Electronic Journal of Biotechnology ISSN: 0717-3458
Vol.6 No.2, Issue of August 15, 2003

Received March 18, 2003 / Accepted July 18, 2003

REVIEW ARTICLE

Antimicrobial peptides: A natural alternative to chemical antibiotics and a potential for applied biotechnology

Sergio H. Marshall*
Laboratorio de Genética e Inmunología Molecular
Instituto de Biología
Facultad de Ciencias Básicas y Matemáticas
Pontificia Universidad Católica de Valparaíso
Avenida Brasil 2950, Valparaíso, Chile
Tel: 56 32 273373
Fax: 56 32 596703
E-mail: smarshal@ucv.cl

Gloria Arenas
Laboratorio de Genética e Inmunología Molecular
Instituto de Biología
Facultad de Ciencias Básicas y Matemáticas
Pontificia Universidad Católica de Valparaíso
Avenida Brasil 2950, Valparaíso, Chile
Tel: 56 32 273205
Fax: 56 32 596703
E-mail: garenas@ucv.cl

Financial support: Project ICA4-2001-10023 (Immunaqua project - European Community).

Keywords: applied biotechnology, innate response, natural antibiotics.

A large group of low molecular weight natural compounds that exhibit antimicrobial activity has been isolated from animals and plants during the past two decades. Among them, cationic peptides are the most widespread. Interestingly, the variety and diversity of these peptides seem to be much wider than suspected. In fact, novel classes of peptides with varying chemical propertiescontinue to be isolated from different vertebrate and invertebrate species, as well as from bacteria. To the early characterized peptides, mostly cationic in nature, anionic peptides, aromatic dipeptides, processed forms of oxygen-binding proteins and processed forms of natural structural and functional proteins can now be added, just to name a few. In spite of the astonishing diversity in structure and chemical nature displayed by these molecules, all of them present antimicrobial activity, a condition that has led researchers to consider them as "natural antibiotics" and as such a new and innovative alternative to chemical antibiotics with a promising future as biotechnological tools. A resulting new generation of anti microbial peptides (AMPs) with higher specific activity and wider microbe-range of action could be constructed, and hopefully endogenously expressed in genetically-modified organisms.

The continuous use of antibiotics has resulted in multi-resistant bacterial strains all over the world and as expected, hospitals have become breeding grounds for human-associated micro organisms (Mainous and Pomeroy, 2001). Nonetheless, the same time-bomb effect is slowly developing with animal-associated pathogens in commercially driven activities, such as aquaculture and confined poultry breeding, where the indiscriminate use of antibiotics is perceivedasessential for industries survival. Consequently, there is an urgent need to search for alternatives to synthetic antibiotics. The discovery of two classes of antimicrobial peptides, non-ribosomally synthesized (Hancock and Chapple, 1999) - present in bacteria - lower eukaryotes and plants - and ribosomally-synthesized peptides, of wider distribution (Boman, 1995; Broekaert et al. 1997; Hancock and Lehrer, 1998; Hoffmann et al. 1999; Thevissen et al. 1999; Zasloff, 2002; Ezekowitz and Hoffmann, 2003), provided a new therapeutic strategy to fight micro organisms. Recent studies show that several cationic and non-cationic peptides expressed in many vertebrate, invertebrate and bacterial species (Lüders et al. 2003) act synergistically to improve immune responses**.**

The knowledge acquired in the past two decades and the discovery of new groups of antimicrobial peptides make natural antibiotics the basic element of a novel generation of drugs for the treatment of bacterial and fungal infections (De Lucca, 2000; Hancock, 2000; Welling et al. 2000; Selitrennikoff, 2001). In addition, the wide spectrum of

*Corresponding author

antimicrobial activities reported for these molecules suggests they potential benefit in the treatment of cancer (Tanaka, 2001) and viral (Chinchar et al. 2001; Andersen et al. 2001; Chernysh et al. 2002) or parasitic infections (Vizioli and Salzet, 2003). Different therapeutic applications of these compounds, from topical administration to systemic treatment of infections, have been developed by several biotechnological companies (Hancock, 2000; http://www.inimexpharma.com; http://biotech.deep13.com/Alpha/alpha.html; http://www.geniconsciences.com/) Interestingly, to date, clinical Phase I and II trials have shown a limited resistance for the bacterial strains tested (Zasloff, 2002). These features make the antibiotic peptides a powerful arsenal of molecules that could be the antimicrobial drugs of the new century as an innovative response to the increasing problem of MDR (http://www.multi-drug-resistance.org; http://www.multi-drug-resistenz.de; http://www.demegen.com.).

