J Mol Med (2007) 85:317–329
DOI 10.1007/s00109-006-0143-4

REVIEW

Multifunctional antimicrobial peptides: therapeutic targets in several human diseases

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Received: 6 August 2006 / Revised: 10 October 2006 / Accepted: 10 November 2006 / Published online: 10 January 2007

Abstract Antimicrobial peptides have emerged as promising agents against antibiotic-resistant pathogens. They represent essential components of the innate immunity and permit humans to resist infection by microbes. These gene-encoded peptides are found mainly in phagocytes and epithelial cells, showing a direct activity against a wide range of microorganisms. Their role has now broadened from that of simply endogenous antibiotics to multifunctional mediators, and their antimicrobial activity is probably not the only primary function. Although antimicrobial peptide deficiency, dysregulation, or overproduction is not known to be a direct cause of any single human disease, numerous studies have now provided compelling evidence for their involvement in the complex network of immune responses and inflammatory diseases, thereby influencing diverse processes including cytokine release, chemotaxis, angiogenesis, wound repair, and adaptive immune induction. The purpose of this review is to highlight recent literature, showing that antimicrobial peptides are associated with several human conditions including infectious and inflammatory diseases, and to discuss current clinical development of peptide-based therapeutics for future use.

Keywords Antimicrobial peptides · Inflammatory diseases · Innate immune system · Infection · Host defense

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Introduction

Antimicrobial peptides (AMPs), also known as genetically encoded antibiotic peptides, are conserved components of the innate immune response [1, 2]. They are produced in many organisms including bacteria, insects, plants, and vertebrates, where they represent a defense system [3–5]. In mammals, these peptides function mainly in phagocytic cells of the immune system to kill engulfed or invasive bacteria and in mucosal epithelial cells to prevent colonization of host tissues by pathogens [1, 6]. In the past two decades, numerous antimicrobial peptide families from many living organisms have been described. In humans, three distinct groups were characterized, including defensins, cathelicidins, and histatins. Additional peptides that have been identified include RNase 7, granulysin, hepcidin, dermacidin, and lactoferrin. As of 2006, the inventory of AMPs comprises about 900 peptides of different origins, and these peptides have been listed in a database in Trieste (http://www.bbcm.univ.trieste.it/~tossi/pag1.htm). Additional information with

regard to these peptides is also available from another website: http://aps.unmc.edu/AP/main.htm. Medline database search using the PubMed interface with "antimicrobial peptides" as key word also shows more than 7,000 references with new references added every week.

AMPs have potent activities against a broad range of microorganisms that covers gram-positive and -negative bacteria, fungi, parasites, and enveloped viruses [7, 8]. Several excellent review articles have examined the role of these peptides in the protection of a variety of human tissues [9–11]. The precise details of their mechanism of action remain unknown. However, it is generally accepted that the positively charged peptides act directly on the negatively charged cellular membranes of bacterial cells, causing an increase in membrane permeability, which then leads to rapid cell death [12, 13]. Other mechanisms of action, including activity on intracellular targets after membrane permeabilization, have been proposed. Examples of intracellular activity include the activation of autolytic enzymes and the inhibition of DNA and protein synthesis [13, 14]. Buforin II has been shown to enter the cell and accumulates in the bacterial cytoplasm where it inhibits cellular functions by binding to DNA and RNA [15]. Attacins block the synthesis of integral membrane proteins [16]. PR-39 was reported to inhibit DNA synthesis [17]. Interestingly, pyrrhocoricin, an insect peptide, was demonstrated to kill bacteria by binding to a protein target called DnaK [18], thereby preventing it from performing its protein-repair function, leading to death of the bacteria. Recently, a different mechanism has been proposed for the θ-defensin, retrocyclin 2, which blocks influenza virus infection by cross-linking and immobilizing surface glycoproteins, resulting in the inhibition of viral entry into the cell [19]. Together, these data make AMPs a very attractive target for clinical development to treat conditions where traditional antibiotics are becoming less effective. As AMP research has proliferated, the repertoire of biological roles of AMPs has expanded to a number of other functions (Fig. 1). These include (1) endotoxin neutralization, a property that allows detoxification of LPS [20], (2) chemokine-like activity, based on chemokine structural motif similarity that allows AMPs such as β-defensins to interact with certain chemokine receptors [21], and (3) immunomodulatory activities linking innate to adaptive immune responses [22, 23].

More recently, there has been growing interest in the role of these peptides in several inflammatory conditions including psoriasis, arthritis, respiratory diseases, atherosclerosis, and wound repair. These novel findings will be summarized in this review. It is hoped that a better understanding of how AMPs contribute to these diseases will provide important clues to the "patho-etiologies" of many of these complex conditions that have often been labeled as idiopathic when no cause is known. These researches would pave the way for the development of novel therapeutic drugs.

