Host Defense Peptides in Wound Healing

Lars Steinstraesser,1,2 Till Koehler,3 Frank Jacobsen,1,2 Adrien Daigeler,1,2 Ole Goertz,1,2 Stefan Langer,1,2 Marco Kesting,4 Hans Steinau,1,2 Elof Eriksson,3 and Tobias Hirsch1,2,3

1Plastic Surgery, Ruhr University Bochum, Germany; 2Burn Center, Ruhr University Bochum, Germany; 3Plastic Surgery, Harvard University, Cambridge, Massachusetts, United States of America; 4Craniofacial Surgery, Technical University Munich, Germany

Host defense peptides are effector molecules of the innate immune system. They show broad antimicrobial action against gram-positive and -negative bacteria, and they likely play a key role in activating and mediating the innate as well as adaptive immune response in infection and inflammation. These features make them of high interest for wound healing research. Non-healing and infected wounds are a major problem in patient care and health care spending. Increasing infection rates, growing bacterial resistance to common antibiotics, and the lack of effective therapeutic options for the treatment of problematic wounds emphasize the need for new approaches in therapy and pathophysiological understanding. This review focuses on the current knowledge of host defense peptides affecting wound healing and infection. We discuss the current data and highlight the potential future developments in this field of research.

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INTRODUCTION

Skin and soft tissue infections account for 7% to 10% of hospitalizations and represent one of the most common indications for the use of antimicrobial therapy in the United States (1). Wound infections and sepsis are an increasing cause of death in severely ill patients (2), and the treatment of chronic and complex wounds puts a significant burden on the health care system and on the economy as a whole (3). The progressive decline of therapeutic efficacies of available antibiotics due to antimicrobial resistance and the limited therapeutic approaches in wound healing emphasize the urgency for further pathophysiological insights and the development of new classes of drugs for the treatment of soft tissue and skin infections (4,5).

The human skin is constitutively colonized with various microorganisms. The epidermal and dermal layers present an important physical barrier against invading microorganisms; disruption of this barrier allows pathogens to invade the body and drastically increases the risk of infection and mortality of severely ill patients (2,6). As an immune reaction, the skin is able to activate the innate immune response (7–9), in which host defense peptides work on the effector side of the immune response of the human host (10,11). Host defense peptides (HDPs) are peptide molecules <100 amino acids, coded by an individual gene (12). They have been identified in all kinds of species, including plants, insects, animals, and humans (13). The total number of known HDPs is increasing every year, with >900 HDPs listed in three databases to date (14–16).

Host defense peptides, also known as antimicrobial peptides (AP), play an important role in the innate immune system as antimicrobial and immunomodulating agents and present an important link between the innate and adaptive immune response (17,18). Owing to their multiple functions, they are considered promising agents for new therapeutic approaches in infectious diseases and wound healing (19–21).

