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REVIEW

Neuroendocrine–immune disequilibrium and endometriosis: an interdisciplinary approach

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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-

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ingly meager, which is attributed in part to the difficulties in studying the disease in humans.

Various forms of endometriosis have been described, such as *endometriosis genitalis interna* with endometrium adjacent to the eutopic endometrium, e.g., within the myometrium (adenomyosis) or the fallopian tubes. Further, *endometriosis genitalis externa* is being referred to if lesions can be located adjoining ovaries (endometriomas, endometriotic cysts), Douglas pouch, uterine ligaments, vagina, vulva, or perineum. Additionally, *endometriosis extragenitalis* mostly occurs within the pelvic cavity, septum rectovaginale, intestine, and ureter [4]. *Deep infiltrating endometriosis*, e.g., in the Douglas pouch, the sacrouterine ligaments, or the septum retrovaginale, is a very painful form of endometriosis characterized by fibrosis, smooth muscle proliferation, and fibromuscular nodules; this clinical entity is also called adenomysis externa [5]. According to Redwine [6], the most common sites of pelvic endometriosis are the Douglas pouch and the uterosacral ligaments.

A widely used classification of endometriosis has been introduced by the American Society for Reproductive Medicine (rASRM) and incorporates number, size, and location of endometrial implants, endometriomas, and/or adhesions as well as their morphology (red, white, and black) yielding to a classification from stage 1 (minimal endometriosis) to stage 4 (severe endometriosis) [7]. The unambiguous diagnosis of endometriosis is generally accepted by clinicians only after laparoscopic macroscopic evaluation and subsequent histomorphometric analyses of biopsies. Noninvasive diagnostic tools are not yet available.

Because the clinical symptoms of endometriosis range from severe dysmenorrhea, dyspareunia, dysuria to severe chronic pelvic pain, it is apparent that the disease rigorously interferes with the patients' quality of life and affects social life, sexuality, and psychological well-being. Accordingly, due to the link between endometriosis and infertility, quality of life is even further reduced if women suffering from the clinical symptoms are surplus childless. It has been proposed that infertility in endometriosis patients might be due to altered folliculogenesis [8], reduced preovulatory steroidogenesis of granulosa cells [9], and decreased capability of fimbrial ovum capture in endometriosis [10]. Furthermore, sperm phagocytosis by peritoneal macrophages [11] and oviductal macrophages [12], anti-sperm antibodies [13], and reduced sperm penetration and velocity [14] have been observed. In addition, an altered egg–sperm interaction [15] and toxicity against early embryonic development [16], as well as defective implantation [17] have been proposed to be responsible for the infertility in endometriosis patients.

Etiology

Different theories have been proposed to explain the etiology of endometriosis: first and foremost, the *transplantation theory* [18], which is based on observations that retrograde menstruation of vital endometrium results in the implantation of such tissue into the peritoneum. To date, this theory is undeniably the most accepted concept. However, it may be challenged by the fact that retrograde menstruation is physiologically occurring in the majority of women, but endometriosis only occurs in approximately one fourth of women in their reproductive years. In this regard, endometriosis may be the result of a failing immune surveillance in the peritoneal cavity in women susceptible to endometriosis. Thus, immunologists should become far more attentive to this disease, as insights on its susceptibility, pathophysiology, and the identification of therapeutic approaches are likely to arise from immunologically based research. Another premise, the so-called *metaplasia theory*, is based on the notion that pluripotent coelomic cells differentiate into endometrial cells [19]. This theory may provide explanation for deep adenomyosis and relatively uncommon locations of endometriosis such as the brain or the lung. On the other hand, the *denervation–reinnervation theory* assumes that damage and denervation during valsalva maneuvers are followed by reinnervation [20] leading to a loss of uterine polar contractility and promoting retrograde menstruation. Spread endometrial tissue then adheres to injured tissue. Here, the extent of denervation and reinnervation is interpreted as primary source for clinical symptoms and their recurrence even after denervatory surgery. This theory is supported by the fact that the majority of patients with pelvic peritoneal defects and a history of pain also suffer from endometriosis [21].

Additionally, various exposure factors are thought to be associated to endometriosis, e.g., dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin) [22]. Here, the idea is that dioxin promotes the development of endometriosis by interfering with immune-mediated mechanisms, more specifically by stimulating the production of pro-inflammatory cytokines. A genetic etiology has also been proposed due to the high prevalence in relatives of affected women [23]. Although a wealth of genetic factors have been anticipated to be linked to an increased susceptibility to endometriosis [24], generally accepted marker gene(s) for endometriosis could not yet be identified. Emerging evidence points towards differential gene expression in eutopic and ectopic endometrium in humans [25, 26], and future research will be needed to provide a more in-depth understanding of the biological significance of this disease.

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 infertile 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 PGE_2 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].

