Antimicrobial peptides – their role in immunity and therapeutic potential

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Abstract

Antimicrobial peptides (AMPs) have a broad antimicrobial spectrum (gram-positive and gram-negative bacteria, fungi, certain viruses) and lyse microbial cells by interaction with biomembranes. AMPs are produced by bacteria and by other species of all kingdoms. Recently, it has been recognized that their function is essential to the immune response. AMPs participate primarily in the innate immune system of many organisms, including plants, insects and vertebrates. They have multiple roles as mediators of inflammation with impact on epithelial and inflammatory cells, influencing diverse processes such as cell proliferation, wound healing, cytokine release, chemotaxis, immune induction. Recently, pharmaceutical companies have started research programmes related to therapeutical usefulness of AMPs. This review summarises the current knowledge about the basic and applied biology of AMPs.

Key words: *antimicrobial peptides, immunity.*

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Nomenclature and tissue distribution of AMPs

Antimicrobial peptides (AMPs) are an extremely diverse group of more than 700 small proteins and can be divided into several categories on the basis of their structures. Most AMPs are cationic (polar) molecules. There are two classes of AMPs based on the mechanism of their cellular synthesis: (1) non-ribosomally synthesised (largely produced by bacteria) and (2) ribosomally synthesised, gene- encoded peptides [1]. Non-ribosomally synthesised AMPs are produced by multifunctional peptide synthetases. These substances are already used in clinical applications: polymyxin B, polymyxin E, colimycin, cyclosporin, tripeptide ACV (precursor of penicillin and cephalosporins) [2, 3]. AMPs, in a more narrow sense, refer to gene-encoded peptides. These AMPs can be grouped according to their size, conformational structure or predominant amino acid structure. On the basis of their gross composition and 3D structure, AMPs can be divided in four main groups: I: linear peptides with an α-helical structure; II: β-sheet structures stabilised by disulphide bridges; III: peptides with predominance of one or more amino acids; IV: peptides with loop structures [4]. This review will concentrate on the biological relevance of two mammalian AMPs- families: defensins (from group II) and cathelicidins (from group I).

The defensins families of AMPs

Defensins are typically 28 to 44 amino acids long and contain 6 to 8 cysteine residues that form characteristic intramolecular disulfide bridges. Defensins are found in mammals, and distantly related peptides appear in insects and plants [5]. They are classified into 3 families- the α-defensins, the β-defensins and the θ-defensins.

• α-defensins

These defensins are 29 to 35 residues in lenght and contain 3 disulfide bridges. The first human α-defensin was isolated from neutrophils in 1985 [6]. At the present time, six α-defensins have been identified from humans. Human neutrophil peptides 1-4 (HNP1-4) are localised in azurophilic granules [7, 8]. The two other α-defensins, human defensins 5 and 6 (HD5-6) are located in Paneth's cells of the small intestine [8] and in epithelial cells of the female urogenital tract [9]. Unlike neutrophils, Paneth's cells do not store defensins as matured peptides; instead, they store them as

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propeptides [10]. Elevated plasma levels of α-defensins were observed in patients with active pulmonary tuberculosis [11] and children with severe sepsis [12].

• β-defensins

These peptides are 36-42 amino acids in lenght and contain 6 cysteine motifs connected by 3 disulfide bridges. The first β-defensin was isolated from cow airway epithelial cells [13]. In human beings, 4 types of β-defensins have been identified so far. The first human β-defensin, called hBD1 was originally isolated from large volumes of hemofiltrate [14] and is expressed constitutively in epithelial cells of the urinary and respiratory tracts [15, 16] and in human skin epidermis [17, 18]. Human β defensin-2 (hBD2) was isolated from psoriatic skin [19] and in contrast to hBD1 is upregulated in cultured keratinocytes by inflammatory stimuli like IL1 and live Pseudomonas aeruginosa [20] or Staphylococcus aureus, S. epidermidis, E. coli [21]. E. coli LPS is a very weak direct inducer of hBD2 mRNA and peptide, but the induction is amplified by IL1-mediated signaling [22]. Inducible human β defensin-3 was isolated from lesional psoriatic scales and investigations of different tissues revealed skin and tonsil to be major hBD3 mRNA- expressing tissues [23]. Human β defensin-4 was identified solely by searches of genomic database [24]. Recently, 28 new genes for human β defensins were identified by screening of human genome database [25]. Peripheral blood monocytes, monocyte-derived macrophages and monocyte-derived dendritic cells all express hBD1mRNA and hBD2mRNA. HBD2 peptide was demonstrated in blood monocytes and alveolar macrophages [26].

