Innate immunity and disorders of the female reproductive tract

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Abstract

Sexually transmitted infections, and their associated sequelae, such as tubal infertility, ectopic pregnancy and preterm labour, are a major worldwide health problem. *Chlamydia trachomatis* infection is thought to be the leading global cause of tubal infertility and tubal ectopic pregnancy. Preterm birth occurs in around 10% of all deliveries, and nearly 30% of preterm deliveries are associated with intrauterine infection. The mucosal innate immune system of the female reproductive tract has evolved to eliminate such sexually transmitted pathogens whilst maintaining its ability to accommodate specialized physiological functions that include menstruation, fertilization, implantation, pregnancy and parturition. The aim of this review was to describe the role and distribution of key mediators of the innate immune system, the natural antimicrobial peptides (secretory leukocyte protease inhibitor, elafin and the defensins) and the pattern recognition toll-like receptors in the normal female reproductive tract and in the context of these pathological processes.

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Introduction

The innate immune system incorporates more rapid and primitive responses to infection than the adaptive immune system, such as surface defences, cytokine elaboration, complement activation and phagocytic responses (Janeway & Medzhitov 2002, Tosi 2005). Together, these effect pathogen elimination and are responsible for the stereotypical inflammatory response. The natural antimicrobial peptides (NAPs) and pattern recognition toll-like receptors (TLRs) are key mediators of the innate immune system (Wira *et al.* 2005). NAPs are released at epithelial surfaces and disrupt the membranes of many microbial pathogens (Ganz 2004). TLRs at mucosal surfaces recognize a range of microbial molecular patterns and generate intra-cellular signals through nuclear factor- κ B (NF κ B) dependent (and independent) pathways to induce chemokine and cytokine expression that activate a range of host responses (Zarembek & Godowski 2002). A summary of the cellular origin, target cell lineages and function of each family of molecules is shown in Fig. 1. The role of macrophages, dendritic, myeloid, natural killer cells and neutrophils in the innate immune system are outwith the scope of this review and have been recently discussed in detail elsewhere (Wira *et al.* 2005). Similarly, this review focuses specifically on data from human studies, except where individual animal work was thought to be especially relevant.

The mucosal innate immune system of the female reproductive tract is uniquely adapted to facilitate specialized physiological functions that include menstruation, fertilization, implantation, pregnancy and parturition, whilst eliminating threatening sexually transmitted and environmental pathogens. Sexually transmitted infections (STIs), and their associated problems, are a major worldwide health problem (see Fig. 2). World Health Organisation figures estimated that 89 million new cases of genital *Chlamydia trachomatis* infections occurred in 1995, highlighting the worldwide prevalence of infections and the economic burden on healthcare delivery (Beagley & Timms 2000). *C. trachomatis* infection is thought to be the leading global cause of tubal infertility and tubal ectopic pregnancy (Faro 1991, Farquhar 2005). One in six couples suffer from infertility, with tubal disease being the direct cause in over 25% of cases (Mardh 2004). Tubal ectopic pregnancy occurs in 1 in 80 pregnancies and remains a common cause of morbidity in early pregnancy and occasional mortality (Tay *et al.* 2000). Preterm birth occurs in around 10% of all deliveries, and nearly 30% of preterm deliveries are associated with intrauterine infection (Ananth & Vintzileos 2006, Romero *et al.* 2007).

Innate immune system competence is of critical importance in preventing microbial penetration. In this context, it is important to consider the differences in the microenvironments of the 'sterile' upper and 'non-sterile'

Figure 1 Summary illustrating the cellular origin, target cell lineages and function of the natural antimicrobial peptides, defensins and toll-like receptors.

lower female reproductive tract (see Fig. 3). Each of these distinct microbial milieu are likely to have a compartmentalized innate immune response that shows temporal changes in their expression of innate immune molecules in response to oestrogen and progesterone (Sonnex 1998, Pioli *et al.* 2004). The vagina and ectocervix harbour a variety of commensal bacteria and are subject to secondary contamination as a result of their proximity to the rectum (Quayle 2002). Despite this constant exposure to microbes, infections are relatively uncommon suggesting effective containment or efficient elimination of pathogens. The endocervix represents a transitional area and it is the epithelia that are most frequently infected with *C. trachomatis* (Quayle 2002). Infection of the endometrium and Fallopian tube can result if pathogens breach the cervical barrier resulting in adverse reproductive consequences (Wiesenfeld *et al.* 2002). During pregnancy, the cervical mucus plug and the placenta and fetal membranes also contribute to the innate immune response, maintaining the sterility of the uterine cavity and protecting the developing fetus.

