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® 2017;107:167–73. ©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

Received May 31, 2016; revised and accepted September 19, 2016; published online October 25, 2016.
G.I. has nothing to disclose. K.K. has nothing to disclose. M.T. has nothing to disclose. T.M. has nothing to disclose. M.N. has nothing to disclose. Y.U. has nothing to disclose. M.H. has nothing to disclose. T.H. has nothing to disclose. Y.H. has nothing to disclose. T.F. has nothing to disclose. Y.O. has nothing to disclose.
Supported by grants from the Ministry of Health, Labor and Welfare and the Ministry of Education (to K.K.).
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Fertility and Sterility® Vol. 107, No. 1, January 2017 0015-0282/$36.00

http://dx.doi.org/10.1016/j.fertnstert.2016.09.036

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

freeze-thaw cycles (inducing necrosis) for inducing death in ESCs because in our preliminary analysis of endometrial cells in the peritoneal cavity obtained during the menstrual period, most annexin-positive cells were double-positive for propidium iodide (PI) (Supplemental Fig. 1, available online at www.fertstert.org), which indicates that the majority of cells are necrotic or late apoptotic, but not early apoptotic.

Preparations of Monocyte-Derived Dendritic Cells

Following in vitro experiments, monocyte-derived dendritic cells (Mo-DCs) were used as an alternative to primary peritoneal DCs (16), owing to the technical limitations in isolating and culturing pure primary peritoneal DCs. Mo-DCs were prepared as reported previously (17, 18). Briefly, peripheral blood was obtained from healthy donors and peripheral blood mononuclear cells were separated by means of density gradient centrifugation with Ficoll-Paque. Monocytes were purified with the use of magnetic microbeads with CD14 antibody (Miltenyi Biotec) and plated at 5×10^6/mL in RPMI 1640 medium with 10% FBS, 20 ng/mL granulocyte-macrophage colony–stimulating factor (R&D Systems) and 20 ng/mL interleukin (IL) 4 (R&D Systems). Mo-DCs were obtained after 4 days of culture.

Culture of Mo-DCs with Dead ESCs

Mo-DCs were resuspended in fresh medium and cultured in the presence or absence of dead ESCs (dESCs) for 6 hours and collected. To block MR, Mo-DCs were cultured with or without 3 mg/dL D-mannan and then suspended in flow cytometry buffer. In the preliminary study, we found that MR expression in Mo-DCs was decreased when D-mannan was added (Supplemental Fig. 2A, available online at www.fertstert.org).

Evaluation of Phagocytosis with the Use of Flow Cytometry

Evaluation of phagocytosis by means of detecting the dye in phagocytes is a classically established method with many references (19, 20). Using this method, the authors originally established an in vitro model that mimics interactions between peritoneal DCs and retrograde menstruation by culturing Mo-DCs in the presence of stained dESCs and detecting the dye in Mo-DCs. Mo-DCs (1×10^6 cells) were cultured for 4 hours in the presence of stained dESCs (1×10^7 cells), stained with anti-CD209-APC (Miltenyi Biotec), and analyzed with the use of flow cytometry. The preliminary study found that the presence of dESCs did not affect the MR expression level in DCs (Supplemental Fig. 2B, available online at www.fertstert.org).

