macrophages, NK cells, and several nonhematopoietic cells produce TNF-α, whereas TNF-β is produced by lymphocytes. These TNFs were initially identified for their ability to kill certain cell lines. But it is now known that they have the ability to initiate the cascade of cytokines and other factors associated with inflammatory responses. *In vitro,* epithelial cell TNF secretion is increased by IL-1, and also modulated by progesterone and plasma protein-14.[124] TNF-α has been shown to increase the adherence of cultured stromal cells to mesothelial cells.[125] This finding suggests that the presence of TNF-α in peritoneal fluid may play a facilitory role in adherence of ectopic endometrial tissue to the peritoneum, allowing implants to develop. Several researchers have found that TNF-α concentrations are increased in the peritoneal fluid of women with endometriosis, and that its level correlates with the stage of the disease.[126] It has also been demonstrated that TNF-α affects sperm motility *in vitro,* but only at very high concentrations.[127] In addition, Hill et al. have tested various cytokines and found significant embryotoxicity only by TNF-α and IFN-γ.[128]

*Vascular endothelial growth factor (VEGF)*. VEGF is a heparin-binding glycoprotein. It is a very potent mitogen for endothelial cells, and induces vascular permeability and acts as a chemoattractant for monocytes. It is also one of the most potent angiogenic factors. It is produced by monocytes, macrophages and smooth muscle cells. VEGF binds to a family of tyrosine kinase receptors including Flt-1 and KDR, leading to dimer formation, autophosphorylation of the receptor, and activation of mitogen-activated protein kinases. It has been shown that VEGF protein is localized predominantly in endometrial glands.[129,130] Estradiol increases the expression of VEGF gene in normal human endometrium.[129] Hypoxia, IL-1, platelet-derived growth factor and TGF-β, epidermal growth factor, and prostaglandin $E_2$ are other factors known to up-regulate VEGF expression.[99,131,132] The expression of VEGF by endometriotic implants provides a mechanism for the neovascularization, which is commonly observed around these lesions.[133] VEGF immunostaining is observed in the epithelium of endometriotic implants,[129] particularly in hemorrhagic red implants.[134] In endometriosis, peritoneal fluid VEGF concentrations are significantly higher in women with moderate to severe endometriosis than in women with minimal to mild endometriosis.[135]

*Impact of Endometriosis on Fertility*

There is also substantial evidence that these immunologic alterations may play a role in endometriosis-associated infertility. There are some studies showing that there is an impairment of implantation in patients with endometriosis, which might be because of the peritoneal fluid inflammation or some abnormalities related to the endometrium or embryos themselves.[136,137] It has been demonstrated that peritoneal macrophages phagocytize spermatozoa *in vitro*[138] Sperm velocity and the proportion of motile spermatozoa decrease when peritoneal fluid from women with endometriosis is added to the medium *in vitro*.[139]

Fertilization of murine oocytes *in vitro* is inhibited by the peritoneal fluid from women with endometriosis more than the peritoneal fluid from women without endometriosis.[140] In addition, peritoneal fluid from women with endometriosis is embryotoxic and decreases mouse embryo cleavage rate.[141] On the other hand, there are also some other reports suggesting that there is no adverse effect of peritoneal fluid from women with endometriosis on fertilization and embryo development in mice.[142,143]

## CONCLUSION

Although there are many gaps in our understanding of the pathogenesis of endometriosis, there is substantial evidence to support that the immunologic factors play a role in the pathogenesis of this disease and endometriosis-associated infertility. Immunologic factors may affect a woman's susceptibility to the implantation of exfoliated endometrial cells. Immune alterations include increased number and activation of peritoneal macrophages, decreased T cell reactivity and NK cytotoxicity, and increased autoantibodies, and changes in the cytokine network (Fig. 2). Owing to the presence of these immune alterations and its resemblance to some autoimmune diseases such as rheumatoid arthritis and Crohn's disease, both of which having elevated levels of autoantibodies and several cytokines, some immune modulatory drugs are also being tried in the treatment of endometriosis. Among these drugs, pentoxifylline and loxoribine have been shown to induce some regression of endometriotic implants in rodent models for endometriosis.[144,145] Furthermore, these drugs do not induce an estrogen deficiency, and therefore it appears that drugs that can modulate immune interactions would become a promising alternative to conventional treatments for endometriosis in the near future. In addition, some of these immune alterations have been used as diagnostic markers, but none of these tests has provided sufficient evidence concerning the disease and stage specificity to be used for screening. This is probably because of the fact that the environment of the peritoneal cavity may also play a decisive role in the development of endometriosis.

