Potential involvement of the immune system in the development of endometriosis

Cleophas M Kyama¹,², Sophie Debrock¹, Jason M Mwenda² and Thomas M D’Hooghe*¹,²

Address: ¹Leuven University Fertility Centre, Department of Obstetrics & Gynaecology, University Hospital Gasthuisberg, Leuven, Belgium and ²Division of Reproductive Biology, Institute of Primate Research, Karen, Nairobi, Kenya

Email: Cleophas M Kyama - kcleophas@yahoo.com; Sophie Debrock - sophie.debrock@uz.kuleuven.ac.be; Jason M Mwenda - jmmwenda@arcc.or.ke; Thomas M D’Hooghe* - thomas.dhooghe@uz.kuleuven.ac.be

* Corresponding author

Abstract

This article presents an overview of immunological factors and their role in the development of endometriosis, with emphasis on inflammatory cytokines, growth and adhesion factors. Although retrograde menstruation is a common phenomenon among women of reproductive age, not all women who have retrograde menstruation develop endometriosis. The development of endometriosis is hypothesised to be a complex process, which may be facilitated by several factors, including the quantity and quality of endometrial cells in peritoneal fluid (PF), increased inflammatory activity in PF, increased endometrial-peritoneal adhesion and angiogenesis, reduced immune surveillance and clearance of endometrial cells, and increased production of autoantibodies against endometrial cells. Potential biomarkers like cytokines and autoantibodies upregulated during development of endometriosis may be useful in the development of a non-surgical diagnostic tool. Although endometriosis can be treated using hormonal suppression, there is need for non-hormonal drugs, which can inhibit the development of endometriosis and alleviate pain or infertility without inhibition of ovulation. New molecules that modulate immune function in endometriosis should be the targets for future research.

Introduction

Endometriosis is a gynaecological disorder characterised by the presence and growth of endometrial tissues in the ectopic site. The endometrial deposits are mostly found in the pelvis (ovaries, peritoneum, uterosacral ligaments, pouch of Douglas and rectovaginal septum). The prevalence of endometriosis among asymptomatic women ranges from 2–22%, while in women with dysmenorrhea, the incidence of endometriosis is 40% to 60% [1]. It is the most common cause of pelvic pain and occurs in 13%-33% of women with infertility [2]. Although endometriosis stands as one of the most investigated disorders of gynaecology [3], our current understanding of aetiology and pathophysiology of the disease remains elusive. Retrograde menstruation is a widely accepted and proposed mechanism that may explain mostly the presence of endometrial cells in ectopic sites [4]. However, it does not account for the fact that these misplaced cells survive in women with endometriosis and not in healthy women. An immunological/inflammatory aetiology has been conjectured, as demonstrated by increased concentrations of activated macrophages, cytokines, T cells and B cells [2]. The endometrial fragments desquamated during menstruation and deposited into peritoneal cavity,
cells from PF are reported to aberrantly suppress cell-mediated immunity by upregulating IL-4 and 10 secretions in PF from women with endometriosis [38,55]. As a result, decreased T cell cytotoxicity may allow implantation of endometrial cells in peritoneum.

**Impaired immune-surveillance and abnormal apoptosis**

The failure of immune cells to transmit death signals to endometrial cells, and/or the ability of endometrial fragments to avoid cell death may be associated with the development of endometriosis. Indeed, in women with endometriosis, it has been hypothesized that endometrial cells in the peritoneal fluid avoid immunosurveillance and implant into peritoneum [56]. It has been speculated that lymphocytes can adhere to endometrial cells through the Lymphocyte Function-Associated Antigen-1 (LFA-1) – Intercellular adhesion molecule-1 (ICAM-1) dependent pathway and present them as a target to NK cells. Soluble forms of ICAM-1 (s-ICAM-1) secreted by PF endometrial cells/endometriotic lesions can also bind to LFA-1 presenting lymphocytes and could prevent the recognition of endometrial cells by these lymphocytes and prevent subsequent NK cell-mediated cytotoxicity [57,58]. Furthermore, IL-6 secreted by endometriotic cells in concert with interferon-γ may upregulate sICAM-1 production by macrophages of patients with endometriosis [59]. As a result, increased secretion of sICAM-1 may allow endometrial fragments to evade immunosurveillance, survive and implant.

Another major pathway in programmed cell death, Fas-Fas Ligand (FasL) system, could also be abnormal in women with endometriosis [60,61]. It has been speculated that the expression of FasL by viable endometrial cells induces apoptosis of T cells through ligation of Fas, allowing endometrial fragments to escape cell death, implant and develop to endometriotic lesions [62]. Interestingly, Garcia-Velasco et al [62] showed that macrophage-conditioned media might stimulate Fas-Fas ligand (FasL) expression by endometrial cells.

