Innate Immunity in the Human Endometrium and Ovary

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Keywords
Infection, inflammation, ovary, toll-like receptor, uterus

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Submitted April 1, 2011;
accepted May 5, 2011.

Citation
Sheldon IM, Bromfield JJ. Innate Immunity in the Human Endometrium and Ovary. Am J Reprod Immunol 2011; 66 (Suppl. 1): 63–71

doi:10.1111/j.1600-0897.2011.01034.x

Problem
Microbial infections of the upper female genital tract perturb the function of the endometrium and ovary. Defense of these tissues is predominantly dependent on innate immunity. This review gives a perspective on innate immunity in the non-pregnant upper female genital tract of women.

Method of study
Literature review of innate immunity in the human endometrium and ovary.

Results
The endometrium is defended against microbes by physical barriers, antimicrobial peptides, complement, Toll-like receptors (TLRs), and other pattern recognition receptors. Endometrial epithelial and stromal cells express TLRs, which sense pathogen-associated molecular patterns (PAMPs), leading to an inflammatory response with the influx of neutrophils and macrophages. Innate immunity in the endometrium is regulated by steroids, eicosanoids, and cytokines. Granulosa cells also express TLRs and respond to PAMPs.

Conclusion
Pattern recognition receptors have roles in endometrial and ovarian inflammation, and innate immunity is central to the defense of the endometrium against pathogens.

Introduction

Microbial infections of the upper female genital tract are common following coitus or parturition in women. The consequences of microbial infections in the endometrium include pelvic inflammatory disease (PID), preterm labor, and puerperal fever.[1–7] The defense of the endometrium against microbial infection appears to rely heavily on innate immunity, which when activated leads to an acute inflammatory response.[8,9] This inflammatory response includes the secretion of cytokines and chemokines, and the recruitment of neutrophils and macrophages to phagocytose the microbes and damaged cells. Awareness of innate immunity has been heightened by the discovery, first in *Drosophila* and then in mammals, of pattern recognition receptors that bind pathogen-associated molecular patterns (PAMPs) to initiate an inflammatory response (reviewed in references[10–12]). While much of the work on pattern recognition receptors has focused on 'professional' cells of the innate immune system such as neutrophils and macrophages, other host cells play roles in immunity, including the endocrine cells of the endometrium.[8] While interactions between host and pathogen are self-evident for the endometrium, it appears that pattern recognition receptors also impact ovarian physiology and pathology.[13] The aim of the present paper is to give a perspective on innate immunity in the non-pregnant upper female genital tract of women.

Mucosal Immunity

Infection, immunity, and host–pathogen interactions at mucosal surfaces are principally studied in the alimentary and respiratory tracts. This bias reflects the frequency and severity of diseases associated with defects in alimentary or respiratory mucosal immunity. In addition, these mucosa and their commensal microbiota are inextricably linked.[14] Indeed, many emerging paradigms about host–pathogen interactions reflect how microbes shape the organization and function of specialized immune cells in the mucosa. Immune cells in the respiratory and alimentary tracts are often organized into aggregates such as mucosa-associated lymphoid tissue (MALT), including Peyer's patches.[15] The vagina may warrant comparison with the alimentary and respiratory mucosa because of the role of the vaginal microbiota, which is dominated by bacteria that produce lactic acid.[14,16,17] However, the endometrium of normal women appears to differ from most other mucosa because a microbiota is rarely reported and bacterial infection of the endometrium is detrimental to fertility.[18–20] In addition, there is little evidence of an organized MALT in the endometrium, perhaps, because the occurrence and size of MALT depend on the exposure to external stimuli such as the presence of microbes.[15] However, temporary aggregates of immune cells have been noted in the endometrium, associated with the presence of infection or during particular stages in the menstrual cycle.[8] The endometrium also differs from other mucosa in that immunity as well as many other physiological functions are closely regulated by ovarian steroids.[8] Finally, immunity in the endometrium is modulated to support implantation of the semi-allogeneic embryo.[21] This latter process has been the focus of much research but the mechanisms remain elusive, and the explanations of how adaptive immunity is managed during pregnancy often appear rather complex.[9,21] The fetal–maternal interface is not a simple case of acceptance or rejection as with artificial transplantation of tissue; instead, this fetal–maternal relationship has clearly evolved robust regulatory mechanisms over the 380 million years since the establishment of viviparity.[21] Putting the matter of pregnancy to one side, an effective immune defense remains important for the endometrium because microbial contamination or infection with pathogenic organisms is common, especially following coitus or parturition.