Antimicrobial peptides against infectious diseases

Why AMPs? Although the discovery and the development of antibiotics during the mid-twentieth century provided potent antimicrobial drugs with high specificity, increasing antibiotic resistance in microorganisms and new emerging pathogens have become a major problem in our society [24]. Several human pathogens are now becoming resistant to a number of clinically significant antibiotics, causing a crisis in the treatment and management of infectious diseases. Strains of *Mycobacterium tuberculosis*, *Enterococcus faecium*, and *Pseudomonas aeruginosa* are no longer easily treated with antibiotics, and increased resistance is observed in other important human pathogens such as *Staphylococcus aureus* and *Streptococcus pneumoniae*. The resistance phenomenon has been attributed to the overuse of antibiotics and the increasing number of immunocompromised patients who have alterations in phagocytic, cellular, or humoral immunity. The diminished effectiveness of current therapies and the widespread resistance has prompted interest in the search for new antibiotics as alternative treatments. A developing area of study using natural peptides with antimicrobial activity may offer the solution for the encountered resistance and hold promise for development of new therapeutic agents.

Evidences for antimicrobial activity of natural peptides

A growing body of evidence has demonstrated that AMPs work as antibiotics. Data from in vitro as well as from in vivo models have provided compelling evidence that these peptides protect against a range of microorganisms including bacteria, enveloped viruses, fungi, and even certain parasites.

AMPs against bacteria Direct antibacterial activity of AMPs has been demonstrated by a number of studies. Depleted levels of antibiotic peptides have been shown to be associated with several pathologic conditions. For instance, in humans, patients with a specific granule deficiency syndrome lack α-defensins and suffer from severe and frequent bacterial infections [25]. Others with a condition known as morbus Kostmann suffer from frequent oral bacterial infections and severe periodontal disease, which correlates with a deficiency in the human cathelicidin peptide LL-37 and human neutrophil peptides (HNP1-3) [26]. Low expression of LL-37, human β-defensin (hBD)-2, and hBD-3 in skin lesions caused by atopic dermatitis coincides with enhanced susceptibility to

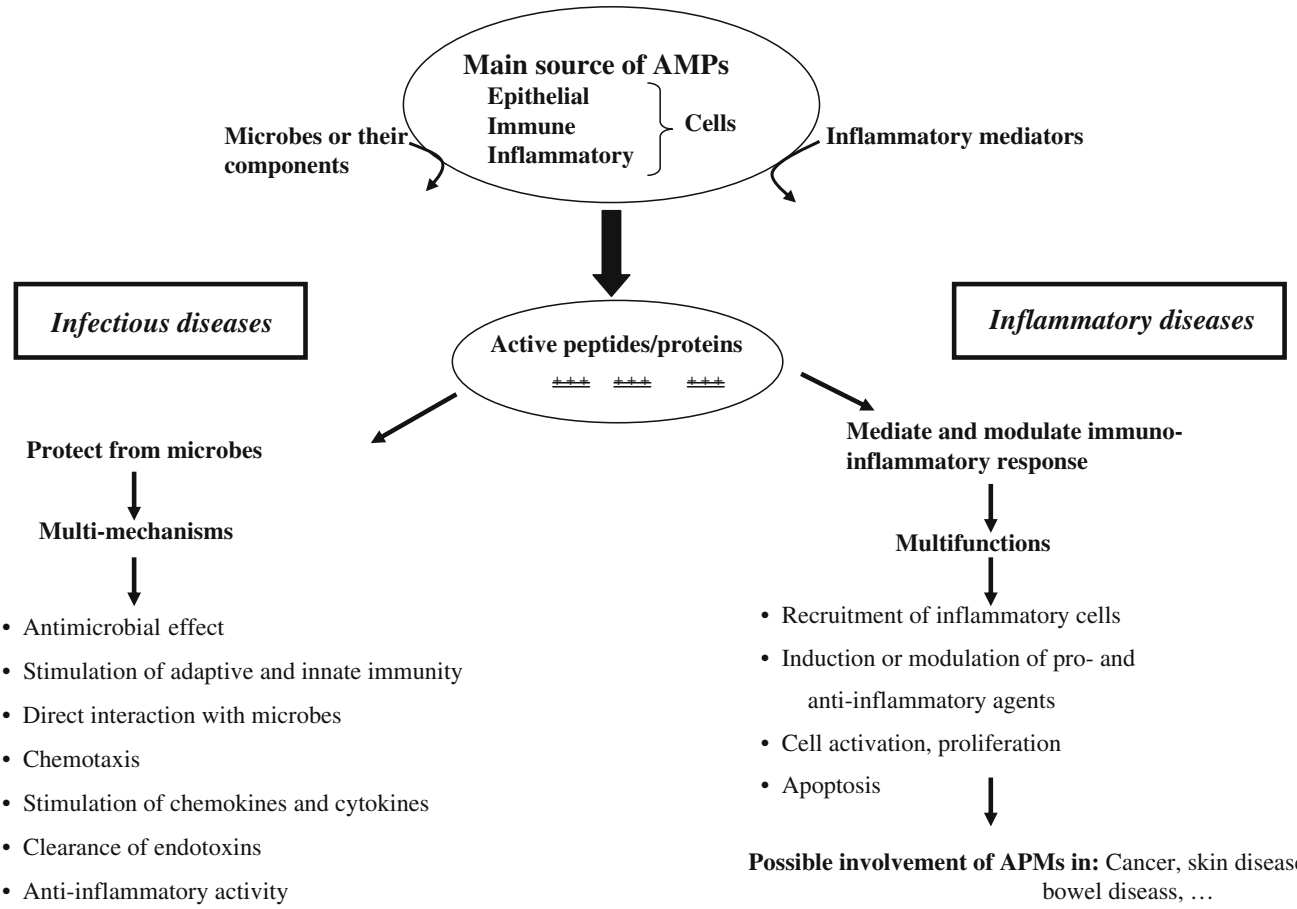

Fig. 1 Multifunctions of antimicrobial peptides in infectious and inflammatory diseases. After their release from various cell types after activation by microbes or inflammatory mediators, AMPs show a number of functions, including antimicrobial activity and modulation of the inflammatory response in different inflammatory diseases. Primary potential mechanisms of action of these peptides are *highlighted*

