Chapter 11
Antimicrobial Peptides and Preterm Birth

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Abstract Preterm birth (delivery before 37 completed weeks of pregnancy) is a major problem worldwide, leading to high mortality and significant long-term morbidity. A complex interaction between ascending lower genital tract infection and the maternal immune system is a likely underlying component of pathogenesis. In this chapter we consider the ways in which expression of antimicrobial peptides in the maternal genital tract may modulate the risk of ascending genital tract infection and thus the risk of preterm birth.

11.1 Preterm Birth and Ascending Lower Genital Tract Infection

Preterm birth (PTB, delivery before 37 completed weeks of gestation) is a major problem in the United Kingdom and worldwide, leading to a high mortality rate and long-term morbidity in babies who survive—particularly those born before 32 weeks (Marlow et al. 2005; Moore et al. 2012). There are currently no proven strategies that both prevent PTB and improve neonatal outcome, making the search for new preventative therapies a priority (Iams et al. 2008). Prematurity is the single largest direct cause of neonatal deaths worldwide (one million, or 35 % of all deaths) and contributes to 50 % of all neonatal deaths (Blencowe et al. 2012): PTB kills more babies than any other single cause).

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The processes leading to preterm delivery are poorly understood. The pathogenesis of PTB is multifactorial, and many mechanisms have been proposed. There is increasing evidence that intrauterine infection in a significant proportion (40–70 % cases of preterm deliveries) is associated with chorioamnionitis (inflammation of the fetal membranes) (Tita and Andrews 2010) Two main models of intrauterine infection leading to PTB have been proposed: (a) pathogens from either a systemic infection or a remote localized infective focus disseminate hematogenously and initiate an immune response in the intrauterine cavity, and/or (b) pathogens from the extensive vaginal microbiome may gain access to the relatively sterile intrauterine cavity via the cervical canal.

The intrauterine cavity is separated from the prolific bacterial load of the vagina by the three centimeters or so of simple columnar epithelium that make up the endocervical canal. As the intrauterine cavity is demonstrably either sterile or has only minimal bacteria present in uncomplicated pregnancies at term (Jones et al. 2009), it is clear that under normal conditions the cervix must act as an effective barrier to the migration of bacteria from the vagina.

11.2 The Cervix as an Antimicrobial Barrier

The integrity of the cervical canal as an antimicrobial barrier is likely to rely on a number of interrelated physical and chemical factors; these include the apical surface lining of the endocervical columnar epithelium and provision of innate and cellular immunity from resident and recruited immune (e.g., macrophages and T-cells) and nonimmune (epithelium/fibroblast) cells via the production of cytokines and chemokines. Many of the "gate-keeper" functions of the cervix are coordinated by the cervical mucus plug (CMP), a large (c.10 g), viscous structure which fills the cervical canal during pregnancy. The CMP is a dynamic structure unique to pregnancy. Scanning electron microscopy reveals that the ultrastructure undergoes significant change during pregnancy, from a largely porous mesh early in the first trimester, to a dense and highly compact mesh at later gestations (Becher et al. 2009).

Molecularly, the CMP is an anionic mucinous glycoprotein skeleton. This serves as a ligand for a variety of molecules, including cytokines and water. In addition, the complex network inhibits the diffusion of larger molecules and pathogens through the CMP by steric exclusion (Becher et al. 2009). The anionic charge of the mucin skeleton also allows the CMP to retain positively charged molecules, including the many highly cationic antimicrobial peptides (AMPs) of the innate immune system.

More than 800 unique AMPs have been identified in a variety of species, but the two main classes of AMP in mammals are cathelicidins and defensins. Despite expressing only one cathelicidin, humans express a range of defensins; these are classified as alpha defensins or beta defensins depending on the cysteine motif of their beta sheet secondary structure (Fellermann and Stange 2001).

