Chapter 9
Antimicrobial Peptides in Host Defense: Functions Beyond Antimicrobial Activity

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Abstract Antimicrobial peptides are well known for their important roles in host defense by enhancing the barrier function and limiting microbial populations of the skin and mucosa. However, many of these peptides are now known to have additional roles assisting innate and adaptive immune functions. To facilitate innate immunity, antimicrobial peptides activate complement, chemoattract cells (e.g., monocytes, macrophages, T cells, neutrophils, immature dendritic cells, and mast cells), enhance phagocytosis, and modulate the production of chemokines and proinflammatory cytokines in other cells. At local sites of initiation, antimicrobial peptides can act as opsonins to enhance phagocytosis by monocytes and phagocytes and can activate cells. In the latter, for example, treatment of osteoblasts and osteoblast-like MG63 cells with human beta-defensin (HBD)2 increases their proliferation rates. Treatment of osteoblast-like MG63 cells with HBD2 and HBD3 increases transcript levels of osteogenic markers for differentiation, increases antileukoprotease (ALP) levels, and enhances mineralized nodule formation. To facilitate adaptive immunity, antimicrobial peptides assist the uptake of antigens by monocytes or other antigen-presenting cells and later direct the process toward a Th1 or Th2 adaptive immune response. More commonly though, antimicrobial peptides induce a mixed response characterized by Th1-/Th2-specific antibodies and Th1/Th2 cytokines from antigen-exposed splenocytes of immunized animals. Finally, antimicrobial peptides can be detected in the margins around both oral and cutaneous wounds, and there is

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growing evidence to suggest they also play a dynamic role in wound healing by improving wound angiogenesis, vascularization, and reepithelialization.

9.1 Introduction

Antimicrobial factors in normal tissues and fluids were described as early as 1888 (Skarnes and Watson 1957). These factors, isolated from extracts of tissues, serum, serous fluids, and leukocytes, later became the well-known members of innate immunity: antibodies, complement, lysozyme, histones, and protamines (Skarnes and Watson 1957). Small, linear, basic peptides (called tissue basic polypeptides) with antimicrobial activity in normal tissues and fluids were also described as early as 1947 (Bloom et al. 1947; Bloom and Prigmore 1952; Bloom and Blake 1948). These peptides contained lysine (29–30 %) and arginine (3.5 %) amino acid residues with isoelectric points between pI 10 and 11.2. They were thought to be attracted to the negatively charged surfaces of microbial cells via electrostatic bonding and to alter microbial membrane integrity. These peptides are likely the group we now know as the antimicrobial peptides (Skarnes and Watson 1957). The early history of antimicrobial peptide discovery and research can be found in two excellent reviews by Skarnes and Watson (1957) and Nakatsuji and Gallo (2012).

Almost immediately after the discovery of cationic peptides with antimicrobial activity, investigators began to assess their secondary functions, and many of these peptides did indeed have additional roles in innate and adaptive immunology (Nakatsuji and Gallo 2012). This was not an unusual finding as the inverse was also found to be true, and some other physiologically important peptides had antimicrobial activity. For example, some neuropeptides, peptide hormones, and chemokines were found to have antimicrobial activities (Brogden et al. 2005; Cole et al. 2001; Yang et al. 2003). These results clearly suggest that peptides with antimicrobial activity are multifunctional in a variety of situations.

In this chapter, we present the alternate functions of peptides with antimicrobial activity, a topic of a number of excellent comprehensive reviews (Yang et al. 2001, 2002, 2004; Yang and Oppenheim 2004; Bowdish et al. 2005; Pingel et al. 2007; Rehaume and Hancock 2008; Semple et al. 2010; Semple and Dorin 2012; Greer et al. 2013). We start by presenting the roles of antimicrobial peptides in innate immunity and their ability to chemoattract and activate cells (Table 9.1). This includes recent discoveries that antimicrobial peptides influence the properties of human mesenchymal stem cells (hMSCs) and osteoblasts in addition to epithelial cells, keratinocytes, and immune cells of myeloid or lymphoid origin. We then present the roles of antimicrobial peptides in adaptive immunity and their ability to influence Th1, Th2, and mixed Th1 and Th2 responses (Table 9.2). Third, we present the ability and conditions of antimicrobial peptides to modulate chemokine and proinflammatory cytokine responses, an exciting area of current research by a variety of investigators (Table 9.3). Finally, we present the roles of antimicrobial peptides in wound healing, angiogenesis, and autoimmune diseases.