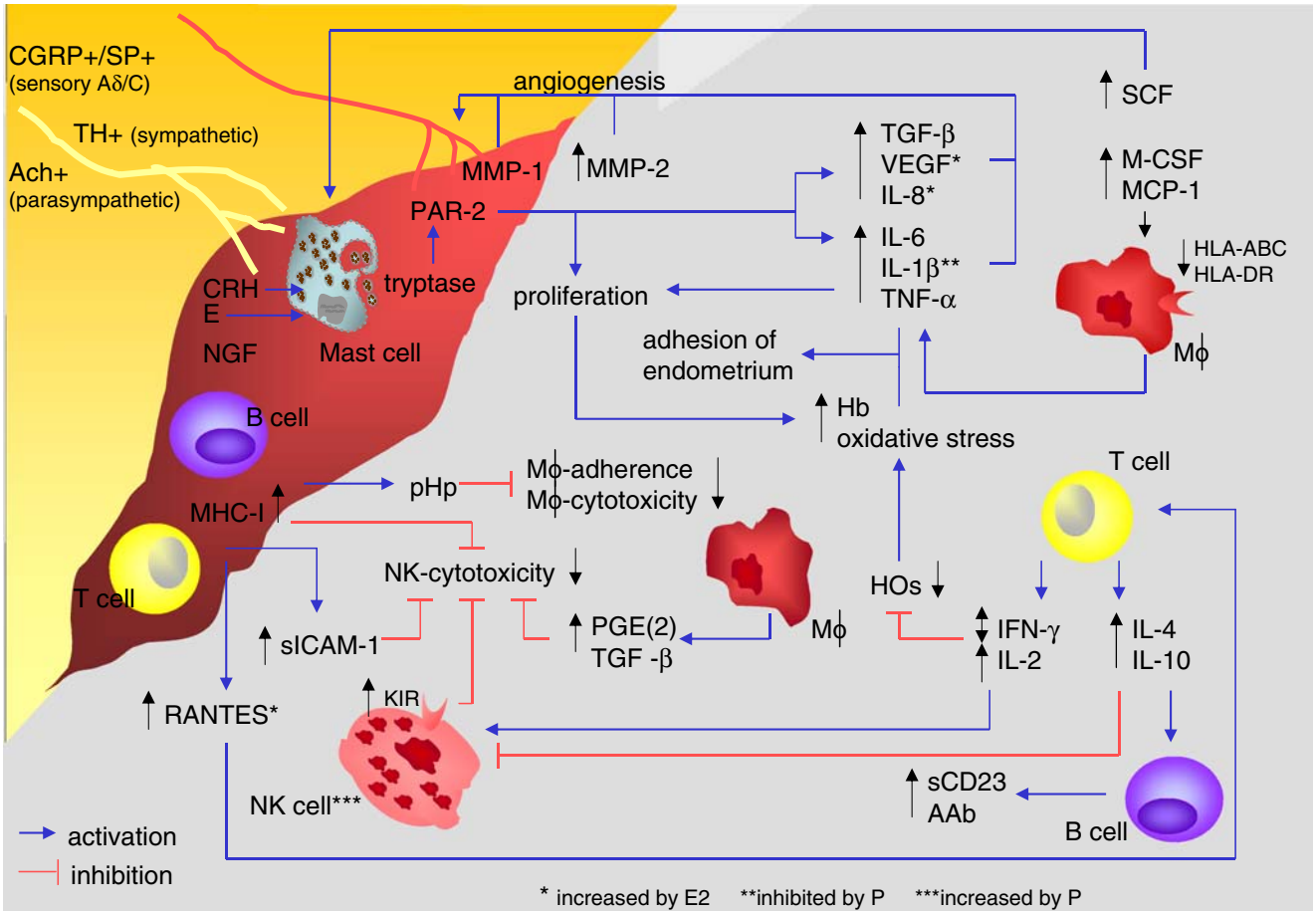

Fig. 1 Neuroendocrine–immune disequilibrium in endometriosis. *AAb* autoantibodies, *Ach* achetylcholine, *CGRP* calcitonin-gene-related peptide, *CRH* corticotropin-releasing hormone, *E* estrogens, *E2* estradiol, *Hb* hemoglobin, *HO* heme oxygenases, *IL* interleukin, *HLA* human leukocyte antigen, *IFN-γ* interferon-γ, *KIR* killer cell inhibitory receptor, *M-CSF* macrophage colony stimulating factor, *MCP-1* monocyte chemotactic protein-1, *MHC-I* major histocompatibility complex class-I, *MMP* matrix metalloproteinase, *NGF* nerve growth factor, *NK* natural killer, *P* progesterone, *PAR-2* protease-activated receptor-2, *PGE(2)* prostaglandin E(2), *pHp* peritoneal haptoglobin, *RANTES* regulated upon activation normal T cell expressed and secreted, *sCD23* soluble CD23, *SCF* stem cell factor, *sICAM-1* soluble intercellular adhesion molecule-1, *SP* substance P, *TGF-β* transforming growth factor-β, *TH* tyrosine hydroxylase, *TNF-α* tumor necrosis factor-α, *VEGF* vascular endothelial growth factor. In endometriosis, elevated levels of M-CSF, MCP-1, RANTES, and SCF in peritoneal fluid might lead to increased numbers of macrophages, T cells, and mast cells. Although markers of antigen presentation on macrophages such as HLA-ABC and HLA-DR are decreased in endometriosis, macrophage-derived IL-6, IL-1β, TNF-α, TGF-β, VEGF, and IL-8 are increased in peritoneal fluid, together with MMP-1 and MMP-2 stimulating angiogenesis. IL-6, IL-1β, and TNF-α support adhesion of endometrial cells to the peritoneum, and TNF-α stimulates the proliferation of ectopic tissue, resulting in high levels of Hb. T cell-derived IL-2 and IFN-γ decrease HO, leading to oxidative stress, and would, in sufficient levels, increase NK cell activity. IFN-γ has been inconsistently described as increased or decreased. Increased T cell-derived IL-4 and IL-10 inhibit cellular immunity and stimulate B cells to AAb production. sCD23 is increased in peritoneal fluid in endometriosis and might derive from activated B cells. Lymphocytes are increased in peritoneal fluid and abundantly present in ectopic tissue. Decreased NK cell cytotoxicity might be due to high anti-inflammatory T cytokines, increased KIR, high macrophage-derived PGE(2) and TGF-β, high MHC-I expression on ectopic cells, and high sICAM-1 levels in peritoneal fluid. Nerve fibers found within lesions are positive for CGRP, SP, TH, and Ach, and NGF and CRH were demonstrated. CRH and accumulated E can activate mast cells to release tryptase, activating PAR-2, which leads to increased secretion of VEGF, IL-8, and IL-6 and proliferation of ectopic tissue. pHp, expressed by ectopic tissue, decreases adherence and, in stage 3 and 4, cytotoxicity of peritoneal macrophages. E2 further increases RANTES, IL-8, and VEGF, whereas P inhibits IL-1β secretion from peritoneal macrophages and increases NK cell numbers

