Cell. Mol. Life Sci. 63 (2006) 1294–1313
1420-682X/06/111294-20
DOI 10.1007/s00018-005-5540-2
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Cellular and Molecular Life Sciences

Review

Human β-defensins

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Received 9 November 2005; received after revision 7 February 2006; accepted 15 March 2006
Online First 15 May 2006

Abstract. The last decade led to the discovery and characterization of several human β-defensins. Analysis of genomic information indicates that the number of β-defensin-like molecules encoded by the human genome may number in the tens. Growing interest in β-defensins steadily enhances our knowledge about various aspects of their gene location, expression patterns and the transcription factors involved in their regulation *in vivo*. The hallmark property of β-defensins, their antimicrobial activity, is clearly only the tip of the iceberg in the extensive network of inter-relations within the immune system in which these peptides function. Structural studies of β-defensins provide the molecular basis for a better understanding of their properties, functions and their potential for practical applications. In this review, we present some recent advances in the studies of human β-defensins, with an emphasis on possible correlations between their structural and functional properties.

Keywords. Human β-defensin, innate and adaptive immunity, antimicrobial and chemotactic activity, β-defensin structure, structure-function relationship.

Introduction

Antimicrobial peptides (AMPs), a diverse group of small molecules (over 800 examples are recognized to date [1]), are classified into several categories based on their primary structures and topologies [2–6]. In humans, one important category of AMPs are defensins [7]. This category encapsulates the group of β-sheet-rich, cationic and amphipathic peptides, usually consisting of six invariant cysteine residues forming characteristic networks of disulfide bridges that assume a conserved structural fold [8–10]. Defensins were identified in many multicellular organisms, including plants [11, 12], invertebrate [13] and vertebrate animals [14], as well as in insects [15]. Over 40 open reading frames (ORFs) with nucleotide sequences bearing signatures defined for defensins have been identified in the human genome [16]. Protein products were confirmed *in vivo* for only 10 of those ORFs, whereas corresponding messenger RNA (mRNA) was identified for an additional 11 sequences (Table 1). Based primarily on the spacing between the cysteine residues and the topology of the disulfide bridges, human defensins are organized into three classes, α-, β- and θ-defensins [10, 17]. It is necessary to mention the provisional nature of the θ-class (the cyclic defensins). The only primate θ-defensin described so far is one isolated from rhesus mon-

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Table 1. Human β-defensins: gene localization and the expression sites[1].

Gene symbols (previous or aliases)	Gene location	Official product of the gene (other designation)	Expression sites	References
DEFB1 (*BD1, HBD1, DEFB-1, DEFB101, MGC51822*)	8p23.2-p23.1	human β-defensin 1 (hBD-1)[2]	the epithelial cells of respiratory and urogenital tract, trachea, uterus, pancreas, kidney, lung, prostate, placenta, thymus, testis, vagina, ectocervix, endocervix, fallopian tubes, gingival tissue, buccal mucosa and tongue, salivary gland, small intestine, conjunctiva, cornea, lacrimal gland, mammary gland, limb joints, astrocytes, microglia, meningeal fibroblast	[37–48]
DEFB4 (*DEFB2, DEFB102, HBD2, DEFB-2, SAP1*)	8p23.1-p22	human β-defensin 2 (hBD2)[2]	skin, oral and pulmonary epithelia, conjunctiva, cornea, astrocytes, gut epithelia, epidermal and gingival keranocytes	[38–41, 47, 49–56]
DEFB103 (*HBD-3, HBP3, DEFB3, HBD-3, HBP-3, DEFB103A*)	8p23	human β-defensin 3 (hBD-3)[2]	oral, respiratory, gastrointestinal, urinary epithelia, fetal thymus, placenta, testis, esophagus, heart, neutrophils, trachea, skeletal muscle, jejunum, tonsils, skin	[38, 40, 57, 58]
DEFB104 (*DEFB4, DEFB-4, DEFB104A*)	8p23	human β-defensin 104 (hBD-4)[2]	testis, epididymis, gastric antrum, uterus, neutrophilis, thyroid gland, lung, kidney, gingival tissues and primary keratinocytes	[59–61]
DEFB105 (*BD-5, DEFB-5, DEFB105A*)	8p23-p22	human β-defensin 105 (hBD-5)[3]	epididymis and testis	[16, 60, 62]
DEFB106 (*BD-6, DEFB-6, DEFB106A*)	8p23-p22	human β-defensin 106 (hBD-6)[3]	epididymis and testis, lung, airway serous cell of submucosal glands and nongoblet cells	[16, 60, 62–64]
DEFB107 (*DEFB-7, DEFB107A*)	8p23-p22	human β-defensin 107 (hBD-7)[3]	gingival tissues and keratinocytes	[16, 62, 63]
DEFB108 (*DEFB-8, DEFB108, DEFB108A*)	8p23-p22	human β-defensin 108 (hBD-8)[3]	gingival tissues and keratinocytes, testis, pancreas	[16, 62–64]
DEFB109 (*DEFB-9, DEFB109, DEFB109A*)[4]	8p23-p22	human β-defensin 109 (hBD-9)[3]	gingival tissues and keratinocytes, heart, brain, placenta, lung, liver, kidney, pancreas, spleen, thymus, prostate, testis, ovary, small intestine, leukocyte	[16, 62–64]
DEFB110 (*DEFB-10*)	6p21	human β-defensin 110 (hBD-10)[4]		[16, 63]
DEFB111 (*DEFB-11*)	6p21	human β-defensin 111 (hBD-11)[3]	gingival tissues and keratinocytes	[16, 63]
DEFB112 (*DEFB-12*)	6p21	human β-defensin 112 (hBD-112)[3]	gingival tissues and keratinocytes	[16, 63]
DEFB113 (*DEFB-13*)	6p21	human β-defensin 113 (hBD-13)[4]		[16]
DEFB114 (*DEFB-14*)	6p21	human β-defensin 114 (hBD-14)[3]	gingival tissues and keratinocytes	[16, 63]

Table 1. (Continued).

Gene symbols (previous or aliases)	Gene location	Official product of the gene (other designation)	Expression sites	References
DEFB115 (*DEFB-15*)	20q11.1	human β-defensin 115 (hBD-15) [5]		[16]
DEFB116 (*DEFB-16*)	20q11.1	human β-defensin 116 (hBD-16) [5]		[16]
DEFB117 (*DEFB-17*)	20q11.1	human β-defensin 117 (hBD-17) [5]		[16]
DEFB118 (*DEFB-18, ESC42, dJ1018D12.3*)	20q11.1-q11.22	human β-defensin 118 (hBD-18, ESC42) [2]	testis and epididymis, sperm, pancreas	[16, 64–67]
DEFB119 (*DEFB-19, DEFB-20, DEFB120, ESC42-RELA, ESC42-RELB, MGC71893*)	20q11.1	human β-defensin 119 (hBD-19, HBD-20) [3]	testis and epididymis	[16, 66]
DEFB120 (*DEFB-19, DEFB-20, DEFB120*)	20q11.1	human β-defensin 120 (hBD-20) [3]	testis and epididymis	[16, 66]
DEFB121 (*DEFB-21, ESC42-RELC*)	20q11.1	human β-defensin 121 (hBD-21) [3]	testis and epididymis	[16, 66]
DEFB122 (*DEFB-22*)	20q11.1	human β-defensin 122 (hBD-22) [4]		[16, 66]
DEFB123 (*DEFB-23, ESC42-RELD*)	20q11.1	human β-defensin 123 (hBD-23) [3]	testis and epididymis	[16, 66]
DEFB124 (*DEFB-24*)	20q11.1	human β-defensin 124 (hBD-24) [4]		[16]
DEFB125 (*DEFB-25*)	20p13	human β-defensin 125 (hBD-25) [3]	epithelial cell layer of the testis and epididymis, skeletal muscle, kidney, liver	[16, 68]
DEFB126 (*DEFB-26, DEFB26, ESP13.2, bA530N10.1, hBD-26*)	20p13	human β-defensin 126 (hBD-26, ESP13.2) [3]	epithelial cell layer of the testis and epididymis, pancreas, skeletal muscle, heart, prostate, brain	[16, 68, 69]
DEFB127 (*DEF-27, DEFB-27, DEFB27, bA530N10.2, hBD-27*)	20p13	human β-defensin 127 (hBD-27) [2]	epithelial cell layer of the testis and epididymis, kidney, liver, pancreas, skel-etal muscle, heart, lung	[16, 68]
DEFB128 (DEFB-28)	20p13	human β-defensin 128 (hBD-28) [2]	epithelial cell layer of the testis and epididymis	[16, 68]
DEFB129 (*DEFB-29, DEFB29, bA530N10.3, hBD-29*)	20p13	human β-defensin 129 (hBD-29) [3]	epithelial cell layer of the testis and epididymis, skeletal muscle	[16, 64, 68]
DEFB130 (*DEFB-30*) [6]		human β-defensin 130 (hBD-30) [4]		[16]
DEFB131 (*DEFB-31*) [6]		human β-defensin 31 (hBD-31) [3]	testis, prostate, small intestine	[16, 64]
DEFB133 (*DEFB-33*) [6]		human β-defensin 33 (hBD-33) [4]		[16]

[1] The symbols of genes and products of the genes are presented according to the recent recommendations on the β-defensin gene nomenclature. (http://www.pnas.org/cgi/content/full/222517899/DC1).
[2] Protein characterized, based on naturally occurring or synthetic preparation.
[3] Expression of the protein product detected on the transcriptional level by either RT-PCR or mRNA hybridization.
[4] Pseudogene, expression of mRNA detected but its sequence codes for a non-functional protein.
[5] Putative protein, deduced from the genomic sequence.
[6] Chromosomal localization is not clearly established.

key leucocytes [17]. Although at least six genes coding for θ-defensins have been found in the human genome, premature stop codons in the genes abort translation and subsequent peptide production [17–20].

In this overview, we intend to highlight the most recent results from studies of human β-defensins. Many excellent reviews on human defensins, including β-class, have already been published [5, 14, 21–32], and avoiding some redundancy is impossible. However, the increasing knowledge of the structural properties of human defensins gathered during last several years has not been reviewed proportionally. We attempt to fill this gap by emphasizing recent advancements in the area of structural studies of human β-defensins.

Classification

The first reported β-defensin, the tracheal antimicrobial peptide (TAP) [33], was a peptide with antimicrobial properties isolated from cow tongue. The assignment of TAP to the defensin family was based on its functional and structural properties, similar to those described earlier for several neutrophil peptides (NPs, referred to as α-defensins) [34]. The spacing between the cysteine residues and the topology of the disulfide bonds in TAP were, however, different compared with α-defensins. Whereas six cysteine residues in α-defensins form the disulfide bonds with the topology Cys^1-Cys^6, Cys^2-Cys^4 and Cys^3-Cys^5 (or 1–6, 2–4, 3–5), the connectivity found in TAP has the topology 1–5, 2–4, 3–6. Comparison of the primary structures for β-defensins from various sources defines them as peptides consisting, in the mature form, of 36 or more amino acid residues with an identifiable consensus sequence: $x_{2-10}\mathbf{C}x_{5-6}(G/A)x\mathbf{C}X_{3-4}\mathbf{C}x_{9-13}\mathbf{C}x_{4-7}\mathbf{CC}x_n$, (where x is any amino acid) [35].

Gene distribution and organization

The first human β-defensin, hBD-1, was identified in 1995 and purified from the plasma of patients with renal disease [36]. Since then, several other human β-defensins have been isolated, or their coding nucleotide sequences have been identified by genome analysis (Table 1, Fig. 1). Human β-defensin 2 (hBD-2) was originally isolated from psoriatic skin lesions [70], whereas hBD-3 was nearly simultaneously purified from psoriatic scales [57, 58] and predicted using bioinformatics [71]. The original reports on hBD-4 through hBD-6 were based solely on genome analyses [59, 60].

Recent, comprehensive searches of the human genome have revealed almost 40 potential coding regions for β-defensins (*DEFBs*) [16, 68]. Because of the high frequency of gene duplication within β-defensin gene clus-

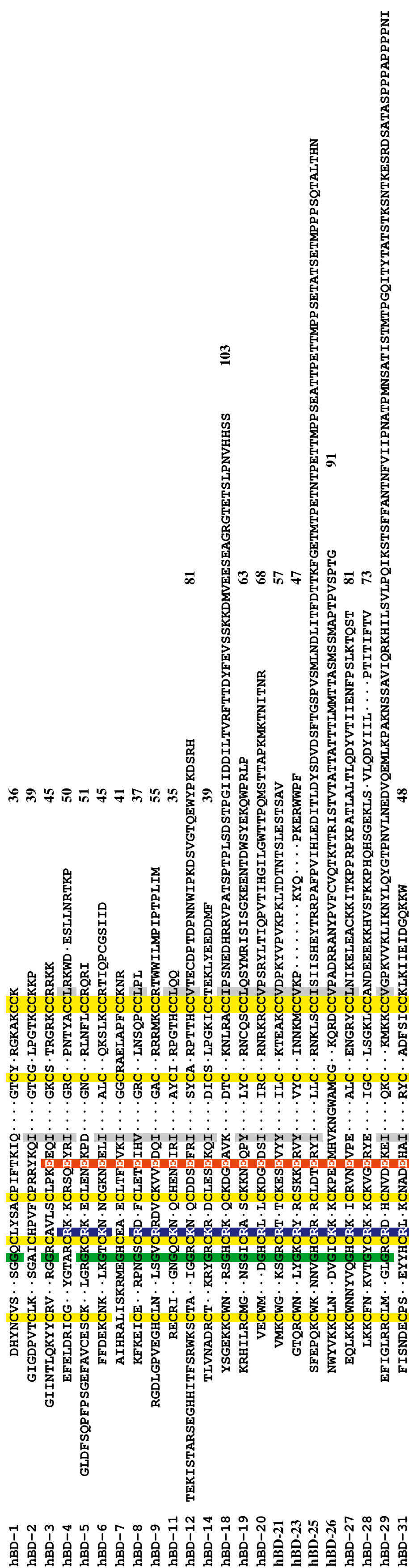

Figure 1. Multiple sequence alignment of mature human β-defensins. The 23 sequences represent peptides which have been purified from natural sources or have been detected at the level of mRNA. The positions of six completely conserved Cys residues are highlighted in yellow. Highly conserved Gly, Glu, basic and hydrophobic residues are shown on green, red, blue and gray background, respectively.

ters, it is possible that the number of *DEFB* genes is even higher [64]. Some of the *DEFB* coding sequences, however, likely represent pseudogenes, since their mRNA products contain premature stop codons [16].
The first gene cluster, coding for β-defensins *DEFB1, DEFB4* and *DEFB103*, is located within chromosomal region 8p21-p23, which also includes all the α-defensin genes [16, 72, 73]. This observation suggested the presence of the common ancestral progenitor defensin gene [16]. Subsequently, three other *DEFB* gene clusters were identified within chromosomes 6p12, 20q11.1 and 20p13. To date, the expression of many of these β-defensins was confirmed by either reverse transcription-polymerase chain reaction (RT-PCR) or mRNA hybridization at the transcriptional level (Table 1).
The pattern of human β-defensin genes shows a common organization. The genomic structure consists of two exons and one intron. The only exception is the gene *DEFB105,* which contains three exons and two introns. In all but the *DEFB1* gene, the first exon encodes the signal peptide and the second carries information about the sequence of mature peptide preceded by a short anionic pro-peptide [14, 57, 73, 74]. In the *DEFB1* gene, the first exon encodes the signal peptide and pro-peptide segment [73]. Post-translational modifications include the proteolytic cleavage of the signal sequence and, subsequently, of the N-terminal pro-piece [75, 76]. A mature peptide is often additionally truncated at the N-terminus, yielding multiple forms released into designated compartments [36, 47, 51, 77]. *In vivo*, processed according to an as yet unknown mechanism, β-pro-defensins are secreted into the immediate surroundings of epithelial cells.
A growing family of proteins with a strong homology to β-defensins, called epididymis-specific secretory proteins (EP2/HE2/SPAG11), comprises the androgen-dependent molecules that are specifically expressed in the epithelium of the male reproductive tract [78–81]. The human gene encoding EP2 is located within the same section of chromosome 8 as several of the β-defensin genes [60, 71]. Through splice variation, several transcripts of the EP2 gene (EP2A-EP2I) are generated in various regions of the human and chimpanzee epididymis [82–84]. Three of these variants, EP2C/D/E, share the cysteine motifs with β-defensins [82, 83]. The members of the EP2 family also share antibacterial properties with β-defensins [85, 86].

