Endometriosis: The Role of Neuroangiogenesis

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Abstract

Endometriosis is a common cause of pelvic pain and infertility, affecting ~10% of reproductive-age women. Annual costs for medical and surgical care in the United States exceed $20 billion. The disorder is characterized by implants of endometrial tissue outside the uterine cavity. Endometriotic lesions induce a state of chronic peritoneal inflammation, accompanied by elevated prostaglandin, cytokine, and growth factor concentrations. The current therapy is surgical ablation of ectopic implants and hormones that block the hypothalamic-pituitary-ovarian axis, but these approaches are expensive, carry perioperative risks, or have unpleasant side effects of hypoestrogenism. Recent evidence indicates that ectopic endometriotic implants recruit their own unique neural and vascular supplies through neuroangiogenesis. It is believed that these nascent nerve fibers in endometriosis implants influence dorsal root neurons within the central nervous system, increasing pain perception in patients. We consider the mechanisms and therapeutic implications of neuroangiogenesis in these lesions and propose potential treatments for the control or elimination of endometriosis-associated pain.

INTRODUCTION

Dysmenorrhea: painful menstrual periods

Dyspareunia: discomfort during intercourse

Dysuria: pain during urination

Dyschezia: pain during defecation

Angiogenesis: the growth of new vessels from preexisting vessels

HPO axis: hypothalamic-pituitary-ovarian axis

Endometriosis is a common gynecological disorder that is characterized by the growth of hormone-responsive endometrial tissue outside the uterine cavity. It is associated with local inflammation and the distressing symptoms of chronic pelvic pain [e.g., painful menstrual periods (dysmenorrhea) and discomfort during intercourse (dyspareunia), urination (dysuria), or defecation (dyschezia)] as well as infertility. As noninvasive tests are not yet available, the diagnosis can be confirmed only at the time of surgical visualization of intraperitoneal lesions. On the basis of this ascertainment, several studies suggest that the overall prevalence of endometriosis among reproductive-age women is approximately 10% (1), but this number is likely an underestimate. Endometriosis is accompanied by pelvic pain or subfertility in >10 million women in the United States and accounts for approximately 400,000 hysterectomies per year (1). Annual estimates for direct and indirect costs for endometriosis care exceed $20 billion nationally, but the impact of endometriosis symptoms on affected women, their families, and society is even more devastating.

The pathophysiology of endometriosis is enigmatic, and the association between observed disease and pain is particularly poor (2). As a result, diagnosis is typically delayed by more than 6 years from the onset of symptoms (3), and clinical management of endometriosis-associated pain is variable, costly, and of suboptimal efficacy. The nature of the pain associated with endometriosis has been inadequately characterized, and the mechanisms involved remain unclear. Although the cause of pain in endometriosis is likely multifactorial (4), critical, common pathways or mechanisms may exist. The resolution of this issue may pave the way to improved therapeutic interventions with the potential of maximizing pain control in patients with endometriosis.

In this focused review, we address the theoretical origins of endometriosis, its clinical presentation, and the main postulated pathophysiological mechanisms responsible for the pain in this condition. We then explore a novel concept, termed neuroangiogenesis, to describe an etiological process that we believe informs our understanding of the cellular and molecular interactions contributing to the pathophysiology of pain in endometriosis. We first review evidence of local peritoneal inflammation, supported by findings of elevated cytokine and growth factor concentrations in the peritoneal fluid of affected patients. We propose a role for angiogenic factors in the establishment and growth of ectopic implants and highlight new data showing that ectopic endometriotic implants recruit and develop their own unique neural and vascular supplies. We then propose that nascent nerve fibers sprouting within endometriosis implants influence the activity of dorsal root neurons within the central nervous system (CNS) to modify pain perception in these patients. We also consider the therapeutic implications of neuroangiogenesis and propose potential treatments that may become useful in the control or elimination of endometriosis pain. It is our hope that this hypothesis will steer research toward more functionally directed therapeutic interventions for endometriosis-associated pain.

Surgical removal of lesions is the mainstay of treatment for endometriosis-associated pain; however, such removal commonly provides only temporary relief with a high recurrence rate. As a result, there have been ongoing attempts to develop new strategies as we gain a better understanding of the disorder's pathophysiology. Our current pharmacological armamentarium for endometriosis pain has a decidedly endocrinological focus, targeting suppression of the hypothalamic-pituitary-ovarian (HPO) axis. This is achieved with birth control pills, danazol (an isoxazole derivative of testosterone), progestins, gonadotropin-releasing hormone analogs (GnRHa), and aromatase inhibitors. Not only do these medications have significant adverse side effects, due predominantly to hypoestrogenism, but they also render the recipients anovulatory, preventing pregnancy during therapy. Our strategy is

to understand other biological mechanisms mediating pain in this disorder so that we can tailor future therapies to more specific nonhormonal targets. In so doing, we expect to achieve effective pain control and moreover to allow patients to have an opportunity to achieve pregnancy while on medications for endometriosis-associated pain. The best available evidence for guiding clinical management of endometriosis-associated pain is also summarized.

THEORIES OF THE ORIGIN OF ENDOMETRIOSIS

The pathogenesis of endometriosis has puzzled scientists for centuries since some of the earliest surgical descriptions of the disease dating back to the seventeenth century (5). Despite the high prevalence of endometriosis and its enormous physical, psychological, and economic burden, we still do not have a clear understanding of its pathogenesis (6, 7). Numerous theories of the histogenesis of endometriosis have been proposed. The most popular hypotheses are retrograde menstruation and implantation of endometrial fragments into the peritoneal cavity, proposed by Sampson (7) in 1927, with consequent abnormalities in immune surveillance mechanisms resulting in implant growth and inflammation of surrounding tissues.