Nevertheless, further studies are still needed to determine whether these changes lead to the disease, are coincidental to it, or result from it.

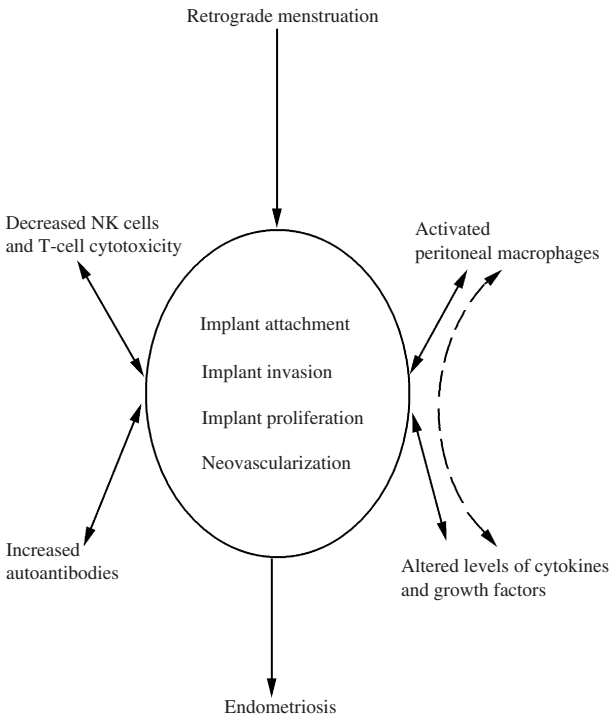


*Fig. 2.* Immune alterations and endometriosis.

## REFERENCES

1. Olive DL, Schwartz LB: Endometriosis. New Engl J Med 1993; 328:1759.
2. Cramer DW: Epidemiology of endometriosis. *In* Endometriosis, EA Wilson (ed.). New York, Alan R. Liss, Inc., 1987, p. 5.
3. Kirshon B, Poindexter AN: Contraception: a risk factor for endometriosis. Obstet Gynecol 1988; 71:829–831.
4. Strathy JH, Molgaard CA, Coulam CB, Melton LJ: Endometriosis and infertility: laparoscopic study of endometriosis among fertile and infertile women. Fertil Steril 1982; 38:667–672.
5. Mahmood TA, Templeton A: Prevalence and genesis of endometriosis. Hum Reprod 1991; 6:544–549.
6. Sangi-Haghpeykar H, Poindexter AN: Epidemiology of endometriosis among parous women. Obstet Gynecol 1995; 85:983–992.
7. Duignan NH, Jordan JA, Coughlan BM, Logan-Edwards R: One thousand consecutive cases of diagnostic laparoscopy. J Obstet Gynecol Br Commonw 1972; 79:1016–1020.
8. Williams TJ, Pratt JR: Endometriosis in 1000 consecutive celiotomies: incidence and management. Am J Obstet Gynecol 1977; 129:245–250.
9. Hasson HM: Incidence of endometriosis in diagnostic laparoscopy. J Reprod Med 1976; 16:135–140.
10. Von Rokitansky C: Zesch. Gesselsch. Aertz 1860; 16:577.
11. Sampson JA: Peritoneal endometriosis due to menstrual dissemination of endometrial tissue into the peritoneal cavity. Am J Obstet Gynecol 1927; 14:422–469.
12. Beyth Y, Jaffe H, Jevij ISH, Sadovsky E: Retrograde seeding of endometrium: sequelia of tubal flushing. Fertil Steril 1975; 26:1094–1097.
13. Bartosik D, Jacob S, Kelly LJ: Endometrial tissue in peritoneal fluid. Fertil Steril 1986; 46:796–800.