Quantitative Reverse-Transcription Polymerase Chain Reaction

The mRNA expressions of tumor necrosis factor (TNF) α, IL-6, transforming growth factor (TGF) β, IL-1β, and IL10 in Mo-DCs cultured with dESCs were evaluated because these are major cytokines known to be involved in the pathogenesis of endometriosis (9,21–28). In this experiment, Mo-DCs (1×10^6 cells) were cultured in the presence or absence of unstained dESCs (5×10^7 cells) for 6 hours. We had confirmed that this culture condition achieved >98% Mo-DC–phagocytized dESCs. Total Mo-DC RNA was isolated from cells with the use of RNA Easy Mini Kit (Qiagen) and then treated with ribonuclease-free recombinant deoxyribonuclease (Roche Diagnostics). Reverse transcription was performed with the use of Revertraace (Toyobo). PCR amplification was performed with the use of the Lightcycler 480 System (Roche Applied Science). TNF-α primers (sense, 5′-TGCCTGCTGCACTTTGGAGTGAT-3′; antisense, 5′-TGGTTATCTCTCAGCTCCACGCCAT-3′), IL-6 primers (sense, 5′-GAACTCCTTCTCCACACAAGCG-3′; antisense, 5′-TTTTCTGCCAGTGCCTCTTT-3′), TGF-β primers (sense, 5′-AGCTCCGAGAAGCGGTACCTGA-3′; antisense, 5′-TCAACCACTGCCGCACAACTCC-3′), IL-1β primers (sense, 5′-AAGTGTCTGAAGCAGCCATGGCA-3′; antisense, 5′-TGAAGCCCTTGCTGTAGTGGTGGT-3′), IL-10 primers (sense, 5′-CCTGGGTTGCCAAGCCTTGTCT-3′; antisense, 5′-ATTCTTCACCTGCTCCACGGCCT-3′), and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) primers (sense, 5′-ACCACAGTCCATGCCATCAC-3′; antisense, 5′-TCCACCACCCTGTTGCTGTA-3′) were used. The conditions for polymerase chain reaction (PCR) were as follows: TNF-α, TGF-β, IL-1β, and IL-10) 45 cycles at 95°C for 10 seconds; 58°C for 10 seconds, and 72°C for 9 seconds; IL-6) 45 cycles at 95°C for 10 seconds, 59°C for 10 seconds, and 72°C for 12 seconds; and GAPDH) 30 cycles at 95°C for 10 seconds, 64°C for 10 seconds, and 72°C for 18 seconds. We confirmed that contamination of Mo-DC RNA with dESC RNA was negligible. All PCRs were followed with melting curve analysis. The expression level of each mRNA was normalized against GAPDH levels in each case.

Data Analysis

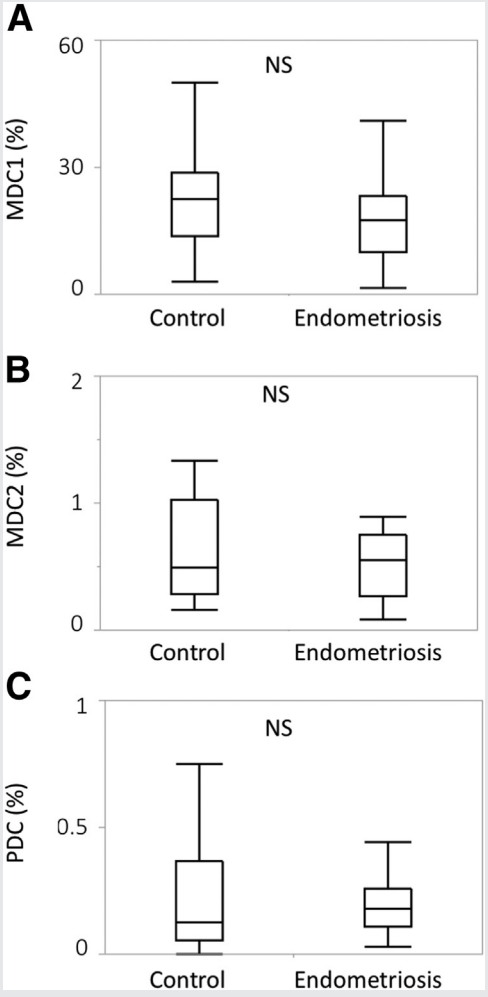
Flow cytometry analysis was conducted with the use of Flowjo (Tree Star). Statistics were calculated with the use of JMP Pro 10.0 and the paired Student t test or Wilcoxon rank sum tests. P values of $<.05$ were regarded as significant. Values in the text indicate the median or the mean ± SEM.

RESULTS

The Proportion of MR-Positive MDC1s Was Significantly Higher in Endometriosis

As shown in Figure 1, the major proportion of peritoneal DCs consisted of MDC1s, whereas MDC2s and PDCs constituted 0%–2% of PFMCs. The proportions of MDC1s (22.3% in control, 17.3% in endometriosis), MDC2s (0.75% in control, 0.58% in endometriosis), and PDCs (0.22% in control, 0.22% in endometriosis) in PFMCs were not significantly different between control and endometriosis samples. The proportions of mature MDC1s (CD83+ MDC1) were not different between the two groups (Fig. 2A). The proportions of MR+ MDC1 were significantly higher in endometriosis samples than in control samples ($P<.05$; Fig. 2B). The MFIs

FIGURE 1



Proportions of peritoneal fluid dendritic cell (DC) subsets are not different between endometriosis patients and nonendometriosis patients. (**A**) The proportions of myeloid DCs type 1 (MDC1s) in peritoneal fluid were not significantly different between groups (n = 63; *P*=.067). (**B**) The proportions of MDC2s in peritoneal fluid mononuclear cells were not significantly different between groups (n = 29; *P*=.74). **C**: The proportions of plasmacytoid DCs (PDCs) were not significantly different between groups (n = 29; *P*=.41). Boxes represent the first (25%) and third (75%) quartiles, horizontal lines in the boxes represent the medians, and whiskers represent the 10th and 90th percentiles. NS = no significant difference.