Table 1 The antimicrobial spectrum of HBD1, HBD2, and HBD3

HBD1	HBD2	HBD3
Escherichia coli	*Escherichia coli*	*Escherichia coli*
Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*
Klebsiella pneumoniae	*Klebsiella pneumoniae*	*Klebsiella pneumoniae*
Staphylococcus aureus	*Staphylococcus aureus*	*Staphylococcus aureus*
HIV-1	*Streptococcus pneumoniae*	*Streptococcus pneumoniae*
–	*Candida albicans*	*Streptococcus pyogenes*
–	*Candida parapsilosis*	*Staphylococcus carnosus*
–	*Candida krusei*	*Burkholderia cepacia*
–	*Enterococcus faecalis*	*Saccharomyces cerevisiae*
–	HIV-1	*Candida albicans*
–	–	*Candida parapsilosis*
–	–	*Candida krusei*
–	–	*Enterococcus faecalis*
–	–	HIV-1

Alpha defensins, also called human neutrophil peptides (HNPs), are small peptides with broad spectrum antimicrobial and/or immunoregulatory properties and are mainly found in the azurophilic granules of neutrophils and in the Paneth cells of the small intestine (Wiesner and Vilcinskas 2010). In contrast, beta defensin expression is not restricted as they can be expressed by all epithelia (Pazgier et al. 2006) and reports indicate expression in circulating immune cells (Jansen et al. 2009).

Defensins were originally described as endogenously occurring antimicrobials. Indeed, collectively they exhibit a considerable antimicrobial spectrum against a range of Gram positive and Gram negative pathogens (Table 11.1). Their mechanism(s) of antimicrobial activity is related to their amphipathic design, in which clusters of hydrophobic and cationic amino acids are spatially organized in discrete sections of the molecule (Zasloff 2002). This design allows the interaction of the cationic, hydrophilic surface of the peptide with the (anionic) bacterial membrane, during which there is displacement of the membrane lipids and subsequent alteration of membrane structure. Many mechanisms of bacterial killing have been proposed, all of which hinge on this single premise of charge-dependent interaction; suggested mechanisms include membrane depolarisation, leakage of intracellular components through compromised bacterial membranes or other disturbances of bacterial membrane function (Zasloff 2002).

Peptides using charge-dependent mechanisms of bacterial killing are able to execute activity in the micromolar concentration range. This capacity is inhibited under conditions of increased ionic strength, for example in a salty environment. HBD3 alone is able to kill in a relatively salt independent fashion (Harder et al. 2001). In addition to this salt insensitivity, HBD3 has potent antimicrobial activity against viruses and fungi (Pazgier et al. 2006). Although the precise mechanisms underlying these properties are not yet clear, the reader is encouraged to read the

accompanying chapters that provide an update to our current understanding of AMP action and function.

In addition to their antimicrobial capacity, HBDs also play an important immunoregulatory role. HBD1 is constitutively expressed but may be upregulated in the context of infection or inflammation (Bajaj-Elliott et al. 2002). This may be IFN-γ dependent (Prado-Montes de Oca 2010). HBD1 induces CCL5/RANTES production by peripheral blood monocytes and, in common with HBD2, can act as a chemoattractant for immature dendritic cells (iDCs) and memory T-cells via CCR6, effectively mediating innate-adaptive immune signaling (Pazgier et al. 2007). HBD3 modulates HIV-1 infectivity via CXCR4, induces antigen presenting cell maturation via TLR1 and TLR2 and is chemoattractant for CCR2 (Röhrl et al. 2010). In addition, HBD3 has been shown to compete with melanocyte stimulating hormone alpha (MSHα), the ligand of the melanocortin 1 receptor (MC1r), in myeloid cells; this competition inhibits the induction of the anti-inflammatory cytokine IL-10 in cells expressing MC1r (Prado-Montes de Oca 2010).

