ORIGINAL ARTICLE: ENDOMETRIOSIS

Mannose receptor is highly expressed by peritoneal dendritic cells in endometriosis

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Objectives: To characterize peritoneal dendritic cells (DCs) in endometriosis and to clarify their role in its etiology.
Design: Experimental.
Setting: University hospital.
Patient(s): Sixty-three women (35 patients with endometriosis and 28 control women) who had undergone laparoscopic surgery.
Intervention(s): Peritoneal DCs from endometriosis and control samples were analyzed for the expression of cell surface markers. Monocyte-derived dendritic cells (Mo-DCs) were cultured with dead endometrial stromal cells (dESCs) to investigate changes in phagocytic activity and cytokine expression.
Main Outcome Measure(s): Cell surface markers and cytokine expression and identification with the use of flow cytometry or reverse-transcription polymerase chain reaction (RT-PCR). Changes in cytokine expression and phagocytic activity of Mo-DCs cultured with dESCs and D-mannan were measured with the use of flow cytometry and RT-PCR.
Result(s): The proportion of mannose receptor (MR)–positive myeloid DC type 1 was higher in endometriosis samples than in control samples. The blocking of MR reduced phagocytosis of dESCs by Mo-DCs. Mo-DCs cultured with dESCs expressed higher levels of interleukin (IL) 1β and IL-6 than control samples.
Conclusion(s): Peritoneal DCs in endometriosis tissue express high levels of MR, which promotes phagocytosis of dead endometrial cells and thereby contributes to the etiology of endometriosis. (Fertil Steril® 2016;■:■–■. ©2016 by American Society for Reproductive Medicine.)
Key Words: Endometriosis, dendritic cell, peritoneal fluid, phagocyte, retrograde menstruation

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Endometriosis is a disease in which the endometrium grows beyond the uterus, commonly into the ovary and/or peritoneal cavity. The prevalence of this condition is 6%–10% in women of reproductive age and 35%–50% in women diagnosed with infertility or menstrual-related pain (1, 2). The cause is still undetermined, although retrograde menstruation and immune abnormalities in the peritoneal cavity are thought to contribute to the onset and progression of this disease (3).

Dendritic cells (DCs) are antigen-presenting cells that recognize and capture antigens, promote differentiation of naïve T cells, and thereby induce antigen-specific immune responses. DCs are extremely heterogeneous and are therefore divided into several subsets according to their origin and functions. Dzionek et al. identified three blood DC antigens (BDCAs): BDCA1 (CD1c), BDCA2 (CD303), and BDCA3 (CD141). They classified DCs into three subsets—myeloid DC type 1 (MDC1), myeloid DC type 2 (MDC2), and plasmacytoid DC (PDC)—on the basis of the presence of BDCA1, BDCA3, and BDCA2, respectively (4, 5).

In addition to these subsets, DCs are further classified according to their expression of cell surface proteins. CD83 and other mature markers are expressed in DCs that have captured pathogens and acquired antigen presentation capacity. The C-type lectin receptors, such as mannose receptor (MR; CD206), DEC205 (CD205), and CD209 (DC-SIGN), are responsible for recognition and uptake of pathogens (6). CD163 is a member of the scavenger

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receptor cysteine-rich (SRCR) superfamily class B, which scavenges haptoglobin-hemoglobin and is known to have a role in regulating immune tolerance (7).

Many studies have demonstrated that various immune cells, such as neutrophils, macrophages, T cells and natural killer (NK) cells (3, 8, 9) are involved in the development of endometriosis. Immune cells in the peritoneal cavity have been extensively studied regarding their frequency and functions, because endometriosis most commonly develops in the peritoneal cavity. Many studies have examined the CD4-CD8 T-cell ratio (10), the cytotoxicity of NK cells (11), and the M1-M2 ratio of macrophages (12) and provided evidence implicating their possible role in the etiology of endometriosis. In contrast, studies that focus on peritoneal DCs in endometriosis are very limited. Until now, peritoneal DCs in endometriosis have been investigated in a single study, which showed that their frequency was not different between the different stages of endometriosis (10). Further evidence, such as characteristics and function(s), of peritoneal DCs in endometriosis is extremely limited.

We hypothesized that peritoneal DCs encounter antigens, such as debris in retrograde menstruation, and induce an immune response in the peritoneal cavity, thereby contributing to the development of endometriosis. To test this hypothesis, we first conducted experiments to characterize peritoneal DCs in endometriosis. With the discovery that MRs were highly expressed on peritoneal DCs from endometriotic tissue, we further attempted to elucidate the possible role of MR in the etiology of endometriosis.