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for DEC205, CD209, and CD163 were not different between the two groups (Fig. 2C–2E).

MR on DCs Is Crucial for Phagocytosis of Dead Endometrial Cells

As shown in Figure 3A, phagocytosis of dESCs by Mo-DCs was evaluated with the use of flow cytometry. Fluorescence in the upper right quadrant (PKH67+ and CD209+) represents Mo-DCs that phagocytosed dESCs. Fluorescence in the upper left quadrant (CD209+) represents Mo-DCs that did not phagocytose dESCs, and in the lower right (PKH67+) represents dESCs. To clarify the involvement of MR on phagocytosis of dESCs, D-mannan was added to the Mo-DC culture. As shown in Figure 3B, the addition of D-mannan significantly reduced phagocytosis of dESCs by Mo-DCs ($P<.05$).

Cytokine mRNA Expressions of DCs Were Altered by the Presence of Dead Endometrial Cells in the Culture

To evaluate the effect of phagocytosis of dESCs by Mo-DCs, RNA expression levels between Mo-DCs cultured with or without dESCs were compared (Fig. 4). The level of IL-6 mRNA expression was significantly higher in Mo-DCs cultured with dESCs ($P<.05$) compared with monocultured Mo-DCs. Similarly, the level of IL-1β mRNA expression was significantly higher in Mo-DCs cultured with dESCs ($P<.05$) compared with monocultured Mo-DCs. The levels of mRNA expression of TNF-α, TGF-β, and IL-10 by Mo-DCs were similar regardless of the presence of dESCs.

DISCUSSION

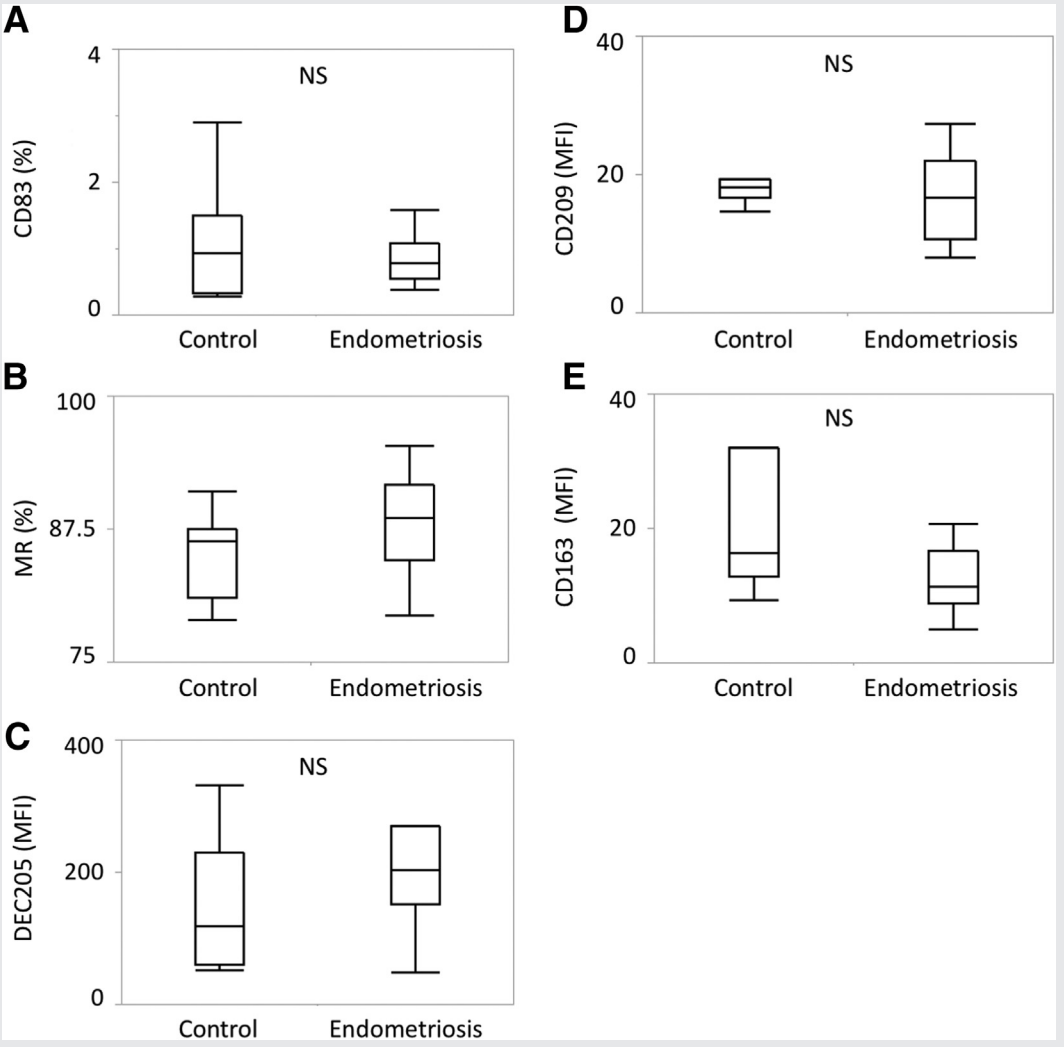
In this study, we characterized the phenotype of peritoneal DCs and compared endometriosis samples with control samples. The proportions of MDC1s, MDC2s, and PDCs were similar between groups. We further analyzed MDC1s, focusing on cell surface protein and discovered that peritoneal MDC1s from endometriosis samples contained a higher proportion of MR+ cells. We also demonstrated that MR on DCs was involved in the phagocytosis of dESCs, and that phagocytosis affected cytokine expression by DCs. These findings implicate peritoneal DCs in the development of endometriosis.

