

Role of the innate immunity in female reproductive tract

Fatemehsadat Amjadi^{1,2}, Ensieh Salehi², Mehdi Mehdizadeh³, Reza Aflatoonian⁴

Fatemehsadat Amjadi and Ensieh Salehi are equally first authors, ¹Applied Physiology Research Center and Department of Physiology, Isfahan University of Medical Sciences, Isfahan, ²Department of Anatomy, Tehran University of Medical Science, ³Department of Anatomy, Cellular and Molecular Research Center, Iran University of Medical Science, Iran, ⁴Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran.

Abstract

The mucosal immune system in the female reproductive tract (FRT) is well equipped to meet the sexually transmitted pathogens, allogeneic sperm, and the immunologically distinct fetus. Analysis of the FRT indicates that epithelial cells provide a physical barrier against pathogens and microbial infections as well as secretions containing anti-microbial peptides, cytokines, and chemokines which recruit and activate immune cells. Epithelial and immune cells confer protection in part through Toll-like receptors. The aim of this literature is to review the diverse components of the innate immune system, contributing to an exclusive protection system throughout the FRT.

Key Words: Anti-microbial peptides, cytokines and chemokines, female reproductive tract, immune cells, Toll-like receptors.

Address for correspondence:

Dr. Reza Aflatoonian, Department of Endocrinology and Female Infertility at Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, P.O. Box: 16635-148, No 12, Eastern Hafez Street, Bani Hashem Street, Resalat Highway, Tehran, Iran. E-mail: R.Aflatoonian@gmail.com

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INTRODUCTION

The main problem of worldwide health in reproductive field is sexually transmitted infections (STIs) and their associated diseases.^[1] In spite of sustained preventive activities, only limited success has been achieved in curtailing the reproductive complexity and mortality related to STIs.^[2]

Some complications of STIs with the largest prevalence and socio-economic burden include

infections by Herpes simplex virus type 2, group B streptococcus, *Treponema pallidum* (syphilis), bacterial vaginosis, Hepatitis B virus (hepatitis), *Neisseria gonorrhoeae* (gonorrhoea), *Chlamydia trachomatis*, and human immunodeficiency viruses (HIVs).^[3] Some of them like *C. trachomatis* are associated with cervicitis, ectopic pregnancy, pelvic inflammatory disease, tubal factor infertility, spontaneous abortion, and chronic pelvic pain.^[4] There are more than 20 pathogens transmissible through sexual intercourse.^[3] Protection against these pathogens and others in the female reproductive tract (FRT) is provided by immune system; thus, knowing better about the immune system can help to design novel strategies which may more effectively treat STIs.

The human immune system is fundamentally divided into two major sub-divisions, the innate or non-specific and the adaptive or specific immune system.

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Although both of them have a protective function against invading pathogens, they differ in time to react, the cells involved, effector mechanisms, type, and specificity of receptors.^[5,6] The innate immune system constitutes the first line of response to infection and incorporates rapidly after dealing with infectious agents, for this reason it has a pivotal role in host defense.^[5-7]

The innate immune system in the FRT consists of mechanical, chemical, and cellular components. Mucus lining and epithelial cells act as the mechanical barrier. The chemical barrier can be divided into natural anti-microbial peptides (AMPs) (NAPs) and pattern recognition receptors (PRRs), specially Toll-like receptors (TLRs).^[8] Briefly, NAPs are mainly produced by epithelial cells and neutrophils. They destroy target cells through abrogation of PH and ionic concentration gradients.^[9] TLRs are expressed on the immune cells, including neutrophils, macrophages, dendritic cells (DCs), dermal endothelial cells, and mucosal epithelial cells.^[10] They detect microbial-associated molecular patterns and gather a number of adapters to initiate intra-cellular signaling pathways in order to recruit the immune cells, to secrete anti-microbial factors eradicating pathogens, and finally, to facilitate adaptive immune responses.^[1,6,8,11]

The cellular components include inflammatory immune cells that migrate into the genital tract, as well as resident epithelial cells and stromal fibroblast.^[8]

The mucosal innate immune system of the FRT is not only involved in specialized physiological events, including menstruation, fertilization, implantation, pregnancy, and parturition, but also in protecting against sexually transmitted pathogens, as well as supporting allogeneic spermatozoa and an immunologically distinct fetus. To meet these challenges, the FRT has unique requirements as mentioned above, briefly.^[6] The purpose of this review article is to examine key mediators of innate immune defense that protect female genital tract against pathogens.

EPITHELIAL CELLS BARRIER, MUCUS

The FRT consists of three compartments: Lower part (vagina and ectocervix), transitional endocervix, and the upper part (endometrium and the fallopian tubes). All of them are covered by epithelial cells which, on one hand, provide a physical and immunological barrier to protect against invading micro-organisms, on the other hand, support the migration of sperm, ovum, and fetus.^[12] Integrity of the mucosal monolayer in the

upper FRT is preserved with tight junctions between columnar epithelial cells. However, the lower part is lined with multiple layers of stratified squamous epithelial cells, containing a loose connection.^[13] Entirety of mucosal epithelial barrier can be directly altered by sex hormones, cytokines, growth factors, TLR agonists, and pathogens.^[12,14,15] A lack of tight junctions in the lower part of the FRT may permit transition of intruder to intra-epithelial, which results in pathogens' counter with immune cells like CD4⁺T.^[16] On the other hand, PRRs, which are located on epithelial cells, detect antigens on these micro-organisms and then induce secretion of AMPs, cytokines, and chemokines. Totally, mucosal epithelial cells play important roles in innate immunity by: (I) formation of a physical and immunological barrier, (II) sending signals to the underlying immune system, (III) production of cytokine and chemokine, (IV) inducing death of infected cell through necrosis, apoptosis, or phagocytosis, (V) activating adaptive immunity, and (VI) development of an acute inflammatory reaction.^[17]

The epithelial cells of endometrium as well as vagina are covered by a layer of mucus, which maintains them from direct contact with infectious agents.^[13]

Most of the components of mucus are water and a family of high-molecular-weight glycoproteins, namely mucin, particularly mucin-1, which traps micro-organisms.^[18] Domino *et al.* showed in an animal model that cervical mucins have a protective role against *Candida albicans*.^[19] Human cervico-vaginal mucus provides a protective barrier blocking the spread of STIs from the vagina toward the upper FRT.^[13] Poor secretion of cervico-uterine mucus seems probably to be related to reduced fertility in women with cystic fibrosis.^[20] The properties and amount of the secreted mucus vary during the menstrual cycle under the influence of the sex hormones. Estrogenic mucus is present at the proliferative stage and increases at mid-cycle. It is less viscous and appears to provide a more favorable environment for sperm migration.^[21] Progestational mucus is present at high level following ovulation and low level during the menstrual and early proliferative phases. It is thick, sticky, and restricts the passage of sperm into the uterus.^[21] It can be concluded that epithelial cells and mucus are two key components of the physiological barrier which protects the FRT against pathogens.

TOLL-LIKE RECEPTORS

Rapid innate immune defense against infection usually involves the detection of pathogen-associated molecular patterns (PAMPs) and endogenous damage-associated molecular patterns (DAMPs) by specific PRRs.^[10,22,23]

Recent studies identified several classes of PRRs family including TLRs, nucleotide oligomerization domain (NOD)-like receptors (NLRs), retinoic acid-inducible gene I (RIG-I)-like receptors (RLRs) and cytosolic DNA sensors.^[24] Toll protein was discovered for its role in dorsoventral patterning of *Drosophila* embryos. Later investigations declared an important role of Toll in the fly's immune response to bacterial and fungal infections that have opened a new window for the mammalian homologues research.^[11,25] The first identified human TLR was TLR4.^[26] Structurally, the TLRs are comprised of an extra-cellular leucine-rich repeat domain that recognizes PAMPs and a cytoplasmic Toll/interleukin 1 receptor domain for downstream signaling transduction.^[27,28] TLRs have a critical role in the induction of immune and inflammatory responses in mammals.^[23] To date, at least 10 human TLRs and 13 mouse TLRs have been described^[11] TLR1, 2, 4, 5, and 6 are located on the plasma membrane and detect pathogen membrane components, while TLR3, 7, 8, and 9 are expressed in cytoplasmic organelles, mainly the endosomes, lysosomes, endolysosomes, and endoplasmic reticulum in order to detect pathogen nucleic acids.^[11,29] TLR1-9 are conserved between human and mice. Mouse TLR 11 is functional, but the human homolog has a stop codon that results in the lack of production.^[23] Each individual TLR has a distinct function in terms of PAMPs detection and immune responses.^[30] Examples of PAMPs include lipopolysaccharide (LPS), the major component of gram-negative bacterial outer membranes, peptidoglycan, and the major component of gram-positive bacterial cell walls, lipoproteins, zymosan, and nucleic acids.^[31] On the other hand, heat-shock protein 60 and 70, polysaccharide fragments of heparin sulfate, hyaluronic acid, fibrinogen, fibronectin DA domain, and mRNA are also categorized as DAMPs.^[8] Table 1 shows an overview of the cognate ligands for TLRs.^[29,30,32-40]