skin infections [27]. In rodent models, cathelicidins can control bacterial load and prevent mortality when administered after bacterial challenge [28, 29]. β-defensin-1 and CRAMP (*cnlp*) gene in knockout mice are more susceptible to and fail to clear infections [30, 31]. Conversely, overexpression of human defensins protected mice against enteric salmonellosis [32]. Systemic administration of nisin, a lanthionine-containing peptide from *Lactococcus lactis*, protected mice against *S. pneumoniae* infection [33]. The antibiotic nature of AMPs was also confirmed by a recent study demonstrating that in transgenic mice, overexpression of the porcine cathelicidin PR-39 enhanced resistance and protection of these animals against group A *Streptococcus* [34]. Lately, a fungal peptide named plectasin, belonging to the family of defensins, has shown antibacterial activity particularly against *S. pneumoniae*, including strains resistant to conventional antibiotics [35].

Although genetically modified mice contribute significantly to the studies of AMPs in vivo, evaluation of how specific peptides contribute to the host response after pathogenic challenge is complicated by a number of issues including (a) presence of large peptide families including 22 alpha-defensins in mice or 35 beta-defensins in humans may lead to functional redundancy, (b) other proteins such as lysozyme and lactoferrin also contribute to host defense, and (c) many AMPs have overlapping antimicrobial activity against gram-positive and gram-negative bacteria. Therefore, to identify the unique contribution of each AMP to disease, specific AMPs in knockout mice and relevant disease models will need to be studied.

Another method used to demonstrate the antibiotic properties of these peptides consisted of gene therapy approach in which mice were inoculated with an adenovirus vector containing the DNA for the human peptide LL-37 [36]. Success of this approach was corroborated by demonstration that overexpression of porcine PR-39 or human LL-37 in keratinocytes transduced with a recombinant lentivirus enhanced bacterial killing [34, 37]. AMP cutaneous gene therapy has also been successfully used in infected wounds and burns [38, 39]. However, although the concept of developing AMPs as antibiotics appears promising, concerns about bacterial resistance to these peptides

have been raised. Experimental analysis has identified a number of mechanisms of bacterial resistance to AMPs including altered cell surface charge, active efflux, production of proteases or trapping proteins, and modification of host cellular processes (reviewed in [40, 41]). Therefore, knowledge of the molecular basis of bacterial resistance to AMPs may provide clues on how to circumvent potential resistance problems. This research will build a basis for the development of AMPs as an alternative therapy to conventional antibiotic agents for the treatment of drug-resistant pathogens.

AMPs against viruses Apart from the antibacterial activity, AMPs such as defensins also possess antiviral activity. Based on the pattern of cysteine connectivity, mammalian defensins are classified into alpha and beta families. Recently, a circular form of AMPs called θ-defensin has been characterized in macaques, whereas in humans, the gene coding this peptide had been silenced by mutation [42]. Different studies have reported that defensins inhibit viral replication [43, 44]. For example, the α-defensins target the human immunodeficiency virus (HIV) activity by directly inactivating viral particles and affecting the ability of the virus to replicate within CD4 cells [45]. Human α-defensins HNP-1 to -3 and HD-5 have been shown to block papillomavirus infection [46]. Retrocyclin 2, a synthetic θ-defensin peptide that humans do not synthesize due to a mutation in the corresponding human gene, has the capacity to block influenza virus infection [19]. Human β-defensins can also block HIV-1 replication, and interestingly, a single-nucleotide polymorphism in a β-defensin gene has been associated with clinical manifestation of HIV-1 infection, suggesting that the human β-defensins play an important role in host defense against HIV [47]. Cathelicidins, in contrast, have an inhibitory effect on lentiviral replication in vitro [48], and LL-37 appears capable of interfering with vaccinia virus replication in vitro and in mice [49]. Dermaseptin S4, a 28-residue AMP isolated from frog skin, attenuates HIV infection in vitro [50]. Other AMPs from frog skin including caerin 1.1, caerin 1.9, and maculatin 1.1 have also demonstrated inhibition of HIV in vitro [48].

How AMPs inhibit viral entry into cells? HIV entry into a target cell is facilitated by its gp120/gp41 glycoprotein (env) interaction with CD14 [51] and a coreceptor, usually CCR5 or CXCR4 [52]. Generally, defensins inhibit HIV entry into the cell by inhibiting one of these mechanisms. For instance, θ-defensin has been shown to prevent HIV-1 env-mediated fusion by binding gp41 and blocking its 6-helix bundle formation [53]. hBD-3 has been reported to inhibit HIV infection by competing with stromal-derived factor, the natural ligand for CXCR4 [54]. Therefore, AMPs, in particular defensins, have clear anti-HIV-1 activity, and their broader anti-viral functions await further exploration.

