Endometriosis: A systemic disorder associated with immunological dysfunction

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INTRODUCTION

Since the beginning of the 1980s numerous studies have been published demonstrating alterations in immune function in monkeys as well as in women with endometriosis. Some of these reported changes in humoral, others in cell mediated immunity. However, in spite of the extensive research it is still not clear whether changes in immune function precede the development of endometriosis and are causative or contributory to the pathogenesis of the disease. Alternatively, immune changes may develop as a result of endometriosis, and are simply an epiphenomenon. In support of the former concept are reports indicating that rhesus monkeys exposed to a whole body single dose proton radiation or treated with polychlorinated biphenyls (PCBs) or dioxin, develop an advanced and aggressive form of endometriosis at least twice as frequently as unexposed controls, following a latent period of approximately 7 to 10 years[1,2,3]. Both radiation and organochlorides such as PCBs or dioxin are known to exert an adverse effect on the immune system, and it has been postulated that it is the immunosuppression that contributes to the development of the disease.

The investigators who have studied immune function in endometriosis, and who consider immune changes associated with this disease more than epiphenomena, are divided in their interpretation of the data. Some propose that humoral changes are the main hallmark of the disease, and suggest that endometriosis is a form of an autoimmune disorder[4]. Others postulate that changes in cell mediated immunity are a primary mechanism leading to the development of this disease[5]. Our group subscribes to the latter concept which will be the basis for this presentation.

DEFECTIVE CELL MEDIATED IMMUNITY

The initial studies in rhesus monkeys and in women with endometriosis reported in 1981 and 1984 suggested that peripheral blood lymphocytes are able to recognize autologous endometrial antigens and cells, and respond with increased proliferation and cytotoxic destruction of these cells[6,7]. This effect was decreased in subjects with endometriosis. We proposed then the hypothesis that defective cell mediated immunity and abnormal immune system response to misplaced endometrial cells lead to the ectopic survival of these cells and endometriosis. Fundamental to this hypothesis were observations that endometrial cells are transported through the Fallopian tubes and can be identified in the peritoneal cavity of all menstruating females.

Our initial studies were subsequently confirmed by Oosterlynck *et al*[8] and by other investigators who attributed decreased cell mediated immunity in endometriosis to the decrease in NK cell cytotoxicity. These investigators reported functional alterations and a decreased differentiation of NK-cells in

endometriosis and the presence of factors inhibiting NK-cell function in the sera and peritoneal fluids of affected women. Oosterlynck *et al*[9] attributed the decrease in NK cytotoxicity to TGFβ which is increased in the peritoneal fluid in endometriosis and is produced by PBM/PM, while Vercellini *et al*[10] proposed that decreased NK function is secondary to the decreased b-endorphin secretion by PBM. According to Oosterlynck *et al*[11] *in vitro* treatment with IL-2 restored NK-cell cytotoxicity.

In addition to the functional changes in CTL and NK cells, circulating PBM also appear to be functionally different in endometriosis. They produce higher levels of reactive oxygen metabolites indicating an increase in their activational status, as we demonstrated using chemiluminescence, and they secrete higher levels of TNFα, IL-6 and IL-8 than PBM of healthy women[12,13]. However, the immune cell most extensively studied in endometriosis are peritoneal macrophages (PM). They are increased in number, size and activational status, and release higher levels of C3, C4, TNFα, IL-l, IL-lra, IL-6, IL-8, IL-10, fibronectin, prostaglandins $F_{2\alpha}$ and E_2, lysozomal enzymes, and growth factors such as TGFβ, EGF, MDGF and PDGF. PM from women with endometriosis also demonstrate increased expression of BCL-2 which indicates decreased apoptosis and increased PM survival[14].