• θ-defensins

This family of defensins has been isolated from rhesus monkey neutrophils [27] and no data about the presence of these molecules in tissues are aviable at this time.

Cathelicidins

The term „cathelicidins" was coined as an attempt to unify a variety of peptides 12 to 80 or more amino acids in length, related by similar „cathelin" precursor domain (cathepsin L inhibitor) [28]. To date, cathelicidins have been found only in mammals [29]: mouse (CRAMP), rat (rCRAMP), pig (protegrin, PMAP-23, PR-39), monkey (rhLL37, RL37), rabbit (CAP-18), sheep (SMAP-29, SMAP-34) and human (hCAP-18/ LL37). Human LL37 was isolated from human bone marrow and is present in neutrophils [30], γδT cells, NK cells, B cells, monocytes/ macrophages from peripheral blood [31]. LL37 is also present in epithelial cells in the respiratory tract, urogenital tract, gastrointestinal tract [32, 33] and skin epidermis, mainly in response to infammatory stimuli [34-36] and cutaneous injury [37]. Human CAP-18/LL37 is also induced in keratinocytes by growth factors like IGF1 and TNFα, both important in wound healing process [38]. In addition, plasma has been reported to contain LL37 [39]. The mouse cathelin related antibicrobial peptide (CRAMP) expression resembles that of LL37 in human [40].

The role of AMPs in immune response

In higher vertebrates such as mammals, there are two types of immunity: innate (or natural) and adaptive (or acquired). The effector branch of innate immunity consists of two major aspects. One is the generation of humoral antibacterial mediators such as complement and AMPs (including defensins and cathelicidins), as well as large antimicrobial substances (lysozyme, cathepsin G, lactoferrin). The other is the recruitment and activation of phagocytic granulocytes, monocytes/ macrophages, and NK cells to sites of microbial (bacteria, fungi, enveloped viruses) invasion. HBD1 and LL37 are produced constitutively by keratinocytes and other epithelial cells and contribute to the barrier functions in the first line of antimicrobial defence. In mice, a deficiency in CRAMP production leads to the increased susceptibility to necrotic skin infections caused by Group A Streptococcus [41]. Neonatal skin in mice and humans expresses increased levels of AMPs compared to adult [42]. Furthermore, hBD1 expression was described in milk and breast tissue during lactation [43]. Recombinant hBD2 produced by baculovirus had a direct antimicrobial activity in vitro [20]. Human defensins, hBD2 and LL37 induce the activation and degranulation of mast cells resulting in the release of histamine and PDG2 [44, 45]. Because mast cell granule products increase neutrophil influx, AMPs can indirectly promote accumulation of these cells at inflammatory sites. Degranulation of neutrophils releases more defensins and upregulates innate host innflammatory defenses against microbial invasion. HNP1 and HNP2 are chemotactic for human monocytes [46] dendritic cells and T cells [47, 48]. Murine β defensin 2 acts directly on immature dendritic cells as an endogenous ligand for Toll-like receptor 4 (TLR4), inducing up-regulation of costimulatory molecules and, subsequently, dendritic cells maturation, acting as the natural adjuvant [49].

In addition to their role in host defense, defensins may also contribute to some pathophysiologic mechanisms. Neutrophil defensins can inhibit fibrinolysis and modulate tissue-type plasminogen activator and plasminogen binding to fibrin and endothelial cells [50]. Cathelicidin LL37 can also play a part in wound closure and its reduction in chronic wounds can impair re-epithelialization [51].

