Dendritic cell populations in the eutopic and ectopic endometrium of women with endometriosis

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BACKGROUND: Immune alterations may be involved in the pathogenesis and progression of endometriosis. Dendritic cells (DCs) are potent antigen presenting cells that are highly involved in the initiation of the immune response. The aim of this study was to investigate DC populations in the eutopic and ectopic endometrium of women with endometriosis compared with controls.

METHODS: Hysterectomy samples were obtained from premenopausal women with (n = 33) and without (n = 28) endometriosis. In addition, paired peritoneal endometriotic lesions and uterine curettings were collected from 32 women with endometriosis. Specimen sections were stained immunohistochemically using antibodies for monoclonal mouse antibodies directed against human CD1a and CD83, which are specific for immature and mature DCs, respectively.

RESULTS: The mean density of endometrial CD1a+ DCs in the basal layer was significantly increased in women with endometriosis compared with controls during the proliferative phase only (P = 0.001). There was a highly significant decrease in the density of endometrial CD83+ DCs in women with endometriosis compared with controls in both layers of the endometrium across all phases of the menstrual cycle (P = 0.001). The density of CD1a+ DCs was significantly increased in peritoneal endometriotic lesions (P = 0.003) and in the surrounding peritoneum (P = 0.001) compared with paired uterine curettings and peritoneum distant from the lesion.

CONCLUSIONS: Both CD1a+ and CD83+ DC populations were altered in the eutopic and ectopic endometrium of women with endometriosis compared with controls. Alterations in these cells, which play a crucial role in the coordination of the immune response, may be involved in pain generation and the pathogenesis of endometriosis.

Key words: endometriosis / endometrium / menstrual cycle / dendritic cells / peritoneal lesions

Introduction

Endometriosis, one of the most common benign gynaecological disorders, is estimated to affect at least 10% of women of reproductive age. The condition is defined as presence of endometrial tissue in ectopic locations outside of the uterus and often characterized by chronic pelvic pain, abnormal bleeding, including premenstrual spotting and heavy menstrual bleeding and subfertility or infertility (Vigano et al., 2004). Although endometriosis is relatively common and disabling for many affected women, the pathogenesis of the disease is not well understood. The retrograde menstruation hypothesis proposed by Sampson (1927) is the most widely accepted theory used to explain the pathogenesis of endometriosis. Retrograde menstruation occurs in the majority of menstruating women with patent fallopian tubes during their reproductive lifetime (Halme et al., 1984). Therefore, in addition to the occurrence of retrograde menstruation, other factors that encourage or allow endometrial cells to escape from the uterus and to attach and proliferate outside of the uterine cavity are likely to be involved in the pathogenesis of endometriosis.

Accumulating evidence suggests that a failure of the immune system at the uterine, systemic or peritoneal level to clear retrograde viable menstrual debris and to protect the peritoneal cavity from the invasion and adhesion of endometrial tissue may be involved in the onset of endometriosis (Lebovic et al., 2001). Although immune cell alterations in peritoneal and ectopic sites in women with endometriosis are well established (Vinatier et al., 1996; Lebovic et al., 2001; Dmowski and Braun, 2004; Siristatidis et al., 2006), it is less clear whether immune cells are altered in the eutopic endometrium of women with endometriosis compared with controls. Several early studies examining uterine leukocyte populations in women with endometriosis have found that
the density of these cells is not altered compared with controls (Fernandez-Shaw et al., 1995; Jones et al., 1995, 1998; Klentzeris et al., 1995), while others have found that some immune cells are altered in quantity or activity in the eutopic endometrium during the menstrual cycle (Mettler et al., 1996; Ota et al., 1996; Gagne et al., 2003; Khan et al., 2004; Antsiferova et al., 2005). Current evidence is not only conflicting, but incomplete; certain leukocyte populations, such as dendritic cells (DCs), have been completely excluded from this research.