Sites of expression and gene regulation

To date, only the first four human β-defensins have been characterized in some detail (Table 1). HBD-1 is primarily expressed in the epithelial lining of the urinary and respiratory tracts [43, 47, 87]. The constitutive expression of hBD-1 is observed in various tissues [48, 49, 88] and may be modulated by inflammation [89–93]. Furthermore, expression of hBD-1 can be induced and upregulated by lipopolysaccharides (LPSs), heat-inactivated *Pseudomonas aeruginosa* and interferon gamma (IFN-γ) [89, 91–94].
The most prevelant expression of hBD-2 is observed in skin and the gastrointestinal and respiratory tracts; however, substantial amounts of this defensin are present throughout the entire epithelia [51, 95]. HBD-3, in addition to the epithelia, was also detected at lower levels in different nonepithelial cells in the heart, liver, fetal thymus and placenta [40, 57, 96]. Isolation of hBD-4 from the natural source has not yet been described [7]. Whereas analyses of the natural distribution of hBD-4 are based on detection of appropriate mRNA (Table 1), a partial characterization of this defensin relies on synthetic/recombinant preparations [59]. HBD-4 is primarily expressed in the testis and epididymis [59]. Although the inducible expression of hBD-4 was detected in primary keratinocytes [61], so far there is no confirmation of its presence in human skin.
Due to a very low basal expression of hBD-2 through hBD-4 in epithelial cells, the expression-regulation of these defensins is somewhat different compared with hBD-1. Up-regulation of their expression can be induced in response to bacterial infection or proinflammatory stimuli [48, 58, 59, 70]. At the transcriptional level, induction of hBD-2 and hBD-3 synthesis was observed in gastric and respiratory epithelium, peripheral blood, dendritic cells and keranatinocytes [5, 84, 94, 95, 97–99]. Stimulators of hBD-2 expression include interleukin-1α (IL-α), IL-1β, tumor necrosis factor-α (TNF-α), IFN-γ, phorbol 12-myristate 13-acetate (PMA), isoleucine, 1,25-dihydroxyvitamin D_3, LPSs and some Gram-negative bacteria [7, 89, 94, 96–98, 100–103]. Similar to hBD-2, expression of hBD-3 is induced in keratinocytes and the respiratory epithelium by TNF-α, IL-1β, IFN-γ, various bacteria and yeast [57, 58, 71]. In contrast to hBD-2, upregulation of hBD-3 expression in keratinocytes was observed in the presence of transforming growth factor alpha (TGF-α) and insulin-like growth factor 1 (IGF-1) [99]. The identified factors upregulating hBD4 expression are similar, as in the case of hBD-2 and hBD-3 [61, 99].
Variable expression and induction patterns of β-defensins suggest involvement of multiple signaling pathways in the regulation of these processes [91, 98, 104, 105]. In various tissues, induction of hBD-2 expression by cytokines and bacterial components is mediated through transcriptional nuclear factor κB (NF-κB) – sometimes assisted by activated protein 1, by mitogen-activated protein kinase (MAP-kinase) and by protein kinase C (PKC) [56, 98, 106–111]. A potential role of the JAK2 (Janus kinase)/signal transducer and activator of transcription (STAT) signaling pathway was suggested in hBD-1 and hBD-3 expression [61, 91]. Also, a contribution of protease-ac-

tivated receptors to hBD-2 upregulation in epithelia was recently described [112].

Induction of antimicrobial peptide proceeds through activation of a distinct group of toll-like receptors (TLRs), which recognize pathogen-associated molecular patterns [104, 113, 114]. Through subsequent activation of NF-κB and/or MAP-kinase signaling pathways, TLRs modulate the expression of numerous genes [104, 113, 114]. The participation of specific toll-like receptors (i.e. TLR2, 4, 6, 9) in the induction of hBD-2 expression, following different bacterial stimuli, was observed in different cells and tissues [113–122]. In all cases, the NF-κB and AP1 sites were also found to be necessary for induction of hBD-2 expression. Together, the reported results demonstrate the complexity of mechanisms underlying upregulation of β-defensins and open a possibility for roles of other molecular partners or signaling pathways in the inducible production of hBDs.

The mechanisms regulating the expression of human β-defensins other than hBD-1 through -4 are currently very poorly understood. The expression of hBD-8 and hBD-14, induced by IL-1β or the *Candida* species, appears to be regulated similarly to hBD-4, involving the NF-κB, MAP-kinase or JAK-STAT signal transduction pathways [63]. The identification of multiple NF-κB-binding sites within the hBD-6 gene points in a similar direction [60]. Understanding the details of the expression patterns, inducing factors and signaling pathways for recently identified β-defensins requires further investigation. An exemplar here could be β-defensins encoded on chromosome 20, misleadingly originally called 'epididymis-specific' [16, 60, 65, 68]. Recent studies show that, in addition to epididymis and testis, these proteins are also secreted in the pancreas, skeletal muscle and kidney (*DEFB118, DEFB125–127)* [64, 68]. However, the biological functions of these peptides are unknown, a particularly interesting fact since there is no evidence for their expression in airways or skin, i.e. the common expression sites for β-defensins that are primarily involved in innate immunity [68].

Diversity of biological activities

In addition to the well-recognized antimicrobial properties of β-defensins, recent reports picture these proteins as potent immunomodulators, indicating their important role in regulating both innate and adaptive immunities [5, 14, 21, 22, 30, 31, 35, 123–130].

Antimicrobial activity

Each of the β-defensins characterized to date has the capacity to kill or inhibit *in vitro* a wide variety of bacteria and fungi, particularly at low concentrations of salt and plasma proteins (Table 2) [10, 14, 31, 36, 125, 131, 132]. The spectrum of this antibacterial activity varies for each of the four proteins. hBD-1 and hBD-2 have been shown to be particularly effective against Gram-negative bacteria and some fungi but relatively less potent against Gram-positive bacteria [51, 70, 87, 133]. hBD-3 is a powerful antimicrobial agent with a broad range of activity toward yeast, Gram-negative and Gram-positive bacteria, including the vanomycin-resistant *Enterococcus faecium* [57, 58].

Studies of the effectiveness of hBD-2 and hBD-3 against oral pathogens showed that both defensins are more potent against aerobic bacteria (100% effective) than anaerobic bacteria (21.4% and 50% effective for hBD-2 and hBD-3, respectively) [134]. Both defensins also demonstrate strain-specific activity against *Candida* species [90]. A synergistic antibacterial effect against *Staphylococcus aureus* was described for hBD1–3, LL-37 and lysozyme [135].

Table 2. Antimicrobial properties of human β-defensins

Defensin	Pathogen	References
hBD-1	*Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella pneumoniae Staphylococcus aureus*	[87, 95]
hBD-2	E. coli, P. aeruginosa, K. pneumoniae S. aureus, *Streptococcus pneumoniae, Candida albicans, Candida parapsilosis*, *Candida krusei, Enterococcus faecalis,* HIV-1	[51, 70, 87, 90, 133, 139, 140]
hBD-3	*E. coli, P. aeruginosa, K. pneumoniae, S. aureus, Streptococcus pyogenes, Ent. faecium, Strep. pneumoniae*, *Staphylococcus carnosus, Burkholderia cepacia, Saccharomyces cerevisiae*, *C. albicans, C. parapsilosis*, *C. krusei,* HIV-1	[57, 58, 90, 133, 139, 140, 143]
hBD-4	*E. coli, S. carnosus*, *P. aeruginosa*, *B. cepacia, Strep. pneumoniae*, *S. aureus, Sacch. cerevisiae*,	[59, 26]
hBD-28	*E. coli*, *P. aeruginosa, K. pneumoniae*, *Strep. pneumoniae*, *S. aureus*	[133]
hBD-18	*E.coli*	[67]
hBD-6 pro-peptide	*E. coli*	[64]

Several studies also described potent antibacterial activity of synthetic hBD-4, including such pathogens as *Burkholderia cepacia*, bacteria resistant to other antibacterial agents and antibiotics [26, 59].

A number of reports showed that the microbicidal activity of β-defensins (with the exception of hBD-3) was strongly inhibited by sodium chloride at physiological concentrations or by various divalent cations [57–59, 87, 95, 136]. These observations suggest the significance of electrostatic interactions for β-defensin activity and seem to restrict sites of such activity to low-salt compartments (i.e. the surface of epithelium or skin) [57–59, 87, 95]. More recent evidence shows, however, that the salt sensitivity of β-defensins *in vivo* may be lower than believed originally [137]. Such salt tolerance as well as structure dependence was observed for a few recently identified β-defensins [64, 67, 133]. Whereas hBD-3-like antibacterial activity was demonstrated for hBD-28, similarly sized hBD-27 was completely inactive against the bacteria tested at concentrations up to 300 µg/ml [133]. High and dose-dependent anti-*Escherichia coli* activity was described for synthetic peptides corresponding to the protein products of *DEFB106* and *DEFB118* [64, 66].

Anti-HIV effect

Several recent reports demonstrated that β-defensins may contribute to controlling the human immunodeficiency virus-type 1 (HIV-1) replication *in vivo* [138–140]. Quinones-Mateu and coworkers [139] demonstrated the HIV-1-induced expression of hBD-2 and hBD-3 in human oral epithelial cells, and dose-dependent inhibition of HIV-1 replication *in vitro* by recombinant hBD-2 and hBD-3. A similar inhibitory effect was observed during R5 and X4 HIV infection of PMBC by chemically synthesized β-defensins [138]. Whereas the former report indicated that hBD-2 and hBD-3 downregulate the HIV-1 coreceptor CXCR4 in PBMC and T lymphocytes, suggesting inhibition of the X4 virus by β-defensins at the viral entry step [139], authors of the latter did not observe receptor down-regulation by hBD-2, and suggested instead that a post-entry, intracellular signaling event is responsible for the inhibition of HIV-1 replication [138]. The idea of intracellular signaling involvement is reminiscent of studies on inhibition of HIV replication by related human neutrophil α-defensins [141, 142]. Despite the differences in the interpretation of inhibition at the cellular level, β-defensins can directly inactivate HIV-1 virions [138, 139]. This activity appears to be a common property of defensins from different families [140].

One study reports the inhibitory effect of the influenza virus fusion into host cells by retrocyclin 2 (θ-defensin RC2), hBD-3 and mannan-binding lectin (MBL) [143]. Authors proposed that inhibition of viral entry by these natural host defense molecules arises due to cross-linking and/or immobilizing of cell-surface glycoproteins. Another report demonstrated the association of a single-nucleotide polymorphism (44C/G) in the *DEFB1* (hBD-1) gene with HIV-1 infection in Italian children [144]. It has been also shown that hBD-1 expression is significantly reduced in alveolar macrophages of HIV-infected patients [145]. Upcoming studies should provide more details on the molecular basis of HIV-1 inhibition by β-defensins.

Chemotactic effects and *in vivo* immunoenhancing activity

In 1999, Yang and coworkers observed the recruitment of human immature dendritic cells (DCs) and memory peripheral blood memory T cells by a concentration gradient of hBD-1 and hBD-2 [35]. The chemotactic activity of hBD2–4 was later described for other blood cells [57, 59, 146, 147]. In another report authors described the hDB-2-dependent activation of mast cells [148]. The chemotactic and immunoenhancing activities of β-defensins are not suppressed by the physiological concentration of salts and serum proteins [35]. The recruitment of immature DCs and memory T cells by hBD-2 and hBD-3 is mediated through the G_i-protein-coupled receptor (GiPCR) CCR6 [35, 147, 149–151]. The competition between hBD-2 and MIP-3α (the chemokine ligand of CCR6) for CCR6 also was recently confirmed for stimulated keratinocytes [152]. Combined, these results not only confirm common signaling pathways in β-defensins and MIP-3α regulation, but also suggest an important and complementary role of β-defensins in innate and adaptive immunity. In addition to CCR6-mediated chemotaxis by hBD1-3, gradient-dependent recruitment of CCR6-deficient monocytes and macrophages observed for hBD-3 and hBD-4 suggests utilization of an additional GiPCR receptor(s) [130].

The ability of β-defensins to chemoattract and/or activate a wide variety of blood cells suggests that β-defensins play roles in both the innate and antigen-specific immunity of the host. Furthermore, the chemotactic activity of β-defensins towards immature DCs also facilitates antigen uptake, processing and presentation, leading to induction of antigen-specific immune responses [123, 153].