AMPs against fungi The increasing incidence and severity of invasive mycoses and the resistance of fungal pathogens to currently available antifungal drugs have led scientists to explore the antifungal properties of AMPs. To date, approximately 100 peptides have been investigated for their capacity to counteract fungal infections, particularly those caused by *Candida spp.* [55]. *Candida spp.* have emerged as the fourth most common cause of bloodstream infections in the USA. Human HNP-1 and HNP-2 have shown fungicidal activity against *Candida albicans* and inhibited significantly the growth of *Cryptococcus neoformans* [56]. Rabbit NP-1, NP-2, and NP-3 also demonstrated good efficacy against *C. albicans* [57]. Histatins are another family of peptides with antifungal activity that are selectively secreted by the human parotid and submandibular glands. Among these peptides, histatins 1, 3 and 5 have been identified and characterized earlier, and their differential fungicidal activity against *C. albicans* has been determined [58]. Additional evidence for the role of AMPs in the protection against fungi has come from a study showing that low levels of histatins from saliva in a group of HIV patients correlated with higher incidence of oral candidiasis [59]. Interestingly, not all strains of *Candida* are sensitive to the action of naturally occurring AMPs. A recent study reported that *Candida glabrata* shows resistance to histatins as well as to magainins [60]. The major mechanism(s) of action by which these peptides inhibit fungi is not clear yet. However, several modes of action have been proposed including binding to and disruption of the outer membrane [61], resulting in leakage of important intracellular contents, and interaction with specific internal targets once they penetrate the cell [62]. Together, these studies have provided evidence that cationic peptides and particularly histatins may represent a new generation of compounds for the treatment of oral fungal infections.

AMPs against protozoa Early studies have demonstrated antiprotozoan activity of AMPs [63, 64]. Since then, considerable research efforts have been directed toward understanding the activities of these peptides against parasites transmitted by insect vectors. Recently, the potential activities of defensins and cathelicidins against the African trypanosome *Trypanosoma brucei* have been explored [65]. *T. brucei* is the vector-borne protozoan parasite that causes sleeping sickness, a disease associated with significant morbidity and mortality in both humans and animals. This study clearly demonstrated the effectiveness of peptides against parasites by disruption of their cell membrane integrity. Further investigation has shown that administration of cathelicidin AMPs to mice with late-stage *T. brucei*

infection rapidly improved parasitemia and prolonged survival [65]. These studies provide evidence for the use of AMPs in the treatment of parasitic diseases and invite more research that examines the role of AMPs in the control of parasitic infections.

Antimicrobial peptides and inflammatory diseases

A number of studies have suggested an association between the activation of innate immune mechanisms and the pathogenesis of inflammatory diseases. Recruitment of inflammatory cells and accumulation of chemokines and proinflammatory cytokines are hallmarks of an inflammatory state. Immune cell infiltrates are the primary source of host antimicrobial agents including AMPs, and released AMPs serve to amplify inflammation. In this context, different studies reported that AMPs including LL-37 may be involved in the regulation of inflammation by activating chemokine release from different cell types [66–68].

Apart from a central role in prevention and clearance of infections, AMPs exhibit an array of ever-expanding functions [69, 70]. The importance of AMPs in inflammatory conditions has now been expanded to include the following: psoriasis, respiratory disorder, inflammatory lung disease, inflammatory bowel disease (IBD), rheumatoid arthritis, and atherosclerosis (Table 1). The potential implication of a role of natural peptides in these diseases will be the major focus of our next discussion.

AMPs and skin diseases

Psoriasis is a common immune-mediated chronic skin disease that comes in different forms and differing levels of severity. Study of psoriatic-scale extracts revealed the presence of several AMPs including LL-37, human neutrophil defensin 1-3, RNase 7, and lysozyme (reviewed in [71, 72]). These peptides are believed to protect inflamed skin from microbial infections, as evidenced by the fact that patients who have psoriasis rarely suffer from skin infections, whereas patients with atopic dermatitis have increased skin infection rate with *S. aureus* [27]. In line with this observation, there exists a good correlation between atopic dermatitis and an impaired innate defense of human skin [73]. High-level expression of AMPs is seen in other pathologic conditions. For instance, hBD-2 and HNP are abundant in lesions of superficial foliculitis, a common skin disease characterized by inflammation of the hair follicle and infection with *S. aureus* [74]. Furthermore, human LL-37 has been shown to be induced in systemic lupus erythematosus and contact dermatitis [75]. From these diverse studies, it is becoming clear that AMPs are involved in skin diseases. However, their role in pathophysiology of inflamed skin tissues is not fully understood. Therefore, future studies must focus on the purported dual functions of AMPS, namely, its antimicrobial activity and its modulation of inflammatory response.