Improved understanding of innate immunity within the female reproductive tract will inform on interventional strategies to protect against disease caused by pathogens

Figure 2 The burden of genital tract infection on reproductive health.

Figure 3 Expression of SLPI, elafin and defensins in the non-pregnant female reproductive tract. *Protein expression has not been reported.

such as *C. trachomatis* (which may help reduce the incidence of infection-related tubal infertility and tubal ectopic pregnancy) and will inform on the clinical management of intrauterine infection during pregnancy.

Natural antimicrobial peptides (NAPs)

Whey acidic protein motif containing proteins: secretory leukocyte protease inhibitor (SLPI) and elafin

Uncontrolled inflammation can result in tissue destruction, in part mediated by proteases (Dallegrì & Ottonello 1997). Anti-proteases, such as SLPI and elafin, counteract the actions of proteases and help prevent resultant damage to host tissues from an over-exuberant response (Sallenave *et al.* 1994, Schalkwijk *et al.* 1999, Sallenave 2000). SLPI inhibits a number of proteases, including neutrophil elastase, trypsin and cathepsin G, whilst elafin appears to be restricted to regulating neutrophil elastase and proteinase 3 (Thompson & Ohlsson 1986, Sallenave & Ryle 1991, Wiedow *et al.* 1991). Both proteins are also potent NAPs, with activity against

Table 1 Natural antimicrobial expression in the female reproductive tract (the menstrual cycle phase showing peak expression of each natural antimicrobial is detailed where this has been reported).

Expression site	Natural antimicrobial					
	HBD1	HBD2	HBD3	HBD4	SLPI	Elafin
Non-pregnant						
Fallopian tube	mRNA (Valore <i>et al.</i> 1998)	Not reported	Not reported	Not reported	mRNA and protein (AW Horne, unpublished observations, Ota <i>et al.</i> 2002)	mRNA and protein (AW Horne, unpublished observations)
Endometrium	mRNA highest in secretory phase (Fleming <i>et al.</i> 2003)	mRNA highest at menstruation (Fleming <i>et al.</i> 2003)	mRNA highest in secretory phase (King <i>et al.</i> 2003b)	mRNA highest in proliferative phase (King <i>et al.</i> 2003b)	mRNA and protein highest in secretory phase (King <i>et al.</i> 2000)	mRNA and protein highest at menstruation (King <i>et al.</i> 2003a)
Cervix	mRNA (Valore <i>et al.</i> 1998)	Not reported	Not reported	Not reported	Protein (Moriyama <i>et al.</i> 1999)	Protein (Pfundt <i>et al.</i> 1996)
Vagina	mRNA (Valore <i>et al.</i> 1998, Narvekar <i>et al.</i> 2007)	mRNA (Narvekar, <i>et al.</i> 2007)	mRNA (Narvekar <i>et al.</i> 2007)	Not reported	Protein – cell line (Fichorova & Anderson 1999, Valore <i>et al.</i> 2006)	Protein (Pfundt <i>et al.</i> 1996) mRNA (Narvekar, <i>et al.</i> 2007)
Pregnant						
Amnion	mRNA and protein (King <i>et al.</i> 2007)	mRNA and protein (King <i>et al.</i> 2007, Stock <i>et al.</i> 2007)	mRNA and protein (King <i>et al.</i> 2007)	Not reported	Protein (Zhang <i>et al.</i> 2001)	mRNA and protein (King <i>et al.</i> 2007)
Chorion	mRNA and protein (King <i>et al.</i> 2007)	mRNA and protein (King <i>et al.</i> 2007)	mRNA and protein (King <i>et al.</i> 2007)	Not reported	Mainly negative (Denison <i>et al.</i> 1999)	mRNA and protein (King <i>et al.</i> 2007)
Placenta	mRNA and protein (King <i>et al.</i> 2007)	mRNA and protein (King <i>et al.</i> 2007)	mRNA and protein (King <i>et al.</i> 2007)	Not reported	Mainly negative (Denison <i>et al.</i> 1999)	mRNA and protein (King <i>et al.</i> 2007)
Decidua	mRNA and protein (King <i>et al.</i> 2007)	mRNA and protein (King <i>et al.</i> 2007)	mRNA and protein (King <i>et al.</i> 2007)	Not reported	mRNA and protein (Denison <i>et al.</i> 1999, King <i>et al.</i> 2000)	mRNA and protein (King <i>et al.</i> 2007)
Cervical mucus plug	Protein (Hein <i>et al.</i> 2002)	Not reported	Not reported	Not reported	Protein (Helmig <i>et al.</i> 1995, Hein <i>et al.</i> 2002)	Not reported
Vaginal secretions	Not reported	Protein (SJ Stock, unpublished observations)	Not reported	Not reported	Protein (SJ Stock, unpublished observations)	Protein (SJ Stock, unpublished observations)

