REVIEW ARTICLE

Antimicrobial Peptides

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ABSTRACT

Over the last decade there has been a rapid increase in the incidence of microbial resistance to commonly available antimicrobial agents. Hence, it has ushered in a spiraling demand for the novel antimicrobial agents effective against the resistant strains of microbes. And, the scientists all over the world have been working hard to develop newer antimicrobial compounds to tackle the looming threat of antimicrobial resistance. Researchers have now recognized human peptides with an inherent ability to kill microbes, which form the essential component of the early innate immune response. These endogenous antimicrobial peptides (AMPs) are also known as host defense peptides exhibit potent killing of a broad range of micro-organisms, including gram-negative and gram-positive bacteria, fungi, viruses and parasites. And, these peptides serve as the first line of defense system, which is present constitutively, but is increased upon injury or acute inflammation. They are generally positively charged short peptides containing between 12 and 50 amino acids. They act either by attaching to or inserting into the membrane bi-layers to form pores. Alternatively they may penetrate into the cell to bind the intracellular molecules, which are crucial for cell living. After gaining entry into the microbial membrane, they act through various mechanisms to kill the target cells. It includes, disrupting the microbial membrane, interfering with the metabolic pathways, and targeting the cytoplasmic components.

Mammals are equipped with diverse combinations of AMPs, these are synthesized and secreted in those tissues that are exposed to environmental microbes, like skin and mucosal epithelium. Mammalian antimicrobial peptides include cathelicidins, defensins, cecropins, histatins, lactoferrins, NK-lysins, and protegrins. Human skin poses wide range of proteins including enzymes, enzyme inhibitors and neuropeptides that have inherent antimicorobial properties. Amongst various peptides discovered for their antimicrobial activites, two major groups: Cathelicidins and Defensins have been widely researched. Apart from this most important function of AMPs, they also help in various other skin conditions like wound healing, atopic dermatitis, psoriasis, acne, rosacea, contact dermatitis etc.

INTRODUCTION

Background

In the last 2 to 3 decades, there has been a rapid increase in the incidence of microbial resistance to commonly available antimicrobial agents from all parts of the world[1]. Hence, the demand for the novel antimicrobial agents effective against the resistant strains of microbes has been spiraling. The decrease in efficacy of the antimicrobial therapy is not just limited to bacteria. It rather extends across all sorts of infection, whether caused by bacteria, virus, fungi or parasites. It has affected almost every major class of antibiotic, and has not even spared the most developed nations[2]. Researchers all over the world have been trying to develop newer antimicrobial compounds to tackle the looming threat of antimicrobial resistance.

Immune Defense Mechanism

Traditionally stratum corneum and the physical barrier were considered as the skin's first line of defense against the invading pathogen.[3] Follow-

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ing disruption of this physical barrier the microbe could enter into the otherwise sterile internal environment and initiate a complex cascade of reactions known as immune defense mechanism based on the bone marrow derived cells. Hence, the initiation of the immune defense was thought to begin with the events of acute inflammation, characterized by increase blood flow, increased vascular permeability, and migration of inflammatory cells. The goal of the immune response was to remove the invader or perceived invader, through the non antigen specific immune response mechanism, and then later mount a antigen specific adaptive immune response. The adaptive immune response is very slow when compared to the evolving ability of many microbes to rapidly multiply in permissive environment, often with a doubling time of 20 minutes or even less. This is much quicker than even some of the non adaptive elements of acute inflammatory response, like recruitment of polymorphs.[4]

But, with the recent finding of the components of the innate immune response that are present and activated even before the development of signs of acute inflammation. This innate immunity refers to all aspects of protection against infection that are genetically determined, and present even before the actual exposure to the invader or perceived invader. The hope to develop a potent antimicrobial class of agents active against the microbes resistant to most of the currently available antimicrobial agents seems within reach.[5]

Researchers have now recognized human peptides with an inherent ability to kill microbes, which form the essential component of the early innate immune response.[6] These endogenous antimicrobial peptides (AMPs) are also known as host defense peptides.[7] AMPs exhibit potent killing of a broad range of micro-organisms, including gram-negative and gram-positive bacteria, fungi, viruses and parasites.[8] These peptides serve as the first line of defense system, which is present constitutively, but is increased upon injury or acute inflammation. Also, AMPs act beyond this, and have an immunomodulatory action that involve clearance of the infection, including the ability to alter host gene expression, act as chemokines and/or induce chemokine production, induce lipopolysaccharide induced pro-inflammatory cytokine production, promote wound healing, and modulating the dendritic cell response in the adaptive immune response.