TLRs do not act alone. Their signal transduction is mediated by the recruitment of different intra-cellular adaptors [Table 1].^[11] Selective usage of these adaptor molecules causes differential responses mediated by these distinguished distinct TLR ligands.^[29] Two intra-cellular signaling cascades can be induced after TLR activation, MyD88-dependent cascades, which lead to secretion of pro-inflammatory cytokines or TRIF-dependent cascades, which induce type 1 interferon (IFN) as well as inflammatory cytokines and chemokines [Figure 1].^[30] After the discovery of TLRs, other PRRs, comprising of NLRs such as RLRs, were identified. Similar to TLRs, NLRs and RLRs have an important role in immune responses; however, in contrast to TLRs, they only detect microbial components in the cytosol. NLR family has

more than 20 members and is involved in response to the various PAMPs and PAMP particles through production of IL-1 β .^[24,30]

TLRs in the female reproductive tract

The mucosal epithelium of the genital tract serves as front line of defense against microbial infections. It has been thought the expression of TLRs on the epithelium plays an important role in antigen detection, initiation of immune response, and connection between innate and adaptive immunity.^[8] Recent studies have also supported the importance of TLRs activation in fertilization and implantation failure through stimulation of the innate immune system.^[41,42] Several research works have been done on the expression and role of these receptors in the FRT.^[2,43,44]

Constitutive expression of TLR1-10 in epithelial cells of fallopian tubes and endometrium has been reported.^[2,43,45,46] The presence of TLR1-9 has also been detected in vagina and cervix.^[43,47-49] TLR1-3, 6, 7, and 10 exist in uterine natural killer (NK) cells^[50,51] and TLR1 is present in vascular endothelial and smooth muscle cells of the cervix and uterus.^[10] Expression of TLR2-4, 7-10 has been shown in endometrial stroma.^[52,53] Other reports have demonstrated the existence of TLR5 in smooth muscle and vascular endothelial cells within the stroma of the vagina and endocervix [Figure 2].^[10]

The presence of TLR2 and TLR4 on amniotic epithelial cells has also been shown during pregnancy; however,

Table 1: Overview of used adaptor proteins by TLRs.

Adaptor protein	Ligand	Receptor number
MyD88/Mal	Triacyl lipopeptides, Pam ₃ Cys-Ser-(Lys) ₄	TLR1
MyD88/Mal	peptidoglycan, lipoprotein, Pam ₃ Cys-Ser-(Lys) ₄ , Zymosan, and lipoteichoic acid	TLR2
TRIF	dsRNA (virus), siRNA, endogenous mRNA, TLR3 and poly (I:C)	TLR3
TRIF/MyD88/Mal/TRAM	LPS, lipid A analogs, cryptococcal capsule, Aspergillus hyphae, respiratory syncytial virus Protein F, heat shock protein 60, 70 and fibronectin, and hyaluronic acid. LPS derived from <i>N. gonorrhoeae</i> , LPS and HSP derived from <i>C. trachomatis</i> and mannan derived from <i>C. albicans</i>	TLR4
MyD88	Flagellin	TLR5
MyD88/Mal	diacyl lipopeptide, soluble tuberculosis factor	TLR6
MyD88	ssRNA, imiquimod, resiquimod and loxoribine (anti-viral and anti-tumoral compounds)	TLR7
MyD88	ssRNA	TLR8
MyD88	Unmethylated CpG DNA, ssRNA	TLR9
MyD88	bacterial lipopeptide ligands	TLR10

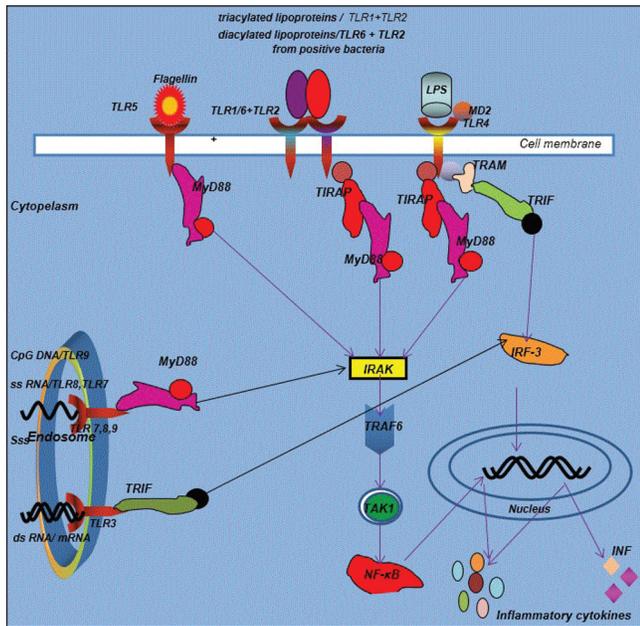


Figure 1: TLRs signaling pathway

the presence of TLR2 seems to be restricted to the basolateral side of these cells.^[54] TLR2 functions as a heterodimer with either TLR1 or TLR6 on the plasma membrane of both innate and adaptive immune cells. The TLR2/TLR1 heterodimers detect triacylated lipoproteins from gram-negative bacteria and mycoplasma, whereas TLR2/TLR6 heterodimers recognize diacylated lipoproteins from gram-positive bacteria and mycoplasma.^[30]

TLR4 proteins have been detected in term decidual inflammatory immune cells, such as neutrophils and macrophages.^[23]

The expression of TLR4 decreases from the upper genital tract toward cervix.

There is controversy about the presence of TLR4 in epithelial cells of the female genital tract. Some reports have declared the presence of TLR4 in the epithelial cells of the fallopian tubes, endometrium, endocervix, and vagina, while others have rejected this report.^[10,43,47,55,56]

TLR4 is involved in the response to LPS of gram-negative bacteria in association with CD14 and MD-2.^[11,57] CD14 is found in endometrial stromal fibroblasts, but not in endometrial and fallopian epithelial cells.^[56,58] Unlike epithelial cells in the upper part of FRT, epithelial cells of cervix and vagina express co-receptor CD14 (55). It was reported that MD2, an ancillary molecule of TLR4-signaling, was missing in cultured epithelial cells derived from normal human vagina, ectocervix, and endocervix.^[8,47] Recently, Packiam *et al.* have

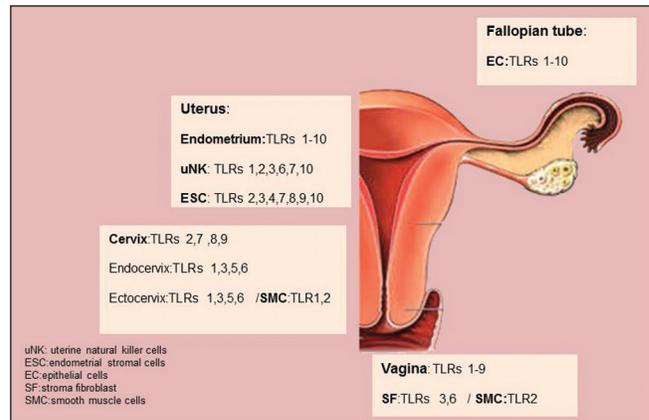


Figure 2: Localization of TLRs in the female reproductive tract

experimentally demonstrated that TLR4 has a protective role against gonococcal infection.^[59]

TLR3 activates by double-stranded RNA and mRNA from killed cells.^[60] It has been shown that TLR3 function can depend on sexual hormones. For example 17- β -estradiol inhibits cytokine and chemokine production which are already induced by TLR3 activation without any effect on TLR3 expression.^[61]

TLR9 recognizes CpG motifs in the genome of bacterial and viral pathogens. Production of IL-8 by cultured epithelial cells of fallopian tubes, uterine, and cervix increases in response to binding CpG oligonucleotides to TLR9.^[49]

Aflatoonian *et al.* have demonstrated the cycle-dependent expression of TLR1-10 in human endometrium. They declared that relative expressions of TLR2-6, 9, and 10 were significantly higher during the secretory phase compared to other phases of the menstrual cycle. According to these findings, we can probably conclude the inhibitory effect of estrogen or the protective effect of progesterone in the genital tract, especially in the endometrium.^[2,62]

NATURAL ANTI-MICROBIAL PEPTIDES

Synthesis of peptides and small proteins with anti-microbial activity is generally emerged as the most ancient primary mechanism of the immune system.^[9] NAPs possess additional functions apart from microbicidal activity, including cell proliferation, cytokine induction, chemotaxis, and modulation of innate and acquired immunity.^[63]

Endogenous AMPs have redundancy and synergism together, so these properties provide better protection in comparison to a single factor.^[64] Major NAPs with different structural and functional characteristics

include defensin, elafin, cathelicidin, secretory leukocyte protease inhibitor (SLPI), lysozyme, and lactoferrin. They are mainly produced by epithelial cells and neutrophils^[9] and regulated by bacterial production and inflammation.^[65] It has shown that AMPs similar to antibodies, cytokines, and chemokines vary at different times during the menstrual cycle reflecting endocrine regulation. Also, it is noticeable that biological activity of antimicrobials can change with pH, salt, serum, and presence of sperm.^[3,66,67] NAPs can interact with cell membrane of pathogens based on the charge, then forming pores that destroy target cell through abrogation of pH and ionic concentration gradients.^[9] Together, NAPs constitute an important chemical barrier which orchestrates immune responses against foreign micro-organisms.