**Relevance in clinical research**

**Biomarkers to predict endometriosis non-surgically**

Presently, the diagnosis of endometriosis can be made only by laparoscopy and biopsy of suspicious lesions with subsequent histological confirmation of endometrial tissue and there is no non-invasive way to diagnose this condition. Laparoscopy is minimally invasive procedure, but requires general anaesthesia and surgical skills with potential complications and procedural costs. Hence, a non-surgical diagnostic tool would be of paramount benefit to both physicians and patients.

Efforts to evaluate the diagnostic value of endometrial markers for endometriosis have been hampered by the lack of easy, reliable and quantitative techniques to assess the expression levels of these markers in sample material. Emerging proteomic techniques offer new approaches to identifying biomarkers for the early detection and follow-up of endometriosis.

Aromatase P450 mRNA has been identified as a candidate diagnostic marker but low sensitivity and specificity impair its application in clinical practice [Reviewed by Brosens et al [63]]. In a recent study [64], the measurement of serum IL-6 levels and PF TNF-α levels could discriminate between patients with endometriosis and those without the disease. Endometriosis could be diagnosed if TNF-alpha levels in PF were higher than 15 pg/ml (100% sensitivity and 89% specificity) and if IL-6 levels in serum were above 2 pg/ml (90% sensitivity and 67% specificity) [64]. Potentially, the quantitation of autoantibodies against endometrial cells could also provide a novel method for the non-invasive diagnosis for endometriosis [65]. However, more studies are needed to confirm that this approach may be clinically useful. The role of Transvagal Ultrasoundonography (TVU) and Magnetic resonance Imaging (MRI) in diagnosis and follow up of small endometriotic lesions is limited at present, but represents an interesting area of research [63].

**New treatment options and future management of endometriosis**

Endometriosis causes pelvic pain and infertility and can be treated by surgery and by hormonal suppression (progestins, continuous use of oral contraceptives, danazol, GnRH agonists), as reviewed recently [66]. A drug like danazol also works as an immunosuppressive agent (reviewed by D’Hooghe and Hill [66]). Indeed, the immunologic effects of danazol have been studied in women with endometriosis and adenomyosis and include a decrease in serum immunoglobulins [67,68], a decrease in serum C3, a rise in serum C4 levels [68], decreased serum levels of autoantibodies against various phospholipid antigens [67,68], and decreased serum levels of CA125 during treatment [69-72]. Danazol inhibits peripheral blood lymphocyte proliferation in cultures activated by T-cell mitogens but does not affect macrophage-dependent T-lymphocyte activation of B lymphocytes [73]. Danazol inhibits interleukin-1 (IL1) and TNF production by monocytes in a dose-dependent manner [74] and suppresses macrophage/monocyte-mediated cytotoxicity of susceptible target cells in women with mild endometriosis [75]. These immunological findings may be important in the remission of endometriosis with danazol treatment and may offer an explanation of the effect of danazol in the treatment of a number of autoimmune diseases, including hereditary angioedema [76], autoimmune hemolytic anemia [77], systemic lupus erythematosus, and idiopathic thrombocytopenic purpura [78,79].
Overall, medical treatment of endometriosis is limited by cost, side effects and recurrence of endometriosis after the cessation of treatment. Therefore, there is need for new drugs that treat endometriosis-associated pain and infertility without inhibition of ovulation. Future potential targets in the treatment or management of endometriosis may be inflammatory cytokines, MMPs, adhesion and growth factors [80,38]. Pentoxifylline has been shown to reduce endometriotic implant growth without inducing hypoestrogenism in both humans and hamsters [38]. In a recent study, Hornung and colleagues [81] demonstrated that Thiazolidinedione (TZD) significantly reduced leukocyte infiltration in the mouse model with endometriosis. Inhibition of TNF-alpha activity has also been a new target in the prevention and treatment of endometriosis. Experimental endometriosis in rats was treated with recombinant human tumour necrosis factor-binding protein-1 (r-hTBP-1), a soluble form of tumour necrosis factor-a receptor type-1 [82]. It was demonstrated that r-hTBP-1 could reduce the size of endometriotic-like peritoneal lesions by 64% [82]. Similarly, a study carried out in baboons showed that r-hTBP-1 effectively inhibited the development of endometriosis and endometriosis-related adhesions [83]. The potential of etanercept, a soluble TNF receptor (TNFR) fusion protein, for the treatment of endometriosis is also being considered [84].

**Conclusion**

Understanding the involvement of the immune system in the development of endometriosis may help to understand the pathogenesis and spontaneous evolution of this condition. At present, most evidence suggests that pelvic inflammation and other immunological changes are a consequence of endometriosis. The development of non-invasive diagnostic tools based on cytokines and autoantibodies could be of great benefit in the clinical management of endometriosis. Therapeutic strategies to eliminate the inflammatory reaction associated with endometriosis could lead to new treatment options for endometriosis.