Retrograde Menstruation and Implantation

The theory of retrograde menstruation and implantation is supported by the common observance of reflux flow and intraperitoneal spillage of viable endometrial tissue in ovulating women during menstruation (8). The incidence of endometriosis is also increased in cases of anatomical menstrual outflow obstruction that predispose to retrograde flow (8). Furthermore, ablation of the eutopic (i.e., intrauterine) endometrium in some women with endometriosis dramatically reduced the risk of recurrence (9). That implants are common in the dependent regions of the pelvic floor and that more than 60% of unilateral lesions occur in the left hemipelvis are also consistent with the accumulation of refluxed cells in this location, due to the position of the mesentery of the sigmoid colon. However, retrograde menstruation occurs in approximately 90% of women (8), with only 10% of women developing endometriosis, leading to the hypotheses that patients with endometriosis have (*a*) an altered eutopic endometrial phenotype, (*b*) aberrant ectopic cellular clearance mechanisms, or (*c*) intrinsic factors that affect the adhesion, proliferation, and invasion of ectopic implants into pelvic peritoneal surfaces. These theories are discussed in more detail below.

Defective Immunosurveillance and Inflammatory Hyperresponsiveness

The pathophysiology of endometriosis has been attributed to changes in both cellular and humoral immunity (10). In normal women, intraperitoneal menstrual debris appears to be eliminated by innate immune cells, particularly macrophages and natural killer (NK) cells, without loss of immune tolerance, presumably mediated by $CD8^+$ T cell production of immunosuppressive cytokines [e.g., interleukin (IL)-13, interleukin 1 receptor agonists (IL-1RA)]. Some researchers have suggested that these mediators of the innate immune system clear regurgitated endometrial cells from the peritoneal cavity via cytotoxicity and phagocytosis (11–14). Oosterlynck et al. (15) first described decreased NK cell cytotoxicity against endometrial and hematopoietic cells in women with endometriosis. The same group showed that peritoneal fluid from women with endometriosis contained more soluble NK cell–suppressive activity than did peritoneal fluid from controls (16).

Although impaired NK cell activity results in inadequate removal of refluxed menstrual debris, the numbers of activated macrophages are increased in the pelvic fluid of women with endometriosis. These macrophages secrete prostaglandins, proinflammatory cytokines, and angiogenic and neurotrophic peptides that stimulate endometriotic cell proliferation

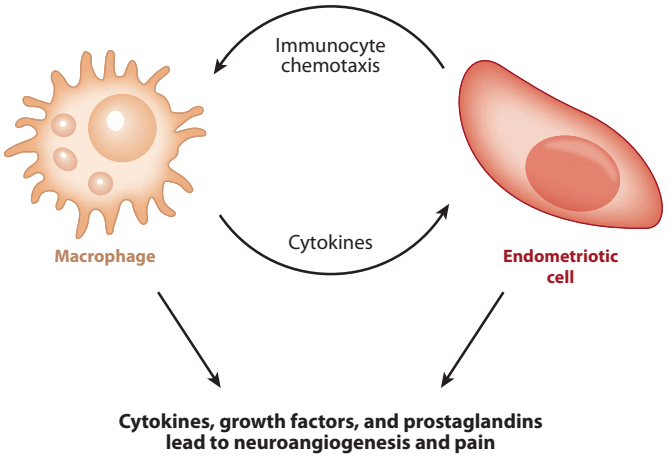

Figure 1

Inflammation and neuroangiogenesis. Chemokines produced by endometriotic cells recruit immune cells (particularly monocytes) to the lesions. Differentiation of the latter cells into activated macrophages is associated with inflammatory cytokine production, creating a feed-forward loop, with the elaboration of growth factors and prostaglandins postulated to mediate endometriosis-associated pain.

and local neuroangiogenesis (**Figure 1**). Our laboratory has been actively engaged in characterizing this local inflammatory milieu. We and others have identified high concentrations of immuno- and bioactive RANTES (regulated on activation, normal T cell expressed and secreted; also known as CCL5) and MCP-1 (monocyte chemoattractant protein-1) in the pelvic fluid of endometriosis cases, and peritoneal macrophages from these women overexpress the chemokine receptor CCR-1 (cognate chemokine receptor type-1) (17–19).

Genetic and Epigenetic Theories of Endometriosis Development

Over the past decade, scholars of endometriosis have integrated several of the classical models of its etiology into a neoclassical perspective that includes the notion that anatomic, genetic, epigenetic, and possibly environmental factors also influence the risk of developing endometriosis (20–22). More recently, other authors have posited that endometriosis may arise from the basal layer of retrogradely refluxed endometrial fragments (23) or may arise from rare bone marrow–derived adult stem cells that migrate to ectopic sites (24). Although exceedingly rare in men (only six cases have been reported in the world's literature), endometriosis-like lesions have been observed in the lower gentiurinary tract, where they may reflect embryological remnants of Müllerian tract origin or possibly stem cell derivatives (25). Future studies will undoubtedly explore these theories.

RANTES (CCL5): regulated on activation, normal T cell expressed and secreted

Fecundity: the ability to reproduce

CLINICAL PRESENTATION OF ENDOMETRIOSIS

Symptoms and Signs

The most common and most specific symptom of endometriosis is pain, typically in the form of progressive, secondary dysmenorrhea. Menstrual pain usually begins before bleeding is noted and continues throughout the duration of menstrual flow. The pain may also manifest as dyspareunia, dysuria, or dyschezia or be referred to musculoskeletal regions, such as the flank or low back. The second-most-common symptom is infertility, with monthly fecundity rates reduced as much as tenfold, resulting in similar per-cycle pregnancy rates as those observed in unexplained infertility patients (0.02 to 0.10). Due to the diffuse and pusillanimous nature of endometriotic lesions, the physical examination is commonly unrevealing. Bimanual palpation of the pelvic structures is of limited precision in either localization or diagnosis of endometriosis. Tender nodules may be palpable along the uterosacral ligaments or within the recto-uterine cul-de-sac of Douglas, especially if the exam is done just before menses. Because disease is found in the dependent areas of the pelvis, performance of a systematic rectovaginal examination is critical. Rarely, impaired renal function and azotemia can develop in women with retroperitoneal ureteric fibrosis.

Diagnosis

Direct visualization of endometriotic implants is the current gold standard for diagnosis. Although endometriosis is associated with some

plasma, cytological, and biochemical characteristics, none of these have adequate sensitivity or specificity for clinical application. Hence, laparoscopy and rarely laparotomy are the most common means to establish a firm diagnosis. Histopathological confirmation of endometriosis is established by the microscopic identification of ectopic endometrial epithelium and stroma, often with fibrosis and the infiltration of hemosiderin-laden macrophages within ectopic lesions. These pathognomonic features are documented in only approximately 70% of clinically suspicious cases.

