

Endometriosis: abnormal endometrium and dysfunctional immune response

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This review highlights recent studies that illuminate the role of the immune system in endometriosis. The findings are discussed in the framework of a model which proposes that endometriosis reflects an immunological selection process. Endometrial cells, which are inherently resistant to apoptosis and immune-mediated elimination, acquire the capacity to utilize the products of an activated immune system to establish ectopic foci of disease. Cyclical inflammatory/immune cell stimulation that fails to eliminate ectopic endometrial implants results in progressive immunological derangement and associated pathophysiological changes which are characteristic of the disease. *Curr Opin Obstet Gynecol* 10:365-369. © 1998 Lippincott Williams & Wilkins.

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Abbreviation

IL interleukin

Introduction

Immune changes in endometriosis are widely documented. What remains controversial is whether these changes are a cause or an effect of the disease. This issue is reminiscent of early theories of cancer development in which it was suggested that immune suppression predisposes the host to neoplasia. However, just as immunosuppression in the absence of genetic changes in host cells does not produce cancer, it is also likely that endometriosis is fundamentally a reflection of a genetic alteration in endometrial cells. While the immune system plays a selective role in determining the survival of individually distinct endometrial cell subtypes (reminiscent of Burnet's clonal selection theory [1]), endometriosis is probably best viewed as arising from abnormal endometrial cells, which chronically stimulate the immune system, leading to its dysregulation, and ultimately to the spectrum of physiological changes in reproductive performance and constitutional symptoms which are characteristic of the disease. This is why distinctions between immune changes that are associated with etiology and changes associated with pathophysiology are somewhat arbitrary. Nevertheless, it appears that much of the symptomatology, as well perhaps as the extent of the disease and its impact on reproduction, might be amenable to control through immune manipulation.

Evidence that endometrial cells from women with endometriosis are fundamentally different from normal endometria

Even though the nature of the genetic changes that are responsible for the potential to develop endometriosis are not known at present, there is ample evidence to demonstrate that endometrial cells from women with endometriosis are functionally distinct from endometrial cells of normal women. We hypothesize that these changes, similar to changes in oncogenes, confer on cells the capacity to behave abnormally in terms of their ability to grow in ectopic locations and outside the constraints of normal physiological controls which are responsible for maintaining peritoneal homeostasis. Nevertheless, we also speculate that these changes in themselves do not guarantee that endometriosis will develop. Rather, we believe that the functional state of the immune system is a major determinant of whether endometriosis develops. Indeed, the capacity of endometrial cells to survive and eventually thrive in the face of an ongoing immunological response may be the single most important factor in determining whether endometrial cells that have the capacity to grow in ectopic locations are permitted to do so.

Recent studies have greatly expanded our appreciation for the differences that exist between eutopic tissues of fertile control individuals and women with endometriosis, as well as the more subtle differences that exist between eutopic endometrium and ectopic endometrium from women with endometriosis. Thus, the cyclic expression of estrogen receptor and progesterone receptor in eutopic endometrium of normal women is prolonged and distorted in eutopic endometrium of patients, and may be constitutively elevated throughout the menstrual cycle in the stroma of implants [2,3], presumably conferring a growth advantage on that subset of cells. This cellular change may occur in concert with additional factors that favor ectopic growth of endometrium, including the selective expression of aromatase, an enzyme that catalyzes the conversion of C19 steroids to estrogens, in stromal cells from eutopic endometrium and implants from women with endometriosis. It is relevant that this enzyme is not seen in endometrium from normal women and can be upregulated by prostaglandin E₂ [4]. Thus, it is possible that endometrium from women with endometriosis has the capacity to provide its own estrogen growth factors locally.

An enhanced capacity of endometrium to proliferate in response to ovarian hormones would not be sufficient to explain the development and persistence of endometriosis. Other intrinsic endometrial cell factors which potentially favor persistence and establishment of ectopic disease that have been reported recently include: (1) an increased expression of insulin growth factor-I in ectopic endometriotic implants, which may, in theory, contribute to the proliferation of fibroblasts in adhesions [5]; (2) an increased expression of fibronectin receptors ($\alpha 4\beta 1$ and $\alpha 5\beta 1$) in glandular cells from endometriotic lesions, which may favor attachment and persistence of endometrium in ectopic sites [6]; (3) increased expression of the hepatocyte growth factor by stromal cells of eutopic endometrium from women with endometriosis, which may directly contribute to endometrial cell proliferation [7*]; and (4) persistent expression of B-cell lymphoma-2 (BCL-2) protein in ectopic endometrium throughout the menstrual cycle [8], which might explain our recent demonstration that eutopic endometrium from women with endometriosis is more resistant to spontaneous apoptosis than eutopic endometrium from normal women [9]. In that study, it was also shown that ectopic endometrial cells are more resistant to spontaneous apoptosis than the matched eutopic endometrium.

