Modeling the early endometriotic lesion: mesothelium-endometrial cell co-culture increases endometrial invasion and alters mesothelial and endometrial gene transcription

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Objective: To determine the role of peritoneal mesothelial cells (PMCs) in the process of endometrial invasion into the peritoneum and to evaluate gene expression after endometrial-PMC co-culture.
Design: In vitro study.
Setting: University laboratory.
Patient(s): Reproductive-age women without endometriosis.
Intervention(s): None.
Main Outcome Measure(s): The rate of endometrial invasion through modeled peritoneum in the presence and absence of PMCs was evaluated. The influence of endometrial-PMC attachment on the expression of target genes, implicated in the pathogenesis of endometriosis, was examined by using reverse transcription polymerase chain reaction.
Result(s): Endometrial stromal cell (ESC) invasion through invasion chambers coated with Matrigel (MTGL) and with growth factor–reduced Matrigel (GFR-MTGL) was increased 10-fold when a PMC monolayer was present. Endometrial epithelioid cell (EM42) invasion increased greater than threefold through the MTGL and GFR-MTGL–coated membranes when a PMC monolayer was present. Endometrial stromal cell, EM42, and PMC transcription of extracellular signal-related kinase, colony stimulating factor-1, *c-fms*, and *c-Met* was increased after endometrial-PMC attachment. Similar changes were not seen when endometrial cells were exposed to PMC-conditioned media and when PMCs were exposed to endometrial cell conditioned media.
Conclusion(s): Peritoneal mesothelial cells increased invasion of ESCs and EM42s through modeled peritoneum. Endometrial-PMC co-culture led to alterations in gene transcription by endometrial cells and PMCs. This study suggests that PMCs contribute to the process of endometrial invasion into the peritoneum. (Fertil Steril® 2008;90:1487–95. ©2008 by American Society for Reproductive Medicine.)

Key Words: Endometriosis, endometrial stromal cell, cell culture, mesothelium, attachment, invasion, migration

The pathogenesis of the early endometriotic lesion is poorly understood (1). Sampson's theory of retrograde menstruation and subsequent implantation of sloughed endometrial fragments remains the most widely accepted theory for the genesis of endometriosis on peritoneal surfaces (2). Many crucial questions concerning the initial interaction of endometrial cells with peritoneal mesothelial cells (PMCs) and invasion into the peritoneum remain unanswered. Recent studies using whole explants of human peritoneum, as well as PMC monolayer cultures, demonstrate that whole fragments of proliferative, secretory, and menstrual phase endometrium, as well as cultured endometrial stromal and epithelial cells (ESCs and EECs, respectively), adhere to intact PMCs within 1 hour (3–5). After attachment to PMCs, endometriotic cells begin to invade PMCs and the basement membrane within 6 hours. By 24 hours, PMC growth over the invaded endometrial cells is well established (4). These studies strongly suggest that PMC attachment and transmesothelial invasion are the initial steps in the genesis of peritoneal endometriotic lesions.

The focus of the present study was to develop a quantitative in vitro model of transmesothelial invasion by endometrial cells. Endometrial invasion through Matrigel, a solubilized protein extract used to model basement membrane, was evaluated in the presence and absence of PMCs. Further studies were then performed to understand the role of endometrial cell-PMC interaction in the process of peritoneal invasion. A preliminary analysis was performed of the transcription of several genes that had been implicated elsewhere in invasion and metastasis and in the pathogenesis of endometriosis, by endometrial cells and PMCs after attachment of endometrial cells to PMCs.

MATERIALS AND METHODS

Approval for this study was granted by the Institutional Review Board of the University of Texas Health Science Center at San Antonio.

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Endometrial Cell Culture

Proliferative phase endometrium was obtained from women without endometriosis by aspiration biopsy using a Pipelle (Prodimed, Unimar Inc., Neuilly-En-Thelle, France) or immediately following hysterectomy performed for benign conditions. Hysterectomy was performed for patients with pelvic prolapse or myomatous uterus, and Pipelle biopsy specimens were obtained from patients undergoing elective interval sterilization or infertility evaluation. Patients had not undergone hormonal treatment for 3 months before collection of endometrium.

Endometrial stromal cells were cultured as described elsewhere (6–8). Briefly, the endometrium was minced and then enzymatically digested with 0.1% collagenase type I and 0.05% DNAse (Worthington Biochemical, Lakewood, NJ). Endometrial epithelial cells were separated from stromal cells by gravity sedimentation. The stromal cell–rich supernatant was placed in a culture flask, and cells were allowed to adhere for 20 minutes and then washed with medium. The adherent stromal cells then were cultured as a monolayer in flasks in Dulbecco's modified Eagle's medium–F12 medium (1:1) (Sigma, St. Louis, MO) containing antibiotics and antimycotics, 5 μg/mL of insulin, and 10% defined fetal calf serum (Hyclone, Logan, UT).