Circulating Markers of Endometriosis

Although they might provide great clinical value, plasma or serum markers of endometriosis have proven to be elusive. The best studied of these is the ovarian cancer tumor marker CA-125 (cancer antigen-125 or carbohydrate antigen-125). Although the sensitivity of preoperative CA-125 for the detection of endometriosis is too low for a screening test, high concentrations may be predictive of advanced disease (26). The relationship between endometriosis and ovarian carcinoma is discussed briefly below. The addition of serum-soluble intercellular adhesion molecule-1 (ICAM-1) only modestly improved the sensitivity of concomitant CA-125 screening for endometriosis (27). Zhang et al. (28) used two-dimensional gel electrophoresis and serum proteomics to identify elevated levels of actin, heat shock, and complement-associated proteins in cases of endometriosis. This unbiased strategy may be a fruitful approach to discover new biomarkers.

Classification

The system most widely used to classify the extent of endometriosis is the surgical staging model promulgated by the American Fertility Society in 1985 (29). This scheme designates disease extent on the basis of total three-dimensional volumes of endometriosis lesions and fibrosis. Importance is placed upon depth of invasion, bilaterality, and ovarian involvement, as well as density of associated adhesions and extent of pelvic cul-de-sac obliteration. Through use of this classification, scores are tallied to objectify and compare natural history and treatment outcomes among different centers and surgeons. Scores of 1 to 15 reflect minimal or mild disease; scores of 16 to 40, moderate disease; and scores of more than 40, severe disease. This staging system was established to predict fertility outcomes, and it has not been shown to correlate well with the more common symptom of pelvic pain (30). Some authors have suggested the incorporation of objective biochemical markers of inflammation as a means to extend the contribution of associated pathological parameters beyond the presence of laparoscopically visible lesions (31, 32). Patients with endometriosis may have other comorbid conditions within the pelvis; approximately 90% of women with endometriosis have adenomyosis (invasion of endometrial tissue into the muscular wall of the uterus) (33), and interstitial cystitis (localized chronic inflammation of the urinary bladder wall) can be observed in at least 60% of women with endometriosis if a careful bladder examination is performed.

CA-125: cancer antigen-125 or carbohydrate antigen-125

Endometrioma: endometriotic ovarian cyst, or chocolate cyst

Radiological Findings

Economic pressures and perioperative risks associated with surgical staging have stimulated attempts to develop noninvasive tests for endometriosis, but these methods have not replaced surgical verification of lesions. Radiological evaluation by magnetic resonance imaging (MRI) and ultrasonography shows some promise.

Compared with other imaging modalities, MRI is the best technique to identify endometriosis. However, it lacks sufficient sensitivity and specificity, especially in the detection of small implants and adhesions. Overall sensitivity of MRI in the detection of endometrial implants is 77%, with a specificity of 78% (34). MRI identification of endometriotic ovarian cysts (endometriomas, or chocolate cysts) is more satisfactory and has high

PCB: polychlorinated biphenyl

sensitivity (90% to 92%) and specificity (91% to 98%) rates (35). Although endometriomas may mimic neoplasms, they typically demonstrate a characteristic sonographic appearance. Due to ease of use, cost-effectiveness, and accessibility, transvaginal ultrasonography has become the imaging of choice when an endometrioma is suspected (36).

CLINICAL EPIDEMIOLOGY OF ENDOMETRIOSIS-ASSOCIATED PAIN

Advances in understanding the epidemiology of endometriosis have lagged behind other diseases because no noninvasive diagnostic methods are available and hence no large-scale, prospective, observational studies have been undertaken. Endometriosis has been found in women between the ages of 12 and 80; the average age at diagnosis is approximately 28 years (37). It presents clinically during the reproductive years, and its symptoms remain stable or progress in 70% of women (38), slowly regressing spontaneously in the other 30%. The prevalence of endometriosis in reproductive-aged women is widely disparate; estimates range from an approximately 4% occurrence of largely asymptomatic endometriosis found in women undergoing tubal ligation to 50% of teenagers with intractable dysmenorrhea. Women with infertility show a prevalence of 21% to 47% (39).

Investigators have described a variety of personal risk factors for endometriosis such as uterine outflow obstruction, as noted above. Other conditions that predispose women to retrograde menstruation and increased exposure to menstrual flow such as early menarche, reduced parity, longer duration of menstrual periods, and shorter cycle length have been reported (40). The risk appears to decrease with personal habits that relate to decreased estrogen levels (e.g., smoking, exercise). Women with endometriosis tend to be taller and thinner than their unaffected counterparts. Results from a single report associating red hair color with endometriosis risk (41) were not confirmed in a larger study (42). Early reports in the 1970s suggested that endometriosis was most prevalent in educated Caucasian women, but Chatman (43) pointed out that this conclusion was confounded by the skewed access of wealthy or well-insured women to diagnostic laparoscopy in that era. More recently, Sangi-Haghpeykar & Poindexter (44) found that relative to Caucasians, the adjusted odds ratio for endometriosis was 0.7 in African-American women, a difference that failed to reach statistical significance. In a study of more than two million women in Sweden, women born in African countries appeared to be protected against hospitalization for endometriosis compared with women born in Scandinavia and other Western countries (45). However, a questionnaire study of 1,500 women seeking infertility care at Brigham and Women's Hospital in Boston, which yielded a 37% response rate overall, indicated endometriosis in 9% of Caucasians and 12% of African-American women (46).

In severe disease there seems to be a familial correlation; however, no clear Mendelian genetic trait has been identified, and most researchers believe endometriosis to be complex and multifactorial (47, 48). Like women, old-world primates also demonstrate familial aggregation in the development of endometriosis (49). Observations of a high prevalence of endometriosis in rhesus monkey colonies exposed to dioxin, a particularly potent polychlorinated biphenyl (PCB) industrial contaminant, have suggested that this compound may influence the risk of endometriosis in women (50–53). Recent case-control studies revealed increased adjusted odds ratios for endometriosis in women exposed to dioxins and other PCB chemicals. In utero exposure to PCBs induced an endometriosis-like uterine phenotype (e.g., reduced progesterone receptor levels) in female mice of the F1 generation. The use of murine models is an active area of endometriosis investigation and is discussed in more detail below.