Dendritic cells (DCs) are a heterogeneous population of antigen presenting cells that are highly involved in the initiation and modulation of the immune response. DCs function in T-cell stimulation through the capture, transport, processing and presentation of antigens to naive T cells, and are also involved in the maintenance of self tolerance (Banchereau and Steinman, 1998). In the human uterus, DCs may act as local mediators of endometrial transformation during the menstrual cycle, leading up to implantation or in the absence of pregnancy, menstruation (Bengtsson et al., 2004). Alterations in DC populations may lead to dysregulation of other types of leukocytes and disruption of the normal endometrial cytokine profile, which may contribute to the development of endometriosis or endometriosis associated infertility. Endometrial DC populations in women with endometriosis may be involved in the development and growth of ectopic lesions through the alteration of the immune response to retrograde endometrial reflux.

Endometriosis has been considered to be an autoimmune disease due to the presence of autoantibodies (Ulcova-Gallova et al., 2002), an association with other autoimmune diseases (Sainii et al., 2002) and links to infertility (Mathur, 2000) in many women with endometriosis. DCs have been implicated in the development of autoimmune diseases, such as systemic lupus erythematosus (SLE) (Ding et al., 2006; Crispin and Alcocer-Varela, 2007) and may play a role in the generation of autoantibodies in some women with endometriosis through the altered presentation of self-antigens to T cells. In addition, accumulating evidence suggests that the female sex steroids, estrogen and progesterone, may directly affect DC function, differentiation, survival, cytokine production and migration, thereby influencing the maintenance of immunity or development of autoimmunity (Hughes and Clark, 2007).

Previously we have examined uterine DC populations in normal women during the menstrual cycle (Schulke et al., 2008). Using this group as a comparison group, we have investigated endometrial DCs in women with endometriosis. We have also examined DC populations within peritoneal endometriotic lesions and in the surrounding peritoneal tissues. The aim of this study was to examine the density of DC populations in the eutopic and ectopic endometrium in women with endometriosis during the menstrual cycle using the immunohistochemical markers CD1a and CD83, two markers which are specific for immature and mature DCs, respectively. CD1a, a cell surface glycoprotein that is structurally related to the MHC molecules and mediates an MHC-independent antigen presentation pathway, is a highly specific and sensitive marker of immature DCs and Langerhans cells, a population of DCs found in the skin (Krenacs et al., 1993). CD83 is a glycoprotein member of the immunoglobulin superfamily that is strongly up-regulated during DC maturation making it a robust marker for mature DC (Lechmann et al., 2002). Increased knowledge about immune aspects of the pathogenesis of endometriosis may allow for the development of novel medical therapies for this debilitating condition.

Materials and Methods

Collection and characterization of hysterectomy specimens

This study was approved by the Human Ethics Committees of the Southwest Sydney Area Health Service and the University of Sydney.

Hysterectomy samples were obtained from 33 premenopausal women with endometriosis confirmed by histological evaluation (mean age 40.9; range 30–50) and 28 premenopausal women without endometriosis with histologically normal endometrium (mean age 43.2, range 36–51). DC populations in this normal group were characterized in detail in a previous study (Schulke et al., 2008). Paraffin-embedded full-thickness hysterectomy samples from the fundus of the uterus containing both endometrium and myometrium were obtained from recent surgical pathology archives. The indications for hysterectomy in the women without endometriosis included uterine prolapse, heavy menstrual bleeding, small intramural fibroids and superficial adenomyosis. All patients included in the control group had histologically normal endometrium and pathology which was expected to have minimal effect on endometrial DC populations. None of the patients with uterine prolapse had a complete prolapse (procidentia).

A second group of paired samples consisting of uterine curettings and peritoneal endometriotic lesions was collected from 43 women with endometriosis (mean age 32.7; range 23–39). Paraffin-embedded uterine curettae samples and peritoneal biopsy samples, which were collected during a single surgical procedure, were obtained from recent surgical pathology archives. Eleven peritoneal samples were excluded due to the insufficient size (n = 7) and inability to clearly identify endometriotic stroma within the biopsy sample (n = 4). Eight samples of normal peritoneum were obtained from premenopausal women without endometriosis during laparoscopic surgery.

Menstrual cycle staging, using an idealized 28 day cycle, was determined by a blinded specialist gynaecological pathologist for all samples. There were no abnormalities of the endometrium determined by pathological examination in any of the included specimens. Of the 28 normal hysterectomy samples, 6 were in the menstrual phase (Days 1–7), 12 were in the proliferative phase (Days 8–14) and 10 were in the secretory phase (Days 15–28) of the cycle. In the hysterecctomy specimens obtained from women with endometriosis, 5 samples were from the menstrual phase, 14 samples were in the proliferative phase and 14 samples were in the secretory phase. In the uterine curetting samples, 10 samples were from the menstrual phase, 19 samples were in the proliferative phase and 14 samples were in the secretory phase. None of the women in this study were on hormonal therapy at the time of surgery or in the 3 months preceding the surgery.