Structure of Antimicrobial peptides(AMPs)

They are unique and diverse group of molecules, classified into subgroups based on their amino acid composition and structure.[9] They are generally short peptides containing between 12 and 50 amino acids. These peptides are generally charged positively. The positively charged residues are by Lysine, Arginine, and in case of acidic environment by Histidine. They contain both hydrophobic and hydrophilic ends, which enables them to be soluble in aqueous environment, and at the same time also retain the capacity to enter lipid rich membranes.[10-11] The secondary structures of these molecules follow 4 themes (Fig. 1),

a- α-helical
b- β-stranded
c- β-hair pin
d- extended

Many of these peptides are unstructured in free solution, and attain their final configuration upon partitioning into biological membranes.

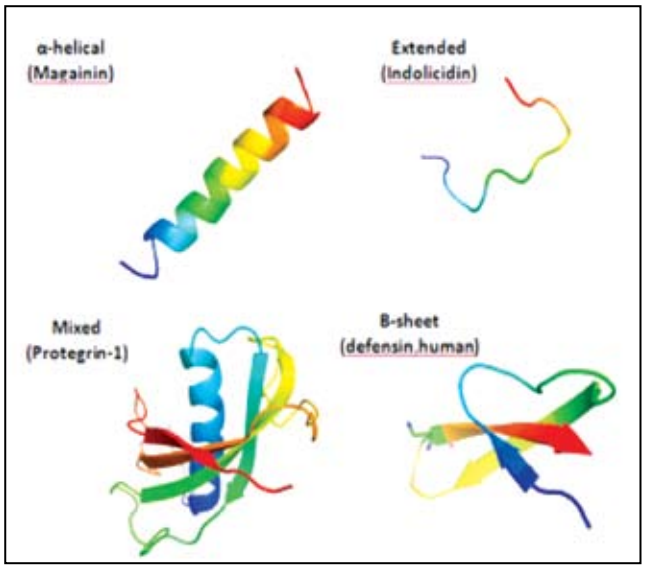

Fig. 1 Showing different basic structure of AMPs
*Source: http://en.wikipedia.org/wiki/file:various AMPs.pns

Mechanism of action

After gaining entry into the microbial membrane, they act through various mechanisms to kill the target cells. It includes, disrupting the microbial membrane, interfering with the metabolic pathways, and targeting the cytoplasmic components (Fig. 2). They achieve this either by attaching to or inserting into the membrane bi-layers to form pores. Alternatively they may penetrate into the cell to bind the intracellular molecules, which are crucial for cell living.[12]

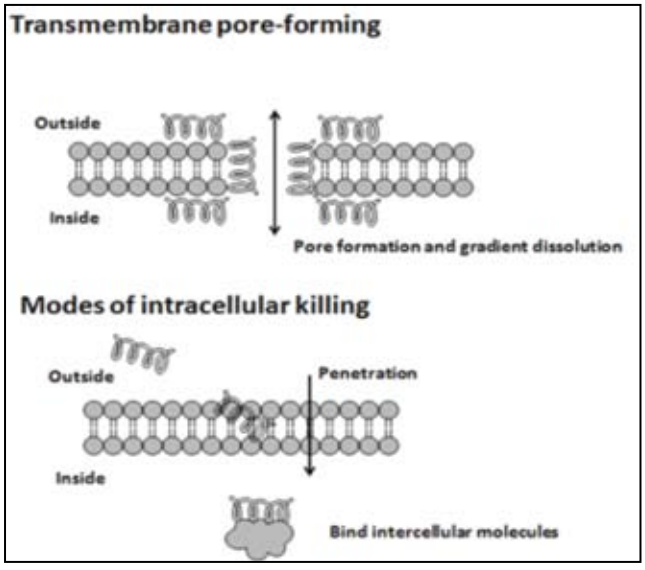

Fig. 2 Depicting various modes of action of AMPs
*Source:
http://en.wikipedia.org/wiki/file:modes_of_action.png

Spectrum of AMPs

The updated version of antimicrobial peptide database (APD, http://aps-unmc.edu/AP/main.php) published online in 2008 has listed 1228 antimicrobial peptides with 65 anticancer, 76 antiviral (53 anti HIV), 327 antifungal and 924 antibacterial peptides. APD is a general database dedicated to antimicrobial peptides from all biological sources, ranging from bacteria, plants, to animals, including humans.[13] This database collects only 'mature and active' peptides (<100 amino acid residues), and is constantly updated keeping in pace with the recent discoveries.