Resistance to chemical antibiotics: an unsolved and growing problem

It is widely accepted among clinicians, medical researchers, microbiologists and pharmacologists, that antibiotic resistance will, in the very near future,leave healthcare professionals without effective therapies for bacterial infections. As an example, it is now estimated that about half of all *Staphylococcus aureus* strains found in many medical institutions are resistant to antibiotics such as methicillin (Roder et al. 1999). The emergence among enterococci of resistance to another useful and widely effective antibiotic, vancomycin (Novak et al. 1999), might accelerate the spread of vancomycin-resistant genes, via plasmids, throughout other species, eventually limiting the efficacy of this drug. Consequently, the priority for the next decades should be focused in the development of alternative drugs and/or the recovery of natural molecules that would allow the consistent and proper control of pathogen-caused diseases. Ideally, these molecules should be as natural as possible, with a wide range of action over several pathogens, easy to produce, and not prone to induce resistance.

The new generation of native peptide molecules, also known as Anti Microbial Peptides (AMPs), isolated from a full range of organisms and species from bacteria to man, seem to fit this description. As a consequence, they have been termed “natural antibiotics”, because they are active against a large spectrum of microorganisms, including bacteria and filamentous fungi - in addition to protozoan and metazoan parasites (Liu et al. 2000; Vizioli and Salzet, 2003). All of these molecules are key elements directly implicated in the innate immune response of their hosts, which includesthe expression of fluid phase proteins that recognize pathogen-associated molecular patterns, instead of specific features of a given agent to promote their destruction. As a result, the response is very fast, highly efficient and applicable to a wide range of infective organisms (Hoffmann and Reichhart, 2002). Additionally, the effect of AMPs can go beyondisolated bacterial cells, as shown by the inhibition they can exert over clusters of pathogenic bacteria, such in biofilm development (Singh et al. 2002).

The importance of the innate immune response in living organisms

In order to survive in a world laden with microorganisms, most multi-cellular organisms ought to depend on a network of host defense mechanisms which in most cases, involves several levels of interacting systems. Since the initial contact of fastidious microorganismswith the host usually occurs at inner or outer body surfaces, they should be the primary site for an immune reaction to occur. Thus, innate immune responses refer to the first line of host defense, which acts within a few hours after microbial exposure to mucosal surfaces. Upon recognition of conserved molecular microbial patterns such as PAMs or Pathogen-Associated Molecular Patterns (e.g. LPS and cell wall components) and Toll-like receptors (TLR) (Hoffman et al. 1999; Aderem and Ulevitch, 2000; Akira et al. 2001) initiate the immune responses of the host. Using the urinary and gastro-intestinal tract as model systems, information has been obtained on how organ- and cell-specific expression patterns of TLR on epithelial cells correlate to the ability of an organ to rapidly respond to bacterial infections has been obtained(BŠckhed et al. 2003). It has become clear now that understanding the innate response to pathogens will certainly provide insights to host defenses as well as the strategies used by pathogens to circumvent these defense mechanisms. Remarkably, the pattern-specific recognition system already acknowledged in animals, has also been reported in plants (Dangl and Jones, 2001).

In complex system suchas humans, an invading microorganism can simply be eliminated by this primary reaction - the innate response - without requiring an activation of the adaptive immunity, the next step in this complex cascade (Bals, 2000). If the invading microbe outgrows the innate host defence, endogenous effector mechanisms of the innate system are up-regulated and have direct antimicrobial activity and mediator function to attract inflammatory cells and cells of the adaptive immune system. In lower eukaryotes, mostly invertebrates, the adaptive system is nonexistent, thus accounting for the versatile and effective role the innate system has in order to control, by itself, the invasiveness of a given pathogen (reviewed by Otvos, 2000).

Differentiating antimicrobial peptides

Memberss of the major groups of antimicrobial peptides have been classified mainly on the basis of their biochemical (net charge) and/or structural features (linear/circular/amino acid composition), looking for common patterns that might help to distinguish them.

(Tossi and Sandri, 2002; Zasloff, 2002). The resulting most important groups are the following:

From Eukaryotes

Cationic peptides: This is the largest group and the first to be reported, being widely distributed in animals and plants. So far, more than a thousand of such peptides have been characterized and over 50 % of them have been isolated from insects (Bulet et al. 1999; Andreu and Rivas, 1998; http://www.bbcm.univ.trieste.it/~tossi/antimic.html). On the basis of their structural features, cationic peptides can be divided as well into three different classes: (1) linear peptides forming-helical structures; (2) cysteine-rich open-ended peptides containing single or several disulfide bridges; and (3) molecules rich in specific amino acids such as proline, glycine, histidine and tryptophan.