Immunohistochemistry

The methodologies used in this investigation were described previously (Schulke et al., 2008). Briefly, tissue sections were cut at 4 μm and immunostained using monoclonal antibodies for mouse anti-human CD1a (dilution 1:100; Dako Cytomation, Carpinteria, CA, USA) and mouse anti-human CD83 (dilution 1:50; Serotec, Raleigh, NC, USA) for 30 min at room temperature. In addition, endometrial peritoneal lesion specimens were stained using CD10 in order to confirm the location of endometriotic stroma. Antigen retrieval was performed prior to immunostaining using a pH 9 target retrieval solution (Dako Australia) for all CD1a slides, a pH 6 target retrieval solution (Dako Australia) for all CD10 slides and
Proteinase K (Dako Australia) for all CD83 slides. Dual endogenous enzyme block (Dako Australia) was applied to all sections for 10 min prior to incubation with the primary antibody. Following the incubation with the primary antibodies, sections were incubated with REAL Link Biotinylated Secondary antibody (Dako Australia) for 15 min. Slides were then incubated with streptavidin alkaline phosphatase (Dako Australia) for 15 min and stained with permanent fast red chromogen (Dako Australia) for 10 min. Slides were counterstained with Mayer's haematoxylin prior to evaluation. Negative and positive control slides were incorporated into each slide run. Skin, small intestine and tonsil were used as positive control tissues for CD1a, CD10 and CD83, respectively. The negative control slides were processed using the same protocol as sample slides, except the primary antibody was omitted. Mouse IgG1 isotype control (Dako Australia) was used at the same dilution as the respective primary antibody to confirm the specificity of staining for CD1a, CD10 and CD83.

All immunostaining was carried out on a Dako Autostainer Model S3400 (Dako USA, Carpinteria, CA, USA). Images of the sections were captured using an Olympus BX51 microscope and DP70 digital camera (Olympus, Tokyo, Japan). DC staining was assessed using the Image Pro Plus Discovery software (MediaCybernetics, MD, USA). For the hysterectomy specimens, images were obtained from the functional and basal layers of the endometrium. Twenty 250 μm² sections from both the functional and basal layers were examined for each hysterectomy specimen. As the functional layer of the endometrium is partially shed during menstruation, data were obtained only from the basal layer for all hysterectomy samples from the menstrual phase.

DCs were quantified in peritoneal lesions using the Dako Automated Cellular Imaging System (ACIS). Within each peritoneal biopsy sample, DCs were quantified within the stroma of the endometriotic lesion, in the peritoneal tissue directly surrounding the endometriotic lesion, and in the histologically normal peritoneal tissue at least 1 mm away from the lesion site. Due to the small size of some biopsy samples, DCs were not able to be quantified in the endometriotic stroma and surrounding peritoneal tissue of every specimen. The endometriotic stroma was identified by histological appearance and confirmed by CD10 staining (Fig. 4). Using the ACIS system, we were able to determine the density of DCs within the variably shaped lesions and surrounding peritoneum. ACIS counts were validated against blinded manual counting, with good correlation. Twenty 200 μm² sections were counted in each endometrial uterine curetting sample and distant peritoneum.

Cells were only counted as positive if they demonstrated both positive staining and DC morphology. DC morphology was defined as nucleated cells of appropriate size with visible processes extending from the cell body (Fig. 1). A visible DC process without an apparent cell body was not counted as a DC. The densities of CD1a+ DCs and CD83+ DCs per square millimeter were calculated for each specimen.

**Statistical analysis**

Results were expressed as the mean ± standard error of CD1a+ or CD83+ DCs per square millimeter. SPSS 13.0 (SPSS Inc., Chicago, IL, USA) was used to perform all statistical analyses and to calculate means and standard errors.

The distribution of the data was examined prior to analysis and the Student’s t-test was used to compare two normally distributed groups. When Levene’s test for equality of variances was significant, the unequal variance t-test result was reported. The Mann–Whitney U-test was used to compare the two groups that were not normally distributed. The Kruskal–Wallis test was used to compare three skewed groups.