The concept of β-defensins contributing to innate and adaptive immunity through the production of pro-inflammatory cytokines, as previously shown for α-defensins [154, 155], was also recently described [156, 157]. The authors demonstrated an individual and synergistic induction of IL-18 as well as other pro-inflammatory cytokines (i.e. IL-20 and IL-8) by hBD-1 through hBD-4, and of the cathelicidin LL-37 in cultured human keratinocytes. IL-18, IL-20 and IL-8 are believed to be involved in the pathogenesis of psoriasis.

Additional activities of β-defensins

Several recent reports also indicate additional physiological functions of β-defensins [156, 158–161]. Significant upregulation of hBD-1 and hBD-3 observed during the maturation of keratinocytes suggests a possible role of these peptides in cell differentiation processes [158, 159].

Another group of studies focused on identification of correlations between the changes in β-defensin expression patterns and the types and/or stages of tumor development [156, 159, 161, 162]. Whereas it was shown that differential gene expression profiles of hBD-1 can be useful for distinguishing the subtypes of both renal cell carcinoma and prostate cancer [156, 161], no similar correlation was observed for hBD-2 in the case of gastric tumors [162].

Observation of hBD-3 expression in osteoarthritic (OA) joints in the absence of bacterial challenge prompted suggestion of the role of this peptide in the tissue remodeling processes taking place in OA cartilages [156, 160].

High expression levels in the male reproductive tract observed for EP2 isoforms and a number of recently discovered β-defensins (Table 1) indicate that these proteins may play roles in the range of protective, adhesive, and regulatory functions, confined to this compartment [60, 68, 80, 124, 163]. However, despite encouraging preliminary results obtained during studies of β-defensins 126, 118, and 119–123, it is still unclear why such a wide range of β-defensins is present in the male reproductive tract [65, 66, 69, 163]. Whereas antimicrobial activities of these defensins have been demonstrated *in vitro*, the nature of their primary activity *in vivo* is still not fully understood.

Structures of human β-defensins

Primary structures

The β-arrangement of disulfide bonds connecting the conserved six cysteines has been confirmed experimentally only for hBD-1, hBD-2 and hBD-3 [36, 51, 58]. With the exception of hBD-5 and hBD-12, the first conserved cysteine residue is situated near the amino termini of the peptides (Fig. 1). The Cys^1-Cys^4 bridge introduces the macrocyclic covalent chain within the core of peptides [28].

Aside from the six cysteine residues, only a few other residues are conserved to most β-defensins. Using the hBD-1 numbering, these residues are Gly10, a cationic residue at position 13; Glu21; and hydrophobic residues 24 and 36. The four most-studied hBDs (hBD-1 through hBD-4) have a high content of cationic residues (Lys, Arg), clustered primarily near the carboxyl termini of the peptides (Fig. 1). Aggregation of these positive charges is important for antimicrobial activity [1, 5, 14, 22, 24, 26, 28, 164].

Several β-defensins encoded on chromosomes 6 and 20 are characterized by an unusually long C-terminal tail (Fig. 1). This extension, rich in anionic residues, has no evident sequence similarity to other β-defensins [67, 68]. Similar motifs are also found in the β-defensin-like family of HE2 peptides [65, 82, 85]. It was suggested that these long C-termini contribute to the decreased salt sensitivity of the antibacterial activity, as observed for β-defensin 118 [67]. The functional significance of this hypothesis remains to be analyzed.

Tertiary structures

To date, the structures of only three human β-defensins (1 through 3) have been determined [165–169]. Appropriate coordinates are deposited in the Protein Data Bank (www.rcsb.org) with the entry codes 1IJV, 1E4S and 1IJU [166, 169] for hBD1; 1FD3, 1FD4, 1E4Q, and 1FQQ [165, 167, 169] for hDB2; and 1KJ6 and 1KJ5 [168] for hBD-3. Despite limited amino acid sequence conservation among the structurally characterized hBDs [168], their tertiary structures are remarkably similar (Fig. 2).

In all structures, the core of the molecule has a common ('defensin-like') topological fold (Structural Classification of Proteins) [174, 175]. The prototype for this fold is the structure of human α-defensin-3 (PDB code 1DFN) [176]. The core of the defensin molecule consists of three β-strands arranged in an antiparallel sheet, which is constrained by three intramolecular disulfide bridges. In β-defensins (but not in the α-class), the β-sheet is flanked by an α-helical segment of a variable length, formed by the N-terminal fragment of the molecule [165, 166, 168]. The orientation of the α-helix in relation to the β-sheet is stabilized by the disulfide bridge (Cys^1-Cys^5). Because of the specific arrangement and linkage of the six conserved cysteine residues in β-defensins, it was recently suggested that the β-defensin fold should be recognized as a distinct topological family of proteins, which would also include some toxins [177–179]. Such a proposal may be somewhat premature, since the previous studies of the hBD-3 isoforms provided evidence contradictory to those observations [180].

A few additional notes may support the role of the β-sheet as central to the defensin's structure and, presumably, its function. Part of the second β-strand in all defensins (including the α-class [181, 182]), consisting of the conserved motif Gly-X-Cys, forms a β-bulge [166, 182]. The primarily role of β-bulges is to accentuate a twist of the β-sheets, and their presence is suggested as necessary for the correct folding and formation of the native structure by mammalian defensins [181]. Recently, it was shown that residues forming β-sheets are less susceptible to either negative or positive selection during the evolution of mammalian β-defensin genes [62].

The size of the N-terminal α-helix, present in hBD-1 through hBD-3 (also found in murine defensins mBD-7 and mBD-8 [169] and in penguin protein (Sphe-2) [183], but not in bovine β-defensin-12 (bBD-12) [184]) is not correlated with the number of amino acids preceding the first cysteine residue (the N-terminus of hBD-3 appears unstructured [168]). The small size and variable length of this helical fragment have prompted suggestions that some hBDs may lack it completely [177]. The specific conformation of the N-termini in β-defensins may be important for the biological properties of these proteins. The primary structures of this region were suggested as contributing to observed differences in the antimicrobial specificity of β-defensins [167].

Despite only moderate sequence conservation, the C-terminal sections in most β-defensins are rich in basic and hydrophobic amino acids (Fig. 1). Consequently, variably shaped molecular surfaces of the C-termini of hBD-1 through hBD-3 have amphiphilic properties, with clearly identifiable, asymmetrically distributed, positively charged motifs (Fig. 2).

Quaternary structures

Human β-defensins display the potential to form various oligomeric structures [165–169], which may play a significant role or influence the biological activities and functions of these proteins [24, 164]. The nature of oligomerization observed in β-defensins appears to be protein-

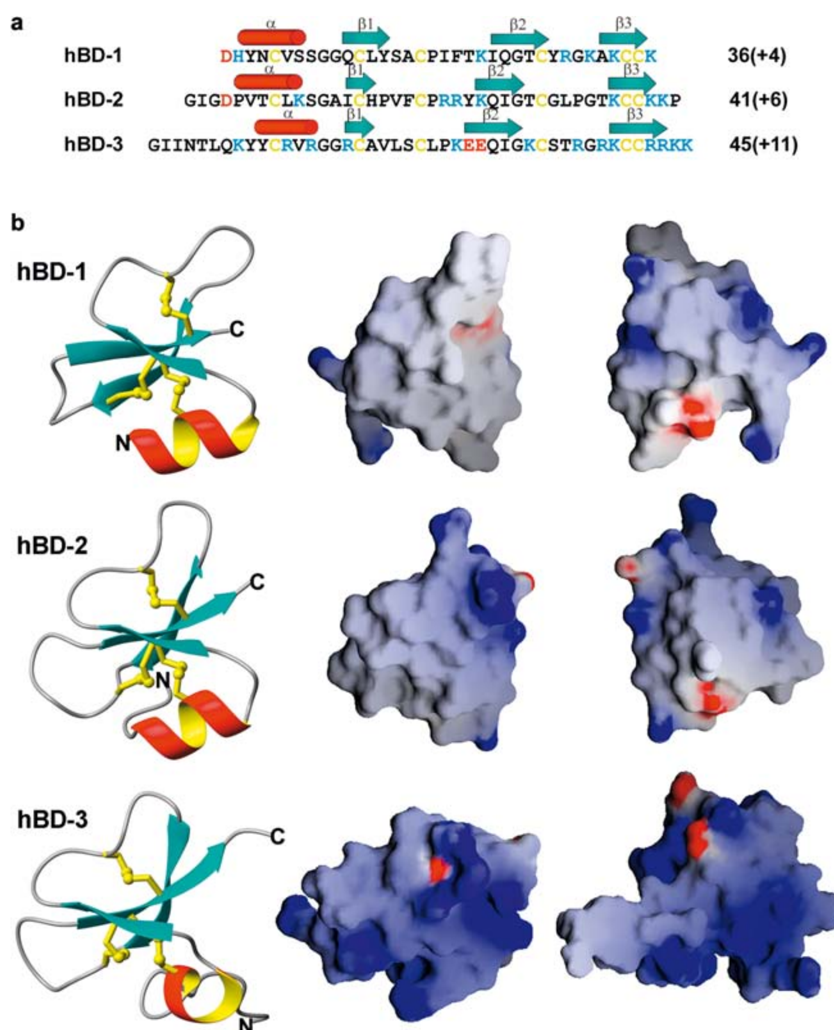

Figure 2. Structural comparison of human β-defensins. (*a*) The amino acid sequence alignment (ClustalW [170]). Numbers in the brackets represent formal charges. Above each sequence is shown the extent of the secondary structure elements. Six conserved cysteine residues are colored in yellow, the anionic residues are shown in red, while the basic residues are shown in blue. (*b*) The ribbon representations of monomeric β-defensins (left side of the panel) are accompanied by the electrostatic potential maps projected on the solvent-accessible surfaces [171] shown in the equivalent orientations (center of the panel) and after 180° rotation (right side of the panel). The positively and negatively charged areas of the solvent-accessible surfaces are colored in blue and red, respectively. Figures were prepared with programs MOLMOL [172] and Grasp [173].

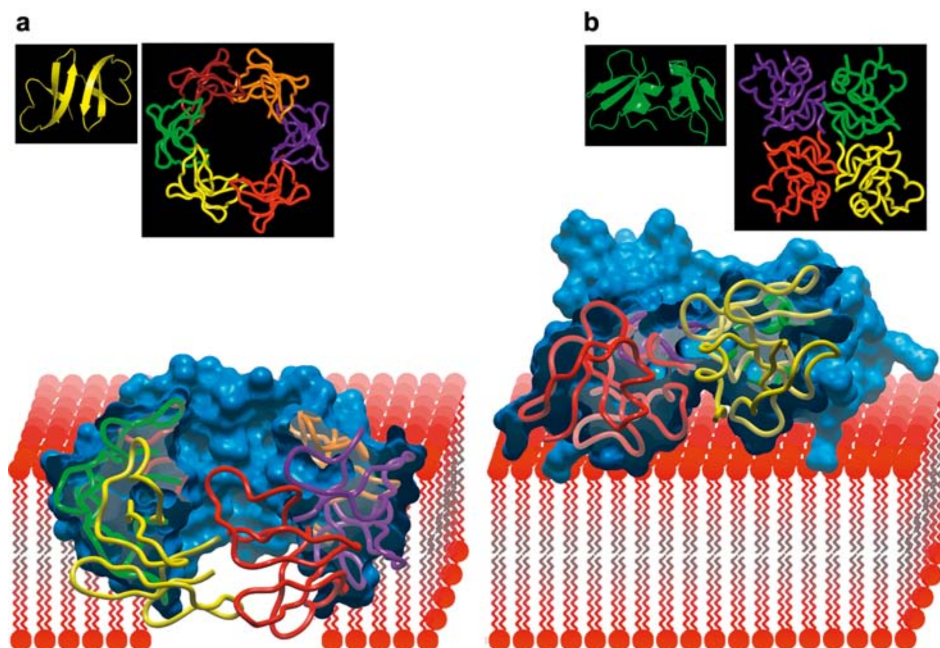

Figure 3. Models of interactions between defensins and bacterial membranes. (*a*) The 'pore-formation' model originally derived from structural studies of hNP-3 [176]. (*b*) The less structurally restricted 'carpet' model, exemplified here by results of crystallographic studies of hBD-2 [165]. The dimeric structures of both defensins, seen in their crystals and shown schematically in the smaller, black panels, are accompanied by models of larger aggregates, theoretically derived 'pores' of hNP-3 and crystallographically determined octamers of hBD-2 (larger black panels). The dimerization mode of hNP-3, a prerequisite for the assembly of the modeled pore, is inaccessible for hBD-2.

specific. The first report of β-defensin oligomerization followed the crystallographic studies of hBD-2 [165]. Results obtained for two different crystal forms describe the octameric assemblies contributed by four noncovalent dimers of hBD-2, stabilized by interactions between the first β-strands of both monomers. The dimers are topologically distinct from those described earlier for the α-defensins [176]. The N-terminal α-helix plays an important role in the stabilization of the hBD-2 octamers [165]. Oligomerization of hBD-2 was not confirmed in subsequent studies in solution by either nuclear magnetic resonance (NMR) spectrometry, light scattering or size exclusion chromatography [167, 169]. Because the aggregation of β-defensins occurs only at elevated protein concentrations (>3 mM) and, possibly, in the sterically constraining environment of a bacterial membrane, the results obtained during the crystallographic studies may be the most adequate of all approaches reported [169]. The flat disk shape of hBD-2 octamers and the charge surface distribution suggest the possible mode of interaction with the surfaces of microbial membranes (Fig. 3).

Only weakly associated dimers (stabilized primarily through the salt bridges) were found in the crystals of hBD-1 [166]. Taking into account the significant reciprocal dependence of the antimicrobial activity of hBD-1 on salt concentration [87, 131], the latter observation may bear functional relevance. A systematic study of hBD-3 showed that this highly cationic molecule creates symmetric dimers that are stabilized by two salt bridges between Glu-28 and Lys-32, and by one hydrogen bond [168]. Subsequent mutational studies confirmed a significant role of Glu-28 in stabilizing the oligomeric structure of hBD-3 [185]. The capacity to create stable dimers in solution, at low concentrations, has been suggested as a possible reason for the high and salt-independent antibacterial activity of hBD-3 [24, 58, 185].

