1-1-2002

Current Status of Defensins and Their Role in Innate and Adaptive Immunity

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Current status of defensins and their role in innate and adaptive immunity

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Abstract
Naturally occurring antimicrobial cationic polypeptides play a major role in innate and adaptive immunity. These polypeptides are found to be either linear and unstructured or structured through disulfide bonds. Among the structured antimicrobial polypeptides, defensins comprise a family of cysteine-rich cationic polypeptides that contribute significantly to host defense against the invasion of microorganisms in animals, humans, insects and plants. Their widespread occurrence in various tissues of these diverse organisms, and their importance in innate and adaptive immunity have led to their identification, isolation and characterization. A large volume of literature is available on defensins’ occurrence, structural characterization, gene expression and regulation under normal and pathological conditions. Much has also been published regarding their antimicrobial, antiviral and chemoattractive properties, and their molecular and cellular interactions. In this review, we describe the current status of our knowledge of defensins with respect to their molecular, cellular and structural biology, their role in host defense, future research paradigms and the possibility of their utilization as a new class of non-toxic antimicrobial agents and immuno-modulators.

Keywords: Antimicrobial agent, α- and β-defensin, Peptide disulfide, β-Sheet peptide, Immuno-modulator, Membrane permeabilizing agent, Amphiphilic molecule

Topics: immune response defensins peptides antimicrobials acquired immunity

Issue Section: Minireview

Introduction
Antimicrobial peptides are widely distributed in nature and represent an ancient mechanism of host defense. Among these naturally occurring antibiotic peptides, defensins form a unique family of cysteine-rich cationic and structured polypeptides with three or four disulfide bridges. Defensins are isolated from mammals, insects, and plants and they serve as effector molecules of innate immunity, providing an efficient initial defense against infectious pathogens.1,2 Mammalian and other vertebrate defensins are quite different from the arthropod defensins in their sequence and structure.3,4 Mammalian defensins comprise genetically distinct α- and β-
subfamilies of cationic tri-disulfide peptides. Most defensins appear to be expressed constitutively, whereas mediators of inflammation induce the biosynthesis of others. Most defensins exhibit remarkable antibacterial, antifungal and antiviral activities against a wide variety of microorganisms, suggesting their role in innate immunity. They also play a major role in adaptive immunity. It is now becoming clear that defensins constitute a primary defense system of the host. The activity of a few α-defensins as specific antagonists of adrenocorticotropic hormone has led to their classification as the corticostatin/defensin family. This review focuses on the current status of defensins in terms of their wide-spread occurrence, their molecular, cellular and structural biology, structure–function relationships, mechanism of their microbicidal activity and future research paradigms.

Occurrence, isolation, identification and classification
Defensins were first identified as a family of peptides in rabbit macrophages and subsequently in rabbit, rat, human and guinea-pig neutrophils, and they all belong to the α-defensin sub-class. Their primary structures, the sequence homology, the conserved cysteine residues and the disulfide linkages of representative α-defensins are provided in Fig. 1. α-Defensins are found in neutrophils of humans, rabbits, guinea pigs, rats, macaques and hamsters, in rabbit alveolar macrophages and in human and rodent small intestinal Paneth cells. β-Defensins were originally isolated as a family of 13 homologous peptides in bovine neutrophils. The folding of the peptide chains and the disulfide motifs (Fig. 2) are distinctly different from α-defensins. Subsequently, β-defensins were reported to be expressed in skin, pancreas, kidney, salivary glands, prostate, placenta, endocervix, and airway and gingival epithelial cells of vertebrates. α-Defensins consist of 29–35 amino acid residues and are shorter than β-defensins, consisting of 38–42 residues. α- and β-defensins differ in the location and position of the cysteine residues in the amino acid sequence and in their disulfide motifs. Two human β-defensins, HBD-1 and HBD-2, were structurally characterized and their presence in human skin, plasma, saliva, and in the urogenital tract have been identified. HBD-3 has been identified and its expression in adult heart, skeletal muscle, placenta, skin, esophagus, gingival keratinocytes, trachea and fetal thymus detected. A new and novel HBD-4 has recently been identified and its tissue-specific inducible and restricted expression in the testis, uterus, thyroid gland, lung and kidney during infection reported.
Amino acid sequences of α-defensins in human neutrophils (HNP), human intestinal Paneth cells (HD), rabbit neutrophils (NP) and rabbit kidney (RK). In HNP sequences, the different amino terminal residue is underlined. The conserved disulfide bridges in α-defensins are indicated in HNP-4. The conserved residues within a species are bold and underlined.
Amino acid sequences of β-defensins in human and bovine neutrophils (HBD and BNBD, respectively), and two Mediterranean mussel defensins (MGD). The conserved disulfide bridges in β-defensins are indicated in HBD-3. The conserved residues in human and bovine neutrophil β-defensins are bold and underlined. In Mediterranean mussel defensins, the residues that are not conserved are underlined. The disulfide motifs of various defensins are provided in the figure. pE: pyroglutamic acid.

