Antimicrobial peptides and pregnancy

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Abstract

Antimicrobial peptides (AMPs) are small proteins produced by epithelial surfaces and inflammatory cells, which have broad-spectrum antimicrobial and immunomodulatory activities. They are known to be important in a number of infectious and inflammatory conditions and have been shown to be present in a number of sites throughout the female reproductive tract. Inflammation and infection are associated with a number of complications of pregnancy including preterm labor, and AMPs may play a key role in maintaining and protecting pregnancy. The aim of this review is to describe the expression and function of AMPs in the pregnant female reproductive tract and their relation to preterm labor.

Reproduction (2011) **141** 725–735

Introduction

Antimicrobial peptides (AMPs) are genetically determined proteins that play a major part in both innate and adaptive immunity. They are an ancient form of host defense first established in lower phyla such as plants and invertebrates but now appreciated as being vital in the mammalian immune response. To date, more than 1600 AMPs have been identified or projected (<http://aps.unmc.edu/AP/main.php>; accessed 25 November 2010). A single species will express a variety of different proteins, either constitutively or after induction by inflammatory stimuli, which can act synergistically to provide broad-spectrum antimicrobial activity against bacteria, fungi, and some viruses (Hancock & Rozek 2002). Although categorized by their antimicrobial activities, it has become clear that many mammalian AMPs are multifunctional molecules, with important roles in the modulation of inflammation and interaction with adaptive immunity (Ganz 2003).

In humans, deficiencies in AMPs have been implicated in pathogenesis of a number of infectious and inflammatory conditions. Deficiencies can result in loss of function of AMPs resulting in increased susceptibility to infection and inflammation, which play a role in many diseases (Beisswenger & Bals 2005). They are likely to have important functions in the female reproductive tract, where infection and inflammation can jeopardize successful pregnancy. In this review, we will discuss the best-characterized human AMPs, defensins, cathelicidin, and the whey acidic protein motif containing proteins secretory leukocyte protease inhibitor (SLPI) and elafin/trappin-2, which have 'defensin-like' activities.

We outline their production and their relevance in the human female reproductive tract in pregnancy.

AMP structure, expression, and regulation

Table 1 summarizes the expression, regulation and functions of AMPs.

Defensins

Defensins are small cationic proteins containing 28–42 amino acids, which form triple-stranded β sheets on a frame of six disulphide-linked cysteines. The classification into α - and β -defensins depends on the position of the cysteine bonds, but they have very similar tertiary structures (Zimmermann *et al.* 1995). A third class, the θ -defensins, has been identified (Tang *et al.* 1999), that has a circular structure and enhanced antiviral activities (Lehrer 2004).

The α -defensins (human neutrophil peptides 1–4; HNP1–4) are a product of neutrophils, forming 30–50% of the protein content of the azurophilic granules (Rice *et al.* 1987). They are also found in the Paneth cells of the small intestine (human defensins 5 and 6; HD5 and HD6; Selsted *et al.* 1992). In contrast, the human β -defensins (HBDs) are predominantly epithelial products. HBD1–4 are the best characterized, although many additional defensin genes have been identified (Scheetz *et al.* 2002, Schutte *et al.* 2002, Yamaguchi *et al.* 2002). HBD1–3 are widely expressed at epithelial surfaces, whereas HBD4 has more restricted expression, found in testis, stomach, uterus, neutrophils,

Table 1 Summary of the expression, regulation and functions of AMPs.