Endometrial Epithelioid Cells (EM42)

The endometrial epithelioid cell line EM42 was used as a surrogate for patient-derived EECs. This cell line has been characterized elsewhere (9–11) and has a similar rate of PMC attachment to patient-derived EECs (Lucid RS, Witz CA, unpublished data). The EM42 cells were grown as monolayers, as described elsewhere (11).

Peritoneal Model

For the model, LP9 PMCs were grown to confluence on Matrigel (MTGL)- and growth factor reduced Matrigel (GFR-MTGL)–coated 24-well invasion chambers containing membranes with 8-μm pores (BD Bioscience, San Jose, CA). Studies elsewhere have demonstrated similar rates of endometrial cell binding to commercially available LP9 PMCs (National Institutes of Health Aging Cell Repository, Coriell Institute for Medical Research, Camden, NJ) and PMCs derived from parietal peritoneum and ovarian surface epithelium (8). This suggests that LP9 PMCs are an appropriate experimental surrogate for patient-derived PMCs.

Evaluation of Modeled Peritoneum and Transmesothelial Invasion

Initial studies were performed to evaluate the suitability of PMCs grown as a monolayer in invasion chambers as a model of endometrial cell attachment and transmesothelial invasion into the peritoneum. Both LP9 PMCs and ESCs were labeled with thiol-reactive CellTracker dyes (Molecular Probes, Eugene, OR). The LP9 cells were labeled with chloromethylbenzoyl-aminotetramethylrhodamine (CellTracker Orange; 10 μM) and grown to confluence on GFR-MTGL–coated membranes. Endometrial stromal cells or EM42 cells were labeled with chloromethylfluoroscein diacetate (CMFDA; CellTracker Green, 10 μM). Labeled ESCs or EM42 cells were placed over the confluent LP9 PMC monolayers (25,000 cells per invasion chamber). Cultures were interrupted at 1, 3, 6, 12, and 24 hours by placing the invasion chamber in cold 3% formaldehyde. Membranes were cut from the invasion chambers, placed between coverslips, and examined with confocal laser scanning microscopy using an Olympus IX70 inverted microscope and the Olympus Fluoview FV500 System (Olympus, Nagano, Japan), as described elsewhere (4). The CellTracker Green and CellTracker Orange emissions were pseudocolorized with green and red, respectively. Optical sections of this modeled peritoneum were taken at 0.5-μm intervals. Orthogonal sections were then reconstructed (4).

Quantification of Endometrial Cell Invasion

We grew LP9 PMCs to confluence on MTGL- and GFR-MTGL–coated invasion chambers. Endometrial stromal cells or EM42 cells were grown to near-confluence, labeled with CellTracker Green, and placed over the LP9-covered membranes (25,000 cells per well). Preliminary experiments demonstrated that 25,000 endometrial cells per well produced a uniform distribution of endometrial cells without crowding or stacking of cells. The membranes were incubated at 37°C in 5% CO_2 in air, and cultures were interrupted at 24 hours. Cells not invaded, on the upper surface of the membranes, were mechanically removed with a cotton tip applicator, and the membranes were fixed in cold formaldehyde. Each invasion assay was run in triplicate.

The membranes were then treated with Hoechst 33342 (Invitrogen, Grand Island, NY), a fluorescent nuclear stain, to identify cell nuclei. The number of invaded cells on the bottom of the coated membranes was determined with a fluorescence microscope with a $\times$20 objective. Images were obtained from eight standardized, nonoverlapping fields representing approximately 40% of the total surface area. The number of invaded endometrial cells was counted by identifying a Hoechst 33342–labeled nucleus that was surrounded by CellTracker Green–labeled cytoplasm.

Evaluation of the Role of PMCs

Replicate cultures of ESCs and EM42 cells were placed on the MTGL- and GFR-MTGL–coated membranes, and invasion was evaluated in the absence of an overlying LP9-PMC monolayer. CellTracker Green–labeled ESCs or EM42 cells, 25,000 per well, were placed in the invasion chamber, and the invasion assay was performed as described in the previous subsection. The rate of invasion of ESCs and EM42 cells through MTGL- and GFR-MTGL–coated membranes with and without an overlying monolayer of LP9 PMCs was compared.