Non-defensin molecules with the defensin-like fold

The broad distribution of peptides that share the defensin-like fold provided cause to identify the structural similarities among these peptides, and possibly to reconstruct the evolutionary tree connecting them to their common ancestor(s) [186]. A possible evolutionary relation is strongly supported by current structural information [177, 186, 187]. The defensin-like fold has been observed for various toxins, including snake toxins [183, 187], sea anemone toxins [188, 189] and platypus venom toxins (defensin-like peptides, DLP1 and DLP-2) [178, 190, 191]. Curiously, platypus DLPs have neither antimicrobial nor mytotoxic properties, and their roles as the components of the venom are unknown [178]. Although there are no reports on reptile defensins, database searches showed a high degree of similarity between both the signal sequences and mature peptides of human β-defensins and avian β-defensins [183, 186] (e.g. gallinacins [187, 192], gallopavins [187, 193]

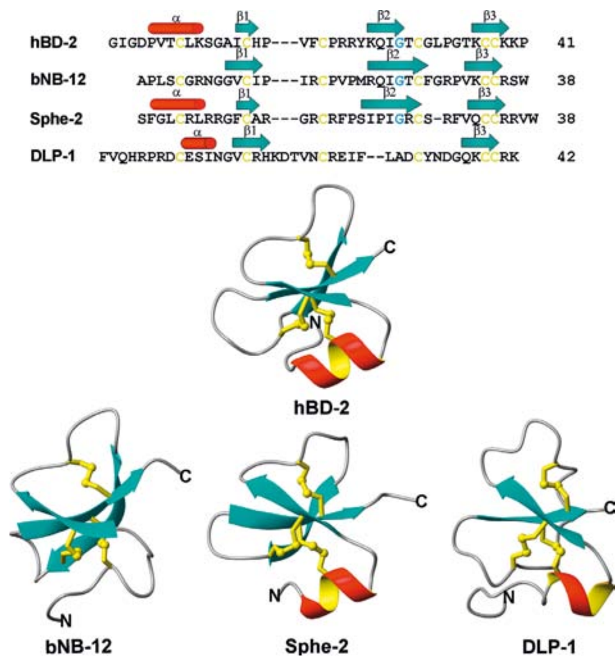

Figure 4. Multiple sequence alignment [170] and ribbon representations of four different molecules from β-defensin-fold family. The PDB accession codes are 1FD3 (hBD-2, [165]), 1BNB (bBD-12, [184]), (Sphe-2, [183]) and 1B8W (DLP-2, [190]). The six conserved Cys residues and moderately conserved Gly residue are shown in yellow and blue, respectively. The red cylinders represent residues of α-helical conformation, while the blue arrows represent residues of β-strand conformation. The distributions of the secondary structure elements are indicated over each sequence. The cartoon part of this figure was prepared with the program MOLMOL [172].

and spheniscins [194]. To date, only β-defensins (but not α) were identified in reptiles and birds, suggesting the appearance of the β-defensin-like molecule prior to the evolutionary split of reptiles, birds and mammals [123, 186]. Possibly, originating from such an ancestor, genes have gradually evolved into β-, α- and θ-defensins, accumulating various biological activities [186]. Figure 4 shows the alignment of amino acid sequences and tertiary structures of representative molecules from the β-defensin-like fold family.

Despite conservation of the overall fold, such differences as lack of the N-terminal α-helix in bovine BD-12 or the presence of only two β-strands in DLP-2 can be easily recognized. Structurally, the most similar to hBDs appears to be the penguin peptide Sphe-2. This highly cationic peptide has a well-defined amphiphilic surface; however, the charge distribution is different than in the case of human β-defensins [183]. Nevertheless, the amphiphilic character and high positive net charge of Sphe-2 have been associated with its substantial antibacterial activity and relatively low salt activity dependence [183].

Structure-function relationship

Antimicrobial activity

The mechanism

An increasing number of studies focus on a better understanding the mechanism governing the microbicidal activities of β-defensins [24, 25, 164, 185, 195, 196]. These studies indicate that β-defensin molecules bind to the negatively charged cytoplasmic membranes and disrupt their integrity, leading to a leakage of intracellular components and inhibition of the DNA, RNA and protein synthesis [3, 6, 22, 23, 26, 125, 132].

Accordingly, the antimicrobial activity and specificity of β-defensins are primarily determined by their physicochemical properties, such as amphiphilic character and a precise balance of positive net charge and hydrophobicity. Additional roles are assigned to the structural properties of β-defensins [14, 24, 185, 195, 196]; oligomerization [25, 165, 168, 197]; and proteolytic stability [7, 198]. Whereas all β-defensins share a very similar structural core, it has been postulated that their microbe specificity results from variable arrangements of nonconserved residues [62, 164]. A broad spectrum of diverse distributions of surface-displayed functions observed for β-defensins is, in turn, a consequence of a pressure posed by constantly evolving bacteria [24, 25].

The central role of interactions between the negatively charged membrane components and β-defensins for the bactericidal properties is supported by the results of experiments with artificial membranes [58, 67, 136, 199, 200], as well as with modified bacterial membranes [29, 201, 202]. The importance of electric forces in defensin action on bacterial membranes was confirmed experimentally for several α-defensins [203–206]. Whereas a partial correlation between the net charge of whole bacteria and the killing potential of β-defensins was demonstrated for some cariogenic and periodontal bacteria, it was limited only to a narrow range of bacterial strains [207]. These results suggest a rather complex mechanism of antibacterial activity of β-defensins, possibly different from the mechanism responsible for the antibacterial activities of other AMPs.

The molecular details of the interactions between β-defensins and membranes are, as yet, not clear. According to one model, β-defensins might form channel-like pores spanning across the membranes [27, 199, 200, 208–213]. Following the initial electrostatic interactions of defensin molecules with anionic phospholipid head groups of the membrane, the peptides are fused into the membrane's interior. The driving force behind the fusion would be the interaction between hydrophobic components of the membrane lipid core and defensin molecules (Fig. 3).

In the second model, the carpet-line layer of β-defensin molecules, formed on the surface of the microbial

membrane, neutralizes negatively charged lipid moieties and the transmembrane electric potential [208, 214–216]. In this model, β-defensins act like classic detergents, binding at high concentrations to the phospholipid head groups on the bacterial surface. Support for the 'pore-formation' model is provided by the amphiphilic character of the β-defensin molecules and, to some extent, by their ability to oligomerize [217]. The weakness of this model stems from the requirement of specific oligomerization needed for channel formation, i.e. a property that as yet has not been directly observed for any defensin. In contrast, neither specific oligomerization nor extensive hydrophobicity of the surface is required, according to the 'carpet model' [23]. Whereas hydrophobic regions of the molecular surface of β-defensin molecules play a role in stabilizing their oligomeric structures, a demonstration of charge-dependent activity of these peptides is in agreement with the 'carpet' model [180].
Most likely, however, neither of these two 'pure' models correctly describes the mechanism of microbial killing, and multiple flavors of the overall scheme may be behind the interactions of different β-defensins and various microbes. Further elucidation of mechanistic details probably will require studies of the interactions between β-defensins and membrane components other than positively charged lipids (i.e. carbohydrates), as well as studies of possible specific interactions with molecular targets, specific to individual microorganisms [3, 14, 24, 132, 164, 180, 217].

Role of oligomerization vs. positive charge
A role of the quaternary structure on the biological activity of β-defensins has been explored by Campopiano and coworkers [218]. They described a positive effect of covalently cross-linked dimerization of Defr1 (a β-defensin-like peptide from mouse heart) on the ability to disrupt bacterial membranes. Broad and salt-independent antimicrobial activity of hBD-3 has been thought to be a result of a high positive charge (+11) and the ability to dimerize [58, 168]. Subsequent studies of the structure-activity relationship of hBD-3, however, have not confirmed a clear role of oligomerization in the antibacterial activity of this peptide [185, 196]. In turn, a high overall hydrophobicity and positive net charge of the hBD-3 molecule was suggested as more important than its ability to oligomerize. In line with these conclusions, introduction of additional tryptophan and tyrosine residues have been shown to enhance activity [196]. Further evidence comes from the observation that the positive selection of the *DEFB103* gene in human and primate species has favored alteration of the positive charge rather than other factors that could affect the antimicrobial properties of hBD-3 [24].

Role of the disulfide array
Several reports indicated that the three disulfide bridges are not required for antimicrobial activity of β-defensins [180, 218–221]. Analyses of the structure-activity relationship also showed that full-length hBD-3 displays very little change in anti-*E. coli* activity, irrespective of the presence or connectivity of disulfides [180, 221]. Similar results have been obtained for the synthetic C-terminal fragments of bovine β-defensin-12, for which the antibacterial activity also did not seem to be related to the number and location of disulfide bonds [219, 220]. Defr1, consisting of only five cysteine residues, has high antimicrobial activity against *P. aeruginosa*, whereas activity of its mutated form, stabilized by a network of disulfide bonds, similar to that of other β-defensins, is significantly impaired [218]. A recent report indicates that the main function of disulfide linkages in defensins is protection from proteolysis due to stabilization of the compact and rigid fold [7]. Although the presence of disulfide bonds may not be required for the antimicrobial properties of β-defensins, a requirement of restrained structures with precisely defined Cys-Cys linkages for other biological properties of these peptides (such as chemotaxis) should be studied further [180].

The role of other structural motifs
Observation that the stability of the secondary structure of hBD-3 may be independent of the disulfide network opens the possibility that the antibacterial activities of β-defensins may be related to some local topological features of these peptides [196]. Identification of such structural motifs in β-defensins appears, however, quite difficult [164]. Previous studies of bBD-12 showed some correlation between the basic charge of the C-terminal tail and high *in vitro* antimicrobial activity of this peptide [219, 220]. Similar observations were also reported for hBD-3 [221]. The latter report also suggested, however, other structural features as possibly important for interactions with the microbial membranes [221]. Data indicate a possible role of the N-terminal α-helix in the antimicrobial properties of hBD-2. The relative orientation of this α-helix in hBD-2 is stabilized by Asp4, the residue earlier demonstrated as a significant factor for this peptide's ability to permeabilize the *E. coli* cytoplasmic membrane [24, 195]. Subsequent work on derivatives of hBD-3 [221] partially confirmed these observations, reporting the peptide corresponding to the N-terminal α-helical fragment as a potent agent against *S. aureus* and *Candida albicans*. Evolutionary analyses of the BD-1 through BD-4 genes in primates indicate several sites, located within the N-terminal helix, as being subject to positive selection, suggesting a possible involvement of this fragment in anchoring the β-defensin molecules to the bacterial membrane [62].

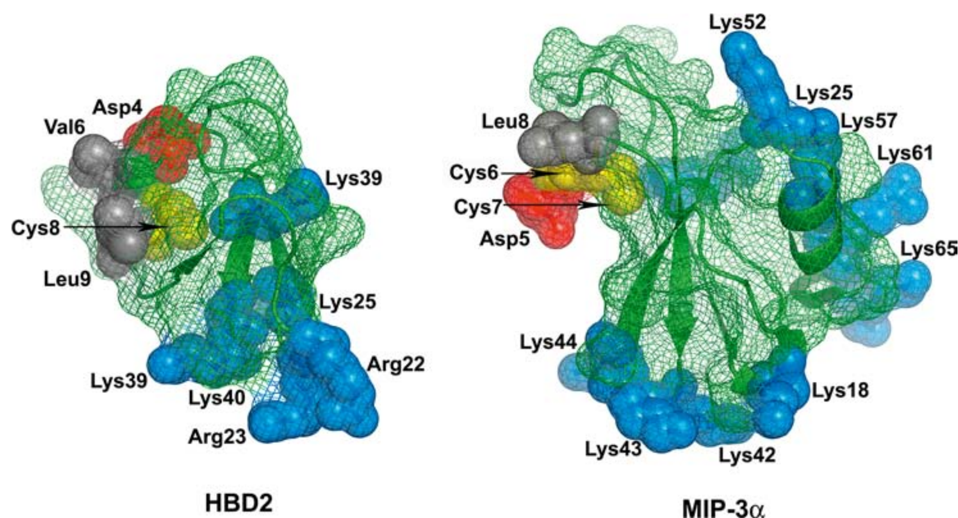

Figure 5. Residues reported as important for the chemotactic activity of hBD-2 and MIP-3α. Monomers of both proteins are shown in the form of the backbone tubes sketched together with the molecular surfaces (figures have been created based on [224, 225]). The aspartate residues, hydrophobic residues (Leu, Tyr or Val) and positively charged residues are shown in red, gray and blue, respectively. The cysteine residues within the DPVTCL and DCCL motifs of hBD-2 and MIP-3α, respectively, are shown in yellow.

Hemolytic properties

The interaction of defensins with membranes is relatively selective [222]. The phosphatidylcholine/phosphatidylethanolamine-rich and less-charged cytoplasmic membranes of eukaryotic host cells are degraded by defensins, as demonstrated in the hemolysis and cytoxicity tests of hBD-3 on various human cells, including erythrocytes [22, 58, 139]. The structural basis of the interactions between β-defensins with eukaryotic membranes is very poorly understood. Limited data suggest the importance of hydrophobic interactions for hemolytic and cytoxic activities of β-defensins [197]. Kluver and coworkers [196] demonstrated that the activity of hBD-3 on eukaryotic cells is dependent on overall hydrophobicity and is not related to the distribution of secondary structure elements.

Chemotactic activity

The molecular details behind the receptor-mediated chemotactic activity of β-defensins are unknown. Studies of the hBD-3 isoforms with alternate disulfide linkages showed alteration of chemotactic activities against monocytes and CCR6(+)HEK293 cells, depending on the disulfide topology [180]. Importantly, the disulfide-deficient variant of hBD-3 was completely inactive in these assays. Enhanced activity of some of the isoforms, as compared with the chemokine ligand of CCR6, MIP-3α, suggests a direct involvement of topological motifs encapsulating the disulfide sites in receptor binding and activation by hBD-3 [180].

Competition between β-defensins and MIP-3α for CCR6 binding has led to suggestions of similarly structured motifs, present in both ligands [35, 223]. While a comparison of the primary structures of hBDs (1 and 2) and MIP-3α revealed only very weak homology, subtle similarities were identified at the level of the three-dimensional structure [224, 225]. These similarities include small topological motifs that could mediate receptor recognition, activation and signal dispatching; shallow grooves on one face of the molecules that potentially create the region of CCR6 binding; and similar distributions of charge residues on the molecular surface areas, which are believed important for receptor binding specificity [224, 225] (see also Fig. 5).

The motif consisting of two N-terminal cysteines and the flanking residues (the DCCL motif in MIP-3α) is believed to be important for the chemotactic activity of many CC chemokines [226–230]. A major role in receptor-ligand recognition, high-affinity binding, activation and signal dispatching was attributed to these N-terminal residues [228, 231, 232]. It has been postulated that molecules of hBD-1 and hBD-2 carry topologically similar motifs, with a single asparate followed by the hydrophobic residue (DHY motif in hBD-1 and DPV or DPVTCL motif in hBD-2 [224, 225]). These residues, being positioned within the well-structured α-helix near a disulfide bond, are also sterically restrained.