Defensins

One of the most prominent NAPs at the mucosal surface is defensins. Two main functional sub-families of them are α and β -defensins. Six α -defensins have been recognized in humans: (I) HNP (human neutrophil peptide) 1-4 and (II) HD5,6 (human defensin). Leukocytes and epithelial cells are the main sources of HDs.^[1] α -Defensins have anti-bacterial activity against gram-negative and gram-positive bacteria, fungi, yeast, and anti-viral effects against HIV-1, 2, and HSV-1; however, α -defensins 5 and 6 increase HIV infection.^[3] Six human β -defensins, HBD1 to 6 have been identified, which are structurally similar to α -defensins. Four of them are expressed by mucosa and epithelial cells of the female genital tract.^[3,68,69] They have anti-viral activity and decrease level of HIV-1 CXCR4 co-receptor.^[70,71] Several studies have examined the presence and role of defensins in the FRT at different stages of menstrual cycle.^[1,72,73] It has been shown that HBDs1-4 and α -defensing 5 are expressed in the endometrial epithelium. HBDs1, 3, and 5 are at maximal concentration during the secretory phase while HBD4 reaches peak in the proliferative phase and HBD2 is highest during the menstruation.^[1,73] Within the cervico-vaginal lavages (CVL), HNPs1-3 and HBD2 are maximum during the proliferative phase and minimum at mid-cycle.^[74]

During pregnancy, endogenous anti-microbials can play a critical role in preservation of uterine health and prevention of its infection. Expression of α - and β -defensins has been detected in the amnion epithelium, chorion, decidua, trophoblast, and cervical mucus plug, during pregnancy. In addition, changes in vaginal microflora are related to defensins at mid-pregnancy.^[75]

Elafin and secretory leukocyte protease inhibitor

SLPI and elafin from whey acidic protein family were introduced as human protease inhibitors.^[76]

SLPI is synthesized by macrophages and epithelial cells. It suppresses elastase and cathepsin G, but not proteinase3, while elafin is inhibitor for elastase and proteinase3. The anti-protease effect of these peptides can restrict host tissue damage from an unregulated inflammation, in part mediated by proteases.^[9]

SLPI has anti-bacterial activity (against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) and anti-fungal activity (against *Aspergillus fumigatus* and *C. albicans*).^[77] Elafin and SLPI also have anti-HIV activity in vaginal fluid that is independent of their protease inhibitory role.^[78,79] SLPI and elafin are present throughout FRT.^[1] SLPI expression has been detected in the vagina, cervix, amnion, vernix caseosa, uterus pregnant, and decidua and at very high level in cervical mucus (1000 $\mu\text{g}/\text{mL}$).^[80,81] Elaphin expresses in the vagina, cervix, uterus pregnant, fetal membranes, and placenta just at term pregnancy.^[3] Endometrial neutrophils are rich source of Elafin during menstruation. SLPI and elafin are expressed during pregnancy probably for anti-inflammatory, anti-protease, and anti-microbial properties.^[1]

Cathelicidin LL37, lactoferrin, and lysozyme

Another component of FRT secretions is cathelicidin. In humans, LL37 is only cathelicidin, which is produced by neutrophils and epithelial cells of the lower FRT.^[3,9] LL37 is found in vaginal fluid and cervical mucus. It counters with bacteria and fungi which may have been introduced by intercourse.^[81]

Main sources of lactoferrin are neutrophils and epithelial secretions. It has been found in vaginal fluid (1 $\mu\text{g}/\text{mL}$) and cervical mucus (100 $\mu\text{g}/\text{mL}$).^[82] It has anti-viral and anti-bacterial effects (against gram-negative bacteria), directly or by sequestration of iron essential for microbes under acidic conditions, such as lower part of FRT.^[81-83] Lactoferrin displays synergism with lysozyme that promotes innate immune protection in the FRT.^[7]

Lysozyme is synthesized by neutrophils and detected in vaginal fluid (13 $\mu\text{g}/\text{mL}$) and mucus plug (1 mg/mL).^[82] In addition to enzymatic lysis of peptidoglycan present on bacterial cell walls, lysozyme can kill bacteria by a non-enzymatic mechanism. Although lysozyme has an anti-bacterial effect against gram-positive species, for example streptococci, but it is ineffective against gram-negative bacteria.^[84] It also blocks HIV-1 viral entry and its replication.^[85,86]

CYTOKINES AND CHEMOKINES

Cytokines are small pleiotropic glycoprotein mediators whose biological actions are locally mediated

by specific receptors.^[75] Chemokines are small chemotactic cytokines, very locally acting, well known for their function in leukocyte recruitment to sites of inflammation and their activation.^[87] Chemokines attract immune cells to the tissue, while cytokines differentiate and activate these cells.^[6] Several studies have demonstrated the constitutive secretion of numerous cytokines, including granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), TNF- α , IL-1, IL-6, leukemia inhibitory factor (LIF), TGF- β , and of chemokines such as MIP-1 β , monocyte chemoattractant protein-1 (MCP-1), and IL-8 by polarized epithelial cell from the cervix, uterus, and fallopian tubes.^[12,88-91] Most of these inflammatory mediators were preferentially secreted into the apical/luminal compartment resulting in a gradient for stimulation and attracting immune cells to the epithelial surface.^[12,92] For example, IL-8 produced by uterine epithelial cells induces neutrophil migration across the epithelium.^[92,93] Also, MCP-1 and MIP-1 β are potent chemoattractants for monocytes and T cells, respectively. Other cytokines, such as TGF β , secreted into the baso-lateral/sub-epithelial compartment of uterus, affect function and development of immune cells.^[94,95] The cytokines TNF α , IL-6, GM-CSF, and G-CSF trigger differentiation of leukocytes to more active pro-inflammatory cells.^[12] In addition to chemotactic activity, IL-8 participates in proliferation and angiogenesis during early to mid-secretory phase, as well as in apoptosis during menstruation.^[91] The type I IFNs are other important cytokine family involved in FRT immunity, particularly against viruses.^[96] IFNs are immediately induced in counter with viral and bacterial pathogens.^[60,97] IFN β is induced in uterine epithelial cells by the double-stranded viral agonist poly (I:C). In the FRT, IFN β induces expression of the anti-HIV molecules, like MIP3 α /CCL20 and hBD2, showing its protective role against HIV-1 infection.^[13] Immune cells, including monocytes, macrophages, NK cells, and DCs, are also the sources of immunoregulatory cytokines and chemokines in the FRT.^[98,99] The secretion of chemokines and cytokines is modulated by autocrine and paracrine manners, as well as sex hormones. The hormonal effect on their secretion is direct or indirect.^[12,13] For instance, progesterone depletion leads to the up-regulation of IL-8, MCP-1, and COX-2, resulting in the activation of monocytes and neutrophils and finally, up-regulation of matrix metalloproteinases for initiation of menstruation.^[100] As an indirect effect, estradiol treatment leads to up-regulation of hepatocyte growth factor (HGF) secretion that in turn regulates TNF α and MIP3 α /CCL20 production by uterine epithelial cells.^[101-103] Thus, concentrations of chemokines and cytokines will vary in the endometrium during

normal physiological processes, as well as pathological conditions, such as infection and endometriosis.^[92] Production of chemokines and cytokines by uterine epithelial cells may associate with pathological conditions during pregnancy. For example, there is a relationship between elevated concentrations of IL-6, IL-8, and MCP-1 and amniotic microbial infection in cervical and amniotic fluids from patients with spontaneous preterm birth.^[104-106] In contrast, low levels of IL-1 β , IL-8, and IL-6 in cervical fluid are correlated with clinical chorioamnionitis in early pregnancy.^[107] So, low concentrations of cytokines create a permissive environment for ascending infection. It has been recommended that the levels of specific chemokine(s) and cytokine(s) in the FRT during pregnancy should be monitored where is a high risk of preterm labor.^[108] Expressions of cytokines, chemokines, and adhesion molecule are also critical for endometrial growth in preparation for fertilization, implantation, and successful pregnancy, but also for the remodeling of the uterus during each menstrual cycle that is regulated by the sex hormones.^[109-111]

Collectively, cytokines and chemokines, as chemical messengers, provide an immunological environment hostile to pathogen survival and maintain the normal homeostatic.^[91] Their secretion leads to rapid communication between the different immune cells which are present in the FRT.^[13] Innate immune cells provide another line of defense.