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 PGE_2 [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 endometric 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'), chemoattractant, 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.

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 autoagressive 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.

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

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 α_2-Heremans Schmidt glycoprotein (α_2-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 $\alpha_{(2-3)}$ to $\alpha_{(2-6)}$ 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*-acetylglucosamine 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*-acetylglucosamine 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 of 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

specific progesterone receptors (PRs) that are members of nuclear receptor superfamily of transcription factors. In contrast to healthy endometrium, hormone receptors remain at constant levels during the menstrual cycle in endometriosis [100], which points at a disruption of the normal cyclic responsiveness to hormonal changes with regular apoptosis of the epithelial stratum functionalis in endometriotic lesions.

The following molecular observations support a role of progesterone in endometriosis. First, a PR gene polymorphism was reported to be associated with endometriosis [101] (Fig. 2b). Second, only low levels of PR isoform A (PR-A) and no PR-B are detectable in extraovarian endometriosis [102] (Fig. 2b). Third, progesterone-dependent regulation of target genes was found to be perturbed in endometriosis. In the normal secretory endometrium, progesterone indirectly induces the 17β-hydroxysteroid dehydrogenase type 2 (17β-HSD-2), which converts estradiol (E2) to estrone (E1) [103] (Fig. 2a). In endometriotic tissue, 17β-HSD-2 is undetectable [104] (Fig 2b). As a consequence, E2 accumulates and probably induces proliferation of endometrial tissue. Moreover, the enzyme aromatase is exclusively found in endometriotic stroma [105] and provides E1, which is further converted to E2 by 17β-HSD type 1 (17β-HSD-1), contributing to the accumulation of E2 (Fig. 2b). The importance of aromatase for the endometriotic growth has been demonstrated by its genetic or enzymatic disruption [106].

Besides, E2 might also be involved in endometriosis-related inflammation, as it stimulates several mediators of inflammation such as IL-8 and RANTES [57, 107] (Fig. 1). Levels of MMP-2 in peritoneal fluid positively correlate with E2, but inversely with progesterone [97], and estrogens can also co-stimulate peritoneal mast cell activation in rats [108] (Fig. 1). Further, both E2 and progesterone increase the release of VEGF from peritoneal macrophages [109] and may hereby promote angiogenesis in endometriotic lesions. Given the high levels of E2 within the lesion, E2 might be more relevant in this context.