For paired data from uterine curettages and peritoneal endometriotic lesions, the Univariate analysis of variance was used to determine if phase of the menstrual cycle affected the mean DC density. The paired t-test was used to compare the two normally distributed paired groups. The Wilcoxon signed ranks test was used to compare two paired groups that were not normally distributed. Statistical significance was established at P-values of <0.05.

**Results**

Endometrial and peritoneal CD1a and CD83 cell populations were analyzed in women with and without endometriosis. Hysterectomy specimens showed that CD1a+ and CD83+ DCs were present in the functional and basal layers of endometrium in all examined...
specimens (Fig. 1). There was a significantly higher density of CD1a+ DCs than CD83+ DCs in both layers of the endometrium throughout the menstrual cycle in both endometriosis cases and controls (P < 0.001) (Table I).

Peritoneal CD1a+ DC and CD83+ DC populations were identified and analyzed within all endometriotic lesions, and adjacent and distant peritoneum of women with endometriosis. In contrast, CD1a+ and CD83+ DC populations were absent from peritoneal biopsies of women without endometriosis.

Comparison of endometrial DC populations in functional and basal layer endometrium of women with and without endometriosis across the menstrual cycle

In the basal layer, the mean density of endometrial CD1a+ DCs was highly significantly increased during the proliferative phase (but not during other phases of the menstrual cycle) in women with endometriosis compared with controls (P = 0.001). In the functional layer, a significant difference in endometrial CD1a+ DCs was not detected between women with and without endometriosis, irrespective of the cycle phase (Fig. 2).

In the eutopic endometrium of women with endometriosis, there was a highly significant decrease in the density of endometrial CD83+ DCs compared with controls. This significant decrease in mature CD83+ DCs was apparent in both layers of the endometrium and across the proliferative (P ≤ 0.001), secretory (P ≤ 0.001) and menstrual (P = 0.02) phases of the menstrual cycle (Fig. 3).

In both the normal and endometriosis groups, the number of CD1a+ DCs in the functional layer remained constant between the proliferative and secretory phases. The density of endometrial CD83+ DCs in the functional and basal layers was also relatively constant during the menstrual cycle in both the normal and endometriosis groups.

The total number of CD1a+ DCs per square millimeter was significantly higher in the basal layer than in the functional layer during the secretory phase in both the normal (P = 0.01) and endometriosis (P = 0.001) groups. In the normal group, the mean density of CD83+ DCs was significantly greater in the basal layer than in the functional layer during both the proliferative (P = 0.01) and secretory phases (P = 0.007). In the endometriosis group, a significant difference in the mean density of CD83+ DCs between the functional and basal layers was only observed in the proliferative phase (P = 0.02).

Table I Mean density of CD1a+ and CD83+ DCs in the functional and basal endometrial layers during the menstrual cycle in women with and without endometriosis

<table>
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<tr>
<th></th>
<th>Proliferative</th>
<th>Secretory</th>
<th>Menstrual</th>
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<tr>
<td></td>
<td>Functional</td>
<td>Basal</td>
<td>Functional</td>
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<tr>
<td>CD1a</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Normal</td>
<td>18.5 ± 1.8</td>
<td>18.9 ± 1.8</td>
<td>16.2 ± 2.2</td>
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<td>Endometriosis</td>
<td>19.1 ± 2.2</td>
<td>34.1 ± 3.5e</td>
<td>17.8 ± 2.9</td>
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<tr>
<td>CD83</td>
<td></td>
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<tr>
<td>Normal</td>
<td>7.9 ± 1.2</td>
<td>12.3 ± 1.2c</td>
<td>9.4 ± 1.2</td>
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<tr>
<td>Endometriosis</td>
<td>4.2 ± 0.5</td>
<td>5.9 ± 0.5d</td>
<td>5.0 ± 0.5</td>
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Data are represented by mean density (cells/mm²) ± SEM.

*Significant increase compared with preceding phase of the menstrual cycle (P < 0.01).

bSignificant increase compared with preceding phase of the menstrual cycle (P < 0.05).

cSignificant increase in the basal layer compared with functional layer (P < 0.01).

dSignificant increase in the basal layer compared with functional layer (P < 0.05).