gram-positive and gram-negative organisms (Simpson *et al.* 1999).

SLPI and elafin are expressed throughout the female genital tract (King *et al.* 2000, 2003c; see Table 1). SLPI and elafin expression has been demonstrated in the vagina (Draper *et al.* 2000) and cervix, with high concentrations of SLPI demonstrated in the cervical mucus (Helmig *et al.* 1995, Pfundt *et al.* 1996). We have shown that endocervical and vaginal cell lines express both SLPI and elafin, and that expression in endocervical cells is increased by treatment with the bacterial wall product lipopolysaccharide (SJ Stock, unpublished observations). Neutrophils within the human endometrium area particularly rich source of elafin during menstruation (see Fig. 4), whereas the endometrial epithelium expresses SLPI during the progesterone-dominant secretory phase of the cycle (King *et al.* 2000, 2003c). *In vitro* studies of polarized endometrial epithelial cells have shown that the presence of an anti-SLPI antibody reduces the antibacterial activity of secretions from the apical surface of these cells (Fahey & Wira 2002). These functional data suggest that SLPI is involved in the endometrial innate immune response at least *in vitro*. In common with the rest of the female reproductive tract, the Fallopian tube morphology undergoes cyclical changes under the influence of oestrogen and progesterone (Sulz *et al.* 1998), and while SLPI and elafin expression have been localized to the Fallopian tube epithelium (Ota *et al.* 2002, Horne *et al.* unpublished data) their temporal expression profiles have not been detailed.

SLPI and elafin are also present during pregnancy. SLPI is present in extremely high concentrations in the cervical mucus plug, a key barrier to upper genital tract infection during pregnancy (Hein *et al.* 2002) and both SLPI and elafin are present in the vaginal secretions in pregnancy (Stock *et al.* unpublished). SLPI is present in first trimester decidua (King *et al.* 2000) and the main

sites of expression within the uterus at term pregnancy are the amnion epithelium and decidua (Denison *et al.* 1999). SLPI concentrations in amniotic fluid are increased in late gestation and particularly with the onset of labour (Denison *et al.* 1999). Elafin is localized to the amnion epithelium, decidua, chorion and placental trophoblast (King *et al.* 2007). The presence of SLPI and elafin at several uterine sites during gestation indicates that they may have an important natural antimicrobial role but also that their anti-protease and anti-inflammatory activity may be involved in preventing excessive inflammation and tissue remodelling during pregnancy.

