Secretion of monocyte chemotactic protein-1 by human uterine epithelium directs monocyte migration in culture

Richard A. Meter, M.D.,[a] *Charles R. Wira, Ph.D.,*[b] *and John V. Fahey, Ph.D.*[b]

[a] Department of Obstetrics and Gynecology, Dartmouth Hitchcock Medical Center; and [b] Department of Physiology, Dartmouth Medical School, Lebanon, New Hampshire

Objective: To determine whether primary human uterine epithelial cells in culture are able to influence monocyte chemotaxis and to establish whether the causal agent of chemotaxis is monocyte chemotactic protein (MCP)–1.
Design: Tissue culture study.
Setting: University medical center.
Patient(s): Women aged 23 to 53 years who were undergoing hysterectomy (n=7).
Intervention(s): Primary human endometrial epithelial cells were acquired from surgical specimens and grown to confluence and high transepithelial resistance. Conditioned media from epithelial cultures were analyzed for the presence of MCP-1 and for capacity to affect monocyte chemotaxis using the THP-1 monocyte line. Antibody neutralization of conditioned media was used to establish the role of MCP-1 in chemotaxis.
Main Outcome Measure(s): Assay of conditioned media for MCP-1, quantitative measurement of monocyte chemotaxis to conditioned media, and inhibition of chemotaxis by antibody neutralization of MCP-1.
Result(s): Primary endometrial epithelial cells in monolayer culture secrete MCP-1 to both the apical and basolateral compartments. Monocyte chemotactic protein-1 was identified as the primary agent of monocyte chemotaxis by antibody neutralization.
Conclusion(s): These findings suggest that biologically active MCP-1 is secreted into both the uterine lumen as well as the underlying stroma and that it mediates the presence of monocytes, macrophages, and other immune cells in the uterine endometrium. (Fertil Steril® 2005;84:191–201. ©2005 by American Society for Reproductive Medicine.)

Key Words: Uterine epithelial cells, endometrium, monocyte chemotaxis, monocyte chemotactic protein, MCP-1, THP-1

The innate immune system is present to structurally impede microbial invasion, recognize potential pathogens, mount a rapid local inflammatory response, and present portions of foreign protein antigens to the adaptive immune system (1). Mucosal epithelium is an integral part of the body's innate immune defense, providing both a passive barrier to potential pathologic organisms, and a sentinel system that detects pathogenic microbial challenge. Endometrial epithelial cells express cell surface and internal receptors to microbes and hormones which, when stimulated, can alter the epithelial cell's secretion of cytokines and chemokines (2). Changes in these cell signal concentrations can then attract, activate, and regulate immune effector cells of both the innate and adaptive immune systems. In addition to responding to potential pathogens and hormone fluctuations, cell signaling in the female reproductive tract is structured so as to provide a level of allogenic tolerance to spermatozoa, the blastocyst at implantation, and the fetus throughout pregnancy (3).

Though historically labeled as sterile, recent studies indicate that the upper female reproductive tract, including the uterus, fallopian tubes, and ovaries, periodically is exposed to bacteria and viruses, including potential pathogenic organisms (4). It is well known that the endometrium and fallopian tubes can be infected by disease-causing pathogens in semen. In addition, the endocervical canal is also often colonized by gram-negative and gram-positive organisms, both aerobes and anaerobes (5). When radiolabeled sperm-sized macrospheres or radio-opaque dye was placed in the vagina of women, transport to the uterus and fallopian tubes occurred as early as 1 minute to 2 hours, with extent of transport under cycle influence (6, 7). Clearly, microbes as well as microbial products and debris frequently move from the vagina or cervix into the uterine lumen.

Received August 31, 2004; revised and accepted January 13, 2005.
Supported by National Institutes of Health grant AI51877.
Reprint requests: Richard A. Meter, M.D., Dartmouth-Hitchcock Medical Center, Department of Obstetrics and Gynecology, Lebanon, New Hampshire 03756 (FAX: 603-650-6130; E-mail: richard.a.meter@hitchcock.org).

Copyright ©2005 American Society for Reproductive Medicine, Published by Elsevier Inc.

To combat this frequent exposure to potential pathogens, a growing body of evidence shows that endometrial epithelial cells form a primary line of defense in the innate immune system by expressing substantial numbers of cell receptors to common pathogen-associated molecular patterns (PAMPs). For example, endometrial epithelial cells express Toll-like receptors (TLRs), which initiate responses to a wide range of microbial antigens (2, 8). We have shown that primary uterine epithelial cells express TLRs 1 to 9 and produce cytokines when exposed to specific TLR agonists (47). In addition, we have shown that primary uterine epithelial cells secrete antimicrobial factors into apical secretions that are bactericidal to both gram-negative and gram-positive microorganisms (9).