Analysis of DC populations in endometrial curettings compared with hysterectomy specimens

The mean density of CD1a+ and CD83+ DCs was compared in endometrial curettings and hysterectomy specimens (functional layer) (Table II). During the proliferative and secretory phases, there were no significant differences between the mean density of CD1a+ DCs and CD83+ DCs in curetting samples and in the functional layer of hysterectomy specimens.

Analysis of DCs in paired peritoneal lesions and uterine curettings during the menstrual cycle

CD1a+ DC populations were shown to be present within the peritoneal lesions in women with endometriosis (Fig. 4). The density of CD1a+ DC was significantly higher within the ectopic endometrium in comparison to paired eutopic endometrium during the proliferative and secretory, but not during the menstrual phase of the cycle (Table III).

CD83+ mature DCs were present in extremely low densities in the peritoneal lesion samples. There was a range of 0–3 CD83+ DCs present in each total biopsy sample irrespective of menstrual cycle phase. No CD83+ DCs were observed in 48.5% of lesions. CD83+ DCs were observed within the endometriotic lesions in 51.5% of samples.

Analysis of DCs within normal and endometriotic peritoneum (adjacent to and distant from the lesion site)

There were no significant differences in the mean density of CD1a+ DCs from endometriotic lesions and from the adjacent peritoneum directly surrounding the lesion. The mean density of CD1a+ DCs was significantly increased in the peritoneum directly surrounding the endometriotic lesion (26.7 ± 2.7; n = 32) compared with peritoneum at least 1 mm away from the lesion (15.5 ± 2.8; n = 15) in 15 paired samples (P = 0.001).
Analysis of CD1a+ and CD83+ DCs within normal peritoneum

No CD1a+ nor CD83+ DCs were observed in peritoneal biopsies from women without endometriosis.

Discussion

We have demonstrated for the first time that CD1a+ and CD83+ DC populations are altered in the eutopic endometrium and ectopic endometrial lesions, as well as in surrounding peritoneum of women with endometriosis compared with controls, with respect to stage of the menstrual cycle, tissue distribution and density. DCs are highly involved in the coordination of the immune response and alterations in these cells could play a role in the pathogenesis or progression of endometriosis. Despite the important role of DCs in the stimulation of T-cell immunity and self tolerance, these cells have not previously been studied in the eutopic or ectopic endometrium of women with endometriosis.

Immature DCs are primarily involved in immune surveillance and antigen capture in peripheral tissues (Banchereau and Steinman, 1998). In normal women, the density of endometrial immature CD1a+ DCs appears to be tightly regulated throughout the menstrual cycle, with migration of CD1a+ DC into the endometrium and an increase in density from the proliferative to menstrual phase of the cycle. This pattern was not observed in the eutopic endometrium of women with endometriosis. We observed a significant increase in CD1a+ DCs in the basal layer of the endometrium during the proliferative phase in women with endometriosis compared with controls. Uterine CD1a+ DCs in normal women are likely to be regulated by steroid hormone-dependent production of chemokines and cytokines (Salamonsen, 2003). Estrogen may modulate the activity of antigen-presenting cells, including DCs and macrophages, thereby altering their functional activity, cytokine production, migration or differentiation (Nalbandian and Kovats, 2005).

Mature CD83+ DCs were significantly decreased in women with endometriosis in both endometrial layers during all phases of the menstrual cycle. The decreased expression of CD83 in women with endometriosis suggests that some aspect of DC maturation is defective in women with endometriosis. Expression of CD83 coincides with DC maturation; mature DCs transit out of peripheral tissues to the secondary lymphoid organs where antigen presentation to T cells occurs (Prechtel and Steinkasserer, 2007). DCs display significant plasticity in their response to pathogens and maturation stimuli. It is possible that the maturation of DCs into CD83+ DCs is occurring at a similar rate in normal women and women with endometriosis, but the rate of transit out of the uterus is increased in women with endometriosis. Hormone or cytokine signals for DC migration may be altered in women with endometriosis, causing these cells to respond abnormally.