SLPI and elafin demonstrate a variety of anti-inflammatory effects in a number of other tissues, which cannot be solely attributed to their anti-proteinase or anti-bacterial activities. In an animal model of chemically induced lung fibrosis, administration of SLPI decreases tissue damage, and even a truncated form that lacks the anti-elastase domain exerts this effect (Mitsuhashi *et al.* 1996). SLPI also decreases cortical damage after ischaemic-induced stroke in rats (Wang *et al.* 2003). Forced expression of elafin in animal models modulates the systemic and pulmonary response to lipopolysaccharide (LPS; Sallenave *et al.* 2003) and reduces inflammation associated with myocardial infarction (Ohta *et al.* 2004). In humans, SLPI and elafin induction appear attenuated in Crohn's disease, characterized by inflammation affecting the full thickness of intestinal wall, when compared with ulcerative colitis, which is an inflammatory condition only involving the mucosa (Schmid *et al.* 2007). Some of the anti-inflammatory effects of the anti-leukoproteins may involve inhibition of NF κ B. *In vitro*, overexpression of SLPI suppresses NF κ B activation (Jin *et al.* 1997, Sano *et al.* 2003, Henriksen *et al.* 2004). It can prevent proteasome-dependent I κ B β degradation (Lentsch *et al.* 1999) and also directly bind to NF κ B consensus sites on DNA, inhibiting transcription (Taggart *et al.* 2005). Wound healing and tissue remodelling are other processes involving the anti-leukoproteins. SLPI and elafin are produced in response to cutaneous injury (van Bergen *et al.* 1996, Wingens *et al.* 1998) and SLPI null mice show impaired cutaneous wound healing and increased inflammation and elastase activity (Ashcroft *et al.* 2000, Angelov *et al.* 2004). SLPI can also modulate production of matrix metalloproteinases from monocytes by inhibiting enzymes involved in PGE₂ (prostaglandin E₂) synthesis (Zhang *et al.* 1997).

Defensins

Leukocytes and epithelial cells are the main sources of human defensins (Klotman & Chang 2006). Human β -defensins (HBD) 1–4 and α -defensin (human defensin 5, HD5) have all been reported to be expressed in the endometrial epithelium, each with their own unique

Figure 4 Immunohistochemical localization of elafin in endometrium from the menstrual phase. Elafin is present in endometrial neutrophils (brown staining). Scale bar = 100 μ m.

temporal expression profile (Svinarich *et al.* 1997, Quayle *et al.* 1998, Valore *et al.* 1998, Fleming *et al.* 2003, King *et al.* 2003a,b). HBD1, HBD3 and HD5 are expressed at highest levels during the secretory phase (Quayle *et al.* 1998, Fleming *et al.* 2003, King *et al.* 2003b; see Table 1). By contrast, HBD2 mRNA expression shows a dramatic peak during menstruation while HBD4 is expressed mainly in the proliferative phase (Fleming *et al.* 2003, King *et al.* 2003b). The functional implications of these different patterns of expression have not been determined and may relate to differences in antimicrobial activity of members of the defensin family or to their other activities such as regulation of leukocyte chemotaxis. HD5 has also been demonstrated in the vagina, cervix and Fallopian tube (Quayle *et al.* 1998). HBD2 is also present in cervicovaginal secretions and is expressed by a vaginal keratinocyte cell line *in vitro* (SJ Stock, unpublished observations). HBD1–3 are widely expressed in the pregnant uterus with expression in the amnion, decidua, chorion and placental trophoblast (King *et al.* 2007, Stock *et al.* 2007). Their presence at key sites suggests that they are likely to be involved in the innate immune response during pregnancy and may act to prevent uterine infection (see Table 1).

Toll-like receptors (TLRs)

TLRs 1–10 are expressed in the female reproductive tract (Darville *et al.* 2003, Pioli *et al.* 2004, Aflatoonian *et al.* 2007). Vaginal epithelial cell lines have been shown to express TLR1–3, TLR5 and TLR6, and primary endocervical epithelial cells have been shown to express TLR1–3 and TLR6 (Fichorova *et al.* 2002). Endometrial epithelial cell lines, primary endometrial epithelial cells and primary decidual cell cultures express TLR1–9, indicating the potential to respond to a wide range of pathogens (Schaefer *et al.* 2004, 2005, Krikun *et al.* 2007). TLRs are present in both endometrial epithelial and stromal cells (Aflatoonian *et al.* 2007, Hirata *et al.* 2007) and have also been reported in uterine NK cells, an endometrial cell with a key role in implantation and early pregnancy (Eriksson *et al.* 2006).

