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DEFENSINS AND BACTERIA, A QUESTION OF "LIVE OR LET DIE"?

LARS-GÖRAN AXELSSON

Microbiology and Tumorbiology Center, Gastrobiology Unit,
Division of Molecular Pathology, Karolinska Institute, Stockholm, Sweden

SUMMARY

Antibacterial peptides have been found in many organs of the body. Defensins are a family of cationic antimicrobial peptides that are found in mammals, insects and plants. The innate immune system consists of the body's own defence mechanisms which can be activated upon exposure to foreign microorganisms without earlier exposure or priming.

One important part of this system seems to be the production of endogenous antimicrobial peptides. Such peptides, e.g., produced in the gastrointestinal tract of humans and animals have been suggested to modulate the acquirement of a bacterial microflora and play an important part in to protect against infections in general.

Antimicrobial peptides have broad-spectrum antibiotic activity against e.g. Gram-positive and Gram-negative bacteria, mycobacteria, fungi, parasites and viruses.

Antimicrobial peptides seem to be less prone to be, or to induce resistance in bacteria, which make them an interesting alternative in the treatment of infections with multiresistant bacteria.

There seem to be hope for the emergence of effective synthetic antimicrobial peptides as drugs for human use in the near future.

INTRODUCTION

Defensins are a family of cationic antimicrobial peptides that are found in mammals, insects and plants (*Del Pero* et al., 2002*; Hancock* and *Diamond*, 2000; *Hancock*, 2001; *Schutte* et al., 2002; *Zasloff*, 2002a) and as a component in venoms (*Corzo* et al., 2001). The defensins are members of the inborn, innate immune system (*Axelsson* and *Mahida*, 2000; *Fellerman* and *Stange*, 2001; *Parkin* and *Cohen*, 2001). The innate immune system consists of the body's own defence mechanisms (*Medzhitov*, 2000) which can be activated upon exposure to foreign microorganisms without earlier exposure or priming (*Boman*, 1995; *Zasloff*, 2002a).

Much of the early knowledge of antimicrobial peptides was gained from studies of the fly *Drosophila* that has been utilised as a model for genetic studies. *Drosophila* lacks adaptive immune system an relies on the inborn, innate, immune system and have been used as a model for the elucidation of the different pathways of the innate immunity (*Hoffman* and *Reichhart*, 2002) especially the Toll-pathway (*Schwartz*, 2002) and the importance of NFκB transactivators (*Luster*, 2002; *Mahida* and *Johal*, 2001).

One important part of this system seems to be the production of endogenous antimicrobial peptides (*Boman*, 1995; *Ganz*, 1994). Such peptides, e.g., produced in the gastrointestinal tract of humans and animals (*Schonwetter*, 1995), have been suggested to modulate the acquirement of a bacterial microflora in neonates (*Sepp*, 1998) and play an important part in the protection against infections in general. Antimicrobial peptides have broad-spectrum antibiotic activity against e.g. Gram-positive and Gram-negative bacteria, mycobacteria, fungi, parasites and viruses (*Boman*, 1995).

Antimicrobial peptides have been shown to be produced by many organs and cells through the body (*Boman*, 1995; *Ganz*, 2000) but this paper will concentrate on the innate immunity of the gastrointestinal tract since it is exposed to an enormous load of microorganisms, some causing disease but others are necessary for our well-being and antimicrobial peptides seem to play an important control function on these microorganisms.

INNATE IMMUNITY OF THE GASTROINTESTINAL TRACT

The innate immunity of the gastrointestinal system is in place when we are born (*Bry* et al., 1994) and will play an important role in defending the body against unwanted microbes, but also in the acquirement and maintenance of a normal and healthy microflora, microbiota (*Bevins* et al., 1999; *Boman*, 2000; *Hooper* and *Gordon*, 2001).

The first sentinels at the gate to our gastrointestinal system are found in the oral cavity. Here we find that mucus is produced, layering the physical border consisting of epithelial cells. This mucus layer is found throughout the gastrointestinal system and has many functions such as being a physical barrier, protecting the epithelial cells, helping in eliminating and transporting unwanted substances out of the body, and also harbouring endogenous protecting bioactive molecules (*Deplanke* and *Gaskins*, 2001).

The antimicrobial peptides are also at place already in the oral cavity (*Bevins*, et al. 1999) and also in the airways (*Moser*, et al. 2002).

The stomach functions as a physical gatekeeper and a producer of chemicals, which will stop and eliminate many microbes to further enter the small and large intestines.