INNATE IMMUNE CELLS

Macrophage

As professional phagocytes, macrophages play an important role in the pathogens recognition, removal of debris, and indirect stimulation of the immune system by cytokines and chemokines production along with all aspects of inflammatory responses. These versatile cells are widely distributed throughout the human FRT (constitute 10% of the leukocytes population in the FRT).^[6,112] The numbers of endometrial macrophages increase prior to menstruation, and also macrophage chemoattractants, like MCP-1, FKN, and MIP-1 β , are up-regulated peri-menstrually.^[6,113] Accumulation of endometrial macrophages also occurs across the mid-secretory phase of the cycle, while numbers of vaginal macrophages remain constant during the menstrual cycle.^[13] Increasing evidences suggest that the migration of macrophages into endometrium is modulated by estradiol and progesterone.^[114-116] Physiologic levels of estrogen induce macrophage proliferation and function.^[117] Tissue macrophages have different phenotypic characteristics, reflecting the unique local micro-environment which they have been exposed.^[6] For example, vaginal macrophages

express higher levels of the HIV-1 receptor CD4, CCRs, and CXCR4.^[118,119] Macrophages have also been identified as key modulators of ovarian function through regulation of folliculogenesis and atresia.^[120] They are most numerous during ovulation as an inflammatory reaction.^[117] Decidual macrophages can participate in diverse activities during pregnancy.^[121] They are classified as M1- and M2-types which take part, respectively, in progression of inflammation and immune tolerance during pregnancy. A balance of them may contribute to the outcome of pregnancy.^[121]

Dendritic cells

DCs, as the major antigen-presenting cells in the FRT, seem to make a link between innate and adaptive immunity. They are present in the sub-epithelial stroma of the endometrium. In contrast, vaginal DCs are localized to the epithelial layer.^[122] Exposure to pathogens and inflammatory stimuli, such as LPS, lead to maturation of DCs which are characterized by CD38 marker expression and IL12 production. Mature DCs facilitate the development of T-helper 1 (Th1) cells.^[122] The roles of DCs are to prevent infection by direct inactivation or the stimulation of adaptive immunity. However, new findings have suggested the expression of DC-SIGN by these cells could increase susceptibility of women to HIV infections.^[123] DCs are not only essential for the induction of primary immune responses, but are also important for the induction of immunological tolerance and maintenance of successful pregnancy.^[124,125] They are recruited into the endometrium and accumulated especially around the implanted embryo.^[124] The function and differentiation of DCs are regulated by the local microenvironment determined by cytokines, chemokines, and estroied hormones.^[124] Estradiol has been shown preferentially through a promotion of a specific sub-set of DCs differentiation, characterized by high surface expression of MHC class II and CD86.^[126]

Natural killer cells

NK cells, as the key innate immune cells, use a variety of effector mechanisms to promote host immune defenses, while eliminating virus-infected cells and tumor cells by secretion of cytotoxic products.^[127] Defects in NK cell activity are associated with increased infections particularly, herpes viral infections, ovarian cancer, uterine cancer, and endometriosis,^[6,128-131] whereas elevated NK-cell activity has been associated with recurrent pregnancy loss.^[132,133] NK cells have the ability to amplify an inflammatory response and to promote macrophage activation, generation of cytotoxic T cells, recognition of fungal infections, and cytokine production.^[6,134,135] Uterine NK cells (uNKs) express several TLRs in particular TLR2, TLR3, and TLR4 which can respond to TLR agonists by producing

cytokines.^[123] Variety of cytokines, including IL12, IL8, IL15, IL1 β , or IFN α in combination with PAMPs will activate NK cell cytokine production, leading to further activation of innate immunity.^[136-138] The number of endometrial NK cells are low in the early proliferative phase and increase as the menstrual cycle progresses, reaching a peak in the late secretory phase.^[112,139,140] However, NK cell numbers in other regions of the FRT are not affected across the menstrual cycle.^[13] Also, an increase in number of endometrial NK cells increase during early pregnancy reaches a maximum at the end of the first trimester and a minimum at term.^[141] It shows an important role of these cells in the establishment and maintenance of pregnancy. At least, two theories have been proposed for the increase of uNK cells within the uterus: *In situ* proliferation and recruitment from the peripheral NK cells blood.^[141-144] Estradiol regulates NK cells activity *via* endogenous TGF β .^[123] Several reports suggest that IL15 is also required for uNK cells survival, proliferation, and differentiation into decidual NK cells.^[144-149] The uNK cells have a distinct phenotype from blood NK cells.^[150] Unlike blood NK cells, uNK cells express CD9 and CD69 on their cell surface.^[99,151-153] It has been shown the cell-surface phenotype of NK cells is different within the FRT. For example, CD69 and CD96 are both expressed by NK cells in the endocervix and endometrium, but not in the ectocervix.^[150] Uterine NK cells, not blood NK cells produce some essential cytokines for implantation such as, angiogenic growth factors (vascular endothelial growth factor (VEGF), Placental growth factor (PLGF), Angiopoietin2) and leukemia inhibitory factor (LIF).^[13,154,155] Finally, it can be concluded that uNK cells are involved in several processes, including host defense, decidualization, implantation, and pregnancy.^[141-143]

Neutrophil

Neutrophils are present in all tissues of the FRT and possess many effector mechanisms for mediating innate immunity.^[156] Under the influence of chemokines gradient,^[157,158] neutrophils can cross the endothelial barrier, eliminate pathogens by phagocytosis, and produce toxic oxygen and nitrogen species, as well as release cytokines and anti-microbial compounds, such as defensin-serine proteases.^[159-161] IL8 is a major neutrophil chemoattractant.^[162] IL8 and GM-CSF, secreted by epithelial cells, cause to bring neutrophils toward the epithelium or cross the epithelial barrier into the lumen.^[6] Insemination also causes a great influx of neutrophils into the uterine lumen to remove superfluous sperm, microorganisms, and seminal debris. This migration is accompanied by accumulation of macrophages, DCs, granulocytes, and lymphocytes in the endometrial stroma to maintain uterine sterility.^[163] In contrast

to NK cells, neutrophil numbers are highest in the fallopian tubes whereas progressively decrease from the upper FRT into the lower regions of the tract.^[112] In spite of the most numerous neutrophil in the fallopian tubes, their exact role remains to be studied.^[164] The number of neutrophils in vagina has been shown to be stable throughout the cycle, similar to T cells and macrophages, except in vaginal fluid from infected women.^[165,166] Also, it has been declared that neutrophils count does not fluctuate across the menstrual cycle, but at menses sharply increase in the endometrium, which is preceded by a surge in IL-8.^[13] At menses, breakdown of endometrial tissue is done by neutrophils *via* the release of elastase which activates matrix-metalloproteinases.^[6,13] Some evidence suggest that exposure to different cytokines within FRT tissue can arise a different neutrophil population by altering their function and receptor expression.^[167] For example, fallopian tube neutrophils express higher level of CD15 marker which may be important in innate immune defense of the fallopian tube.^[168]

Our knowledge regarding immune defense mechanisms in the FRT remains limited. By further studies, new avenues may be identified both to protect against pathogens and to improve the quality of woman's reproductive health.

CONCLUSIONS AND FUTURE PERSPECTIVES

Growing body of data about the FRT demonstrates the presence of a complex system of immune protection. Mucus lining, a tight epithelial barrier, the secretion of AMPs and cytokines by epithelial and innate immune cells, and expression of TLRs throughout the reproductive tract indicate that the FRT has evolved to meet the challenges of STIs and to minimize the risk of infection in order to support an allogeneic fetus. This review confers the opportunity of understanding the unique immunological characteristics of the female genital tract, and also highlights the need for further researches. Finally, we hope to provide new approaches into design novel therapeutic means for the female reproductive diseases associated with the innate immune system.

REFERENCES

- Horne AW, Stock SJ, King AE. Innate immunity and disorders of the female reproductive tract. *Reproduction* 2008;135:739-49.
- Aflatoonian R, Tuckerman E, Elliott SL, Bruce C, Aflatoonian A, Li TC, et al. Menstrual cycle-dependent changes of Toll-like receptors in endometrium. *Hum Reprod* 2007;22:586-93.
- Wira CR, Patel MV, Ghosh M, Mukura L, Fahey JV. Innate immunity in the human female reproductive tract: Endocrine regulation of endogenous antimicrobial protection against HIV and other sexually transmitted infections. *Am J Reprod Immunol* 2011;65:196-211.
- Morré SA, Rozendaal L, van Valkengoed IG, Boeke AJ, van Voorst Vader PC, Schirm J, et al. Urogenital *Chlamydia trachomatis* serovars in men and women with a symptomatic or asymptomatic infection:

An association with clinical manifestations? *J Clin Microbiol* 2000;38:2292-6.