14. Keettel WC, Stein RJ: The viability of cast-off menstrual endometrium. Am J Obstet Gynecol 1951; 61:440–442.
15. Telinde RW, Scott RB: Experimental endometriosis. Am J Obstet Gynecol 1950; 60:1147–1173.
16. Ridley JH, Edwards IK: Experimental endometriosis in the human. Am J Obstet Gynecol 1958; 76:783–790.
17. Jenkins S, Olive DL, Haney AF: Endometriosis: pathogenic implications of the anatomic distribution. Obstet Gynecol 1986; 67:335–338.
18. Blumenkrantz MJ, Gallagher N, Bashore RA, Tenckhoff H: Retrograde menstruation in women undergoing chronic peritoneal dialysis. Obstet Gynecol 1981; 57:667–670.
19. Halme J, Hammond MG, Hulka JF, Raj SG, Talbert LM: Retrograde menstruation in healthy women and in patients with endometriosis. Obstet Gynecol. 1984; 64:151–154.
20. Houston DE, Noller KL, Melton LJ III, Selwyn BJ, Hardy RJ: Incidence of pelvic endometriosis in Rochester, Minnesota, 1970–1979. Am J Epidemiol 1987; 125:959–969.
21. Kjerulff KH, Erickson BA, Langenberg PW: Chronic gynecological conditions reported by US women: findings from the National Health Interview Survey, 1984 to 1992. Am J Public Health 1996; 86:195–199.
22. Senturk LM, Arici A: Immunology of endometriosis. J Reprod Immunol 1999; 43:67–83.
23. Dmowski WP, Steele RN, Baker GF: Deficient cellular immunity in endometriosis. Am J Obstet Gynecol 1981; 141:377–383.
24. Steele RW, Dmowski WP, Mormer DJ: Immunologic aspects of human endometriosis. Am J Reprod Immunol 1984; 6:33–36.
25. Oosterlynck DJ, Cornillie FJ, Waer M, Vandeputte M, Koninckx PR: Women with endometriosis show a defect in natural killer activity resulting in a decreased cytotoxicity to autologous endometrium. Fertil Steril 1991; 56:45–51.
26. Wilson TJ, Hertzog PJ, Angus D, Munnery L, Wood EC, Kola I: Decreased natural killer cell activity in endometriosis patients: relationship to disease pathogenesis. Fertil Steril 1994; 62:1086–1088.
27. Oosterlynck DJ, Meuleman C, Waer M, Koninckx PR, Vandeputte M: Immunosuppressive activity of peritoneal fluid in women with endometriosis. Obstet Gynecol 1993; 82:206–212.
28. Kanzaki H, Wang HS, Kariya M, Mori T: Suppression of natural killer cell activity by sera from patients with endometriosis. Am J Obstet Gynecol 1992; 167:257–261.
29. Ho HN, Wu MY, Yang YS: Peritoneal cellular immunity and endometriosis. Am J Reprod Immunol 1997; 38:400–412.
30. Wu MY, Yang JH, Chao KH, Hwang JL, Yang YS, Ho HN: Increase in the expression of killer cell inhibitory receptors on peritoneal natural killer cells in women with endometriosis. Fertil Steril 2000; 74:1187–1191.
31. Maeda N, Izumiya C, Yamamoto Y, Oguri H, Kusume T, Fukaya T: Increased killer inhibitory receptor KIR2DL1 expression among natural killer cells in women with pelvic endometriosis. Fertil Steril 2002; 77:297–302.
32. Hsu C, Lin Y, Wang S, Huang K: Immunomodulation in women with endometriosis receiving GnRH agonist. Obstet Gynecol 1997; 89:993–998.
33. Hill JA, Faris HM, Schiff I, Anderson DJ: Characterization of leukocyte subpopulations in the peritoneal fluid of women with endometriosis. Fertil Steril 1988; 50:216–222.
34. Hsu C, Lin Y, Wang S, Huang K: Immunomodulation in women with endometriosis receiving GnRH agonist. Obstet Gynecol 1997; 89:993–998.
35. Garzetti GG, Ciavattini A, Provinciali M, Muzzioli M, Di Stefano G, Fabris N: Natural cytotoxicity and GnRH agonist administration in advanced endometriosis: positive modulation on natural killer activity. Obstet Gynecol 1996; 88:234–240.
36. Zeller JM, Henig I, Radwanska E, Dmowski WP: Enhancement of human monocyte and peritoneal macrophage chemiluminescence activities in women with endometriosis. Am J Reprod Immunol Microbiol 1987; 13:78–82.
37. Haney AF, Muscato JJ, Weinberg JB: Peritoneal fluid cell populations in infertility patients. Fertil Steril 1981; 35:696–698.
38. Halme J, Becker S, Hammond MG, Raj S: Pelvic macrophages in normal and infertile women: the role of patent tubes. Am J Obstet Gynecol1982; 142:890–895.
39. Halme J, Becker S, Wing R: Accentuated cyclic activation of peritoneal macrophages in patients with endometriosis. Am J Obstet Gynecol 1984; 148:85–90.
40. Halme J, Becker S, Haskill S: Altered maturation and function of peritoneal macrophages: possible role in the pathogenesis of endometriosis. Am J Obstet Gynecol 1987; 156:783–789.
41. Dunselman GAJ, Hendrix MGR, Bouckaert PXJ, Evers JLH: Functional aspects of peritoneal macrophages in endometriosis of women. J Reprod Fertil 1988; 82:707–710.
42. Lebovic DI, Mueller MD, Taylor RN: Immunobiology of endometriosis. Fertil Steril 2001; 75:1–10.
43. Ramprasad MP, Fischer W, Witztum JL et al.: The 94- to 97-kDa mouse macrophage membrane protein that recognizes oxidized low-density lipoprotein and phosphatidylserine-rich liposomes is identical to macrosialin, the mouse homologue of human CD68. Proc Natl Acad Sci USA 1995; 92:9580–9584.
44. Ottnad E, Parthasarathy S, Sambrano GR et al.: A macrophage receptor for oxidized low-density lipoprotein distinct from the receptor for acetyl low-density lipoprotein: partial purification and role in recognition of oxidatively damaged cells. Proc Natl Acad Sci USA 1995; 92:1391–1395.
45. Stanton LW, White RT, Bryant CM et al.: A macrophage Fc receptor for IgG is also a receptor for oxidized low-density lipoprotein. J Biol Chem 1992; 267:22446–22451.
46. Endemann G, Stanton LW, Madden KS et al.: CD36 is a receptor for oxidized low-density lipoprotein. J Biol Chem 1993; 268:11811–11816.
47. Moulton KS, Wu H, Barnett J et al.: Regulated expression of the human acetylated low-density lipoprotein receptor gene and isolation of promoter sequences. Proc Natl Acad Sci USA 1992; 89:8102–8106.
48. Wu H, Moulton K, Horvai A et al.: Combinatorial interactions between AP-1 and Ets domain proteins