This study also aimed to investigate the presence of peritoneal DC populations in women with and without endometriosis as well as to compare the density of these cell populations at the lesion site, adjacent and distant peritoneum of women with the disease. Paired eutopic and ectopic endometrial samples were analyzed. An association was observed in the density of DC within the functional layer of hysterectomy samples and the endometrial curetting samples, as expected. This study provides evidence that CD1a+ DC populations are present within the endometriotic lesions and within the peritoneum in women with endometriosis. While CD83+ DC were present in negligible numbers within the peritoneal lesions of women with endometriosis, it is important to note that in the normal peritoneum of women without endometriosis, CD1a+ and

Figure 2 Comparison of the mean density of CD1a+ DCs in normal women and women with endometriosis in the functional (A) and basal (B) layers of the endometrium during the menstrual cycle. Data are represented by mean ± SEM. *P = 0.001.
CD83$^+$ DC populations were not detected at all. CD1a$^+$ DCs were most abundant at the lesion site, and their density progressively decreased as the distance from lesion increased (from adjacent to distant peritoneum). These results suggest that in endometriosis, ectopic endometrial cells themselves, or a microenvironment of diseased peritoneum may be signaling recruitment of immature CD1a$^+$ DC, in order to initiate uptake and subsequent clearance of foreign ectopic antigens. The stage of the menstrual cycle did not appear to have a substantial effect on CD1a$^+$ DC density at the lesion site. However CD1a$^+$ DC density at lesion site was the highest during the secretory stage. These data are in accordance with the previously published results, which showed that GM-CSF (granulocyte-macrophage colony-stimulating factor which induces differentiation of monocytes towards DC lineage) is highly expressed in the lesion during the secretory phase in women with endometriosis (Sharpe-Timms et al., 1994).

A very recent study found that peritoneal fluid from women with endometriosis favorably induces differentiation of peripherally isolated
monocytes toward macrophage rather than DC lineage, thereby disturbing the normal balance of monocyte differentiation (Na et al., 2008). In endometriosis, increased populations of proliferative phase CD1a+ DCs, as observed by this study, may be attributed to alterations in local hormone or cytokine concentrations in the uterus of women with endometriosis.

DCs have been implicated in the development of autoimmune disease (Cravens and Lipsky, 2002). Interestingly, the decreased expression of CD83 was also observed in SLE (Ding et al., 2006). DCs isolated and cultured from peripheral blood mononuclear cells from patients with SLE, displayed down-regulation of CD83 maturation marker following induction of DC maturation. Other DC maturation and differentiation markers, including CD86, CD80 and HLA-DR, were significantly higher in SLE than healthy controls in the absence of maturation stimuli (Ding et al., 2006). The significant decrease in CD83+ DCs endometrium in women with endometriosis observed in this study is similar to the decreased expression of CD83 in patients with SLE, which suggests a possible link between endometriosis and autoimmunity. The significance of defective CD83 DC maturation in SLE and endometriosis is unknown, but indicates that the immune response to immunogenic stimuli is altered in these patients.

Changes in DC density or activity in women with endometriosis may lead to widespread alterations in the immune response. Gene expression profiling studies have identified several cytokines and chemokines that are produced by DCs during different stages of maturation, including the interleukins, IL-6; IL-10, IL-12, TNF-α (tumor necrosis factor-α); RANTES (Regulated upon Activation, Normal T-cell Expressed and Secreted) and MCP-1 (monocyte chemotactic protein-1) (Nagorsen et al., 2004). DCs influence other leukocyte populations through the secretion of these soluble factors. Alterations in DC populations could directly contribute to aberrant T-cell function in women with endometriosis. Several studies have reported changes in T-cell populations in the eutopic endometrium of women with endometriosis compared with controls (Mettler et al., 1996; Ota et al., 1996; Antsiferova et al., 2005).

Cytokine and chemokine release may also be important in pain generation in women with endometriosis. Increased local release of proinflammatory chemokines causes increased pain perception through the down-regulation of opioid receptors (Szabo et al., 2002). DCs are also capable of producing the neurotrophins, nerve growth factor and brain-derived neurotrophic factor (Noga et al., 2007), which may be involved in the growth of nerve fibers in the endometrium and endometriotic lesions of women with endometriosis (Tokushige et al., 2006). The increase in CD1a+ DCs in the basal layer endometrium during the proliferative phase may have a role in regeneration and repair of nerve fibers in endometriosis. This contention is supported by in-vitro studies which suggest that neuropeptides secreted by nerve fibers can attract immature DCs (Dunzendorfer et al., 2001). Such neuropeptides also appear to have inhibitory effect on mature DCs (Dunzendorfer et al., 2001). This is consistent with our finding of highly significantly decreased numbers of CD83+ mature DC in endometriosis. Furthermore, changes in DC populations in women with endometriosis could be involved in the generation of pain in women with endometriosis through the altered secretion of chemokines and cytokines which encourage neurogenesis and altered neural sensitivity in the endometrium and in lesions.