Further immunomodulatory properties of HBD3 include the activation of monocytes via TLR1 and TLR2 to produce IL-8, IL-6 and IL-1β but not IL-10. In contrast to these largely proinflammatory properties, HBD3 can also neutralize lipopolysaccharide (LPS) and inhibit TNFα and IL-6 accumulation (Semple et al. 2010, 2011). The net effect of HBD3 action is therefore difficult to discern as it can be proinflammatory and/or anti-inflammatory. The available information raises the hypothesis that HBD3 pro- and/or anti-inflammatory function in vivo may be context dependent.

11.3 Antimicrobial Peptides and Preterm Birth

The potential role of AMPs, secretory leukocyte protease inhibitor (SLPI), and elafin in the pathogenesis of PTB has been investigated. The CMP itself displays direct antimicrobial activity in vitro, and both peptides have been identified as components of the CMP (Hein et al. 2002; Stock et al. 2009). Low cervicovaginal levels of elafin have been associated with bacterial vaginosis (BV) (Stock et al. 2009); conversely, it has been suggested that high concentrations of elafin in cervicovaginal fluid are associated with cervical shortening and may be predictive of PTB (Abbott et al. 2014). High cervicovaginal concentrations of the human cathelicidin LL37 have also been associated with bacterial vaginosis in pregnancy (a risk factor for PTB) (Frew et al. 2014).

HBDs have also been identified in the CMP (Frew and Stock 2011). Numerous studies (Cobo et al. 2011; Polettini et al. 2011; Buhimschi et al. 2009) have focused on the expression of HBDs in the amniotic fluid, fetal membranes, and the placenta in women who deliver preterm. Although these studies suggest that there may be an association between increased expression (transcriptional and translational) of these peptides and PTB, currently no mechanistic studies have been reported. There are

also no reports detailing cervicovaginal HBD expression in women at increased risk of PTB, although two studies report an association between increased alpha defensins in cervicovaginal fluid and PTB (Xu et al. 2008; Lucovnik et al. 2011).

11.4 Progesterone, AMPs and Preterm Birth

Significant data showing that progesterone can prolong pregnancy in women at risk of PTB have raised questions about its mode of action (Maggio and Rouse 2014). The gestation extending effects of progesterone are most pronounced in those women who have both a history of prior preterm deliveries and a reduced cervical length (below 25 mm when measured by transvaginal ultrasound) in the pregnancy in question. It is clear that women with a reduced cervical length will also have a reduced surface area of endocervical epithelium. The precise mechanism(s) by which progesterone treatment may compensate for this reduced surface area remains ambiguous, and the long-term outcome of children born to women treated with progesterone has yet to be determined.

The risk of ascending genital tract infection is highest when serum progesterone is at its lowest in the menstrual cycle (Wira et al. 2015), and limited data suggest that progesterone may modulate HBD protein expression in primary endometrial cells and transformed cell cultures (King et al. 2003). Furthermore, vaginal progesterone has been shown to increase the expression of HBD1 in mice, albeit not at the protein level (Nold et al. 2013). This has clear implications for the regulation of mucosal immunity in pregnancy. Progesterone receptors A and B are expressed by the cervix in vivo, and it has recently been shown that the ectocervical cell line Ect1/E6E7 and vaginal cell line VK2/E6E7 also express these receptors (Africander et al. 2011). It therefore seems probable that HBD expression by cervical epithelia may also be progesterone sensitive. It is probable that the explanation for the gestation extending effects of progesterone will include a combination of actions, but this limited evidence suggests that regulation of lower genital tract antimicrobial peptide expression may play a role, perhaps by reducing the risk of ascending infection.

11.5 Conclusion

Emerging evidence is providing a tantalizing glimpse linking increased risk of ascending infection and preterm birth. In addition to the mother's adaptive and innate immune response, the cervical antimicrobial barrier is likely to be the key determinant of the status quo. Further studies are needed to confirm the potential protective role of AMPs in reducing the risk of ascending infection in susceptible (those with a history of preterm births) individuals. If confirmed, manipulation of AMP expression may be a viable future therapeutic option.

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