Several defensins isolated from arthropods have been found to display interesting sequence homology to defensins isolated from Mediterranean mussels, even though they belong to markedly distinct phylogenetic groups. Defensins isolated from a variety of plants display high sequence identity with each other and their amphiphilic β-sheet structure resembles that of animal defensins shown in Fig. 3. Plant defensins with four disulfide linkages at highly conserved locations are designated as γ-thionins. Even though defensins with four disulfide bridges have been isolated and characterized from insects and plants, such defensins have not yet been identified in vertebrates. Most defensins are primarily linear polypeptides, folded and...
stabilized by three to four disulfide bonds. However, a defensin with the amino and carboxy terminals cyclized by a peptide bond has been recently isolated from primate leukocytes.19

A perspective view of the three-dimensional basket-like amphiphilic structure of NP defensin human neutrophil-3 deduced from the crystal structure [31]. The side-chains are not included for clarity. The yellow color indicates the disulfide bridge. The N- and C-terminal residues form the polar face at the top, whereas the middle region assumes the apolar face (the lower portion) of the amphiphilic structure. Differences in the sequence and in the folding pattern of mammalian, insect and plant defensins result primarily in the alteration of polarity and hydrophobicity of the polar and apolar faces, respectively, leading to variation in the amphiphilicity of the structure. This amphiphilicity variation reflects in defensins’ diversity in specificity and microbicidal potency.

Gene expression, regulation and biosynthesis
Increasing evidence suggests that human α- and β-defensins might descend from a shared ancestral gene. The genes encoding HBD-1 and HBD-2 have been assigned to human chromosome region 8p23 and 8p22-8p23.1, respectively, close to the α-defensins.12 The 5’-flanking region of HBD-1 contains nuclear factor (NF)-interleukin (IL)-6 and γ-interferon consensus sites, suggesting that inflammatory mediators could regulate its expression.14 The cDNA and genomic sequences of the two rabbit macrophage defensins are highly homologous and identical to rabbit neutrophil defensins NP1 and NP2, respectively. These genes are closely linked within 13 kb, suggesting that they evolved by a recent tandem gene duplication. The rabbit macrophage defensin mRNA is found in bone marrow and spleen and organs that contain immature polymorphonuclear leukocytes (PMNs).20 While matured PMNs lack defensin mRNA, the possibility of a certain degree of mRNA and protein synthesis in activated PMNs has not yet been ruled out.
α-Defensins are produced and stored as pre-propeptides in matured PMNs and Paneth cells. β-Defensins are constitutively expressed in the epithelial compartment and can be induced to higher levels of expression upon infection or inflammation. Metabolic labeling studies in the promyelocytic cell line HL-60 and in chronic myeloid leukemia cells indicate that human neutrophil pre-prodefensins are processed to mature defensins over 4–24 h via a 75-residue prodefensin generated by the cleavage of the signal sequence and a 56-residue prodefensin resulting from a subsequent proteolytic cleavage. The post-translational and enzymatic processing of pre-prodefensins to mature peptides with storage in cytoplasmic granules is a specialized feature of granulocytic lineage cells. The α-defensins in Paneth cells are also synthesized as pre-prodefensins, but they are secreted into the lumen of the small intestine where they are proteolytically cleaved into active antimicrobial peptides. The expression of human Paneth cell defensins HD-5 and HD-6 is found to be minimal in oral keratinocytes, whereas HD-5 mRNA is expressed infrequently and to varying degrees in bronchial and nasal epithelial cells. The expression of human α- and β-defensin mRNA in gastrointestinal epithelia has been found to be tissue- and peptide-specific, and the defensins are expressed with high inter-individual variability. Cloning and expression of bovine neutrophil β-defensins indicate that the precursor structures of α- and β-defensins are quite different. The structural differences of the two defensin families suggest that they could be stored and packaged by distinct intracellular pathways and mobilized differently from the respective cells.