Table 9.1 Antimicrobial peptides regulate innate immunity by chemoattracting inflammatory cells, enhancing phagocytosis, enhancing the production of proinflammatory mediators, and regulating complement activation

Chemoattract cells
Cathelicidins LL-37 and CRAMP chemoattract monocytes, neutrophils, macrophages, and peripheral blood leukocytes (Kurosaka et al. 2005; An et al. 2005)
α-, β-defensins chemoattract monocytes, immature dendritic cells, neutrophils, macrophages, $CD4^+$ T cells (CD45 RA+), and $CD8^+$ T cells (Chertov et al. 1996; Fleischmann et al. 1985; Ichinose et al. 1996; Territo et al. 1989; Yang et al. 1999, 2000; Li et al. 2014)
Enhance phagocytosis
α-defensins enhance macrophage phagocytosis in a variety of species (Ichinose et al. 1996; Fleischmann et al. 1985)
Enhance the production of proinflammatory mediators
α-, β-defensin-treated epithelial cells and monocytes produce IL-1, IL-8, IL-10, and TNF-α (Chertov et al. 1996; Van Wetering et al. 1997; Chaly et al. 2000)
HBD3 induces production of Gro-α, MDC, MCP-1, MIP-1α, MIP-1β, and VEGF in monocytes and macrophages (Petrov et al. 2013)
LL-37 induces production of Gro-α, MDC, MCP-1, MIP-1α, MIP-1β, and VEGF in monocytes and macrophages (Petrov et al. 2013)
Degranulate mast cells
α-, β-defensins degranulate mast cells and release histamine and prostaglandin D2 (Yamashita and Saito 1989; Befus et al. 1999; Chertov et al. 2000; Niyonsaba et al. 2001)
Regulate complement
Defensins regulate complement activation (Prohaszka et al. 1997; van den Berg et al. 1998)

9.2 Antimicrobial Peptides in Innate Immunity

By definition, innate immunity is a nonspecific defense against mechanical injury and damage, chemical exposure, or microbial infection in barrier surfaces like the skin or mucosa. It also protects from internal exposure to abnormal cells (Martin 2014). Cellular components such as macrophages, dendritic cells, neutrophils, and granulocytes and humoral components such as lactic acid, fatty acids, lysozyme, and complement are often involved. Cells produce inducible humoral components after stimulation of surface receptors with microbe-associated molecular pattern (MAMP) or pathogen-associated molecular pattern (PAMP) molecules that include lipopolysaccharides, peptidoglycans, or nucleic acids. Exposed cells release chemokines and proinflammatory cytokines via a variety of receptor signaled pathways. Cells also release antimicrobial peptides after exposure to PAMPs directly or can release antimicrobial peptides after exposure to chemokines and proinflammatory cytokines (Liu et al. 2013b; Jan et al. 2006). Antimicrobial peptides activate complement, attract neutrophils, enhance phagocytosis, and complete the cycle by enhancing the production of chemokines and proinflammatory cytokines in other cells (Yang et al. 2002).

Antimicrobial peptides can enhance the barrier function of the skin or mucosa. For example, in the skin, HBD3 regulates cell permeability and membrane tight junctions in keratinocytes. HBD3 enhances the expression of claudins (e.g., 1–5, 9,

Table 9.2 Antimicrobial peptides influence Th1, Th2, and mixed Th1/Th2 adaptive immune responses, often with adjuvant-like activities