HOST DEFENSE (ANTIMICROBIAL) PEPTIDES

According to their molecular composition, size, conformational structure, or predominant amino acid structure, host defense peptides can be divided into four main classes: linear α-helical structure, β-sheet structure stabilized by characteristic disulfide bridges, peptides with predominance of one or more amino acids, and loop-structured peptides (Table 1) (22–25). In humans, two main classes of host defense peptides have been identified: cathelicidins and defensins. Cathelicidins were first described in 1995 to classify peptides containing both a cathelin and a c-terminal antimicrobial peptide domain expressed in mammalian myeloid cells (26,27). The molecular structure of cathelicidins is a bipartite molecule, characterized by a
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<th>Host defense peptide</th>
<th>Pathogen directed activity</th>
<th>Immunomodulatory function</th>
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<td>Human cathelicidin (cationic, α-helical structure) hCAP18/LL-37</td>
<td><em>Escherichia coli; Pseudomonas aeruginosa; Staphylococcus aureus; Enterococcus faecalis; group A, B, and C Streptococcus; Klebsiella pneumoniae; Listeria monocytogenes; Salmonella typhimurium; Proteus vulgaris; Proteus mirabilis; Burkholderia cepacia; Actinobacillus actinomycetemcomitans; Capnocytophaga spp; Candida albicans; HIV-1</em></td>
<td>Broad antimicrobial activity, antiviral and antifungal activity, endotoxin-binding properties, modulation of pro-inflammatory response, chemotactic, influence of cell proliferation and differentiation, promotion of wound healing and angiogenesis, induction of gene expression, induction of adaptive immunity</td>
<td>Neutrophils, keratinocytes, epithelial cells of the skin and testis, gastrointestinal and respiratory tract, mast cells, monocytes/macrophages, CD4+ cells, myelocytes, wound and blister fluid, airway fluid, seminal fluid, cervix, vagina, esophagus, mouth, tongue</td>
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<td>Human β-defensins (cationic, disulfide bridge, β-sheet structure) hBD-1</td>
<td><em>Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus</em></td>
<td>Broad antimicrobial activity; antiviral and antifungal activity; chemotactic; induction of chemokines and cytokines; recruitment of immune cells; induction of adaptive immunity and pro-inflammatory cytokines such as IL-8, -18, and -20; degranulation of mast cells; promotion of phagocytosis; induction of dendritic cell maturation by TLR-4; LPS and ITS binding properties; inhibition of TNF-α production; induction of matrix-metalloproteinase (MMP); inhibition of MMP-inhibitors (TIMP-1/-2)</td>
<td>CD4+ and CD8+ T cells; dendritic cells; epithelial cells of skin, respiratory, gastrointestinal, and urogenital tract; trachea; uterus; pancreas; kidney; lung; prostate; placenta; thymus; testis; vagina; gingival intestine; conjunctiva; cornea; lachrymal and buccal mucosa; tongue; salivary gland; mammary glands; limb joints; astrocytes; microglia</td>
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<td>hBD-2</td>
<td><em>Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae, Enterococcus faecalis, Candida albicans, Candida parapsilosis, Candida cruzei, HIV-1</em></td>
<td>mast cells, CD4+ and CD8+ T cells; dendritic cells; skin; oral, pulmonal, and gastric epithelia; conjunctiva; cornea; astrocytes</td>
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<td>hBD-3</td>
<td><em>Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus carnosus, Streptococcus pyogenes, Streplococcus pneumoniae, Enterococcus faecalis, Burkholeria cepacia, Saccharomyces cerevisiae, Candida albicans, Candida parapsilosis, Candida cruzei, HIV-1</em></td>
<td>Monocytes; CD4+ T cells; oral, respiratory, gastrointestinal, urinary, and skin epithelial cells; uterus; placenta; testis; esophagus; heart; neutrophils; trachea; skeletal muscle; tongue; kidney; liver; gastrointestinal tract; pharynx; tonsils; salivary glands</td>
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stable N-terminal cathelin domain and an antimicrobial C-terminal domain (28). To date, hCAP18/LL-37 is the only human cathelicidin described. The cathelicidin family has great variance but only the hCAP-18, with a cathelicidin gene on chromosome 3, can be produced as a propeptide. It is stored as a precursor in human neutrophil granules (29) and various cells and tissues such as T cells, monocytes, lymphocytes, natural killer (NK) cells, B cells, and mast cells. The epithelia of the airways, mouth, tongue, esophagus, intestine, cervix, vagina, salivary glands, epididymis, and testis have been shown to express LL-37 (30,31). Furthermore, LL-37 is secreted in human wound, sweat, and airway surface fluids (28,32–36) and is upregulated in response to cutaneous infection or injury (36,37). Its C-terminal antimicrobial domain, LL-37, can be liberated by proteinase 3 after degranulation and secretion (27,38).

Cathelicidin has broad activity against bacterial, viral, and fungal pathogens (39,40). Inflammation or injury seems to be the trigger for upregulation of the LL-37/hCAP-18 gene, particularly in keratinocytes and leukocytes (27,41). An indirect immune-modulating effect of LL-37/hCAP-18 is its chemotactic effect in the peripheral blood flow on monocytes, neutrophils, and CD4– cells (42,43). In an animal model, a mast cell degranulation effect by LL-37 (43) and a chemoattractant effect of neutrophils with the initiation of a specific immune response by phagocytosis of opsonized bacteria (44) have been shown. LL-37 shows the direct binding of lipopolysaccharide (LPS) and the inhibition of LPS-induced cell responses like release of tumor necrosis factor (TNF)-α, nitric oxide, and tissue factor (45–47).