Another feature in hBD-2, resembling the groove in the molecule of MIP-3α created by the N-loop and the β2-β3 hairpin, is formed by the N-terminal helix, the loop between first two β-strands and the residues at the C-terminus [68, 224, 228]. Mutagenesis and receptor binding studies have indicated the importance of this groove for recognition and high-affinity ligand binding in chemokines [231, 233].

Beyond the strictly structural similarities between β-defensins and MIP-3α, both types of molecules share some similarity in the distribution of positively charged residues on the molecular surface (Fig. 5) [224, 225]).

Interestingly, antimicrobial activity was also reported for MIP-3α [234].

Closing remarks

Current evidence shows multiple and complementary roles of β-defensins. In addition to the hallmark, antimicrobial properties of these proteins, recent studies provide numerous examples of other activities, although their relevance *in vivo* is not yet completely established. The large number of these structurally and functionally similar molecules encoded by a single genome suggests redundancies and complementarities of their biological roles. Whereas mice deficient in a single β-defensin are more susceptible to infections by specific pathogens, their antimicrobial resistance is not grossly compromised [235, 236]. However, despite accelerated efforts in research on defensins, numerous questions still remain unanswered. What is the molecular mechanism of their antibacterial and antiviral activity? Are their chemotactic properties physiologically relevant? How, if at all, are the structural features of these peptides related to their biological properties and specificities? These are just a few, yet very important, questions that certainly need to be addressed. Current interest in human defensins promises that in the near future, more than just a handful of the proteins will be fully characterized, leading to a better understanding of their multifaceted roles in human physiology and pathophysiology. It is already apparent that the properties of defensins warrant an exploration of their potential in therapeutic and preventive (i.e. topical antimicrobial) applications. Their diversity of biological functions makes β-defensins attractive candidates for the development of novel therapeutic strategies [237, 238]. Different mechanisms of action, as compared with those of traditional antibacterial and antiviral drugs, has already led to the consideration of β-defensins as substitutes in the treatment of various microbial infections [140, 143, 239], thermal injury [21] and Crohn's disease [30]. The anticancer activity of these proteins indicates a possibility for their use in cancer treatment [222], while their role as modulators and/or enhancers of immune responses hints at the development of novel vaccines [240].

1 Izadpanah A. and Gallo R. L. (2005) Antimicrobial peptides. J. Am. Acad. Dermatol. **52:** 381–390
2 Beisswenger C. and Bals R. (2005) Functions of antimicrobial peptides in host defense and immunity. Curr. Protein Pept. Sci. **6:** 255–264
3 Brogden K. A. (2005) Antimicrobial peptides: pore formers or metabolic inhibitors in bacteria? Nat. Rev. Microbiol. **3:** 238–250
4 Gordon Y. J., Romanowski E. G. and McDermott A. M. (2005) A review of antimicrobial peptides and their therapeutic potential as anti-infective drugs. Curr. Eye Res. **30:** 505–515
5 Harder J. and Schroder J. M. (2005) Antimicrobial peptides in human skin. Chem. Immunol. Allergy **86:** 22–41
6 Toke O. (2005) Antimicrobial peptides: new candidates in the fight against bacterial infections. Biopolymers **80:**717–35
7 Selsted M. E. and Ouellette A. J. (2005) Mammalian defensins in the antimicrobial immune response. Nat. Immunol. **6:** 551–557
8 Mallow E. B., Harris A., Salzman N., Russell J. P., DeBerardinis R. J., Ruchelli E. et al. (1996) Human enteric defensins. Gene structure and developmental expression. J. Biol. Chem. **271:** 4038–4045
9 Ouellette A. J. and Lualdi J. C. (1990) A novel mouse gene family coding for cationic, cysteine-rich peptides. Regulation in small intestine and cells of myeloid origin. J. Biol. Chem. **265:** 9831–9837
10 Selsted M. E., Tang Y. Q., Morris W. L., McGuire P. A., Novotny M. J., Smith W. et al. (1993) Purification, primary structures, and antibacterial activities of beta-defensins, a new family of antimicrobial peptides from bovine neutrophils. J. Biol. Chem. **268:** 6641–6648
11. Thomma B. P., Cammue B. P. and Thevissen K. (2002) Plant defensins. Planta **216:** 193–202
12 Lay F. T. and Anderson M. A. (2005) Defensins – components of the innate immune system in plants. Curr. Protein Pept. Sci. **6:** 85–101
13 Rodriguez de la Vega R. C. and Possani L. D. (2005) On the evolution of invertebrate defensins. Trends Genet. **21:** 330–332
14. Lehrer R. I. and Ganz T. (2002) Defensins of vertebrate animals. Curr. Opin. Immunol. **14:** 96–102
15 Hoffmann J. A. and Hetru C. (1992) Insect defensins: inducible antibacterial peptides. Immunol. Today **13:** 411–415
16 Schutte B. C., Mitros J. P., Bartlett J. A., Walters J. D., Jia H. P., Welsh M. J. et al. (2002) Discovery of five conserved beta-defensin gene clusters using a computational search strategy. Proc. Natl. Acad. Sci. USA **99:** 2129–2133
17 Tang Y. Q., Yuan J., Osapay G., Osapay K., Tran D., Miller C. J. et al. (1999) A cyclic antimicrobial peptide produced in primate leukocytes by the ligation of two truncated α-defensins. Science **286:** 498–502
18 Nguyen T. X., Cole A. M. and Lehrer R. I. (2003) Evolution of primate theta-defensins: a serpentine path to a sweet tooth. Peptides **24:** 1647–1654
19 Cole A. M., Wang W., Waring A. J. and Lehrer R. I. (2004) Retrocyclins: using past as prologue. Curr. Protein Pept. Sci. **5:** 373–381
20 Selsted M. E. (2004) Theta-defensins: cyclic antimicrobial peptides produced by binary ligation of truncated alpha-defensins. Curr. Protein Pept. Sci. **5:** 365–371
21 Steinstraesser L., Oezdogan Y., Wang S. C. and Steinau H. U. (2004) Host defense peptides in burns. Burns **30:** 619–627
22 Ganz T. (2003) Defensins: antimicrobial peptides of innate immunity. Nat. Rev. Immunol. **3:** 710–720
23 Reddy K. V., Yedery R. D. and Aranha C. (2004) Antimicrobial peptides: premises and promises. Int. J. Antimicrob. Agents **24:** 536–547
24 Crovella S., Antcheva N., Zelezetsky I., Boniotto M., Pacor S., Verga Falzacappa M. V. et al. (2005) Primate beta-defensins – structure, function and evolution. Curr. Protein Pept. Sci. **6:** 7–21
25 Ganz T. (2005) Defensins and other antimicrobial peptides: a historical perspective and an update. Comb. Chem. High Throughput Screen. **8:** 209–217
26 Schneider J. J., Unholzer A., Schaller M., Schafer-Korting M. and Korting H. C. (2005) Human defensins. J. Mol. Med. **83:** 587–595
27 Rausch J. M. and Wimley W. C. (2001) A high-throughput screen for identifying transmembrane pore-forming peptides. Anal. Biochem. **293:** 258–263

28 Lehrer R. I. (2004) Primate defensins. Nat. Rev. Microbiol. **2:** 727–738
29 Papo N. and Shai Y. (2005) A molecular mechanism for lipopolysaccharide protection of Gram-negative bacteria from antimicrobial peptides. J. Biol. Chem. **280:** 10378–10387
30 Wehkamp J., Fellermann K. and Stange E. F. (2005) Human defensins in Crohn‘s disease. Chem. Immunol. Allergy **86:** 42–54
31 Zasloff M. (2002) Antimicrobial peptides in health and disease. N. Engl. J. Med. **347:** 1199–1200
32 Chen H., Xu Z., Peng L., Fang X., Yin X., Xu N. et al. (2005) Recent advances in the research and development of human defensins. Peptides Oct. 11 [Epub ahead of print]
33 Diamond G., Zasloff M., Eck H., Brasseur M., Maloy W. L. and Bevins C. L. (1991) Tracheal antimicrobial peptide, a cysteine-rich peptide from mammalian tracheal mucosa: peptide isolation and cloning of a cDNA. Proc. Natl. Acad. Sci. USA **88:** 3952–3956
34 Selsted M. E. and Harwig S. S. (1989) Determination of the disulfide array in the human defensin HNP-2. A covalently cyclized peptide. J. Biol. Chem. **264:** 4003–4007
35 Yang D., Chertov O., Bykovskaia S. N., Chen Q., Buffo M. J., Shogan J. et al. (1999) Beta-defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science **286:** 525–528
36 Bensch K. W., Raida M., Mägert H. J., Schulz-Knappe P. and Forssmann W. G. (1995) hBD-1: a novel β-defensin from human plasma. FEBS Lett. **368:** 331–335
37 Boe R., Silvola J., Yang J., Moens U., McCray P. B. Jr, Stenfors L. E. et al. (1999) Human beta-defensin-1 mRNA is transcribed in tympanic membrane and adjacent auditory canal epithelium. Infect. Immun. **67:** 4843–4846
38 Dunsche A., Acil Y., Siebert R., Harder J., Schroder J. M. and Jepsen S. (2001) Expression profile of human defensins and antimicrobial proteins in oral tissues. J. Oral Pathol. Med. **30:** 154–158
39 Hao H. N., Zhao J., Lotoczky G., Grever W. E. and Lyman W. D. (2001) Induction of human beta-defensin-2 expression in human astrocytes by lipopolysaccharide and cytokines. J. Neurochem. **77:** 1027–1035
40 Hattenbach L. O., Gumbel H. and Kippenberger S. (1998) Identification of beta-defensins in human conjunctiva. Antimicrob. Agents Chemother. **42:** 3332
41 Haynes R. J., Tighe P. J. and Dua H. S. (1999) Antimicrobial defensin peptides of the human ocular surface. Br. J. Ophthalmol. **83:** 737–741
42 Krisanaprakornkit S., Weinberg A., Perez C. N. and Dale B. A. (1998) Expression of the peptide antibiotic human beta-defensin 1 in cultured gingival epithelial cells and gingival tissue. Infect. Immun. **66:** 4222–4228
43 McCray P. B. Jr and Bentley L. (1997) Human airway epithelia express a beta-defensin. Am. J. Respir. Cell Mol. Biol. **16:** 343–349
44 Paulsen F., Pufe T., Petersen W. and Tillmann B. (2001) Expression of natural peptide antibiotics in human articular cartilage and synovial membrane. Clin. Diagn. Lab Immunol. **8:** 1021–1023
45 Paulsen F. P., Pufe T., Schaudig U., Held-Feindt J., Lehmann J., Schroder J. M. et al. (2001) Detection of natural peptide antibiotics in human nasolacrimal ducts. Invest. Ophthalmol. Vis. Sci. **42:** 2157–2163
46 Tunzi C. R., Harper P. A., Bar-Oz B., Valore E. V., Semple J. L., Watson-MacDonell J. et al. (2000) Beta-defensin expression in human mammary gland epithelia. Pediatr. Res. **48:** 30–35
47 Valore E. V., Park C. H., Quayle A. J., Wiles K. R., McCray P. B. Jr and Ganz T. (1998) Human beta-defensin-1: an antimicrobial peptide of urogenital tissues. J. Clin. Invest. **101:** 1633–1642
48 Zhao C., Wang I. and Lehrer R. I. (1996) Widespread expression of beta-defensin hBD-1 in human secretory glands and epithelial cells. FEBS Lett. **396:** 319–322
49 Fulton C., Anderson G. M., Zasloff M., Bull R. and Quinn A. G. (1997) Expression of natural peptide antibiotics in human skin. Lancet **350:** 1750–1751
50 Liu A. Y., Destoumieux D., Wong A. V., Park C. H., Valore E. V., Liu L. et al. (2002) Human beta-defensin-2 production in keratinocytes is regulated by interleukin-1, bacteria, and the state of differentiation. J. Invest. Dermatol. **118:** 275–281
51 Bals R., Wang X., Wu Z., Freeman T., Bafna V., Zasloff M. et al. (1998) Human beta-defensin 2 is a salt-sensitive peptide antibiotic expressed in human lung. J. Clin. Invest. **102:** 874–880
52 Hiratsuka T., Nakazato M., Date Y., Ashitani J., Minematsu T., Chino N. et al. (1998) Identification of human beta-defensin-2 in respiratory tract and plasma and its increase in bacterial pneumonia. Biochem. Biophys. Res. Commun. **249:** 943–947
53 Mathews M., Jia H. P., Guthmiller J. M., Losh G., Graham S., Johnson G. K. et al. (1999) Production of beta-defensin antimicrobial peptides by the oral mucosa and salivary glands. Infect. Immun. **67:** 2740–2745
54 Chadebech P., Goidin D., Jacquet C., Viac J., Schmitt D. and Staquet M. J. (2003) Use of human reconstructed epidermis to analyze the regulation of beta-defensin hBD-1, hBD-2, and hBD-3 expression in response to LPS. Cell Biol. Toxicol. **19:** 313–324
55 Krisanaprakornkit S., Kimball J. R., Weinberg A., Darveau R. P., Bainbridge B. W. and Dale B. A. (2000) Inducible expression of human beta-defensin 2 by Fusobacterium nucleatum in oral epithelial cells: multiple signaling pathways and role of commensal bacteria in innate immunity and the epithelial barrier. Infect. Immun. **68:** 2907–2915
56 Krisanaprakornkit S., Kimball J. R. and Dale B. A. (2002) Regulation of human beta-defensin-2 in gingival epithelial cells: the involvement of mitogen-activated protein kinase pathways, but not the NF-kappaB transcription factor family. J. Immunol. **168:** 316–324
57 Garcia J. R., Jaumann F., Schulz S., Krause A., Rodriguez-Jimenez J., Forssmann U. et al. (2001) Identification of a novel, multifunctional beta-defensin (human beta-defensin 3) with specific antimicrobial activity. Its interaction with plasma membranes of *Xenopus* oocytes and the induction of macrophage chemoattraction. Cell Tissue Res. **306:** 257–264
58 Harder J., Bartels J., Christophers E. and Schroder J. M. (2001) Isolation and characterization of human beta -defensin-3, a novel human inducible peptide antibiotic. J. Biol. Chem. **276:** 5707–5713
59 Garcia J. R., Krause A., Schulz S., Rodriguez-Jimenez F. J., Kluver E., Adermann K. et al. (2001) Human beta-defensin 4: a novel inducible peptide with a specific salt-sensitive spectrum of antimicrobial activity. FASEB J. **15:** 1819–1821
60 Yamaguchi Y., Nagase T., Makita R., Fukuhara S., Tomita T., Tominaga T. et al. (2002) Identification of multiple novel epididymis-specific beta-defensin isoforms in humans and mice. J. Immunol. **169:** 2516–2523
61 Harder J., Meyer-Hoffert U., Wehkamp K., Schwichtenberg L. and Schroder J. M. (2004) Differential gene induction of human beta-defensins (hBD-1, -2, -3, and -4) in keratinocytes is inhibited by retinoic acid. J. Invest. Dermatol. **123:** 522–529
62 Semple C. A., Rolfe M. and Dorin J. R. (2003) Duplication and selection in the evolution of primate beta-defensin genes. Genome Biol. **4:** R31
63 Premratanachai P., Joly S., Johnson G. K., McCray P. B., Jia H. P. and Guthmiller J. M. (2004) Expression and regulation of novel human -defensins in gingival keratinocytes. Oral Microbiol. Immunol. **19:** 111–117