Studies in rodent models of endometriosis suggest that DCs may play a role in the pathogenesis and progression of endometriosis. Interestingly, immature DCs were found to infiltrate peritoneal lesions and to encourage angiogenesis and lesion growth in a murine model (Fainaru et al., 2008). Treatment with the immune modulators Figure 4 Presence of endometriotic stroma and glands in peritoneum of women with endometriosis is confirmed by CD10 staining of the stroma with the chromogen permanent fast red, under 100× magnifications (A) and under ×200 magnification (B). Presence of CD1a+ DCs, characterized by chromogen permanent fast red is observed in peritoneal endometriotic lesions, under 400× magnifications (C).
recombinant interleukin-2 (Velasco et al., 2007), loxoribine and levamisole (Keenan et al., 1999) resulted in decreased implant size and an increased number of certain leukocyte populations, including OX-62+ DCs. DCs are an extremely heterogeneous cell population and it is difficult to ascertain the relationship between animal and human DC populations due to the diversity in DC markers. Further human studies are needed to confirm these findings from the animal model.

The changes in endometrial DC populations observed in this study may represent a primary defect in immune cell function that predisposes these women to develop endometriosis or a secondary occurrence due to the presence of ectopic lesions and other related anomalies. Irrespective of whether changes in DC populations are primary or secondary to the development of endometriosis, these changes may lead to disturbances in the local regulation of the uterine environment contributing to the occurrence of infertility or implantation defects in women with endometriosis. DCs have been implicated in embryonic implantation and the maintenance of pregnancy (Blois et al., 2007). A recent randomized trial demonstrated an increased rate of pregnancy in women with endometriosis associated with infertility following flushing of the fallopian tubes during a hysterosalpingogram using the oil-soluble contrast medium lipiodol (Johnson et al., 2004). The mechanism by which lipiodol enhances infertility is unknown, but lipiodol was found to alter murine uterine DC populations, which suggests that lipiodol treatment may alter the human uterine immune response (Johnson et al., 2005). Future studies should explore the possible role of DCs in endometriosis-related infertility in women.

This study utilized tissue samples from women undergoing hysterectomy in order to allow for examination and orientation of the full-thickness endometrium during the menstrual cycle. Women having a hysterectomy are likely to be near the end of their reproductive lifetime, and correspondingly the mean age of women included in this study was over 40 (40.9 in endometriosis group and 43.2 in normal group). While we excluded any patients with major endometrial pathology, many of the women in our control group had some minor pathology diagnosed at the time of hysterectomy. This study was also limited to the use of the immunohistochemical markers, CD1a and CD83, which are two of the most robust markers for human DC populations. Future studies should examine other DC markers, which may identify other changes in DC subpopulations in women with endometriosis.

We have found that DC populations are altered in the eutopic endometrium of women with endometriosis compared with controls. Our results also suggest that, immature CD1a+ DC populations are altered within the peritoneal lesions, as well as in surrounding peritoneum in women with endometriosis. These novel findings have raised numerous questions about the possible involvement of DCs in the pathogenesis and progression of endometriosis. Future therapeutic treatments for endometriosis may focus on the immunomodulation of potentially pathogenic immune cell populations, including DCs.

**Acknowledgements**

The authors thank Professor Peter Russell (Department of Pathology, University of Sydney, Australia) for his assistance in obtaining the pathology blocks used in this study and for blindly reviewing the tissue sections; Lawrence Young (Dako, Australia) for his technical support; and Georgina Luscombe (University of Sydney, Australia) for her statistical expertise.

**Funding**

This study was supported by research funding from the Department of Obstetrics and Gynaecology, University of Sydney.

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Submitted on February 5, 2008; resubmitted on February 9, 2009; accepted on February 11, 2009.