β-Defensins are essentially synthesized in the epithelial compartment with the exception of bovine neutrophils. In epithelial cells, HBD genes are found to be both constitutively expressed and inducible. Gene expression has been shown to be induced in vitro by stimulation with bacterial lipopolysaccharide (LPS) as well as inflammatory mediators. In vivo, up-regulation of β-defensin genes has been shown to occur in both infectious and inflammatory states. Gene regulation appears to proceed via signal-transduction pathways utilizing NFs including NF-κB and NF-IL-6. The amino acid L-isoleucine and several of its analogs can specifically induce epithelial β-defensin expression involving the activation of the NF-κB/rel family of trans-activating factors, suggesting that isoleucine analogs may serve as immuno-stimulants to bolster the defense barrier of mucosal surfaces.

Characterization and transcriptional profiles of a *Drosophila* gene encoding an insect defensin suggest that insect and mammalian defensins might have evolved independently. Though it is commonly believed that insect defensin gene expression is only induced upon septic injury and infection, the constitutive expression of defensin peptides in insects has recently been observed. These results suggest two modes of defense against infection in insects. The first one is the transcription of the genes encoding defensin-like peptides, mainly in the fat body after septic injury, with a rapid release of the peptides into the hemolymph, and the second mode is the constitutive production and storage of the defensin peptides, particularly in hemocytes with subsequent release into the hemolymph after immune challenge.

Plant defensins also form part of the permanent and inducible defense barriers. In plants, both small and large cysteine-rich defensin genes are induced through different signal-transduction pathways during fruit ripening to protect the reproductive organs against biotic and abiotic
stress. These data suggest that defensins are constitutively and inducibly expressed across the phylogenetic spectrum.

Defensins – key components of innate and adaptive immunity
The presence of defensins in lower organisms clearly indicates their ancient origins, and argues that they developed as a part of a primordial immune protective mechanism. Defensins exhibit broad-spectrum antimicrobial activity against bacteria, fungi, mycobacteria and enveloped viruses. Constitutive expression of defensins provides a first line of defense against colonization by pathogens. Normal gingival epithelial cells express β-defensins, indicating their importance in host–pathogen interaction at the oral mucosal barrier and their relevance in oral health. The deficiency of neutrophil defensins has been associated with the risk for invasive bacterial infections observed in the newborn. Mucoid *Pseudomonas aeruginosa*, tumor necrosis factor (TNF)-α and IL-1β, but not IL-6, induce HBD-2 expression in respiratory epithelia, suggesting the protective role of HBD-2 in lung infection caused by mucoid *P. aeruginosa*. Defensins can also activate the classical complement pathway, and they have the potential to modify the inflammatory response through the regulation of cytokine production and adhesion-molecule expression. All these observations suggest a key role for defensins in innate immunity.