We first characterized peritoneal DCs with the use of BDCA1–3 markers. The proportions of MDC1s, MDC2s, and PDCs were similar between endometriosis and control samples. Tariverdian et al. (10) previously analyzed the frequency of peritoneal DCs identified by Lin−HLA−DR+CD11c+CD123+ cells, a myeloid phenotype, and found no statistically significant difference between control subjects and samples from different stages of endometriosis (10). The present results are consistent with this report; on the basis of the myeloid/plasmacytoid–based classification, no difference in peritoneal DC phenotypes was detected in endometriosis samples.

Because MDC1s constitute a major proportion of peritoneal DCs, we then characterized the expression of MDC1 cell surface proteins. The proportion of MR+ MDC1s was significantly higher in endometriosis samples than in control samples, whereas no significant differences were detected for DEC205, CD209, and CD163 expressions by CD83+ cells between the two groups. The high expression of MR by DCs has been reported for patients with allergen sensitivity (29), and MR expression by DCs was reduced after allergen-specific immunotherapy (30). The high proportion of MR+ peritoneal MDC1s found in samples from endometriosis patients implicates MR in the development of endometriosis.

Regarding CD163, Bacci et al. (12) discovered that CD163 expression by peritoneal macrophages was enhanced in

FIGURE 2



Myeloid dendritic cell type 1 cell surface marker expression in endometriosis samples. (**A**) No significant difference was detected between control and endometriosis sample median proportions for CD83 (n = 28; 0.93 vs. 0.78; *P*=.89). (**B**) The median proportion of mannose receptor (MR)–positive cells was significantly higher in endometriosis samples than in control samples (n = 31; 88.2% vs. 86.8%, respectively; *P*<.05). (**C**) No significant difference was detected in MFI medians for DEC205+ cells (n = 12; 118.9 vs. 203.6; *P*=.22). (**D**) No significant difference was detected in mean fluorescence index (MFI) medians for CD209+ cells (n = 17; 18.1 vs. 16.6; *P*=.43). (**E**) No significant difference was detected in MFI medians for CD163+ cells (n = 19; 16.3 vs. 11.3; *P*=.07). Boxes represent the first (25%) and third (75%) quartiles, horizontal lines in the boxes represent medians, and whiskers represent the 10th and 90th percentiles.

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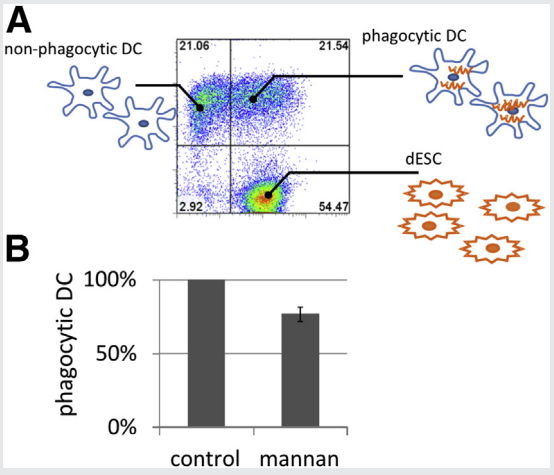
endometriosis patients, but similar results were not observed for DCs. It has been suggested that CD163 expression by macrophages contributes to the progression of endometriosis by transferring iron and provoking an immune response (12), but the function of CD163 on DCs may be different from CD163 on macrophages.