Recent studies that illustrate exploitation of the immune response in the etiology and pathophysiology of endometriosis

None of the aforementioned intrinsic endometrial cell factors would be meaningful if the cells destined to give rise to endometriosis were not able to survive and thrive in the presence of an ongoing immunological response. Although several decades of investigation have documented immunological changes in women with endometriosis, the

more recent studies are notable because of their attempt to address the causative relationships between a specific immunological event and the etiology or pathophysiology of the disease. They provide evidence to suggest that the presence of ectopic endometrial cells within the peritoneal cavity leads to substantial changes in the immunological milieu present within the cavity.

These studies extend and clarify the observation that the relative and absolute numbers of immune cell subtypes and their activational status are altered in women with endometriosis. One of the more interesting new findings is that the T cell impairment that has been described in women with endometriosis [10] is caused primarily by reduction in the CD4 cell subset [11,12]. Based on studies from Ho *et al.* [11,12], it appears that peritoneal CD4⁺ T cells in women with endometriosis are predominantly of the type 1 subset. That is, they produce type 1 cytokines such as gamma interferon or interleukin (IL)-2 to a substantially greater extent than their production of the type 2 cytokines, such as IL-4 or IL-10. The balance between type 1 and type 2 cytokines is thought to determine the magnitude and direction of the immune response in terms of cell-mediated immunity (principally a type 1 phenomenon) or humoral immunity (primarily a type 2 phenomenon). However, these type 1 CD4 cells appear to be suppressed based on a reduced expression of HLA-DR antigen and reduced capacity to synthesize IL-2. Interestingly, in a study by Martinez-Roman *et al.* [10], the reductions in T cell percentages were predominantly seen in endometriosis patients with infertility but not in fertile individuals with endometriosis. This reduction could, in theory, reflect localization of T cells to sites of ectopic disease, as was shown in a study by Chiang and Hill [13]. In that study, CD3⁺ cells were found to aggregate around IFN- γ ⁺ and HLA-DR⁺ cells in glandular epithelium, suggesting major histocompatibility complex class II-restricted antigen specific recognition of epitopes on ectopic endometrial cells. Because recognition by the CD4 subset of T cells is specific for antigens expressed in the context of the major histocompatibility complex class II molecules (HLA-DR in humans) this finding could also explain why the reduction in peritoneal T cell counts observed in women with endometriosis are attributed primarily to the CD4 subset. That these cells may be comprised, at least in part, by the $\gamma\delta$ subset of T cells is suggested by the studies of Ota *et al.* [14]. Although generally regarded as a minor subset of T cells in the circulation, $\gamma\delta$ T cells (so named because of the genes used to form the T cell receptor) were found in significant numbers in endometrial stroma [15]. Ota *et al.* found that expression of the heat shock protein hsp 27 is increased in eutopic endometrium of women with endometriosis [14]. They suggest that this protein may play a role either as a ligand for the $\gamma\delta$ T cells in this site, or in antigen processing and expression within the endometrial tissues of women with endometriosis. Not surprisingly, and in agreement with other studies, recent studies [11] also

found that natural killer cell numbers are reduced while B cell numbers are normal in peritoneal fluids of women with endometriosis.

The decline in T cells and natural killer cells in endometriosis occurs coincident with an increase in macrophage numbers and activation status. An increase in peritoneal fluid volume, macrophage content and macrophage activation is generally considered to be a hallmark of this disease. Several recent studies confirm this observation and include additional information regarding the phenotypic and functional characteristics of these activated peritoneal macrophages that may be directly relevant to the etiology and pathophysiology of endometriosis. Thus, a recent study by McLaren *et al.* [16**] in endometriosis revealed that the peritoneal macrophages have increased expression of the B-cell lymphoma-2 gene, a regulatory gene that is positively associated with resistance to apoptosis. Because B-cell lymphoma-2 activity can prolong the survival of cells even in the absence of growth factors, this finding helps to explain, in part, the presence of increased numbers of peritoneal macrophages in endometriosis. Similarly, the relevance of macrophage activation was illustrated in recent investigations by Martinez-Roman *et al.* [17] that characterized the expression of the transferrin receptor (CD71) as a marker of activation on peritoneal macrophages. The results demonstrate increased CD71 expression on peritoneal macrophages from infertile women with endometriosis. Significantly, CD71 expression was not increased in either fertile women with endometriosis or infertile women without endometriosis, suggesting a direct association between macrophage activation and endometriosis-related subfertility.