One of the best-characterized families of host defense peptides in vertebrates are defensins, which represent the second important class of HDPs in humans (48). Defensins are small, cationic cystine-rich peptides with a characteristic molecular β-sheet structure, including three disulfide bridges with an amphipathic charge distribution (49,50). Defensins are subclassified into α-defensins, also called human neutrophil peptides (HNP)s, and β-defensins (hBD). Their molecular configuration enables defensins to interact with cell membranes of target cells and disrupt them (51). It ensures broad antimicrobial activity that functions by forming channels in the target membrane, leading to cell lysis and consecutive cell death (17,52–54).

The human α-defensin was isolated from myeloid-derived cells like neutrophils and macrophages (55). Five different human α-defensin genes and six different human α-defensin molecules are called HNP-1 to -6 (56–59). HNP-1 to -4 are localized in azurophilic granules of neutrophil granulocytes, where they contribute to the oxygen-independent killing of phagocytosed microorganisms (55,60). HNP-5 and -6 are enteric defensins, first discovered in Paneth cells. As immune modulators, HNP-1, -2, and -3 upregulate TNF-α and interleukin (IL)-1 in human monocytes that have been activated by bacteria (61,62). Furthermore, HNP-1 and -2 have the ability to directly kill gram-negative and -positive bacteria (48), Candida albicans (63), and enveloped viruses such as members of the herpes family (64,65). HNP-5 has concentration-dependent microbicidal activity against Escherichia coli, Listeria monocytogenes, Salmonella typhimurium, and C. albicans (66).

The human β-defensin (hBD-1) was identified and purified in 1995 from blood plasma of patients with renal disease (67). HBD-1 is constitutively expressed in different tissues with primary expression in the epithelial lining of the respiratory and urinary tracts (7,68–72). Different studies have shown that hBD-1 expression can be upregulated by LPS, heat-inactivated Pseudomonas aeruginosa, and interferon (IFN)-γ (73–78). In contrast to many other antimicrobial peptides in cutaneous wounds, hBD-1 does not seem to be involved in a specific manner. However, it shows special activity against gram-negative bacterial strains like E. coli and P. aeruginosa (71).

The initial isolation of human β-defensin-2 (hBD-2) occurred in 1997 from psoriatic skin lesions (79). The most prevalent expression of hBD-2 is observed in keratinocytes, the gastrointestinal tract, and the respiratory tract (80,81). Stored in lamellar bodies of keratinocytes (82), hBD-2 can be upregulated directly by bacterial pathogens (62,83) or inflammatory cells like monocyte-derived, macrophage-derived (73,84–86), and lymphocyte-derived (76,87) cells. Several mechanisms and signaling pathways are involved in the expression of hBD-2. Detection of bacterial LPS by CD14 and Toll-like receptor 2 and subsequent activation of the NF-κB cascade induces human β-defensin 2 (88,89). Furthermore, human Toll-like receptor 2 mediates induction of the antimicrobial peptide hBD-2 in response to bacterial lipoprotein (88,90,91). HBD-2 signaling pathways involve NF-κB (89) and mitogen-activated protein kinase (92), including Src-dependent Raf-MEK1/2-ERK (93). The promoter of hBD-2 has binding sites for NF-κB and putative binding sequences for AP-1, NF-IL6, and STATs (85,89,91). After upregulation, hBD-2 shows immune-stimulating properties by chemoattracting immature dendritic cells and T cells to modify the adaptive immune reaction (94).

As an inducible HDP, hBD-2 seems to be involved in wound repair by activating the intrinsic immunity after destruction of epidermal skin layers and inflammation (85). Mediators upregulating hBD-2 in epithelial tissue are proinflammatory cytokines like IL-1 (84), IL-22 (96), bacterial LPS (97), and direct bacterial contact with epithelial cells (79,98,99). After activation, hBD-2 shows direct activity against P. aeruginosa, E. coli, and C. albicans (100). Furthermore, hBD-2 shows a synergistic effect with LL-37 in increased activity against S. aureus (101). In the setting of chronic skin disorders, Ong et al. (101) showed a continuous upregulation of hBD-2 in psoriatic skin scale with a low susceptibility for skin infections.