Important subfamilies of cationic peptides include:

- *Cecropins:* This is a family of 3 - 4 kDa linear amphipatic peptides described in the haemolymph of insects in the early 1980s (Hultmark et al. 1980; Andreu and Rivas, 1998; Boman 1998; Zheng and Zheng , 2002). These molecules are devoid of cysteine residues and contain two distinctive helical segments: a strongly basic N-terminal domain and a long hydrophobic C-terminal helix, linked by a short hinge. Shortly thereafter, other linear amphipatic peptides such as the magainins isolated from *Xenopus* skin, were isolated from vertebratesand included in the same group (Zasloff, 1987; Bechinger et al. 1993; Simmaco et al. 1998). These were the first molecules used to evaluate their biomedical applications (Hancock, 2000; http://www.genaera.com; http://www.inimexpharma.com; http://biotech.deep13.com/Alpha/alpha.html; http://www.geniconsciences.com/).

- *Defensins:* This isa highly complex group of 4-kDa open-ended cysteine-rich peptides arranged with different structural motifs. They have been mostly isolated from mollusc, acari, arachnids**,** insects, mammals and plants. Defensins are arranged in families, based on their structural differences. Invertebrates (Hubert et al. 1996; Andreu and Rivas, 1998; Dimarcq et al. 1998; Bulet et al. 1999; Mitta et al. 1999; Silva et al. 2000; Nakajima et al. 2001) and plant (Broekaert et al. 1997; García-Olmedo et al. 1998; Segura et al. 1998; Liu et al. 2000) defensins are characterized by three and four disulfide bridges, respectively. They show a common structure comprising an ά-helix linked to a β-sheet by two disulfide bridges, distinctive structure known as the CSab motif.In mammals, ά - and β-defensins are characterized by an antiparallel -β sheet structure, stabilized by three disulfide bridges (Zasloff, 2002). Some of them naturally exist as cyclic molecules such as the theta-defensins (Tang et al. 1999; Lehrer and Ganz, 2002). It has been difficult to determine whether all molecules are homologous or have independently evolved similar features, but evidences are in favour of a distant relationship. The best evidence of this relationship is structural, particularly from their overall three-dimensional structure and from the spacing of half-cystine residues involved in intra-chain disulfide bonds.

- *Thionins:* These are antimicrobial, and generally basic, plant peptides with a molecular weight of 5000 Da, which contain 6 or 8 conserved cysteine residues. Their *in vitro* toxicity against plant pathogenic bacteria and fungi indicates a role in the resistance of plants (Bohlmann, 1999). Ligatoxin B, a new basic thionin containing 46 amino acid residues has been recently isolated from the mistletoe *Phoradendron liga* (Li et al. 2002). Similarities observed by structural comparison of the helix–turn–helix (HTH) motifs of the thionins and the HTH DNA-binding proteins, led the authors to propose that thionins might represent a new group of DNA-binding proteins.

- *Amino acid-enriched class:* This is a distinctive class of antibacterial and antifungal cationic peptides, enriched in specific amino acids, with distinctive features depending on the organism from which they are isolated . Those proline- and glycine-rich are mostly from insects and active against Gram-negative bacteria (Bulet et al. 1999; Otvos, 2000); while cysteine-rich peptides, not related to defensins, represent the most diverse family among arthropods (Dimarcq et al. 1998). On the other hand those enriched in histidine are particularly basic, mostly from mammals (Pollock et al. 1984). Among them, histatin recovered from saliva from humans and primates and primarily directed against fungal pathogens, outstands for its distinctive mechanism of action which does not involve channel formation in the fungal cytoplasmic membrane but rather translocates efficiently into the cell and targets the mitochondrion (Tsai and Bobek, 1998). Those enriched in histidine and glycine are quite large, also affecting fungal pathogens and a distinctive feature is that their residues are arranged in approximately regular but different structural repeats (Tossi and Sandri, 2002). Finally, only two peptides enriched in tryptophan residues have been described, both derived from porcine cathelicidin precursors (Schibli et al. 2002). The outstanding feature though, is broad spectrum of activity including hundreds of Gram-positive and negative clinical isolates in addition of fungi and even the enveloped HIV virus (Gennaro and Zanetti, 2000).