Knowing that MR is enhanced in peritoneal DCs from endometriosis tissue, we attempted to clarify the function of MR on peritoneal DCs. MR is responsible for recognition and phagocytosis of antigens (6), and it is reported to induce T_H2 differentiation in DCs by mediating antigen uptake (31, 32). Given that C-type lectin receptor recognizes foreign antigen and dead cells (33–36), we hypothesized that MR on peritoneal DCs engages in the clearance of dESCs in retrograde menstruation, a well known risk factor for endometriosis (37–39). To test this hypothesis, we established an in vitro model that mimics interactions between peritoneal DCs and retrograde menstruation, by culturing Mo-DCs with dESCs, and evaluated the function of MR on DCs. We found that phagocytosis of dESCs by Mo-DCs was significantly decreased by blocking MR, which indicates that MR on DCs is responsible for the phagocytosis of dESCs. Taken together with the finding that MR is enhanced in peritoneal DCs in endometriosis, this result implies that peritoneal DCs in endometriosis patients are more capable of phagocytosing dESCs compared with control DCs.

FIGURE 3



Mannose receptor plays a crucial role in the phagocytosis of dead endometrial stromal cells (dESCs). (**A**) Phagocytosis of monocyte-derived dendritic cells (Mo-DCs) was evaluated with the use of flow cytometry. The *x*-axis indicates levels of fluorescence intensity for PKH67+ cells (dESCs). The *y*-axis indicates fluorescence intensity levels for CD209+-APCs (Mo-DCs). CD209+-APCs are represented in the upper quadrants. PKH67+ cells are represented in the lower right quadrant. Fluoresence in the upper right quadrant represents Mo-DCs that have phagocytosed dESCs. The ratio of fluorescence in the upper right quadrant to the upper half is the proportion of phagocytic MoDCs. This figure is represents the results of three independent experiments. (**B**) The proportion of phagocytic MoDCs decreased when Mo-DCs were cultured with D-mannan (75.7 ± 4.9% compared with control; $P<.05$).

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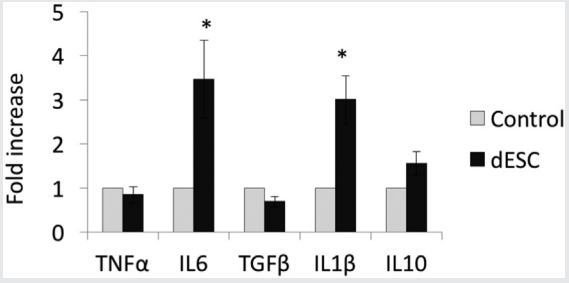
Finally, we evaluated the effect of phagocytosis of dESCs on cytokine expression by Mo-DCs, given that activated DCs express various cytokines to provoke immune responses (40). We found that Mo-DCs cultured with dESCs expressed significantly higher IL-1β and IL-6 mRNA, whereas expression levels of TNF-α, TGF-β, and IL-10 mRNA were not significantly changed by the coculture. IL-1β promotes endometriosis by up-regulating angiogenesis (22) and escaping immunosurveillance (23). IL-6 has also been shown to promote endometriosis progression (24, 25). In addition, in the presence of TGF-β, IL-6 secreted from DCs triggers the differentiation of naïve T cells to T_H17 (41, 42), which implicates IL-6 in the pathogenesis of endometriosis (26, 43, 44). In contrast, TNF-α, which promotes inflammatory response in endometriosis (9), and IL-10, which induces immune tolerance, are not increased in Mo-DCs cultured with dESCs. Taken together with the present findings, peritoneal DCs may contribute to the pathogenesis of endometriosis by phagocytosing cells from retrograde menstruation, thereby provoking various immune responses.

FIGURE 4



mRNA expression by monocyte-derived dendritic cells (Mo-DCs) cultured with dead endometrial stromal cells (dESCs). Interleukin (IL) 6 mRNA expression was significantly increased in Mo-DCs cultured with dESCs (3.26 ± 0.88–fold increase; $P<.05$; n = 3). Mean IL-1β mRNA expression was significantly increased in Mo-DCs cultured with dESCs (3.00 ± 0.54–fold increase; $P<.05$; n = 6). The mRNA expressions of tumor necrosis factor (TNF) α, transforming growth factor (TGF) β, and IL- 10 did not differ significantly between Mo-DCs cultured with and without dESCs ($P=.43$, $P=.05$, and $P=.16$, respectively; n = 6). *$P < .05$.