contribute to the developmental regulation of the macrophage scavenger receptor gene. Mol Cell Biol 1994; 14:2129–2139.
49. De Villiers WJ, Fraser IP, Gordon S: Cytokine and growth factor regulation of macrophage scavenger receptor expression and function. Immunol Lett 1994; 43:73–79.
50. Sidell N, Han SW, Parthasarathy S: Regulation and modulation of abnormal immune responses in endometriosis. Ann N Y Acad Sci 2002;955:159–173 (discussion 199–200, 396–406).
51. Kim JG, Keshava C, Murphy AA et al.: Fresh mouse peritoneal macrophages have low scavenger receptor activity. J Lipid Res 1997; 38:38:2207–2215.
52. Olive DL, Weinberg JB, Haney AF: Peritoneal macrophages and infertility: the association between cell number and pelvic pathology. Fertil Steril 1995; 44:772–777.
53. Badawy SZ, Cuenca V, Stitzel A, Tice D: Immune rosettes of T and B lymphocytes in infertile women with endometriosis. J Reprod Med 1987; 32:194–197.
54. Gleicher N, Dmowski WP, Siegel I, Liu TL, Friberg J, Radwansea E et al.: Lymphocyte subsets in endometriosis. Obstet Gynecol 1984; 63:463–466.
55. Dudley DJ: The immune system in health and disease. Clin Obstet Gynecol 1992; 6:393–416.
56. D'Hooghe T, Hill J, Oosterlynck D et al.: Effect of endometriosis on white blood cell subpopulations in peripheral blood and peritoneal fluid of baboons. Hum Reprod 1996; 11:1739–1740.
57. Dmowski WP, Gebel HM, Braun DP: The role of cell-mediated immunity in pathogenesis of endometriosis. Acta Obstet Gynecol Scand Suppl 1994; 159:7–14.
58. Witz CA, Montoya IA, Dey TD, Schenken RS: Characterization of lymphocyte subpopulations and T cell activation in endometriosis. Am J Reprod Immunol 1994; 32:173–179.
59. Mettler L, Volkov NI, Kulakov VI, Jurgensen A, Parwaresch MR: Lymphocyte subsets in the endometrium of patients with endometriosis throughout the menstrual cycle. Am J Reprod Immunol 1996; 36:342–348.
60. Viganò P, Gaffuri B, Somigliana E, Busacca M, Di Blasio AM, Vignali M: Expression of intercellular adhesion molecule (ICAM)-1 mRNA and protein is enhanced in endometriosis versus endometrial stromal cells in culture. Mol Hum Reprod 1998; 4:1150–1156.
61. Garcia-Velasco JA, Arici A, Zreik T, Naftolin F, Mor G: growth factors modulate Fas ligand expression in cultured endometrial stromal cells: a role in endometriosis. Mol Hum Reprod 1999; 5:642–650.
62. Selam B, Kayisli UA, Garcia-Velasco JA, Arici A: Extracellular matrix-dependent regulation of Fas ligand expression in human endometrial stromal cells. Biol Reprod 2002; 66:1–5.
63. Startseva NV: Clinical immunological aspects of genital endometriosis. Akush Ginekol (Mosk) 1980; 3:23–26.
64. Weed JC, Arquembourg PC: Endometriosis: can it produce an autoimmune response resulting in infertility? Clin Obstet Gynecol 1980; 23:885–893.
65. Mathur S, Garza DE, Smith LF: Endometrial autoantigens eliciting immunoglobulin (Ig)G, IgA, and IgM responses in endometriosis. Fertil Steril 1990; 54:56–63.