Evidence is accumulating to support the role of defensins in the activation and in the recruitment of the cells and machinery of the adaptive immune response. Defensins provide a critical link between the innate immune system, which is phylogenetically ancient, and the adaptive immune response that is found only in vertebrates. The innate immune system relies on germline-encoded receptors on epithelial cells and phagocytes as sentinels of the host–environment boundary. These pattern-recognition receptors (PRRs) recognize conserved pathogen-associated molecular patterns (PAMPs) such as LPS, peptidoglycan, zymosan and possibly isoleucine. These PRRs initiate an appropriate antimicrobial response to try to contain infection, which involves the up-regulation of specific antimicrobial peptides including defensins. The second major task for the innate immune system is to activate an appropriate adaptive immune response against the invading organism. Recent data suggests that defensins may play a key role in the recruitment and activation of the appropriate adaptive effector response. Examples of the stimulatory effects of defensins on humoral and cell-mediated immune elements illustrate this connection.

Human α-defensins are chemotactic to monocytes, dendritic and T-cells at ~10^{-10} M concentration, suggesting their importance in shaping the adaptive immune response. β-Defensins are chemotactic for dendritic and memory T-cells through the chemokine receptor CCR6. In a murine model, human neutrophil defensins delivered intra-nasally with ovalbumin have been shown to enhance the systemic adaptive immune response by increasing antigen-specific IgG and IgM levels in serum. β-Defensin gene expression in vertebrate epithelia, including that of humans, and their up-regulation in the presence of bacterial LPS and TNF-α emphasize that these molecules might function to protect the host against microbial pathogenesis at the critical confrontation sites. These studies provide convincing evidence that defensins play a key role in directing and augmenting an adaptive immune response.
Defensins as antimicrobial and antiviral agents

Defensins exhibit remarkable antibacterial, antifungal and antiviral activity against a wide variety of microorganisms, as established by in vitro studies.\textsuperscript{2,8} The in vitro minimal inhibitory concentrations against a panel of microorganisms range between $\sim 0.5$ and 10 $\mu$M for most defensin peptides.\textsuperscript{8} In vitro studies, however, do not mimic the in vivo environment, where inflammatory exudates, phagosomes, ions, serum factors and proteases might alter the efficacy of these peptides. Hence, the microbicidal efficacy observed for defensins cannot be directly related to in vivo applications. However, defensin concentration in vivo might reach significant levels at the site of production and secretion (5 $\mu$g/10$^{-6}$ human granulocytes). Neutrophil defensin concentration has been elevated by 500–10 000 fold in bronchoalveolar lavage of cystic fibrosis (CF) individuals. The concentration of human neutrophil peptide (HNP-1) in the saliva of patients with oral diseases and oral inflammation are found to be significantly higher than those in healthy subjects.\textsuperscript{27} The local concentration of defensins has been estimated to be in the mg ml$^{-1}$range in human gingival crevicular fluid. These observations suggest that defensins are present in vivo in significant amounts that are likely to have major effects on the microbiology of the host. In addition to their high concentration, the microbicidal activity of defensins in vivo could be enhanced due to the synergistic interaction with other proteins such as lactoferrin and cathelicidins. Defensin and lactoferrin levels are found to be elevated in the cerebrospinal fluid of children with meningitis.\textsuperscript{8} The synergistic action of defensins with other host-protective proteins is likely to be relevant in the host–pathogen interaction in vivo.

Defensins exhibit remarkable antiviral activity against recombinant adeno-associated virus and Herpes simplex virus. Direct binding of defensins appears to prevent envelope virus infectivity.\textsuperscript{1,28}