The TLRs are differentially expressed in each individual reproductive tract tissue and also show cycle-dependent expression in the endometrium. For example, TLR2 and TLR4 show higher expression in the Fallopian tube and endometrium compared with that in the cervix (Pioli *et al.* 2004). Studies detailing cycle-dependent expression in endometrium are contradictory with one study suggesting that *TLR2–4* and *TLR9* mRNA expression is higher during the perimenstrual phase (Hirata *et al.* 2007), while another shows peak expression of *TLR2–6*, *TLR9* and *TLR10* mRNA in the secretory phase (Aflatoonian *et al.* 2007). However, there are differences in the cycle phases compared in these studies and also in the way that menstrual cycle phase was confirmed.

TLRs have been shown to be functional in cell types from throughout the reproductive tract in *in vitro* experiments. Endometrial, cervical and Fallopian tube epithelial cells have both been shown to respond to the TLR3 ligand, poly I:C, with increased cytokine output (Lesmeister *et al.* 2005, Andersen *et al.* 2006, Ghosh *et al.* 2008, Nasu *et al.* 2007). Fallopian tube fibroblasts and endometrial epithelial and stromal cells have been shown to increase inflammatory mediator output in response to LPS (lipopolysaccharide), a TLR4 ligand (Hirata *et al.* 2005, Itoh *et al.* 2006). These *in vitro* data suggest that TLRs on reproductive tract cells will be capable of responding to pathogen products *in vivo*.

TLRs are also present in the pregnant uterus. Several studies have examined TLR expression both in the first trimester and in late pregnancy. TLR2 and TLR4 are widely reported to be present on trophoblast cells (Holmlund *et al.* 2002, Abrahams *et al.* 2004, Kumazaki *et al.* 2004, Beijar *et al.* 2006, Ma *et al.* 2007). The related, intracellular innate immune receptors nucleotide oligomerization domain (NOD) 1 and 2 are also present in the first trimester trophoblast (Costello *et al.* 2007). *In vitro* studies with the first trimester trophoblast have shown TLR3 and TLR4 to respond to their ligands by increasing cytokine and chemokine output and increasing chemotaxis of monocytes and neutrophils (Abrahams *et al.* 2004, 2005, 2006). Similar studies have shown TLRs on syncytiotrophoblast and in villous explants from term pregnancies to signal responses to ligand binding (Holmlund *et al.* 2002, Ma *et al.* 2007). These studies suggest that the trophoblast is capable of mounting an innate immune response and may also initiate activation of the adaptive immune response.

Disorders of the female reproductive tract

Sexually transmitted infection

The potential role of natural antimicrobials in preventing STI is unclear and there are very few studies investigating the activity of natural antimicrobials against genital tract pathogens (see Table 2). We have shown that bacterial vaginosis, a condition associated with increased transmission of STIs, is associated with low levels of both elafin and SLPI in cervicovaginal secretions (SJ Stock, unpublished observations). In addition, low levels of SLPI in vaginal secretions are associated with the presence of various lower reproductive tract infections, including *Chlamydia* (Draper *et al.* 2000). This study suggested that these low levels of SLPI may result from degradation by pathogens or reduced expression as a consequence of damage to mucosal surfaces due to infection. An alternative possibility is that defective natural antimicrobial production or function may predispose to genital tract infection. This remains to be investigated.

Table 2 The role of natural antimicrobials and pattern recognition receptors in disorders of the female reproductive tract.