Enhance Th1 responses
Murine β-defensin 2 (mDF2β) induces potent cell-mediated responses and antitumor immunity when genetically fused with nonimmunogenic tumor antigens (Biragyn et al. 2001, 2002; Biragyn 2005)
Mice receiving L1210 cells expressing mDF2β have responses strong NK and CTL responses with enhanced IL-12 and IFN-γ production, protecting them from lethal challenge with L1210 cells (Ma et al. 2006)
Zebra fish immunized with zebra fish β-defensin 2 (zfBD2) develops a Th1 immune response with an upregulated IFN-γ response (Garcia-Valtanen et al. 2014)
Enhance Th2 responses
Cationic peptide KLKL5KLK with ovalbumin induces a Th2 response. Immunized mice produce ovalbumin-specific IgG1, but not IgG2, and splenocytes from immunized mice, stimulated with ovalbumin, produce IL-4 and IL-5, but not IFN-γ (Fritz et al. 2004)
Enhance mixed Th1/Th2 responses
Melittin enhances a mixed Th1/Th2 response to tetanus toxoid in mice. Total IgG and IgG2a responses are increased (Bramwell et al. 2003)
CRAMP enhances ovalbumin-specific IgG1, IgG2a, IgG2b, and IgG3 and Th1/Th2 cellular ovalbumin-specific responses in mice (Kurosaka et al. 2005)
LL-37 enhances Th1 and Th2 humoral, cytotoxic, and protective responses in mice when fused with M-CSF receptor cloned from J6-1 leukemia cells (M-CSFRJ$_{6\text{-}1}$). Splenocytes from immunized mice, stimulated with M-CSFRJ$_{6\text{-}1}$, produce IFN-γ (An et al. 2005)
HNP-1, HNP-2, HNP-3 enhance keyhole limpet hemocyanin-specific and ovalbumin-specific IgG1, IgG2a, and IgG2b responses, and splenocytes from immunized mice stimulated with keyhole limpet hemocyanin produce KLH-specific IFN-γ (Th1 cytokine) and IL-4 (Th2 cytokine), and splenocytes from immunized mice stimulated with ovalbumin produce greater amounts of IL-5, IL-6, IL-10, and IFN-γ (Th1 and Th2 cytokines) (Lillard et al. 1999; Tani et al. 2000)

11, 14–17, 20, 23, and 25) and the location of claudins in cell membranes and elevates transepithelial electrical resistance (Kiatsurayanon et al. 2014). This occurs via HBD3-mediated pathways involving Rac1, atypical protein kinase C, glycogen synthase kinase, and phosphatidylinositol 3-kinase (Kiatsurayanon et al. 2014).

Once produced, cathelicidins and defensins then attract a variety of cells to their sites of induction (Table 9.1). LL-37, CRAMP, α-defensins, and β-defensins all are reported to chemoattract monocytes, macrophages, T cells, neutrophils, immature dendritic cells, and mast cells.

At the site of initiation, antimicrobial peptides then can act as opsonins (Fleischmann et al. 1985), enhance phagocytosis by monocytes and phagocytes (Ichinose et al. 1996), and activate cells. They can also degranulate mast cells. For example, human, rabbit, and guinea pig α-defensins activate and degranulate mast cells releasing histamine and prostaglandin D2 (Yamashita and Saito 1989; Befus et al. 1999; Niyonsaba et al. 2001).

An additional and well-known function is the ability of antimicrobial peptides, particularly defensins, to regulate complement activation (Prohaszka et al. 1997; van den Berg et al. 1998). Here, C1q of C1 binds to HNP-1, HNP-2, and HNP-3,

Table 9.3 Peptides and proteins in oral secretions with antiinflammatory and proinflammatory properties

Antiinflammatory activities
LL-37
Attenuates agonist-induced, chemokine, and proinflammatory cytokine responses in macrophages (Scott et al. 2000), lung epithelial cells (Scott et al. 2000), peripheral blood mononuclear cells (Molhoek et al. 2009), and whole blood leukocytes (Walters et al. 2010)
Attenuates MAPK pathway activation of p38 and ERK responses in gingival fibroblasts (Inomata et al. 2010)
α-defensins
Inhibit the production of proinflammatory cytokines from macrophages (Miles et al. 2009)
Attenuate a chemokine and proinflammatory cytokine response in mice (Kohlgraf et al. 2010)
β-defensins
HBD3 attenuates agonist-induced, chemokine, and proinflammatory cytokine responses in dendritic cells (Pingel et al. 2008; Harvey et al. 2013), in THP-1 human myelomonocytic cells (Semple et al. 2010), peripheral blood monocyte-derived macrophages (Semple et al. 2010), and in RAW264.7 murine macrophages (Semple et al. 2010)
DEFB114 attenuates MAPK pathway p42/44 response and attenuates an agonist-induced TNF-α response in RAW264.7 murine macrophages (Yu et al. 2013)
DEFB123 attenuates an agonist-induced MAPK pathway activation of p42/44 and p38 and attenuates an agonist-induced TNF-α response in RAW264.7 murine macrophages (Motzkus et al. 2006)
DEFB126 attenuates an agonist-induced proinflammatory response in RAW264.7 murine macrophages (Liu et al. 2013a)
Attenuate a chemokine and proinflammatory cytokine response in mice (Kohlgraf et al. 2010)
θ-*defensins*
Retrocyclin RTD-1 attenuates agonist-induced, chemokine, and proinflammatory cytokine responses in human peripheral blood leukocytes (Schaal et al. 2012)
Histatins
Histatin 5 attenuates agonist-induced, chemokine, and proinflammatory cytokine responses in gingival fibroblasts (Imatani et al. 2000) and dendritic cells (Borgwardt et al. 2014)
CEMA (*cecropin-melittin hybrid*)
CEMA blocks the binding of LPS to LPS-binding protein, attenuates agonist-induced, chemokine, and proinflammatory cytokine responses in murine macrophages (Scott et al. 2000)
Proinflammatory activities
LL-37
50–100 μg/ml enhances an agonist-induced IL-8 response in epithelial cells (Scott et al. 2002)
β-defensins
Pre-stimulation or post-stimulation of dendritic cells and mice with HBD3 enhances an agonist-induced chemokine and proinflammatory cytokine response (Harvey et al. 2013)
Pre-stimulation of macrophages with MBD14 enhances an agonist-induced chemokine and proinflammatory cytokine response (Barabas et al. 2013)
HD5 upregulates expression of genes involved in cell survival and inflammation in an NF-kB-dependent fashion in epithelial cells (Lu and de Leeuw 2013)