MECHANISMS OF ENDOMETRIOSIS-ASSOCIATED PAIN: CONVENTIONAL THEORIES

In advanced stages of endometriosis, in which large endometriotic cysts are present or when distortion of the pelvic anatomy results from extensive scarring and adhesions, symptoms of pelvic pain are readily explicable. However, in cases of minimal or mild endometriosis, in which only scattered, small implants are observed, the mechanisms underlying pain perception remain controversial. The three main hypotheses of pain production that have been proposed are reviewed below.

Cyclical Bleeding Within Lesions

Ectopic endometriotic lesions, because they retain their endocrine responsiveness, undergo episodes of intraperitoneal bleeding during menstruation. This phenomenon is supported by the observation of visible intraperitoneal bleeding when laparoscopy is performed during menses and by the presence of localized hemorrhage and hemosiderin-laden macrophages in lesions examined microscopically. Cyclical bleeding within the implants is believed to result in a chronic inflammatory nidus that causes pelvic pain, and such bleeding may explain why medical therapies such as progestins, danazol, and GnRHa that induce amenorrhea are partially effective in the relief of this symptom.

Inflammation in the Peritoneal Fluid

A key concept that has led to much of our current understanding of endometriosis and pain is that of endometriosis as a peritoneal inflammatory process. It is hypothesized that in situ menstruation within endometriotic lesions elicits a sterile, low-grade inflammatory response within the peritoneal cavity.

Halme et al. (11) first identified peritoneal macrophage activation as a central contributor to the pathogenesis of endometriosis. These macrophages are derived from circulating monocytes that migrate into the peritoneal cavity. In normal women, these cells are usually few in number and remain in a quiescent state. However, they can be actively recruited to sites of inflammation by chemokines and induced to undergo activation by cytokine stimulation or in response to foreign antigens (**Figure 1**). During the activation process, the macrophages secrete a cascade of cytokines, which can contribute to a feed-forward loop, exacerbating local inflammation. Increased concentrations of macrophages and T lymphocytes exist in the peritoneal fluid of endometriosis patients (54), and an increased number of these leukocytes have an activated phenotype.

We were among the first to put forth the hypothesis that monocyte recruitment into the peritoneal cavity of women with endometriosis occurs through the local production of chemokines (13). RANTES was originally identified, but the concentrations of several chemokines are elevated in the pelvic fluid of women with endometriosis (**Table 1**). In turn, the activated leukocytes that infiltrate the peritoneal cavity secrete a rather complex array of cytokines, growth factors, and proinflammatory prostanoids that contribute to the pathophysiology of endometriosis. Prostaglandins offer a particularly plausible mechanism for endometriosis-associated pain. These mediators have intrinsic vasoactive and nociceptive properties and are thought to activate a critical common pathway involved in the symptoms of endometriosis (55). Scholl et al. (56) reported that peritoneal fluid concentrations of tumor necrosis factor (TNF)-α, RANTES, and

Nociception: the neural process of encoding and processing noxious stimuli

Table 1 Chemokines identified in pelvic peritoneal fluid

Chemokine	Reference
Eotaxin	118
Epithelial cell–derived neutrophil–activating peptide (ENA)-78	119
Growth-related protein (GRO)-1α	120
Interferon-γ-induced protein (IP)-10	121
Interleukin (IL)-8	122
Monocyte chemoattractant protein (MCP)-1	123
Regulated on activation T cell expressed and secreted (RANTES) (also known as CCL5)	13

glycodelin were highest in those endometriosis subjects reporting the most severe menstrual pain. Important sequelae of the localized inflammation include neuroangiogenesis at the site of ectopic implants, which is the main thesis of this review and is discussed in more detail below.

Hyperalgesia: increased sensitivity to pain, which may be caused by damage to nociceptors or peripheral nerves

Neurogenesis: the process by which neurons are generated

Irritation and Invasion of Pelvic Nerves

Via secretion of matrix-degrading enzymes (57) and acquired migratory behavior of endometriotic cells, irritation or direct invasion of pelvic floor nerves by infiltrating implants, particularly in the cul-de-sac, is currently the most popular thesis explaining endometriosis-associated pain (58). In 2000 Anaf et al. (59) demonstrated that patients with the highest preoperative pain scores displayed the highest density of nerve encapsulation within endometriotic lesions and more frequent peri- and endoneurial invasion by endometriotic cells than patients with lower preoperative pain scores.

MECHANISMS OF ENDOMETRIOSIS-ASSOCIATED PAIN: NOVEL CONCEPTS

The above hypotheses notwithstanding, there is evidence that other, more elusive mechanisms are likely to contribute to pain associated with endometriosis. The existence of such other mechanisms may explain why, in conventional medical or surgical therapies of endometriosis, pain relief for more than 6 months can be expected in only 40% to 70% of the affected women (60). Furthermore, there is poor correlation between the extent or morphological characteristics of the disease and the intensity and character of the pain symptoms; widespread endometriosis can be found in largely asymptomatic women, whereas small amounts of endometriosis appear to cause intractable pelvic pain in others. In general, the pains of endometriosis are at least partially responsive to therapies that suppress estrogen production. However, the hormonal mechanisms that maintain the ectopic growths and how these might promote pain symptoms are poorly understood. Recent studies in women and animal models suggest that estrogen action exacerbates pain sensitivity by stimulating the growth of a nerve supply (neurogenesis), in parallel with the growth of new blood vessels (angiogenesis) into the ectopic endometrial tissue. In the following section, we develop two novel concepts based on the recent literature that may contribute to endometriosis-associated pain: neuroangiogenesis and neuropathic hyperalgesia.

