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Innate immunity and the sensing of infection, damage and danger in the female genital tract

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Abstract

Tissue homeostasis in the female genital tract is challenged by infection, damage, and even physiological events during reproductive cycles. We propose that the evolutionarily ancient system of innate immunity is sufficient to sense and respond to danger in the non-pregnant female genital tract. Innate immunity produces a rapidly inducible, non-specific response when cells sense danger. Here we provide a primer on innate immunity and discuss what is known about how danger signals are sensed in the endometrium and ovary, the impact of inflammatory responses on reproduction, and how endocrinology and innate immunity are integrated. Endometrial epithelial and stromal cells, and ovarian granulosa cells express pattern recognition receptors, similar to cells of the innate immune system. These pattern recognition receptors, such as the Toll-like receptors, bind pathogen-associated or damage-associated molecular patterns. Activation of pattern recognition receptors leads to inflammation, recruitment of immune cells from the peripheral circulation, and phagocytosis. Although the inflammatory response helps maintain or restore endometrial health, there may also be negative consequences for fertility, including perturbation of oocyte competence. The intensity of the inflammatory response reflects the balance between the level of danger and the systems that regulate innate immunity, including the endocrine environment. Understanding innate immunity is important because disease and inappropriate inflammatory responses in the endometrium or ovary cause infertility.

Keywords

Innate immunity; inflammation; uterus; Toll-like receptors; damage

Abbreviations

DAMP, damage-associated molecular pattern; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; MyD88, Myeloid differentiation primary response gene 88; NF-ĸB, Nuclear Factor Kappa B; NLR, nucleotide-binding domain and leucine-rich repeat; NOD, nucleotide-binding oligomerization domain; PAMP, pathogen-associated molecular pattern; RIG-I, retinoic acid-inducible gene I; STAT3, signal transducer and activator of transcription-3; TLR, Toll-like receptor.

1. Introduction

The female genital tract needs to sense danger because it faces disruption to tissue homeostasis by a wide range of challenges including infection, damage, and even insemination. Understanding innate immunity in the female genital tract is important because sexually transmitted infections are a world-wide health problem in animals and humans, and infertility is caused by disorders such as endometritis and endometriosis. The objectives of this review are to provide a primer on innate immunity, and discuss the evidence for how danger signals are sensed in the non-pregnant female genital tract, the subsequent impact of the inflammatory responses on reproductive biology, and how the endocrine environment integrates with innate immunity.

The uterus is an immune-privileged site, and modulation of adaptive immunity is necessary for fertilization of the mammalian oocyte by allogenic spermatozoa, and formation of the placenta to nurture the semi-allogenic foetus (Beagley and Gockel 2003, Chaouat *et al.* 2010). Coevolution of adaptive immunity and viviparity are clearly successful, but innate immunity has its origins well before the development of viviparity at 340 million years ago (Long *et al.* 2008, Chaouat *et al.* 2010, Ronald and Beutler 2010). The female genital tract is exposed to a range of challenges to tissue homeostasis, including infections, damage to the surface of the ovary during ovulation, and damage to the endometrium during menstruation (Fig. 1). Both microbial infections and tissue damage require defence responses by the affected tissues (Chovatiya and Medzhitov 2014). The role of innate immunity in pregnancy, parturition and the puerperal period are reviewed elsewhere (Gomez-Lopez *et al.* 2014, Kourtis *et al.* 2014, Sheldon *et al.* 2014); and here we will focus on the non-pregnant female genital tract. We propose that innate immunity is sufficient to cope with most of the dangers challenging the female reproductive tract, with adaptive immunity providing a supplemental defence system.

2. General aspects of innate immunity

Innate immunity is a rapidly inducible cellular response to danger, if homeostasis is challenged by microbes or damage (for reviews see: (Kawai and Akira 2010, Moresco *et al.* 2011, Chovatiya and Medzhitov 2014)). A characteristic of innate immunity is that sensing of microbes is non-specific, whereas adaptive immunity is highly specific. The innate immune response leads to inflammation, recruitment of immune cells from the peripheral circulation, phagocytosis, and engagement of adaptive immunity. However, this inflammatory response is only necessary when cell and tissue resilience is compromised.