AMPs as therapeutic agents

The broad spectrum of activity and the low incidence of bacterial resistance are attractive features of AMPs. Animal

studies provided first proof that AMPs can be used to modify the course of infection and inflammatory diseases. Small biotech companies in association with larger pharmaceutical companies carried out human studies using AMPs of animal origin. AMPs from the skin of frogs, called magainins, have been one of the first substances to go through a drug development process. Its derivative, pexiganan was investigated in phase III trials of 926 patients and topical use has been found to show equivalence to oral ofloxacin against polymicrobic diabetic foot ulcer [52]. The porcine neutrophil-derived protegrins are the first of the mammalian cathelicidin peptides used in clinical trials [53]. A phase II study showed that this peptide used orally by patients undergoing bone marrow transplantation significantly reduced mucositis and number of febrile days. A phase III study of protegrin is now underway [54]. In future, animal studies may resolve the role of AMPs in tissue protection, wound healing and interaction with the aquired immune system. Better understanding of AMPs relationship between the innate and the aquired immune systems is the critical step to give us insite into how we naturally prevent infections and how to design new drugs to fight diseases.

Concluding remarks

AMPs have emerged as effector substances of the innate immune system involving not only activities as endogenous antibiotics but also as mediators of inflammation. Several important topics will have to be addressed in the future: (I) AMPs might contribute to the development of diseases as pro- or anti- inflammatory substances; (II) clinical useful of AMPs as novel therapeutic agents promises to revolutionize treatment of many inflammatory and infectious diseases.

References

1. Zasloff M (2002): Antimicrobial peptides of multicellular organisms. Nature 415 (6870): 389-395.
2. Evans ME, Feola DJ, Rapp RP (1999): Polymyxin B sulfate and colistin: old antibiotics for emerging multiresistant gram- nrgative bacteria. Ann Pharmacother 33 (9): 960-967.
3. Zhang J, Demain AL (1992): ACV synthetase. Crit Rev Biotechnol 12 (3): 245-260.
4. Hancock RE (1997): Peptide antibiotics. Lancet 349 (9049): 418-422.
5. Raj PA, Dentino AR (2002): Current status of defensins and their role in innate and adoptive immunity. FEMS Microbiol Lett 206: 9-18.
6. Ganz T, Selsted ME, Szklarek D, et al. (1985): Defensins: natural peptide antibiotics of human neutrophils. J Clin Invest 76 (4): 1427-1435.
7. Selsted ME, Harwig SS, Ganz T, et al. (1985): Primary structures of three human neutrophil defensins. J Clin Invest 76 (4): 1436-1439.
8. Selsted ME, Miller SI, Henschen AH, Ouellette AJ (1992): Enteric defensins: antibiotic peptide components of intestinal host defense. J Cell Biol 118: 929-936.
9. Quayle AJ, Porter EM, Nussbaum AA, et al. (1998): Gene expression, immunolocalization and secretion of human defensin 5 in human female reproductive tract. Am J Pathol 152: 1247-1258.