Therapeutic potential of AMPs

AMPs are the excellent candidates for the development of new therapeutic agents, and complement to the existing armamentarium of currently available antibiotics.[14] Because in contrast to the conventional antibiotics these peptides have broad range of activity, have bactericidal properties, require short contact time to induce killing and do not appear to induce antibiotic resistance amongst the microbes.[15] A number of naturally occurring peptides and their derivatives have been developed as novel anti-infective therapies for various kinds of infections.[16-17]

Mammalian AMPs

Mammals are equipped with diverse combinations of AMPs, these are synthesized and secreted in those tissues that are exposed to environmental microbes, like skin and mucosal epithelium.[18-22] Mammalian antimicrobial peptides include cathe-

licidins, defensins, cecropins, histatins, lactoferrins, NK-lysins, and protegrins.[23] These antimicrobial peptides provide a powerful defense system that can both protect the skin from infection and signal host cells to change their behavior in response to injury. This was apparent by investigations that denoted in addition to killing bacteria, viruses, fungi, and protozoa, some of these antimicrobial peptides function in regulating cell proliferation[24], and extracellular matrix production.[25] Antimicrobial peptides also have been shown to be important in such diverse functions as: angiogenesis, wound healing, and chemotaxis.[26]

Human skin poses wide range of proteins including enzymes, enzyme inhibitors and neuropeptides that have inherent antimicrobial properties. Amongst various peptides discovered for their antimicrobial activites, two major groups: Cathelicidins and Defensins have been widely researched.

Cathelicidins

Cathelicidins are a family of polypeptides found in lysosomes of macrophages and polymorphonuclear leukocytes.[27] These are highly heterogeneous ranging in size from 12 to 80 amino acid residues and have different structures.

1. Linear peptides of 23-37 amino acid residues that fold into amphipathic α helices are the most common of these AMPs.[28]
2. Then there are 12-18 amino acid residues with β-hair pin structures stabilized by disulphide bonds, and a 13- residue linear peptide with high proportion of tryptophan.
3. Finally, there are 39-80 amino acid residue sized large molecules with repetitive proline motifs forming extended polyproline like structures.[29]

But all three groups of molecules despite having marked structural diversity, are all stored in cells in an unprocessed form with a fairly conserved N-terminal prosequence known as the "Cathelin" domain (hallmark feature of these molecules), and a structurally variable cationic antimicrobial peptide at the C-terminus. The cathelin domain functions as both a protease inhibitor and as an antimicrobial peptide in humans. Mature cathelicidin peptides show in addition to their rapid, potent, antimicrobial activity, an immunomodulatory functions.[30] Cathelicidin family components have been isolated in almost every mammal species investigated so far. Currently identified cathelicidins include the following:-

a- Human: hCAP-18/LL-37
b- Rhesus monkey: RL-37
c- Mice: CRAMP-1/2
d- Rats: rCRAMP
e- Rabbits: CAP-18
f- Guinea pigs: CAP-11
g- Pigs: PR-39, Prophenin, PMAP-23,36,37
h- Cattles: BMAP-27, 28, 34, Bac5, Bac7
i- Sheeps
j- Goats
k- Horses

The 18-kDa human cationic antimicrobial protein (hCAP) is the only human member of cathelicidin family of AMPs known to date.[31-32] LL-37 is the C-terminal part of the hCAP. LL-37 derives its name from its primary structure containing 37 amino acids residue with two leucine residues at its N-terminal. LL-37 assumes α-helical structure in solutions as plasma, or interstitial fluids. It should be activated from its precursor by neutrophil proteases such as proteinase 3.[33] LL-37 and the other Cathelin peptide formed as a result are

both active.[34] The "Cathelin" domain functions as both antimicrobial and as a protease inhibitor. LL-37 is also an antimicrobial with a different spectrum of action than "Cathelin" domain, but it can also directly modify the immune response by being cytotoxic to the host cell, by acting as chemoattractant of neutrophils, monocytes and T cells.[35] It is also chemotactic for mast cells, which by positive feed back cycle produce LL-37. It is produced in eccrine structures and is secreted in sweat suggesting barrier function against topical skin infections.[36] In addition, LL-37 stimulates endothelial cells proliferation.[37]

Defensins

Defensins are another group of cationic molecules with a structure based on a common beta sheet core stabilized by three disulfide bonds.[38] These molecules contain 6-8 cystine residues that form the characteristic disulfide bridges. On the basis of arrangement of disulfide bridges, they are subdivided into distinct subfamilies:

a) α-defensin
b) β-defensin
c) θ-defensin

Like, cathelicidins, they are also expressed by epidermal cells in skin and epithelial cells in mucosae. Out of the 3 sufamilies, only α-defensin and β-defensin are found in humans.