- *Histone derived compounds:* This is a family of cationic helical peptides corresponding to cleaved forms of histones originally isolated from toad – (buforin) (Park et al. 1996) and fish epithelia (parasin)

(Park, 1998). These molecules are structurally similar to cecropins and quite active against bacteria and fungi. In the case of buforin II, at least, it was demonstrated that this molecule penetrates bacterial membranes and bind to nucleic acids thus interfering with cell metabolism and leading to rapid cell death (Park et al. 1998). AMPs are important factors in fish innate immunity (Iwanaga et al. 1994; Lemaitre et al. 1996; Zhou et al. 2002) and new contributions tend to demonstrate it. Recently, an active peptide was identified both in coho salmon mucus and blood, which display full identity with the N-terminus of trout H1 histone (Patrzykat et al. 2001). This is an indication that histone proteins may be a relatively ubiquitous component of host defenses (Hirsh, 1958). This assumption has been strengthened in recent years by the isolation of histone-like proteins in the cytoplasm of murine macrophages (Hiemstra et al. 1993) and the characterization of histone H2B fragments in human wound fluids (Frohm et al. 1996).

- *Beta-hairpin:* The third class of cationic peptides known includes a wide range of 2 to 8-kDa compounds containing beta-hairpin cross-linked by disulphide bridge(s) . The smallest members of this class with one disulfide bridge, is represented by thanatin and brevinin. Those containing two disulfide bridges are represented by androctonin (Mandard et al. 1999) tachyplesin and protegrin I (Mandard et al. 2002). Members of this latter group are 2-kDa hairpin-structured peptides, isolated from both invertebrates and vertebrates and show preferential antibacterial and antifungal activities (Dimarcq et al. 1998; http://www.sanger.ac.uk/Users/sgj/thesis/node53.html).

- *Other natural structural and functional proteins:* Cationic peptides have been successfully recovered from precursor proteins others than hemocyanin, such as hemoglobin in tick (Fogaca et al. 1999) and lactoferrin in human (Andersen et al. 2001). Recently, a fraction enriched in a novel antibacterial domain from the N-terminal part of caprine lactoferrin (fragment 14 – 42) has been recovered from its precursor protein bound to a cation-exchange membrane, followed by *in-situ* enzymatic cleavage with an appropriate enzyme and referred as lactoferricin-C (Jones et al. 1994). Additionally, the *Lactoccocus lactis* lantibiotic nisin was also successfully released from its precursor polypeptide by the same procedure (Recio et al. 2003). The purification procedure described above could be used to isolate cationic peptides produced in bacteria as inactive fusion proteins or from naturally occurring antibacterial peptides by specific digestion from their precursors.

Two other forms of precursor-derived peptides are represented by cathelicidins and thrombocidins. The formers are quite abundant in mammals and generated from precursor proteins bearing an amino-terminal cathepsin L inhibitor domain (cathelin) (Lehrer and Ganz, 2002). The latters are compounds released from platelets and arise from deletions of the CXC chemokines neutrophil-activating peptide 2 and connective tissue-activating III in humans (Krijgsveld et al. 2000).

In plants, a similar picture is slowly emerging. A new family of antimicrobial peptides has been described from *Macadamia integrifolia* of which the first purified member has been termed MiAMP2c (Marcus et al. 1997). The peptide, active against a number of plant pathogens *in vitro,* derives from a precursor protein similar to vicilins 7S globulin proteins, suspected of a putative participation in defense during seed germination (Marcus et al. 1997). The novel peptide is inserted in the highly hydrophilic N-proximal region of the precursor, where three additional cysteine-containing MiAMP2c-like patterns exist, suggestive of three additional peptide isoforms, a pattern already described for fish AMPs (Lauth et al. 2002).

Proposed mechanism of action of cationic peptides

In spite of the fact that the mechanism of action is not satisfactory established for all cationic peptides, the structural model established by Shai-Matzusaki-Huang (Matzusaki, 1999) provides a reasonable explanation for most antimicrobial activities of these compounds (Zasloff, 2002). The model proposes that these linear amphipatic-helical peptides interact with bacterial membranes and increase their permeability, either by the effect of their positive charges with anionic lipids of the target membrane or by membrane destabilization through lipid displacements due to the drastic changes in the net charge of the composed system. A similar mechanism has been proposed for the cysteine-rich peptides such as defensins, which are suggested to form ion-permeable channels in the lipid bilayer. In contrast, some peptides penetrate into cells to exert their action over target molecules (Kragol et al. 2001). Several additional hypotheses have been proposed to explain the mechanisms by which peptides kill target cells; such hypotheses include induction of hydrolases which degrade the cell wall, disturbance of membrane functions and damage to crucial intracellular targets after internalization of the peptide (Zasloff, 2002).

Anionic peptides: This is a smaller novel group of molecules displaying antimicrobial activity which, up to now, have been mostly isolated from mammals.