Disorder	Natural antimicrobials	Pattern recognition receptors
Sexually transmitted infection	Bacterial vaginosis results ↓ SLPI and elafin in cervicovaginal secretions (S) Stock, unpublished observations) ↓SLPI in vaginal secretions when lower tract infection is present (Draper <i>et al.</i> 2000)	TLR2 knockout mice have ↓ cytokine concentrations in genital tract secretions and ↓ pathology in the Fallopian tube when they are infected with <i>Chlamydia</i> (Darville <i>et al.</i> 2003) in comparison TLR2 normal mice TLR2 (O'Connell <i>et al.</i> 2006) and TLR4 (Bulut <i>et al.</i> 2002) have a role in chlamydial infection of human cells <i>in vitro</i> The presence of ≥ SNPs in <i>TLR9</i> , <i>TLR4</i> , <i>CD14</i> and <i>NOD2</i> is associated with an ↑ risk of tubal pathology as a result of chlamydial infection (den Hartog <i>et al.</i> 2006) No studies
Tubal damage, infertility and tubal ectopic pregnancy	Chlamydial infection of HeLa and human trophoblast cells results in ↑ SLPI expression (N Wheelhouse, personal communication) SLPI mRNA expression is ↑ in uterine decidua from women with tubal pregnancies compared with those with failed intrauterine pregnancies (Dalgetty <i>et al.</i> 2008)	
Preterm labour	HBD3 protein expression is ↑ in fetal membranes from women with preterm labour associated with chorioamnionitis (Buhimschi <i>et al.</i> 2004) Elafin mRNA and protein is ↑ in fetal membranes from women with preterm labour associated with chorioamnionitis (Tromp <i>et al.</i> 2004) SLPI concentrations in amniotic fluid are ↓ in cases of preterm premature rupture of membranes (Helmig <i>et al.</i> 2002) Elafin mRNA and protein expression is ↓ in cases of preterm premature rupture of membranes (Tromp <i>et al.</i> 2004)	TLR4 normal mice treated with heat-killed <i>Escherichia coli</i> deliver preterm while there is no effect on TLR4 mutant mice (Wang & Hirsch 2003) Activation of TLR2 or TLR3 also causes preterm labour in mice (Ilievski <i>et al.</i> 2007) TLR2 expression is ↓ in placentae (Rindsjo <i>et al.</i> 2007) and ↑ in fetal membranes (Kim <i>et al.</i> 2004) in pregnancies complicated by chorioamnionitis TLR4 expression is ↑ in placental macrophages in pregnancies complicated by chorioamnionitis (Kumazaki <i>et al.</i> 2004) Chlamydial heat shock protein 60 treatment causes apoptosis of primary trophoblast, placental fibroblasts and the JEG3 cell line and this may lead to placental dysfunction and poor pregnancy outcome (Equils <i>et al.</i> 2006) A TLR4 polymorphism in infants is associated with a ↑ risk of preterm birth (Lorenz <i>et al.</i> 2002) Infants with two copies of a variant TLR2 are at ↑ risk of preterm birth (Krediet <i>et al.</i> 2007)

There are data to suggest that TLRs may be involved in the pathology of STIs (see Table 2), particularly *C. trachomatis* and *Neisseria gonorrhoeae*. Infection of TLR2 knockout mice with *Chlamydia muridarum* (previously classified as the mouse biovar of *C. trachomatis*) results in reduced levels of the cytokines, tumour necrosis factor α and MIP2 (macrophage inflammatory protein), in genital tract secretions and reduced chronic pathology in the Fallopian tube relative to normal mice, although the progression of infection does not differ (Darville *et al.* 2003). This suggests that, at least in the mouse, TLR2 plays a central role in Fallopian tube pathology as a result of chlamydial infection. Mice deficient in TLR4 did not show changes relative to the wild type. Studies in mice also suggest that NOD1 does not play a major role in the genital tract response to chlamydial infection. While the presence of a dominant negative mutant of *NOD1* partially inhibited NF κ B activation as a result of chlamydial infection of the cervical epithelial HeLa cell line, proinflammatory mediator expression in the genital tracts of NOD1-deficient mice after chlamydial infection was

comparable with that in wild-type mice (Welter-Stahl *et al.* 2006). However, these data may not be representative of infection with *C. trachomatis* as the pathology of infection has been reported to differ between *C. muridarum* and *C. trachomatis* (Morre *et al.* 2000).

There are few studies examining the role of TLRs in the infection of human cells with *C. trachomatis* and these have yielded conflicting information depending on the cell type examined. However, there is evidence to suggest that TLR2 and TLR4 may both be involved in the response to *Chlamydia*. O'Connell *et al.* (2006) have reported that TLR2 is recruited to intracellular *Chlamydiae* in HEK293 cells transfected with TLR2 and that TLR2 is required for cellular activation (determined by IL-8 measurement) during infection. In human microvascular cells, chlamydial heat shock protein 60 (HSP60) acts via TLR4 to activate NF κ B and increase IL-8 secretion (Bulut *et al.* 2002).