2.1 Cell and tissue resilience

The resilience of cells and tissues in the female genital tract is an important first barrier to infection and damage. Physical barriers to infection include the vulva and cervix, the epithelial surface of the mucosa lining the female genital tract, the basement membrane of ovarian follicles, and the zona pellucida of the oocyte. In addition, mucus in the female genital tract provides a hostile barrier to infections, enhanced by the presence of anti-microbial peptides and the complement protein systems. At a cellular level, endometrial epithelial cells are more resilient to microbes and their toxins than the underlying stromal cells (Healy *et al.* 2015). For example, endometrial stromal cells are far more sensitive to cholesterol-dependent cytolysins produced by bacteria than the overlying epithelial cells, so pathology is dependent on bacteria penetrating beyond the epithelium (Amos *et al.* 2014). In

addition, the resilience of cells can be manipulated. For example, depleting cholesterol from endometrial stromal cells enhances the resilience to bacterial pore-forming toxins (Amos *et al.* 2014). Once the resilience of the cells or tissue is overcome by infection or trauma, the innate immune system senses and responds to danger.

2.2 Danger signals

Innate immunity is predicated on cellular pattern recognition receptors detecting danger signals. The most well defined danger signals are molecules that are non-self; typically molecules found in prokaryotes that are not present in eukaryotes (for reviews see: (Kawai and Akira 2010, Moresco *et al.* 2011). Such molecular signatures are often called pathogen-associated molecular patterns (PAMPs); although, as the molecules are often not unique to pathogens, they are alternatively called microbial-associated molecular patterns. The most widely studied PAMPs are lipopolysaccharide (LPS), bacterial lipopeptides, and viral nucleic acids (Table 1). Many of these PAMPs are found in the female reproductive tract and are associated with a range of infections.

Innate immunity also has a role in resolving tissue damage. The danger hypothesis proposes that tissues sense and respond to cell death, damage and stress, in a similar manner as they might to pathogens (for reviews see: (Matzinger 2002, Chen and Nunez 2010). Briefly, the molecular signatures that alert tissues and cells to damage are called damage-associated molecular patterns (DAMPs; Table 1). These signals are “self” and so this paradigm is less easy to rationalize than PAMPs. However, the DAMPs are typically molecules that are processed into an unexpected form or found in a location where they are not normally encountered. Release of DAMPs is associated with damage and trauma to tissues, and infections with bacteria that cause cell damage by releasing pore-forming toxins, or viruses that cause cytopathic effects. Additional causes of tissue damage in the female genital tract are labour, menstruation, endometriosis and ovulation (Fig. 1). It could be argued that as menstruation is physiological it is more typical of a stress response leading to para-inflammation, rather than a pathological response (Chovatiya and Medzhitov 2014). Yet, the abundant influx of neutrophils points more towards a *bona fide* inflammation, perhaps because menstruation has not fully evolved to be integrated with innate immunity.

2.3 Pattern recognition receptors

The trans-membrane Toll-like receptors (TLRs; Table 2) were the first functional mammalian pattern recognition receptors to be discovered (Poltorak *et al.* 1998, Hoshino *et al.* 1999). Most other pattern recognition receptors are cytosolic, including the nucleotide-binding oligomerization domain (NOD) receptors, the nucleotide-binding domain and leucine-rich repeat containing family members (NLRs; also called NOD-like receptors), retinoic acid-inducible gene I (RIG-I)-like family, and C-type lectin receptors. The function of most of these receptors was first identified by studying knock-out or mutant mice, which appear to be fertile (for reviews see: (Kawai and Akira 2010, Moresco *et al.* 2011, Lamkanfi and Dixit 2014).