and complement in normal human serum is activated by HNP-released C4b (Prohaszka et al. 1997).

Antimicrobial peptides clearly influence the properties of a variety of cell types including epithelial cells, keratinocytes, and immune cells of myeloid or lymphoid origin, and these properties are listed in Table 9.1. They also influence the properties of hMSCs and osteoblasts. Treatment of hMSCs, osteoblasts, and osteoblast-like MG63 cells with HBD2 increases their proliferation rates (Warnke et al. 2013; Kraus et al. 2012), and treatment of osteoblast-like MG63 cells with HBD2 and HBD3 increases their transcript levels of osteogenic markers for differentiation, increases ALP levels, and enhances mineralized nodule formation (Kraus et al. 2012).

9.3 Antimicrobial Peptides in Adaptive Immunity

Adaptive immunity is an acquired resistance produced after antigenic exposure in the form of antibody production together with the development of cell-mediated immunity. The adaptive immune system is organized around highly specialized cells including antigen-presenting cells and two classes of specialized lymphocytes, T and B cells, with a variety of functions (Dunkelberger and Song 2010). These cells display a diverse repertoire of antigen-specific recognition receptors. This enables specific identification and elimination of pathogens, tailoring of immune responses, and long-lived immunological memory.

Antimicrobial peptides are known to play roles in adaptive immune responses and exert their influence at numerous steps in the process. Early in the process, antimicrobial peptides can facilitate the uptake of antigen by monocytes or other antigen-presenting cells (Fritz et al. 2004) and later direct the process toward a Th1, Th2, or mixed Th1/Th2 adaptive immune response (Table 9.2).

9.3.1 Induced Th1 Responses

Cells expressing defensins or vaccines containing defensins induce strong Th1 responses resulting in protection from lethal cell challenges and appear to have clinical promise in combating cancer (Biragyn 2005). There are three nice examples of antimicrobial peptide-induced Th1 responses. In one example, mice receiving L1210 cells expressing murine β-defensin 2 (mDF2β) developed strong CTL and NK cell responses with enhanced IL-12 and IFN-γ production, which protected them from lethal challenge with L1210 cells (Ma et al. 2006). In another example, mDF2β-based vaccines elicited potent cell-mediated responses and antitumor immunity when genetically fused with another nonimmunogenic tumor antigen (Biragyn et al. 2001). The fusion proteins, consisting of mDF2β linked to a tumor antigen, acted directly on immature dendritic cells as an endogenous ligand for TLR-4 and upregulated co-stimulatory molecules, induced dendritic cell maturation, and induced the production of lymphokines (Biragyn et al. 2002).

Zebra fish immunized with a plasmid encoding zebra fish β-defensin 2 (zfBD2), and the glycoprotein G of the spring viremia of carp virus (gpG_{svcv}) developed a Th1 immune response with an upregulated IFN-γ response (Garcia-Valtanen et al. 2014). Expression of zfBD2 upregulated IS *mx* genes related to the activation of the type I IFN system. It also induced the transcription of proinflammatory cytokine genes *tnf*α and *il*1β, increased the presence of *mhc*2 transcripts related to MHC class II presentation of antigens, enhanced granzyme and NK lysine transcripts related to immune cytotoxic responses cytotoxic responses, and mediated recruitment of Th cells at the injection site.