**References**

29. Steinleitner A, Lambert H, Roy S: Immunomodulation with pentoxifylline abrogates macrophage-mediated infertility in an vivo model: a paradigm for a novel approach to the treat-


Neuroendocrine–immune disequilibrium and endometriosis: an interdisciplinary approach

Nadja Tariverdian · Theoharis C. Theoharides · Friederike Siedentopf · Gabriela Gutiérrez · Udo Jeschke · Gabriel A. Rabinovich · Sandra M. Blois · Petra C. Arck

Received: 16 February 2007 /Accepted: 15 April 2007 / Published online: 15 May 2007 © Springer-Verlag 2007

Abstract Endometriosis, a chronic disease characterized by endometrial tissue located outside the uterine cavity, affects one fourth of young women and is associated with chronic pelvic pain and infertility. However, an in-depth understanding of the pathophysiology and effective treatment strategies of endometriosis is still largely elusive. Inadequate immune and neuroendocrine responses are significantly involved in the pathophysiology of endometriosis, and key findings are summarized in the present review. We discuss here the role of different immune mechanisms particularly adhesion molecules, protein–glycan interactions, and pro-angiogenic mediators in the development and progression of the disease. Finally, we introduce the concept of endometrial dissemination as result of a neuroendocrine-immune disequilibrium in response to high levels of perceived stress caused by cardinal clinical symptoms of endometriosis.

Keywords Corticotropin-releasing hormone · Ectopic endometrium · Inflammation · Progesterone · Sickness behaviour

Introduction

Endometriosis, first described in 1860 by von Rokitansky, is a chronic disease that is characterized by the occurrence of endometrial glands and stroma outside the uterine cavity [1]. Endometriosis affects up to 22% of women in their reproductive age [2] and is associated with chronic pelvic pain. Additionally, endometriosis is closely linked to severely impaired fertility, as it can be diagnosed in 68% of patients suffering from infertility [3]. To date, insights into the pathophysiology of endometriosis, and thus the development of effective treatment strategies, are surpris-
Immunological aspects in endometriosis

Because endometrial lesions are frequently present in the peritoneal cavity, they are in direct contact with peritoneal fluid, which bathes the pelvic cavity, uterus, fallopian tubes, and ovaries. However, in endometriosis, ectopic endometrial cells escape pathways involved in immune-mediated surveillance. In search for a better understanding of the pathogenesis of endometriosis, here, we analyzed different immune-mediated mechanisms and mediators that might be involved in disease development and resolution.

Role of innate immune responses in endometriosis

Macrophages

Macrophages are the main population of peritoneal leukocytes and—according to the published evidence so far—comprise up to 90% of peritoneal fluid cells [27]. In women suffering from endometriosis as well as in baboons with spontaneously occurring mild endometriosis, the concentration of peritoneal macrophages is increased as compared to healthy or fertile controls or animals with normal pelvis, respectively [27, 28] (Fig. 1). However, their percentage of total mononuclear cells seems to be decreased in favor of lymphocytes [29]. Further, an increased percentage of peritoneal macrophages is positive for the cell activation marker acid phosphatase in mild endometriosis [30]. Increased cell counts may be attributable to elevated levels of macrophage colony-stimulating factor (M-CSF) [31] and monocyte chemotactic protein (MCP)-1 [32] (Fig. 1). Here, M-CSF and MCP-1 have been proposed to derive from endometrial/endometriotic cells or peritoneal macrophages, respectively.

In vitro studies revealed that peritoneal macrophages derived from patients with endometriosis produce increased levels of the cytokines interleukin (IL)-6 [33], IL-1β, and tumor necrosis factor (TNF)-α [34], compared to peritoneal macrophages of women with other benign gynecological disorders (Fig. 1). Because IL-6, IL-1β, and TNF-α promote the adhesion of endometrial cells to peritoneum, increased secretion of these cytokines by peritoneal macrophages in patients with endometriosis might contribute to the development and progression of the disease [35] (Fig. 1). It is further noteworthy that IL-6, IL-1, and TNF-α correlate with infertility and embryotoxicity when secreted at high levels [36, 37]. Further, TNF-α induces proliferation of ectopic stromal cells, which would subsequently result in the growth of endometriotic lesions [38] (Fig. 1). Moreover, TNF-α target genes have been proposed to be overexpressed in experimental endometriosis in rats [39].

Elevated prostaglandins (PG), particularly PGE₂, in the peritoneal fluid of endometriosis patients [40] (Fig. 1), may result from macrophage activation and have been proposed to subsequently aggravate endometriosis-associated pain by altering uterine and tubal contractility and cause infertility due to a delayed ovum transport [41]. On the other hand, emerging evidence addresses the decrease in human leukocyte antigen (HLA)-ABC and HLA-DR on peritoneal macrophages in endometriosis patients (Fig. 1), suggesting defective antigen presentation [42]. In advanced stages, macrophage-mediated cytotoxicity against endometrial cells is reduced as compared to early stages [43] (Fig. 1). Interestingly, cytotoxic activity could be restored by application of the non-steroidal, PG-inhibiting drug indomethacin, which points towards a dampening effect of PG on macrophage cytotoxicity in endometriosis [43] (Fig. 1). In early stages, however, the cytotoxicity of peritoneal macrophages is increased compared to fertile controls.