Mechanism of antimicrobial activity

The antibacterial activity of defensins is generally ascribed to their effects on microbial membranes. Defensin-like peptides, being positively charged, interact with negatively charged components of microbial membranes that include LPS in Gram-negative bacteria, polysaccharides (teichoic acid) in Gram-positive bacteria, and phospholipids (phosphotidylglycerol). Since these membrane components are PAMPs that are not found in mammalian cells, defensins in general appear to be electrostatically specific for prokaryotic cells \cite{5}. Human defensin HNP-1 has been shown to mediate bactericidal activity against \textit{Escherichia coli} by permeabilizing the outer and inner membranes. An insect defensin with $\beta$-sheet structure has been reported to permeabilize the cytoplasmic membrane of \textit{Micrococcus luteus}. Defensins in \textit{M. luteus} cells cause depolarization of cytoplasmic membrane, inhibition of respiration, and loss of cytoplasmic potassium and ATP, suggesting that defensins target the bacterial cytoplasmic membrane. All research regarding modes of action of antibiotic peptides has been carried out with lipid model planar bilayer membranes or small unilamellar vesicles formed from lipid mixtures of various compositions of negatively charged to neutral lipids.\textsuperscript{5,8} These studies have led to the hypothesis that in Gram-positive bacteria, the monomeric peptides aggregate to form multimeric pores in the cell's single cytoplasmic membrane. In the case of Gram-negative bacteria, defensins have to traverse the outer membrane. The high affinity of defensins for LPS facilitates competitive displacement of divalent cations (Ca$^{2+}$ or Mg$^{2+}$) that
serve as a bridge between the LPS molecules.\textsuperscript{29} The initial interaction of defensin with a target membrane causes rapid permeabilization of the inner membrane in \textit{E. coli}. A small defensin-like dodecapeptide with a disulfide bridge permeabilizes the outer membrane of \textit{P. aeruginosa} and \textit{Salmonella typhimurium} and this membrane activity appears to correlate with its microbicidal activity. This peptide also interacts with the cytoplasmic membrane of an outer membrane-defective mutant \textit{E. coli}. However, a recent study of cationic antimicrobial peptides of varying structures with a planar bilayer and with the cytoplasmic membrane of \textit{E. coli} suggests the possibility that the cytoplasmic membrane is not the target for some or even most cationic antimicrobial peptides. Stimulation of autolytic enzymes, interference with bacterial DNA and/or protein synthesis, inhibition of DNA synthesis leading to filamentation, or binding to and inhibition of cellular nucleic acids have been suggested as the possible mechanism of action. Interaction with cellular nucleic acids has been suggested as a credible alternative mechanism. Increasing evidence indicates that the antimicrobial mechanism and the cytoplasmic target could vary with the primary sequence and the amphiphilicity of the antimicrobial peptide.\textsuperscript{30}

Electrostatic interactions between defensins and the target cell membrane ruling out any specific membrane-bound receptor could account for the rare occurrence of resistant bacterial strains. However, specific binding sites for plant defensins on fungal cells are required for antifungal activity. The resistant strains of \textit{S. typhimurium} and \textit{Staphylococcus aureus} have been reported to have modified membrane structure involving LPS and teichoic acid, respectively, resulting in the neutralization of electrostatic charge, thereby minimizing the interaction with defensin peptides. These studies provide convincing evidence that defensins’ initial interaction is with the LPS and teichoic acid of Gram-negative and Gram-positive bacteria, respectively. This is also consistent with the role of defensins in innate immunity and a possible adjuvant role in adaptive immunity. The emergence of resistant strains may have implication directly on the antimicrobial activity and/or on the efficacy of defensins to activate the immune system. Further research in this direction is required to clarify the function of defensins under these circumstances.

Defensins are expected to be minimally active on host-cell membranes. Their selective toxicity is associated with the anionic phospholipid of microbial membranes. In contrast, zwitterionic phospholipids and the presence of cholesterol in mammalian cell membranes partly account for the minimal cytotoxicity to host cells.\textsuperscript{8} However, in vitro studies indicate that human defensins can have lytic effects on human lymphocytes and endothelial cells as well as murine lymphoid cells. The cytolytic mechanism involves an early step of membrane binding, followed by a series of cytoskeletal-dependent events that are sensitive to concentration, energy, and temperature. The first stage of membrane binding in the defensin-mediated cytotoxicity is inhibited under conditions of low temperature and by the presence of serum and heparin.\textsuperscript{8} The tolerance of human erythrocytes, epithelial and endothelial cells to defensins at physiological concentrations, temperature, pH and ionic strength has not yet been systematically established. These studies would provide invaluable information for defensins’ application in pharmaceutical industries.
Structure–function correlations