ABNORMAL ENDOMETRIAL RESPONSE

Further studies of the immune and ectopic, as well as eutopic endometrial cells and their interactions, have revealed alterations in the endometrial cells in endometriosis and suggested that it is the ectopic endometrium that escapes the immune detection and destruction. Endometrial cells in the ectopic locations produce a variety of immuno-suppressive factors as demonstrated by Wang *et al* in 1987[15] and subsequently Hirata *et al* in 1994[16], and Somigliana *et al* in 1996[17]. Furthermore, the decrease in the lymphocyte proliferation in response to autologous endometrial cells which we demonstrated in 1981[6] may also be a reflection of abnormal endometrial, rather than the immune cell function as recently suggested by Helvacioglu *et al*[18]. Endometrial stromal cells in advanced endometriosis may escape immunosurveillance through increased shedding of ICAM-l adhesion molecules, as suggested by Somigliana *et al*[19]. Endometrial cells also demonstrate a decreased expression of E-cadherins, an invasion suppressing molecule, which may be a mechanism to increase their invasive properties[20]. We have also reported recently a decrease in the cytolysis of ectopic as compared to eutopic endometrial cells by the PBM/PM[21].

Alterations in the endometrial-immune cell interactions may be related to the differences in antigenic/immunogenic properties of the endometrium in endometriosis. Over the years, numerous investigators have reported the presence of unique endometrial antigens of different molecular weights in the endometriotic endometrium, and absence of these antigens in the endometrium of healthy women. These antigens seem to stimulate the immune system to produce abnormal autoantibodies, the presence of which has been reported extensively in women with endometriosis. It may be relevant that increased expression of HLA-DR and upregulation of MHC class l molecules in the ectopic endometrial cells has also been reported[22,23]. Lessey *et al*[24] and others reported aberrant expression of integrins in the endometrium of women with endometriosis, and Isaacson *et al*[25], estradiol independent C3 and C4 secretion. Furthermore, endometrium in endometriosis secretes a variety of proteins, qualitatively and quantitatively different than normal endometrium[26,27].

Moreover, the endometrium in ectopic locations seems to acquire a certain growth autonomy. The expression of estradiol and progesterone receptors in the ectopic endometrium is increased with loss of cyclic variability, resulting in the increased ectopic

endometrial cell proliferation without characteristic cyclicity[28,29]. The C-19 aromatase which converts androgens to estrogens and is absent in the normal endometrium is present in eutopic and ectopic endometrial cells of women with endometriosis and is upregulated by PGE_2[30,31]. The growth factors such as EGF, IGF-1, IGF-2, HGF and their corresponding receptors are expressed abnormally in the ectopic endometrial cells and there is aberrant secretion of IL-6, IL-6ra and other growth regulatory cytokines[32–36].

Other studies suggest that the endometrial cells in endometriosis not only modify the immune response, they also seem to subvert that response to their advantage. Several investigators have demonstrated that endometriotic cells produce chemotactic substances for macrophages and lymphocytes, and secrete higher levels of complement factors which are chemotactic for lymphocytes and macrophages[37,38]. This results in a higher number of stromal lymphocytes in endometriosis[39]. We have reported that both eutopic and ectopic endometrial cells in endometriosis respond with increased proliferation in co-culture with PBM or to PBM/PM secretions or TNFα[40–42]. In contrast, eutopic endometrial cells from healthy women decrease proliferation in response to PBM/PM, their secrections or TNFα.

CLINICAL CHANGES

Endometriotic lesions in the peritoneal cavity have a characteristic appearance. However, even more characteristic are peritoneal changes surrounding these lesions. Peritoneal defects, 'peritoneal windows' and adhesions, as well as increased congestion of the peritoneum and dilatation of the peritoneal vessels, have long been recognized as the 'tell-tale' signs of endometriosis in the absence of endometriotic lesions[43]. These 'tell-tale' signs of endometriosis seem to develop as the result of the peritoneal-endometrial-immune cell interactions at the molecular level. In endometriosis, peritoneal mesothelial cells abnormally express ICAM-1 and VCAM-1 adhesion molecules, which are further upregulated by TNFα[44,45]. The endometriotic cells in turn abnormally express cadherins, integrins and ICAM-1 molecules[46]. Ectopic endometrial cells and PM produce TGFβ and IL-1ra, and PM high levels of TNFα[47,48]. Peritoneal macrophages in endometriosis release high levels of fibronectin, and fibronectin receptors are abnormally expressed on endometriotic cells[49]. Mesothelial cells and PM secrete IL-8 which is upregulated by IL-1 and TNFα, and increased levels of VEGF are produced by PM[50]. Interactions between the cells and molecules described above lead to the peritoneal congestion, angiogenesis and adhesion development.