- Mackay I, Rosen FS. Advances in immunology. *N Engl J Med* 2000;343:338-44.
- Wira CR, Fahey JV, Sentman CL, Pioli PA, Shen L. Innate and adaptive immunity in female genital tract: Cellular responses and interactions. *Immunol Rev* 2005;206:306-35.
- Janeway CA Jr, Medzhitov R. Innate immune recognition. *Annu Rev Immunol* 2002;20:197-216.
- Nasu K, Narahara H. Pattern recognition *via* the toll-like receptor system in the human female genital tract. *Mediators Inflamm* 2010;2010:976024.
- Wiesner J, Vilcinskis A. Antimicrobial peptides: The ancient arm of the human immune system. *Virulence* 2010;1:440-64.
- Fazeli A, Bruce C, Anumba DO. Characterization of Toll-like receptors in the female reproductive tract in humans. *Hum Reprod* 2005;20:1372-8.
- Thompson MR, Kaminski JJ, Kurt-Jones EA, Fitzgerald KA. Pattern recognition receptors and the innate immune response to viral infection. *Viruses* 2011;3:920-40.
- Ochiel DO, Fahey JV, Ghosh M, Haddad SN, Wira CR. Innate Immunity in the female reproductive tract: Role of sex hormones in regulating uterine epithelial cell protection against pathogens. *Curr Womens Health Rev* 2008;4:102-17.
- Hickey DK, Patel MV, Fahey JV, Wira CR. Innate and adaptive immunity at mucosal surfaces of the female reproductive tract: Stratification and integration of immune protection against the transmission of sexually transmitted infections. *J Reprod Immunol* 2011;88:185-94.
- Capaldo CT, Nusrat A. Cytokine regulation of tight junctions. *Biochim Biophys Acta* 2009;1788:864-71.
- Fahey JV, Wright JA, Shen L, Smith JM, Ghosh M, Rossoll RM, et al. Estradiol selectively regulates innate immune function by polarized human uterine epithelial cells in culture. *Mucosal Immunol* 2008;1:317-25.
- Hladik F, Sakchalathorn P, Ballweber L, Lentz G, Fialkow M, Eschenbach D, et al. Initial events in establishing vaginal entry and infection by human immunodeficiency virus type-1. *Immunity* 2007;26:257-70.
- Farage MA, Miller KW, Gerberick GF, Saito FH, Ledger WJ, Witkin SS. Innate immunity in the lower female mucosal tract. *J Steroids Hormon Sci* 2011;2:106.
- Carson DD, DeSouza MM, Kardon R, Zhou X, Lagow E, Julian J. Mucin expression and function in the female reproductive tract. *Hum Reprod Update* 1998;4:459-64.
- Domino SE, Hurd EA, Thomsson KA, Karnak DM, Holmen Larsson JM, Thomsson E, et al. Cervical mucins carry $\alpha(1,2)$ fucosylated glycans that partly protect from experimental vaginal candidiasis. *Glycoconj J* 2009;26:1125-34.
- Muchekehu RW, Quinton PM. A new role for bicarbonate secretion in cervico-uterine mucus release. *J Physiol* 2010;588:2329-42.
- Vigil P, Cortés ME, Zúñiga A, Riquelme J, Ceric F. Scanning electron and light microscopy study of the cervical mucus in women with polycystic ovary syndrome. *J Electron Microscop* (Tokyo) 2009;58:21-7.
- Kumar H, Kawai T, Akira S. Pathogen recognition by the innate immune system. *Int Rev Immunol* 2011;30:16-34.
- Aflatoonian R, Fazeli A. Toll-like receptors in female reproductive tract and their menstrual cycle dependent expression. *J Reprod Immunol* 2008;77:7-13.
- Kanneganti TD, Lamkanfi M, Núñez G. Intracellular NOD-like receptors in host defense and disease. *Immunity* 2007;27:549-59.
- Kaisho T, Akira S. Toll-like receptor function and signaling. *J Allergy Clin Immunol* 2006;117:979-87; quiz 88.
- Medzhitov R, Preston-Hurlburt P, Janeway CA Jr. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature* 1997;388:394-7.
- Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: Update on Toll-like receptors. *Nat Immunol* 2010;11:373-84.