Acne vulgaris is a chronic inflammatory disorder of the pilosebaceous unit that widely affects adolescents and young adults (reviewed in [76]). The main events in the development of an inflammatory acne vulgaris lesion involve *Propionibacterium acnes* colonization and proliferation, which has shown resistance to conventional antibiotics. Because of their ability to neutralize endotoxins and consequently to inhibit the secretion of proinflammatory cytokines (e.g., TNF-α, IL-1) by host cells, cationic peptides could be potential candidates for treatment of acne. Granulysin is an antimicrobial peptide of the saposin-like family reported to be present in acne lesions. Recently, it has been reported that granulysin-derived peptides kill *P. acnes* effectively [77] and possess anti-inflammatory effects, as demonstrated by the suppression of *P. acnes*-stimulated cytokine release. Study of another peptide designated MX-594AN, which is under clinical development by Migenix, has demonstrated significant decrease in lesion counts of acne vulgaris in humans [78]. Taken together, these studies suggest that AMPs may be useful as agents for the treatment of acne. Hence, development of peptide-based drugs, preferentially applied topically, should be one area of research focus to resolve skin infections such as acne infection and other worse conditions.

AMPs and respiratory and lung disorder

Respiratory secretions have microbicidal properties mediated by their constituent antimicrobial peptides/proteins including lysozyme, lactoferrin, secretory leukocyte protease inhibitor (SLPI), defensins, and cathelicidins. These molecules are produced by airway epithelial and inflammatory cells and are involved in many processes such as host defense, stimulation of adaptive immune response, and modulation of inflammatory response of lung disease [79]. Evidence for their role in lung disorder came from a number of recent studies describing the alteration of antimicrobial concentration in several pulmonary disease states [80]. Increased defensin levels were observed in cystic fibrosis, diffuse panbroncheolitis, idiopathic pulmonary fibrosis, acute respiratory distress syndrome, and lung transplant [81, 82]. Cystic fibrosis, a genetic disease associated with recurrent bacterial infections of the airways and inflammation, represents one illustration of the implication of AMPs in human pathologies. The defective chloride channel causing the disease increases salinity of the alveolar fluid and thus impairs the bactericidal activity against *P. aeruginosa* of β-defensins, which are salt

Table 1 Expression level and proposed functions of antimicrobial peptides/proteins in various human inflammatory diseases

Disease state	Peptides	Expression levels and potential functions
Skin inflammatory diseases		
Psoriasis	LL-37, defensins	Overexpressed, absence of *S. aureus* [27]
Atopic dermatitis	LL-37, defensins	Downregulated, presence of *S. aureus* [27]
Lupus, erythrematous, and contact dermatitis	LL-37	Increased [75]
Acne vulgaris	MX-594 AN	Inhibits *P. acne* [78]
	Granulysin	Kills *P. acne*, anti-inflammatory action [77]
Respiratory diseases		
Cystic fibrosis	LL-37, β-defensins	Reduced antimicrobial activity due to salt accumulation [7]
Periodontal disease	Defensins	Reduced in saliva of patients with oral candidiasis [142]
	LL-37	Absent in patients with congenital neutropenia [26]
	Histatin 5	Protects periodontium from bacterial infection [89] and prevents biofilm formation [90]
Inflammatory bowel disease		
Crohn's disease	HD5 and HD6	Deficient expression in Paneth cells
	HD5 and HD6	Reduced in CD patients with Nod2 mutation [91]
	LL-37	Expression is altered [91]
Ulcerative colitis	HD5, 6; hBD2–4	Upregulated in patients with UC [95]
Cancer	Magainin II	Toxic effect against cancer cell lines melanoma, breast and lung cancer, lymphoma, and leukemia [97, 98]
	Insect cecropins	Lyse tumour cells [100]
	Bovine lactoferrin	Inhibits lung and liver metastasis of murine melanomas and lymphomas [101] and cytotoxic toward neuroblastoma cells [103]
Atherosclerosis	Defensins	Involved in lipoprotein metabolism [111, 113], exhibit antifibrolytic activity [111], and regulate angiogenesis [115]
	LL-37	Increased expression in human lesions [118]
Inflammatory articular joints	hBD-3, LL-37	Upregulated in osteoarthritis [124]

sensitive [83]. In this context, overexpression of human LL-37 in lungs of mice inhibited bacterial load and inflammatory response after pulmonary challenge with *P. aeruginosa* [84]. In a further investigation, gene transfer approach restored the expression of LL-37 in a cystic fibrosis xenograft model and restored bacterial killing [85]. Overall, these peptides/proteins act as the first line of defense against pulmonary infection and may amplify the adaptive immune response by inducing the production of cytokines, which promote T cell-dependent cellular immunity and antigen-specific Ig production [86], or by enhancing both cellular (Th1-dependent) and humoral (Th2-dependent) cytokine production and immune responses [87]. Future studies should identify AMPS active against cystic fibrosis-associated pathogens including *P. aeruginosa*, *S. aureus*, and *Burkholderia cepacia*. Additionally, these peptides need to be selected or engineered to retain their activities at a high salt concentration.