MATERIALS AND METHODS

Tissue and Peritoneal Cell Sampling

Peritoneal fluid (PF) was obtained from patients who underwent laparoscopy for a benign gynecologic condition and/or infertility. Women who did not have regular menstrual cycles, had taken hormonal or immunosuppressive medication, or had a history of pelvic inflammatory disease or hysterosalpingography were excluded from the study. The stage of endometriosis was established according to the revised American Society of Reproductive Medicine classification. Sixty-three women were recruited for this study. Twenty-eight of the 63 women had never been diagnosed with endometriosis and 35 were diagnosed with stage III–IV endometriosis. The mean ages of nonendometriosis patients and endometriosis patients were 34.0 and 37.1 years, respectively; there was no significant difference of age between groups. At the time of surgery, 14 of the 28 nonendometriosis patients and 16 of the 35 endometriosis patients were in the proliferative phase, and there was no significant difference between groups. Endometrial tissues were obtained from patients during hysterectomies for benign disease. The experimental procedures were approved by the Institutional Review Board of the University of Tokyo and signed informed consent was obtained from each woman.

Isolation of Peritoneal Fluid Mononuclear Cells

Peritoneal fluid was aspirated from the pouch of Douglas immediately after the insertion of trocars to minimize contamination with blood. Grossly hemorrhagic specimens were excluded. PF was heparinized and centrifuged at 300*g* for 10 minutes and supernates were discarded. The cell pellet was resuspended in phosphate-buffered saline solution (PBS), layered onto Ficoll-Paque (GE Healthcare Bio-Sciences KK), and centrifuged at 900*g* for 30 minutes. The cells in the middle layer were collected and identified as peritoneal fluid mononuclear cells (PFMCs). Red blood cells were removed completely by means of lysis with NH_4Cl lysing buffer. PFMCs were then washed with the use of PBS and resuspended in flow cytometry buffer (PBS with 0.1% bovine serum albumin and 0.04% NaN_3).

Characterization of Dendritic Cells in PFMCs

Dendritic cells in PFMCs were characterized as previously described (4, 13). PFMCs were incubated with the following antibodies: anti-BDCA1-FITC, anti-BDCA3-FITC, anti-BDCA2-FITC, anti-CD19-APC, and anti-CD14-APC (Miltenyi Biotec), and analyzed with the use of flow cytometry (FACSCaliber; Bekton Dickinson). BDCA1+CD19− cells were defined as myeloid dendritic cells type 1 (MDC1s), and BDCA3+CD14− cells as myeloid dendritic cells type 2 (MDC2s). BDCA2+ cells were defined as plasmacytoid dendritic cells (PDCs). The proportions of MDC1s, MDC2s, and PDCs in PFMC samples were compared between endometriosis and control groups.

MDC1s were found to constitute a major proportion of PF; therefore, subanalysis of MDC1s was performed. First, the level of maturation was evaluated by incubating PFMCs with anti-CD83-PE, and the proportions of CD83+ MDC1s were compared between endometriosis and control samples. To detect pattern recognition receptors, cells were identified with the use of anti-MR-PE (Miltenyi Biotec), anti-DEC205-PE (Biolegend), CD209-PE (Miltenyi Biotec), and anti-CD163-PE (R&D Systems). The proportions of MR+ cells in MDC1s were compared between groups. Mean fluorescence intensities (MFI; the mean of fluorescent intensity of all analyzed cells) for DEC205, CD209, and CD163 were compared between endometriosis and control samples, because these receptors were detected in all MDC1s.

Isolation, Culture, Staining, and Cell Preparation of Endometrial Stromal Cells

Endometrial stromal cells (ESCs) were isolated and cultured as previously reported (14, 15). Briefly, endometrial tissue was minced and digested with the use of 0.25% type I collagenase (Sigma-Aldrich) and 15 IU/mL deoxyribonuclease I (Takara Shuzo). The dispersed endometrial cells were filtered with the use of a 40-μm-pore-size nylon cell strainer (BD Biosciences). Stromal cells in the flow-through were collected and cultured in DMEM/F12 with 5% fetal bovine serum (FBS). At first passage, cells were tagged with fluorescence with the use of PKH67 Green Fluorescent Cell Linker Kit (Sigma-Aldrich) according to the manufacturer's protocol. To induce cell death, cultured ESCs were collected, suspended in RPMI 1640 medium (Invitrogen), and subjected to three freeze-thaw cycles. We chose