The role of TLRs and NODs expressed by cells of the female reproductive tract (e.g. Fallopian tube epithelium) in the response to chlamydial infection, or any STI, remains poorly understood. However, the

aforementioned differences in the expression pattern of the TLRs in individual reproductive tract microenvironments suggest they may contribute, at least in part, to a compartmentalized immune response towards pathogen invasion (Pioli *et al.* 2004). Furthermore, a recent study examining single nucleotide polymorphisms in *TLR9*, *TLR4*, *CD14* and *NOD2* in women with chlamydial infections indicated that women with SNPs in two or more of these genes have a trend towards an increased risk of tubal pathology (den Hartog *et al.* 2006) suggesting that pattern recognition receptors are involved in the progression of the infection.

Tubal damage, infertility and tubal ectopic pregnancy

Although it has been suggested that chlamydial infection is one of the major causes of tubal damage (Odland *et al.* 1993), the innate immunopathogenic events that lead to infertility and tubal ectopic pregnancy following infection are unclear (see Table 2). *In vitro* studies have demonstrated that *Chlamydia* induces expression of SLPI in HeLa and a human trophoblast cell line (N Wheelhouse, personal communication). It is possible that *Chlamydia* acts via NF κ B by way of the above signalling cascade, and that persistent, or repeated, infection causes atypical expression of SLPI in the uterus and Fallopian tube leading to tubal damage, and/or blockage, or to a susceptibility to tubal implantation. To our knowledge, there have been no studies performed examining the expression pattern of NAPs or TLRs in these pathologies.

With tubal ectopic pregnancy, direct comparison of the expression of factors important for innate immunity between tubal and intrauterine sites is difficult due to the anatomical differences of the two implantation locations. However, similar to the endometrial response to an intrauterine pregnancy, with a tubal gestation, there is a decidual reaction in the uterine cavity but not usually in the Fallopian tube (Stock 1991). The uterine decidua contains innate immune leukocytes, such as CD56+ uterine natural killer cells, macrophages and mast cells (Bulmer *et al.* 1988, King & Loke 1991, Clark *et al.* 1994, Hunt 1994, Marx *et al.* 1999). We therefore compared SLPI and elafin expression in the uterine decidua of women with ongoing intrauterine (women undergoing surgical termination of pregnancy), failed intrauterine (women with miscarriage) and tubal gestations and demonstrated that *SLPI* (but not elafin) mRNA expression was significantly higher in the uterine decidua of women with a tubal compared with a failed intrauterine gestation (miscarriage; Dalgetty *et al.* 2008). If the uterine environment truly reflects that of the Fallopian tube, the abnormal expression of SLPI in the uterine decidua may simply mirror the biological changes occurring in the Fallopian tube due to extra-uterine implantation. Alternatively, the abnormal expression of

SLPI may predispose to, rather than be a consequence of, tubal pregnancy.

Preterm birth

There is evidence to suggest that expression of natural antimicrobials is altered in preterm labour, particularly in cases of chorioamnionitis (see Table 2). HBD3 protein (Buhimschi *et al.* 2004) and elafin mRNA and protein expression (Tromp *et al.* 2004) have both been reported to increase in the fetal membranes of pregnancies complicated with chorioamnionitis suggesting that these natural antimicrobials are upregulated as a result of infection. In addition, amniotic fluid concentrations of SLPI (Helmig *et al.* 2002) and mRNA and protein expression of elafin in fetal membranes (Tromp *et al.* 2004) are reduced in cases of preterm premature rupture of membranes. This reduced expression may result in decreased anti-protease activity promoting a shift in favour of protease-mediated degradation of membranes. Their reduced expression may, however, also reduce antimicrobial protection in the uterus, allowing the establishment of detrimental infections. It should be noted that the studies detailed above do not address whether the changes described are a cause or effect of the pathology and there are no functional data showing a role for natural antimicrobials in preterm labour.