Thus, hypothetically, activated macrophages in the peritoneal fluid may control the number and size of peritoneal endometriotic lesions. On the other hand, a wealth of mediators derived from peritoneal macrophages such as the abovementioned cytokines may promote adherence and proliferation of endometrial cells and angiogenesis, thus facilitating the dissemination of endometriotic lesions. In conclusion, additional work aiming to dissect the role of peritoneal macrophages in endometriosis is needed.

Mast cells

Mast cells play a pivotal role within innate immune responses. In addition, these cells play a critical role in sustaining Th2-mediated responses by secreting high levels of IL-4. Because mast cells are predominantly resident cells of loose connective tissue, mast cells’ presence and function have been investigated in endometriotic tissue [44]. Here, Kempuraj et al. observed increased numbers of highly activated mast cells in the stroma of peritoneal endometriotic lesions as compared to eutopic endometrium (Fig. 1). Mast cell migration and proliferation may be related to increased levels of stem cell factor (SCF) in the peritoneal fluid of women with early stage endometriosis and the expression of its cognate receptor on ectopic endometrium [45], whereby SCF may derive from fibroblasts, endothelial, and granulosa cells [46]. Activated mast cells release enzymes, such as tryptase, which stimulate protease-activated receptor (PAR)-2. Strikingly, PAR-2 agonist induces proliferation of purified endometriotic stromal cells and the release of IL-6 and IL-8 in vitro [47] (Fig. 1). Especially in deep infiltrating endometriosis, mast cells were found near nerve fibers. This led to the suggestion that mast cells might play a pivotal role in endometriosis-related pain [48].
Natural killer cells

Natural killer (NK) cells play a pivotal role at the crossroads of innate and adaptive immunity not only through their ability to lyse infected or tumor cells but also by the secretion of cytokines that contribute to direct selective adaptive responses. Published data indicate that NK cells derived from peripheral blood, characterized by markers such as CD16 and CD57, are involved in the cytotoxicity against endometrial cells in vitro [49]. The cytotoxic activity of peritoneal NK cells derived from endometriosis patients against NK-cell-sensitive K562 target cells was shown to
be reduced [50, 51] (Fig. 1). The literature varies with regards to the percentages of NK cells within the peritoneal fluid among different patients. Nevertheless, the alterations in cytotoxicity seem to depend on a functional defect of NK cell activity in endometriosis rather than differences in the total cell number [50]. One explanation for such a decrease in NK cell activity might rely on the increased levels of transforming growth factor (TGF)-β [52] and PGE2 [40] in peritoneal fluid of women with endometriosis, which both inhibit NK cell activity and are derived from macrophages (Fig. 1). A second explanation for the reduced function of these cells in endometriosis involves a group of receptors referred to as killer cell inhibitory receptors (KIR). These receptors recognize major histocompatibility complex class I (MHC-I) antigens, which inhibit cytotoxicity against MHC-I-expressing target cells. Interestingly, the expression of KIRs on peritoneal NK cells of women with endometriosis is increased [53] (Fig. 1). Insights on MHC expression of endometriotic lesions are surprisingly meager; however, in a recent study using cDNA microarray technique, an up-regulation of genes encoding MHC antigens could be detected on ovarian endometrial cysts [54], which would support the notion of an escape of NK-cell-mediated cytotoxicity induced by the endometriotic lesion itself. Strikingly, in severe endometriosis, endometriotic cells are also more resistant to lysis by heterologous NK cells derived from peripheral blood [49], which further supports the immune escape theory.

Role of adaptive immune responses in endometriosis

The absolute number as well as the relative percentage of lymphocytes in peritoneal fluid of endometriosis patients have been shown to be significantly augmented [29].

T cells

Endometriotic lesions may be considered as an ‘autologous transplant’; nonetheless, one might expect T cells in the peritoneal fluid to be a candidate population in the rejection of endometrial tissue. Indeed, T cells expressing markers such as CD3, CD4, and CD8 have been described to be elevated in endometriotic tissue [55, 56] (Fig. 1). RANTES (short for ‘regulated upon activation normal T cell expressed and secreted’), chemottractant, e.g., to T cells, is expressed in ectopic endometrium [57] and elevated in the peritoneal fluid in endometriosis [58] (Fig. 1), which might explain the high number of T cells within the lesion. However, an in-depth analysis of the frequency of T cells and, in particular, their functional differentiation into cytotoxic (CD8+) or helper (Th1, Th2, or Th17 cells) cell subsets in peritoneal fluids of endometriosis patients still remains elusive.