9.3.2 Induced Th2 Responses

In the presence of an antigen, some cationic peptides induce immunized animals to produce primary Th2-specific antibodies and Th2 cytokines from antigen-exposed splenocytes of immunized animals. Cationic peptide $KLKL_5KLK$ is one of these peptides. Mice immunized with $KLKL_5KLK$ with ovalbumin produce ovalbumin-specific IgG1, but not IgG2, and splenocytes from immunized mice, stimulated with ovalbumin, produce IL-4 and IL-5, but not IFN-γ (Fritz et al. 2004).

9.3.3 Induced Th1/Th2 Responses

More commonly than the above two examples, antimicrobial peptides induce immunized animals to produce a mixed response characterized by producing Th1-/Th2-specific antibodies and Th1/Th2 cytokines from antigen-exposed splenocytes of immunized animals. Melittin from bees enhances a mixed Th1/Th2 response to tetanus toxoid in mice. Melittin increases tetanus toxoid total IgG and IgG2a antibody responses (Bramwell et al. 2003). Similarly CRAMP enhances mixed Th1/Th2 antigen-specific immune responses to ovalbumin in mice. CRAMP increases ovalbumin IgG1, IgG2a, IgG2b, and IgG3 antibody responses (Kurosaka et al. 2005). LL-37 enhances a mixed Th1/Th2 humoral, cytotoxic, and protective response in mice when LL-37 was fused with $M\text{-}CSFRJ_{6\text{-}1}$, an M-CSF receptor cloned from J6-1 leukemia cells ($M\text{-}CSFRJ_{6\text{-}1}$). Splenocytes from immunized mice, stimulated with $M\text{-}CSFRJ_{6\text{-}1}$, produced IFN-γ (An et al. 2005).

A mixed Th1/Th2 response is also induced by defensins. HNP-1, HNP-2, and HNP-3 enhance keyhole limpet hemocyanin (KLH) IgG1, IgG2a, and IgG2b antibody responses (Tani et al. 2000), and HNP-1, HNP-2, and HNP-3 and human β-defensins enhance ovalbumin-specific IgG1, IgG2a, and IgG2b antibody responses (Lillard et al. 1999; Brogden et al. 2003). T cells from KLH-immunized mice, stimulated with KLH, produce KLH-specific IFN-γ (Th1 cytokine) and IL-4 (Th2 cytokine) (Tani et al. 2000). T cells from ovalbumin immunized mice, stimulated with ovalbumin, produce greater amounts of $CD4^+$ Th1 and Th2 cytokines (IFN-γ, IL-5, IL-6, and IL-10) (Lillard et al. 1999; Brogden et al. 2003).

More recently, a human adenovirus vector expressing mDF2β (e.g., HAd-mDF2β) was found to chemoattract murine bone marrow-derived immature dendritic cells and increase their surface expression levels of CD40, CD80, and CD86 activation markers (Vemula et al. 2013). Immunization with the HAd-mDF2β vector prior to immunization with a human adenovirus-hemagglutinin-nucleoprotein vaccine significantly increases hemagglutinin inhibition antibody titers and increases nucleoprotein-147 epitope-specific $CD8^+$ T cells. Immunization also decreases virus titers of VNH5N1-PR8/CDC-RG in the lungs of challenged mice.

9.4 Antimicrobial Peptides Modulate Chemokine and Cytokine Responses

Cells treated with antimicrobial peptides alone or with a microbial antigen have both proinflammatory and antiinflammatory activities: a dichotomy that is not entirely well understood (Harvey et al. 2013). These proinflammatory and antiinflammatory activities appear to be dependent upon a number of conditions that include antimicrobial peptide concentration, antimicrobial peptide association with proinflammatory agonists, or the temporal order of peptide exposure to cells, with respect to agonist exposure to cells.

9.4.1 Exposure of Cells to Low Concentrations of Antimicrobial Peptides Is an Antiinflammatory Event

Generally, cells exposed to <1.0–10.0 μg/ml antimicrobial peptide do not produce much of a chemokine and proinflammatory cytokine response. 0.003–0.03 μg/ml HNP alone does not induce TNF-α or IL-1β expression in resting monocytes (Chaly et al. 2000), and 5.0 μg/ml mBD14, HBD2, or HBD3 does not produce TNF-α in murine bone marrow-derived macrophages (Barabas et al. 2013).