Bacterial Infections of the Female Genital Tract

Bacterial infections of the female genital tract that are frequently considered in clinical practice include bacterial vaginosis, sexually transmitted infections, PID, preterm labor, and puerperal fever.[1–7] The prevalence of bacterial vaginosis among reproductive age women in the USA is about 29%.[3] Bacterial vaginosis is often associated with a change in the vaginal microbiota with fewer of the predominant *Lactobacilli* species, and the emergence of a range of pathogenic organisms such as *Gardnerella vaginalis*.[17,22] Sexually transmitted infections, particularly *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis*, are widespread across the world with an estimated 340 million new infections each year.[2] As well as causing infertility, sexually transmitted infections can lead to PID, which affects about 1% of women each year in developed countries.[1] The symptoms of PID range from mild to severe, and complications include endometritis, chronic pain, tubal damage, infertility, and ectopic pregnancy.[7] Preterm labor accounts for approximately 10% of births in the Western world and 70% of infant mortality, and microbial infections of the female genital tract are associated with 25–40% of these cases.[4] Finally, infections of the uterus post partum leading to puerperal fever were a common cause of maternal mortality for women in the developed world until the dawn of the twentieth century, and even today, puerperal fever causes 75,000 maternal deaths every year across the world, mostly in developing countries.[5,6] However, immune mechanisms in the female genital tract mean that microbial infections do not proceed unopposed.

Innate Immunity in the Female Genital Tract

Innate immunity and reproduction are highly conserved, ancient, and key drivers during evolution from flies and worms to humans. Thus, we expect innate immunity and reproduction are highly integrated and likely share common pathways. Our central paradigm is that innate rather than acquired immunity is the primary defense mechanism against microbes in the endometrium. The term 'innate immunity' encompasses many non-specific mechanisms by which organisms defend themselves against microbes. At the simplest level, the anatomical barriers formed by the vulva, vagina, and cervix present considerable hurdles for ascending microbes. Of

course, sexually transmitted infections circumvent much of the barrier by transmission during coitus. Further barriers to ascending infections include the low vaginal pH, the resident vaginal microbiota, and the presence of mucus.[16,17] In addition, the stratified pseudosquamous epithelium lining the vagina likely provides a greater barrier to microbial invasion than the single columnar epithelium of the endometrium. However, there are a variety of more active defense mechanisms in the endometrium, including antimicrobial peptides, complement, and pattern recognition receptors.

Antimicrobial Peptides

Antimicrobial peptides are an ancient component of the immune system expressed mainly by epithelial cells and leukocytes. Defensins are a family of particular importance for mucosal immunity because they non-specifically kill bacteria.[23] Transcription of antimicrobial peptides is increased when pattern recognition receptors are activated and in response to cytokines. As well as killing microbes, antimicrobial peptides also help to protect epithelia against microbial proteases and aid in the resolution of inflammation.[23,24] The human endometrial epithelium expresses β-defensins 1–4, α-defensin (human defensin 5), elafin, and secretory leukocyte protease inhibitor.[24,25] Although the expression of antimicrobial peptides in the female genital tract varies with the specific location and stage of the menstrual cycle, they likely play an important role in limiting the ascension of pathogens.[16,24,25] Mucin-1 (MUC1) is another endometrial epithelial cell protein that may have a role in microbial defense of the endometrium, as well as having a more established role in implantation.[26] Finally, acute phase proteins such as C-reactive protein and haptoglobin (normally produced in the liver in response to cytokines such as IL-6) may help to protect the endometrium against microbes;[27] there is even evidence of localized transcription of haptoglobin in the endometrium.[28]

Complement System

The complement system represents a complex pathway of more than 30 proteins and receptors that are activated by recognizing 'non-self' molecules.[29,30] The key step is activation of the central C3 component by the classical pathway (antibody mediated), the lectin pathway, or the alternative pathway in which C3 promiscuously binds to a wide range of receptor sites on 'foreign' cells, including bacteria. The uterus and oviduct epithelia have abundant C3 because there is local biosynthesis.[31,32] Activation of C3 on cell surfaces stimulates neutrophil recruitment and formation of the membrane attack complex on target cells leading to lysis. To avoid self-destruction, the host cells are protected by several membrane-bound regulatory molecules such as CD46, CD55, and CD59.[30,33] The complement regulatory proteins are important because differences in the expression of these molecules are associated with bacterial infection and cervical cancer.[30,33] However, as well as regulating complement, CD46 is also involved in sperm–egg binding.[34]