10. Cunliffe RN, Rose FR, Keyte J, et al. (2001): Human defensin 5 is stored in precursor form in normal Paneth cells and is expressed by some villous epithelial cells and by metaplastic Paneth cells in the colon in inflammatory bowel disease. Gut 48: 176-185.
11. Ashitani J, MukaeH, Hiratsuka T, et al. (2002): Elevated levels of α-defensins in plasma and BAL fluid of patients with active pulmonary tuberculosis. CHEST 121 (2): 519-526.
12. Thomas NJ, Carcillo JA, Doughty LA, et al. (2002): Plasma concentrations of defensins and lactoferrin in children with severe sepsis. Pediatr Infct Dis J 21 (1): 34-38.
13. Diamond G, Zasloff M, Eck H, et al. (1991): Tracheal antimicrobial peptide, a cysteine- rich peptide from mammalian tracheal mucosa: peptide isolation and cloning of cDNA. Proc Natl Acad Sci USA 88: 3952- 3956.
14. Bensch K, Raida M, Magert HJ, et al. (1995): hBD-1: a novel β-defensin from human plasma. FEBS Lett 368: 331-335.
15. McCray JP, Bentley L (1997): Human airway epithelia express a beta-defensin. Am J Respir Cell Mol Biol 16 (3): 343-349.
16. Valore EV, Park CH, Quayale AJ, et al. (1998): Human beta-defensin-1: an antimicrobial peptide of urogenital tissues. J Clin Invest 101 (8): 1633-1642.
17. Fulton C, Anderson GM, Zasloff M, et al. (1997): Expression of natural peptide antibiotics in human skin. The Lancet 350: 1750-1751.
18. Ali RS, Falconer A, Ikram M, et al. (2001): Expression of the peptide antibiotics human β defensin-1 and human β defensin-2 in normal human skin. J Invest Dermatol 117: 106-111.
19. Harder J, Bartels J, Christofers E, Schroeder JM (1997): A peptide antibiotic from human skin (letter). Nature 387: 861.
20. Liu AY, Destoumieux D, Wong AV, et al. (2002): Human β defensin-2 production in keratinocytes is regulated by IL1, bacteria, and the state of differentiation. J Invest Dermatol 118: 275-281.
21. Dinulos JGH, Mentele L, Fredericks LP, et al. (2003): Keratinocyte expression of human β defensin 2 following bacterial infection: role in cutaneous host defence. Clin Diagnost Lab Immunol 10 (1): 161-166.
22. Liu L, Roberts AA, Ganz T (2002): By IL1 signaling, monocyte-derived cells dramatically enhance the epidermal antimicrobial response to lipopolysaccharide. J Immunol 170: 575-580.
23. Harder J, Bartels J, Christophers E, Schroeder JM (2001): Isolation and characterization of human β defensin-3, a novel human inducible peptide antibiotic. J Biol Chem 276 (8): 5707-5713.
24. Garcia JR, Krause A, Schulz S, et al. (2001): Human β defensin-4: a novel inducible peptide with a specific salt- sensitive spectrum of antimocrobial activity. FASEB J 15 (10): 1819-1821.
25. Schutte BC, Mitros JP, Bartllet JA, et al. (2002): Discovery of five conserved beta defensin gene clusters using a computational search strategy. Proc Natl Acad Sci USA 99 (4): 2129-2133.
26. Duits LA, Ravensbergen B, Rademaker M, et al. (2002): Expression of β defensin 1 and 2 mRNA by human monocytes, macrophages and dendritic cells. Immunol 106: 517-525.
27. Tang YQ, Yaun J, Osapay G, et al. (1999): A cyclic antimicrobial peptide produced in primate leukocytes by the ligation of two truncated alpha defensins. Science 286: 498-502.
28. Zanetti M, Gennaro R, Romeo D (1995): Cathelicidins: a novel protein family with a common proregion and a variable C-terminal antimicrobial domain. FEBS Lett 374: 1-5.