In contrast to estrogens, progesterone or synthetically produced progestogens called progestins exert potent im-

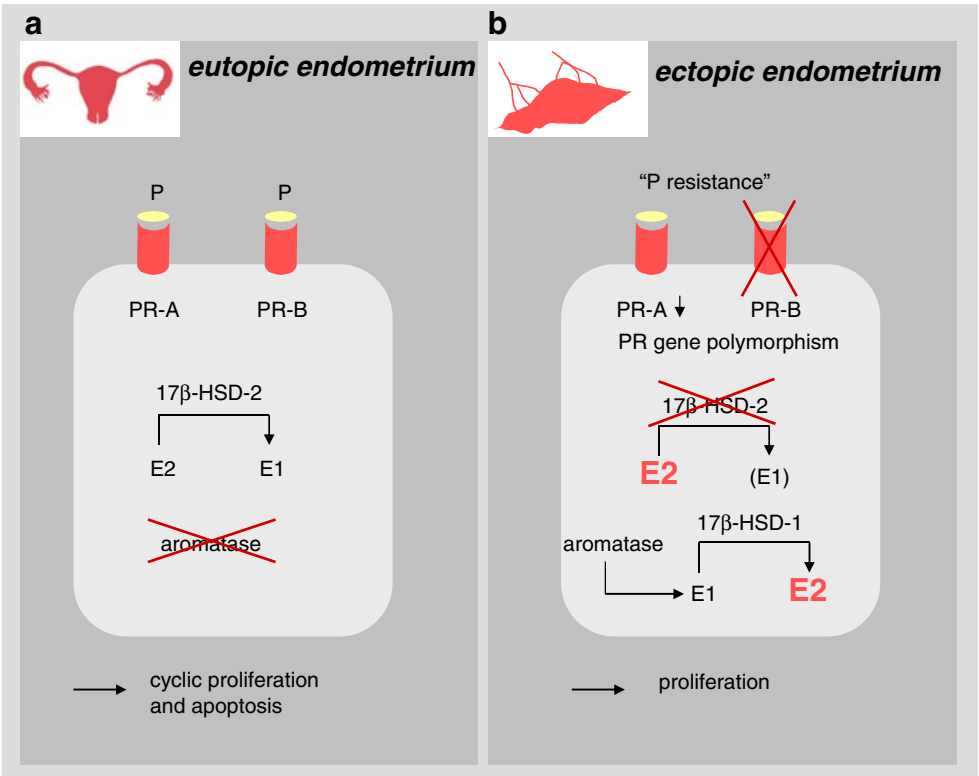

Fig. 2 Progesterone resistance and estradiol accumulation in endometriosis. *E1* estrone, *E2* estradiol, *P* progesterone, *PR* progesterone receptor, *17β-HSD-1* 17β-hydroxysteriod dehydrogenase type 1, *17β-HSD-2* 17β-hydroxysteriod dehydrogenase type 2. **a** In normal endometrium, P actions are mediated by PR; its isoforms PR-A and PR-B are both expressed on the tissue. P indirectly induces 17β-HSD-2, which converts E2 to E1; aromatase is not expressed. Tissue proliferation is followed by cyclic apoptosis. **b** In endometriotic tissue, only low expression of PR-A and the lack of PR-B have been observed, which leads to a partial resistance to P actions. Additionally, a PR gene polymorphism has been described to be more frequent in patients with endometriosis, which may be additionally involved in the partial resistance to P actions. Low levels of 17β-HSD-2 along with the enzymatic activity of aromatase—which is exclusively found in endometriotic stroma—lead to an accumulation of E2. Hence, physiological cyclic regulation of proliferation and apoptosis is disrupted in endometriosis

munosuppressive properties, and mechanisms of dampening inflammation have been demonstrated for the progestin dydrogesterone [110–112]. Using other progestins available to date, a reduction in transplant growth, a degeneration of transplants, an increase in NK cells in peritoneal fluid, and a decrease in IL-1β secretion from peritoneal macrophages could be demonstrated [113–115] (Fig. 1). It would be interesting to investigate the cross talk between hormones and regulatory T cells in this process.

One might conclude from these observations that E2 promotes endometriosis and progesterone/progestins dampen it, which is the rationale why progestins are widely used to treat endometriosis. In general, they act as agonists on the PR and have a functional anti-proliferative effect on endometrial tissue and endometriotic lesions. In addition, progestin treatment is generally better tolerated than other endometriosis therapies, which are associated with rather severe side effects [116]. Importantly, progestins are known to be effective in the control of pain symptoms in general or related to endometriosis [112, 116, 117]. However, not all patients respond to this regimen [118]. This clinical observation may be explicable by the partial resistance of endometriosis tissue to progesterone action due to the down-regulation of its receptors, as outlined earlier.

Neurological aspects in endometriosis

The female reproductive organs are innervated by sympathetic, parasympathetic, and sensory afferent nerves. Their distribution and the immunoreactivity to various peptides point at a role in hemodynamics and smooth muscle contraction. Uteri of patients with advanced endometriosis—similar to chronic pelvic pain—show increased numbers of nerve fibers and perivascular nerve fiber proliferation [119]. In addition, small nerve fibers were detected in the functional layer of the eutopic endometrium in endometriosis patients but not in controls [120].