AMPs	Gene(s)	Regulation	Source	Main function
SLPI	<i>SLPI</i>	Inducible expression by IL1B, LPS, TNF, EGF, mycobacteria, and viral dsRNA (POLY I:C)	Epithelial cells: respiratory, intestinal and genital tracts, oral cavity Neutrophils, macrophages, and mast cells Decidua	Broad-spectrum antibacterial activity against Gram (+) and Gram (–) bacteria Antiviral activity against HIV Protease inhibition against cathepsin G, trypsin, chymotrypsin, chymase and neutrophil elastase
Elafin/trappin-2	<i>PI3</i>	Constitutive and inducible expression by LPS, IL1B, and TNF	Epithelial cells: respiratory and intestinal tract, skin Neutrophils and macrophages Decidua Placental and chorion trophoblasts	Broad-spectrum antibacterial activity against Gram (+) and Gram (–) bacteria Induction of chemotactic agents, neutrophils Protease inhibition against human neutrophil elastase and proteinase-3
LL37	<i>CAMP</i>	Constitutive and inducible expression by vitamin D and LPS	Epithelial cells: gut, urinary tract, respiratory tract, skin Neutrophils, monocytes, mast and myeloid cells	Broad-spectrum antimicrobial activity with specific activity against Group A and B <i>Streptococcus</i> , <i>Staphylococcus aureus</i> , <i>Escherichia coli</i> Induction of chemotactic agents, monocytes, neutrophils Direct chemotactic activity Angiogenesis
β -Defensins HBD1	<i>DEFB1</i>	Constitutive expression	Epithelial cells: genitourinary tract, lung, skin, gut	Broad-spectrum antibacterial activity, specifically active against <i>Escherichia coli</i> , <i>Pseudomonas aeruginosa</i> Antiviral activity against HIV
HBD2	<i>DEFB4</i>	Inducible expression by IL1B, Gram (+) and Gram (–) bacteria, TNF, LPS and viral dsRNA (POLY I:C)	Placental trophoblasts	
HBD3	<i>DEFB103</i>	Inducible expression by Gram (+) and Gram (–) bacteria, TNF	Decidua	Induction of cytokines IL8, IL6, CCL2 (MCP-1), and IL10
α -Defensins HNP1	<i>DEFA1, 3</i>	Constitutive expression	Neutrophil azurophilic granules	Broad-spectrum antibacterial activity against Gram (+) and Gram (–) bacteria Antiviral activity Angiogenesis
HNP2	<i>DEFA1, 3</i>	Constitutive expression		
HNP3	<i>DEFA1, 3</i>	Constitutive expression		
HNP4	<i>DEFA 4</i>	Constitutive expression		

thyroid, and kidney (Garcia *et al.* 2001b). HBD3 is also expressed in some non-epithelial tissues including skeletal and cardiac muscle and leukocytes (Garcia *et al.* 2001a).

Unlike the HNPs, which are stored, HBD concentrations are generally governed by synthesis and secretion rates. Production of HBD1 is generally constitutive, whereas HBD2 and HBD3 tend to be upregulated by a variety of inflammatory stimuli including bacteria, bacterial products, and inflammatory cytokines (Lai & Gallo 2009). The 5'-flanking region of the *HBD2* gene contains NFkB, C/EBP, and AP-1 binding sites (Tsutsumi-Ishii & Nagaoka 2002), whereas the region of the *HBD3* gene has C/EBP and AP-1 sites (Jia *et al.* 2001), suggesting that these elements are important in HBD expression. *In vivo*, HBD2 and HBD3 tend to be found in epithelia in association with infection and inflammation, whereas HBD1 is often intrinsically

present in healthy tissues (Lai & Gallo 2009). HBDs can be downregulated by glucocorticoids and psychological stress, increasing susceptibility to infections (Aberg *et al.* 2007).

Most HBD genes are found in a cluster at 8p23.1 (Schutte *et al.* 2002), which is polymorphic in copy number (Hollox *et al.* 2005). Individuals have 2–12 copies of this repeat per diploid genome. The copy number is reflected in mRNA expression levels, and Crohn's disease has been associated with both a decreased copy number of *DEFB4* and a decreased intestinal mRNA expression of HBD2 (Fellermann *et al.* 2006). A more recent study has failed to replicate these findings (Aldhous *et al.* 2010), and copy number polymorphisms are not associated with severity of lung disease in cystic fibrosis (Hollox *et al.* 2005). It remains to be observed whether the copy number correlates with other infectious or inflammatory conditions.

Until recently, it was thought that humans had lost the capacity to produce θ -defensin peptides, which are expressed in old world primates (Doss *et al.* 2009). Although humans express a gene with almost 90% identity with the θ -defensin gene from the rhesus macaque (Venkataraman *et al.* 2009), a premature stop codon in the upstream signaling sequence normally prevents translation (Klotman & Chang 2006). Recently, Venkataraman *et al.* (2009) showed that human pro-myelocytic cells transfected with plasmids containing repaired retrocyclin-like genes had the ability to produce the cyclic antiviral peptides θ -defensins. Aminoglycoside antibiotics also had the ability to read-through the premature stop codon and produce functional peptides confirming that human cells do retain the ability to produce θ -defensins (Venkataraman *et al.* 2009). This is of particular relevance in reproductive biology, as produced θ -defensins have important anti-HIV activities (see below).