9.4.2 Exposure of Cells to High Concentrations of Antimicrobial Peptides Is a Proinflammatory Event

Generally, cells exposed to 10.0 to >100.0 μg/ml antimicrobial peptide produce higher amounts of chemokines and proinflammatory cytokines in a dose-related fashion. Monocytes and macrophages treated with 20.0 μg/ml HBD3 or 20.0 μg/ml of LL-37 produce Gro-α, MDC, MCP-1, MIP-1α, MIP-1β, and VEGF (Petrov et al. 2013). Keratinocytes treated with 30 μg/ml HBD2, HBD3, or HBD4 produce elevated IL-6, IL-10, IP-10 (CXCL10), MCP-1 (CCL2), MIP-3α (CCL20), and RANTES (CCL5) (Niyonsaba et al. 2007). RAW264.7 murine macrophages treated

with 50.0–100.0 μg/ml LL-37 produce 200, 400, and >1,000 pg/ml MCP-1 (CCL2) (Scott et al. 2002); and A549 human epithelial cells treated with 10–100 μg/ml LL-37 produce 300–1,200 pg/ml IL-8 (Scott et al. 2002). Epithelial cells treated with 100.0 μg/ml HNP-1, HNP-2, and HNP-3 induce ~17,000 pg/ml IL-8 (Van Wetering et al. 1997).

9.4.3 Antimicrobial Peptide Binding to Proinflammatory Agonists Is an Antiinflammatory Event

Antimicrobial peptides readily bind to microbial lipopolysaccharides, adhesins, and toxins with rapid association rate constants, slower dissociation rate constants, and high affinity (Caccavo et al. 2002; Wang et al. 2003, 2006; Owen et al. 2004a, b; Liu et al. 2013a; Yu et al. 2013; Dietrich et al. 2008; Pingel et al. 2008). This binding generally alters the physiological properties of the agonist (Gough et al. 1996; Bowdish and Hancock 2005; Motzkus et al. 2006; Scott et al. 2011; Kim et al. 2005, 2006; Giesemann et al. 2008; Yeom et al. 2011).

The binding of antimicrobial peptides to microbial lipopolysaccharides, adhesins, and toxins also alters binding of these agonists to cell surface receptors (Gallo and Hooper 2012). Lactoferrin is a good example. It inhibits the interaction of LPS with CD14 on cell surfaces by competing with the LPS-binding protein (Molhoek et al. 2009), and it blocks DC-SIGN-gp120 interaction and prevents dendritic cell-mediated HIV type 1 transmission (Groot et al. 2005). HBD3 alters the binding of *Porphyromonas gingivalis* hemagglutinin B (HagB) to the surface of dendritic cells (Van Hemert et al. 2012) and HNP-1, HBD1, HBD2, HBD3, DEFB104, and LL-37, all inhibit binding of Alexa Fluor 546-labeled *P. intermedia* and *T. forsythia* LPS to THP-1 human monocytes (Lee et al. 2010).

It also appears that this changes agonist-induced signal transduction. Antimicrobial peptides can selectively attenuate agonist-induced signal transduction including MAPK pathways involving p38, c-Jun NH_2-terminal protein kinases (JNK), or extracellular signal-regulated kinase (ERK). DEFB114 attenuates an LPS-induced activation of p42/44 responses in RAW264.7 murine macrophages (Yu et al. 2013), and DEFB123 (Motzkus et al. 2006) and DEFB126 (Liu et al. 2013a) attenuate an LPS-induced activation of p42/44 and p38 responses in RAW264.7 murine macrophages. LL-37 attenuates a *P. gingivalis* extract-induced activation of p38 and ERK responses in human gingival fibroblasts (Inomata et al. 2010). Modulation of the TLR response by LL-37 occurs at least partly through inhibition of p38 phosphorylation (Walters et al. 2010).

The resulting chemokine and proinflammatory cytokine output is noticeably reduced, and this is a very popular area of research. Lactoferrin attenuates an LPS-induced IL-1β, IL-6, and ICAM-1 mRNA response in bovine aortic endothelial cells (Yeom et al. 2011). Cathelicidins also attenuate chemokine and proinflammatory cytokine output. LL-37 attenuates periodontopathogenic LPS-induced IL-8 responses in human periodontal ligament fibroblasts and gingival fibroblasts

(Suphasiriroj et al. 2013); *P. aeruginosa* LPS-induced IL-8 response in THP-1 human monocyte cells (Scott et al. 2011); LPS-induced TNF-α response in mouse bone marrow-derived macrophages and tissue macrophages (Brown et al. 2011); LPS-induced, *S. aureus* lipoteichoic acid-induced, and *Mycobacterium* lipoarabinomannan-induced TNF-α response in RAW264.7 murine macrophages and IL-8 and MCP-1 (CCL2) response in A549 human lung epithelial cells (Scott et al. 2000); and *P. gingivalis* extract-induced IL-6, IL-8, and IP-10 (CXCL10) responses in human gingival fibroblasts (Inomata et al. 2010).