Neuroangiogenesis

In any transplanted tissue or tumor metastasis, the development of a new blood supply is critical for the survival of the explants (61). This scenario has also been postulated to hold true for shed endometrial fragments that implant on the peritoneum or in the rectovaginal septum (62). The endometrium has intrinsic angiogenic potential, and endometriotic lesions tend to grow in areas with rich vascularization, suggesting that angiogenesis is a prerequisite for endometriosis development (63). Indeed, increased vasculature is observed clinically around implants, often manifested in a radial or circumferential pattern; this feature can be an indication of the presence of endometriosis, even when the implant itself is not obvious, as in puckered peritoneal lesions. This pattern is seen in particular in the subgroup of patients with deeply infiltrative (subperitoneal) endometriosis, in which the histology shows a dramatic increase in microvascular density that is highly correlated with angiogenic growth factor expression (64). **Table 2** summarizes the findings of a number of studies and their references that characterized different angiogenic proteins in the peritoneal fluid of women with endometriosis. Angiogenesis is important not only for implant establishment but also for supporting ongoing lesion growth and progression, as several rodent models of endometriosis have shown. Lesions were initially established in the

animals, and subsequently angiostatic treatments were administered. These drugs almost uniformly reduced the surface area or volume of the preexisting lesions (63, 65, 66), indicating that after implantation of ectopic endometrial implants a continuous angiogenic process is required for survival of the lesion. In one model, Dinulescu et al. (67) induced oncogenic K-*ras* expression in conditional transgenic mice carrying a transcriptionally silenced K-*ras* allele via direct injection of adenovirus Cre recombinase into the ovarian bursa. Local activation of the K-*ras* oncogene induced ovarian and peritoneal lesions that resembled endometriosis. When the same model was applied in mice carrying floxed alleles of the *Pten* tumor suppressor gene, invasive endometrioid ovarian carcinomas that were histologically similar to the endometriosis-associated ovarian cancers observed rarely in women were induced (68).

Recent elucidation of developmental mechanisms of embryonic neurovascular patterning provides substantial evidence for a direct link between neurogenesis and angiogenesis (69, 70). Anatomically, peripheral nerves have long been known to track alongside blood vessels in discrete neurovascular bundles. But it is now known that the patterning and branching of vessels and nerves are more molecularly linked than anticipated, with signals from each influencing the migration of the other. During zebra fish development, nascent arteries and veins grow posteriorly from the dorsal aorta and posterior cardinal vein, respectively, in response to gradients of angiogenic factors (**Figure 2*a***). As a result, intersomitic arteries and veins are formed (**Figure 2*b–d***). Administration of morpholinos that inhibit semaphorin receptor production disrupts anteroposterior boundaries, resulting in misguided vessels (**Figure 2*e***). Similarly, expression of netrin along the neural tube–notochord boundary is thought to coordinate the growth of interconnecting parachordal and ventral vessels longitudinally with axonal migration (**Figure 2*f*,*g***).

Ligand-receptor pairs implicated in axonal and vessel guidance include ephrins and their Eph receptors, slit ligands and their roundabout (Robo) receptors, semaphorins and their plexin and neuropilin receptors, and netrins and their DCC/neogenin and Unc5 receptors. Ephrins and Ephs form a large family of transmembrane and membrane-associated proteins capable of bidirectional signaling. Eph-ephrin signaling directs topographical axonal projection in the CNS. We observed that ephrin A1 mRNA transcripts were reduced in cDNA microarray studies of matched endometrium of women with endometriosis compared with patients with no evidence of disease, but differences were not noted in follow-up reverse transcription polymerase chain reaction (RT-PCR) analyses (71). Slits are secreted proteins that act primarily as neuronal repellents, preventing ipsilateral axon crossing. In a recent publication, Shen et al. (72) performed an immunohistochemical survey of ovarian endometriosis cases and noted higher expression of Slit and Robo1 proteins, as well as increased microvascular density, in cases of endometrioma recurrence. As summarized in **Figure 2**, semaphorins consist of seven structurally diverse families of membrane-associated and secreted ligands that act as short-range cues to direct axons and vessels through specific corridors. By gene expression microarrays and as confirmed by RT-PCR, we found that semaphorin E (now referred to as semaphorin 3c) is an upregulated transcript in the endometrium of women with endometriosis (71). Class 3 semaphorins bind to neuropilin-plexin receptor complexes on the axonal surface membranes of neurons and endothelial cells and stimulate cell migration. The neuropilins, first described as semaphorin receptors

Table 2 Angiogenic factors identified in pelvic peritoneal fluid

Factor(s)	Reference
Glycodelin	124
Insulin-like growth factors	125
Interleukin (IL)-6	126
IL-8	122
Leptin	127
Transforming growth factor (TGF)-β	128
Vascular endothelial growth factor (VEGF)	62