64 Kao C. Y., Chen Y., Zhao Y. H. and Wu R. (2003) ORFeome-based search of airway epithelial cell-specific novel human [beta]-defensin genes. Am. J. Respir. Cell Mol. Biol. **29:** 71–80
65 Liu Q., Hamil K. G., Sivashanmugam P., Grossman G., Soundararajan R., Rao A. J. et al. (2001) Primate epididymis-specific proteins: characterization of ESC42, a novel protein containing a trefoil-like motif in monkey and human. Endocrinology **142:** 4529–4539
66 Radhakrishnan Y., Hamil K. G., Yenugu S., Young S. L., French F. S. and Hall S. H. (2005) Identification, characterization, and evolution of a primate beta-defensin gene cluster. Genes Immun. **6:** 203–210
67 Yenugu S., Hamil K. G., Radhakrishnan Y., French F. S. and Hall S. H. (2004) The androgen-regulated epididymal sperm-binding protein, human beta-defensin 118 (DEFB118) (formerly ESC42), is an antimicrobial beta-defensin. Endocrinology **145:** 3165–3173
68 Rodriguez-Jimenez F. J., Krause A., Schulz S., Forssmann W. G., Conejo-Garcia J. R., Schreeb R. et al. (2003) Distribution of new human beta-defensin genes clustered on chromosome 20 in functionally different segments of epididymis. Genomics **81:** 175–183
69 Yudin A. I., Generao S. E., Tollner T. L., Treece C. A., Overstreet J. W. and Cherr G. N. (2005) Beta-defensin 126 on the cell surface protects sperm from immunorecognition and binding of anti-sperm antibodies. Biol. Reprod. **73:**1243–52
70 Harder J., Bartels J., Christophers E. and Schröder J. M. (1997) A peptide antibiotic from human skin. Nature **387:** 861
71 Jia H. P., Schutte B. C., Schudy A., Linzmeier R., Guthmiller J. M., Johnson G. K. et al. (2001) Discovery of new human β-defensins using a genomics-based approach. Gene **263:** 211–218
72 Bevins C. L., Jones D. E., Dutra A., Schaffzin J. and Muenke M. (1996) Human enteric defensin genes: chromosomal map position and a model for possible evolutionary relationships. Genomics **31:** 95–106
73 Liu L., Zhao C., Heng H. H. and Ganz T. (1997) The human β-defensin-1 and α-defensins are encoded by adjacent genes: two peptide families with differing disulfide topology share a common ancestry. Genomics **43:** 316–320
74 Michaelson D., Rayner J., Couto M. and Ganz T. (1992) Cationic defensins arise from charge-neutralized propeptides: a mechanism for avoiding leukocyte autocytotoxicity? J. Leukoc. Biol. **51:** 634–639
75 Ganz T., Liu L., Valore E. V. and Oren A. (1993) Posttranslational processing and targeting of transgenic human defensin in murine granulocyte, macrophage, fibroblast, and pituitary adenoma cell lines. Blood **82:** 641–650
76 Valore E. V. and Ganz T. (1997) Laboratory production of antimicrobial peptides in native conformation. Methods Mol. Biol. **78:** 115–131
77 Diamond D. L., Kimball J. R., Krisanaprakornkit S., Ganz T. and Dale B. A. (2001) Detection of beta-defensins secreted by human oral epithelial cells. J. Immunol. Methods **256:** 65–76
78 von Horsten H. H., Schafer B. and Kirchhoff C. (2004) SPAG11/isoform HE2C, an atypical anionic beta-defensin-like peptide. Peptides **25:** 1223–1233
79 Kirchhoff C. (1999) Gene expression in the epididymis. Int. Rev. Cytol. **188:** 133–202
80 Kirchhoff C., Osterhoff C., Pera I. and Schroter S. (1998) Function of human epididymal proteins in sperm maturation. Andrologia **30:** 225–232
81 Osterhoff C., Kirchhoff C., Krull N. and Ivell R. (1994) Molecular cloning and characterization of a novel human sperm antigen (HE2) specifically expressed in the proximal epididymis. Biol. Reprod. **50:** 516–525
82 Fröhlich O., Po C. and Young L. G. (2001) Organization of the human gene encoding the epididymis-specific EP2 protein variants and its relationship to defensin genes. Biol. Reprod. **64:** 1072–1079
83 Fröhlich O., Ibrahim N. M. and Young L. G. (2003) EP2 splicing variants in rhesus monkey (Macaca mulatta) epididymis. Biol. Reprod. **71:** 1453–1460
84 Hamil K. G., Sivashanmugam P., Richardson R. T., Grossman G., Ruben S. M., Mohler J. L. et al. (2000) HE2beta and HE2gamma, new members of an epididymis-specific family of androgen-regulated proteins in the human. Endocrinology **141:** 1245–1253
85 Yenugu S., Hamil K. G., Birse C. E., Ruben S. M., French F. S. and Hall S. H. (2003) Antibacterial properties of the sperm-binding proteins and peptides of human epididymis 2 (HE2) family; salt sensitivity, structural dependence and their interaction with outer and cytoplasmic membranes of *Escherichia coli*. Biochem. J. **372:** 473–483
86 von Horsten H. H., Derr P. and Kirchhoff C. (2002) Novel antimicrobial peptide of human epididymal duct origin. Biol. Reprod. **67:** 804–813
87 Goldman M. J., Anderson G. M., Stolzenberg E. D., Kari U. P., Zasloff M. and Wilson J. M. (1997) Human beta-defensin-1 is a salt-sensitive antibiotic in lung that is inactivated in cystic fibrosis. Cell **88:** 553–560
88 Ali R. S., Falconer A., Ikram M., Bissett C. E., Cerio R. and Quinn A. G. (2001) Expression of the peptide antibiotics human beta defensin-1 and human beta defensin-2 in normal human skin. J. Invest. Dermatol. **117:** 106–111
89 Duits L. A., Ravensbergen B., Rademaker M., Hiemstra P. S. and Nibbering P. H. (2002) Expression of beta-defensin 1 and 2 mRNA by human monocytes, macrophages and dendritic cells. Immunology **106:** 517–525
90 Feng Z., Jiang B., Chandra J., Ghannoum M., Nelson S. and Weinberg A. (2005) Human beta-defensins: differential activity against candidal species and regulation by *Candida albicans*. J. Dent. Res. **84:** 445–450
91 Joly S., Organ C. C., Johnson G. K., McCray P. B. Jr and Guthmiller J. M. (2005) Correlation between beta-defensin expression and induction profiles in gingival keratinocytes. Mol. Immunol. **42:** 1073–1084
92 Sorensen O. E., Thapa D. R., Rosenthal A., Liu L., Roberts A. A. and Ganz T. (2005) Differential regulation of beta-defensin expression in human skin by microbial stimuli. J. Immunol. **174:** 4870–4879
93 Zhu B. D., Feng Y., Huang N., Wu Q. and Wang B. Y. (2003) Mycobacterium bovis bacille Calmette-Guerin (BCG) enhances human beta-defensin-1 gene transcription in human pulmonary gland epithelial cells. Acta Pharmacol. Sin. **24:** 907–912
94 Fang X. M., Shu Q., Chen Q. X., Book M., Sahl H. G., Hoeft A. et al. (2003) Differential expression of alpha- and beta-defensins in human peripheral blood. Eur. J. Clin. Invest. **33:** 82–87
95 Singh P. K., Jia H. P., Wiles K., Hesselberth J., Liu L., Conway B. A. et al. (1998) Production of β-defensins by human airway epithelia. Proc. Natl. Acad. Sci. USA **95:** 14961–14966
96 Dunsche A., Acil Y., Dommisch H., Siebert R., Schroder J. M. and Jepsen S. (2002) The novel human beta-defensin-3 is widely expressed in oral tissues. Eur. J. Oral Sci. **110:** 121–124
97 O'Neil D. A., Cole S. P., Martin-Porter E., Housley M. P., Liu L., Ganz T. et al. (2000) Regulation of human beta-defensins by gastric epithelial cells in response to infection with *Helicobacter pylori* or stimulation with interleukin-1. Infect. Immun. **68:** 5412–5415
98 Tsutsumi-Ishii Y. and Nagaoka I. (2003) Modulation of human beta-defensin-2 transcription in pulmonary epithelial cells by lipopolysaccharide-stimulated mononuclear phagocytes via proinflammatory cytokine production. J. Immunol. **170:** 4226–4236
99 Sorensen O. E., Cowland J. B., Theilgaard-Monch K., Liu L., Ganz T. and Borregaard N. (2003) Wound healing and expression of antimicrobial peptides/polypeptides in human

keratinocytes, a consequence of common growth factors. J. Immunol. **170:** 5583–5589
100 Fehlbaum P., Rao M., Zasloff M. and Anderson G. M. (2000) An essential amino acid induces epithelial beta -defensin expression. Proc. Natl. Acad. Sci. USA **97:** 12723–12728
101 Wang T. T., Nestel F. P., Bourdeau V., Nagai Y., Wang Q., Liao J. et al. (2004) Cutting edge: 1,25-dihydroxyvitamin D3 is a direct inducer of antimicrobial peptide gene expression. J. Immunol. **173:** 2909–2912
102 Dale B. A. and Krisanaprakornkit S. (2001) Defensin antimicrobial peptides in the oral cavity. J. Oral Pathol. Med. **30:** 321–327
103 Harder J., Meyer-Hoffert U., Teran L. M., Schwichtenberg L., Bartels J., Maune S. et al. (2000) Mucoid Pseudomonas aeruginosa, TNF-alpha, and IL-1beta, but not IL-6, induce human beta-defensin-2 in respiratory epithelia. Am. J. Respir. Cell Mol. Biol. **22:** 714–721
104 Froy O. (2005) Regulation of mammalian defensin expression by Toll-like receptor-dependent and independent signalling pathways. Cell Microbiol. **7:** 1387–1397
105 Liu L., Wang L., Jia H. P., Zhao C., Heng H. H., Schutte B. C. et al. (1998) Structure and mapping of the human β-defensin HBD-2 gene and its expression at sites of inflammation. Gene **222:** 237–244
106 McDermott A. M., Redfern R. L., Zhang B., Pei Y., Huang L. and Proske R. J. (2003) Defensin expression by the cornea: multiple signalling pathways mediate IL-1beta stimulation of hBD-2 expression by human corneal epithelial cells. Invest Ophthalmol. Vis. Sci. **44:** 1859–1865
107 Tsutsumi-Ishii Y. and Nagaoka I. (2002) NF-kappa B-mediated transcriptional regulation of human beta-defensin-2 gene following lipopolysaccharide stimulation. J. Leukoc. Biol. **71:** 154–162
108 Jang B. C., Lim K. J., Paik J. H., Kwon Y. K., Shin S. W., Kim S. C. et al. (2004) Up-regulation of human beta-defensin 2 by interleukin-1beta in A549 cells: involvement of PI3K, PKC, p38 MAPK, JNK, and NF-kappaB. Biochem. Biophys. Res. Commun. **320:** 1026–1033
109 Chung W. O. and Dale B. A. (2004) Innate immune response of oral and foreskin keratinocytes: utilization of different signaling pathways by various bacterial species. Infect. Immun. **72:** 352–358
110 Wehkamp J., Harder J., Wehkamp K., Wehkamp-von Meissner B., Schlee M., Enders C. et al. (2004) NF-kappaB- and AP-1-mediated induction of human beta defensin-2 in intestinal epithelial cells by *Escherichia coli* Nissle 1917: a novel effect of a probiotic bacterium. Infect. Immun. **72:** 5750–5758
111 Donnarumma G., Paoletti I., Buommino E., Orlando M., Tufano M. A. and Baroni A. (2004) Malassezia furfur induces the expression of beta-defensin-2 in human keratinocytes in a protein kinase C-dependent manner. Arch. Dermatol. Res. **295:** 474–481
112 Chung W. O., Hansen S. R., Rao D. and Dale B. A. (2004) Protease-activated receptor signaling increases epithelial antimicrobial peptide expression. J. Immunol. **173:** 5165–5170
113. Birchler T., Seibl R., Buchner K., Loeliger S., Seger R., Hossle J. P. et al. (2001) Human Toll-like receptor 2 mediates induction of the antimicrobial peptide human beta-defensin 2 in response to bacterial lipoprotein. Eur. J. Immunol. **31:** 3131–3137
114 Wang X., Zhang Z., Louboutin J. P., Moser C., Weiner D. J. and Wilson J. M. (2003) Airway epithelia regulate expression of human beta-defensin 2 through Toll-like receptor 2. FASEB J. **17:** 1727–1729
115 Hertz C. J., Wu Q., Porter E. M., Zhang Y. J., Weismuller K. H., Godowski P. J. et al. (2003) Activation of Toll-like receptor 2 on human tracheobronchial epithelial cells induces the antimicrobial peptide human beta defensin-2. J. Immunol. **171:** 6820–6826
116 Macredmond R. E., Greene C. M., Taggart C. T., McElvaney N. G. and O'Neill S. (2005) Respiratory epithelial cells require Toll-like receptor 4 for induction of Human b-defensin 2 by lipopolysaccharide. Respir. Res. **6:** 116
117 Kawai K., Shimura H., Minagawa M., Ito A., Tomiyama K. and Ito M. (2002) Expression of functional Toll-like receptor 2 on human epidermal keratinocytes. J. Dermatol. Sci. **30:** 185–194
118 Nagy I., Pivarcsi A., Koreck A., Szell M., Urban E. and Kemeny L. (2005) Distinct strains of Propionibacterium acnes induce selective human beta-defensin-2 and interleukin-8 expression in human keratinocytes through toll-like receptors. J. Invest. Dermatol. **124:** 931–938
119 Pivarcsi A., Nagy I., Koreck A., Kis K., Kenderessy-Szabo A., Szell M. et al. (2005) Microbial compounds induce the expression of pro-inflammatory cytokines, chemokines and human beta-defensin-2 in vaginal epithelial cells. Microbes. Infect. **7:**1117–27
120 Vora P., Youdim A., Thomas L. S., Fukata M., Tesfay S. Y., Lukasek K. et al. (2004) Beta-defensin-2 expression is regulated by TLR signaling in intestinal epithelial cells. J. Immunol. **173:** 5398–5405
121 Platz J., Beisswenger C., Dalpke A., Koczulla R., Pinkenburg O., Vogelmeier C. et al. (2004) Microbial DNA induces a host defense reaction of human respiratory epithelial cells. J. Immunol. **173:** 1219–1223
122 Schaefer T. M., Fahey J. V., Wright J. A. and Wira C. R. (2005) Innate immunity in the human female reproductive tract: antiviral response of uterine epithelial cells to the TLR3 agonist poly(I:C). J. Immunol. **174:** 992–1002
123 Yang D., Biragyn A., Hoover D. M., Lubkowski J. and Oppenheim J. J. (2004) Multiple roles of antimicrobial defensins, cathelicidins, and eosinophil-derived neurotoxin in host defense. Annu. Rev. Immunol. **22:** 181–215
124 Dacheux J. L., Gatti J. L. and Dacheux F. (2003) Contribution of epididymal secretory proteins for spermatozoa maturation. Microsc. Res. Tech. **61:** 7–17
125 Ganz T., Oren A. and Lehrer R. I. (1992) Defensins: microbicidal and cytotoxic peptides of mammalian host defense cells. Med. Microbiol. Immunol. (Berl) **181:** 99–105
126 Medzhitov R. and Janeway C. A. Jr (1999) Innate immune induction of the adaptive immune response. Cold Spring Harb. Symp. Quant. Biol. **64:** 429–435
127 Boman H. G. (2000) Innate immunity and the normal microflora. Immunol. Rev. **173:** 5–16
128 Fiuza C. and Suffredini A. F. (2001) Human models of innate immunity: local and systemic inflammatory responses. J. Endotoxin. Res. **7:** 385–388
129 Hoffmann J. A., Kafatos F. C., Janeway C. A. and Ezekowitz R. A. (1999) Phylogenetic perspectives in innate immunity. Science **284:** 1313–1318
130 Oppenheim J. J. and Yang D. (2005) Alarmins: chemotactic activators of immune responses. Curr. Opin. Immunol. **17:** 359–365
131 Zucht H. D., Grabowsky J., Schrader M., Liepke C., Jurgens M., Schulz-Knappe P. et al. (1998) Human beta-defensin-1: a urinary peptide present in variant molecular forms and its putative functional implication. Eur. J. Med. Res. **3:** 315–323
132 Ganz T. and Lehrer R. I. (1998) Antimicrobial peptides of vertebrates. Curr. Opin. Immunol. **10:** 41–44
133 Schulz A., Kluver E., Schulz-Maronde S. and Adermann K. (2005) Engineering disulfide bonds of the novel human beta-defensins hBD-27 and hBD-28: differences in disulfide formation and biological activity among human beta-defensins. Biopolymers **80:** 34–49
134 Joly S., Maze C., McCray P. B. Jr and Guthmiller J. M. (2004) Human beta-defensins 2 and 3 demonstrate strain-selective