Compared to normal peritoneum, nerve fibers in human peritoneal endometriotic lesions are increased [121]. In addition, artificially induced endometriotic cysts in rats are robustly innervated by fibers accompanying blood vessels and extending in patterns similar to the uterus [122]. Because endometriotic tissue may be considered as a 'vascularized autologous transplant', its innervation has been suggested to occur via sprouting of para- and perivascular nerve fibers. This coordination of vascularization and innervation has also been observed in other transplanted organs such as the parathyroid glands [123] and skin [124]. Immunostaining of endometriotic tissue revealed calcitonin-gene-related peptide $(CGRP)^+$ and substance P $(SP)^+$ sensory C and Aδ [121, 122], acetylcholine $(Ach)^+$ parasympathetic as well as tyrosine hydroxylase $(TH)^+$ sympathetic [121] fibers (Fig. 1). $CGRP^+$ and SP^+ fibers even reach the epithelium of the ectopic tissue [122]. Endometriosis-related pain might be mediated by these pro-inflammatory neuroactive agents. They are increased by nerve growth factor (NGF) [125, 126], which also sensitizes terminals of sensory nerve fibers [127]. Interestingly, intense immunoreactivity for NGF and its receptor NGFRp75 was demonstrated near endometriotic glands [121] (Fig. 1). Whether and how this might be connected with immunological alterations in endometriosis will be discussed in the course of the following section.

Stress perception, endometriosis, and sickness response

As previously outlined, cardinal symptoms of endometriosis are chronic pain and infertility, which may severely interfere with the patient's quality of life and be perceived as a persistent stressor. To date, it is well established that high perception of stress may trigger or aggravate the incidence or exacerbation of diseases such as inflammatory bowel disease [128, 129], immune dermatoses [130, 131], or pregnancy complications such as spontaneous abortion and pre-eclampsia [132]. Further, high levels of perceived stress have been proposed to contribute to the progression of endometriosis [133].

Nowadays, it is well established that high levels of perceived stress activate the release of neurohormones such as corticotropin-releasing hormone (CRH) as well as adrenocorticotropic hormone (ACTH) and glucocorticoids (GCs) largely via the hypothalamus–pituitary–adrenal (HPA) axis [134–136]. Via these stress-related hormones, accompanied by additional stress response mediators like neuropeptides or neurotrophins [137, 138], immune responses are profoundly altered [135, 136]. For example, GCs dampen the release of IL-12, IFN-γ, and TNF-α by antigen-presenting cells and Th1 cells, but up-regulate the production of IL-4, IL-10, and IL-13 by Th2 cells [139, 140]. Thus, it has been proposed that the release of CRH, ACTH, and subsequently GCs induces the selective suppression of the Th1-mediated cellular immunity and elicits a skew in the direction of Th2-dominated immunity. Indeed, it has also been postulated that such Th2 shift may actually protect the organism from systemic surpassing of pro-inflammatory cytokines, which could have severe tissue-injuring features [141].

However, besides such well-described immunosuppressive effects of GCs, relevant examples of pro-inflammatory actions of peripheral CRH have recently been introduced, e.g., in the synovia of rheumatoid arthritis patients [142]. This peripheral CRH leads to proliferation of immunocytes [143, 144] and production of IL-1, IL-2, and IL-6 [145, 146]. Moreover, CRH antagonizes some of the immuno-

regulatory effects of serotonin (5-HT), e.g., the suppression of TNF-α production [147]. Inflammation can be dampened by blocking CRH at the periphery [148], as CRH receptors occur on immune cells, e.g., on T cells, monocytes [149], and mast cells [150]. Peripheral CRH may, thus, be referred to as "immune" CRH. Immune cells such as T cells, B cells [151], and mast cells [152] may be the source of peripheral CRH. Besides, CRH-like immunoreactivity was demonstrated in splenic nerve fibers [153], in the dorsal horn of the spinal cord, and dorsal root ganglia as well as sympathetic nerve fibers [154] pointing at a neuronal source of "immune" CRH in descending nerve fibers.

Intriguingly, CRH is highly expressed in endometriotic lesions [44] and has been proposed to stimulate mast cells to secrete VEGF [150] (Fig. 1). This would facilitate angiogenesis in endometriotic lesions and perpetuate the dissemination of the disease. Thus, it may be proposed that high levels of perceived stress promote the dissemination of endometriosis via CRH-dependent pathways, as peripheral CRH is found increased in response to psychological stress [155]. It remains to be elucidated whether and how CRH can be made attributable for the peritoneal inflammation present in the peritoneal cavity of patients suffering from endometriosis. In contrast to endometriotic tissue, CRH levels in peritoneal fluid of endometriosis patients were not different from controls; however, CRH-binding protein (CRH-BP) is increased in advanced stages of endometriosis, compared to early stages or healthy controls [156], which may be interpreted as an attempt of the body to down-regulate high CRH levels.