Pattern Recognition Receptors

The initial defense of the endometrium against microbes is now thought to be highly dependent on pattern recognition receptors.[8,9] Immune cells possess pattern recognition receptors such as the ten Toll-like receptors (TLRs) and several nucleotide-binding domain (NOD)-like receptors (NLRs), which bind molecules specific to microbial organisms–often called PAMPs or microbial-associated molecular patterns.[11,12] Briefly, TLR1, TLR2, and TLR6 recognize bacterial lipids such as lipoteichoic acid from Gram-positive bacteria, and TLR5 binds flagellin. Nucleic acids, often from viruses, are recognized by TLR3, TLR7, TLR8, and TLR9, although TLR9 also recognizes bacterial DNA. The NLRs are also intracytoplasmic receptors that recognize bacterial peptidoglycans and components of viruses. Finally, TLR4, in complex with CD14 and MD-2, binds lipopolysaccharide (LPS, endotoxin), the cell wall component of Gram-negative bacteria.[12] Activation of pattern recognition receptors initiates the production of pro-inflammatory cytokines, chemokines, and prostaglandins, often via mitogen-activated protein kinase (MAPK) and nuclear factor kappa B (NFκB) pathways, leading to the recruitment of neutrophils and monocytes to the site of infection.[11,12]

Most of the TLRs and NLRs have been detected in primary epithelial and stromal cells isolated from the human endometrium (Table I). Furthermore, several of these epithelial and stromal cell pattern recognition receptors can detect PAMPs, which lead to inflammatory responses akin to those of professional immune cells such as macrophages.[8] In particular, the endometrial cells appear to secrete cytokines and

Table I Primary Human Endometrial Epithelial Cell and Stromal Cell Expression of mRNA, Protein and Functional Responses for the Toll-Like Receptor (TLR) and Nucleotide-Binding Domain (NOD)-Like Receptors (NLR) Pattern Recognition Receptors, with Exemplar References (ND, Not Detected)

Pattern recognition receptor	mRNA	Protein	Function
TLR1	Epithelial[88]	Epithelial[89]	
TLR2	Epithelial[88,90]	Epithelial[36,89,90]	
	Stromal[35,90]	Stromal[90]	
TLR3	Epithelial[37,88,90]	Epithelial[89,91]	Epithelial[37]
TLR4	Epithelial[35,88]	Epithelial[35,36,89,91]	Epithelial[35]
	Stromal[35,90]	Stromal[35,90]	Stromal[35]
TLR5	Epithelial[88]	Epithelial[89]	
TLR6	Epithelial[37,88]	Epithelial[89]	
TLR7	Epithelial[37]	Epithelial[92]	
		Stromal[92]	
TLR8	Epithelial[37]	Epithelial[92]	
		Stromal[92]	
TLR9	Epithelial[88]	Epithelial[92]	
	Stromal[92]	Stromal[92]	
TLR10	ND – Epithelial[37,88]	Epithelial[92]	
		Stromal[92]	
NOD1	Epithelial[38]	Epithelial[38]	Epithelial[38]
	Stromal[38]	Stromal[38]	Stromal[38]
NOD2	Epithelial[38]	Epithelial[38]	Epithelial[38]
	Stromal[38]	Stromal[38]	Stromal[38]

chemokines when they are cultured with ligands that bind TLR4,[35,36] TLR3,[37] or NODs.[38] The importance of TLR4 in the endometrium has been confirmed *in vivo* using TLR4 null mice, which are protected against LPS-induced PID.[39] As well as PAMPs, endogenous danger-associated molecular patterns (DAMPs) released by necrotic or damaged cells induce a mild inflammatory response, possibly by binding to pattern recognition receptors.[40] Common DAMPs include HMGB1 (high-mobility group protein B1), heat shock proteins, hyaluronan, and nucleotides such as ATP.[40] Another nucleotide released by necrotic cells is UDP-glucose, which is detected by a purinergic membrane receptor (P2RY14) on human endometrial epithelial but not stromal cells, and expression of this receptor was increased during PID.[41] While there appears to be a role for epithelial and stromal cells in innate immunity, the relative contribution of these cells and professional immune cells *in vivo* to endometrial pathology is not yet clear.