28. Heil F, Hemmi H, Hochrein H, Ampenberger F, Kirschning C, Akira S, *et al.* Species-specific recognition of single-stranded RNA *via* toll-like receptor 7 and 8. *Science* 2004;303:1526-9.
29. Akira S, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006;124:783-801.
30. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: Update on Toll-like receptors. *Nat Immunol* 2010;11:373-84.
31. Medzhitov R, Janeway CA Jr. Decoding the patterns of self and nonself by the innate immune system. *Science* 2002;296:298-300.
32. Wetzler LM. The role of Toll-like receptor 2 in microbial disease and immunity. *Vaccine* 2003;21(Suppl 2):S55-60.
33. Naumann M, Wessler S, Bartsch C, Wieland B, Meyer TF. *Neisseria gonorrhoeae* epithelial cell interaction leads to the activation of the transcription factors nuclear factor κ B and activator protein 1 and the induction of inflammatory cytokines. *J Exp Med* 1997;186:247-58.
34. Netea MG, Van der Graaf C, Van der Meer JW, Kullberg BJ. Recognition of fungal pathogens by Toll-like receptors. *Eur J Clin Microbiol Infect Dis* 2004;23:672-6.
35. Ohashi K, Burkart V, Flohé S, Kolb H. Cutting edge: Heat shock protein 60 is a putative endogenous ligand of the toll-like receptor-4 complex. *J Immunol* 2000;164:558-61.
36. Kawai T, Akira S. Innate immune recognition of viral infection. *Nat Immunol* 2006;7:131-7.
37. Pasare C, Medzhitov R. Toll-like receptors: Linking innate and adaptive immunity. *Microbes Infect* 2004;6:1382-7.
38. Zhang D, Zhang G, Hayden MS, Greenblatt MB, Bussey C, Flavell RA, *et al.* A toll-like receptor that prevents infection by uropathogenic bacteria. *Science* 2004;303:1522-6.
39. Chuang T, Ulevitch RJ. Identification of hTLR10: A novel human Toll-like receptor preferentially expressed in immune cells. *Biochim Biophys Acta* 2001;1518:157-61.
40. Hemmi H, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, *et al.* A Toll-like receptor recognizes bacterial DNA. *Nature* 2000;408:740-5.
41. Caballero I, Al Ghareeb S, Basatvat S, Sanchez-Lopez JA, Montazeri M, Maslehat N, *et al.* Human trophoblast cells modulate endometrial cells nuclear factor κ B response to flagellin *in vitro*. *PLoS One* 2013;8:e39441.
42. Fujita Y, Mihara T, Okazaki T, Shitanaka M, Kushino R, Ikeda C, *et al.* Toll-like receptors (TLR) 2 and 4 on human sperm recognize bacterial endotoxins and mediate apoptosis. *Hum Reprod* 2011;26:2799-806.
43. Pioli PA, Amiel E, Schaefer TM, Connolly JE, Wira CR, Guyre PM. Differential expression of Toll-like receptors 2 and 4 in tissues of the human female reproductive tract. *Infect Immun* 2004;72:5799-806.
44. Darville T, O'Neill JM, Andrews CW Jr, Nagarajan UM, Stahl L, Ojcius DM. Toll-like receptor-2, but not Toll-like receptor-4, is essential for development of oviduct pathology in chlamydial genital tract infection. *J Immunol* 2003;171:6187-97.
45. Schaefer TM, Desouza K, Fahey JV, Beagley KW, Wira CR. Toll-like receptor (TLR) expression and TLR-mediated cytokine/chemokine production by human uterine epithelial cells. *Immunology* 2004;112:428-36.
46. Ghosh M, Schaefer TM, Fahey JV, Wright JA, Wira CR. Antiviral responses of human fallopian tube epithelial cells to toll-like receptor 3 agonist poly(I:C). *Fertil Steril* 2008;89(5 Suppl):1497-506.
47. Fichorova RN, Cronin AO, Lien E, Anderson DJ, Ingalls RR. Response to *Neisseria gonorrhoeae* by cervicovaginal epithelial cells occurs in the absence of toll-like receptor 4-mediated signaling. *J Immunol* 2002;168:2424-32.
48. Andersen JM, Al-Khairi D, Ingalls RR. Innate immunity at the mucosal surface: Role of toll-like receptor 3 and toll-like receptor 9 in cervical epithelial cell responses to microbial pathogens. *Biol Reprod* 2006;74:824-31.
49. Hart KM, Murphy AJ, Barrett KT, Wira CR, Guyre PM, Pioli PA. Functional expression of pattern recognition receptors in tissues of the human female reproductive tract. *J Reprod Immunol* 2009;80:33-40.
50. Sentman CL, Wira CR, Eriksson M. NK cell function in the human female reproductive tract. *Am J Reprod Immunol* 2007;57:108-15.
51. Gregg CR, Melly MA, Hellerqvist CG, Coniglio JG, McGee ZA. Toxic activity of purified lipopolysaccharide of *Neisseria gonorrhoeae* for human fallopian tube mucosa. *J Infect Dis* 1981;143:432-9.
52. Pivarcsi A, Nagy I, Koreck A, Kis K, Kenderessy-Szabo A, Szell M, *et al.* Microbial compounds induce the expression of pro-inflammatory cytokines, chemokines and human β -defensin-2 in vaginal epithelial cells. *Microbes Infect* 2005;7:1117-27.
53. Hirata T, Osuga Y, Hamasaki K, Hirota Y, Nose E, Morimoto C, *et al.* Expression of toll-like receptors 2, 3, 4, and 9 genes in the human endometrium during the menstrual cycle. *J Reprod Immunol* 2007;74:53-60.
54. Abrahams VM, Bole-Aldo P, Kim YM, Straszewski-Chavez SL, Chaiworapongsa T, Romero R, *et al.* Divergent trophoblast responses to bacterial products mediated by TLRs. *J Immunol* 2004;173:4286-96.
55. Herbst-Kralovetz MM, Quayle AJ, Ficarra M, Greene S, Rose WA 2nd, Chesson R, *et al.* Quantification and comparison of toll-like receptor expression and responsiveness in primary and immortalized human female lower genital tract epithelia. *Am J Reprod Immunol* 2008;59:212-24.
56. Itoh H, Nasu K, Nishida M, Matsumoto H, Yuge A, Narahara H. Human oviductal stromal fibroblasts, but not oviductal epithelial cells, express Toll-like receptor 4: The site-specific mucosal immunity of the human fallopian tube against bacterial infection. *Am J Reprod Immunol* 2006;56:91-101.
57. Akashi-Takamura S, Miyake K. TLR accessory molecules. *Curr Opin Immunol* 2008;20:420-5.
58. Hirata T, Osuga Y, Hirota Y, Koga K, Yoshino O, Harada M, *et al.* Evidence for the presence of toll-like receptor 4 system in the human endometrium. *J Clin Endocrinol Metab* 2005;90:548-56.
59. Packiam M, Wu H, Veit SJ, Mavrogiorgos N, Jerse AE, Ingalls RR. Protective role of Toll-like receptor 4 in experimental gonococcal infection of female mice. *Mucosal Immunol* 2012;5:19-29.
60. Schaefer TM, Fahey JV, Wright JA, Wira CR. Innate immunity in the human female reproductive tract: Antiviral response of uterine epithelial cells to the TLR3 agonist poly(I:C). *J Immunol* 2005;174:992-1002.
61. Lesmeister MJ, Jorgenson RL, Young SL, Misfeldt ML. 17β -estradiol suppresses TLR3-induced cytokine and chemokine production in endometrial epithelial cells. *Reprod Biol Endocrinol* 2005;3:74.
62. Aboussahou W, Aflatoonian R, Bruce C, Elliott S, Ward J, Newton S, *et al.* Expression and function of Toll-like receptors in human endometrial epithelial cell lines. *J Reprod Immunol* 2009;84:41-51.
63. Bowdish DM, Davidson DJ, Hancock RE. Immunomodulatory properties of defensins and cathelicidins. *Curr Top Microbiol Immunol* 2006;306:27-66.
64. Agerberth B, Gudmundsson GH. Host antimicrobial defence peptides in human disease. *Curr Top Microbiol Immunol* 2006;306:67-90.
65. Tjabringa GS, Vos JB, Olthuis D, Ninaber DK, Rabe KF, Schalkwijk J, *et al.* Host defense effector molecules in mucosal secretions. *FEMS Immunol Med Microbiol* 2005;45:151-8.
66. Hazlett L, Wu M. Defensins in innate immunity. *Cell Tissue Res* 2011;343:175-88.
67. Mackewicz CE, Yuan J, Tran P, Diaz L, Mack E, Selsted ME, *et al.* α -Defensins can have anti-HIV activity but are not CD8 cell anti-HIV factors. *AIDS* 2003;17:F23-32.
68. Doss M, White MR, Teclé T, Hartshorn KL. Human defensins and LL-37 in mucosal immunity. *J Leukoc Biol* 2009;87:79-92.
69. Ganz T. Defensins: Antimicrobial peptides of innate immunity. *Nat Rev Immunol* 2003;3:710-20.
70. Sun L, Finnegan CM, Kish-Catalone T, Blumenthal R, Garzino-Demo P, La Terra Maggiore GM, *et al.* Human β -defensins suppress human immunodeficiency virus infection: Potential role in mucosal protection. *J Virol* 2005;79:14318-29.
71. Weinberg A, Quiñones-Mateu ME, Lederman MM. Role of human β -defensins in HIV infection. *Adv Dent Res* 2006;19:42-8.
72. Valore EV, Park CH, Igrati SL, Ganz T. Antimicrobial components of vaginal fluid. *Am J Obstet Gynecol* 2002;187:561-8.
73. King AE, Critchley HO, Sallenave JM, Kelly RW. Elafin in human endometrium: An antiprotease and antimicrobial molecule expressed during menstruation. *J Clin Endocrinol Metab* 2003;88:4426-31.
74. Keller MJ, Guzman E, Hazrati E, Kasowitz A, Cheshenko N, Wallenstein S, *et al.* PRO 2000 elicits a decline in genital tract immune mediators