29. Zaiou M, Gallo RL (2002): Cathelicidins, essential gene – encoded mammalian antibiotics. J Mol Med 80: 549-561.
30. Sorensen O, Arnljots K, Cowland JB, et al. (1997): The human antibacterial cathelicidin, hCAP-18, is synthesized in myelocytes and metamyelocytes and localized to specific granules in neutrophils. Blood 90: 2796-2803.
31. Agerberth B, Charo J, Werr J, et al. (2000): The human antimicrobial and chemotactic peptides LL37 and α defensins are expressed by specific lymphocytes and monocytes populations. Blood 96: 3086-3093.
32. Bals R, Wang X, Zasloff M, Wilson JM (1998): The peptide antibiotic LL37/ hCAP18 is expressed in epithelia of the human lung where it has broad antimicrobial activity at the airway surface. Proc Natl Acad Sci USA 95: 9541-9546.
33. Frohm Nilsson M, Sandstedt B, Sorensen O, et al. (1999): The human cationic antimicrobial protein (hCAP18), a peptide antibiotic, is widely expressed in human squamous epithelia and colocalizes with IL6. Infect Immunol 67: 2561-2566.
34. Hase K, Eckmann L, Leopard JD, et al. (2002): Cell differntiation is a key determinant of cathelicidin LL37/ human cationic antimicrobial protein 18 expression by human colon epithelium. Infect Immun 70 (2): 953-963.
35. Frohm M., Agerberth B, Ahangari G, et al. (1997): The expression of the gene coding for the antibacterial peptide LL37 is induced in human keratinocytes during inflammatory disorders. J Biol Chem 272 (24): 15258-15263.
36. Ong PY, Ohtake T, Brandt C, et al. (2002): Endogenous antimicrobial peptides and skin infections in atopic dermatitis. Nengl J Med 347 (15): 1151-1160.
37. Dorschner RA, Pestonjamasp VK, Tamakuwala S, et al. (2001): Cutaneous injury induces the release of cathelicidin antimicrobial peptides active against group A Streptococcus. J Invest Dermatol 117: 91-97.
38. Sorensen OE, Cowland JB, Theilgaard-Monch K, et al. (2003): Wound healing and expression of antimicrobial peptides/ polypeptides in human keratinocytes, a consequence of common growth factors. J Immunol 170: 5583-5589.
39. Sorensen O, Bratt T, Johnsen AH, et al. (1999): The human antibacterial cathelicidin, hCAP18, is bound to lipoproteins in plasma. J Biol Chem 274: 22445-22451.
40. Gallo RL, Kim KJ, Bernfield M, et al. (1997): Identification of CRAMP, a cathelin related antimicrobial peptide expressed in the embryonic and adult mouse. J Biol Chem 272: 13088-13093.
41. Nizet V, Ohtake T, Lauth X, et al. (2001): Innate abtimicrobial peptide protects the skin from invasive bacterial infection. Nature 414: 454-457.
42. Dorschner RA, Lin KH, Murakami M, Gallo RL (2003): Neonatal skin in mice and humans expresses increased levels of antimicrobial peptides: innate immunity during development of the adaptive response. Pediatr Res 53 (4): 566-572.
43. Jia HP, Starner T, Ackermann M, et al. (2001): Abundant human beta defensin 1 expression in milk and mammary gland epithelium. J Pediatr 138: 109-112.
44. Befus AD, Mowat C, Gilchrist M, et al. (1999): Neutrophil defensins induce histamine secretion from mast cells: mechanisms of action. J Immunol 163: 947-953.
45. Niyonsaba F, Someya A, Hirata M, et al. (2001): Evolution of the effect of peptide antibiotics human beta defensin 2 and LL37 on histamine release and prostaglandin D2 production from mast cells. Eur J Immunol 31: 1066-1075.
46. Territo MC, Ganz T, Selsted ME, Lehler R (1989): Monocyte-chemotactic activity of defensins from human neutrophils. J Clin Invest 84: 2017-2020.
47. Yang D, Chertov O, Bykovskaia SN, et al. (1999): β defensins: linking innate and adaptive immunity through dendritic and T cell CCR6. Science 286: 525-528.
48. Yang D, Chen Q, Chertov O, et al. (2000): Human neutrophil defensins selectively chemoattract naive and immature dendritic cells. J Lukocyte Biol 68: 9-14.
49. Biragyn A, Ruffini PA, Leifer CA, et al. (2002): Toll-like receptor 4-dependent activation of dendritic cells by β defensin 2. Science 298: 1025-1029.
50. Higazi A, Ganz T, Kariko K, Cines DB (1996): Defensin modulates tissue-type plasminogen activator and plasminogen binding to fibrin and endothelial cells. J Biol Chem 271 (30): 17650-17655.
51. Heilborn JD, Frohm Nilsson M, Kratz G, et al. (2003): The cathelicidin anti-microbial peptide LL37 is involved in re-epithelialization of human skin wound and its lacking in chronic ulcer epithelium. J Invest Dermatol 120: 379-389.
52. Jacob L, Zasloff M (1994): Potential therapeutic applications of magainins and other antimicrobial agents of animal origin. Ciba Found Symp 186: 197-216.
53. Mosca DA, Hurst MA, SO W, et al. (2000): IB-367, a protegrin peptide with in vitro and in vivo activities against the microflora associated with oral mucositis. Antimicrob Agents Chemother 44: 1803-1808.
54. Chen J, Falla TJ, Liu H, et al. (2000): Development of protegrins for the treatment and prevention of oral mucositis: structure-activity relationships of synthetic protegrin analogues. Biopolymers 55: 88-98.