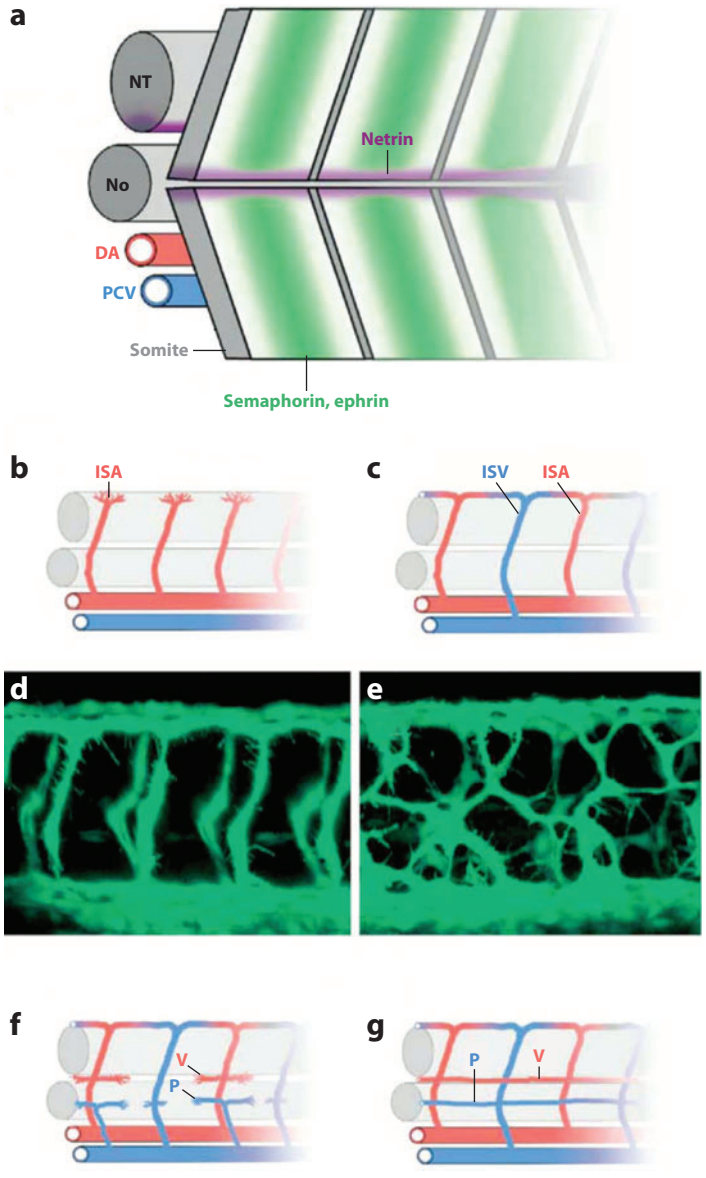

Figure 2

Coordination of neuroangiogenesis. Angiogenic factors (*a*) stimulate the dorsal aorta (DA) and posterior cardinal vein (PCV) to bud off intersomitic arteries (ISA) (*b*) and intersomitic veins (ISV) (*c*), respectively. Normal anteroposterior vessel growth (*d*) can be disrupted by administration of morpholinos that inhibit semaphorin receptor production at the somite boundaries, resulting in misguided vessels (*e*). Similarly, expression of netrin along the neural tube (NT)-notochord (No) boundary (*a*) is thought to coordinate the growth of interconnecting parachordal (P) and ventral (V) vessels longitudinally with axonal migration (*f*,*g*). Reproduced with permission from Weinstein (70).

responsible for neuronal guidance, also have nanomolar affinities for vascular endothelial growth factor (VEGF) isoforms, particularly the predominant $VEGF_{165}$ isoform, and can mediate angiogenesis (73). Netrins are secreted proteins that can have either attractive or repulsive activity to steer neurons toward their final targets. Although the expression or action of netrin has not been reported in endometriosis, its activity is consistent with the increased neuroangiogenic phenotype hypothesized in endometriosis. Knockout mice with selective deletion of axonal and vessel guidance ligand-receptor pairs may be attractive new models to test this hypothesis.

Other factors may coordinate the close physical association between larger nerves and vessels. Nerve-derived VEGF is essential for the formation of arteries in skin (74). In vivo, limb skin arteries closely coalign with peripheral nerves, and in the mouse, loss of nerve or Schwann cells prevents proper arteriogenesis. Secretoneurin, a neuropeptide expressed in nerve fibers that are found in close apposition to blood vessels, stimulates endothelial cell migration and angiogenesis in in vitro and in vivo assays (75). This protein has been identified in capsaicin-sensitive C-afferent nerve fibers in rat endometrium (76), although loss-of-function studies are not yet available to definitively assign its role. Conversely, vessels also provide cues for the growth and alignment of adjacent nerves. The blood vessel–derived artemin protein, a member of the glial cell–derived neurotrophic factor (GDNF) family of ligands, acts as a guidance factor for the growth of sympathetic nerve fibers along blood vessels in a variety of mouse tissues, including the gastrointestinal tract (77). Knockouts of artemin or its preferred receptor GFRα cause severe defects in the migration and axonal projection of sympathetic neurons but not other types of neurons. Although artemin expression in endometriosis is unknown, increased artemin expression in endometrial carcinoma was recently reported; in the endometrium, artemin appears to increase cancer cell migration and invasiveness (78). Ciliary neurotrophic factor (CNTF)

concentrations were quantified in the peritoneal fluid of women with and without endometriosis. CNTF was detectable in 43% of the subjects tested, but no differences in levels were noted between the two groups (79).

Several researchers have identified nerve fibers in endometriotic lesions (59, 80) and more recently in the eutopic endometrium of women with endometriosis (81, 82). Berkley et al. (83, 84) reported that ectopic endometriotic implants developed a sensory and sympathetic nerve supply both in rats and in women. These are generally characterized as sensory Aδ, sensory C, adrenergic, and cholinergic fibers. Pain-conducting substance-P-positive nerves were directly colocalized with endometriotic lesions in 74.5% of all cases (85), and a correlation between nerve fiber density in endometriotic lesions and pain severity was demonstrated. Moreover, hormonal therapies widely used to treat endometriosis may work by reducing nerve fiber density (86, 87). These results support the hypothesis that the observed nerve fibers play a causative role in the etiology of endometriosis-associated pelvic pain.

Nerve growth factor, basic fibroblast growth factor (FGF), brain-derived neurotrophic factor (BDNF), and neurotrophin-3 are all expressed by endometrial cells. However, only the latter two proteins appear to be quantitatively upregulated in women with endometriosis (88). Growth-associated protein 43, a marker of neural outgrowth and regeneration, is expressed in endometriosis-associated nerve fibers but not in existing peritoneal nerves. The fibers appear to sprout from para- and perivascular nerve fibers that accompany the blood vessels as they vascularize the ectopic growths (85).

Neuropathic Hyperalgesia: Centrally Mediated Pain

The small, unmyelinated nerve fibers observed in the functional layer of the endometrium of women with endometriosis (81) have been identified as nociceptive in experimental and clinical studies (83, 89, 90), and similar nerves have been noted in ectopic endometriotic lesions (81, 84, 91). It is believed that this nascent endometriosis-associated neural system has a widespread influence on the activity of neurons in the CNS and hence on pain perception in the patient. Nociceptors on these neurons transmit noxious stimuli and propagate these messages to the CNS. In the case of neuropathic pain, tactile afferents acquire synapses within the CNS, which enable the afferents to trigger central pain activity. Sympathetic (as well as classical sensory) nerve fibers contribute to hyperalgesia (92), and the former are rich in the peritoneum of women with endometriosis. Persistent nociceptive input from endometriotic lesions is postulated to lead to central sensitization via increased responsiveness of spinal cord dorsal horn neurons processing input from the implants and affected adjacent viscera. Increased excitability of viscero-visceral convergent neurons to the spinal cord has been associated with persistent neuropathic pain and hyperalgesia in this setting (93).