- *Neuropeptide derived molecules:* This is the first class of anionic compounds recently found in infectious exudates of cattle and humans. They mostly include peptides derived from the processing of neuropeptide precursors such as pro-enkephalin-A, to yield active peptide B and enkelytin; some of them are phosphorylated (Salzet and Tasiemsky, 2001). These peptides are mainly active against Gram-positive bacteria at micromolar concentrations, likecationic

peptides, and similar products have been reported in some invertebrate species (Salzet, 2001).

- *Aspartic-acid-rich molecules:* Peptides of this class have been isolated and characterized primarily fromcattle pulmonary surfactants (Brogden et al. 1996; Bals, 2000; Fales-Williams et al. 2002). They have a structure similar to the charge-neutralizing pro-peptides of Group I serine proteases and have been proposed to regulate the activity of pulmonary enzyme systems in these animals. Recently, a novel anionic 47-amino-acid peptide, named dermicidin, has been identified in human sweat, in response to a variety of pathogenic Gram-positive bacteria and ascribed to this class of molecules (Schittek et al. 2001).

- *Aromatic dipeptides:* The aromatic dipeptides comprise low molecular weight antibacterial compounds primarily isolated from dipteran larvae. There areonly two well characterized members: the N--alanyl-5-*S*-glutathionyl-3,4-dihydroxy-phenylalanine (573 Daltons), identified in the flesh fly *Sarcophaga peregrina* (Leem, 1999; Akiyama et al. 2000), and the *p*-hydroxycinnamaldehyde, isolated from the saw fly *Acantholyda parki* (Leem et al. 1999). The mode of action of these molecules is, at present, unknown.

- *Oxygen-binding proteins:* Peptides derived from oxygen-binding proteins, or hemocyanin derivatives (Destoumieux-Garzon et al. 2001; Muñoz et al. 2002; Muñoz et al. 2003), are the first representatives of the group of peptides derived from oxygen-binding proteins recently isolated from the hemolymph of arthropods and annelids species. Another molecule, detected in tick hemolymph, is a cleaved form of vertebrate hemoglobin, processed by the parasite after blood meal ingestion (Fogaca et al. 1999). These proteins have been reported as bactericidal compounds and might be considered as a reservoir of defense molecules to be used as integrative weapons to fight pathogens (Vizioli and Salzet, 2002). Bactericidal activity of anionic peptides, oxygen-binding protein derivatives and aromatic dipeptides are not as potent as cationic peptides, and their physiological relevance remains to be established in order to define their importance as components of the innate response (Decker et al. 2001).These molecules, whose mode of action could differ from that of cationic peptides and other antibiotics, could complement the activity of other compounds and constitute a useful base to develop novel synthetic derivatives.

From Prokaryotes:

The antimicrobial peptides produced by bacteria have been grouped into different classes based upon the producer organisms, molecular size, chemical structure and mode of action, which resulted in different names for putative compounds which turned out to be identical: (thiolbiotics, lantibiotic microcin, colicin, bacteriocin, to name a few) (Kolter and Moreno, 1992). The most relevant active-membrane peptides among them are produced by gram-positive bacteria and classified taxonomically as bacteriocins (Oscáriz and Pisabarro, 2001). Some of them have been the center of attention because of their application as food preservatives (Schillinger et al. 1996).

Bacteriocins, cationic, neutral and anionic in chemical nature, are all in the range of 1.9 (Actagardine) and 5.8 (Lactococcin B) kDa in molecular mass (Jack et al. 1995), cationic, neutral and anionic in chemical nature (Oscáriz and Pisabarro, 2001). The most thoroughly studied bacteriocins are those produced by lactic-acid bacteria, of which sakacins seem to be most unique (Jack et al. 1995; Simon et al. 2002), and the lantibiotics, which contain modified amino acid residues (Oscáriz and Pisabarro, 2001). Another representative, pediocins, are usually co-transcribed with a gene encoding a cognate-immunity protein (Fimland et al. 2002). The 44-amino acid pediocin produced by *Pediococcus acidilactici* strains is encoded in an 8.9 kb plasmid.

The importance of AMPs in humans

Peptides of the defensin, cathelicidin, thrombocidin and histatin classes are found in humans protecting epithelia against invading microorganims and assisting neuthrophils and platelets (Peschel, 2002). In the airways, α-and-β-defensins and the cathelicidin LL-37/hCAP-18 are produced by the respiratory epithelium and alveolar macrophages and then secreted into the airway surface fluid (Wang et al. 1999). Beyond their antimicrobial function, these peptides are known to be multi-functional. In fact, it has been demonstrated their multiple roles as mediators of inflammation with effects on epithelial and inflammatory cells, and the impact these roles have over such diverse processes as proliferation, immune induction, wound healing, cytokine release, chemotaxis, protease–antiprotease balance, and redox homeostasis (Ganz, 2002; Cole et al. 2003; Com et al. 2003; Liu et al. 2003).