The sequence homology is more than 90% conserved for defensins isolated from a specific tissue within a given species. For instance, human neutrophil α-defensin sequences are conserved except for one amino acid at the N-terminus. Rabbit neutrophil defensins mostly differ only at the C- and N-terminal residues. Similarly, bovine neutrophil β-defensin sequences exhibit high sequence homology (Fig. 2). The size and sequence homology of defensins vary to a greater extent when compared with those isolated from different biological sources. The sequences of human and rabbit neutrophil defensins differ widely (Fig. 1). The minor variations within a family of defensins affect significantly their specificity and microbicidal potency. HNP-1 is a potent antifungal agent against *Candida albicans*, whereas HNP-3 has minimal activity. These two defensins differ only in one amino acid at the N-terminus (Fig. 1). Similarly, HNP-2 is significantly less active against *C. albicans* as compared with HNP-1. The only difference between these two molecules is one alanine residue at the N-terminus (Fig. 1). Interestingly, human defensins HNP-1 and HNP-2 are equipotent in their cidal activity against *Capnocytophaga* species, and their activity is significantly greater than that of HNP-3. Similar variations in the bactericidal activity of rat defensins have also been observed. This wide functional diversity observed in the family of similarly structured defensins is clearly a consequence of minute alterations in sequence, chain length, backbone conformation and side-chain topography.

The three-dimensional structures of a few α-defensins reported in the crystalline state and in solution\(^4,\)\(^3\)\(^1\) indicate amphiphilic triple-stranded β-sheet structures (Fig. 3). The N- and C-terminal residues form the polar face, and the middle portion of the sequence constitutes the apolar face of the amphiphilic structure (Fig. 3). The polar and apolar faces of the β-sheet structure show distinct differences in the amphiphilicity of α-, β-, plant, and insect defensins. These variations presumably account for their diversity in specificity and antimicrobial activity. The residues at the polar face are found to alter significantly the microbicidal potency and specificity of human neutrophil defensins.\(^3\)\(^2\) This was demonstrated by the observation that the Gram-negative bacteria *Actinobacillus actinomycetemcomitans* and *Porphyromonas gingivalis* and the Gram-positive bacteria *Streptococcus gordonii* and *Streptococcus mutans* are insensitive to human neutrophil defensins. However, these same microbes are susceptible to rabbit neutrophil defensins that differ significantly from human neutrophil defensins. Analogs of human neutrophil defensins incorporating two additional cationic residues at the N- and C-terminals have been shown to exhibit high microbicidal potency against these organisms, suggesting the importance of the N- and C-terminal residues for microbicidal activity. The enhanced microbicidal activity observed for defensin analogs could be due to optimization of the amphiphilicity of the structure, facilitating specific interactions with microbial membranes.\(^3\)\(^2\)

The structure of HBD-2 reported previously suggests that its dimeric structure is distinctly different from human α-defensins. The higher-order octamer of HBD-2 supports an electrostatic charge-based membrane permeabilization mechanism rather than that based on formation of bilayer-spanning pores.\(^3\)\(^3\) The structure of bovine neutrophil β-defensin has been shown to be a triple-stranded β-sheet, the β-bulge preceding the hairpin turn and the
amphiphilic character. The structural features are consistent with those of other β-defensins.
The three-dimensional structure of defensins has been used to design simple analogs that mimic the structure of native molecules. The constructed analogs have been found to elicit microbicidal potency comparable to that of native defensins.\textsuperscript{34} The microbicidal potency of small molecules seem to support the electrostatic charge-based membrane permeabilization mechanism. It appears that the amphiphilic structure of defensins stabilized by three or four disulfide bridges is critical for membrane permeabilization and antimicrobial activity.

