Decreased human leukocyte antigen–DR expression in the lipid raft by peritoneal macrophages from women with endometriosis

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Objective: To investigate the macrophage response in endometriosis by determining the expression and localization of human leukocyte antigen (HLA)-ABC and HLA-DR by the peritoneal fluid (PF) macrophages and PF concentrations of interferon (IFN)-γ that regulate HLA expression.
Design: Case-control study.
Setting: University hospital.
Patient(s): 64 Japanese endometriosis patients, and 65 women with other laparoscopic diagnoses.
Intervention(s): Venipuncture and laparoscopic peritoneal fluid collection.
Main Outcome Measure(s): Expression and localization of HLA-ABC and HLA-DR in PF macrophages were determined by flow cytometry and confocal microscopy. The concentration of IFN-γ in PF was determined by enzyme-linked immunosorbent assay.
Result(s): In women with endometriosis, expression of HLA-ABC and HLA-DR by PF macrophages, and the IFN-γ concentrations in PF were statistically significantly lower than in controls. Women with endometriosis showed a statistically significant positive correlation between HLA expression and IFN-γ concentration. By confocal microscopy, HLA-ABC was distributed homogenously on the macrophage surface whereas HLA-DR expression on these cells corresponded to the lipid raft.
Conclusion(s): In women with endometriosis, low HLA expression and particularly reduced HLA-DR in the lipid raft may be influenced by low IFN-γ and may compromise antigen presentation, limiting the immune response to peritoneal cavity antigens such as implanted or metaplastic endometrial tissue. (Fertil Steril® 2008;89:52–9. ©2008 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, peritoneal macrophage, human leukocyte antigen, lipid raft, antigen presentation, natural killer cell, interferon gamma

Investigation of cellular events in the peritoneal cavity, the major site involved by endometriosis, is important to understanding the condition's pathogenesis. Most cells in peritoneal fluid (PF) are macrophages, accompanied by smaller numbers of lymphocytes, natural killer (NK) cells, and mesothelial cells. As macrophages, lymphocytes, and NK cells all are immunocompetent, impairment of their local immune function is considered important in initiation and progression of endometriosis (1, 2).

After peripheral blood (PB) monocytes migrate into the peritoneal cavity, they become PF macrophages, functioning locally in phagocytosis, antigen presentation, and cytokine production. These events, which are central to the inflammatory process and reactions to foreign bodies, are carried out in concert with other types of immunocompetent cells (3, 4). Increased concentrations of macrophage-derived proinflammatory cytokines (5–7) have been found in the PF of women with endometriosis.

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Also, we recently found PF macrophages from women with endometriosis showed significantly decreased expression of human leukocyte antigen (HLA) (8). Antigen presentation to T cells by macrophages, which represents the immunologic communication between these cells, involves HLA antigenic determinants together with costimulatory adhesion molecules (9). This interaction transmits information concerning the antigen from macrophages to T cells through HLA and T-cell receptors (TCR) (10). Antigen presentation by HLA in this "immunologic synapse" is important for processing foreign antigens in the peritoneal cavity (11). Decreased expression of HLA suggests depressed antigen presentation capacity in PF macrophages.

Interferon gamma (IFN-γ), produced by lymphocytes and mainly NK cells through their cytotoxic action, regulates HLA expression on macrophages (12, 13).

We investigated the HLA expression by PF macrophages as an index of antigen presentation capability and the relationship of expression to IFN-γ concentration in PF from women with endometriosis. We also determined localization of HLA-ABC and HLA-DR on PF macrophages. Implications of the findings concerning the relationship of antigen-

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presenting capability to initiation and progression of endometriosis then were considered.

MATERIALS AND METHODS

Patients

We studied 64 Japanese women with endometriosis (10 with stage I disease according to the revised classification of the American Society for Reproductive Medicine [ASRM] [14, 15]; eight with stage II; 23 with stage III; and 23 with stage IV), as well as 65 women with a normal menstrual cycle and no endometriosis. Laparoscopic diagnoses in the latter control group included 21 cases of uterine myoma, 29 of benign ovarian cyst, seven of unexplained infertility, six of chronic abdominal pain, and two of paraovarian cysts. All patients were examined laparoscopically at the Department of Obstetrics and Gynecology of Kochi Medical School between January 2004 and May 2006. Mean ages ($\pm$ standard deviation) in the endometriosis and control groups, respectively, were 34.2 ± 7.1 and 34.2 ± 7.5 years (no statistically significant difference).