There are ten TLRs in humans, with more in mice, and each TLR has a repertoire of factors that can be sensed (Table 2). Some receptors sensing extracellular signals, whilst other receptors principally detect intracellular factors (for reviews see: (Kawai and Akira 2010, Moresco *et al.* 2011). Surface-expressed TLRs, such as TLR1, TLR2, TLR4, TLR5 and TLR6, have extracellular leucine-rich repeats, whilst the remaining TLRs are associated with

intracellular membranes. The leucine-rich repeats bind to PAMPs and DAMPs directly or in association with co-receptors. Upon ligand binding, the TLRs form homodimers, or in some case heterodimers (TLR1/TLR2 and TLR2/TLR6), leading to conformational changes that trigger intracellular signalling via the Toll/interleukin-1 receptor (TIR) homology domain, prompting inflammatory responses. Intracellular signalling pathways that are activated after ligand binding have been determined for most TLRs (for reviews see: (Kawai and Akira 2010, Ronald and Beutler 2010, Ting *et al.* 2010, Lamkanfi and Dixit 2014, Philpott *et al.* 2014). Briefly, apart from TLR3, all TLRs can use Myeloid differentiation primary response gene 88 (MyD88)-dependent intracellular signalling pathways, which trigger the Nuclear Factor Kappa B (NF-ƙB) and mitogen-activated protein kinase (MAPK) cascades to induce expression of genes encoding inflammatory mediators. In addition, some signals activate the interferon regulatory transcription factor 7 pathway and induction of type 1 interferons, to counter viral infections. The TIR-domain-containing adapter-inducing interferon-β pathway associated with TLR3 also activates NF-ƙB and interferon regulatory transcription factor 3. Using these multiple intracellular signalling pathways amplifies the inflammatory response when pattern recognition receptors are activated.

Nucleotide-binding oligomerization domain-containing proteins (NOD1 and NOD2) detect peptidoglycan components (Philpott *et al.* 2014). The NLRs are intracytoplasmic, where they assemble multiprotein inflammasome complexes, which activate caspases (Lamkanfi and Dixit 2014). The main canonical inflammasome molecules include NLRP3, NLRP1, NLRC4 and AIM2, which convert procaspase-1 into the active caspase-1, leading to cleavage of pro-IL-1β into active IL-1β, and induction of pyroptosis, with release of DAMPs such as IL-1α. In addition, noncanonical procaspase-11 in the mouse (procaspase-4 and -5 in humans) stimulates pyroptosis via activation of gasdermin D (Man and Kanneganti 2016). It should be noted that the NLRs involved in innate immunity are distinct from NLRs that have roles in reproduction and embryo development. Similarly, the inflammatory caspases are also different from those caspases that cause apoptosis (Man and Kanneganti 2016).

2.4 Cellular responses to activation of pattern recognition receptors

Inflammation is the principal response to activation of pattern recognition receptors during infection and tissue damage (Kawai and Akira 2010, Moresco *et al.* 2011, Chovatiya and Medzhitov 2014). This inflammation is particularly driven by cells of the innate immune system, such as macrophages, neutrophils and dendritic cells. However, epithelial and stromal cells also participate in the innate immune response in endometrium of humans and animals (Schaefer *et al.* 2004, Hirata *et al.* 2005, Herath *et al.* 2006, Soboll *et al.* 2006, Cronin *et al.* 2012, Turner *et al.* 2014). Furthermore, within the immunologically privileged site of the ovarian follicle, the granulosa cells initiate inflammation (Shimada *et al.* 2006, Herath *et al.* 2007, Price *et al.* 2013). Macrophages principally produce TNFα, IL-1β and IL-6 (Moresco *et al.* 2011); whereas endometrial and granulosa cells produce IL-6 and IL-8 (Schaefer *et al.* 2004, Soboll *et al.* 2006, Cronin *et al.* 2012, Turner *et al.* 2014). Chemokines, such as IL-8, attract further immune cells to migrate to the site of infection or damage. Cytokines increase the permeability of the vascular endothelium; stimulate the release of antimicrobial peptides, complement proteins, acute phase proteins, prostaglandins and reactive oxygen species; and, regulate the inflammatory response. Ultimately the activated macrophages and neutrophils phagocytose microbes and damaged cells to resolve the insult to the tissue.