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This study has several limitations. First, we used Mo-DCs as an alternative to peritoneal primary DC for evaluating the interaction between DCs and dESCs. It is possible that Mo-DCs act differently from peritoneal DCs, and this may have affect the results. Second, we only detected only mRNA expressions of various cytokines in DCs, and their secretion levels were undetermined. Although the authors think that this cytokine profile may have certain contributions to the local immune environment, further studies are warranted to elucidate the impact of these cytokines expressed by DCs on the entire peritoneal cavity, or on neighboring cellss such as T cells.

In conclusion, this study demonstrates that peritoneal DCs from endometriotic tissue express high levels of MR and increase the ability of these cells to phagocytose dESCs, thereby contributing to the development of endometriosis. Controlling MR activity or expression by peritoneal DCs may be a potential strategy for managing endometriosis.

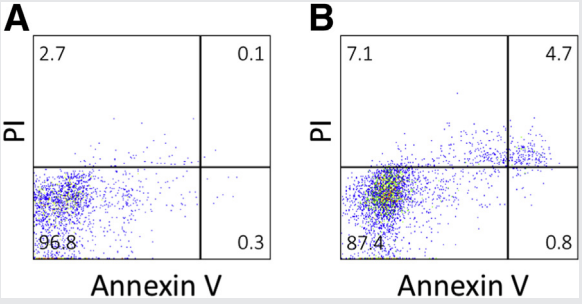
Acknowledgments: The authors thank their colleagues at the University of Tokyo Hospital for collecting clinical data and Dr. Kate Hale for editing the manuscript.

REFERENCES

1. Sensky TE, Liu DT. Endometriosis: associations with menorrhagia, infertility and oral contraceptives. Int J Gynaecol Obstet 1980;17:573–6.
2. Houston DE. Evidence for the risk of pelvic endometriosis by age, race and socioeconomic status. Epidemiol Rev 1984;6:167–91.
3. Giudice LC, Kao LC. Endometriosis. Lancet 2004;364:1789–99.
4. Dzionek A, Fuchs A, Schmidt P, Cremer S, Zysk M, Miltenyi S, et al. BDCA-2, BDCA-3, and BDCA-4: three markers for distinct subsets of dendritic cells in human peripheral blood. J Immunol 2000;165:6037–46.
5. Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, et al. Nomenclature of monocytes and dendritic cells in blood. Blood 2010;116:e74–80.
6. Kerrigan AM, Brown GD. C-Type lectins and phagocytosis. Immunobiology 2009;214:562–75.
7. Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK, et al. Identification of the haemoglobin scavenger receptor. Nature 2001;409:198–201.
8. Barrier BF. Immunology of endometriosis. Clin Obstet Gynecol 2010;53:397–402.
9. Lebovic DI, Mueller MD, Taylor RN. Immunobiology of endometriosis. Fertil Steril 2001;75:1–10.