α-defensin: They are stored in azurophil granules in neutrophils. The disulfide bond in this subfamily is between cysteines 1-6, 2-4, and 3-5. Four different α-defensins are expressed by human neutrophils, referred to as human neutrophil peptide 1-4.[39] Two other molecules found in humans are expressed in Paneth cells of the small intestine and in epithelial cells of the female genital tract.[40-41] In addition to their antimicrobial role, they also upregulate TNF-α and IL-1 in monocytes.[42] Defensins are virtually absent in normal skin, while their expression in human keratinocytes requires stimulation by cytokines or bacteria.[43]

β-defensin: Similar to α subgroup, they too contain 6 cysteine motifs. But the location of bridges in this group is between cysteine residues at 1-5, 2-4, 3-6. So far, 4 β-defensin molecules (HBD-1 to HBD-4) have been identified in humans. In addition to their antimicrobial properties, they also perform various immune related functions. HBD-2 promotes the release of PG-D2 and histamine from the mast cells,[44] and is also chemotactic for memory specific T cells.[45-46]

Granulysin

It is another distinct AMP molecule isolated in humans. It has α- helix structure and belongs to Saposin like family of proteins.[47] Its active form is a 9-kd cationic peptide, and is primarily found in cytotoxic T lymphocytes and natural Killer cells.[48-49] It has broad antimicrobial spectrum, similar to other two AMPs. But its main difference from other AMPs lies in the fact that unlike, cathelicidin and defensin group of AMPs, it is not produced locally in the skin.[50] Rather it is brought to the site through T-cells. Hence, more appropriately it should be classified as a component of adaptive immune response. Apart from having a antimicrobial action, it has been shown to lyse variety of tumor cells and induce apoptosis, hence helping in tumor surveilience.[51-52]

Other Antimicrobial Peptides and Proteins

Whereas, the cathelicidins and defensins are best known for their antimicrobial properties, many

other peptides also demonstrate antimicrobial activities including proteinase inhibitors, chemokines and neuropeptides.[53] However, the dependence of antimicrobial activity of these peptides on their original activity varies. For example, P-cystatin α inhibits bacterial proteinase activity as a mechanism of microbial growth inhibition, whereas cystatin C antimicrobial activity does not depend on its ability to inhibit bacterial proteinase.[54] Also α-MSH tripeptide that is important for antimicrobial activity, while this is not true for sequences involved in learning and memory.[55]

ANTIMICROBIAL PEPTIDES AND SKIN

The skin's first line of defense against invasion by microbial agents is the stratum corneum, however, this physical barrier is susceptible to injuries that allow the entry of opportunistic microbial agents into the skin. In the event of injury to this barrier, the AMPs in the skin act immediately to suppress the microbial proliferation, and limit the spread of infection.[56] This process is very important, because it helps to gain time for the adaptive immune response to take over in the form of leucocyte migration, phagocytosis by neutrophils and macrophages, and their production of reactive oxygen intermediates that kill microbial agents. The activation of antimicrobial peptides is essential for the ability of the skin to resist bacterial infections.[57] Apart from this most important function of AMPs, they also help in various other skin conditions[58] like:

Wound Healing

AMPs were first discovered by researchers during their search for a wound repair modifying molecules. The pig cathelicidin PR-39 stimulates dermal fibroblasts to produce more extracellular matrix proteoglycans syndecan-1 and 4. Lack of syndecan has been shown to inhibit normal wound healing process.[59-60] Similarly, human cathelicidin LL-37 has also been shown to be associated with hosts of stimulatory events considered necessary for the process of wound healing. LL-37 also stimulates keratinocyte proliferation,[61] and induces angiogenesis. Thus, AMPs serve the dual role in wound healing by killing the microbe, and stimulating wound repair process.[62-63]

Atopic dermatitis

Atopic dermatitis is often complicated by recurrent infections of the skin lesions by bacterial, viral and fungal pathogens.[64] *Staph aureus* has been isolated from the skin lesions of most of the patients with atopic dermatitis.[65] About 30% of patients with atopic dermatitis develop bacterial or viral cutaneous infections as compared with only 7% of patients with psoriasis, even though both diseases are characterized by defective skin barrier.[66] The answer to this question might be explained by lower concentration of human beta defensin 2 and the canthelicidin LL-37 observed in inflammatory skin lesions from patients with atopic dermatitis. Whereas, in the patients with psoriasis show increased concentration of AMPs in lesional skin. Furthermore, the inability of the skin of patients with atopic dermatitis to increase the concentrations of beta defensin 2 and LL-37 in response to inflammation makes them unable to kill *staph aureus*, and this may explain the susceptibility of atopic dermatitis patients to skin infections.[67]