3. Innate immunity in the endometrium

Evidence for functional roles of pattern recognition receptors in the endometrium is available from animal and *in vitro* studies. Murine epithelial and stromal cells express TLR4, and *Ttlr4*-null mice are protected against induction of endometritis with LPS (Sheldon and Roberts 2010). Similarly, siRNA has been used to provide evidence for functional activity of TLR1, TLR2, TLR4, and TLR6 in bovine endometrial cells (Cronin *et al.* 2012, Turner *et al.* 2014). Addition of PAMPs to endometrial tissue or component cells usually stimulates inflammatory responses akin to those of macrophages (Pioli *et al.* 2004, Hirata *et al.* 2005, Schaefer *et al.* 2005, Wira *et al.* 2005). The endometrial cells produce abundant IL-6 and IL-8 in response to a range of PAMPs. In addition, the IL-6 and IL-8 response is augmented via paracrine signaling and a positive feedback loop, via the IL-6 receptor and signal transducer and activator of transcription-3 (STAT3) (Cronin *et al.* 2016). Interestingly, there is evidence for vectorial deployment of inflammatory mediators in the endometrium. Polarized endometrial epithelial cells secrete IL-8 towards the direction of challenge, prostaglandins are predominantly released basolaterally, whilst IL-6 is only secreted apically, irrespective of whether PAMPs are encountered apically or basolaterally (Healy *et al.* 2015).

Although endometrial cells have roles in defense against microbes, they are not as important as macrophages. For example, bovine endometrial cells do not sense DAMPs, such as hyaluronan, HMGB1 or necrotic cells, although they produce and sense IL-1α, which is released from damaged cells after infection with bacteria (Healy *et al.* 2014). Furthermore, human endometrial epithelial but not stromal cells sensed uridine 5'-diphosphoglucose via a purinergic membrane receptor, and this receptor was more abundant during uterine infection (Arase *et al.* 2009).

Beyond sensing of pathogens and damage, pattern recognition receptors have other roles in the uterus. First, they contribute to the inflammatory response to seminal plasma that is found in many species after insemination (Schjenken *et al.* 2015). There is evidence using *Tlr4*-null mice that sensing of seminal plasma after insemination is dependent on TLR4 pathways. Another intriguing twist for the role of innate immunity in reproduction is that TLR7 and TLR8 recognize endogenous retroviruses in the ovine endometrium during implantation and early conceptus development (Ruiz-Gonzalez *et al.* 2015). However, whilst innate immunity might contribute to physiological events in reproductive biology, one always has to consider that mutation or disruption of genes that encode pattern recognition receptors is not reported to cause reproductive failure.

4. Innate immunity in the ovary

The role of innate immunity in the mammalian ovary is an emerging area of interest, which has focused on follicle development and ovulation, formation of the corpus luteum, and development of the oocyte. The abundance of macrophages in the ovary changes with the stage of ovarian cycle, and most are found in the corpus luteum and atretic follicles (Brannstrom *et al.* 1994, Takaya *et al.* 1997). Macrophages resident in the vascular bed of the ovarian follicle contribute to ovulation and the formation and function of the corpus luteum (Pate 1995). A more intriguing area of investigation is the healthy ovarian follicle, because immune cells are absent within the basement membrane (Bulmer 1964, Kirsch *et al.* 1981, Bromfield and Sheldon 2011). Human and bovine granulosa cells express TLR1-9 (Zhou *et al.* 2009, Price *et al.* 2013, Price and Sheldon 2013). Similarly, murine cumulus cells express TLR2 and TLR4, and activation of these pattern recognition receptors induces

events associated with ovulation such as cumulus expansion (Shimada *et al.* 2006, Shimada *et al.* 2008, Jang *et al.* 2015). Furthermore, endocrine regulation of TLRs was evident in bovine and murine granulosa cells using FSH or EGF (Shimada *et al.* 2006, Shimada *et al.* 2008, Price and Sheldon 2013). However, TLRs are not required for ovarian function as the *Tlr4* mutant or *Tlr4*-null mice breed successfully (Poltorak *et al.* 1998, Hoshino *et al.* 1999).