The small intestine harbours a relative small number of bacteria compared to the large intestine (*Hooper* and *Gordon*, 2001; *Skar et* al., 1986, 1989). The large intestine, which includes the caecum and colon, is the part of the gastrointestinal tract where a large number of bacteria, as well as many different species are found (*Cunliffe* et al., 2001). These bacteria can be commensals, invading pathogenic bacteria or, as recently suggested, opportunistic semipathogens (*Gillespie*, 2002; *Medzhitov* and *Janeway*, 2002). One important cell type producing antimicrobial peptides and other antimicrobial substances is the Paneth cell (*Porter* et al., 2002; *Zasloff*, 2002b). Apart from antimicrobial peptides there are several other defence mechanisms in the small intestine (Figure 1).

There are, for example, antimicrobial phospholipase A2, lysozyme and trypsin. The trypsin seems to have multiple functions since recently it was shown that trypsin is the enzyme that activate HD-5 (*Gosh* et al., 2002), one of the enzymes that are able to activate prodefensins. Matrilysin has been advocated as another activating enzyme (*Wilson* et

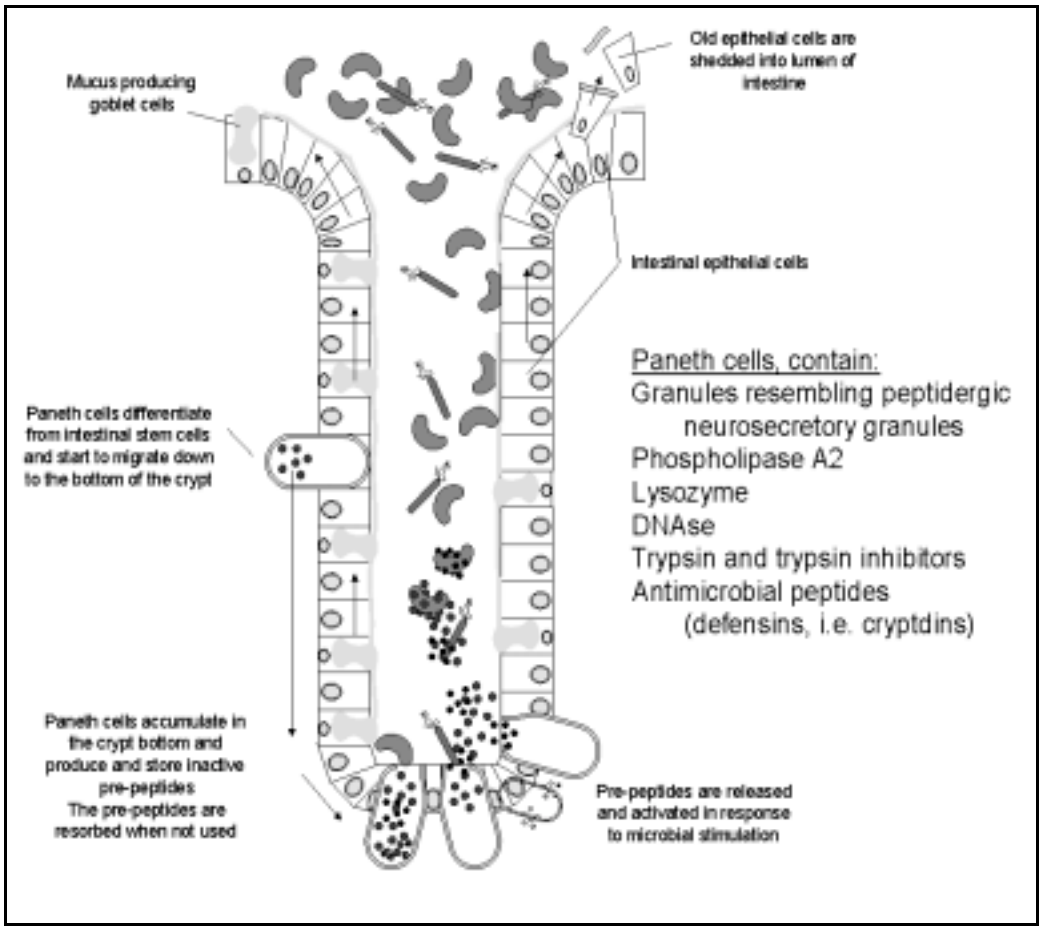

Figure 1: Defence mechanisms in the small intestine.

al., 1999), however this enzyme could not be detected in germfree animals (*López-Boado* et al., 2000) while others have shown actual activation of prodefensins to mature bioactive antimicrobial peptides in germfree animals (*Axelsson* et al., 1999; *Pütsep* et al. 2000).