Endometriosis was diagnosed by laparoscopic examination and by histopathologic examination of resected tissues. The revised ASRM scores were determined by the same expert on the basis of intra-operative observation and were finalized by postoperative review of video and photo materials. Peripheral blood and PF samples were taken in either the follicular or the luteal phase of the menstrual cycle (31 follicular and 33 luteal among women with endometriosis, and 26 follicular and 39 luteal among controls). The menstrual cycle was determined from the date of onset of last menstrual period and from serum estradiol and progesterone concentrations.

Women with a history of pregnancy within 12 months or a history of treatment with gonadotropin-releasing hormone (GnRH) agonists within a similar period as well as those with complications of apparent pelvic inflammatory disease were excluded from analysis. Peritoneal fluid samples contaminated by fresh blood during the procedure also were excluded. As a consequence, control samples of this fluid could not be obtained from patients undergoing laparoscopic tubal ligation because in Japan this procedure usually is performed shortly after delivery. Control samples therefore were collected from women with no evidence of endometriosis who were undergoing laparoscopy for a variety of benign gynecologic disorders.

Informed consent for collection of PB and PF samples was obtained before laparoscopy. This study was approved by the institutional review board at Kochi Medical School.

Handling of Samples

Peripheral blood (2 mL) was collected in heparinized Hank's buffer before laparoscopy. At initiation of laparoscopy, PF identified within the abdominal cavity was collected in heparinized Hank's buffer.

Monoclonal Antibodies

Fluorescein isothiocyanate (FITC)-labeled anti-CD14 mAb (CD14-FITC; Beckman-Coulter, Fullerton, CA) was used as a marker for monocytes/macrophages. Phycoerythrin (PE)-labeled anti-HLA-ABC mAb (HLA-ABC-PE) and anti-HLA-DR mAb (HLA-DR-PE) (Beckman-Coulter) were used to assess expression of HLA-ABC and HLA-DR on monocytes/macrophages. The controls were FITC-labeled IgG2a mAb (IgG2a-FITC) and PE-labeled IgG2a mAb (IgG2a-PE) (Beckman-Coulter). After PB mononuclear cells (PBMC) and PF mononuclear cells (PFMC) were allowed to react with mAbs, these cells were evaluated by flow cytometry to determine any differences between women with and without endometriosis.

For the confocal microscopic investigation, FITC-labeled cholera toxin B subunit (Sigma-Aldrich, St. Louis, MO) was used to stain endogenous glycosphingolipids on PF macrophages. Anti-cholera toxin-B antibody (Calbiochem, San Diego, CA) was added to patch the lipid rafts. Mouse anti-HLA-ABC and HLA-DR mAbs (Immunotech, Marseille, France) and Texas Red-labeled anti-mouse IgG mAb (Molecular Probes, Eugene, OR) were used to detect HLA-ABC and HLA-DR.

Flow Cytometry

Double staining of samples with FITC-labeled and PE-labeled mAbs was carried out before flow cytometry. Pairs of mAbs (5 μL each) including IgG2a-FITC with IgG2a-PE, CD14-FITC with HLA-ABC-PE, or CD14-FITC with HLA-DR-PE were added to a 200-μL aliquot of PB sample and allowed to react at 4°C for 1 hour. The PF cells were resuspended in phosphate-buffered saline (PBS) after pelleting by centrifugation at $400 \times g$ for 7 minutes. Then mAb pairs were added to the resuspended cells and allowed to react at 4°C for 1 hour. After the mAb reaction, erythrocytes were hemolyzed in ammonium chloride; each cell sample then was centrifuged and resuspended in 0.5 mL of PBS. An EPICS Elite flow cytometer (Beckman Coulter) was used for analysis. The PBMC and PFMC fractions in each sample were gated with forward-scattered and side-scattered light, and fluorescence intensities of cell populations in the monocyte/macrophage fractions in the PBMC and PFMC were measured.

Expression of HLA-DR by monocytes and macrophages was measured by flow cytometry. We used CD14-FITC and HLA-DR-PE for detection of HLA-DR on these cells. Mean fluorescence intensities (FI) for HLA-DR on $CD14^+$ $HLA\text{-}DR^+$ cells were measured as an index of HLA-DR expression.

Confocal Microscopy

The PF macrophages were attached to coverslips by incubation on ice for 45 minutes. After washing in PBS, FITC-labeled cholera toxin B subunit was added for incubation on ice for 30 minutes. After washing in PBS, anti-cholera toxin B antibody was added as described by James et al. (16). After