Similarly, defensins attenuate agonist-induced chemokine and cytokine responses. HNP-1 attenuates an LPS-induced IL-1β, but not TNF-α, response in human monocytes (Shi et al. 2007); DEFA1-3 attenuates *P. aeruginosa*-induced TNF-α, IL-8, IL-6, and IL-1β responses in human monocyte-derived macrophages (Miles et al. 2009); and HNP-1 and HNP-3 attenuate *P. intermedia* LPS-induced IL-1β, IL-8, and ICAM-1 responses in THP-1 human monocytes and HGF cells (Lee et al. 2010). HBD1, HBD2, HBD3, and DEFB104A attenuate *P. intermedia* LPS-induced IL-1β, IL-8, and ICAM-1 responses in THP-1 human monocytes and HGF cells (Lee et al. 2010); HBD3 attenuates the IL-6, IL-10, GM-CSF, and TNF-α responses of HagB-induced human myeloid dendritic cells (Pingel et al. 2008); DEFB114 attenuates an LPS-induced TNF-α response in RAW264.7 murine macrophages (Yu et al. 2013); and DEFB123 (Motzkus et al. 2006) and DEFB126 (Liu et al. 2013a) attenuate an LPS-induced IL-6 (e.g., DEFB126) and TNF-α (e.g., DEFB123, DEFB126) response in RAW264.7 murine macrophages. Finally, θ-defensin retrocyclin RTD-1 inhibits a TLR2, 4, and 5 agonist-induced TNF-α, IL-1α, IL-1β, IL-6, IL-8, MCP-1 (CCL2), MIP-1α (CCL3), and MIP-1β (CCL4) responses in human peripheral blood leukocytes (Schaal et al. 2012).

Histatin 5 suppresses the induction of IL-6 and IL-8 in *P. gingivalis* outer membrane protein-induced human gingival fibroblasts, and this activity is more effective when outer membrane protein is incubated with histatin 5 before addition to the cell culture (Imatani et al. 2000). Histatin 5 also attenuates a *P. gingivalis* hemagglutinin B (HagB)-induced chemokine and proinflammatory cytokine response in dendritic cells (Borgwardt et al. 2014). 20.0 mM histatin 5, mixed with 0.02 mM HagB, attenuates an HagB-induced CCL3/MIP-1α, CCL4/MIP-1β, and TNF-α responses.

9.4.4 The Order of Peptide Exposure to Cells, with Respect to Agonist Exposure to Cells, Is a Proinflammatory Event

This, too, is a recent and very exciting area of research. Antimicrobial peptides given before or after a proinflammatory agonist induce cells to produce higher concentrations of proinflammatory mediators. When given together, LL-37 attenuates LPS-induced TNF-α responses (Scott et al. 2011; Brown et al. 2011). However, when THP-1 monocytes are treated with LL-37 for 1 h before the addition of LPS (Scott et al. 2011) or 3 h after incubation with LPS (Brown et al. 2011), attenuation is abolished. In yet another example, pre-mixing of leukocytes and *E. coli* for up to

2 h, followed by addition of retrocyclin RTD-1, led to a reduction of TNF-α levels (46–93 %) by RTD-1 at each time point (Schaal et al. 2012).

In our work, HBD3 given before or after a proinflammatory agonist induces human dendritic cells, murine JAWSII dendritic cells and mice to produce higher concentrations of proinflammatory mediators (Harvey et al. 2013). HBD3 (0.2, 2.0, or 20.0 μM) given to human myeloid dendritic cells pre- (1 h before), co-, or post- (1 h after) HagB treatment (0.02 or 0.2 μM) displayed a concentration-dependent ability to both attenuate and enhance the chemokine and proinflammatory cytokine response. Timing is important, and MIP-1α (CCL3), MIP-1β (CCL4), and TNF-α responses to 0.02 μM HagB are both enhanced and attenuated when 0.2 and 2.0 μM HBD3 is given pre-/post- or co-HagB exposure, respectively.