Cathelicidin

Like the defensins, cathelicidins are small basic peptides. In humans, only one cathelicidin gene has been identified, *CAMP*, which encodes a 18 kDa precursor protein hCAP18. This is enzymatically processed to release the active form LL37, which has a α -helix structure (Lai & Gallo 2009). Cathelicidin is expressed in neutrophils, macrophages, and epithelia (Sorensen 2005). In contrast to the defensins, expression is not directly influenced by inflammatory stimuli and found to be mediated through vitamin D (Bucki *et al.* 2010). The active form of vitamin D, 1,25 D₃ increases transcription of hCAP18 directly, as well as upregulating inflammatory signaling components such as Toll-like receptors (TLRs) and CD14, thus further increasing other AMP expression and innate immune responses.

LL37, the active form of hCAP18, is released from the C-terminus by proteolytic enzymes such as proteinase 3 and kallikrein (Lai & Gallo 2009). In human neutrophils, hCAP18 is usually processed to release LL37, whereas epithelial LL37 may be further processed into smaller peptides such as RK-31, KS-30, and K20 by bacterial proteases from microflora (Murakami *et al.* 2004). These derivatives exhibit significant heterogeneity in their antimicrobial activities.

Secretory leukocyte protease inhibitor

SLPI is a 11.7 kDa protein consisting of 107 amino acids including 16 cysteine residues that form eight disulphide bridges (Seemuller *et al.* 1986). It is constitutively expressed by many epithelia, including the mucosa of the respiratory, intestinal and genital tracts, and oral cavity (Williams *et al.* 2006) as well as neutrophils, macrophages and mast cells (Bohm *et al.* 1992, Jin *et al.*

1997, Sallenave *et al.* 1997, Westin *et al.* 1999). The *SLPI* gene is localized on chromosome 20q12–13.2 (Kikuchi *et al.* 1997) and is a non-polymorphic, stable gene but can be modulated at both the transcriptional and the translational levels (Maruyama *et al.* 1994). The promoter region has not been extensively studied, but early investigations demonstrated an AP-1 and AP-2 consensus site (Abe *et al.* 1991). Epithelial expression of SLPI appears to be tissue specific. It is increased by a variety of inflammatory stimuli including LPS, IL1B, TNF, EGF, HNP, and human neutrophil elastase whereas antiinflammatory mediators such as TGF β and IL10 can decrease production (Williams *et al.* 2006).

Elafin/trappin-2

Elafin is a 9.9 kDa protein composed of 95 amino acids and shows 40% sequence identity with the SLPI molecule (Sallenave & Silva 1993). Encoded by the *PI3* gene, expression is constitutive in a number of epithelial barriers constantly exposed to foreign antigens and pathogens, including skin, airway, and intestinal mucosa (Pfundt *et al.* 1996). *PI3* is also expressed in neutrophils and macrophages and can be transcriptionally upregulated by LPS and pro-inflammatory cytokines such as IL1B and TNF (Sallenave *et al.* 1994, Pfundt *et al.* 2000, Simpson *et al.* 2001). *PI3* has 5'-regulatory sites for NF κ B and AP-1 (Zhang *et al.* 1995), and the response to cytokines appears to involve MAPK pathways in the skin (Pfundt *et al.* 2000) and NF κ B pathways in the lung (Bingle *et al.* 2001). The *PI3* gene is highly polymorphic, with 23 single-nucleotide polymorphisms (SNPs), 11 of which are in the promoter region (Chowdhury *et al.* 2006), and polymorphisms have recently been associated with low circulating elafin levels and increased risk of adult respiratory distress syndrome in critically ill patients (Tejera *et al.* 2009).

Elafin is released by proteolysis from a precursor protein, trappin-2, containing an N-terminal 'cementoin' domain that forms covalent bonds in extra-cellular matrix via tissue transglutaminases, anchoring the molecule to tissues (Nara *et al.* 1994). Its C-terminal domain contains an antiproteinase site that is similar to that of SLPI (Nara *et al.* 1994). There is some evidence that tissue bound trappin-2 is more effective at preventing elastase-mediated tissue damage *in vivo* (Tremblay *et al.* 2002).