Neurohormonal responses to stress also include an activation of the sympathetic nervous system with the subsequent increase in catecholamines [137], and published evidence strongly supports that the immune response can be regulated via the sympathetic nervous system/catecholamines at regional, local, and systemic levels [134]. For example, lymphocytes express adrenergic receptors and respond to catecholamine stimulation with the development of stress-induced lymphocytosis, inflammation, and distinct changes in lymphocyte trafficking, circulation, proliferation, and cytokine production [139, 157, 158]. Because sympathetic nerve fibers are present in endometriotic tissue, it appears likely that—besides CRH—catecholamines contribute to the peritoneal inflammation in endometriosis, and an increased release of catecholamines in response to high levels of stress could additionally perpetuate local inflammation.

Besides the classical stress-related neurohormones—the players of the HPA axis and catecholamines—NGF and the neuropeptide SP are now recognized as pivotal mediators of the stress responses [131, 159–161]. SP co-functions as neurotransmitter in the central and peripheral nervous system in pain, anxiety, and emotional centers besides stress [131]. Additionally, SP is a potent pro-inflammatory mediator, as it induces the production and release of cytokines such as IL-1, IL-6, and TNF-α and NGF [162, 163] and promotes angiogenesis during inflammation [164]. NGF also enhances pro-inflammation and has been proposed to increase the release of SP [161, 165, 166]. SP and NGF derived from nerve endings induce leukocyte recruitment and release of pro-inflammatory cytokines, which is generally referred to as neurogenic inflammation. Vice versa, pro-inflammatory cytokines may induce the expression of neuropeptides such as SP and NGF and their cognate receptors [167, 168], which might provide an explanation for the up-regulation of NGF expression in inflamed areas, e.g., in peritoneal endometriosis [121]. Interestingly, the activation of PAR-2 on afferent neurons, e.g., by proteases such as mast cell tryptase, leads to subsequent release of pro-inflammatory CGRP and SP [169], which might provide explanation for pain during inflammation. To date, published evidence on the expression of SP in endometriosis is very limited; some authors report no changes of SP in endometriosis, which could be due to the difficulties in detecting SP because it is a rather small peptide of no more than 11 amino acids [170]. Hence, future studies employing novel technologies such as gene or protein arrays may allow to investigate the expression of pain-related neurotransmitters as well as their receptors, e.g., SP high affinity receptor neurokinin (NK)-1R [112] on endometrial tissue. However, as outlined earlier, Tokushige et al. [121] recently identified an increased number of nerve fibers in peritoneal endometriotic lesions using immunohistochemistry; in fact, these nerve fibers were immunoreactive for SP, CGRP, Ach, NGF, and TH (Fig. 1).

In conclusion, a hypothetical scenario on how high stress perception might be related to endometriosis is depicted in Fig. 3. It may be postulated, in terms of a "brain-body cross talk", that psychologically stressful conditions promote the aggravation of inflammatory processes, angiogenesis, pain, and infertility via pathways that most likely involve catecholamines, "immune" CRH, NGF, SP, and CGRP (Fig. 3 right), whereby the dependancy between NGF/SP and CRH should be addressed in future experimental settings.

Peripheral immunological events in turn signal certain brain regions to induce the so-called sickness response including (neuro)hormonal and behavioral changes [171]. These profound behavioral alterations are referred to as sickness behavior, which comprises depressive-like behavior and is characterized by reduced locomotor activity, fatigue, hypophagia or anorexia, diminished social interactions, and female sexual behavior [171–173]. Interestingly, such sickness behavior can be experimentally provoked by peripheral administration of IL-1 or TNF-α [172, 173]. In healthy humans, levels of anxiety, depressed mood, and memory