Another function of pattern recognition receptors in granulosa cells is to detect PAMPs in ovarian follicular fluid, and LPS and viral nucleic acids are found in the follicular fluid of infected cattle and humans (Devaux *et al.* 2003, Herath *et al.* 2007). Uterine bacterial infection perturbs ovarian follicle growth and function, LPS accumulates in follicular fluid, and LPS stimulates inflammatory responses and reduces oestradiol secretion (Sheldon *et al.* 2002, Herath *et al.* 2007, Bromfield and Sheldon 2011, Price *et al.* 2013). Similarly, LPS increases follicle atresia and granulosa cell apoptosis, and reduces oestradiol secretion from granulosa cells in rats (Taylor and Terranova 1996, Besnard *et al.* 2001). In mice, TLR3, MDA5 and RIG-I in granulosa cells have roles in sensing viral PAMPs, and double stranded RNA stimulates inflammation, whilst suppressing endocrine function (Yan *et al.* 2014). During and after formation of the corpus luteum, which is associated with the immigration of immune cells and considerable angiogenesis, corpus luteum function is also compromised by activation of innate immunity during infections (Pate 1995, Luttgenau *et al.* 2016).

As granulosa cells sense PAMPs and DAMPs, a logical question is whether oocyte development and maturation might also be impacted. Indeed, cumulus oocyte complexes produce IL-6 in response to LPS, and LPS reduces oocyte developmental competence, with increased germinal vesicle breakdown failure, and abnormal spindle formation (Bromfield and Sheldon 2011). Oocyte development in antral follicles may be influenced by LPS directly, or inflammatory mediators that are required for oocyte development, such as IL-6. Activation of innate immunity might also impact earlier events during oocyte and follicle development. Primordial follicles are restrained in a quiescent state by endogenous proteins, such as phosphatase and tensin homolog and forkhead box O3a. However, LPS stimulated loss of these proteins in primordial follicles, and spontaneous activation of primordial follicles to primary and secondary follicles (Bromfield and Sheldon 2013).

5. Regulation of innate immunity

As we outline above, the value of an innate immune response is to counter stressful challenges and return tissues to their homeostatic state. However, the intensity of the inflammatory response should reflect the severity of the challenge to the tissues. Furthermore, once the initial danger is contained, it is important that inflammation is resolved and the innate immune response truncated, otherwise chronic inflammation persists to the detriment of tissue function.

The main focus of research has been induction of inflammation by individual PAMPs and DAMPs. However, beyond this experimental approach, several factors increase the inflammatory response to meet the challenge to the tissues (Fig. 2). First, in a typical infection there are not one but several PAMPs and DAMPs, which likely provoke a greater response than individual molecular patterns. Second, live microbes generate greater inflammatory responses than dead microbes, and some PAMPs are indicative of a live pathogen, such as bacterial mRNA, which is termed a vita-PAMP (Sander *et al.* 2011). Thirdly, microbial virulence factors can enhance the inflammatory response. For example,

bacterial secretion systems can translocate PAMPs into the cytoplasm of host cells to disrupt their cytoskeleton, and pore-forming toxins disrupt host cell membranes. However, one has to be cautious in assuming all bacterial systems stimulate inflammation because some microbes have coevolved factors to subvert or evade the innate immune system, which limits the inflammatory response (Baxt *et al.* 2013).

Host cell signalling and metabolic pathways can also amplify or regulate inflammatory responses. For example, in endometrial stromal cells the inflammatory response to LPS is amplified via the IL-6 receptor and STAT3 pathway (Cronin *et al.* 2016). An indirect mechanism that regulates the inflammatory response is the impact of infection on cellular metabolism. In mouse macrophages, LPS induces the Warburg effect and increases the accumulation of succinate in the Krebs cycle, which leads to increased IL-1β secretion (Tannahill *et al.* 2013). Other metabolic pathways play a part in innate immunity. The inflammatory response to LPS is blunted in bovine endometrial cells when the mevalonate pathway is inhibited using statins (Healey *et al.* 2016). However, the mechanistic link to TLR4-mediated responses includes the isoprenoid mevalonate pathway-intermediates, because farnesyl diphosphate and geranylgeranyl diphosphate also reduced endometrial cellular inflammatory responses (Healey *et al.* 2016).