activity against oral microorganisms. J. Clin. Microbiol. **42:** 1024–1029

135 Chen X., Niyonsaba F., Ushio H., Okuda D., Nagaoka I., Ikeda S. et al. (2005) Synergistic effect of antibacterial agents human beta-defensins, cathelicidin LL-37 and lysozyme against *Staphylococcus aureus* and *Escherichia coli*. J. Dermatol. Sci. **40:** 123–132

136 Lehrer R. I., Barton A., Daher K. A., Harwig S. S., Ganz T. and Selsted M. E. (1989) Interaction of human defensins with *Escherichia coli*. Mechanism of bactericidal activity. J. Clin. Invest. **84:** 553–561

137 Huang G. T., Zhang H. B., Kim D., Liu L. and Ganz T. (2002) A model for antimicrobial gene therapy: demonstration of human beta-defensin 2 antimicrobial activities *in vivo*. Hum. Gene Ther. **13:** 2017–2025

138 Sun L., Finnegan C. M., Kish-Catalone T., Blumenthal R., Garzino-Demo P., Terra Maggiore G. M. et al. (2005) Human β-defensins suppress human immunodeficiency virus infection: potential role in mucosal protection. J. Virol. **79:** 14318–14329

139 Quinones-Mateu M. E., Lederman M. M., Feng Z., Chakraborty B., Weber J., Rangel H. R. et al. (2003) Human epithelial beta-defensins 2 and 3 inhibit HIV-1 replication. AIDS **17:** F39–F48

140 Chang T. L. and Klotman M. E. (2004) Defensins: natural anti-HIV peptides. AIDS Rev. **6:** 161–168

141 Chang T. L., Francois F., Mosoian A. and Klotman M. E. (2003) CAF-mediated human immunodeficiency virus (HIV) type 1 transcriptional inhibition is distinct from alpha-defensin-1 HIV inhibition. J. Virol. **77:** 6777–6784

142 Chang T. L., Vargas J. Jr, DelPortillo A. and Klotman M. E. (2005) Dual role of alpha-defensin-1 in anti-HIV-1 innate immunity. J. Clin. Invest **115:** 765–773

143 Leikina E., Delanoe-Ayari H., Melikov K., Cho M. S., Chen A., Waring A. J. et al. (2005) Carbohydrate-binding molecules inhibit viral fusion and entry by crosslinking membrane glycoprotein. Nat. Immunol. **6:** 995–1001

144 Braida L., Boniotto M., Pontillo A., Tovo P. A., Amoroso A. and Crovella S. (2004) A single-nucleotide polymorphism in the human beta-defensin 1 gene is associated with HIV-1 infection in Italian children. AIDS **18:** 1598–1600

145 Alp S., Skrygan M., Schlottmann R., Kreuter A., Otte J. M., Schmidt W. E. et al. (2005) Expression of beta-defensin 1 and 2 in nasal epithelial cells and alveolar macrophages from HIV-infected patients. Eur. J. Med. Res. **10:** 1–6

146 Niyonsaba F., Iwabuchi K., Matsuda H., Ogawa H. and Nagaoka I. (2002) Epithelial cell-derived human beta-defensin-2 acts as a chemotaxin for mast cells through a pertussis toxin-sensitive and phospholipase C-dependent pathway. Int. Immunol. **14:** 421–426

147 Niyonsaba F., Ogawa H. and Nagaoka I. (2004) Human beta-defensin-2 functions as a chemotactic agent for tumour necrosis factor-alpha-treated human neutrophils. Immunology **111:** 273–281

148 Niyonsaba F., Someya A., Hirata M., Ogawa H. and Nagaoka I. (2001) Evaluation of the effects of peptide antibiotics human beta-defensins-1/-2 and LL-37 on histamine release and prostaglandin D(2) production from mast cells. Eur. J. Immunol. **31:** 1066–1075

149 Conejo-Garcia J. R., Benencia F., Courreges M. C., Kang E., Mohamed-Hadley A., Buckanovich R. J. et al. (2004) Tumor-infiltrating dendritic cell precursors recruited by a beta-defensin contribute to vasculogenesis under the influence of Vegf-A. Nat. Med. **10:** 950–958

150 Biragyn A., Surenhu M., Yang D., Ruffini P. A., Haines B. A., Klyushnenkova E. et al. (2001) Mediators of innate immunity that target immature, but not mature, dendritic cells induce antitumor immunity when genetically fused with nonimmunogenic tumor antigens. J. Immunol. **167:** 6644–6653

151 Zlotnik A. and Yoshie O. (2000) Chemokines: a new classification system and their role in immunity. Immunity **12:** 121–127

152 Pernet I., Reymermier C., Guezennec A., Branka J. E., Guesnet J., Perrier E. et al. (2003) Calcium triggers beta-defensin (hBD-2 and hBD-3) and chemokine macrophage inflammatory protein-3 alpha (MIP-3alpha/CCL20) expression in monolayers of activated human keratinocytes. Exp. Dermatol. **12:** 755–760

153 Brogden K. A., Heidari M., Sacco R. E., Palmquist D., Guthmiller J. M., Johnson G. K. et al. (2003) Defensin-induced adaptive immunity in mice and its potential in preventing periodontal disease. Oral Microbiol. Immunol. **18:** 95–99

154 Chaly Y. V., Paleolog E. M., Kolesnikova T. S., Tikhonov I. I., Petratchenko E. V. and Voitenok N. N. (2000) Neutrophil alpha-defensin human neutrophil peptide modulates cytokine production in human monocytes and adhesion molecule expression in endothelial cells. Eur. Cytokine Netw. **11:** 257–266

155 Lillard J. W. Jr, Boyaka P. N., Chertov O., Oppenheim J. J. and McGhee J. R. (1999) Mechanisms for induction of acquired host immunity by neutrophil peptide defensins. Proc. Natl. Acad. Sci. USA **96:** 651–656

156 Donald C. D., Sun C. Q., Lim S. D., Macoska J., Cohen C., Amin M. B. et al. (2003) Cancer-specific loss of beta-defensin 1 in renal and prostatic carcinomas. Lab. Invest. **83:** 501–505

157 Niyonsaba F., Ushio H., Nagaoka I., Okumura K. and Ogawa H. (2005) The human {beta}-defensins (-1, -2, -3, -4) and cathelicidin LL-37 induce IL-18 secretion through p38 and ERK MAPK activation in primary human keratinocytes. J. Immunol. **175:** 1776–1784

158 Abiko Y., Nishimura M., Kusano K., Yamazaki M., Arakawa T., Takuma T. et al. (2003) Upregulated expression of human beta defensin-1 and -3 mRNA during differentiation of keratinocyte immortalized cell lines, HaCaT and PHK16–0b. J. Dermatol. Sci. **31:** 225–228

159 Shiba H., Mouri Y., Komatsuzawa H., Ouhara K., Takeda K., Sugai M. et al. (2003) Macrophage inflammatory protein-3alpha and beta-defensin-2 stimulate dentin sialophosphoprotein gene expression in human pulp cells. Biochem. Biophys. Res. Commun. **306:** 867–871

160 Varoga D., Pufe T., Harder J., Schroder J. M., Mentlein R., Meyer-Hoffert U. et al. (2005) Human beta-defensin 3 mediates tissue remodeling processes in articular cartilage by increasing levels of metalloproteinases and reducing levels of their endogenous inhibitors. Arthritis Rheum. **52:** 1736–1745

161 Young A. N., Oliveira Salles P. G., Lim S. D., Cohen C., Petros J. A., Marshall F. F. et al. (2003) Beta defensin-1, parvalbumin, and vimentin: a panel of diagnostic immunohistochemical markers for renal tumors derived from gene expression profiling studies using cDNA microarrays. Am. J. Surg. Pathol. **27:** 199–205

162 Markeeva N., Lisovskiy I., Lyzogubov V., Usenko V., Soldatkina M., Merentsev S. et al. (2005) Expression of beta-defensin-2 in human gastric tumors: a pilot study. Exp. Oncol. **27:** 130–135

163 Zhou C. X., Zhang Y. L., Xiao L., Zheng M., Leung K. M., Chan M. Y. et al. (2004) An epididymis-specific beta-defensin is important for the initiation of sperm maturation. Nat. Cell Biol. **6:** 458–464

164 Sahl H. G., Pag U., Bonness S., Wagner S., Antcheva N. and Tossi A. (2005) Mammalian defensins: structures and mechanism of antibiotic activity. J. Leukoc. Biol. **77:** 466–475

165 Hoover D. M., Rajashankar K. R., Blumenthal R., Puri A., Oppenheim J. J., Chertov O. et al. (2000) The structure of human β-defensin-2 shows evidence of higher order oligomerization. J. Biol. Chem. **275:** 32911–32918

166 Hoover D. M., Chertov O. and Lubkowski J. (2001) The structure of human β-defensin-1: new insights into structural properties of beta-defensins. J. Biol. Chem. **276:** 39021–39026

167 Sawai M. V., Jia H. P., Liu L., Aseyev V., Wiencek J. M., McCray P. B. Jr et al. (2001) The NMR structure of human beta-defensin-2 reveals a novel alpha-helical segment. Biochemistry **40:** 3810–3816

168 Schibli D. J., Hunter H. N., Aseyev V., Starner T. D., Wiencek J. M., McCray P. B. Jr et al. (2002) The solution structures of the human β-defensins lead to a better understanding of the potent bactericidal activity of HBD3 against *Staphylococcus aureus*. J. Biol. Chem. **277:** 8279–8289

169 Bauer F., Schweimer K., Kluver E., Conejo-Garcia J. R., Forssmann W. G., Rosch P. et al. (2001) Structure determination of human and murine beta-defensins reveals structural conservation in the absence of significant sequence similarity. Protein Sci. **10:** 2470–2479

170 Thompson J. D., Higgins D. G. and Gibson T. J. (1994) CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:** 4673–4680

171 Connolly M. L. (1983) Solvent-accessible surfaces of proteins and nucleic acids. Science **221:** 709–713

172 Koradi R., Billeter M. and Wuthrich K. (1996) MOLMOL: a program for display and analysis of macromolecular structures. J. Mol. Graph. **14:** 51–32

173 Nicholls A., Sharp K. A. and Honig B. (1991) Protein folding and association: insights from the interfacial and thermodynamic properties of hydrocarbons. Proteins **11:** 281–296

174 Andreeva A., Howorth D., Brenner S. E., Hubbard T. J., Chothia C. and Murzin A. G. (2004) SCOP database in 2004: refinements integrate structure and sequence family data. Nucleic Acids Res. **32:** D226-D229

175 Murzin A. G., Brenner S. E., Hubbard T. and Chothia C. (1995) SCOP: a structural classification of proteins database for the investigation of sequences and structures. J. Mol. Biol. **247:** 536–540

176 Hill C. P., Yee J., Selsted M. E. and Eisenberg D. (1991) Crystal structure of defensin HNP-3, an amphiphilic dimer: mechanisms of membrane permeabilization. Science **251:** 1481–1485

177 Torres A. M. and Kuchel P. W. (2004) The beta-defensin-fold family of polypeptides. Toxicon **44:** 581–588

178 Torres A. M., de Plater G. M., Doverskog M., Birinyi-Strachan L. C., Nicholson G. M., Gallagher C. H. et al. (2000) Defensin-like peptide-2 from platypus venom: member of a class of peptides with a distinct structural fold. Biochem. J. **348** 649–656