AMPs and periodontal disease

Human periodontitis is a multifactorial inflammatory oral disease with a complex pathogenesis. It is initiated by a group of predominantly gram-negative anaerobic bacteria such as *Porphyromonas gingivalis*, *Bacteroides forsythus*, and *Actinobacillus actinomycetemcomitans*. One primary barrier to infection is the oral mucosa with its innate and adaptive immune processes. Disruption of this tissue can lead to infection by a variety of microbial challenges that can cause periodontal diseases. In line with this observation, patients with a severe congenital neutropenia have AMP deficiency, which is associated with the occurrence of infection and periodontal diseases [26]. A recent study has shown the expression of hBD-1 and hBD-2 peptides in gingival epithelia from periodontally healthy subjects, as well as patients with unsolved chronic periodontitis, suggesting a role of these peptides in periodontal health and disease [88]. Histatin 5, a salivary peptide, was reported to protect the periodontium from host and bacterial enzymes implicated in periodontal disease [89]. More recently, histatin 5 was shown to prevent biofilm formation, thereby reducing denture-induced stomatitis [90]. Thus, salivary AMPs could play a primary role in blocking of periodontal disease initiated by microbes. The knowledge gained from these and future studies will help identify the role of AMPs in periodontal pathogenesis and will provide support for the ongoing attempts to develop these peptides as drugs for the prevention of oral diseases.

AMPs and chronic inflammatory bowel diseases

In the small intestine, Paneth cells are the major source of AMPs that purportedly play a crucial role in intestinal immunity. A breakdown of this functional barrier may lead to aberrant production of defense molecules and consequently to IBD. IBD is a chronic inflammation of the intestinal mucosa often grouped into two major entities: Crohn's disease (CD) and ulcerative colitis (UC).

Crohn's disease is attributed in part to intestinal bacteria that may initiate and perpetuate mucosal inflammation in genetically susceptible individuals [91]. Studies of transgenic and knockout mice support a pivotal role of Paneth cell α-defensins in protection from bacterial pathogens. New data suggest that deficient expression of Paneth cell α-defensins (HD5 and HD6) may contribute to the pathophysiology of CD [91, 92]. These observations are supported by recent findings showing that mice lacking Nod2, an intracellular sensor of bacteria-derived muramyl dipeptide, fail to express cryptidins, equivalents of human α-defensins [93]. Similarly, human α-defensin expression is diminished in CD patients, particularly in those with Nod2 mutations [91]. Besides defensins, cathelicidin peptides also showed alteration of their expression profile in IBD [94].

Ulcerative colitis is considered a consequence of a failing adaptive and/or innate immune system, in particular AMPs, to cope with pathogenic microorganisms in the intestine. Its has been demonstrated that the expression of hBD-2 to -4 is upregulated in colonic enterocytes in patients with UC [95] and that HD5, HD6, and lysozyme are overexpressed due to metaplastic Paneth cell differentiation in UC colon [96]. Overall, these data provide compelling evidence supporting the hypothesis that AMPs play an important role in intestinal defense, and reduction in their expression may compromise host defense and initiate inflammatory diseases. The discovery of factors such as Nod2 [93] that have a direct impact on the expression of AMPs may contribute to development of future preventive and treatment strategies against IBD.

AMPs and cancer

Cancer treatment using classical chemotherapy approach presents a number of limitations, including toxicity and development of multi-drug resistance by cancer cells. Several reports have demonstrated that AMPs are emerging as a promising class of new natural drugs with toxic activity towards cancer cells. In this aspect, magainin II was shown to exert cytotoxic effect against a wide range of cancer cell lines including melanoma, breast and lung cancers, as well as lymphomas and leukemias [97, 98]. Further studies carried out in vivo have shown that magainin peptides improve survival of animals with ascites-producing tumors [97]. More recently, magainin II was reported to exert cytotoxic and antiproliferative efficacy by pore formation in bladder cancer cells but had no effect on normal murine or human fibroblasts [99]. Cecropins, insect-derived cationic peptides, were also found to be effective in lysis of tumor cells including multidrug-resitant tumor cell lines [100]. In contrast, bovine lactoferricin inhibited liver and lung metastasis of both murine melanomas and lymphomas [101] and induced apoptosis in human leukaemia and carcinoma cell lines [102]. In another study, Eliassen et al. [103] reported that lactoferricin B shows cytotoxicity to neuroblastoma cells in vitro and inhibits the growth of neuroblastoma xenograft in nude rats. Interestingly, lactoferricin seems to induce a rapid destabilization of tumor cell cytoplasmic membrane, triggering apoptosis cascades at a dose that does not affect normal fibroblasts or erythrocytes [104]. Moreover, α-defensins were localized to solid epithelial tumors, specifically oral squamous cell carcinoma, lung tumors, and renal cell tumors, suggesting an association of these peptides with cancer development [105–107]. Recently, an association between the overexpression of α-defensin peptides and bladder cancer invasiveness was reported [108]. However, a physiological approach involving animal models will help to better define the biological activity of these peptides toward cancer cells and to clarify the molecular mechanism by which these cells become susceptible to some of the AMPs. So far, at least three mechanisms have been proposed, (1) cell membrane lytic effect, (2) activation of intrinsic pathways of apoptosis via mitochondrial membrane disruption (reviewed in [109]), and (3) certain peptides are potent inhibitors of blood vessel development that is associated with tumor progression; extensive studies need to be performed to prove that these peptides do not destroy vital organs and have lower toxicity toward normal cells. If all these desirable properties are proven, then AMPs could be the next generation of drugs for cancer therapy. Hence, the development of a strategy that can be used to efficiently deliver apoptosis-promoting and/or anti-angiogenic peptides to both tumor cells and tumor vasculature should be another issue to take in consideration.