10. Tariverdian N, Siedentopf F, Rucke M, Blois SM, Klapp BF, Kentenich H, et al. Intraperitoneal immune cell status in infertile women with and without endometriosis. J Reprod Immunol 2009;80:80–90.
11. Ho HN, Chao KH, Chen HF, Wu MY, Yang YS, Lee TY. Peritoneal natural killer cytotoxicity and CD25+ CD3+ lymphocyte subpopulation are decreased in women with stage III–IV endometriosis. Hum Reprod 1995; 10:2671–5.
12. Bacci M, Capobianco A, Monno A, Cottone L, di Puppo F, Camisa B, et al. Macrophages are alternatively activated in patients with endometriosis and required for growth and vascularization of lesions in a mouse model of disease. Am J Pathol 2009;175:547–56.
13. Wertel I, Polak G, Bednarek W, Barczynski B, Rolinski J, Kotarski J. Dendritic cell subsets in the peritoneal fluid and peripheral blood of women suffering from ovarian cancer. Cytometry B Clin Cytom 2008;74:251–8.
14. Miyashita M, Koga K, Izumi G, Makabe T, Hasegawa A, Hirota Y, et al. Drospirenone induces decidualization in human eutopic endometrial stromal cells and reduces DNA synthesis of human endometriotic stromal cells. Fertil Steril 2015;104:217–24.e2.
15. Izumi G, Koga K, Nagai M, Urata Y, Takamura M, Harada M, et al. Cyclic stretch augments production of neutrophil chemokines, matrix metalloproteinases, and activin a in human endometrial stromal cells. Am J Reprod Immunol 2015;73:501–6.
16. Ohyagi H, Onai N, Sato T, Yotsumoto S, Liu J, Akiba H, et al. Monocyte-derived dendritic cells perform hemophagocytosis to fine-tune excessive immune responses. Immunity 2013;39:584–98.
17. Pickl WF, Majdic O, Kohl P, Stockl J, Riedl E, Scheinecker C, et al. Molecular and functional characteristics of dendritic cells generated from highly purified CD14+ peripheral blood monocytes. J Immunol 1996;157:3850–9.
18. Bender A, Sapp M, Schuler G, Steinman RM, Bhardwaj N. Improved methods for the generation of dendritic cells from nonproliferating progenitors in human blood. J Immunol Methods 1996;196:121–35.
19. Sauter B, Albert ML, Francisco L, Larsson M, Somersan S, Bhardwaj N. Consequences of cell death: exposure to necrotic tumor cells, but not primary tissue cells or apoptotic cells, induces the maturation of immunostimulatory dendritic cells. J Exp Med 2000;191:423–34.
20. Albert ML, Pearce SF, Francisco LM, Sauter B, Roy P, Silverstein RL, et al. Immature dendritic cells phagocytose apoptotic cells via $\alpha v\beta 5$ and CD36, and cross-present antigens to cytotoxic T lymphocytes. J Exp Med 1998; 188:1359–68.
21. Suen JL, Chang Y, Chiu PR, Hsieh TH, Hsi E, Chen YC, et al. Serum level of IL-10 is increased in patients with endometriosis, and IL-10 promotes the growth of lesions in a murine model. Am J Pathol 2014;184:464–71.
22. Lebovic DI, Bentzien F, Chao VA, Garrett EN, Meng YG, Taylor RN. Induction of an angiogenic phenotype in endometriotic stromal cell cultures by interleukin-1β. Mol Hum Reprod 2000;6:269–75.
23. Vigano P, Gaffuri B, Somigliana E, Busacca M, di Blasio AM, Vignali M. Expression of intercellular adhesion molecule (ICAM)-1 mRNA and protein is enhanced in endometriosis versus endometrial stromal cells in culture. Mol Hum Reprod 1998;4:1150–6.
24. Salmassi A, Acil Y, Schmutzler AG, Koch K, Jonat W, Mettler L. Differential interleukin-6 messenger ribonucleic acid expression and its distribution pattern in eutopic and ectopic endometrium. Fertil Steril 2008;89:1578–84.
25. Sharpe-Timms KL, Zimmer RL, Ricke EA, Piva M, Horowitz GM. Endometriotic haptoglobin binds to peritoneal macrophages and alters their function in women with endometriosis. Fertil Steril 2002;78:810–9.
26. Takamura M, Koga K, Izumi G, Hirata T, Harada M, Hirota Y, et al. Simultaneous detection and evaluation of four subsets of CD4+ T lymphocyte in lesions and peripheral blood in endometriosis. Am J Reprod Immunol 2015; 74:480–6.
27. Hirata T, Osuga Y, Takamura M, Kodama A, Hirota Y, Koga K, et al. Recruitment of CCR6-expressing T_H17 cells by CCL 20 secreted from IL-1β–, TNF-α–, and IL-17A–stimulated endometriotic stromal cells. Endocrinology 2010;151:5468–76.
28. Dela Cruz C, Reis FM. The role of TGFβ superfamily members in the pathophysiology of endometriosis. Gynecol Endocrinol 2015;31:511–5.
29. Deslee G, Charbonnier AS, Hammad H, Angyalosi G, Tillie-Leblond I, Mantovani A, et al. Involvement of the mannose receptor in the uptake of Der p 1, a major mite allergen, by human dendritic cells. J Allergy Clin Immunol 2002;110:763–70.
30. Lundberg K, Rydnert F, Broos S, Andersson M, Greiff L, Lindstedt M. Allergen-specific immunotherapy alters the frequency, as well as the FcR and CLR expression profiles of human dendritic cell subsets. PLoS One 2016;11:e0148838.
31. Royer PJ, Emara M, Yang C, Al-Ghouleh A, Tighe P, Jones N, et al. The mannose receptor mediates the uptake of diverse native allergens by dendritic cells and determines allergen-induced T cell polarization through modulation of IDO activity. J Immunol 2010;185:1522–31.
32. Salazar F, Hall L, Negm OH, Awuah D, Tighe PJ, Shakib F, et al. The mannose receptor negatively modulates the Toll-like receptor 4-aryl hydrocarbon receptor–indoleamine 2,3-dioxygenase axis in dendritic cells affecting T helper cell polarization. J Allergy Clin Immunol 2015;137:1841–51.e2.
33. Sancho D, Joffre OP, Keller AM, Rogers NC, Martinez D, Hernanz-Falcon P, et al. Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. Nature 2009;458:899–903.
34. Ogden CA, deCathelineau A, Hoffmann PR, Bratton D, Ghebrehiwet B, Fadok VA, et al. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. J Exp Med 2001;194:781–95.
35. Yamasaki S, Ishikawa E, Sakuma M, Hara H, Ogata K, Saito T. Mincle is an ITAM-coupled activating receptor that senses damaged cells. Nat Immunol 2008;9:1179–88.
36. Shrimpton RE, Butler M, Morel AS, Eren E, Hue SS, Ritter MA. CD205 (DEC-205): a recognition receptor for apoptotic and necrotic self. Mol Immunol 2009;46:1229–39.
37. Halme J, Hammond MG, Hulka JF, Raj SG, Talbert LM. Retrograde menstruation in healthy women and in patients with endometriosis. Obstet Gynecol 1984;64:151–4.
38. Nunley WC Jr, Kitchin JD 3rd. Congenital atresia of the uterine cervix with pelvic endometriosis. Arch Surg 1980;115:757–8.
39. d'Hooghe TM. Clinical relevance of the baboon as a model for the study of endometriosis. Fertil Steril 1997;68:613–25.
40. Steinman RM, Hemmi H. Dendritic cells: translating innate to adaptive immunity. Curr Top Microbiol Immunol 2006;311:17–58.
41. Hubbell JA, Thomas SN, Swartz MA. Materials engineering for immunomodulation. Nature 2009;462:449–60.
42. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, et al. Reciprocal developmental pathways for the generation of pathogenic effector T_H17 and regulatory T cells. Nature 2006;441:235–8.
43. Hirata T, Osuga Y, Hamasaki K, Yoshino O, Ito M, Hasegawa A, et al. Interleukin (IL)-17A stimulates IL-8 secretion, cyclooxygenase-2 expression, and cell proliferation of endometriotic stromal cells. Endocrinology 2008;149:1260–7.
44. Hirata T, Osuga Y, Takamura M, Saito A, Hasegawa A, Koga K, et al. Interleukin-17F increases the secretion of interleukin-8 and the expression of cyclooxygenase 2 in endometriosis. Fertil Steril 2011;96:113–7.