The concept of neuropathic pain has been invoked in the past decade to explain frequent and often severe forms of chronic pain. Whereas classic nociceptive pain originates from peripheral tissues, neuropathic pain is modulated via effects within the CNS and may be central or peripheral. Central neuropathic pain is found in spinal cord injury, multiple sclerosis, fibromyalgia, and some strokes. The common causes of painful peripheral neuropathies include autoimmune diseases (e.g., multiple sclerosis), metabolic diseases (e.g., diabetic neuropathy), infection (e.g., shingles and the sequel, postherpetic neuralgia), HIV-related neuropathies, nutritional deficiencies, toxins, remote manifestations of malignancies, and genetic and immune-mediated disorders. The perpetrating lesion is almost always associated with the nociceptive pathways and subsequently involves CNS neurons. Central sensitization in dorsal horn neurons can be induced by an increase in excitatory synaptic transmission, mediated via the glutamate *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) receptors, or by a loss of inhibitory

VEGF: vascular endothelial growth factor

BDNF: brain-derived neurotrophic factor

Neuropathic pain: a type of pain caused by damage to or dysfunction of the nervous system that cannot be explained by a single specific location of damage

GABA: γ-aminobutyric acid

Photosensitization: the process of transferring the energy of absorbed light

synaptic transmission (disinhibition), mediated via γ-aminobutyric acid (GABA) and glycine receptors (94). Such sensitization is particularly important for the persistence of neuropathic pain and spread of pain beyond the initial injury site (95). Prevailing work has focused on the dorsal horn as the site for this sensitization, but more rostral pathways may be involved as well.

Neuropathic pain is usually accompanied by physical nerve injury or invasion of cells into the nerve fiber; however, it can also be induced by inflammatory stimuli in the absence of de facto injury (96). Several lines of evidence indicate that immune mechanisms are involved both peripherally and centrally, including cytokine activation of microglial cells in the dorsal horn. Moreover, activated immune cells in the peritoneum may contribute directly to neuropathic hyperalgesia by releasing histamine, tryptase (97), TNF-α (98), prostaglandins (99), serotonin, and interleukin-1β, which can sensitize the endometriosis-associated nerve fibers and transmit sharp localized pain to the CNS.

TREATMENT OF ENDOMETRIOSIS-ASSOCIATED PAIN

The optimal treatment of endometriosis-associated pain remains unknown, and the efficacy of various medical and surgical options continues to be a source of ongoing controversy. Complete eradication of endometriosis symptoms is rarely possible, even following surgical excision. Current therapy aims to reduce the pain and to delay recurrence for as long as possible. Typically, the disease recurs after cessation of treatment, underlining the importance of developing new treatment strategies.

Surgical Therapy for Endometriosis-Associated Pain

Due to their operatively accessible, superficial peritoneal location, most endometriotic lesions are amenable to laparoscopic extirpation via implant excision, electrosurgical coagulation, or laser vaporization. It is rare for laparotomy or hysterectomy to be necessary as a treatment modality for women who wish to preserve their uterus, except for cases of severe, advanced stages of disease or cases involving coexisting malignancy.

Two randomized trials have substantiated the role of laser laparoscopy in the management of endometriosis pain. Sutton and colleagues (100) in the early 1990s conducted a randomized, prospective, double-blind study of 63 patients with minimal to moderate endometriosis. Half of the subjects underwent laser ablation of endometriotic deposits and laparoscopic uterine nerve ablation (interruption of the uterosacral ligament and associated pelvic nerves), whereas the others received a diagnostic laparoscopy and expectant management without tissue destruction. Pain symptoms quantified by a visual analog scale were significantly improved in 62.5% of those receiving laser ablation, compared with 22.6% in the expectant group at 6 months after surgery. In a long-term follow-up study, approximately 72 months after the original surgery, continued symptom relief was reported in 55.3% of the respondents in the laser-treated cohort; the median time to pain recurrence after surgical treatment was 20 months.

Abbott et al. (101) reported an 80% success rate in pain relief after surgical excision of endometriotic implants compared with an only 30% response to sham surgery. The effects were independent of disease extent. Future innovations in endoscopic surgery for endometriosis may include targeted energy absorption such as photosensitization of the lesions using hematoporphyrin derivatives (102) or autofluorescence to detect more subtle nonpigmented endometriotic lesions (103).

Ovarian endometriomas respond very poorly to medical therapy, and hence operative extirpation is the preferred approach. According to a recent Cochrane review (104), excision was better than drainage and ablation of the cyst wall epithelium with respect to resolution of the mass, recurrence of pain symptoms, and improved fertility outcome. As noted above, although rare, definitive surgical treatment of

endometriosis consists of hysterectomy with the removal of both ovaries. This commonly leads to the dilemma of postoperative hormonal therapy (to correct hypoestrogenemia) versus the risk of exacerbating residual endometriosis. The recurrence rate with hormonal therapy is 3.5%, or approximately 0.9% per year, and there is no greater risk of recurrence of pain if estrogen is started immediately after surgery compared with a 6-month delay (105).

Medical Therapy for Endometriosis-Associated Pain

Evidence-based assessments of endometriosis-associated pelvic pain in two randomized, placebo-controlled trials indicated that treatment with medroxyprogesterone acetate, danazol, or GnRHa was more effective than placebo. Pain relief of more than 6 months' duration was noted in 40% to 70% of women (60).