Studies in mice suggest that activation of TLRs results in preterm labour. Administration of heat-killed *Escherichia coli* to TLR-normal pregnant mice results in preterm labour, while identical treatment of TLR4-mutant mice has a reduced effect. In this study, TLR4 stimulation reduced prostaglandin dehydrogenase expression in fetal membranes and myometrium resulting in increased local concentrations of prostaglandins; mediators with a well-established role in the onset of labour (Wang & Hirsch 2003). Preterm delivery also occurs in mice upon activation of TLR2 or TLR3 (Ilievski *et al.* 2007) with TLR3 activation resulting in increased levels of CCL5 and IFN γ in uterine tissues. Zhang *et al.* (2007) have reported pregnancy failure and abnormal uterine spiral artery development in mice as a result of administration of the TLR3 agonist, poly I:C, earlier in gestation. These studies suggest that the presence of TLR ligands in the uterus stimulate local inflammatory pathways resulting in preterm labour.

Studies in human pregnancies also suggest that TLRs may be involved in the onset of preterm labour associated with infection. It has been reported that TLR2 and TLR4 protein expression is altered in pregnancies complicated with chorioamnionitis (inflammation between the amnion and the chorion). TLR2 expression is reported to be reduced in placenta affected by chorioamnionitis (Rindsjo *et al.* 2007), while it is increased in chorioamnion (Kim *et al.* 2004). TLR4 expression in placental macrophages (Hofbauer cells) is also reported to increase in preterm delivery

complicated with chorioamnionitis (Kumazaki *et al.* 2004). While these studies suggest that TLR expression changes with chorioamnionitis, it remains unclear whether this is a cause or effect of inflammation and/or parturition. Additionally, changes in expression may not be reflected in functional activity of the TLRs although some studies have suggested that there may be an association between TLR function and pregnancy failure or preterm labour. The chlamydial HSP60 has been shown to cause apoptosis of primary first trimester trophoblast cells, placental fibroblasts and the JEG3 trophoblast cell line via TLR4 (Equils *et al.* 2006). It has been suggested that *TLR4* activity may cause placental apoptosis and possibly compromise a pregnancy in women with chlamydial infection. In addition, Lorenz *et al.* (2002) have reported that when an Asp299Gly polymorphism is present in the *TLR4* gene of infants they have a higher risk of preterm birth. This polymorphism causes impaired receptor function suggesting that a reduced response to gram-negative bacteria increases the likelihood of infection and subsequent preterm labour. It has also been reported that infants carrying two copies of a variant *TLR2* gene are at increased risk of premature delivery (Krediet *et al.* 2007).

Conclusion

Infection and inflammation can have devastating consequences to fertility and pregnancy. To date, most interventions directed at preventing and treating such infections have focused on pathogen elimination through the use of antibiotics. Unfortunately, these strategies have been disappointing in eradicating such infections, or decreasing the burden of related complications, such as tubal infertility, ectopic pregnancy and preterm birth.

The study of the innate immune response has highlighted the importance of host-related factors in the response to infectious agents. Genetic variation in innate immunity can explain individual differences in responses to infection. A suboptimal innate immune response may result in a permissive environment for pathogen colonization, whereas an over-exuberant response will cause excessive inflammation and tissue damage. This may be why a 'one-size fits all' approach has been hitherto unsuccessful and modulation of the host response to infection is an attractive alternative or adjuvant approach to antibiotic therapies in treatment of genital tract infections. TLR agonists and antagonists are currently under clinical development for the treatment of cancer, allergies and as vaccines and vaccine adjuvants (Kanzler *et al.* 2007) raising the possibility that in the future interventions may be aimed at modulating the innate immune response in the reproductive tract. Further research into the innate immune response may elucidate pathological

mechanisms underlying infectious morbidities such as tubal infertility and preterm labour, but as yet few studies have examined the functional effects of innate immune components such as NAPs and TLRs in the reproductive tract. While existing SLPI (Ashcroft *et al.* 2000) and TLR-null (Sugawara *et al.* 2003, Andersen-Nissen *et al.* 2007) mutant mouse models show impaired wound healing and altered responses to infection, they have provided little information relating directly to the reproductive tract. In addition, fundamental differences in key physiological reproductive processes, such as implantation and placentation, exist between mouse and human limiting the relevance of these rodent models.

It is anticipated that a greater understanding of the function of the innate immune system in the reproductive tract and the development of TLR agonists and antagonists for clinical use may lead to advances in the management of clinically important conditions, such as tubal infertility, ectopic pregnancy and preterm labour.

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