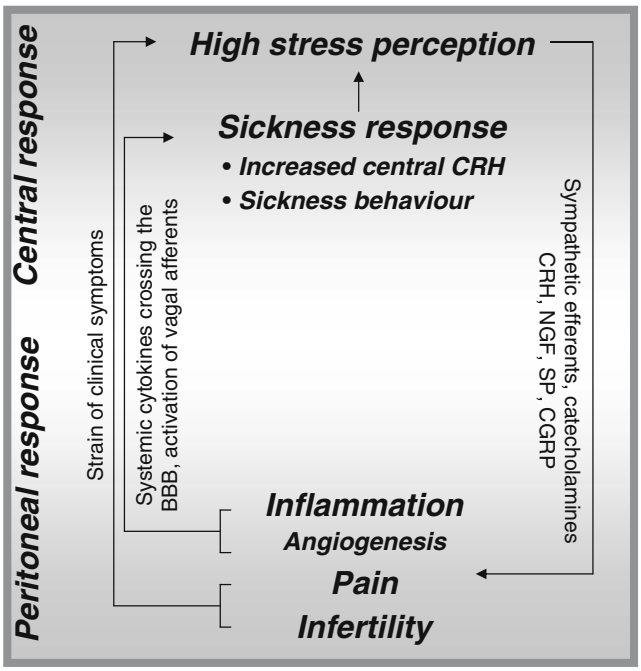

Fig. 3 Hypothetical scenario for endometriosis: the 'brain–body–brain cross talk'. *BBB* blood–brain barrier, *CGRP* calcitonin-gene-related peptide, *CRH* corticotropin-releasing hormone, *NGF* nerve growth factor, *SP* substance P. Perceived stress aggravates peritoneal inflammation and angiogenesis, chronic pain, and infertility in patients with endometriosis via neuronal pathways involving catecholamines, CRH, NGF, SP, and CGRP; this may be referred to as 'brain–body cross talk' because it involves central stress response pathways. Clearly, the aspect of infertility—a frequent clinical symptom in patients with endometriosis—may be perceived as an additional stressor besides stressors such as daily hassles. Peripheral 'inflammatory stress'—as described in endometriosis—and the strain of clinical symptoms such as chronic pain may in turn induce a sickness response, which can be entitled 'body–brain cross talk'. Such 'body–brain cross talk' may subsequently perpetuate stress perception and trigger the release of central CRH and the onset of behavioral alterations, i.e., sickness behavior, via elevated circulating pro-inflammatory cytokines bypassing/crossing the BBB or via stimulation of vagal afferents by peritoneal pro-inflammatory cytokines. As a result, a vicious circle of 'brain–body–brain cross talk' is closed in patients suffering from endometriosis

function correlate with levels of TNF-α, its soluble receptors, and IL-6 [174]. Brain regions identified to date comprise structures associated with the central stress system, namely, the hypothalamus, hippocampus, frontal/prefrontal structures, and the limbic system including the amygdala [175].

Intravenous as well as intraperitoneal applications of IL-1 result in an increased CRH production in the hypothalamus [176, 177]. Because elevated levels of central CRH have been associated to the abovementioned behavioral changes during sickness [178], non-cognitive 'inflammatory stress' can induce behavioral and emotional alterations via increase in central CRH. As mentioned earlier in this review, IL-1, IL-6, and TNF-α are elevated in peritoneal fluid of endometriosis patients. Evidently, due to the up-regulation of pro-inflammatory cytokines not only in the peritoneal fluid but also in the peripheral blood of patients with endometriosis [179], this 'inflammatory stress' may increase stress perception in affected patients due to the onset of the sickness response, which includes an increase in central CRH as well as behavioral changes and which would aggravate the strain generated by symptoms such as pain and infertility in terms of a 'body–brain cross talk' (Fig. 3, left).

Undoubtedly, the question that arises is how peripheral 'inflammatory stress' can reach the central nervous system. Here, it has been proposed that peripheral blood cytokines bypass the blood–brain barrier (BBB) entering in the circumventricular organs [180]. Secondly, these cytokines might cross the BBB, especially when its permeability is enhanced, e.g., in stressful conditions [181]. Thus, perceived stress might predispose certain individuals to develop sickness behavior during illness. Finally, vagal nervous afferents might be involved, as suggested by experiments in rats where behavior has been investigated before and after vagotomy and upon intraperitoneal application of IL-1β [182], and IL-1β-induced inhibition of social exploration was attenuated in vagotomized rats. Because Ach^{+} nerve fibers were found in endometriotic tissue, as illustrated above, behavioral changes mediated by vagal afferents might play a relevant role in endometriosis (Fig. 3, left).

In conclusion, dissemination of endometriosis may be sustained and advanced by high levels of perceived stress accompanying neuroendocrine-immune disequilibrium in affected patients. This, in turn, fosters additional stressors such as pain and infertility and induces sickness response. As a result, stress perception is even higher, which induces a vicious cycle of 'brain–body–brain cross talk' in affected women (Fig. 3).

Animal models of endometriosis

As evident from the previous paragraphs, an in-depth knowledge on the pathophysiology of endometriosis is limited, mostly due to the difficulty in studying the disease in the human [(a) need for laparoscopy, (b) painful lesions are often very small in size and invisible from the peritoneal cavity, and (c) high individual patient variation]. To overcome this gap, convincing animal models are needed in addition to clinical studies. Endometriosis is a primate-specific disease, but primate studies can only be performed adequately in a limited number of laboratories [183] and are rather time-consuming. To date, the rat is one of the most thoroughly studied laboratory animal species, and in spite of its phylogenetic distance to the human, surgically generated transplants of uterine fragments at different sites of the peritoneal cavity have been shown to resemble

human endometriotic lesions in many respects [184]. Advantages and disadvantages of such rat models have been vividly discussed, e.g., the absence of menstruation into the peritoneal cavity in rats and consequently the lack of retrograde menstruation as one of the key prerequisites for the development of endometriosis. On the other hand, rat endometrium undergoes hormone-dependent tissue remodeling; surgically transplanted uterine tissue in the rat mirrors human endometriotic lesions, and endometrial lesions in rats are responsive to steroid treatment. The benefits of using the rat model are also supported by observations that, in rats with surgically induced endometriosis—similar to humans—fertility and fecundity are also impaired [41, 185].