An ultimate marker of danger is likely when the infection is combined with tissue damage. Microbial invasion of tissues often breaches physical barriers such as the epithelial layer of the endometrium, leading to sensing of damage as well as exposing underlying stromal cells to unexpected challenges. In the endometrium, microbial damage to cells and tissues releases DAMPs such as IL-1α and uridine 5'-diphosphoglucose, which act to further scale the inflammatory response (Arase *et al.* 2009, Healy *et al.* 2014).

The potential for regulation of innate immunity by the endocrine environment is a feature of the endometrium that is perhaps distinct from most other tissues in the body. In particular, the ovarian steroids oestradiol and progesterone modulate the susceptibility to infection and the inflammatory response to microbes (Beagley and Gockel 2003, Wira *et al.* 2015). For example, the abundance of immune cells and expression of TLRs varies during the menstrual cycle (Wira *et al.* 2015). In addition, steroids have indirect effects on innate immunity, as progesterone increases endometrial vascular permeability (Goddard *et al.* 2014). Furthermore, there are several examples of crosstalk between endocrine and inflammatory pathways. In particular, the NF-κB pathway crosstalks with steroid nuclear receptors in human endometrial cells (King *et al.* 2010). In endometriosis, estradiol receptor β interacts with NLRP3 to enhance caspase-1 activity and IL-1β secretion in ectopic endometrium (Han *et al.* 2015). Oestrogen or oestrus also induce expression of interferon ε in endometrial epithelial cells, which is a type 1 interferon, that is protective against viral and Chlamydia infections (Fung *et al.* 2013). Endometrial prostaglandin E_2 is regulated by the ovarian cycle, with roles in implantation and menstruation, but prostaglandin E_2 is also induced by LPS in endometrial epithelial and stromal cells in mice and cattle (Herath *et al.* 2006, Sheldon and Roberts 2010). In addition, Prostaglandin E_2 suppresses macrophage inflammatory responses to LPS (Uematsu *et al.* 2002). However, a mechanistic link between hormones and innate immunity is not always evident. *Ex vivo* bovine endometrium inflammatory responses were not affected by the stage of ovarian cycle, exogenous oestradiol or progesterone, or inhibition of the steroid nuclear receptors (Saut *et al.* 2014).

Factors other than hormones are important for regulation of endometrial innate immunity. Cytokines such as IL-4 and IL-10 inhibit TNFα-induced chemokine production, and IL-10 suppress TNFα-induced IL-6 production by endometrial stromal cells (Arima *et al.* 2000, Tagashira *et al.* 2008). Crosstalk between cells is also important as uterine macrophage and natural killer cell secretions modulate endometrial cell function; whilst, endometrial epithelial cells regulate dendritic cell differentiation and responses to PAMPs (Germeyer *et al.* 2009, Ochiel *et al.* 2010).

Resolution of inflammation is a surprisingly active process. For example, a wide range of lipids such as lipoxins and resolvins dampen the inflammatory response after microbes are cleared from tissues (Serhan *et al.* 2008). 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 (Serhan *et al.* 2008).

6. Perspective and conclusions

Much of the details about sensing of danger signals and the intracellular signalling of pattern recognition receptors have been worked out using mice *in vivo*, or their immune cells *in vitro*. Whilst the role of innate immunity in the intestinal mucosa is widely explored, there is relatively little research on innate immunity in the female reproductive tract. However, infections of the genital tract are very common, and an important cause of infertility and disease across the world. Much work remains to be done to fully understand how innate immunity contributes to disease such as sexually transmitted infections and endometriosis. Furthermore, the role of innate immunity in physiological events such as menstruation and ovulation remains unclear. Although it is unlikely that innate immunity is essential for reproduction, we propose that innate immunity is sufficient to sense and respond to danger in the female reproductive tract.