AMP functions

Although recognized for their direct antimicrobial properties, it is increasingly clear that AMPs can help protect against infection by virtue of indirect immunomodulatory activities. Indeed, in some instances, other AMP functions, such as chemokine and antiendotoxin activities, may be more important than their direct

antimicrobial functions in the resolution of infection. The functions of AMPs are illustrated in Fig. 1.

Antimicrobial effects

In vitro, AMPs have broad-spectrum antimicrobial activity against bacteria, viruses (including HIV), and fungi (reviewed by Reddy *et al.* (2004)). These effects appear to be synergistic with each other and other classes of AMPs (Chen *et al.* 2005). AMPs may have enhanced antimicrobial efficiency at low pH (4.6) (Valore *et al.* 1998) and *in vitro* activity is salt dependent (Jenssen *et al.* 2006).

It has been postulated that microbial killing by AMPs may be effected via a variety of mechanisms including membrane depolarization, membrane permeabilization, induction of hydrolases, disruption of membrane functions, and/or damage of critical intracellular proteins (Zasloff 2002, Lai & Gallo 2009). Crucial to all these processes are selective interactions with the membranes of microorganisms. Increasing ionic strength generally decreases antimicrobial activity, probably via reducing the strength of initial interactions. Mammalian cell membranes are mainly constructed by zwitterionic phospholipids, which have no net charge overall, and any phospholipids with anionic head groups tend to face inward. They are also stabilized by high cholesterol content. In contrast, prokaryote membranes have many highly anionic phospholipids orientated outward which interact with positively charged, hydrophobic antimicrobials. AMPs may form aggregates in the lipid bilayer, physically disrupting it, and, in some cases, allowing

diffusion of peptides to intracellular targets (Matsuzaki 1999, Shai 1999, Yang *et al.* 2000).

In vivo models have shown increased susceptibility to infection with AMP knockdown (Gallo & Nizet 2008) or improved resolution of infection with forced expression (Simpson *et al.* 2001). However, the minimal inhibitory concentrations required for bactericidal activity *in vitro* are often considerably higher than normal physiological AMP concentrations (Lai & Gallo 2009). Various explanations have been suggested to explain this apparent contradiction, including that AMPs act synergistically, or that high concentrations of AMPs accumulate locally in association with inflammation. However, AMPs also have indirect effects that may help to eradicate infection.

Immunomodulation

AMPs can act as opsonins to aid clearance of bacteria by inflammatory cells (Wilkinson *et al.* 2009). AMPs have also been variously shown to be chemotactic for monocytes, macrophages, neutrophils, mast cells, dendritic cells, and T-cells (Oppenheim *et al.* 2003). They can indirectly recruit leukocytes through upregulation of inflammatory cytokines and other chemokines including IL8, IL6, and CCL2 (MCP-1) (Lai & Gallo 2009).

AMPs also exhibit antiinflammatory or pro-resolution functions, being capable of inducing production of antiinflammatory cytokines such as IL10 and TGFB (Lai & Gallo 2009). They can interact with pattern recognition receptors such as TLRs. These are expressed in cells involved in the first line of host defense including neutrophils, macrophages, and mucosal epithelial cells (Fazeli *et al.* 2005). There are ten TLRs in humans with each one having specificity for different pathogen products, which work collectively to alert the immune system to detection of a pathogen (Fazeli *et al.* 2005). TLRs generate intra-cellular signals through NFκB-dependent and independent pathways to mediate inflammation and elimination of pathogens. Cathelicidin has been shown to inhibit TLR-mediated induction of cytokine release and maturation of dendritic cells (Di Nardo *et al.* 2007). AMPs can also modulate components of TLR signaling pathways such as NFκB (Lai & Gallo 2009) and bind and neutralize LPS, a TLR ligand, thus indirectly altering TLR responses (McMichael *et al.* 2005, Rosenfeld *et al.* 2006).