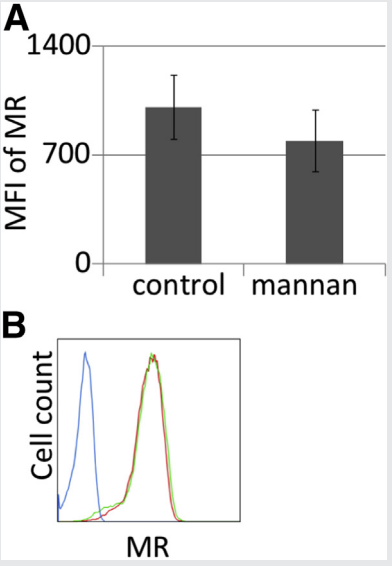
SUPPLEMENTAL FIGURE 1



Apoptosis/necrosis analysis of endometrial cells in the peritoneal cavity. Endometrial cells in the peritoneal cavity were collected from peritoneal fluid during the menstrual period by negative selection of CD45+ cells with the use of magnetic cell sorting, and their apoptosis/necrosis was analyzed with the use of propidium iodide (PI) and annexin V staining. (**A**) unstained; (**B**) stained. Most of annexin V–positive cells were double-positive for PI, which indicates that the majority of endometrial cells in the peritoneal cavity are necrotic or late apoptotic, but not early apoptotic.

Izumi. Peritoneal dendritic cells in endometriosis. Fertil Steril 2016.

SUPPLEMENTAL FIGURE 2



(**A**) Mannose receptor (MR) expression on monocyte derived dendritic cells (Mo-DCs) cultured in the presence or the absence of D-mannan. Mean fluorescence index (MFI) of MR on Mo-DCs cultured in the presence of D-mannan was significantly lower than on Mo-DCs cultured in the absence of D-mannan (792 ± 199 vs. 1,009 ± 205, respectively; $P<.05$). (**B**) MR expression of Mo-DCs cultured in the presence or absence of dead endometrial stromal cells (dESCs). The presence of dESCs did not significantly change the MR expression on MoDC. *Blue*: isotype control; *red*: Mo-DCs cultured in without dESCs; *green*: Mo-DCs cultured with dESCs.

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