66. Mathur S, Peres MR, Williamson HO, Youmans CD, Maney SA, Garvin AJ et al.: Autoimmunity to endometrium and ovary in endometriosis. Clin Exp Immunol 1982; 50:259–266.
67. Badawy SZ, Cuenca V, Stitzel A, Jacobs RD, Tomar RH: Autoimmune phenomena in infertile patients with endometriosis. Obstet Gynecol 1984; 63:271–275.
68. Gleicher N, el-Roeiy A, Confino E, Friberg J: Is endometriosis an autoimmune disease? Obstet Gynecol 1987; 70:115–122.
69. Kennedy SH, Sargent IL, Starkey PM, Hicks BR, Barlow DH: Localization of anti-endometrial antibody binding in women with endometriosis using a double-labeling immunohistochemical method. Br J Obstet Gynecol 1990; 97:671–674.
70. Confino E, Harlow L, Gleicher N: Peritoneal fluid and serum autoantibody levels in patients with endometriosis. Fertil Steril 1990; 53:242–245.
71. Taylor PV, Maloney MD, Campbell JM, Skerrow SM, Nip MM, Parmar R, Tate G: Autoreactivity in women with endometriosis. Br J Obstet Gynecol 1991; 98:680–684.
72. Nothnick WB: Treating endometriosis as an autoimmune disease. Fertil Steril 2001; 76:223–231.
73. Hang L, Slack JH, Amundson C, Izui S, Theofilopoulos AN, Dixon FJ: Induction of murine autoimmune disease by chronic polyclonal B cell activation. J Exp Med 1983; 157:874–883.
74. Prud'homme GJ, Balderas RS, Dixon FJ, Theofilopoulos AN: B cell dependence on and response to accessory signals in murine lupus strains. J Exp Med 1983; 157:1815–1827.
75. Malinak LR, Buttram VCJr, Elias S, Simpson JL: Heritage aspects of endometriosis: II: clinical characteristics of familial endometriosis. Am J Obstet Gynecol 1980; 137:332–337.
76. Maxwell C, Kilpatrick DC, Haining R, Smith SK: No HLA-DR specificity is associated with endometriosis. Tissue Antigens 1989; 34:145–147.
77. Isaacson KB, Coutifaris C, Garcia CR, Lyttle CR: Production and secretion of complement component 3 by endometriotic tissue. J Clin Endocrinol Metab 1989; 69:1003–1009.
78. Isaacson KB, Galman M, Coutifaris C, Lyttle CR: Endometrial synthesis and secretion of complement component-3 by patients with and without endometriosis. Fertil Steril 1990; 53:836–841.
79. Meek SC, Hodge DD, Musich JR: Autoimmunity in infertile patients with endometriosis. Am J Obstet Gynecol 1988; 158:1365–1373.
80. Switchenko AC, Kauffman RS, Becker M: Are there antiendometrial antibodies in sera of women with endometriosis? Fertil Steril 1991; 56:235–241.
81. Mathur S, Chihal HJ: Antiendometrial antibodies? Fertil Steril 1992; 57:704–705.
82. Moncayo H et al.: Ovarian failure and autoimmunity: detection of autoantibodies directed against both the unoccupied luteinizing hormone/human chorionic gonadotropin receptor and the hormone-receptor complex of bovine corpus luteum. J Clin Invest 1989; 84:1857–1865.
83. D'Cruz OJ et al.: Antibodies to carbonic anhydrase in endometriosis: prevalence, specificity, and relationship to clinical and laboratory parameters. Fertil Steril 1996; 66:547–556.
84. Barbieri RL: CA-125 in patients with endometriosis. Fertil Steril 1986; 45:767–769.