- without compromising intrinsic antimicrobial activity. *AIDS* 2007;21:467-76.
75. Balu RB, Savitz DA, Ananth CV, Hartmann KE, Miller WC, Thorp JM, *et al.* Bacterial vaginosis and vaginal fluid defenses during pregnancy. *Am J Obstet Gynecol* 2002;187:1267-71.
 76. Moreau T, Baranger K, Dadé S, Dallet-Choisy S, Guyot N, Zani ML. Multifaceted roles of human elafin and secretory leukocyte proteinase inhibitor (SLPI), two serine protease inhibitors of the chelonianin family. *Biochimie* 2008;90:284-95.
 77. Baranger K, Zani ML, Chandener J, Dallet-Choisy S, Moreau T. The antibacterial and antifungal properties of trappin-2 (pre-elafin) do not depend on its protease inhibitory function. *FEBS J* 2008;275:2008-20.
 78. Pillay K, Coutoudis A, Agadzi-Naqvi AK, Kuhn L, Coovadia HM, Janoff EN. Secretory leukocyte protease inhibitor in vaginal fluids and perinatal human immunodeficiency virus type 1 transmission. *J Infect Dis* 2001;183:653-6.
 79. Iqbal SM, Ball TB, Levinson P, Maranan L, Jaoko W, Wachih C, *et al.* Elevated elafin/trappin-2 in the female genital tract is associated with protection against HIV acquisition. *AIDS* 2009;23:1669-77.
 80. King AE, Paltoo A, Kelly RW, Sallenave JM, Bocking AD, Challis JR. Expression of natural antimicrobials by human placenta and fetal membranes. *Placenta* 2007;28:161-9.
 81. Cole AM. Innate host defense of human vaginal and cervical mucosae. *Curr Top Microbiol Immunol* 2006;306:199-230.
 82. Hein M, Valore EV, Helmig RB, Ulbjerg N, Ganz T. Antimicrobial factors in the cervical mucus plug. *Am J Obstet Gynecol* 2002;187:137-44.
 83. Swart PJ, Kuipers EM, Smit C, Van Der Strate BW, Harmsen MC, Meijer DK. Lactoferrin. Antiviral activity of lactoferrin. *Adv Exp Med Biol* 1998;443:205-13.
 84. Ganz T. Antimicrobial polypeptides. *J Leukoc Biol* 2004;75:34-8.
 85. Lee-Huang S, Maiorov V, Huang PL, Ng A, Lee HC, Chang YT, *et al.* Structural and functional modeling of human lysozyme reveals a unique nonapeptide, HL9, with anti-HIV activity. *Biochemistry* 2005;44:4648-55.
 86. Cole AM, Cole AL. Antimicrobial polypeptides are key anti-HIV-1 effector molecules of cervicovaginal host defense. *Am J Reprod Immunol* 2008;59:27-34.
 87. Salamonsen LA, Hannan NJ, Dimitriadis E. Cytokines and chemokines during human embryo implantation: Roles in implantation and early placentation. *Semin Reprod Med* 2007;25:437-44.
 88. Duerst RJ, Morrison LA. Innate immunity to herpes simplex virus type 2. *Viral Immunol* 2003;16:475-90.
 89. Kaushic C, Grant K, Crane M, Wira CR. Infection of polarized primary epithelial cells from rat uterus with *Chlamydia trachomatis*: Cell-cell interaction and cytokine secretion. *Am J Reprod Immunol* 2000;44:73-9.
 90. Kaushic C, Zhou F, Murdin AD, Wira CR. Effects of estradiol and progesterone on susceptibility and early immune responses to *Chlamydia trachomatis* infection in the female reproductive tract. *Infect Immun* 2000;68:4207-16.
 91. Kayisli UA, Mahutte NG, Arici A. Uterine chemokines in reproductive physiology and pathology. *Am J Reprod Immunol* 2002;47:213-21.
 92. Fahey JV, Schaefer TM, Channon JY, Wira CR. Secretion of cytokines and chemokines by polarized human epithelial cells from the female reproductive tract. *Hum Reprod* 2005;20:1439-46.
 93. Carolan EJ, Mower DA, Casale TB. Cytokine-induced neutrophil transepithelial migration is dependent upon epithelial orientation. *Am J Respir Cell Mol Biol* 1997;17:727-32.
 94. Eriksson M, Meadows SK, Wira CR, Sentman CL. Endogenous transforming growth factor- β inhibits toll-like receptor mediated activation of human uterine natural killer cells. *Am J Reprod Immunol* 2006;56:321-8.
 95. Ochiel DO, Ghosh M, Fahey JV, Guyre PM, Wira CR. Human uterine epithelial cell secretions regulate dendritic cell differentiation and responses to TLR ligands. *J Leukoc Biol* 2010;88:435-44.
 96. Le Bon A, Tough DF. Links between innate and adaptive immunity via type I interferon. *Curr Opin Immunol* 2002;14:432-6.
 97. Trinchieri G. Type I interferon: Friend or foe? *J Exp Med* 2010;207:2053-63.
 98. Cooper MA, Fehniger TA, Turner SC, Chen KS, Ghaehri BA, Ghayur T, *et al.* Human natural killer cells: A unique innate immunoregulatory role for the CD56(bright) subset. *Blood* 2001;97:3146-51.
 99. Eriksson M, Meadows SK, Wira CR, Sentman CL. Unique phenotype of human uterine NK cells and their regulation by endogenous TGF- β . *J Leukoc Biol* 2004;76:667-75.
 100. Critchley HO, Kelly RW, Brenner RM, Baird DT. The endocrinology of menstruation – A role for the immune system. *Clin Endocrinol (Oxf)* 2001;55:701-10.
 101. Coleman KD, Wright JA, Ghosh M, Wira CR, Fahey JV. Estradiol modulation of hepatocyte growth factor by stromal fibroblasts in the female reproductive tract. *Fertil Steril* 2009;92:1107-9.
 102. Grant-Tschudy KS, Wira CR. Paracrine mediators of mouse uterine epithelial cell transepithelial resistance in culture. *J Reprod Immunol* 2005;67:1-12.
 103. Haddad SN, Wira CR. Keratinocyte growth factor stimulates macrophage inflammatory protein 3 α and keratinocyte-derived hemokine secretion by mouse uterine epithelial cells. *Am J Reprod Immunol* 2010;64:197-211.
 104. Jacobsson B, Holst RM, Wennerholm UB, Andersson B, Lilja H, Hagberg H. Monocyte chemotactic protein-1 in cervical and amniotic fluid: Relationship to microbial invasion of the amniotic cavity, intra-amniotic inflammation, and preterm delivery. *Am J Obstet Gynecol* 2003;189:1161-7.
 105. Matsuda Y, Kouno S, Nakano H. Effects of antibiotic treatment on the concentrations of interleukin-6 and interleukin-8 in cervicovaginal fluid. *Fetal Diagn Ther* 2002;17:228-32.
 106. Goepfert AR, Goldenberg RL, Andrews WW, Hauth JC, Mercer B, Iams J, *et al.* The Preterm Prediction Study: Association between cervical interleukin 6 concentration and spontaneous preterm birth. National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network. *Am J Obstet Gynecol* 2001;184:483-8.
 107. Simhan HN, Caritis SN, Krohn MA, Martinez de Tejada B, Landers DV, Hillier SL. Decreased cervical proinflammatory cytokines permit subsequent upper genital tract infection during pregnancy. *Am J Obstet Gynecol* 2003;189:560-7.
 108. Romero R, Chaiworapongsa T, Kuivaniemi H, Tromp G. Bacterial vaginosis, the inflammatory response and the risk of preterm birth: A role for genetic epidemiology in the prevention of preterm birth. *Am J Obstet Gynecol* 2004;190:1509-19.
 109. Tabibzadeh S. Evidence of T-cell activation and potential cytokine action in human endometrium. *J Clin Endocrinol Metab* 1990;71:645-9.
 110. Dominguez F, Pellicer A, Simón C. Paracrine dialogue in implantation. *Mol Cell Endocrinol* 2002;186:175-81.
 111. Tabibzadeh S. The signals and molecular pathways involved in human menstruation, a unique process of tissue destruction and remodelling. *Mol Hum Reprod* 1996;2:77-92.
 112. Givan AL, White HD, Stern JE, Colby E, Gosselin EJ, Guyre PM, *et al.* Flow cytometric analysis of leukocytes in the human female reproductive tract: Comparison of fallopian tube, uterus, cervix, and vagina. *Am J Reprod Immunol* 1997;38:350-9.
 113. Jones RL, Hannan NJ, Kaitu'u TJ, Zhang J, Salamonsen LA. Identification of chemokines important for leukocyte recruitment to the human endometrium at the times of embryo implantation and menstruation. *J Clin Endocrinol Metab* 2004;89:6155-67.
 114. DeLoia JA, Stewart-Akers AM, Brekosky J, Kubik CJ. Effects of exogenous estrogen on uterine leukocyte recruitment. *Fertil Steril* 2002;77:548-54.
 115. Jones RL, Kelly RW, Critchley HO. Chemokine and cyclooxygenase-2 expression in human endometrium coincides with leukocyte accumulation. *Hum Reprod* 1997;12:1300-6.
 116. Starkey PM, Clover LM, Rees MC. Variation during the menstrual cycle of immune cell populations in human endometrium. *Eur J Obstet Gynecol Reprod Biol* 1991;39:203-7.
 117. Carruba G, D'Agostino P, Miele M, Calabro M, Barbera C, Bella GD, *et al.* Estrogen regulates cytokine production and apoptosis in PMA-differentiated, macrophage-like U937 cells. *J Cell Biochem* 2003;90:187-96.
 118. Shen R, Richter HE, Clements RH, Novak L, Huff K, Bimczok D, *et al.* Macrophages in vaginal but not intestinal mucosa are monocyte-like