9.5 Wound Healing and Angiogenesis

Wound healing occurs in three phases: an inflammatory phase, a proliferative phase, and a maturational phase (Sinno and Prakash 2013), all involving multiple steps in hemostasis, inflammation, remodeling, formation of granulation tissue, and reepithelialization (Ramos et al. 2011). The process involves various cells like fibroblasts, keratinocytes, endothelial cells, growth factors, extracellular matrix components, and chemokines and cytokines. Antimicrobial peptides can be detected in the margins around both oral and cutaneous wounds, and there is a growing body of evidence to suggest that they also play a dynamic role in the wound healing process at multiple steps. Furthermore, a large number of antimicrobial peptides do have properties that have the ability to speed wound healing and angiogenesis.

Early steps involve cell migration and proliferation. Histatins and LL-37 induce fibroblast migration, HBD2 promotes keratinocyte migration (Niyonsaba et al. 2007), and LL-37 induces human microvascular endothelial cell and human umbilical vein endothelial cell migration (Ramos et al. 2011). Histatins and LL-37 also induce fibroblast proliferation, HBD2 increases keratinocyte proliferation (Niyonsaba et al. 2007; Warnke et al. 2013), and LL-37 induces human microvascular endothelial cell and human umbilical vein endothelial cell proliferation (Ramos et al. 2011).

Later steps involve wound angiogenesis, vascularization, and reepithelialization, and LL-37 again is particularly active. LL-37 stimulates angiogenesis (Nakatsuji and Gallo 2012) and induces the formation of tubule-like structures (Ramos et al. 2011). Another cathelicidin CRAMP has angiogenic properties, too (Kurosaka et al. 2005). LL-37 also stimulates reepithelialization (Heilborn et al. 2003; Ramos et al. 2011; Nakatsuji and Gallo 2012).

One unique mechanism, discussed in detail below, involves LL-37 produced by keratinocytes in injured skin (Lande et al. 2007). Here LL-37 combines with self-DNA released from injured or dead tissues and initiates immune responses in damaged skin enhancing resistance to infection and initiating wound healing.

9.6 Autoimmune Functions

Defensins and LL-37 bind to CpG, self-DNA, and RNA (Tewary et al. 2013; Frasca and Lande 2012; Lande et al. 2007; Gilliet and Lande 2008). This interaction forms complexes. HBD3 + human genomic DNA forms large complex DNA nets (Tewary et al. 2013), and LL-37 + DNA forms aggregated and condensed structures (Lande et al. 2007). These complexes are readily taken up by plasmacytoid dendritic cells in a TLR9 (e.g., HBD3/DNA; LL-37/DNA)-dependent manner in the endocytotic pathway and induce the production of IFN-α (Tewary et al. 2013; Lande et al. 2007). In mice, CpG + HBD3 complexes administered intravenously alone induce proinflammatory cytokines in serum, administered subcutaneously alone induce the formation of local inflammatory cell infiltrates, and administered intraperitoneally with an immunogen like ovalbumin enhance both cellular and humoral responses to ovalbumin. Nakatsuji and Gallo suggest that this is an important normal physiological and immunological function leading to the attraction of various immune cells (Nakatsuji and Gallo 2012). Tewary and colleagues suggest that these complexes could improve vaccine formulations and enhance immune responses (Tewary et al. 2013). However, they also point out that these complexes are found to be a constituent of circulating immune complexes isolated from sera in patients with autoimmune diseases (Tewary et al. 2013). Frasca and Lande (2012) and Gilliet and Lande (2008) also suggest that this mechanism may lead to autoimmune and autoinflammatory diseases.

There is a growing body of evidence that suggests defensins and LL-37 have roles in autoimmune and autoinflammatory diseases like psoriasis, rosacea, ulcerative colitis, rheumatic joint disease, and systemic lupus erythematosus (Frasca and Lande 2012; Vordenbaumen et al. 2010). Subjects with autoimmune disease have increased circulating concentrations of α- and β-defensins (Vordenbaumen et al. 2010). In the sera of subjects with systemic lupus erythematosus, concentrations of HBD2 correlate with red blood cell count, dsDNA antibody titers, systemic lupus erythematosus disease activity index, and clinical transverse myelitis and myositis (Vordenbaumen et al. 2010). Similarly, serum HNP concentration correlates with subject white blood cell counts and clinical transverse myelitis and rash (Vordenbaumen et al. 2010). The relative amounts of HNP mRNA from neutrophils correlate with C3c concentrations, systemic lupus erythematosus disease activity index, and clinical renal involvement and rash (Vordenbaumen et al. 2010).

Clearly these are interesting findings, and further work is needed to clarify the roles of defensins and LL-37 (and other antimicrobial peptides) in the pathophysiology of autoimmune and autoinflammatory diseases (Frasca and Lande 2012; Gilliet and Lande 2008).

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