85. Mathur SP et al.: Levels of antibodies to transferrin and α 2-HS glycoprotein in women with and without endometriosis. Am J Reprod Immunol 1998; 40:69–73.
86. Pillai S et al.: Antibodies to endometrial transferrin and α 2-Heremans Schmidt (HS) glycoprotein in patients with endometriosis. Am J Reprod Immunol 1996;35:483–494. (Erratum: Am J Reprod Immunol 1997;37: 277.)
87. Lang GA, Yeaman GR: Autoantibodies in endometriosis sera recognize a Thomsen–Friedenreich-like carbohydrate antigen. J Autoimmun 2001; 16:151–161.
88. Yeaman GR, Collins JE, Lang GA: Autoantibody responses to carbohydrate epitopes in endometriosis. Ann N Y Acad Sci 2002; 955:174–182 (discussion 199–200, 396–406).
89. Gleicher N, el-Roeiy A, Confino E, Friberg J: Reproductive failure because of autoantibodies: unexplained infertility and pregnancy wastage. Am J Obstet Gynecol 1989; 160:1376–1380.
90. Glinoer D, Soto MF, Bourdoux P, Lejeune B, Delange F, Lemone M, Kinthaert J, Robijn C, Grun JP, de Nayer P: Pregnancy in patients with mild thyroid abnormalities: maternal and neonatal repercussions. J Clin Endocrinol Metab 1991; 73:421–427.
91. el-Roeiy A, Dmowski WP, Gleicher N, Radwanska E, Harlow L, Binor Z et al.: Danazol but not gonadotropin-releasing hormone agonists suppress autoantibodies in endometriosis. Fertil Steril 1988; 50:864–871.
92. Kennedy SH, Starkey PM, Sargent IL, Hicks BR, Barlow DH: Antiendometrial antibodies in endometriosis measured by an enzyme-linked, immunosorbent assay before and after treatment with danazol and nafarelin. Obstet Gynecol 1990; 75:914–918.
93. Dmowski WP, Rana N, Michalowska J, Friberg J, Papierniak C, el-Roeiy A: The effect of endometriosis, its stage and activity, and of autoantibodies on in vitro fertilization and embryo transfer success rates. Fertil Steril 1995; 63:555–562.
94. Kayisli UA, Mahutte NG, Arici A: Uterine chemokines in reproductive physiology and pathology. Am J Reprod Immunol 2002; 47:213–221.
95. Hill J, Anderson D: Lymphocyte activity in the presence of peritoneal fluid from fertile women and infertile women and without endometriosis. Am J Obstet Gynecol 1989; 161:861–864.
96. Fakih H, Bagget B, Holtz G et al.: Interleukine-1: a possible role in the infertility associated with endometriosis. Fertil Steril 1987; 47:213–217.
97. Mori H, Sawairim M, Nakagawa M: Peritoneal fluid interleukin-1β and tumor necrosis factor in patients with benign gynecologic disease. Am J Reprod Immunol 1991; 26:62–67.
98. Keenan J, Chen T, Chadwell N: IL-1β, TNF-α and IL-2 in peritoneal fluid and macrophage-conditioned media of women with endometriosis. Am J Reprod Immunol 1995; 34:381–385.
99. Lebovic DI, Bentzien F, Chao VA, Garett EN, Meng YG, Taylor RN: Induction of an angiogenic phenotype in endometriotic stromal cell cultures by interleukine-1β. Mol Hum Reprod 2000; 6:269–275.
100. Zarmakoupis PN, Rier SE, Maroulis GB, Becker JL: Inhibition of human endometrial stromal cell proliferation by interleukin 6. Hum Reprod 1995; 10:2395–2399.