Regulation of Innate Immunity in the Endometrium

Excessive tissue inflammation is detrimental and so innate immunity must be carefully regulated.[11,12] The obvious candidates for the regulation of immunity in the endometrium are the ovarian steroids. Estradiol and progesterone secreted by the ovary during the menstrual cycle act on endometrial cells and immune cells in the reproductive tract to modulate immunity and the inflammatory response to microbes.[42] The steroids act by binding their respective nuclear receptors, estrogen receptors, and progesterone receptors, which bind DNA at response elements or modify the function of transcription factors such as NFκB.[43,44] Indeed, there is cross-talk between NFκB pathways and steroid receptors in endometrial cells.[45] Furthermore, there is regulation of TLR expression in endometrial samples collected at different stages of the menstrual cycle.[46]

Immuno-regulatory cytokines such as IL-4, IL-10, IL-13 and transforming growth factor beta (TGFβ) are also active in the endometrium. For example, IL-4 and IL-10 inhibit TNFα-induced chemokine production by stromal cells[47] and IL-10 suppresses TNFα-induced IL-6 production by stromal cells.[48] The regulatory cytokines are also subject to endocrine influences; for example, estradiol can stimulate the secretion of IL-13 by human endometrial epithelial and stromal cells.[49] Macrophages, stromal cells, and glandular cells in the endometrium express TGFβ, which has important roles in modulating immunity in the endometrium at the time of insemination and implantation, as well as being associated with diseases such as endometriosis.[50,51] Endogenous TGFβ also alters the cytokine responses of uterine natural killer cells in response to TLR agonists.[52]

Although not the focus of the present review, it is important to recognize that immunity in the endometrium may also be regulated by uterine natural killer cells, regulatory T cells, and macrophages, although much of the work has focused on the role of these cells during pregnancy.[52–54] Of particular importance is the two-way interaction between immune cells and endometrial cells. Mediators from uterine macrophages modulate endometrial epithelial cell function[55] and paracrine mediators from uterine natural killer cells modulate stromal cell function, particularly the accumulation of chemokines such as IL-8.[56] Conversely, endometrial epithelial cell secretions regulate dendritic cell differentiation and responses to TLR ligands.[57]

Prostaglandin E_2 is synthesized in the endometrium and has roles in implantation and menstruation.[58,59] However, at least in murine immune cells, prostaglandin E_2 limits the inflammatory responses to LPS.[60] In addition, prostaglandin E_2 was induced by LPS in endometrial epithelial or stromal cells in wild-type but not TLR4-null mice.[39] There is a wide range of other lipid regulators of immunity such as lipoxins and resolvins.[61] Lipoxins are produced by lipoxygenase-mediated metabolism of arachidonic acid, and resolvins are synthesized from the essential omega-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid. There is evidence that fatty acids play roles in modulating inflammatory pathways in the endometrium[62] but their role during infection of the endometrium is not clear.

MicroRNAs (miRNAs) are emerging as important regulators of innate immunity.[63] The 70- to 90-bp RNA precursors are generated and processed in the nucleus before being transported to the cytoplasm where they are cleaved by Dicer to mature 17- to 23-bp miRNAs. Mature miRNAs interfere with target mRNA translation to regulate the expression of proteins, including pattern recognition receptors and many of their effector pathways, to fine tune innate immune responses.[63] Several miRNAs that target cytokine and chemokine mRNAs are found in the endometrium and are differentially regulated by endometrial disease or cancer.[64,65] Furthermore, these miRNAs also target estradiol and progesterone receptors and appear to be regulated by the endocrine environment.[65]

Innate Immunity In The Ovary

The mammalian ovary contains a finite number of oocytes required for the development of the next generation. The process required for the release of a healthy oocyte from the Graafian follicle (ovulation) is considered a controlled inflammatory response.[66] The ovary contains small, but important populations of macrophages required for tissue homeostasis and normal ovarian function. The number of macrophages changes with the stage of ovarian cycle but most are associated with the corpus luteum and with atretic but not health follicles.[67–70] Macrophages resident around the vascular bed of the Graafian follicle are of particular importance in ovulation and in the formation and function of the corpus luteum.[71] However, the intra-follicular environment that encompasses the oocyte is devoid of professional immune cells.[72,73] This leads to the question of whether innate immunity has a role within the ovarian follicle.