DISCUSSION

Is there an induced resistance to AMPs?

Considering that AMPs are natural barriers to bacterial infections, pathogens ought to have developeda variety of strategies that render them resistant to antimicrobial host defenses. The only currently available structural model explaining the mechanism of action of AMPs (Shai-Matzusaki-Huang) (Matzusaki, 1999), the action of these peptides is from the outside and over the pathogen's membrane either by increasing their permeability or by destabilizing membranes by changing the net charge of the composed system. Since biological membranes are indeed dynamic fluids, the generation of resistance appears to be less likely to occur. Nonetheless, pathogens have evolved countermeasures not to resist, but at least to limit AMPs'

effectiveness, such as chemical modifications and/or alternation of energy-dependent pumps at the membrane level (Peschel, 2002). The same is true for intracellular bacterial pathogens, in which resistance-limitation is less effective against mostly cationic peptide-driven antimicrobial activity existing in the phagosomes of circulating monocytes, neutrophils and some mucosal epithelial cells (Ernst et al. 1999). Additionally, the fact that the common features for most peptides are a net positive charge and an amphipathic nature, allows them to persist at water-lipid interfaces and then to disturb microbial membrane components (Ruissen et al. 2001).

AMPs and biotechnology: Is there a promising future?

Good progress has been achieved with respect to defining the rules by which the immune system works and its complexity and interconnections are being slowly understood. In this perspective, the innate immune response has been neglected, but the consolidation of new discoveries in the field is slowly repositioning it (Fearon, 2000; Nathan, 2002). Nonetheless, the potential massive use of these natural compounds is hampered by the limited amount that can be extracted *in vivo* as well as non-optimal specific activities, which would require huge amounts for clinical and therapeutical application. This is the point where biotechnology should play a pivotal role in the near future, independent that chemical synthesis of peptides could also be a non exclusive alternative**.** Classically, these peptides are encoded by small genes, with conserved sequences and patterns that make their cloning easy, and should allow easy expression and both small- and large scale purification (Uteng et al. 2002). From a more innovative point of view, gene amplification and transgenesis seem like feasible ways to increase production and enhance specific activity of selected molecules. But, is this possible to achieve *in vivo*? The answer is, once again, yes. Biosynthetic and preparative production of AMPs have been successfully reported (Haught et al. 1998; Martemyanov et al. 2001), as have synthetic forms of AMP analogues displaying enhanced antimicrobial activity (Cudic et al. 2003). There are some additional examples: Since AMPs were first characterized in insects, a great deal of complementary work comes from that area of applied research. One of the most notable pieces of work deals with *Drosophila* mutants not expressing any known endogenous AMP genes and, as a consequence, highly susceptible to bacterial infections. Genetic manipulation of these mutants complemented with a single constitutively expressed AMP gene can rescue susceptibility to infections (Tzou et al. 2002). In plants, as expected, tobacco has been thetarget for successful engineered-production of mammalian AMPs (Morassutti et al. 2002), as well as amphibian anti microbial peptides, where vertical transmission of resistance occurs (Ponti et al. 2003). In addition, AMPs from other origins have been added to confer disease resistance in transgenic tobacco and banana (Chakrabarti et al. 2003) and potato (Osuky et al. 2000), thus opening unsuspected alternatives to provide agronomically relevant levels of disease control worldwide (Van der Biesen, 2001).

Relevancy of AMPs: Is there more to come?

Although at present AMPs are believed to exerttheir primary activity on bacterial membranes, new evidence is suggesting that AMP activity might be broader, including selective inhibition of intracellular targets (Cudic and Otvos, 2002). It is thought that cationic peptides might induce genomic responses in bacteria treated with AMPs, in addition to any lethal effect on the bacterial membrane**.** This appears to be the case, as recently demonstrated (Hong et al. 2003). These authors have shown that the transcription profiles of at least 26 *Escherichia coli* genes change specifically and significantly after exposure to lethal and sub lethal concentrations of Cecropin A, an emblematic cationic peptide. Moreover, half of these transcripts corresponded to proteins of unknown function, which makes these observations quite intriguing.