AMPs and the inflammatory process of atherosclerosis

Atherosclerosis is a complex and chronic inflammatory disease process characterized by the retention and modification of low-density lipoproteins within the wall of blood vessels [110] and the development of local inflammation event. AMPs produced by endothelial cells and neutrophils are present within atheromatous plaques in the cerebral and coronary circulation [111, 112]. The potential role of these peptides in the development of atherosclerosis, including

endothelium dysfunction, has been investigated. In this context, defensins were found to have multiple functions. They participate in the lipoprotein uptake and degradation in the vessel wall [111, 113]. They possess antifibrolytic activity [114] and regulate angiogenesis [115, 116]. Lately, animal studies have shown that neutrophil α-defensins cause endothelial cell dysfunction in porcine coronary arteries by reducing endothelium-dependent vasorelaxation [117]. Besides defensins, cathelicidins have also been investigated in the atherosclerosis development process. A sixfold increase in LL-37 transcripts in human atherosclerotic lesions compared with normal arteries was reported [118]. Using gene transfer approach, adenoviral PR-39 was reported to improve blood flow and myocardial function in a pig model of chronic myocardial ischemia by enhancing collateral formation [119]. Antimicrobial peptides/proteins have also been associated with an emerging global problem of obesity and diabetes, a complication that leads to increased burden of heart disease. In diabetic patients, an interesting theory on lactoferrin and lysozyme inactivation proposes specific binding of these proteins to glucose-modified proteins bearing advanced glycation end products [120]. These motifs inhibit endogenous bacterial proteins, thereby increasing susceptibility to bacterial infections in the diabetic population. However, a number of questions have not been answered convincingly. For instance, what is the biological significance of these findings? Is increased expression of these peptides beneficial or harmful to the vessel wall? Is there a link between AMP expression and modified lipids known as a major risk factor for the development of atherosclerosis? Because of the high prevalence of atherosclerosis and the resulting complications, clinical studies are needed to explain the significance of the above-mentioned findings.

AMPs and articular joints

For the last couple of years, AMPs have been investigated in articular joint inflammation including pyogenic arthritis, rheumatoid arthritis, and osteoarthritis. Their presence in human articular cartilage has been reported previously [121]. Further studies have revealed an alteration in the expression of these peptides in synovial membrane from patients with inflammatory joint disease [122], suggesting a role in the protection against microbial infection. However, the upregulation of hBD-3 and LL-37 in osteoarthritis without bacterial challenge has led scientists to believe that they have functions other than the ability to kill microbes. This hypothesis was confirmed later by experiments using animal model for osteoarthritis showing an induction of mouse AMPs in the pathogenesis of osteoarthritis in vivo [123, 124]. Of interest is the finding of a report by Varoga and his colleagues [125], showing overexpression of hBD-3 in osteoarthritis cartilage without bacterial challenge. The observed induction of defensins was attributed to their modulation by proinflammatory cytokines including TNF-alpha and IL-1, known to regulate AMPs [126, 127]. Together, these findings widen our knowledge of the functional spectrum of AMPs, particularly that of hBD-3, which is a multifunctional peptide with the ability to link host defense mechanisms and inflammation with tissue-remodeling processes in articular cartilage. A full knowledge of the exact function of these intrinsic peptides in articular cartilage and synovial membrane will provide further insight into prevention of human articular joint disease.

AMPs and injury, angiogenesis and wound healing

Angiogenesis is one of the complex biological processes of inflammation and wound repair [128]. For instance, when skin epithelial barrier function is breached as a result of injury, wound repair process requires both recruitment and coordination of numerous cell types including inflammatory cells. Neutrophils and macrophages are among cells that invade wound areas and constitute the major source of several angiogenic growth factors and AMPs such as LL-37, which exerts chemoattractant and angiogenic activity. In fact, the expression of LL-37 was reported to be increased in wounded skin [129]. The biological significance of this alteration is probably the protection of injured tissue until it is restored. Growth factors are of major importance in wound healing. Insulin-like growth factor I and TGF-alpha have been shown to induce the expression of the antimicrobial protein hCAP-18/LL-37 (human cationic antimicrobial protein of 18 kDa), hβD-3, neutrophil gelatinase-associated lipocalin, and SLPI in human keratinocytes [130]. In this context, mice deficient in SPLI have a deficiency in cutaneous wound repair that is associated with increased inflammation [131]. Another compelling evidence of the role of AMPs in wound repair comes from a study by Heilborn and his colleagues [132] showing that LL-37 peptide is involved in the re-epithelialization of human skin wound, and its reduction is associated with chronic ulcer epithelium. Another potential effect of AMPs is seen in burn wounds. In severely burned patients, there is a relative deficiency in certain AMPs, and these patients have greater risk of infection [133, 134]. A study by Steinstraesser et al. [135] investigated the in vivo antimicrobial activity of protegrin-1 using an animal burn model. This study showed a rapid decrease in bacterial counts compared to the controls infected in burn wounds.