Similar to rats, mice do not menstruate and—similar to rats—murine endometrium undergoes hormone-dependent tissue remodeling. Further, mouse models are commonly used in immunological research. Hence, based on the growing evidence of a failing immune surveillance in endometriosis, the need for a murine mouse model to study immunological aspects of endometriosis is becoming more and more evident. To date, a few mouse models have already been introduced and are summarized in Table 1. In some of these models, human or murine endometrium is injected into the peritoneal cavity [66, 186, 187] or fixed on the mesentery or the peritoneum [188, 189] of either intact or ovariectomized mice of different strains. In other murine models, human endometrioma tissue was either injected subcutaneously in nude mice [190] or injection is performed upon transducing whole fragments of human endometrium in vitro by adenoviral infection with the green fluorescent protein (GFP) cDNA before transplantation into nude mice. Implantation and growth of endometriotic-like lesions could be here non-invasively followed and repeatedly documented by in vivo imaging [191]. Such rodent models may provide tools for drug testing and/or gene target validation in endometriosis.

Clinical considerations

Current approaches to therapy for endometriosis are surgical, directed at resecting endometriotic tissue, and pharmacologically, aiming at inhibiting estrogenic stimulation, thus reducing dissemination and disease burden. However, despite such therapeutical approaches, endometriosis is still a progressive disease, and recurrences are observed in up to 74.4% after medical treatment depending on the stage of disease [192].

Hormonal therapeutic approaches currently used for amelioration of endometriosis-associated pelvic pain include GnRH agonists and daily oral contraceptives used in a cyclic or continuous regimen [118]. Because these interventions inhibit ovulation and interfere with the cyclic remodeling of the endometrium, the onset of pregnancy is impossible, and one potential stressor of endometriosis, the infertility, is not targeted. In line with such hormonal approach in endometriosis treatment, clinical studies suggest an apparent resolution of endometriosis-related symptoms during pregnancy, supporting the rationale of treatments aiming at a pseudo-pregnancy regime [193]. As mentioned earlier in this review, published evidence suggests that progestins are effective therapies in the treatment of pain symptoms associated with endometriosis and ameliorate symptoms of endometriosis, as the pro-inflammatory setting in the peritoneal cavity may be dampened and pain receptors such as the NK1 receptor are down-regulated [112, 194]. Clearly, the future holds promise for highly specific therapeutic agents aiming at targeting the pro-inflammatory setting in endometriosis. Such approaches may include aromatase inhibitors, progestins, and progesterone receptor modulators. Therapeutic approaches aiming at diminishing inflammation also include cytokine antagonists such as the aforementioned application of recombinant human TNF-α-binding protein-1 [65]. Further, MMP inhibitors and mast cell stabilizers such as the flavonoid quercetin may have the potential to ameliorate symptoms in endometriosis, as they have been shown to inhibit the release of pro-inflammatory mediators [195]. Interestingly, progesterone is known to suppress MMPs and to enhance the expression of MMP inhibitors (TIMPs) during endometrial differentiation [196]. Hence, a combinational therapy of progestins and MMP inhibitors may be envisioned to be highly effective in endometriosis.

Table 1 Recently published mouse models of endometriosis

Mouse strain	Ovariectomy	Initial tissue placement	Reference #
ncr/nude (athymic)	+	Subcutaneous injection of human endometrioma biopsies	[190]
C57BL/6 Balb/c	+	Intraperitoneal inoculation of murine endometrial fragments	[66]
ncr/nude	−/+	Intraperitoneal injection of human endometrioma biopsies	[186]
Progesterone receptor −/−	+	Intraperitoneal fixation of murine endometrium on mesentery	[188]
C57BL/6	−	Intraperitoneal fixation of murine endometrial punches on peritoneum and mesentery	[189]
C57BL/6	+	Intraperitoneal injection of GFP-labeled murine endometrial fragments	[187]

GFP Green fluorescent protein, *PR* progesterone receptor

Conclusions

Profound and comprehensive insights on neuroendocrine-immunological disequilibrium are required to fully understand the pathophysiology of endometriosis and to develop primary and secondary prevention strategies. Clearly, multidisciplinary research activities are needed, and future basic science and clinical trials must refrain from analyses of single pathways, but aim at identifying currently known key protagonists in endometriosis. In this regard, markers and mediators introduced and summarized in the present review may just reflect the often quoted 'tip of the iceberg', but foster vivid scientific discussions and mutual research endeavors between basic scientists and physicians aiming at successfully targeting symptoms of this complex and multifactorial disease that affects up to 22% of women in their reproductive age. Obviously, the effect of psychosocial counseling or psychotherapy of patients with endometriosis—which has noticeably been underrated in previous studies—may be beneficial because an improved stress management may disrupt the vicious cycle of cross talk between peritoneal inflammation, sickness response, pain perception, and stress.

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