Conflict of interest

All authors declare that they have no conflicts of interest.

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Table 1 Common danger signals that stimulate innate immune responses

Common pathogen-associated molecular patterns (PAMPs) and damage-associated molecular patterns (DAMPs) that are signs of danger to cells.

PAMPs	DAMPs
Bacteria	**Cellular**
Lipopolysaccharide	HMGB1
Lipopeptides	IL-1α
Flagellin	IL-33
Peptidoglycan	ATP
Unmethylated CpG-rich DNA	DNA, nuclear and mitochondrial
	UDP-glucose
	Heat shock proteins
	S100 proteins
Viruses	**Extracellular matrix**
Single-stranded RNA	Hyaluronan fragments
Double-stranded RNA	Fibronectin
DNA	Biglycan
	Uric acid crystals
Fungi	**Plasma membrane ion fluxes**
Zymosan	Potassium efflux
β-glucan	Calcium influx

Table 2 Principal pattern-recognition receptors of the innate immune system, and examples of their common ligands

Pattern recognition receptors	Exemplar ligands
Toll-like receptors	
TLR1, TLR2, TLR6	Bacterial lipopeptides
TLR4	LPS, hyaluronan
TLR5	Bacterial flagellin
TLR3	Double-stranded RNA
TLR7, TLR8	Single-stranded RNA
TLR9	DNA
TLR10	Unknown
NODs	
NOD1, NOD2	Peptidoglycans
NLRs	
NLRP1	Muramyldipepetide, peptidoglycan
NLRP3	Nucleic acids, uric acid, ATP
NLRC4	Flagellin, bacterial secretion systems
AIM2	Double-stranded RNA
CLRs	
Dectin-1	Fungal β-glucans
Mincle	*Mycobacterium tuberculosis*
RLRs	
RIG-I	Viral RNA
MDA5	Viral RNA
LGP2	Viral RNA