Leprosy

Granulysin was also supposed by some researchers to have a role in leprosy, where granulysin

expressing T-cells were found to be 6 folds more in tuberculoid leprosy cutaneous lesions as compared to lepromatous leprosy lesions. Given the broad antimicrobial spectrum of granulysin, these data provide evidence that T-cell release of granulysin contributes to host defense in leprosy and can reflect the outcome of leprosy in patients.[68]

Psoriasis

Recently, a shift in the predominance from one type that normally predominates in normal skin to another type was noted in psoriatic skin lesions. This was attributed to the cathelicidin LL-37 that binds to self DNA and the resultant complex signals to the production of a very potent immunomodulatory cytokine. The resultant cytokine may pave the way to the production of psoriatic lesions in a process where vitamin D is involved.[69]

Acne

The effect of granulysin, the antimicrobial peptide on *propionibacterium acnes*, considered as the key therapeutic target in acne vulgaris, was investigated by some authors. They concluded that the antimicrobial peptide can kill *propionibacterium acnes* more effectively than traditionally used bacteriostatic agents. Moreover, they demonstrated the anti-inflammatory properties of graulysin through reduction of *propionibacterium acnes*-stimulated production of cytokines and chemokines. This combined antimicrobial and anti-inflammatory properties make this peptide as an ideal therapeutic agent for acne vulgaris.[70]

Contact dermatitis

Cathelicidin can alter host immune responses including stimulating pro-inflammatory events.[71] On the other hand, cathelicidin can also exert an anti-inflammatory role by inhibiting Toll like receptor 4 and this was shown in a study investigating cases with allergic contact dermatitis in animal models, and using cathelicidin or modulators of cathelicidin expression in clinical diseases pathogenesis as atopic dermatitis and allergic contact dermatitis was suggested.

Viral diseases

Epidermal cathelicidin expression has been shown to be induced in cases of verruca vulgaris and condyloma acuminate[72] (infections caused by human papilloma viruses). Though, the evidence to suggest that their increased expression is directly related to either the resistance or limits the spread of virus is not yet available. Thus, AMPs may offer explanation into the understanding of the pathogenesis of viral skin infections, and the increased susceptibility to these viral infections observed in otherwise healthy, and immunocompetent individuals.

Summary

All multicellular organisms share the formidable task of maintaining a barrier against the physical and microbiologic challenges of the environment. Given this challenge, a system for efficient control of microbial proliferation must exist in mammalian skin. Such a system would have two distinct functions. First, it would regulate the colonization of the intact skin surface. Second, this defense system would respond to disruption of epidermal integrity with an increase in antimicrobial activity. In recognizing the importance of protecting epithelial integrity, multicellular organisms respond to barrier compromise with panoply of host defense mechanisms aimed at controlling microbial proliferation. This is the innate immu-

nity that provides broad spectrum recognition and rapid elimination of invading micro-organisms. This is distinct from the adaptive immunity where a host response is initiated only following antigen stimulation of specific lymphocyte sub-populations.[73]

Furthermore, elements of the innate immune system may regulate the adaptive immune response through identification of microbial proteins as foreign antigens and by activation of naïve T cells. This function may be particularly important when the microbial load threatens the overwhelm local defenses and interfere with the wound repair process.[74]

Antimicrobial peptides are effectors of innate immune system. These peptides directly kill a broad spectrum of bacteria, fungi, and viruses. In addition, these peptides modify the local inflammatory response and activate mechanisms of cellular and adaptive immunity. In skin some of these peptides are constitutively present, while others are expressed only by injury or inflammation in the skin as a trigger. Antimicrobial peptides may have a crucial role in many cutaneous diseases as atopic dermatitis, psoriasis, allergic contact dermatitis, acne vulgaris, and leprosy.

Future

As these AMPs are continued to be investigated, we would probably better understand their exact function, and importance in various human skin diseases. The scope of the potential clinical application of the technology being developed for the better understanding of AMPs is enormous. Foremost is the development of new class of antibiotics, resistant to most known human pathogens. Also, since these newer antibiotics would be derived from human products, the likelihood of developing drug reactions to these agents would be minimal. Also, the recent finding of their role of in angiogenesis, tissue repair, and inflammation, could provide a newer and safer approach to many common skin diseases like atopic dermatitis, acne, rosacea, and psoriasis.

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