Table II Expression of Toll-Like Receptors (TLRs) in the Ovary of Mammals

Species	Detected TLRs	Tissue	Cancer
Human	1–9	Ovarian epithelium and granulosa[74]	2, 3, 4, 5 increased[74]
	2, 4	Cumulus[77]	
	9		Resident dendritic cells are TLR9 non-responsive in ovarian cancer[93]
Cattle	4	Granulosa[80]	
Mouse	4	Granulosa[76]	
	6	Ovary[94]	
	2, 4, 8, 9	COC[76,77]	
Buffalo	2–10	Ovary[95]	
Pig	4	Ovary[96]	

Few studies have investigated the presence or role of TLRs within the ovary, and current data are summarized in Table II. In women, TLRs 1–9 are expressed by granulosa cells and the ovarian surface epithelium;[74] granulosa cells from *in vitro* fertilisation (IVF) patients express *TLR4*.[75] Changes in *TLR* gene expression have also been identified in ovarian cancer cells, with increased expression of *TLRs 2–5*.[74] In mice, *Tlr2* and *Tlr4* gene expression and function have been demonstrated in cumulus cells.[76,77] Also in the mouse, cumulus cell *Tlr4* gene expression was positively regulated by follicle stimulating hormone (FSH) and the epidermal growth factor receptor ligand amphiregulin. Both FSH and amphiregulin are essential for normal ovarian follicle function and oocyte development, which suggests an intrinsic role for TLR4 within the ovary in mice.[76] In addition, TLR4 was proposed to play a role in oocyte ovulation and fertilization in the mouse owing to its association with endogenous ligands such as the DAMP hyaluronan, which is found in abundance surrounding the ovulated cumulus–oocyte complex (COC).[77] However, when housed in a clean environment, the *Tlr4* mutant or *Tlr4*-null mice breed successfully.[78,79]

The TLRs could have an alternative role for the detection of PAMPs within the ovarian follicle. Among the PAMPs, LPS and viral nucleic acids have been isolated from the follicular fluid of infected cattle and humans, respectively;[80,81] Furthermore, the

presence of LPS in IVF culture media dramatically reduces conception rates in women.[82,83] For example, the incidence of pregnancy was 8% when LPS was >1 ng/mL in the IVF culture medium, compared with 32% if no LPS was detected.[82] In addition, LPS stimulated an increase in TNFα expression when human granulosa cells were treated with endotoxin.[84] In cattle, uterine bacterial infection perturbs ovarian follicle function, and TLR4 was expressed and functional in granulosa cells.[80,85] In rats, LPS increases follicle atresia and granulosa cell apoptosis[86] and *in vitro* LPS suppresses steroid secretion from granulosa cells.[87] Whether PAMPs or DAMPs have a role in human ovarian pathology or physiology remains to be determined.

Conclusions

The endometrium is unusual among the mucosa because a commensal microbiota is not evident, there is no well-organized MALT, and the endometrium supports implantation of a semi-allogeneic embryo. However, the endometrium often has to combat infections with pathogenic organisms. The defense of the human endometrium appears to rely heavily on innate immunity, which non-specifically senses microbial infection by binding PAMPs. The innate immune response appears to be carried out by the epithelial and stromal cells of the endometrium, which then recruit more professional immune cells to the site of infection. However, there is close integration between endometrial endocrine and immune function, particularly by the ovarian steroids. The ovarian follicles may also be exposed to PAMPs but healthy growing ovarian follicles are devoid of immune cells, which to leads to the question of what protects the oocyte? As for the endometrium, the answer may be innate immunity. Future work will likely focus on the interactions between the innate and adaptive immune system in the female genital tract and the regulation of immunity to develop improved therapeutics for uterine disease.

Acknowledgments

Work in the Centre for Reproductive Immunobiology is funded at least in part by the Biotechnology and Biological Sciences Research Council (Grant Numbers: BB/F005121/1 and BB/I017240/1).

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