Now, regarding the wide variety and diverse classes of natural peptides, we must add necessarily, the processing alternatives, which are slowly being reported that might make these molecules incommensurable, approaching the diversity of immunoglobulins. The case of lactoferricin-C, generated as a functional internal domain of caprine lactoferrin in a manner mimicking the generation of inteins (selfish DNA elements inserted in-frame and translated together with their host proteins: http://bioinfo.weizmann.ac.il/~pietro/inteins), opens a new and broad area of research. Something similar occurs with milk-derived compounds, where it is clear that milk contains a group of proteins, which perform a protective function. These proteins harbor in their primary sequence, peptides that are inactive in the parent protein and that are released during gastrointestinal digestion or food processing (Yamauchi, 1992). In contrast, the generation of thrombocidin, arising from carboxy-terminal deletions of key neuthrophil- and connective tissue-activating peptides in humans, broadens the spectrum of alternative for processing associated with the generation of AMPs. Additionally, slight variations in the structure of preexisting peptides might broaden their potential as AMPs. A good example is that of histatin-5, a naturally occurring anti-fungal peptide in human saliva, which presents at least two variants (dhvar4 and dhvar5) displaying increased anti-microbial activity by subtle changes in their amphipathicity, a good indicator of membrane destroying activity, which allows them to be internalised showing a more destructive effect on mitochondria than on external membranes (Ruissen et al. 2001). Therefore, it is reasonable to think that a number of existing functional proteins, unrelated to immune responses, might contain potential and fully active AMPs This is a complementary strategy to that of natural anti-microbial peptides, which by themselves might adjust to potential bacterial adaptations to counteract their pathogenicity. This is only the tip of the iceberg in this

appealing topic. The recent proposalthat antibody multi specificity can be mediated by conformational diversity of pre existing isomers to increase the effective size of the antibody repertoire (James et al. 2003), is perfectly applicable to understand diversity of existing AMPs as well as the potential of those derived from multiple and heterogeneous type of precursors.Only time will verify these assumptions.

REFERENCES

ADEREM, A. and ULEVITCH, R. Toll-like receptors in the induction of the innate immune response. *Nature*, 2000,vol. 406, no. 6797, p. 782-787.

AKIRA, S.; TAKEDA, K. and KAICHO, T. Toll-like receptors: Critical proteins linking innate and acquired immunity. *Nature Immunology*, 2001, vol. 2, no. 8, p. 675-680.

AKIYAMA, N.; HIJIKATA, M.; KOBAYASHI, A.; YAMORI, T.; TSURUO, T. and NATORI, S. Anti-tumor effect of N-beta-alanyl-5-S-glutathionyl dihydroxyphenylalanine (5-S-GAD), a novel anti-bacterial substance from an insect. *Anticancer Research*, 2000, vol. 20, no. 1A, p. 357-362.

ANDERSEN, J.; OSBAKK, S.; VORLAND, L.; TRAAVIK, T. and GUTTEBERG, T. Lactoferrin and cyclic lactoferricin inhibit the entry of human cytomegalovirus into human fibroblasts. *Antiviral Research*, 2001, vol. 51, no. 2, p. 141-149.

ANDREU, D. and RIVAS, L. Animal antimicrobial peptides: an overview. *Biopolymers,* 1998, vol. 47, no. 6, p. 415-433.

BALS, R. Epithelial antimicrobial peptides in host defence against infection (Review). *Respiratory Research,* 2000, vol. 1, no. 3, p. 141-150.

BECHINGER, B.; ZASLOFF, M. and OPELLA, S.J. Structure and orientation of the antibiotic peptide magainin in membranes by solid-state nuclear magnetic resonance spectroscopy. *Protein Sciences*, 1993, vol. 2, no. 12, p. 2077-2084.

BOHLMANN, H. The role of thionins in the resistance of plants. In: DATTA, S.K., MUTHUKRISHNAN, S. eds. *Pathogenesis-related proteins in plants*, CRC Press, 1999, p. 207-234.

BOMAN, H. Gene-encoded peptide antibiotics and the concept of innate immunity: an update review. *Scandinavian Journal Immunology*, 1998, vol. 48, no. 1, p. 15-25.

BOMAN, H. Peptide antibiotics ant their role in innate immunity. *Annual Review of Immunology*, 1995, vol. 13, p. 61-92.

BROEKAERT, W.; CAMMUE, B.; DE BOLLE, M.; THEVISSEN, K.; DE SAMBLANX, G. and OSBORN, R. Antimicrobial peptides from plants. C*ritical Reviews in Plant Sciences*, 1997, vol. 16, no. 3, p. 297-323.

BROEKAERT, W.; MARIEN, W.; TERRAS, F.; DE BOLLE, M.; PROOST, P.; VAN DAMME, J.; DILLEN, L.; CLAEYS, M.; REES, S. and VANDERLEYDEN, J. Antimicrobial peptides from *Amaranthus caudatus* seeds with sequence homology to the cysteine/glycine-rich domain of chitin-binding proteins. *Biochemistry*, 1992, vol. 31, no. 17, p. 4308-4314.