In burn wounds, decreased hBD-2 activity was shown, indicating that innate immune defects contribute to the risk of burn wound infection and sepsis (102).
The human β-defensin-3 (hBD-3) was originally discovered from psoriatic skin lesions and isolated nearly simultaneously from two groups in 2001 (103, 104). hBD-3 was further detected in many other tissues such as heart, liver, fetal thymus, and placenta cells (103, 105,106). In skin, hBD-3 is stored like hBD-2 in lamellar bodies of keratinocytes (107). TNF-α, transforming growth factor (TGF)-α, insulin-like growth factor 1 (IGF-1), Toll-like receptor 5, IL-1β, IFN-γ, TGF-α, and IGF-1 as well as various bacteria play an important role in activation of the synthesis of hBD-3 (103,108).

After testing a large number of bacterial strains, the broad bactericidal activity of hBD-3 against gram-positive and -negative bacteria was reported, including multidrug-resistant strains of S. aureus and P. aeruginosa (109,110).

Human β-defensin-4 is primarily expressed in testis and epididymis (111) and inducible in primary keratinocytes (112). These data are based on detection of mRNA, and a partial characterization of this defensin relies on recombinant preparation by Garcia et al. (111). The activation of hBD-4 seems similar to that of hBD-2 and hBD-3 (112).

Human β-defensins promote histamine release and prostaglandin-2 production in mast cells (43,113), connect the innate and adaptive immune system by chemoattraction of immature dendritic cells and T cells (94), and increase the expression of TNF-α and IL-1 in human monocytes following activation by bacterial stimulus (61).

Host defense peptides in human skin are primarily produced by keratinocytes, eccrine glands, and neutrophile granulocytes (114). Constitutively produced HDPs of the human skin are dermicidin (115), protease inhibitor antileucoprotease (ALP) (116,117), RNase 7 (118), psoriasin (119), lysozyme (120), β-defensin-1, (69) and secretory phospholipase A2 (121). Inducible HDPs of the human skin are cathelicidin LL-37 (35) produced in keratinocytes, α-defensins (HNP 1–4, human neutrophil peptide) produced by neutrophils (55,122), and β-defensins-2 and -3 (79,103,104).

PROPERTIES OF HOST DEFENSE PEPTIDES
Host defense peptides show great variance of effects and interactions. One major function of HDPs is to inactivate microbes, including bacteria, fungi, parasites, and viruses, through multiple direct effects on their membranes (9,23,36,123) (Table 1).

HDPs have the ability to attack specific external targets simultaneously, such as the cytoplasmic membrane of bacteria, by building perturbing peptides and attacking internal targets by invading the barrier of the bacterial cell wall and permitting passage of various molecules into the cell (23,124).

Another major function is their active role in the transition to the adaptive immune response by being chemotactic for human monocytes, neutrophils, and T cells and by exhibiting adjuvant and polarizing effects in influencing dendritic cell development (125).

A high endotoxin-neutralization capacity after bacterial infections slows down the acute immune reaction and prevents septic shock in severely ill patients (17,23,126–128); host defense peptides have a direct influence on the adaptive immune responses by activation of different immune factors such as TNF-α, IL-1, and IFN-γ without employing the NF-κB pathway (129–131).

HOST DEFENSE PEPTIDES IN WOUNDS
Host defense peptides, synthesized in the skin at sites of potential microbial entry, provide a soluble barrier that acts as an impediment to infection (62). If the skin is intact, bacterial growth will be controlled by bacteriostatic and bactericidal compounds such as psoriasin and RNase 7 (6). However, in injury and infection of the skin, expression of host defense peptides will be upregulated owing to increased synthesis by keratinocytes and deposition from degranulation of recruited neutrophils. Constitutive and inducible expression of human cathelicidin (hCAP18/LL-37), as well as hBD-2 and -3, have been observed in epidermal keratinocytes (36,41,104).

In a wound, IGF-1 and TGF-α are stimulators for the human cathelicidin hCAP18/LL-37 to a comparable level as the proinflammatory cytokine IL-1 (36,132). Both play important roles in wound healing by activating epidermal cells and fibroblasts to form granulation tissue, mediate angiogenesis, and chemoattract macrophages and fibroblasts (133–135). In a feedback mechanism, cathelicidin from activated leukocytes in pigs (PR-39) has shown a direct influence on dermal fibroblasts by increasing synthesis of the extracellular matrix proteoglycans, syndecan-1 and -4 (136), which are required for the activity of many growth factors (137–140). In an animal model, syndecan production was delayed and ineffective wound repair (141,142) was reported.