ANTIMICROBIAL PEPTIDES, MOLECULAR PROPERTIES AND MODE OF ACTION

The antimicrobial effect of antimicrobial peptides on bacteria is commonly tested and expressed as for conventional antibiotics. The effective concentration range lies in the micro- to nanomolar range (*Zasloff,* 2002a). The exact mechanism by which antimicrobial peptides kill microbes is not known. Several models have been proposed and generally some kind of permeabilisation of the bacterial membrane is depicted (*van 't Hof* et al., 2001). This is accomplished by utilising electrostatic binding and the different hy-

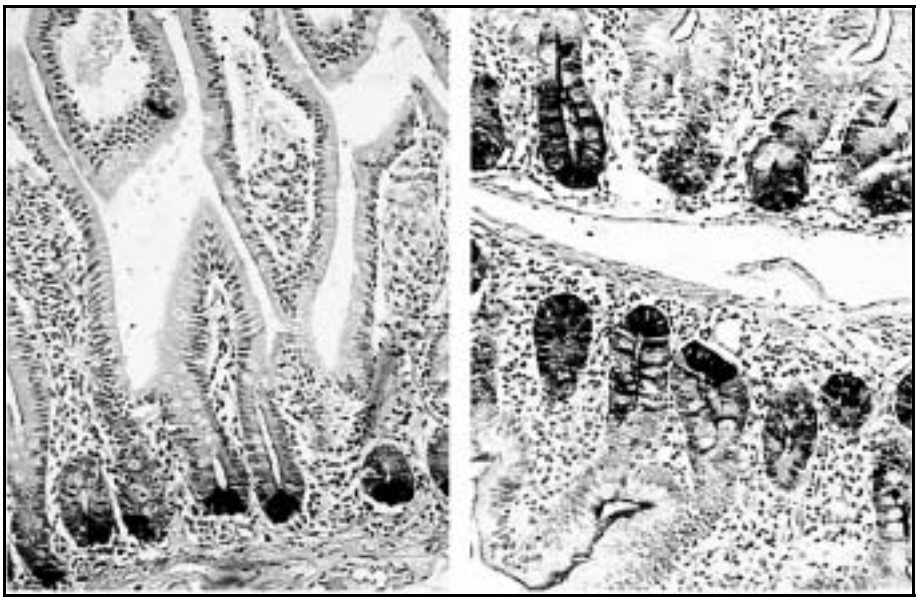

Figure 2: Sections of small intestine stained with antibody to HD-5. Left: Positive Paneth cells in the very bottom of the crypt. Right: Positive cells are found higher up in the crypt and also positive material secreted into the lumen of the crypt.

drophilic and hydrophobic properties of the peptide (*van 't Hof* et al., 2001, *Zasloff*, 2002a). Interactions with lipids have also been advocated and could play in concert with scavenger receptors (*Peiser* et al., 2002). Certain antimicrobial peptides, lactoferricin B and magainin 2, have also been found to cross over the bacterial membrane into the cytoplasm (*Haukland* et al., 2001).

The range of microbes that are sensitive to the antimicrobial peptides is quite broad and the term broad-spectrum antibiotic applies to many of the peptides (*Periathamby* and *Dento*, 2002; *Porter* et al., 1997). Some strains show resistance but this is due to the membrane structure as such (*Zasloff*, 2002a) and acquired resistance has been postulated as unlikely to occur (*Peschel*, 2002).

SMALL INTESTINAL MICROBIAL PEPTIDES

Antimicrobial peptides seem to be important to maintain a relatively microbe-free small intestine (*Ganz*, 2000; *Ouelette* et al., 2000; *Ouelette* and *Bevins*, 2001). There is differential presence of antimicrobial peptides throughout the gastrointestinal channel (*Frye* et al, 2000). In mouse small intestine, the nematode *Trichinella spiralis* induces atrophy of the villi, hyperplasia of the crypts of Lieberkühn and of the mucus-producing goblet cells (*Kamal* et al., 2001). This infection also lead to an increase of Paneth cell-number, a more widespread presence of Paneth cells and intermediate cells expressing mouse antimicrobial α-defensins, cryptdins (*Ayab*e et al., 2002a). The modulation of the mouse cryptdins has been shown to be dependent of Ca^{2+}-activated potassium channels (*Ayabe* et al., 2002b).

A similar effect on Paneth cells has been seen in humans after Roux-en-y Gastric bypass surgery (*Sundbom* et al., 2002). This is a standard surgical procedure for morbid obesity where food and oral-nasal-pharyngeal secretion pass directly into the small bowel without passing through the acid environment of the normal stomach. Immunostaining of human intestinal antimicrobial α-defensins, defensin-5 (HD-5), showed an up-regulation of the antibacterial peptide in the Paneth cells and a spread of anti-defensin positive material upwards in the crypt wall and also a release inside the crypt lumen (Figure 2).