- and permissive to human immunodeficiency virus type 1 infection. *J Virol* 2009;83:3258-67.
119. Cassol E, Cassetta L, Alfano M, Poli G. Macrophage polarization and HIV-1 infection. *J Leukoc Biol* 2010;87:599-608.
 120. Wu R, Van der Hoek KH, Ryan NK, Norman RJ, Robker RL. Macrophage contributions to ovarian function. *Hum Reprod Update* 2004;10:119-33.
 121. Nagamatsu T, Schust DJ. The contribution of macrophages to normal and pathological pregnancies. *Am J Reprod Immunol* 2010;63:460-71.
 122. Iijima N, Thompson JM, Iwasaki A. Dendritic cells and macrophages in the genitourinary tract. *Mucosal Immunol* 2008;1:451-9.
 123. Wira CR, Fahey JV, Ghosh M, Patel MV, Hickey DK, Ochiel DO. Sex hormone regulation of innate immunity in the female reproductive tract: The role of epithelial cells in balancing reproductive potential with protection against sexually transmitted pathogens. *Am J Reprod Immunol* 2010;63:544-65.
 124. Plaks V, Birnberg T, Berkutzi T, Sela S, BenYashar A, Kalchenko V, *et al.* Uterine DCs are crucial for decidua formation during embryo implantation in mice. *J Clin Invest* 2008;118:3954-65.
 125. Kämmerer U, Schoppet M, McLellan AD, Kapp M, Huppertz HI, Kämpgen E, *et al.* Human decidua contains potent immunostimulatory CD83(+) dendritic cells. *Am J Pathol* 2000;157:159-69.
 126. Paharkova-Vatchkova V, Maldonado R, Kovats S. Estrogen preferentially promotes the differentiation of CD11c⁺ CD11b(intermediate) dendritic cells from bone marrow precursors. *J Immunol* 2004;172:1426-36.
 127. Lee JY, Lee M, Lee SK. Role of endometrial immune cells in implantation. *Clin Exp Reprod Med* 2011;38:119-25.
 128. Rebmann V, Regel J, Stolke D, Grosse-Wilde H. Secretion of sHLA-G molecules in malignancies. *Semin Cancer Biol* 2003;13:371-7.
 129. Ma D, Gu MJ, Liu BQ. A preliminary study on natural killer activity in patients with gynecologic malignancies. *J Tongji Med Univ* 1990;10:159-63.
 130. Yang JH, Chen MJ, Chen HF, Lee TH, Ho HN, Yang YS. Decreased expression of killer cell inhibitory receptors on natural killer cells in eutopic endometrium in women with adenomyosis. *Hum Reprod* 2004;19:1974-8.
 131. Maeda N, Izumiya C, Yamamoto Y, Oguri H, Kusume T, Fukaya T. Increased killer inhibitory receptor KIR2DL1 expression among natural killer cells in women with pelvic endometriosis. *Fertil Steril* 2002;77:297-302.
 132. Thum MY, Bhaskaran S, Abdalla HI, Ford B, Sumar N, Shehata H, *et al.* An increase in the absolute count of CD56dimCD16 + CD69 + NK cells in the peripheral blood is associated with a poorer IVF treatment and pregnancy outcome. *Hum Reprod* 2004;19:2395-400.
 133. Dosiou C, Giudice LC. Natural killer cells in pregnancy and recurrent pregnancy loss: Endocrine and immunologic perspectives. *Endocr Rev* 2005;26:44-62.
 134. Peritt D, Robertson S, Gri G, Showe L, Aste-Amezaga M, Trinchieri G. Differentiation of human NK cells into NK1 and NK2 subsets. *J Immunol* 1998;161:5821-4.
 135. Lidstrom C, Matthiesen L, Berg G, Sharma S, Ernerudh J, Ekerfelt C. Cytokine secretion patterns of NK cells and macrophages in early human pregnancy decidua and blood: Implications for suppressor macrophages in decidua. *Am J Reprod Immunol* 2003;50:444-52.
 136. Chalifour A, Roger J, Lemieux S, Duplay P. Receptor/ligand avidity determines the capacity of Ly49 inhibitory receptors to interfere with T-cell receptor-mediated activation. *Immunology* 2003;109:58-67.
 137. Sivori S, Parolini S, Marcenaro E, Millo R, Bottino C, Moretta A. Triggering receptors involved in natural killer cell-mediated cytotoxicity against choriocarcinoma cell lines. *Hum Immunol* 2000;61:1055-8.
 138. Krieg AM. Now I know my CpGs. *Trends Microbiol* 2001;9:249-52.
 139. Hunt JS. Immunologically relevant cells in the uterus. *Biol Reprod* 1994;50:461-6.
 140. King A, Wellings V, Gardner L, Loke YW. Immunocytochemical characterization of the unusual large granular lymphocytes in human endometrium throughout the menstrual cycle. *Hum Immunol* 1989;24:195-205.
 141. King A, Balendran N, Wooding P, Carter NP, Loke YW. CD3-leukocytes present in the human uterus during early placentation: Phenotypic and morphologic characterization of the CD56⁺ population. *Dev Immunol* 1991;1:169-90.
 142. Tabibzadeh S. Proliferative activity of lymphoid cells in human endometrium throughout the menstrual cycle. *J Clin Endocrinol Metab* 1990;70:437-43.
 143. Kämmerer U, Marzusch K, Kröber S, Ruck P, Handgretinger R, Dietl J. A subset of CD56⁺ large granular lymphocytes in first-trimester human decidua are proliferating cells. *Fertil Steril* 1999;71:74-9.
 144. Manaster I, Mandelboim O. The unique properties of uterine NK cells. *Am J Reprod Immunol* 2010;63:434-44.
 145. Waldmann TA, Tagaya Y. The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens. *Annu Rev Immunol* 1999;17:19-49.
 146. Rosmaraki EE, Douagi I, Roth C, Colucci F, Cumano A, Di Santo JP. Identification of committed NK cell progenitors in adult murine bone marrow. *Eur J Immunol* 2001;31:1900-9.
 147. Ye W, Zheng LM, Young JD, Liu CC. The involvement of interleukin (IL)-15 in regulating the differentiation of granulated metrial gland cells in mouse pregnant uterus. *J Exp Med* 1996;184:2405-10.
 148. Okada S, Okada H, Sanzumami M, Nakajima T, Yasuda K, Kanzaki H. Expression of interleukin-15 in human endometrium and decidua. *Mol Hum Reprod* 2000;6:75-80.
 149. Barber EM, Pollard JW. The uterine NK cell population requires IL-15 but these cells are not required for pregnancy nor the resolution of a *Listeria monocytogenes* infection. *J Immunol* 2003;171:37-46.
 150. Mselle TF, Meadows SK, Eriksson M, Smith JM, Shen L, Wira CR, *et al.* Unique characteristics of NK cells throughout the human female reproductive tract. *Clin Immunol* 2007;124:69-76.
 151. Verma S, King A, Loke YW. Expression of killer cell inhibitory receptors on human uterine natural killer cells. *Eur J Immunol* 1997;27:979-83.
 152. Koopman LA, Kopcow HD, Rybalov B, Boyson JE, Orange JS, Schatz F, *et al.* Human decidual natural killer cells are a unique NK cell subset with immunomodulatory potential. *J Exp Med* 2003;198:1201-12.
 153. Ho HN, Chao KH, Chen CK, Yang YS, Huang SC. Activation status of T and NK cells in the endometrium throughout menstrual cycle and normal and abnormal early pregnancy. *Hum Immunol* 1996;49:130-6.
 154. Li XF, Charnock-Jones DS, Zhang E, Hiby S, Malik S, Day K, *et al.* Angiogenic growth factor messenger ribonucleic acids in uterine natural killer cells. *J Clin Endocrinol Metab* 2001;86:1823-34.
 155. Stewart CL, Kaspar P, Brunet LJ, Bhatt H, Gadi I, Kontgen F, *et al.* Blastocyst implantation depends on maternal expression of leukemia inhibitory factor. *Nature* 1992;359:76-9.
 156. Paul TR, Knight ST, Raulston JE, Wyrick PB. Delivery of azithromycin to *Chlamydia trachomatis*-infected polarized human endometrial epithelial cells by polymorphonuclear leucocytes. *J Antimicrob Chemother* 1997;39:623-30.
 157. Godaly G, Bergsten G, Hang L, Fischer H, Freundés B, Lundstedt AC, *et al.* Neutrophil recruitment, chemokine receptors, and resistance to mucosal infection. *J Leukoc Biol* 2001;69:899-906.
 158. Gale LM, McColl SR. Chemokines: Extracellular messengers for all occasions? *Bioessays* 1999;21:17-28.
 159. Faurischou M, Sørensen OE, Johnsen AH, Askaa J, Borregaard N. Defensin-rich granules of human neutrophils: Characterization of secretory properties. *Biochim Biophys Acta* 2002;1591:29-35.
 160. Nathan CF. Neutrophil activation on biological surfaces. Massive secretion of hydrogen peroxide in response to products of macrophages and lymphocytes. *J Clin Invest* 1987;80:1550-60.
 161. Brown EJ. The role of extracellular matrix proteins in the control of phagocytosis. *J Leukoc Biol* 1986;39:579-91.
 162. Salamonsen LA, Woolley DE. Menstruation: Induction by matrix metalloproteinases and inflammatory cells. *J Reprod Immunol* 1999;44:1-27.
 163. Robertson SA. Seminal fluid signaling in the female reproductive tract: Lessons from rodents and pigs. *J Anim Sci* 2007;85(13 Suppl):E36-44.

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164. Johnson RM. Murine oviduct epithelial cell cytokine responses to *Chlamydia muridarum* infection include interleukin-12-p70 secretion. *Infect Immun* 2004;72:3951-60.
165. Patton DL, Thwin SS, Meier A, Hooton TM, Stapleton AE, Eschenbach DA. Epithelial cell layer thickness and immune cell populations in the normal human vagina at different stages of the menstrual cycle. *Am J Obstet Gynecol* 2000;183:967-73.
166. Fidel PL Jr, Barousse M, Espinosa T, Ficarra M, Sturtevant J, Martin DH, *et al.* An intravaginal live *Candida* challenge in humans leads to new hypotheses for the immunopathogenesis of vulvovaginal candidiasis. *Infect Immun* 2004;72:2939-46.
167. Zhao Y, Chegini N. The expression of granulocyte macrophage-colony stimulating factor (GM-CSF) and receptors in human endometrium. *Am J Reprod Immunol* 1999;42:303-11.
168. Lund-Johansen F, Olweus J, Horejsi V, Skubitz KM, Thompson JS, Vilella R, *et al.* Activation of human phagocytes through carbohydrate antigens (CD 15, sialyl-CD 15, CDw17, and CDw65). *J Immunol* 1992;148:3221-9.

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