Perhaps the greatest number of recent immunological studies in women with endometriosis have been concerned with cytokines. These molecules, which are the principal mediators of immunological reactions, have pleiotropic effects on multiple physiological reactions. Not surprisingly, recent studies confirmed that the levels of many different cytokines are abnormal in women with endometriosis. Cytokines with chemotactic activity for leukocytes such as IL-8 [18,19], RANTES (regulated upon activation: normal T cell expressed/secreted) [20*], monocyte chemoattractant protein-1 [21*], and an uncharacterized, protease-sensitive chemotactic protein [22] were found to be increased, providing additional explanations for why peritoneal leukocytes are increased in number in women with endometriosis. In some cases, these chemotactic molecules were correlated with increased biosynthesis by leukocytes in the peritoneal cavity or in the blood [18,19], while in other cases, these activities were produced by endometrial tissues [20*,22] or mesothelial cells [21*]. Some of these studies also demonstrated a correlation between the level of chemotactic activity and the severity of the endometriosis. It is significant that a number of these studies showed that other cytokines such as TNF α or IL-1 β have the capacity to

increase the synthesis of chemotactic factors from leukocytes or endometrial cells. This is because inflammatory cytokines such as TNF α , IL-1 β and IL-12 were also shown to be elevated in women with endometriosis [11,12,18,19]. The significance of these results as an explanation for some of the pathophysiological features of endometriosis is obvious in terms of adhesion formation, angiogenesis, symptomatology and infertility.

The balance between type 1 (cellular immunity-mediating) and type 2 (humoral immunity-mediating) cytokines also appears to be of interest in women with endometriosis based on recent studies. Of the type 2 cytokines studied to date, the well documented increase in IL-6 in women with endometriosis was shown to be cyclical and sensitive to modulation by TNF α and IL-1 β [23,24], and to be attributable to both increased synthesis by peritoneal macrophages [18] and peritoneal endometriotic tissues [24]. However, other type 2 cytokines were also shown to be elevated in women with endometriosis in recent studies. Thus, levels of IL-4 [25] and IL-10 [12,18] were increased in peritoneal fluid or peritoneal macrophages and mRNA for both IL-6 and IL-10 were detected in ovarian implants [26]. The significance of increased IL-4 as a negative regulator of cellular immunity in the peritoneal cavity was inferred by showing a negative correlation between IL-4 levels and gamma interferon, a potent type 1 cytokine with macrophage, T cell and natural killer cell activating properties, which was also correlated in recent studies with increased levels of the heat shock protein hsp60 in peritoneal fluids of women with endometriosis [27]. Similarly, the significance of increased IL-10 was inferred by demonstrating a negative correlation between IL-10 levels and CD4 counts [12]. In contrast, the type 2 cytokine IL-13 which inhibits macrophage activation, inflammatory cytokine synthesis and prostaglandin biosynthesis was reported to be decreased significantly in the peritoneal fluids of endometriosis patients [28]. It appears, therefore, that the balance between type 1 and type 2 cytokines in the peritoneal cavity and systemically has a significant impact on the pathophysiology of endometriosis as well as its severity.

While changes in cytokine levels and other regulatory elements have significant implications for the pathophysiology of endometriosis, the contribution of these changes to the etiology of the disease remains obscure. There are, however, some recent studies that directly illustrate the capacity of endometrial cells to evade immunological surveillance mechanisms. This capacity, coupled with the apparent resistance of subsets of endometrial cells to undergo programmed cell death normally, is perhaps the most important characteristic of those endometrial cells destined to establish ectopic sites of disease. It is significant that endometrial cells from women with endometriosis were recently shown to be capable of subverting the actions of both natural killer cells, T cells and peritoneal macrophages, the principal effector cells likely to be responsible for the

destruction of ectopic endometrium. Thus, in the study by Somigliana *et al.* [29], conditioned media from endometrial stromal cells were shown to inhibit natural killer-mediated destruction of stromal cell targets with increased inhibitory activity produced by endometrial cultures from endometriosis patients. Similarly, eutopic and ectopic endometrial cells from women with endometriosis were shown to stimulate significantly less autologous lymphocyte proliferation than in control individuals even though nonspecific blastogenic responses to phytohemagglutinin were normal [30]. Finally, the cytolysis of autologous eutopic and ectopic endometrial cells was shown to be inhibited in peritoneal macrophages from women with endometriosis, with the ectopic cells being almost completely resistant to macrophage-mediated destruction [31]. The impairment in cytolysis was attributed to resistance of the ectopic endometrial cells and not to generalized macrophage impairment because the same peritoneal macrophages were able to kill a non-endometrial target cell line normally.

Conclusion

This concise review of recent studies in endometriosis permits a more unified understanding of the impact of immunological changes on the etiology and pathophysiology of the condition. The proposed model suggests that the immune response of women with endometriosis is not intrinsically abnormal. Nevertheless, the immune response can become disturbed both locally and systemically because of altered homeostasis within the peritoneal cavity caused, in part, by the failure of endometrial cells to undergo programmed cell death normally, and a failure of the immune response to eliminate all cells with the potential to establish ectopic foci of disease. A key concept here is that the changes inherent in endometrium from women with endometriosis may permit subsets of cells to utilize the immune response to enhance survival. In the presence of normal endometrial cells, the immune response is expected to maintain normal homeostasis, but in the presence of abnormal endometrial cells, the immune response may select for cells with the capacity to exploit the response and establish ectopic sites of disease. This raises the possibility that endometrial cells, particularly ectopic endometrial cells that have been selected for survival by the immune response, may ultimately acquire the capacity to control the qualitative and quantitative nature of the response, locally and systemically. Clarification of these issues may provide the rationale for immunological intervention as a component of patient management.

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