The characteristic chronic pelvic pain symptoms of endometriosis have long been attributed to abnormal prostaglandin production, although abnormal prostaglandin levels have been difficult to demonstrate. Nevertheless, these symptoms improve during treatment with prostaglandin synthetase inhibitors. Abnormal prostaglandin production has been demonstrated in both eutopic and ectopic endometrial cells in endometriosis, but the concentration of prostaglandins in the peritoneal fluid appears to be variable and dependent on a multitude of factors. Interestingly, however, Karck *et al*[51] have demonstrated that PM in endometriosis produce higher levels of PGE_2 and $PGF_{2\alpha}$. It is possible that the same applies to the circulating PBM.

Both PM and PBM in endometriosis produce higher levels of TNFα, IL-6, IL-8, and perhaps other cytokines. It is quite likely that the generalized symptoms of aches and pains, general malaise, low grade fever, nausea, vomiting and diarrhea, frequently experienced by women with endometriosis, and in the past referred to as somatizations of the disease, are caused by abnormal cytokine and prostaglandin secretion, not only by the endometrial, but also by the immune cells.

The peritoneal fluid in endometriosis contains a variety of substances produced by the ectopic endometrial cells as well as the

immune cells, and is known to adversely affect several steps in the reproductive function. Increased sperm phagocytosis, abnormal sperm survival, defective ovum capture by the fimbriae of the Fallopian tubes, abnormal fertilization, and embryotoxic effects of the peritoneal fluid in endometriosis, have all been reported. It is generally agreed that these effects play a role in the decreased reproductive performance associated with endometriosis. Abnormal autoantibodies to endometrial cells, as well as to a variety of cell derived antigens, are present in the circulation, peritoneal, follicular, and other body fluids in endometriosis. The presence of such autoantibodies has been associated with infertility, embryo implantation failure, and increased frequency of spontaneous abortions.

CELL CYCLE-CELL DEATH EVENTS

We have reported recently that programmed cell death or spontaneous apoptosis in the eutopic endometrium is lower in endometriosis than in healthy controls[52]. Furthermore, in women with endometriosis, spontaneous apoptosis is lower in matched ectopic than eutopic endometria. These changes overshadow cycle related variability and confirm prior results by Watanabe *et al*[53] indicating persistent expression of BCL-2 in the ectopic endometrium during the menstrual cycle.

It is well-recognized that in healthy women there is an increase in endometrial cell apoptosis at the end of the cycle[54]. It is quite likely that PBM/PM, PBM/PM secretions or TNFα suppress endometrial cell proliferation and signal apoptosis in the healthy endometrium[54]. In endometriosis, it appears that PBM/PM, their secretions and TNFα stimulate endometrial cell proliferation rather than apoptosis. Furthermore, PM are less effective than PBM in both eutopic and ectopic endometrial cell lysis and ectopic endometrial cells are more resistant than eutopic to both PBM and PM mediated cell lysis[21].

TNFα is a major secretory product of PBM and PM, and its secretion is significantly upregulated in endometriosis. It has been demonstrated previously that TNFα, after binding to a cell membrane receptor, activates the sphingomyelin pathway leading to the hydrolysis of sphingomyelin by sphingomyelinase and the generation of ceramide[55]. Ceramide, a second messenger, can activate, in the same cells, either proliferation/inflammation or apoptosis pathways. Considering a divergent effect of TNFα on the endometrial cells in women with and without endometriosis, one can speculate that in endometriosis activation of the proliferation/inflammation pathway allows survival of the misplaced endometrial cells, while in the normal women activation of the apoptosis pathway leads to the endometrial cell death regardless of their location.

CONCLUSION/SPECULATION

The data reviewed above indicate profound changes in the immune and endometrial cell functions in endometriosis and dysregulated immune-endometrial cell communications. It is possible that dysregulated immune-endometrial cell communications and abnormal signal transduction in the sphingomyelin and other cell cycle/cell death pathways of the endometrial cells may be the cause of endometriosis.

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