Defensins and infectious diseases

In vitro studies suggest that bacterial challenge causes an increase in defensin synthesis and secretion by epithelial cells. In vivo studies also indicate elevated defensins concentration in plasma, blood, and body fluids from patients with bacterial infection, suggesting defensins’ physiological significance in infection. Interestingly, microbial infection does not seem to be a problem in the skin disorder, psoriasis. This could be due to the fact that psoriatic epithelium up-regulates the expression of a cathelin-class antimicrobial peptide, designated as LL-37, and HBD-2 as compared to that of normal skin.\textsuperscript{5} The synergistic activity of these molecules may account for the rare occurrence of infection in this skin disorder.\textsuperscript{8}

Cystic fibrosis (CF) patients (on the other hand) are highly susceptible to upper-respiratory infection by bacterial and fungal pathogens. This is the result of a defective tracheal epithelial cell gene product, namely, the CF trans-membrane conductance regulator that causes elevated levels of Na\textsuperscript{+} and Cl\textsuperscript{−} ions in the airway surface fluid. The high cationic concentration associated with the airway surface fluid in CF patients appears to inactivate β-defensins, thereby enhancing microbial colonization, leading to inflammation and tissue destruction.\textsuperscript{5,8} It appears that only the monovalent and divalent cations inactivate defensins and not the anions. Since defensins’ initial interaction with microorganisms involves electrostatic forces, the altered ionic environment might compromise the initial interaction of defensins with microbial membranes.

Periodontitis is one of the most common chronic infectious diseases of humans and is a major cause of tooth loss. This oral disease is strongly associated with the formation of a predominantly Gram-negative anaerobic biofilm in the gingival crevice or periodontal pocket. \textit{P. gingivalis} and \textit{A. actinomycetemcomitans} are the most well-documented periodontal pathogens having multiple virulence factors that could circumvent normal host-defense mechanisms. In healthy individuals, the secretion of β-defensins from oral epithelium and the constant influx of neutrophil α-defensins along with other salivary defense molecules serve as an efficient barrier against the formation of a pathogenic sub-gingival plaque or biofilm. The disruption of this barrier can lead to the progression of periodontal disease. \textit{P. gingivalis} uses a mechanism known as chemokine paralysis to inhibit the expression of IL-8 by gingival epithelial cells, which may indirectly decrease the defensin barrier by inhibiting neutrophil emigration.\textsuperscript{35} Bacterial DNA can also down-regulate the production of human defensins in epithelial cells. Moreover, studies have demonstrated that \textit{P. gingivalis} can survive the non-oxidative killing mechanisms of PMNs, which may be of greater importance in the anaerobic environment of the periodontal pocket. This finding may be in part due to the lack of microbicidal activity of human
neutrophil α-defensins against oral pathogens including *P. gingivalis* and *A. actinomycetemcomitans*.32

Another mechanism that may be very important in the host–pathogen interaction, particularly in the periodontium, is the direct deactivation or clearance of defensins by substances such as dermatan sulfate and α 2-Macroglobulin. Approximately 60% of the glycosaminoglycan content of healthy human gingiva is made of dermatan sulfate, which has recently been shown to bind to defensins and completely neutralize their microbicidal activity.36 Similarly, defensin levels may be decreased by activated α 2-macroglobulin. α 2-Macroglobulin is the major serum protease inhibitor, which upon activation, will bind to defensin peptide.37 In the environment of the periodontal pocket, bacterial tissue invasion and high levels of protease activity could cause increased levels of activated α 2-macroglobulin. The defensin–α 2-macroglobulin interaction may constitute an important mechanism in the host–pathogen interaction that warrants further investigation.

Additional regulatory functions

Human α-defensins inhibit fibrinolysis by modulating tissue-type plasminogen activator and plasminogen binding to fibrin and endothelial cells, which could have significant implications in thrombotic pathology. In pathological conditions, defensins released in the circulation might adhere to the endothelium and their presence in the vascular tissues could contribute to the pathological consequences of inflammation. In vitro studies have shown that neutrophil defensins interact with ACTH receptors, inhibiting ACTH-induced steroidogenesis. Neutrophil defensins induce histamine secretion from mast cells mediated by a G-protein-dependent response, which is distinctly different from antigen-IgE-mediated activation.8 α-Defensins are found to induce proteoglycan-dependent catabolism of low-density lipoprotein (LDL) by vascular cells, leading to a new class of inflammatory apolipoprotein, suggesting that they could possibly contribute to atherogenesis.8 They also regulate secretory leukocyte protease inhibitor and elafin release from bronchial epithelial cells, indicating their role in the dynamic regulation of the antiprotease secretion in the lung at the site of inflammation. Defensins increase proliferation of epithelial cells, suggesting their involvement in wound healing.38 These important biological functions establish that defensins are multifunctional cell-effector molecules in addition to their role in integrating the innate and adaptive immune responses.