Protease inhibition

Although the innate immune response to pathogens is designed to protect the host, the inflammatory response mediated through this system can potentially result in considerable damage to the host tissue. This is in part mediated by proteases that are produced by a number of phagocytic inflammatory cells to degrade ingested

Figure 1 Summary illustrating the function of epithelial AMPs.

pathogens (Dallegrì & Ottonello 1997, Williams *et al.* 2006). The host responds to this by secreting antiprotease molecules in order to protect the host tissue and neutralize any excess protease (Williams *et al.* 2006). SLPI and elafin/trappin-2 have antiprotease activity (Sallenave 2010). SLPI can inhibit a variety of human neutrophil proteases including cathepsin G, trypsin, chymotrypsin, and chymase; however, its greatest activity seems to be against neutrophil elastase (Thompson & Ohlsson 1986, Boudier & Bieth 1992). Trappin-2 has more restricted antiprotease activity to SLPI, inhibiting porcine pancreatic elastase, human neutrophil elastase, and proteinase-3 (Schalkwijk *et al.* 1999).

Tissue remodeling

Wound healing and tissue remodeling are other processes involving AMPs. AMPs are produced in response to cutaneous injury (vanBergen *et al.* 1996, Wingens *et al.* 1998), and AMP knockout mice show impaired cutaneous wound healing and increased inflammation and elastase activity (Ashcroft *et al.* 2000, Angelov *et al.* 2004). AMPs present at the site of wound repair may also be protecting the wound from potential infection, with HBD1–3 shown to be present in human wound fluid (Frohm *et al.* 1996).

AMPs in pregnancy

The female reproductive tract is split into upper (endocervix and uterus) and lower (ectocervix and vagina) compartments, which are distinct in their morphology, microenvironment, and function (Kaushic 2011). The lower compartment of the genital tract is continuously exposed to the external environment and must be able to distinguish between commensal microflora and pathogens (Kaushic 2011). In the

non-pregnant female genital tract, expression of AMPs varies with site, which may promote tolerance to certain bacteria in the vagina, but effect elimination of pathogens in the uterine cavity (Soboll *et al.* 2006). Production has been shown to be influenced by steroid hormones, and expression varies with the menstrual cycle (King *et al.* 2003a, 2003b), suggesting a protective effect at key times such as menstruation and implantation. The production of AMPs may be involved in the prevention of a variety of reproductive complications including subfertility and ectopic pregnancy (Horne *et al.* 2008). Studies using PCR of bacterial DNA have shown that in pregnancy, the upper genital tract is also usually sterile prior to the onset of labor (Jones *et al.* 2009). Infection resulting from the ascension of vaginal bacteria to the uterus is associated with pregnancy complications including preterm labor, neonatal infection, and *post partum* endometritis. Viral infections, such as HIV, herpes simplex and human papilloma virus may jeopardize the health of mother and babies. The expression and regulation of AMPs in pregnancy suggest that they may have a role in preventing such complications, and there is increasing evidence that deficient or defective production is associated with disease.

Expression of AMPs

The expression of natural antimicrobial RNA and protein by maternal and fetal tissues in pregnancy are summarized in Figs 2 and 3.

HBD1–3 are widely expressed in the pregnant uterus, with expression detected in the amnion, deciduas, chorion, and placental trophoblasts (Feng *et al.* 2003, Buhimschi *et al.* 2004, King *et al.* 2007a, 2007b, Stock *et al.* 2007). *HBD1* and *HBD2* mRNA expression has also been located in the chorion, placenta, and umbilical cord (Feng *et al.* 2003). HBD1–3 peptides have also been identified in amniotic fluid obtained

Figure 2 Expression of AMP RNA by maternal and fetal tissue in pregnancy.

Figure 3 Expression of AMP protein by maternal and fetal tissue in pregnancy.

from term pregnancy (Akinbi *et al.* 2004). Detection of mRNA corresponding to HNP1 or HNP3 had been found in amnion, chorion, and placental tissue (Svinarich *et al.* 1997a), and *HD5* mRNA was also found in samples from the chorion and cervix (Svinarich *et al.* 1997b). The expression of HNP1-3 has been identified in amniotic fluid, with levels significantly higher in patients presenting with an intrauterine infection (Heine *et al.* 1998). The expression of HNP1-3 has also been shown in the vernix caseosa, the substance covering and protecting the skin of the fetus (Akinbi *et al.* 2004).