Heilborn et al. (143) described a receptor K67–dependent, continuous increase of LL-37 produced by keratinocytes and granulocytes (36), with a peak maximum after 48 h and high expression in the wound fluid and wound tissue of healing skin. Expression decreased after wound closure, and a lack of LL-37 in chronic wounds was reported (143). Different authors have shown a protective function of LL-37 from invasive bacterial skin infections, particularly against P. aeruginosa, S. aureus, and group A Streptococcus (36,101,144,145). Comparing wild-type mice with Cnlp-deficient mice (targeted deletion of the cathelicidin gene), a prolonged period of wound healing and an increase in bacterial colonization for Cnlp-deficient mice was reported (145). These findings were confirmed by Nizet et al. (144), who reported a better outcome for wild-type mice versus Cnlp-deficient mice after challenge with necrotic skin infections of Group A Streptococcus.

Ong et al. (101) showed better immune response against S. aureus in patients with psoriasis caused by a higher LL-37 expression level, whereas patients suffering from topical dermatitis showed decreased expression (146). These findings may provide an explanation for the sus-
ceptibility of patients suffering from atopic dermatitis to skin infection compared with patients with psoriasis (147).

We demonstrated a bactericidal effect of LL-37 in a rat animal model following transient adenoviral gene therapy to P. aeruginosa–infected burn wounds (148).

LL-37 has a direct effect on wound healing by promoting neovascularization and angiogenesis. Koczulla et al. (149) showed the impact of LL-37 to angiogenesis in a chorioplastic anocutaneous membrane assay and by a revascularization model in an animal after hind-limb ischemia. The authors found a direct effect of LL-37 by activating vessel growth in cultivated epithelial cells, and after injection of LL-37 in the ischemic limb of a rabbit, they noted increased blood supply. They found direct participation of the formyl peptide receptor like 1 (FPRL1) in activation of hCAP-18 and following neovascularization (149). We confirmed the angiogenic effect of LL-37 in a skinfold chamber model in mice (150).

Another study analyzed visualization and localization of the cathelicidin LL-37, neutrophil defensin α (human neutrophil peptide), and human β-defensin-1, -2, and -3 in normal and burned skin and determined the cell types in which these host defense peptides were localized using fluorescence microscopy. The authors showed that in normal skin, human β-defensin-1 was localized to the perinuclear region of keratinocytes and human β-defensin-2 was primarily localized to the stratum germinativum; human β-defensin-3 was detected in the stratum spinosum, whereas human α-defensins (HNPs) were randomly distributed in the papillary dermis. The cathelicidin LL-37 was concentrated in the stratum corneum and along ducts.

In burned skin, human β-defensin-1 was expressed in dermal glands, including hair shafts; human β-defensin-2 and -3 were found in the remaining keratin layers and glands of the lower dermis; human neutrophil peptides were localized to hair shafts and in residual keratin layers. Interestingly, LL-37 was detected in very high concentrations in the epithelium of sweat ducts. The authors concluded that the cells in the lower dermal and subdermal regions of burned skin produce host defense peptides after burn injury to maintain a barrier against infection (151,152).

HNPs promote wound healing. Oono et al. (153) showed that synthetic HNP-1 increases the expression of pro-collagen mRNA and protein in dermal fibroblast cultures. In contrast, the expression of matrix-metalloproteinase-1 was decreased. The authors suggest that HNP-1 may promote wound repair by enhancing extracellular matrix deposition (153). Another study showed mitogenic activity of HNPs in epithelial and fibroblast cell lines in vitro (154).

For β-defensins, Supp et al. (155) showed expression of the β-defensin-1, -2, and -3 in keratinocyte cultures and split skin grafts from healthy and burned donors. Later studies reported that β-defensins stimulate migration and proliferation of epidermal keratinocytes and thus might promote cutaneous wound healing (156). In chronic and acute wounds, β-defensin-2 seems to be upregulated, whereas it is not detectable in healthy skin (157).

Expression of human β-defensin-3 in keratinocytes is induced by skin infection with S. aureus via Toll-like receptor 2 and epidermal growth factor receptor (EGFR) (76,158), and Kisich et al. (159) demonstrated that the capacity of human keratinocytes to fight bacterial infections (S. aureus) depends on its β-defensin-3 expression. We have demonstrated that gene transfer of human β-defensin-3 to infected diabetic porcine wounds enhances wound closure by 25% (unpublished data).