Future paradigms and summary

Increasing evidence unambiguously establishes that defensins play a major role in adaptive and innate immunity. However, less research has been focused on the pharmaceutical application of defensins as an alternative to circumvent microbial adaptive resistance and toxicity associated with conventional antibiotics. Though defensins have been suggested as therapeutic agents for several infectious diseases, the development has been hampered by difficulties and the expenses involved in their large-scale production. However, large-scale synthesis of human neutrophil defensins and their analogs has recently been reported.32 Recombinant technology for the production of defensins is yet to emerge, though there are a few reports that describe the synthesis of small amounts of defensins. Interestingly, it has been demonstrated that defensin gene transformation and its expression impart disease resistance in plants, which is
equivalent to the conventional fumigation practice. Similar research in humans will be invaluable to control or prevent infectious and inflammatory diseases. The toxicity of naturally occurring defensins to human cells at physiological conditions has to be thoroughly examined for pharmaceutical applications.

The studies on the structural biology of defensins have identified simple amphiphilic molecules that mimic the structure of defensins and elicit similar antimicrobial potency.\textsuperscript{34} Peptide detergents can be generated by alteration of amphiphilicity of these simple molecules. These detergents could selectively lyse microbial membranes and target infectious pathogens. As mentioned earlier, high ionic strength of the airway fluid associated with CF leads to the impairment of β-defensins’ activity. However, a cyclic defensin, wherein amino and carboxy terminals are cyclized by a peptide bond, has been reported to be active even at high ionic strength.\textsuperscript{19} This suggests a novel approach to overcome the salt-sensitive deactivation of defensins in CF patients. Research should focus on this class of cyclic defensins for pharmaceutical applications when the natural defense barrier is compromised in a pathological state. Structure–function studies could identify the functional domain for defensins’ chemotactic activity to monocytes and other adaptive immune cells. Such studies might facilitate the design and synthesis of simple molecules that could activate or direct the adaptive immune response, and serve as immuno-modulators.

The interaction between activated α 2-macroglobulin and defensin peptides is an undeveloped area that warrants further investigation. With the observations that α 2-macroglobulin functions as an extra-cellular chaperone as well as a major serum protease inhibitor, the interplay between defensins and α 2-macroglobulin becomes more intriguing. Defensins appear to be recycled in vivo by activated α 2-macroglobulin via receptor-mediated endocytosis. Recycling may occur through either the LDL receptor-related protein, or CD91, the heat shock protein receptor. Since CD91 plays a role in antigen presentation, and α 2-macroglobulin acts as a T-cell adjuvant, one could speculate that defensins might aid in the delivery of non-self lipoprotein or glycolipid antigens and help direct the adaptive immune response accordingly. Further research in this area might clarify the precise role of α 2-macroglobulin in the activity of defensins.

In summary, this review has attempted to describe the current status of defensins as natural barriers at the host–microbe interface, linking adaptive and innate immunity. The current knowledge of defensins envisages the possibility of their utilization as a new class of natural antibiotics to overcome microbial adaptive resistance to existing conventional antibiotics. They might serve as immuno-modulators to activate the immune system suppressed by infection and inflammation.

**Acknowledgements**
The authors would like to apologize for not citing many articles on defensins in this MiniReview primarily because of the publisher’s page limitations. The authors would like to thank Ms. Kavitha J. Antonyraj, Georgetown University, Washington, DC, USA, for critically reading the manuscript.
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