Using in vitro and in vivo models for wound healing, Koczulla et al. [136] have identified another novel biologic activity of human LL-37, which consists of induction of angiogenesis and arteriogenesis. Interestingly, the porcine cathelicidin PR-39, has been shown to induce a robust

angiogenic response in in vivo and in vitro experimental models [137]. In conclusion, the multiple links among AMP expression, increased angiogenesis, and tissue repair involve a number of pathways, including those of toll-like receptors, inflammatory cytokines, and putative "danger" signals. Together, these complicated processes could have several implications, including protection of vulnerable tissue against infection, growth promoting activities, and epithelialization events.

Clinical development of antimicrobial peptides

The challenge at hand is to use current knowledge of AMPs in the design and development of useful drugs. In this respect, biotechnology firms have begun development of new peptide-based compounds that show different and potentially improved resistance profiles compared to early antibiotics. A comprehensive summary of AMP-based therapeutics under development has been published recently [11]. Representative examples of peptides going preclinical or clinical trials are discussed next:

- *Plectasin* (Novozymes) is a defensin peptide that shows good microbicidal activity against antibiotic-resistant bacteria such as those responsible for diseases like pneumonia [35]. Unlike other clinically tested AMPs, plectasin appears to be tolerated at high doses and shows efficacy in treatment of systemic infections. The development of plectasin as a therapeutic agent is currently entering preclinical phase with the anticipation that a product will be approved for clinical use in 8–10 years.
- *P-113* (Dermegen) is a 12-amino acid fragment derived from histatin 5 with anti-candidal activity comparable to that of the parent form. This peptide is in phase I/II clinical trials and is used as mouth rinse for treatment of plaques and gingivitis. Data from a human experimental model showed that P-113 is active against gingivitis and plaques [138].
- *MBI-226* (Migenix) is an Idolicidin analogue in phase III clinical trials. It will be used for the treatment of catheter-related blood stream infection [139].
- *MX-594AN* (Migenix) is an antimicrobial cationic peptide in development that has been formulated as topical treatment for mild to moderate acne vulgaris. MX-594AN is under clinical trials and appears to demonstrate efficacy against all types of acne lesion. Migenix has also developed MX-594AN under the name CLS001, which is tested for topical treatment of rosacea. Phase II trials are due to be completed in 2007.
- *PG-1 protegrin* (Intrabiotics) is a peptide in phase III clinical trial designed for the treatment of peritoneal infections caused by *P. aeruginosa, S. aureus*, and methicillin-resistant *S. aureus*.
- *rBPI-21* (Xoma) is a recombinant form of human bactericidal/permeability-increasing (BPI) protein that has reached phase II/III clinical trials and will be used for treatment of meningococcaemia and Crohn's disease.
- *Heliomycin* (EntoMed) is a peptide derived from insects with antifungal activity, currently in preclinical trials.

In summary, although several clinical trials underlined the broad therapeutic potential of AMP-based drugs, these agents are still at an early stage of technologic maturation, and many hurdles have yet to be overcome.

Future perspectives

Although no peptide has yet reached the drug market, pharmaceutical companies remain enthusiastic about the prospect of developing these novel agents as a new generation of medications. Additionally, researchers are now convinced that these natural peptides have functions beyond those of antimicrobial activities and represent an attractive target for the future design of therapeutic agents. The previous section has provided examples of the promising use of AMPs in the clinical setting. However, there are a few drawbacks to consider. As AMPs are of peptidic nature, they could present the following problems: (1) high manufacturing costs, (2) short half life, (3) lost of activity in physiological conditions [140], (4) application problems, (5) unwanted systemic reactions (aggregation, half-life, or immunoreactivity), and (6) interference with normal flora bacteria that may arise when trying to use those peptides as antibacterial agents [141]. Additionally, there remains a number of unresolved issues to consider: (1) Standardized techniques to assess the activity of these peptides is still unavailable. (2) Molecular regulation mechanisms of certain alpha-defensin genes are poorly defined. Detailed knowledge of how AMPs are regulated will allow researchers to create drugs that directly modulate the expression of specific AMPs to ameliorate the specific disease condition. 3) The ability to target these future medications to the site of disease remains a challenge. AMP gene therapy could be a promising approach to revolutionize treatment of many inflammatory and infectious diseases once strategies such as gene delivery are optimized. In this sense, one of the goals will be to design a system that simultaneously achieves high efficiency, prolonged gene expression, and low toxicity. However, as it was mentioned above, formidable challenges related to AMP properties are still to be faced. 4) Tolerance and toxicity issues of these

peptides need to be addressed, especially as their cationic property make them prone to interact with anionic components of the host cells. An ideal peptide drug would be one that can be tolerated at high dose, resists degradation, and is suitable for topical as well as intravenous administration. 5) Finally, understanding of the role and expression of AMPs both in health and disease remains a challenging area of research.

In conclusion, research that focuses on the above issues should provide further important insights into the pathophysiological roles AMPs play in complex diseases. This knowledge will lay the foundation for the use of these peptides as prototypes of innovative drugs and/or as potential sensors and biomarkers for early detection and prevention of diseases.

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