Natural killer T (NKT) cells are also cells that bridge innate and adaptive immune mechanisms. They produce a wide range of cytokines, mainly IL-4, driving the development of Th2-mediated responses and recognize glycolipids associated to CD1d molecules. Therefore, it appears rather puzzling that no published evidence is currently available indicating the presence or function of such cells in peritoneal fluid in the context of endometriosis.

Various authors have addressed the issue of Th1/Th2 cytokine balance in the peritoneal fluid of patients with endometriosis. Several of these cytokines may be produced by cells of the adaptive immune response, and especially the ratio of pro-inflammatory Th1-like and anti-inflammatory Th2-like cytokines has been in the center of scientific attention. In endometriosis, the production of pro-inflammatory interferon (IFN)-γ and IL-2 as well as the anti-inflammatory cytokines IL-4 and IL-10 was found to be increased in peritoneal fluids, although controversial data have been published especially with regard to IFN-γ [29, 60, 61] (Fig. 1). Th1 cytokines such as IFN-γ and IL-2 are well known to lead to the activation of T cell-mediated and delayed-type hypersensitivity (Fig. 1). On the other hand, Th2 cytokines such as IL-4 and IL-5 stimulate antibody-mediated immunity via B cell differentiation to plasma cells and inhibit Th1-mediated responses (Fig. 1). Whether endometriosis is a typical Th1- or Th2-mediated disease is not clear. In addition, future studies are warranted on the role of the recently identified pro-inflammatory Th17 cells, which secrete high levels of IL-17A, IL-17F, IL-6, and TNF-α and sustain tissue damage.

A pro-inflammatory status has been proposed to result in the down-regulation of heme oxygenases (HOs) [62] (Fig. 1). HOs are required to degrade heme into biliverdin and carbon monoxide (CO) to avoid toxic heme effects such as oxidative stress; hence, HOs have been proposed to

Surprisingly, no published evidence is currently available whether or not regulatory T cells (Treg) [59] are involved in the undesired ‘immunological tolerance’ of endometriotic lesions. Autoreactive T cells are capable of encountering self-peptide/MHC complexes. Hence, the basic importance of Tregs in the maintenance of immune cell homeostasis is to suppress such autoreactive T cells. One might speculate that, in the context of endometriosis, it may be desirable to have autoreactive T cell-like populations in the peritoneal fluid, which may be capable of targeting the autologous endometriotic transplant. Hence, the presence of peritoneal Treg—which could suppress such autoaggressive cells—would be undesired. However, no published data indicate the existence of autoreactive T cell-like populations in the peritoneal cavity of women without endometriosis, which could provide an explanation for the successful deletion of endometrial tissue entering the peritoneal cavity by retrograde menstruation in these women.
be involved in tissue protection. Increased proliferation of endometrial tissue leads to high levels of hemoglobin (Fig. 1) as well as heme, which requires adequate HO activity to sustain tissue protection. Hemoglobin [63] as well as markers of oxidative stress such as lipid peroxides [64] are elevated in patients with endometriosis (Fig. 1). Thus, it has been suggested that the HO system might be insufficient to detoxify heme in women with endometriosis. Such insufficiency of the HO system may result from the continuous pro-inflammatory environment in the peritoneal cavity of patients with endometriosis. As a consequence of the insufficient HO system, high levels of oxidative stress may further enhance adhesion of more refluxed endometrial cells onto the peritoneum during menstruation and perpetuate the progression of endometriosis (Fig. 1).

Interestingly, recombinant human TNF-binding protein-1 reduces experimental endometriosis in rats [65], supporting the notion of an adverse effect of this pro-inflammatory cytokine. In contrast, intraperitoneal injection of the Th1-like cytokine IL-12 also reduces endometriotic lesions in vivo [66], probably by enhancing cytotoxic activity. It is, thus, not finally resolved whether pro- or anti-inflammatory responses or both of them should be suspected to contribute to the development of endometriosis.

**B cells**

B cells are involved in antibody-mediated adaptive immune responses. While B2 cells can give rise to classical plasma cells producing many distinct immunoglobulin (Ig) isotypes (IgG, IgA), other B cell types including B1 cells, which are present in the peritoneal cavity, and BZM cells, which are located in the marginal zone of the spleen, produce only high levels of IgM to T-independent antigens. An increase in activated CD20⁺ B cells could be demonstrated in ectopic endometrium [67] (Fig. 1). Soluble CD23, which may be derived from mature B cells and also from activated macrophages, eosinophils, follicular dendritic cells, and platelets, is increased in serum and peritoneal fluid of endometriosis patients, which may be suggestive of an exacerbated B cell activation in endometriosis [68] (Fig. 1). In addition, an increase in autoantibodies (AAb) such as anti-phospholipid and anti-histone IgG could be detected in the peritoneal fluid of endometriosis patients [69] (Fig. 1). Serum and cervical secretions of women with endometriosis contain organ-specific anti-endometrial and anti-ovarian specific IgG and IgA, suggesting the activation and plasma cell differentiation of B2 cells [70].