Although useful in the management of chronic pain with endometriosis, GnRHa treatment has been limited to 6 months by the United States Food and Drug Administration due to hypoestrogenic side effects induced by ovarian suppression. Side effects resulting from an inhibition of the HPO axis by GnRHa can now be ameliorated with add-back of exogenous steroids. Dose-seeking trials have identified low-dose regimens of norethindrone acetate combined with estrogen that reverse vasomotor symptoms and bone mineral loss. These add-back strategies can safely extend pain relief and bone preservation for at least 1 year, and one trial with a limited number of participants found no ill effects, even after 10 years of therapy (106). Two randomized, clinical trials (107, 108) demonstrated that 6 months of subcutaneous progestin were as effective as GnRHa in diminishing endometriosis-associated pain. Subcutaneous depot medroxyprogesterone has the benefits of decreased cost, easier administration, and a more favorable effect on bone mineral density.

According to a recent Cochrane review (109), the effectiveness of nonsteroidal anti-inflammatory drugs (NSAIDs) (e.g., naproxen) in managing pain caused by endometriosis is inconclusive, and there was no evidence that a particular NSAID class was more effective than any other.

NSAID: nonsteroidal anti-inflammatory drug

Experimental Therapeutic Options

In the past few years, as our understanding of the pathophysiology of endometriosis at the cellular and molecular levels has improved, there has been a corresponding evolution of new medical strategies with theoretical promise for the treatment of endometriosis-associated pain. New pharmaceutical agents affecting estrogen production, inflammation, immune response, angiogenesis, and matrix metalloproteinase activity are being used or investigated to prevent or inhibit the development of endometriosis.

Through their effects on estrogen production and action, aromatase inhibitors are being utilized in endometriosis (110). Preliminary clinical studies have demonstrated the efficacy of third-generation nonsteroidal aromatase inhibitors (e.g., anastrozole and letrozole) in reducing the intensity of pain symptoms associated with the presence of endometriosis. However, because of their ability to stimulate gonadotropin secretion and ovarian cyst formation in cycling women, these drugs need to be administered with oral contraceptive pills. Acceptance of such combination therapy awaits larger clinical trials.

Increased concentrations of activated pelvic macrophages and lymphocytes, and concurrently elevated levels of specific cytokines and growth factors, support the hypothesis that the immune response is activated in endometriosis. It is not known at this time whether components of the innate and adaptive immune systems play primary, causative roles or merely reflect a reaction to the presence of ectopic implants. Therapeutic manipulation of the immune system has shown promise in rodent and baboon models of endometriosis using agents such as rosiglitazone (111), imiquimod

(a Toll-like receptor agonist), recombinant human interferon-α-2b, leflunomide, levamisole (112–114), recombinant TNF-binding protein-1, or anti-TNF-α monoclonal antibodies (115, 116).

Antiangiogenic treatments aimed at inhibiting new vessel formation also have proven efficacy in experimental endometriosis models (117). However, as many endometriosis sufferers seek fertility correction, an important concern remains the risk of teratogenic effects associated with antiangiogenic therapies in the case of pregnancy. These agents may be most useful in women not interested in fertility.

It is yet to be determined through rigorous clinical trials if these new therapeutic agents are superior to current standard medical treatments for endometriosis pain. It is our opinion that future endometriosis therapeutics should ideally spare the function of a woman's eutopic endometrium and her reproductive cycle and be compatible with pregnancy. The identification of relatively specific targets within pathways that influence the activity of neurons in the CNS and hence pain perception in the patient should be aggressively explored to develop drugs that inhibit endometriosis-associated neural hyperexcitability. Tricyclic antidepressants, dual-reuptake inhibitors of serotonin and norepinephrine, calcium channel α(2)-δ ligands (e.g., gabapentin and pregabalin), and NMDA receptor antagonists that have been designed to target neurotransmission may prove useful in treating endometriosis pain. Another strategy is to target neurotrophic factors or those that guide neuroangiogenesis.

CONCLUSIONS

Endometriosis is a common gynecological disease resulting from implantation of exfoliated menstrual endometrial tissue in locations outside of the uterus. The dominant symptom of this condition is pelvic pain, but the precise mechanisms that link the presence of lesions and pain perception remain enigmatic.

A complex network of locally produced steroids, cytokines, autacoids, and possibly environmental toxins induces endometriotic implant establishment and growth via angiogenesis. Microvascular density and functional blood flow appear to correlate directly with pain symptoms, particularly in ovarian endometriomas and deeply invasive rectovaginal disease. New therapeutic strategies that target VEGF block the establishment or progressive growth and invasion of endometriotic lesions in animal models, but the teratogenic actions of antiangiogenic drugs make them potentially risky in affected women who desire fertility.

Medical therapeutics directed toward the HPO axis and cyclooxygenase, as well as surgical ablation of ectopic implants, are likely to remain therapeutic standards for the treatment of endometriosis-associated pain. In this review we provide an integrated view of neurovascular development within the nascent endometriotic lesion as a novel target for new therapies to control and eliminate endometriosis-associated pain. It is our hope and expectation that a deeper understanding of the regulation of neuroangiogenesis in these lesions will inform future models of endometriosis pathogenesis, drug discovery, and clinical trials for this common and debilitating gynecological malady.

SUMMARY POINTS

1. Endometriosis is a common gynecological disease resulting from implantation of exfoliated menstrual endometrial tissue in locations outside of the uterus.
2. The dominant symptom of this condition is pelvic pain, but the precise mechanisms that link the presence of lesions and pain perception remain enigmatic.

3. The current therapy is surgical ablation of ectopic implants and hormones that block the hypothalamic-pituitary-ovary axis, but these approaches are expensive, carry perioperative risks, or have unpleasant side effects of hypoestrogenism.
4. Our strategy is to understand other biological mechanisms mediating pain in this disorder so that we can tailor future therapies to more specific nonhormonal targets.
5. Recent evidence indicates that ectopic endometriotic implants recruit their own unique neural and vascular supplies through neuroangiogenesis.
6. It is believed that nascent nerve fibers in endometriosis implants influence dorsal root neurons within the central nervous system, increasing pain perception.
7. We provide an integrated view of neurovascular development within the nascent endometriotic lesion as a novel target for new therapies to control and eliminate endometriosis-associated pain.
8. It is our hope and expectation that a deeper understanding of the regulation of neuroangiogenesis in these lesions will inform future models of endometriosis pathogenesis, drug discovery, and clinical trials for this common and debilitating gynecological malady.

DISCLOSURE STATEMENT

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