101. Yoshioka H, Harada T, Iwabe T, Nagano Y, Taniguchi F, Tanikawa M, Terakawa N: Menstrual cycle-specific inhibition of the proliferation of endometrial stromal cells by interleukin 6 and its soluble receptor. Am J Obstet Gynecol 1999; 180:1088–1094.
102. Tabibzadeh SS, Santhanam U, Sehgal PB, May LT: Cytokine-induced production of IFN-beta 2/IL-6 by freshly explanted human endometrial stromal cells: modulation by estradiol-17 beta. J Immunol 1989; 142:3134–3139.
103. Rier SE, Zarmakoupis PN, Hu X, Becker JL: Dysregulation of interleukin-6 responses in ectopic endometrial stromal cells: correlation with decreased soluble receptor levels in peritoneal fluid of women with endometriosis. J Clin Endocrinol Metab 1995; 80:1431–1437.
104. Schroder W, Gaetje R, Baumann R: Interleukin-6 and soluble interleukin-6 receptor in peritoneal fluid and serum of patients with endometriosis. Clin Exp Obstet Gynecol 1996; 23:10–14.
105. Tseng JF, Ryan IP, Milam TD et al.: Interleukin-6 secretion in vitro is up-regulated in ectopic and eutopic endometrial stromal cells from women with endometriosis. J Clin Endocrinol Metab 1996; 81:1118–1122.
106. Arici A, Head J, MacDonald PC, Casey ML: Regulation of interleukin-8 gene expression in human endometrial cells in culture. Mol Cell Endocrinol 1993; 94:195–204.
107. Arici A, Tazuke SI, Attar E et al.: Interleukin-8 concentration in peritoneal fluid of patients with endometriosis and modulation of interleukine-8 expression in human mesothelial cells. Mol Hum Reprod 1996; 2:40–45.
108. Arici A, Seli E, Senturk LM, Gutierrez LS, Oral E, Taylor HS: Interleukin-8 in the human endometrium. J Clin Endocrinol Metab 1998; 83:1783–1787.
109. Iwabe T, Harada T, Tsudo T, Tanikawa M, Onohara Y, Terakawa N: Pathogenetic significance of levels of interleukin-8 in peritoneal fluid of patients with endometriosis. Fertil Steril 1998; 69:924–929.
110. Arici A, Seli E, Zeyneloglu HB, Senturk LM, Oral E, Olive DL: Interleukin-8 induces proliferation of endometrial stromal cells: a potential autocrine growth factor. J Clin Endocrinol Metab 1998; 83:1201–1205.
111. Iwabe T, Harada T, Tsudo T, Nagano Y, Yoshida S, Tanikwa M, Terakawa N: Tumor necrosis factor-α promotes proliferation of endometriotic stromal cells by inducing interleukin-8 gene and protein expression. J Clin Endocrinol Metab 2000; 85:824–829.
112. Gazvani MR, Christmas S, Quenby S et al.: Peritoneal fluid concentrations of interleukine-8 in women with endometriosis: relationship to stage of disease. Hum Reprod 1998; 13:1957–1961.
113. Iwabe T, Harada T, Tsudo T et al.: Pathogenic significance of increased levels of interleukin-8 in the peritoneal fluid of patients with endometriosis. Fertil Steril 1998; 69:924–930.
114. Garcia-Velasco JA, Arici A: Interleukin-8 stimulates the adhesion of endometrial stromal cells to fibronectin. Fertil Steril 1999; 72:336–340.
115. Garcia-Velasco JA, Arici A: Interleukin-8 expression in endometrial stromal cells is regulated by integrin-dependent cell adhesion. Mol Hum Reprod 1999; 5:1135–1140.