Since the acid environment of the stomach is by-passed, there is a possibility for microbes to invade the otherwise protected small intestine. This could lead to overgrowth of bacteria in these patients. However, these patients display an almost normal microflora, which could be the result of an activation of anti-microbial peptides in response to an increased load of ingested bacteria.

HD-5 is normally stored in precursor form and is activated upon stimulation, as discussed above, by bacteria but also by inflammation (*Axelsso*n, 1999; *Cunliffe* et al., 2001). However, in inflammatory bowel disease the epithelial barrier is defect and the intestinal tissue is exposed to bacteria and bacterial products which could be part of the mechanism of this activation. Similar changes in Paneth cell distribution as in the patients having gastric by-pass surgery could also be detected in patients with active inflammatory bowel diseases pointing to that similar mechanisms could be at play, possibly involving bacterial interference with the mucosa (*Cunliffe* et al., 2002)

This precursor form can be found in both individuals having a normal microflora or being exposed to bacterial products, and in germfree animals. It has been shown that germfree mice, which are bred for many generations in an sterile environment after they have been born under aseptic conditions and then maintained germfree, generate the same products from enteric prodefensins (*Pütsep* et al., 2000). So, animals which are naive to microbes have precursor forms of antimicrobial peptides which can be activated and momentarily exert their antimicrobial functions (*Ayabe* et al., 2002c). However, there is a possibility that these animals are exposed to bacterial products which have been left unaffected by sterilisation in their animal feed. For example there can be bacterial LPS originating in the raw material or from the manufacturing process.

Experiments which compare germfree animals with animals having a conventional commensal microflora or with animals mono-associated with a specific bacterial strain or species, can give valuable information of the interplay between intestinal microflora and the individual or animal (*Pütsep* et al, 2000). The intestinal microflora has profound effects on the development and maintenance of a healthy intestinal mucosa (*Falk* et al., 1998). In the newborn there is a succession of microbial habitants building up this “normal” flora. Starting with bacteria acquired from the mother during labour, neonates acquire for example *Clostridium spp*. and *Bifidobacterium spp*. and the resultant flora is in part determined by environmental factors such as food and eating habits, country of living, sociological factors and level of sanitation (*Falk* et al., 1998). Postnatal studies have shown that the Paneth cells are found early and differentiate to mature cells around postnatal day 14-28 (*Bry* et al., 1994).

PHARMACEUTICAL APPLICATIONS

During the last years there has been a mounting problem with bacterial strains that have become resistant to commonly used antibiotics. Some strains have even acquired resistance to the antibiotics that are used as a last means to treat life-threatening infections. Biotechnological companies have seen the potential in using antimicrobial peptides for treatment of multi-resistant bacteria. For example, Magainin Pharmaceuticals Inc., Philadelphia, USA, was founded in 1997 with one of the early peptide researchers, Dr. Michael Zasloff, as Executive Vice President. Clinical testing has been done of some investigational drug but no real break-through has been seen so far. One problem has been the handling and administration of the drug, another problem, the very high cost of manufacturing synthetic antimicrobial peptides.

One approach has been to develop manufacturing processes to construct the complex structures of biologically effective antimicrobial peptides. Recently, reports of successful synthesis of bioactive polymers displaying antimicrobial activity have been publicised. One approach has been to use amphiphilic acrylamide polymers resembling some of the properties of the β-peptide class. These acrylamide polymers showed a bactericidal activity against *E. coli* and minimal inhibitory concentration (MIC) values for the Gram-negative *Klebsiella pneumoniae*, ampicillin and streptomycin-resistant *E. coli*, and the Gram-positive *Bacillus subtilis* were established (*Tew* et al., 2002). The most effective polymer was additionally tested with good results against ampicillin and streptomycin-resistant *E. coli* and tetracycline resistant *Salmonella typhimurium*. The ability to interact and disrupt phospholipid bi-layers was also confirmed (*Tew* et al., 2002).

Another approach was used by self-assembly of amino acids in synthetic membranes into tubular structures (*Fernandez-Lopez* et al., 2001). Antibiotic activity was established against several bacteria including methicillin resistant *S. aureus* (MRSA) and the membrane disrupting ability was confirmed in a membrane depolarisation assay (*Fernandez-Lopez* et al., 2001).

Another antimicrobial peptide has been identified in the mouse, cathelin-related antimicrobial peptide, CRAMP (*Gallo* et al., 1997). Analogues of this structure have now been designed and shown to have strong antibacterial activity, but without the endogenous CRAMPs haemolytic properties (*Shin* et al., 2000).

With these developments there seem to be hope for the emergence of effective synthetic antimicrobial peptides for human use in the near future. These developments give the possibility to synthesise peptides that are suitable for production at an industrial scale and at reasonable prices. However, clinical testing is now needed to find out whether they have adverse effects that make them not suitable for use in man or animals.

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