Some of the antigens from endometriotic tissue, which are recognized by serum AAb, have been identified; these include mainly glycoproteins including the human chorionic gonadotropin (hCG) receptor [71], carbonic anhydrase isoforms I and II [72, 73], transferrin, and α₂-Heremans Schmidt glycoprotein (α₂-HSG) [74]. Interestingly, Lang and Yeaman [73] demonstrated that removal of carbohydrate moieties from endometrial antigens prevented antibody binding via Thomsen–Friedenreich disaccharide-dependent pathways. Thomsen–Friedenreich antigens, which are expressed on epithelial cells of the uterus, bind to a variety of glycan-binding proteins including galectin (Gal)-1 and Gal-3, which are also expressed in endometrial tissue. Gal-1 and Gal-3 are evolutionarily conserved glycan-binding proteins that have been shown to contribute to cell–cell and cell–matrix interactions, cell migration, and angiogenesis [75]. In addition, galectins have been shown to modulate T cell apoptosis and immune privilege in vivo [76], thus contributing to autoimmunity by dampening antigen-specific immune responses. Because levels of AAb against Thomsen–Friedenreich antigen are significantly up-regulated in endometriosis tissue, it is possible that these AAb might block or mimic some of the biological functions of galectins. However, to date, no data are available indicating the regulated expression of Gal-1 and Gal-3 in endometriotic tissue.

In conclusion, AAb might be directly involved in the pathogenesis of endometriosis supporting an autoimmune etiology. However, AAb against Thomsen–Friedenreich antigen could also be an autoimmune epiphenomenon due to aberrantly glycosylated endometrial antigens. If the latter assumption is correct, it would be of great interest to investigate cells of the adaptive immune response with regard to the recognition of such altered self-antigens.

**Adhesion molecules and protein–glycan interactions in endometriosis**

Due to the prevailing inflammation in endometriosis, adhesion molecules have been suggested to be involved, e.g., by mediating the migration of leukocytes into the peritoneal cavity. In this context, published data point towards altered cell adhesion mechanisms, which might interfere with leukocyte function. The following scenarios might—at least in part—provide an explanation for the failing immune surveillance in endometriosis. First, leukocyte function antigen (LFA)-1⁺ effector cells interact with intercellular adhesion molecule (ICAM)-1 expressed by target cells, whereby soluble ICAM-1 (sICAM-1) may interfere with such interactions. In endometriosis, the severe decrease in NK cell-mediated lysis of endometrial cells could be manifested when endometrial supernatants contained high levels of sICAM-1 (Fig. 1), whereby it remains to be fully elucidated whether peritoneal NK cells express LFA-1 [66]. Strikingly, the expression of sICAM-1 by ectopic endometrium is increased as compared to eutopic endometrium [77], and the levels of sICAM-1 are elevated in peritoneal fluid of
endometriosis patients [78] (Fig. 1). Hence, endometriotic lesions may ‘neutralize’ peritoneal LFA-1+ effector leukocytes via sICAM-1, which results in impaired immune surveillance of such effector cells.

A second putative scenario involves the glycoprotein peritoneal haptoglobin (pHp) or endometriosis protein-I (Endo-I). This glycoprotein is highly expressed in peritoneal endometriotic lesions [79] (Fig. 1) and interacts with various lectins or glycan-binding proteins [80]. Increased interaction of pHp with different lectins might be due to variations in the ratios of α(2,3) to α(2,6) sialic acid and fucose [80]. These alterations could be connected to increased expression of glycans mainly involved in carbohydrate–selectin interactions and cell–cell adhesion. Some studies report increased expression of E- and P-selectins in endometriosis [39, 81]. Because several reports are controversial [82], one might hypothesize that the function of selectins, determined by the binding to their epitopes, is more important than their quantitative levels. Sharpe-Timms et al. [83] showed that peritoneal macrophages from women with endometriosis bind more pHp in vivo than those from women without the disease, although the concentration of pHp is similar in both groups. The authors speculate that this effect may be due to altered forms of haptoglobin. Glycoproteins with increased numbers of glycans containing Sialyl-Lewis X structures are able to block adhesions of immune cells [84, 85], which may provide an explanation for the decreased ability of peritoneal macrophages to mediate cytolysis of misplaced endometrial tissues, as detectable in the peritoneal cavity (Fig. 1), which is further associated with an increased resistance of these cells to apoptosis in women with endometriosis [83].

Glycosylation and morphological changes were further investigated in a baboon model of endometriosis, and an increased lectin binding to fucosylated N-acetylgalcosamine residues could be detected in early stages of the disease, whereas such binding—accompanied by a late secretory phenotype of endometrial glands—was decreased in later stages of the disease [86]. A clear evidence of an asynchrony between the estimated day of the menstrual cycle and the observed histological/ultrastructural appearance of the glands could be identified. Fucosylated N-acetylgalcosamine residues are essential for the adhesion of the hatched blastocyst to the endometrium [87]. Glycosylation is important in the regulation of embryo attachment, and differential glycosylation can be regulated in the endometrium by the action of several hormones mainly during the secretory phase [88]. Therefore, one might speculate that abnormal glycosylation in endometriosis may be one of the factors leading to reduced fertility and other clinical and pathological manifestations of the disease.