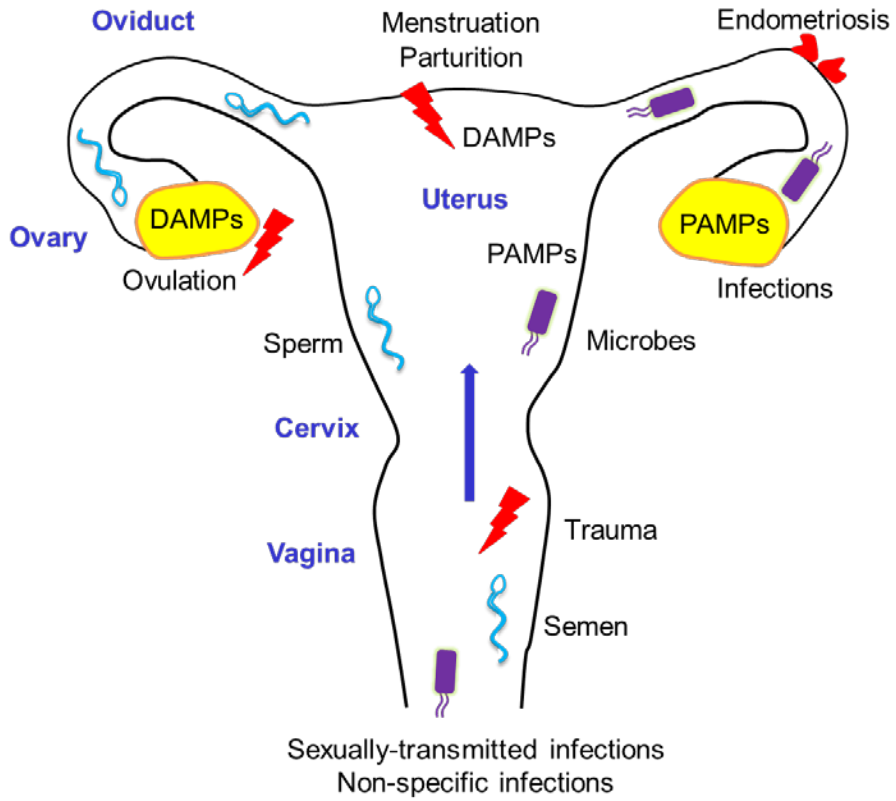

Figure 1 Challenges to tissue homeostasis in the female genital tract

The vagina, cervix, endometrium, oviduct and ovary face a range of challenges to their tissue homeostasis. Sexually-transmitted and non-specific infections are associated with microbes ascending the genital tract, although other microbes can also infect the tissue via the haematogenous route. These infections expose the tissues to pathogen-associated molecular patterns (PAMPs), pore-forming toxins, and cell damage. Trauma of the genital tract is caused by physical damage, menstruation, and ovulation, with the release of damage-associated molecular patterns (DAMPs). Inflammation is also associated with the presence of ectopic endometrium (endometriosis), and even after insemination, which exposes the genital tract to sperm and seminal plasma.

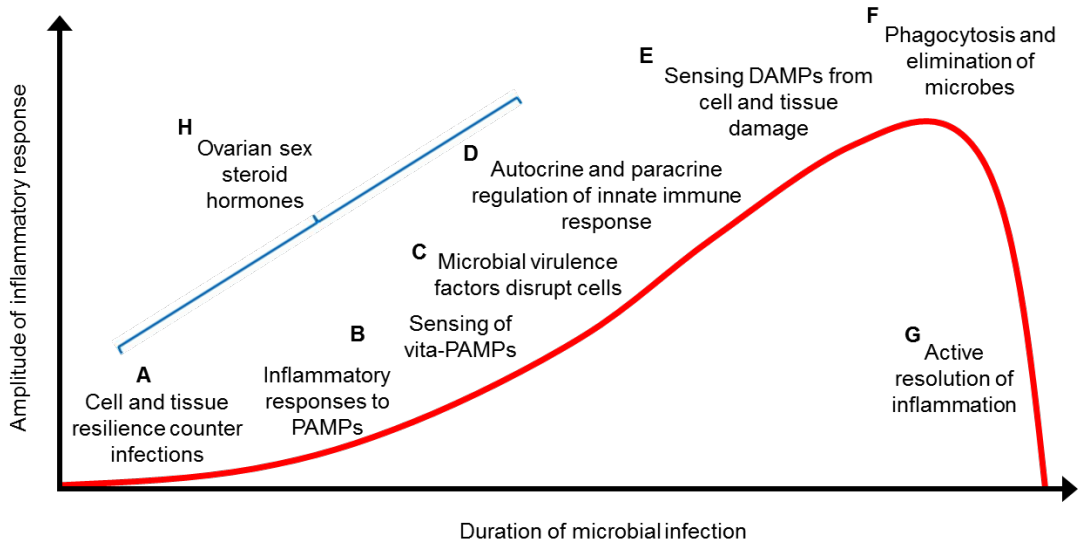

Figure 2 Progression of the innate immune response in the endometrium

During an infection, microbes must first overcome the resilience of the endometrial tissue and cells (A). Sensing of PAMPs and vita-PAMPs leads to inflammation, with the secretion of cytokines such as IL-6, and chemokines such as IL-8 (B). The inflammatory response to PAMPs is further increased when host cells additionally sense microbial virulence factors (C), such as pore-forming toxins and bacterial secretion systems. The inflammation is scaled by autocrine and paracrine signalling (D); for example, via IL-6 receptor and STAT3 signalling to endometrial cells. Finally, cell damage and sensing of DAMPs also enhances the inflammatory response (E). The consequences of inflammation include the immigration of phagocytes to help clear the microbes; phagocytes are attracted to the site of infection along a chemokine gradient, and regulated by cytokines such as IL-6 (F). Once the infection is resolved, there is active resolution of the inflammation, and restoration of endometrial tissue homeostasis (G). All these stages are modulated by hormones, particularly by the ovarian steroid hormones oestradiol and progesterone (H).