In a rat burn model, we showed that the host defense peptide histone 1.2 is effective against P. aeruginosa wound infection, with a threefold reduction in bacterial burden (160). In a recent published study, we analyzed host defense peptide expression in burned skin in humans. In this study, we showed that concentrations of LL-37 and human β-defensin-1, -2, and -3 change significantly in burned traumatized skin. Whereas human β-defensin-1 showed only a moderately lower expression in burn wounds compared with healthy tissue, hBD-2 expression changed drastically: burn wound tissue showed an upregulation of 380-fold compared with controls. hBD-3 showed a 10-fold increase in mRNA expression.

Tissue sections taken from the center of burn wounds showed no direct changes in LL-37 expression compared with comparable sections from unburned patients. However, it became evident that in the edges of burn wounds a 10-fold reduction in LL-37 expression occurs. This might be due to the presence of more viable, traumatized cells, whereas in the center, cells are already dead (161). These combined data show that host defense peptides play a major role in wound healing and wound infection. In contrast to clinically used antibiotics, HDPs have interesting features for topical application to treat wound infection and promote healing. However, there are few data investigating their role in wounds in detail. Thus, more experimental and clinical studies are needed to gain further insights into this important field of research.

FROM BENCH TO BEDSIDE

Despite the seemingly successful application of host defense peptides in in vitro experiments and animal models, cytotoxicity could be a serious limitation to clinical studies. We demonstrated that application of the host defense peptide protegrin-1, a mammalian HDP, led to increased bacterial reduction but decreased survival in a sepsis model in mice. These data indicate that protegrin-1 induces extensive endotoxin release and thus elicits large alterations in host innate immune response (20). Another study showed that neonatal rats that developed LPS-induced sepsis died within 5–8 h when more than 300 μg/kg LL-37 was applied (162). However, mortality was significantly reduced in groups that received lower doses of LL-37 (162). In fact, LL-37 has shown nonselective cell
toxicity and hemolytic activity (163) and causes DNA fragmentation in cell cultures (164). Recently, we detected high cytotoxicity of human β-defensin-3 after gene transfer to primary keratinocyte cell cultures (unpublished data). Many other host defense peptides were shown to be cytotoxic (165).

These setbacks led to increased efforts to develop novel synthetic host defense peptides with less cytotoxic effects and improved antimicrobial activity. We demonstrated that the designer peptide proline-novispirin G10 possesses low hemolytic and cytotoxic activities combined with broad-spectrum microbicidal activities against gram-positive and -negative bacteria in vitro. These findings were confirmed in a large animal wound healing model against *S. aureus* (166) and in a *P. aeruginosa*-infected rat burn model (21).

Ciornei et al. (167) presented a modified truncated LL-37 peptide. Although LPS binding and neutralization was not affected and concentration-dependent chemotactic activity was similar, it caused significantly less hemolysis than the native LL-37 (167). Another study described an active domain of LL-37, and demonstrated the possibility to use shorter and less-toxic variants of LL-37 with retained or improved antimicrobial and endotoxin-neutralizing activities (168). Another strategy was reported by Braunstein et al. (169) in which D-amino acids substituted several of the L-amino acids in host defense peptides, thus markedly reducing toxicity in mice (170).

Bacterial resistance to conventional antibiotics is a major concern and the main reason for extensive, ongoing research to develop new therapeutics. Microorganisms have also developed ways to escape direct killing by human host defense peptides (171–174), which emphasizes their important and ancient role in the innate immune system (172). However, resistance mechanisms are generally related to the direct killing of bacteria and are not comparable to the resistance mechanisms of conventional antibiotics. Furthermore, the potent immunomodulatory functions are not hampered.

A promising aspect in improving antimicrobial therapy is the combination of common therapeutic antibiotics with host defense peptides. The application of the host defense peptide temporin L combined with β-lactams in a murine model of gram-negative sepsis led to the lowest plasma endotoxin and TNF-α levels measured in the study, the highest antimicrobial activities, and the highest survival rates (175).