116. Zeyneloglu HB, Senturk LM, Seli E et al.: The peritoneal fluid levels of interleukin-12 in women with endometriosis. Am J Reprod Immunol 1998; 39:152–156.
117. Mazzeo D, Vigano P, Di Blasio AM, Sinigaglia F, Vignali M, Panina-Bordignon P: Interleukin-12 and its free p40 subunit regulate immune recognition of endometrial cells: potential role in endometriosis. J Clin Endocrinol Metab 1998; 83:911–916.
118. Arici A, Oral E, Attar E, Tazuke S, Olive DL: Monocyte chemotactic protein-1 concentration in peritoneal fluid in patients with endometriosis and its modulation in human mesothelial cells. Fertil Steril 1997; 67:1065–1072.
119. Taketani Y, Kuo TM, Mizuno M: Comparison of cytokine levels and embryo toxicity in peritoneal fluid in infertile women with untreated or treated endometriosis. Am J Obstet Gynecol 1992; 167:265–270.
120. Khorram O, Taylor RN, Ryan IP et al.: Peritoneal fluid concentrations of the cytokines RANTES correlate with the severity of endometriosis. Am J Obstet Gynecol 1993; 169:1545–1549.
121. Hornung D, Ryan IP, Chao VA, Vigne JL, Schriock ED, Taylor RN: Immunolocalization and regulation of the chemokine RANTES in human endometrial and endometriosis tissues and cells. J Clin Endocrinol Metab 1997; 82:1621–1628.
122. Ryan IP, Tseng JF, Schriock ED, Khorram O, Schall TJ, Landers DV et al.: RANTES chemokine expression is up-regulated in stromal cellscultured from human endometriosis tissues. J Soc Gynecol Invest 1996; 3:74A.
123. Oosterlynck DJ, Meuleman C, Waer M, Koninckx PR: Transforming growth factor β activity is increased in peritoneal fluid from women with endometriosis. Obstet Gynecol 1994; 83:287–292.
124. Laird SM, Tuckerman EM, Saravelos H, Li TC: The production of tumour necrosis factor alpha (TNF-alpha) by human endometrial cells in culture. Hum Reprod 1996; 11:1318–1323.
125. Zhang RJ, Wild RA, Ojago J: Effect of tumor necrosis factor-alpha on adhesion of human endometrial stromal cells to peritoneal mesothelial cells: an in vitro system. Fertil Steril 1993; 59:1196–1201.
126. Eisermann J, Gast MJ, Pineda J, Odem RR, Collins JL: Tumor necrosis factor in peritoneal fluid of women undergoing laparoscopic surgery. Fertil Steril 1988; 50:573–579.
127. Hill JA, Haimovici F, Anderson DJ: Products of activated lymphocytes and macrophages inhibit mouse embryo development in vitro. J Immunol 1987; 139:2250–2254.
128. Hill JA, Haimovici F, Politch J, Anderson DJ: Effect of soluble products of activated lymphocytes and macrophages (lymphokines and monokines) on human sperm motion parameters. Fertil Steril 1987; 47:460–465.
129. Shifren JL, Tseng JF, Zaloudek CJ, Ryan IP, Meng YG, Ferrara N et al.: Ovarian steroid regulation of vascular endothelial growth factor in the human endometrium: implications for angiogenesis during the menstrual cycle and in the pathogenesis of endometriosis. J Clin Endocrinol Metab 1996; 81:3112–3118.
130. Li XF, Gregory J, Ahmed A: Immunolocalization of vascular endothelial growth factor in human endometrium. Growth Factors 1994; 11:277–282.
131. Brogi E, Wu T, Namiki A: Indirect angiogenic cytokines upregulate VEGF and bFGF gene expression in vascular smooth muscle cells, whereas hypoxia upregulates VEGF expression only. Circulation 1994; 90:649–652.
132. Ben-Av P, Crofford LJ, Wilder L, Hla T: Induction of vascular endothelial growth factor in synovial fibroblasts by prostaglandin E and interleukin-1: a potential mechanism for inflammatory angiogenesis. FEBS Lett 1995; 372:83–87.
133. Nisolle M, Casanas-Roux F, Anaf V, Mine J-M, Donnez J: Morphometric study of the stromal vascularization in peritoneal endometriosis. Fertil Steril 1993; 59:681–684.
134. Donnez J, Smoes P, Gillerot S, Casanas-Roux F, Nisolle M: Vascular endothelial growth factor (VEGF) in endometriosis. Hum Reprod 1998; 13:1686–1690.
135. McLaren J, Prentice A, Charnock-Jones DS et al.: Vascular endothelial growth factor is produced by peritoneal fluid macrophages in endometriosis and is regulated by ovarian steroids. J Clin Invest 1996; 98:482–489.
136. Arici A, Oral E, Bukulmez O, Duleba A, Olive DL, Jones EE: The effect of endometriosis on implantation: results from the Yale University in vitro fertilization and embryo transfer program. Fertil Steril 1996; 65:603–607.
137. Simon C, Gutierrez A, Vidal A, de los Santos MJ, Tarin JJ, Remohi J, Pellicer A:Outcome of patients with endometriosis in assisted reproduction: results from in-vitro fertilization and oocyte donation. Hum Reprod 1994; 9:725–729.
138. Muscato JJ, Haney AF, Weinberg JB: Sperm phagocytosis by human peritoneal macrophages: a possible cause of infertility in endometriosis. Am J Obstet Gynecol 1982; 144:503–510.
139. Burke RK: Effect of peritoneal washings from women with endometriosis on sperm velocity. J Reprod Med 1987; 32:743–746.
140. Sueldo CE, Lambert H, Steinleitner A, Rathwick G, Swanson J: The effect of peritoneal fluid from patients with endometriosis on murine sperm–oocyte interaction. Fertil Steril 1987; 48:697–699.
141. Marcos RN, Gibbons WE, Findley WE: Effect of peritoneal fluid on in vitro cleavage of two-cell mouse embryos: possible role in infertility associated with endometriosis. Fertil Steril 1985; 44:678–683.
142. Awadalla SG, Frieman CI, Haq AU, Roh SI, Chin NW, Kim MH: Local peritoneal factors: their role in infertility associated with endometriosis. Am J Obstet Gynecol 1987; 157:1207–1214.
143. Dodds WG, Miller FA, Friedman CI, Lisko B, Goldberg JM, Kim MH: The effect of preovulatory peritoneal fluid from cases of endometriosis on murine in vitro fertilization, embryo development, oviduct transport and implantation. Am J Obstet Gynecol 1992; 166:219–224.
144. Nothnick WB, Curry TE, Vernon MW: Immunomodulation of rat endometriotic implant growth and protein production. Am J Reprod Immunol 1994; 31:151–162.
145. Keenan JA et al.: Regression of endometrial explants in a rat model of endometriosis treated with the immune modulators loxoribine and levamisole. Fertil Steril 1999; 72:135–141.