Cathelicidin LL37 has also been identified in high concentration within the vernix caseosa (Tollin *et al.* 2005) as well as on fetal skin, in much higher concentration than found in healthy adults (Marchini *et al.* 2002). Protein expression of LL37 has also been identified in amniotic fluid (Yoshio *et al.* 2003) and in non-pregnant vaginal fluid (Valore *et al.* 2002).

SLPI has been localized in first trimester deciduas (King *et al.* 2000) and in the amnion epithelium and deciduas at term (Denison *et al.* 1999, Zhang *et al.* 2001). SLPI is also found in amniotic fluid, with levels shown to rise between the second and the third trimester and continuing to increase at the time of term labor (Denison *et al.* 1999, Zhang *et al.* 2001). High concentrations of SLPI have been identified in the vernix caseosa (Akinbi *et al.* 2004) and in the cervical mucus plug and cervical tissue (Helmig *et al.* 1995, Hein *et al.* 2002). Protein expression of both SLPI and elafin/trappin-2 has also been identified in cervicovaginal secretions from the pregnant tract (Stock *et al.* 2009). Elafin/trappin-2 is found at low levels in first trimester deciduas (King *et al.* 2003a). Protein expression has been identified in both the fetal membranes and the placenta at term, identified strongly in the amnion epithelium, deciduas, chorion trophoblasts, and placenta syncytiotrophoblasts (Hein *et al.* 2002, King *et al.* 2007a, 2007b).

Expression at mRNA of elafin/trappin-2 has been also been shown in both vaginal and cervical cells (Stock *et al.* 2009).

AMPs and preterm labor

Preterm birth is the leading cause of neonatal morbidity and mortality and results in a substantial burden of long-term complications (Beck *et al.* 2010). All parturition is associated with inflammation of the intrauterine tissues, and in preterm labor inflammatory response is particularly vigorous (Romero *et al.* 2006). A causal link between infection and prematurity has been established, with at least 40% of all preterm births associated with infection and lower gestational age being linked to a higher frequency of intrauterine infection (Romero *et al.* 2003). The majority of infections found in the uterine cavity associated with preterm labor are of vaginal origin (Epstein *et al.* 2000, Jones *et al.* 2009); however, antibiotics have been disappointing in preventing preterm birth (Simcox *et al.* 2007). Alterations in AMP levels have been associated with infections that may lead to preterm birth as well as preterm labor and rupture of membranes. They make attractive therapeutic targets for the prediction and prevention of infection associated with preterm labor (Horne *et al.* 2008).

The α -defensins have been associated with increased risk of premature delivery; women showing elevated levels of neutrophil defensins in vaginal fluid had a greater risk of premature delivery before 32 weeks compared with women with no levels of defensins present (Balu *et al.* 2003). A SNP has also been identified within the maternal α -defensin *HD5* gene that has been identified as part of a locus that is associated with preterm premature rupture of the membranes (Romero *et al.* 2010). The presence of bacterial vaginosis in the reproductive tract has been shown to reduce the

concentration of AMPs compared with cervicovaginal fluid from healthy women; however, when effective treatment of the bacterial vaginosis is administered, the levels of antimicrobials are normalized suggesting that reduction is due to the presence of the disease (Valore *et al.* 2006).

Epithelially produced defensins have also been found to be altered in association with preterm labor. HBD2 expression in the placenta and the fetal membranes is upregulated by inflammatory cytokines (King *et al.* 2007b, Stock *et al.* 2007), and HBD2 concentrations are elevated in amniotic fluid from patients with preterm delivery due to microbial invasion of the amniotic cavity (Soto *et al.* 2007). Mid-trimester amniotic fluid concentrations of HBD2, but not HBD3, have also been associated with preterm premature rupture of membranes (Xu *et al.* 2008). However, HBD3 protein expression is increased in fetal membranes of patients with preterm delivery associated with chorioamnionitis (Buhimschi *et al.* 2004).