Angiogenesis in endometriosis

The establishment of a new blood supply is essential for the survival of endometrium attached to the peritoneum and the maintenance of endometriosis. Increased microvessel density in endometriotic tissue with high proliferative activity has been demonstrated compared to lesions with low proliferative activity [89], which could be inhibited by blocking vascular endothelial growth factor (VEGF) [90]. High levels of VEGF and more specifically VEGF-A are present in peritoneal fluid of endometriosis patients [89] (Fig. 1). Other macrophage- or mast cell-derived factors, which either directly or indirectly induce angiogenesis and are up-regulated in endometriosis, include IL-1, IL-6, TNF-α, and TGF-β [91–93] (Fig. 1). In addition, IL-8 is increased in peritoneal fluid in endometriosis [94] and contributes to angiogenesis [95] (Fig. 1). Finally, matrix metalloproteinases (MMP) are involved in tissue remodeling and angiogenesis. The expression of MMP-1 by endometriotic cells is increased as compared to eutopic endometrium of patients and controls [96], and levels of MMP-2 were found to be elevated in peritoneal fluid of endometriosis patients [97] (Fig. 1). In contrast, another study reported MMP-1 and MMP-2 to be highly expressed in eutopic but not ectopic endometrium of women with endometriosis and a decrease in the MMP-2 inhibitor (TIMP-2) [98], suggesting a role of MMP in the very early development of the disease.

It may be concluded that immune cells in the peritoneal fluid and probably within the lesions do not only fail to reject endometriotic lesions but also support their growth by promoting vascularization and thus nourishing the tissue with their secreted factors such as cytokines.

The role of steroid hormones in endometriosis

Circumstantial and laboratory evidences are indicative of critical roles of steroid hormones in the establishment and maintenance of endometriosis. A lower incidence of the disease is noted in women with decreased endogenous estrogen production due to extensive exercise or smoking [99]. Although the development of endometriosis has historically been viewed as an estrogen-dependent disease, recent studies suggest that a failure of progesterone to appropriately regulate the expression of genes during endometrial differentiation might be a critical component of the disease process. It is well accepted that progesterone inhibits estradiol (E2)-dependent proliferation in the uterine epithelium. In humans and other vertebrates, the biological activities of progesterone are mediated by interaction with
Immunotherapy - a New Approach in the Treatments of Endometriosis

What is Endometriosis?
Endometriosis is a condition where the cells that are normally found lining the uterus are also found in other areas of the body: usually within the pelvis. Each month this ectopic tissue, under normal hormonal control, proliferates and is shed in the same cyclic way as the endometrium. The monthly internal bleeding in the pelvis, unlike the period, has no outlet from the body, which leads to inflammation, pain and the formation of scar tissue.

Fig. 1. The possible localizations of endometriomas

The term endometriosis is derived from the ancient Greek: end- inside, metra- womb and osis- disease. According to different sources, from 6 to 44% of the women in reproductive ages suffer from endometriosis [1]. Moreover, endometriosis is observed in 30-40% of cases among the infertile women [2, 3]. The "nodules" or "tumors" of endometrial tissue (also called as endometriomas) are found mainly in peritoneum linings of pelvis and ovaries, which appear either in the form of small superficial 'islands' or in the form of epithelial "chocolate" cysts. The growth and progression of endometriomas, just like endometrium, respond to the cyclic changes of estrogen levels. The basic clinical symptoms of endometriosis are dysmenorrhea, dyspareunia (pains during sexual intercourse), pelvic and abdominal pains in correlation with menstrual cycles, leading to one of the frequent complications – infertility [3, 4]. In the last decade, a considerable rise in malignancy preceded by endometriosis has been observed.

In accordance to the American Society of Reproductive Medicine (formerly the American Fertility Society) endometriosis is classified into the following stages:

- The stage of endometriosis is based on the location, mass, depth, and size of the endometrial implants, i.e. the specific criteria include:
  - the extent of the spread of endometrial implants;
  - the extent of pelvic adhesions;
  - the involvement of pelvic structures in the disease;
  - the degree of the fallopian tube occlusion.

The stage of the endometriosis does not necessarily reflect the intensity of pain experienced, risk of infertility, or other symptoms present. For example, it is possible for a woman in Stage I to be in tremendous pain, while a woman in Stage IV may be asymptomatic. It has been noticed that women who received treatment during the first two stages of the disease had the greatest chance of regaining their ability to become pregnant following treatment.