179 Arolas J. L., Popowicz G. M., Lorenzo J., Sommerhoff C. P., Huber R., Aviles F. X. et al. (2005) The three-dimensional structures of tick carboxypeptidase inhibitor in complex with A/B carboxypeptidases reveal a novel double-headed binding mode. J. Mol. Biol. **350:** 489–498

180. Wu Z., Hoover D. M., Yang D., Boulegue C., Santamaria F., Oppenheim J. J. et al. (2003) Engineering disulfide bridges to dissect antimicrobial and chemotactic activities of human beta-defensin 3. Proc. Natl. Acad. Sci. USA **100:** 8880–8885

181 Xie C., Prahl A., Ericksen B., Wu Z., Zeng P., Li X. et al. (2005) Reconstruction of the conserved β-bulge in mammalian defensins using D-amino acids. J. Biol. Chem. **280:** 32921–32929

182 Yount N. Y. and Yeaman M. R. (2004) Multidimensional signatures in antimicrobial peptides. Proc. Natl. Acad. Sci. USA **101:** 7363–7368

183 Landon C., Thouzeau C., Labbe H., Bulet P. and Vovelle F. (2004) Solution structure of spheniscin, a beta-defensin from the penguin stomach. J. Biol. Chem. **279:** 30433–30439

184 Zimmermann G. R., Legault P., Selsted M. E. and Pardi A. (1995) Solution structure of bovine neutrophil beta-defensin-12: the peptide fold of the beta-defensins is identical to that of the classical defensins. Biochemistry **34:** 13663–13671

185 Boniotto M., Antcheva N., Zelezetsky I., Tossi A., Palumbo V., Verga Falzacappa M. V. et al. (2003) A study of host defence peptide beta-defensin 3 in primates. Biochem. J. **374:** 707–714

186 Sugiarto H. and Yu P. L. (2004) Avian antimicrobial peptides: the defense role of beta-defensins. Biochem. Biophys. Res. Commun. **323:** 721–727

187 Zhao C., Nguyen T., Liu L., Sacco R. E., Brogden K. A. and Lehrer R. I. (2001) Gallinacin-3, an inducible epithelial beta-defensin in the chicken. Infect. Immun. **69:** 2684–2691

188 Fogh R. H., Kem W. R. and Norton R. S. (1990) Solution structure of neurotoxin I from the sea anemone *Stichodactyla helianthus*. A nuclear magnetic resonance, distance geometry, and restrained molecular dynamics study. J. Biol. Chem. **265:** 13016–13028

189 Pallaghy P. K., Scanlon M. J., Monks S. A. and Norton R. S. (1995) Three-dimensional structure in solution of the polypeptide cardiac stimulant anthopleurin-A. Biochemistry **34:** 3782–3794

190 Torres A. M., Wang X., Fletcher J. I., Alewood D., Alewood P. F., Smith R. et al. (1999) Solution structure of a defensin-like peptide from platypus venom. Biochem. J. **341:** 785–794

191 Nicastro G., Franzoni L., de Chiara C., Mancin A. C., Giglio J. R. and Spisni A. (2003) Solution structure of crotamine, a Na+ channel affecting toxin from *Crotalus durissus terrificus* venom. Eur. J. Biochem. **270:** 1969–1979

192 Harwig S. S., Swiderek K. M., Kokryakov V. N., Tan L., Lee T. D., Panyutich E. A. et al. (1994) Gallinacins: cysteine-rich antimicrobial peptides of chicken leukocytes. FEBS Lett. **342:** 281–285

193 Evans E. W., Beach F. G., Moore K. M., Jackwood M. W., Glisson J. R. and Harmon B. G. (1995) Antimicrobial activity of chicken and turkey heterophil peptides CHP1, CHP2, THP1, and THP3. Vet. Microbiol. **47:** 295–303

194 Thouzeau C., Le Maho Y., Froget G., Sabatier L., Le Bohec C., Hoffmann J. A. et al. (2003) Spheniscins, avian beta-defensins in preserved stomach contents of the king penguin, *Aptenodytes patagonicus*. J. Biol. Chem. **278:** 51053–51058

195 Antcheva N., Boniotto M., Zelezetsky I., Pacor S., Verga Falzacappa M. V., Crovella S. et al. (2004) Effects of positively selected sequence variations in human and *Macaca fascicularis* beta-defensins 2 on antimicrobial activity. Antimicrob. Agents Chemother. **48:** 685–688

196 Kluver E., Schulz-Maronde S., Scheid S., Meyer B., Forssmann W. G. and Adermann K. (2005) Structure-activity relation of human beta-defensin 3: influence of disulfide bonds and cysteine substitution on antimicrobial activity and cytotoxicity. Biochemistry **44:** 9804–9816

197 Hwang P. M. and Vogel H. J. (1998) Structure-function relationships of antimicrobial peptides. Biochem. Cell Biol. **76:** 235–246

198 Maemoto A., Qu X., Rosengren K. J., Tanabe H., Henschen-Edman A., Craik D. J. et al. (2004) Functional analysis of the alpha-defensin disulfide array in mouse cryptdin-4. J. Biol. Chem. **279:** 44188–44196

199 Kagan B. L., Selsted M. E., Ganz T. and Lehrer R. I. (1990) Antimicrobial defensin peptides form voltage-dependent ion-permeable channels in planar lipid bilayer membranes. Proc. Natl. Acad. Sci. USA **87:** 210–214

200 Wimley W. C., Selsted M. E. and White S. H. (1994) Interactions between human defensins and lipid bilayers: evidence for formation of multimeric pores. Protein Sci. **3:** 1362–1373

201 Brissette C. A. and Lukehart S. A. (2002) *Treponema denticola* is resistant to human beta-defensins. Infect. Immun. **70:** 3982–3984

202 Peschel A. (2002) How do bacteria resist human antimicrobial peptides? Trends Microbiol. **10:** 179–186

203 Xie C., Zeng P., Ericksen B., Wu Z., Lu W. Y. and Lu W. (2005) Effects of the terminal charges in human neutrophil alpha-

defensin 2 on its bactericidal and membrane activity. Peptides **26:** 2377–83
204 Tanabe H., Qu X., Weeks C. S., Cummings J. E., Kolusheva S., Walsh K. B. et al. (2004) Structure-activity determinants in paneth cell alpha-defensins: loss-of-function in mouse cryptdin-4 by charge-reversal at arginine residue positions. J. Biol. Chem. **279:** 11976–11983
205 Satchell D. P., Sheynis T., Kolusheva S., Cummings J., Vanderlick T. K., Jelinek R. et al. (2003) Quantitative interactions between cryptdin-4 amino terminal variants and membranes. Peptides **24:** 1795–1805
206 Raj P. A., Antonyraj K. J. and Karunakaran T. (2000) Large-scale synthesis and functional elements for the antimicrobial activity of defensins. Biochem. J. **347:** 633–641
207 Ouhara K., Komatsuzawa H., Yamada S., Shiba H., Fujiwara T., Ohara M. et al. (2005) Susceptibilities of periodontopathogenic and cariogenic bacteria to antibacterial peptides, β-defensins and LL37, produced by human epithelial cells. J. Antimicrob. Chemother. **55:** 888–896
208 Fujii G., Selsted M. E. and Eisenberg D. (1993) Defensins promote fusion and lysis of negatively charged membranes. Protein Sci. **2:** 1301–1312
209 Zanich A., Pascall J. C. and Jones R. (2003) Secreted epididymal glycoprotein 2D6 that binds to the sperm‘s plasma membrane is a member of the beta-defensin superfamily of pore-forming glycopeptides. Biol. Reprod. **69:** 1831–1842
210 Lohner K., Latal A., Lehrer R. I. and Ganz T. (1997) Differential scanning microcalorimetry indicates that human defensin, HNP-2, interacts specifically with biomembrane mimetic systems. Biochemistry **36:** 1525–1531
211 Tossi A., Sandri L. and Giangaspero A. (2000) Amphipathic, alpha-helical antimicrobial peptides. Biopolymers **55:** 4–30
212 Oren Z. and Shai Y. (1998) Mode of action of linear amphipathic alpha-helical antimicrobial peptides. Biopolymers **47:** 451–463
213 Matsuzaki K. (1998) Magainins as paradigm for the mode of action of pore forming polypeptides. Biochim. Biophys. Acta **1376:** 391–400
214 Trabi M., Schirra H. J. and Craik D. J. (2001) Three-dimensional structure of RTD-1, a cyclic antimicrobial defensin from Rhesus macaque leukocytes. Biochemistry **40:** 4211–4221
215 Hristova K., Selsted M. E. and White S. H. (1996) Interactions of monomeric rabbit neutrophil defensins with bilayers: comparison with dimeric human defensin HNP-2. Biochemistry **35:** 11888–11894
216 Cociancich S., Ghazi A., Hetru C., Hoffmann J. A. and Letellier L. (1993) Insect defensin, an inducible antibacterial peptide, forms voltage-dependent channels in *Micrococcus luteus*. J. Biol. Chem. **268:** 19239–19245
217 Shai Y. (1999) Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by alpha-helical antimicrobial and cell non-selective membrane-lytic peptides. Biochim. Biophys. Acta **1462:** 55–70
218 Campopiano D. J., Clarke D. J., Polfer N. C., Barran P. E., Langley R. J., Govan J. R. et al. (2004) Structure-activity relationships in defensin dimers: a novel link between beta-defensin tertiary structure and antimicrobial activity. J. Biol. Chem. **279:** 48671–48679
219 Mandal M., Jagannadham M. V. and Nagaraj R. (2002) Antibacterial activities and conformations of bovine β-defensin BNBD-12 and analogs: structural and disulfide bridge requirements for activity. Peptides **23:** 413–418
220 Krishnakumari V., Sharadadevi A., Singh S. and Nagaraj R. (2003) Single disulfide and linear analogues corresponding to the carboxy-terminal segment of bovine beta-defensin-2: effects of introducing the beta-hairpin nucleating sequence d-pro-gly on antibacterial activity and biophysical properties. Biochemistry **42:** 9307–9315
221 Hoover D. M., Wu Z., Tucker K., Lu W. and Lubkowski J. (2003) Antimicrobial characterization of human beta-defensin 3 derivatives. Antimicrob. Agents Chemother. **47:** 2804–2809
222 Papo N. and Shai Y. (2005) Host defense peptides as new weapons in cancer treatment. Cell. Mol. Life Sci. **62:** 784–790
223 Skelton N. J., Aspiras F., Ogez J. and Schall T. J. (1995) Proton NMR assignments and solution conformation of RANTES, a chemokine of the C-C type. Biochemistry **34:** 5329–5342
224 Hoover D. M., Boulegue C., Yang D., Oppenheim J. J., Tucker K., Lu W. et al. (2002) The structure of human macrophage inflammatory protein-3alpha /CCL20. Linking antimicrobial and CC chemokine receptor-6-binding activities with human beta-defensins. J. Biol. Chem. **277:** 37647–37654
225 Perez-Canadillas J. M., Zaballos A., Gutierrez J., Varona R., Roncal F., Albar J. P. et al. (2001) NMR solution structure of murine CCL20/MIP-3alpha, a chemokine that specifically chemoattracts immature dendritic cells and lymphocytes through its highly specific interaction with the beta-chemokine receptor CCR6. J. Biol. Chem. **276:** 28372–28379
226 Gong J. H., Uguccioni M., Dewald B., Baggiolini M. and Clark-Lewis I. (1996) RANTES and MCP-3 antagonists bind multiple chemokine receptors. J. Biol. Chem. **271:** 10521–10527
227 Harrison J. K., Fong A. M., Swain P. A., Chen S., Yu Y. R., Salafranca M. N. et al. (2001) Mutational analysis of the fractalkine chemokine domain. Basic amino acid residues differentially contribute to CX3CR1 binding, signaling, and cell adhesion. J. Biol. Chem. **276:** 21632–21641
228 Hemmerich S., Paavola C., Bloom A., Bhakta S., Freedman R., Grunberger D. et al. (1999) Identification of residues in the monocyte chemotactic protein-1 that contact the MCP-1 receptor, CCR2. Biochemistry **38:** 13013–13025
229 Jarnagin K., Grunberger D., Mulkins M., Wong B., Hemmerich S., Paavola C. et al. (1999) Identification of surface residues of the monocyte chemotactic protein 1 that affect signaling through the receptor CCR2. Biochemistry **38:** 16167–16177
230 Clark-Lewis I., Kim K. S., Rajarathnam K., Gong J. H., Dewald B., Moser B. et al. (1995) Structure-activity relationships of chemokines. J. Leukoc. Biol. **57:** 703–711
231 Mayer M. R. and Stone M. J. (2001) Identification of receptor binding and activation determinants in the N-terminal and N-loop regions of the CC chemokine eotaxin. J. Biol. Chem. **276:** 13911–13916
232 Ott T. R., Lio F. M., Olshefski D., Liu X. J., Struthers R. S. and Ling N. (2004) Determinants of high-affinity binding and receptor activation in the N-terminus of CCL-19 (MIP-3 beta). Biochemistry **43:** 3670–3678
233 Schall T. J. (1991) Biology of the RANTES/SIS cytokine family. Cytokine **3:** 165–183
234 Yang D., Chen Q., Hoover D. M., Staley P., Tucker K. D., Lubkowski J. et al. (2003) Many chemokines including CCL20/MIP-3alpha display antimicrobial activity. J. Leukoc. Biol. **74:** 448–455
235 Morrison G., Kilanowski F., Davidson D. and Dorin J. (2002) Characterization of the mouse Beta defensin 1, defb1, mutant mouse model. Infect. Immun. **70:** 3053–3060
236 Moser C., Weiner D. J., Lysenko E., Bals R., Weiser J. N. and Wilson J. M. (2002) beta-Defensin 1 contributes to pulmonary innate immunity in mice. Infect. Immun. **70:** 3068–3072
237 Gallo R. L. and Nizet V. (2003) Endogenous production of antimicrobial peptides in innate immunity and human disease. Curr. Allergy Asthma Rep. **3:** 402–409
238 Eckmann L. (2005) Defence molecules in intestinal innate immunity against bacterial infections. Curr. Opin. Gastroenterol. **21:** 147–151
239 Adermann K., John H., Standker L. and Forssmann W. G. (2004) Exploiting natural peptide diversity: novel research tools and drug leads. Curr. Opin. Biotechnol. **15:** 599–606
240 Biragyn A. (2005) Defensins – non-antibiotic use for vaccine development. Curr. Protein Pept. Sci. **6:** 53–60