In rat models, β-lactams even increased plasma endotoxin and TNF-α levels, whereas the combination with cecropin B proved to be the most effective treatment affecting survival rates, antimicrobial activity, and reduction in plasma endotoxin and TNF-α levels (176). Cirioni et al. (177) tested the efficacy of a combination of two α-helical antimicrobial peptides (cecropin A and maigainin II) and vancomycin against *S. aureus* with intermediate resistance to glycopeptides in a murine sepsis model. The authors demonstrated that the combination showed low lethality rates (5%–15%), low bacterial blood counts, and the strongest reduction in TNF-α and IL-6 in the plasma (177). Host defense peptides seem to be impaired by bacteriostatic antibiotics. If the growth of *E. coli* and *S. aureus* is suppressed by chloramphenicol and erythromycin, the susceptibility of bacteria to cathelicidin antimicrobial peptides is markedly diminished (178). These findings indicate the importance and relevance of the development of new therapeutic strategies, including host defense peptides, in the treatment of wound infections.

To gain a better understanding of the role of host defense peptides, it is crucial to determine their kinetics in systemic and local infection and inflammation. In general, only a few clinical studies deal with host defense peptides to date (179); however, four host defense peptides were successful in therapeutic clinical trials (180).

Children suffering from meningococcal sepsis were treated with the designer host defense peptide rBPI3 [Neuprex, derived from bacterial/permeability-increasing protein [BPI]], a human host defense protein synthesized by polymorphonuclear leukocytes (181). The investigators reported significantly reduced morbidity and improved functional outcome; however, there was no significant benefit in mortality (182). Currently, there is an ongoing clinical trial investigating therapeutic effects of rBPI2 in burn patients. Other studies investigated the treatment of diabetic foot ulcers and impetigo with the modified host defense peptide MSI-78. The bovine indolicin-derived Migenix MX-226 has already proceeded to a phase 3b study for the treatment and prevention of catheter-associated infections (179,180, 183). Promising candidates for the treatment of acne are the designer peptides XOMA 629 and MBI 594AN with bactericidal effects against *Propionibacterium acnes* (184). This agent is expected to be available for clinical therapy in the near future.

To date, no clinical trial for the treatment of wounds using host defense peptides is published. Other ongoing trials are promising, however, and clinical studies using host defense peptides for the treatment of impaired and infected wounds are expected in the near future.

**CONCLUSION**

Despite the loss of epidermal barrier function and subsequent exposure to environmental microorganisms, superficial epidermal wounds in vertebrates usually heal without major complications. One reason is that the skin innate immune defense function creates a soluble antimicrobial peptide barrier. After injury, host defense peptide levels in the skin rise rapidly due to increased synthesis by keratinocytes and deposition from degranulation of recruited neutrophils. In addition to the direct antimicrobial effect, host defense peptides have emerged as important effectors in infection and inflammation by modulating the innate immune reaction and activating adaptive immunity.

The growing problem of resistance to conventional antibiotics and the need for new ones has stimulated interest in the development of antimicrobial peptides as
potential therapeutic agents. The main impediments to the development of host defense peptides as systemic therapy include that many of the naturally occurring peptides (such as magainin), although active in vitro, are effective in animal models of infection only at very high doses, often at close to toxic concentrations. Most pharmaceutical research has been devoted to the development of topically applied agents, such as the magainin analog pexiganan, largely because of the relative safety of topical therapy and the uncertainty surrounding the long-term toxicology of any new class of drug administered systemically (17). The potential use of HDPs as future therapeutics depends on factors such as toxicity, stability, and immunogenicity of the substances in the host.

Protegrin-1, a member of the cathelicidin family in pigs, and novospirin G10, a modified ovospirin-1, were applied as a topical ointment, successfully reducing bacterial load and promoting healing of burn wounds in an animal model (21,185). By using the neovascularization and antibacterial effect of LL-37, we could show a high potential of LL-37 in skin reconstruction in a biopolymer assay as a future option for clinical therapy.

Beyond single-therapy regimens, there is a promising future approach: the application of new complex therapeutic regimens, such as host defense peptides combined with conventional and established antibiotic agents or the application of multiple host defense peptides. These therapies could both affect the pathogens and simultaneously activate and modulate innate and adaptive immune systems of the host.

These developments and insights in host defense peptides and are promising and may contribute significantly to the therapeutic endeavors to fight systemic and local infection and inflammation in the near future.

REFERENCES

38. Frohm M, et al. (1996) Biochemical and antibac-