Cervicovaginal levels of elafin/trappin-2 in pregnancy have been associated with bacterial vaginosis, itself associated with preterm labor (Stock *et al.* 2009). Trappin-2 mRNA and protein expression in fetal membranes of pregnancies complicated with chorioamnionitis have an increase, suggesting upregulation of trappin-2 in response to infection (Tromp *et al.* 2004). In addition, the concentration of SLPI in amniotic fluid (Helmig *et al.* 2002) and mRNA of protein expression of trappin-2 is reduced in cases of prelabor rupture of membranes compared with women with intact membranes (Tromp *et al.* 2004).

AMPs and neonatal infection

Neonatal sepsis is particularly associated with preterm delivery (Gonzalez *et al.* 2003); however, intrauterine infection and inflammation increase neonatal morbidity even when gestation is adjusted. Constitutive expression of AMPs is developmentally controlled and influenced by gestation (Starnner *et al.* 2005). The developing fetus expresses AMPs in skin, lung, gut, and vernix, and it has been hypothesized that they may have a specialized role in protecting the developing fetus and neonate from infection. Pulmonary infections in preterm neonates are associated with significantly increased concentrations of LL37, HBD1, and HBD2 in tracheal aspirates (Schaller-Bals *et al.* 2002). There is some evidence that cathelicidin is transferred transplacentally from mother to fetus (Mandic Havelka *et al.* 2010). Levels are higher in cord blood after normal delivery compared with caesarean section indicating release at this vulnerable time. The presence of fetally derived HNP1 and HNP2 in amniotic fluid is associated with a fetal inflammatory response (Buhimschi *et al.* 2005).

AMPs and HIV infection

Several AMPs present in the reproductive tract are emerging as effective inhibitors of HIV-1 infection *in vitro*, and recent evidence implicates α -defensins in resistance of HIV-1 progression *in vivo* (Cole & Lehrer 2003). Cervicovaginal secretions measured from healthy HIV-positive and HIV-negative women contained the antimicrobials HBD2, elafin/trappin-2, and SLPI, with HBD2 correlated with anti-HIV activity in the HIV-positive patients (Ghosh *et al.* 2010a). Elafin/trappin-2 has also been shown to have an inhibitory activity against HIV-1 *in vitro* when incubated with epithelial cells of the female reproductive tract directly with the virus, but not if the AMP was added before or after infection with the virus, suggesting a direct mechanism of inhibition between elafin/trappin-2 and HIV-1 (Ghosh *et al.* 2010b).

AMPs as therapeutic agents

The broad range of antimicrobial, antiviral, and immunomodulatory effects that AMPs possess make them potential novel therapeutic agents for the treatment of infectious and inflammatory diseases. AMPs are under development as a treatment in the lung and skin, and could be developed as topical antiinflammatory and antimicrobial agents in the lower genital tract. This could be an important alternative strategy to the prevention of infection and inflammatory complications.

AMP-based treatments offer several advantages over currently used classes of drugs (Jenssen *et al.* 2006). One of the main advantages that AMP treatment may have is the substantially reduced risk of resistance that is currently associated with antibiotics. AMPs have several targets on microbial cells allowing them to be less specific than some antibiotics that allow for a lower risk for the emergence of resistance. AMP therapy could be applied in combination with antibiotics to give an additive effect and relieve the pressure of a single molecular target. Synthetic AMPs can be modified to enhance antimicrobial activity further. Additionally, the ability of AMPs such as elafin/trappin-2 to bind to tissues via transglutaminase-catalyzed covalent bonds increases its potential to be retained in the cervix.

Summary

AMPs are expressed at a number of sites throughout the pregnant female reproductive tract where they have the capacity to carry out a number of functions that may play key roles in maintaining and protecting the fetus during pregnancy. AMPs have many responses to infectious and inflammatory stimuli with elevated expression observed at a number of sites in response to intrauterine infection, a factor associated with an increased risk of preterm delivery. Further understanding of the function of AMPs

and their mechanisms of actions will allow them to be considered in new strategies as therapeutic agents in controlling human diseases including pregnancy complications such as preterm labor.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

Funding

Funding was provided by a Clinical Lecturer Starter Grant from the Academy of Medical Sciences and Wellcome Trust and Tommy's (www.tommys.org).

Acknowledgements

The authors would like to thank Ronnie Grant (CRB Visual Aids, University of Edinburgh) for graphic design.

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Received 23 December 2010

First decision 21 February 2011

Revised manuscript received 21 March 2011

Accepted 7 April 2011