Leukocytes and lymphocytes including neutrophils, macrophages, dendritic cells, CD56+ natural killer (NK) cells, CD4+ T cells, and CD8+ T cells have been demonstrated in the endometrium throughout the menstrual cycle (3, 10). Both leukocytes and lymphocytes, as well as epithelial cells, elaborate a spectrum of chemokines and cytokines as part of the body's response to bacterial and viral pathogens (11). It has been demonstrated previously that endometrial epithelial cells are capable of secreting numerous cytokines (12), as well as presenting antigen to T cells (13, 14).

Providing a relative barrier to cell migration, tight junctions enable the epithelial cells to form a contiguous monolayer. Immediately below the epithelial cells providing a second relative barrier is the basement membrane. Below these two laminar physical structures, various immune cells traffic in and out of the subepithelial areas, and there is evidence that their relative abundance and activity is under hormone influence (3). In the subepithelium, some of these various immune effector cells congregate to form lymphoid aggregates consisting of monocytes and macrophages, T cells, and B cells (10).

As an interface between the innate and adaptive immune systems, peripheral blood monocytes migrate into tissues and respond to chemokines secreted by epithelial and other cells at sites of injury or infection. Monocyte chemotactic protein (MCP)–1 is one of the major chemokines that is expressed by various sites throughout the body by epithelial cells, endothelial cells, smooth muscle cells, stromal cells, adipocytes, monocytes, lymphocytes, and fibroblasts (15–17). Monocyte chemotactic protein-1 is known to induce directional movement (chemotaxis) of immune effector cells including monocytes, macrophages, basophils, mast cells, dendritic cells, T cells, and NK cells (18–21). Once at the site of inflammation, monocytes mature into effector macrophages or dendritic cells to process and present antigens to local or distant immune effector cells (18).

Within the female reproductive tract of healthy women, endometrial epithelial and stromal cells express MCP-1 mRNA (22). As determined by immunohistochemistry, MCP-1 was also found in the endometrial glands of women with endometriosis (23) as well as in the endometrial glands of a group of infertility patients undergoing uterine pretransfer hormone treatment (16). When stimulated in culture with cytokines TNFα and IL-1β, MCP-1 has been detected by immunoprecipitation in secretions of uterine epithelial cells obtained from women with endometriosis; however, none was detected in healthy women (24). Monocyte chemotactic protein-1 also has been detected by ELISA in secretions of cultured endometrial epithelial cells from infertility patients, after patient stimulation with exogenous estrogen and progesterone (P) for donor embryo transfer (16). Most recently, MCP-1 has been detected in secretions from endometrial epithelial cell lines (25, 26). Acting as a chemoattractant, MCP-1 has been implicated in the physiologic processes of embryo implantation, maintenance or failure of pregnancy, preterm and term labor, preterm premature rupture of membranes, preterm chorioamnionitis, and the pathogenesis of endometriosis (24, 27–34).

The overall objective of the present study was to examine the role of uterine epithelial cells in regulating monocyte chemotaxis. The goals of this study were to [1] evaluate whether epithelial cells in culture secrete products which influence chemotaxis, [2] examine these secretions to identify the factor(s) responsible for chemotaxis, and [3] establish the role of MCP-1 in regulating chemotaxis.

MATERIALS AND METHODS

Uterine Tissue Collection

Uterine mucosal tissue was obtained immediately after surgery from women who had undergone hysterectomies at Dartmouth-Hitchcock Medical Center. Tissues used in this study were distal to the sites of pathology and were determined to be unaffected by disease upon inspection by a trained pathologist. Pathologists also determined the stage of the menstrual cycle of the patients. Tissues were transported from the pathology department on ice, and procedures to prepare purified epithelial sheets began within 2 hours of surgery. Approval to use these tissues was previously obtained from the Committee for the Protection of Human Subjects, Dartmouth Hitchcock Medical Center, and informed consent was obtained from the patients before surgery.

Isolation of Uterine Epithelial Cells

Epithelial cells were isolated as described elsewhere (35, 36). Briefly, tissues were minced under sterile conditions into 1- to 2-μm fragments and were subjected to enzymatic digestion using a PHC enzyme mixture, containing final concentrations of 3.4 mg/mL of pancreatin (Life Technologies, Grand Island, NY), 0.1 mg/mL of hyaluronidase (Worthington Biochemical Corporation, Freehold, NJ), 1.6 mg/mL of collagenase (Worthington), and 2 mg/mL of D-glucose, in Hanks' balanced salt solution (HBSS; Life Technologies) containing 50 U/mL of penicillin and 50 mg/mL of streptomycin. Enzymes were chosen to maximize digestion of the extracellular matrix while minimizing diges-

Download English Version:

https://daneshyari.com/en/article/9325234

Download Persian Version:

https://daneshyari.com/article/9325234

Daneshyari.com