Endometriosis only a gynecological manifestation of a systemic pathology – it may emerge like tip of a much larger invisible iceberg, one that represents a whole range of health problems that have underlying hormonal and/or immune disorders. Besides the basic symptoms – dysmenorrhea, dyspareunia and infertility which are traditionally associated with endometriosis, women with this disease may develop a whole range of other symptoms – high degrees of allergies, chemical sensitivities, susceptibility to infections, mitral valve prolapse and chronic fatigue syndrome [5]. A recently completed study by the American Endometriosis Association has provided strong evidence that there is an increased risk of breast cancer, ovarian cancer, melanoma and non-Hodgkin's lymphoma in women with endometriosis and in their relatives [6]. Women with endometriosis have a higher incidence of thyroid disease, including, active thyrotoxicosis (hyperthyroidism), hypothyroidism, Hashimoto's Thyroiditis and Hashimoto's Thyroiditis [7]. In addition, other autoimmune diseases such as Rheumatoid Arthritis, Lupus, Multiple Sclerosis, and Ménier's disease are seen somewhat more frequently in women with endometriosis and in their close families, which once again approves that there is an important immune component in endometriosis developments.

Endometriosis and Immune system

Endometriosis can be considered analogous to tumor diseases with the similar involvement of immune system controlling over them. Usually, the normally functioning immune regulatory system of a women checks the outgrowths of ectopic endometrium in any places of the body other than in the endometrial cavity. If immune system can destroy the ectopic endometrial cells just like any other neoplastic cells,
Endometriosis and the neoplastic process

Rajesh Varma1,2, Terrance Rollason3, Janesh K Gupta2 and Eamonn R Maher1

1Section of Medical and Molecular Genetics, 2Academic Department of Obstetrics and Gynaecology and 3Department of Histopathology, Birmingham Women’s Hospital, Birmingham B15 2TG, UK

Correspondence should be addressed to R Varma; Email r.varma@bham.ac.uk

Abstract

Endometriosis is a frequent disorder that commonly presents with infertility and pelvic pain. Although the precise aetiology of endometriosis is unclear, it is generally considered to involve multiple genetic, environmental, immunological, angiogenic and endocrine processes. Genetic factors have been implicated in endometriosis but the susceptibility genes remain largely unknown. Although endometriosis is a benign disorder, recent studies of endometriosis suggest endometriosis could be viewed as a neoplastic process. Evidence to support this hypothesis includes the increased susceptibility to develop ovarian clear-cell and endometrioid cancers in the presence of endometriosis, and molecular similarities between endometriosis and cancer. In this article we discuss (i) the evidence suggesting that endometriosis might be viewed as a neoplastic process, and (ii) the implications of this hypothesis for elucidating the pathogenesis of endometriosis and developing novel methods of diagnostic classification and individualised treatments.


What is endometriosis?

Endometriosis is defined as the implantation of endometrium-like glandular and stromal cells outside their normal location in the uterus. Endometriotic lesions are usually identified at laparoscopy localised to ovaries and the pouch of Douglas (Fig. 1). Endometriosis is diagnosed in 30% of cases referred for infertility investigations (Lapp 2000) and in 10–70% of women with pelvic pain (Lapp 2000). Overall, studies estimate that endometriosis may affect around 7–15% of women of reproductive age, thus making this a common condition.

Endometriosis has been considered a ‘disease’ because it is often identified when investigating women with infertility, pelvic pain, dyspareunia (pain on intercourse) and dysmenorrhoea (painful periods). Traditionally the classification of endometriosis has been made by anatomical (surgical staging by revised American Fertility Society score) and histopathological (atypical and non-atypical endometriosis) criteria (Roberts & Rock 2003). However, this combined approach of classification does not correlate closely with pelvic pain or reproductive outcome, and is prone to inter-observer error. Furthermore, the emphasis on targeting the endometriotic lesion, by surgical removal or hypo-oestrogenic inactivation, does not necessarily correct the aberrant underlying molecular mechanism(s). This explains why current endometriosis treatment does not alleviate clinical symptoms in all cases, and recurrence is common (Donnez et al. 2002). These disparities suggest that endometriosis may not be a true ‘disease’ but a heterogeneous entity with differing subtypes. One subtype may be capable of causing symptomatic disease directly consequent to endometriotic pathology (e.g. ovarian endometriomas, pelvic adhesions). While another subtype may be associated with symptoms without an obvious endometriotic-lesion basis. Another subtype may be clinically asymptomatic and its presence be considered a normal ‘non-pathogenic’ phenomena. Consequently the current focus on treating the endometriotic lesion should be reconsidered, and efforts to understand the pathogenesis of endometriosis, and its temporo-spatial relationship with symptomology, should be increased.

Endometriosis and the neoplastic process

For some time, endometriosis research has focused on comparisons of various physiological processes in the endometrium (ectopic vs eutopic) of women affected by endometriosis, against unaffected women (Sharpe-Timms 2001). This has identified multiple anomalies in genetic, environment, angiogenic, endocrine, metabolic and immunological mechanisms. Some of these correlate with the severity of endometriosis and/or associated clinical