BROGDEN, K.; DE LUCCA, A.; BLAND, J. and ELLIOTT, S. Isolation of an ovine pulmonary surfactant-associated anionic peptide bactericidal for *Pasteurella haemolytica. Proceeding National Academy of Sciences USA*, 1996, vol. 93, no. 1, p. 412-416.

BŠCKHED, F.; SŠDERHŠLL, M.; EKMAN, P.; NORMARK, S. and RICHTER-DAHLFORS, A. Induction of innate immune responses by *E. coli* and purified LPS correlate to organ- and cell-specific expression of Toll-like receptors within the human urinary tract. *Cellular Microbiology*, vol. 5. In press, 2003.

BULET, P.; HETRU, C.; DIMARCQ, J. and HOFFMANN, D. Antimicrobial peptides in insects; structure and function. *Developmental Comparative Immunology,* 1999, vol. 23, no. 4-5, p. 329-344.

CHAKRABARTI, A.; GANAPATHI, T.R.; MUKHERJEE, P.K. and BAPAT, V.A. MSI-99, a magainin analogue, imparts enhanced disease resistance in transgenic tobacco and banana. *Planta,* 2003, vol. 216, no. 4, p. 587-596.

CHERNYSH, S.; KIM, S.I.; BECKEER, G.; PLESKACH, V.A.; FILATOVA, N.A.; ANIKIN, V.B.; PLATONOV, V.G. and BULET, P. Antiviral and antitumor peptides from insects. *Proceedings of the National Academy of Science USA,* 2002, vol. 99, no. 20, p. 12628-12632.

CHINCHAR, V.G.; WANG, J.; MURTI, G.; CAREY, C. and ROLLINS-SMITH, L. Inactivation of frog virus 3 and channel catfish virus by esculentin-2P and ranatuerin-2P, two antimicrobial peptides isolated from frog skin. *Virology,* 2001, vol. 288, p. 351-357.

COLE, A.M.; DAROUICHE, R.O.; LEGARDA, D.; CONNELL, N. and DIAMON, G. Characterization of a fish antimicrobial peptide: gene expression subcellular localization and spectrum of activity. *Antimicrobial Agents Chemotherapy,* 2000, vol. 44, no. 8, p. 2039-2045.

COLE, A.M.; LIAO, H.I.; GANZ, T. and YANG, O.O. Antibacterial activity of peptides derived from envelope glycoproteins of HIV-1. *FEBS Letters*, 2003, vol. 535, no. 1-3, p. 195-199.

COM, E.; BOURGEON, F.; EVRARD, B.; GANZ, T.; COLLEU, D.; JEGOU, B. and PINEAU, C. Expression of antimicrobial defensins in the male reproductive tract of rats, mice, and humans. *Biology of Reproduction,* 2003, vol. 68, p. 95-104.

CUDIC, M.; CONDIE, B.A.; WEINER, D.J.; LYSENKO, E.S.; XIANG, Z.Q.; INSUG, O.; BULET, P. and OTVOS, JR. L. Development of novel antibacterial peptides that kill resistant isolates. *Peptides*, vol. 24. In press, 2003.

CUDIC, M. and OTVOS, JR. Intracellular targets of antibacterial peptides. *Current Drug Targets*, 2002, vol. 3, p. 101-106.

DANGL, J.L. and JONES, J. D. Plant pathogens and integrated defense responses to infection. *Nature,* 2001, vol. 411, no. 6839, p. 826-833.

DECKER, H.; RYAN, M.; JAENICKE, E. and TERWILLIGER N. SDS-induced phenoloxidase activity of hemocyanins from *Limulus polyphemus, Eurypelma californicum*, and *Cancer magister*. *Journal of Biological Chemistry,* 2001, vol. 276, no. 2, p. 17796-17799.

DE LUCCA, A.J. Antifungal peptides: potential candidates for the treatment of fungal infections. *Expert Opinion on Investigational Drugs*,2000, vol. 9, no. 2, p. 273-299.

DESTOUMIEUX-GARZON, D.; SAULNIER, D.; GARNIER, J.; JOUFFREY, C.; BULET, P. and BACHÈRE, E. Crustacean immunity. Antifungal peptides are generated from the C terminus of shrimp hemocyanin in response to microbial challenge. *Journal of Biological Chemistry,* 2001, vol. 276, no. 45, p. 47070-47077.

DIMARCQ, J.L.; BULET, P.; HEBRU, CH. and HOFFMANN, J. Cysteine-rich antimicrobial peptides in invertebrates. *Biopolymers*, 1998, vol. 47, p. 465-477.

ERNST, R.K.; GUINA, T. and MILLER, S.I. How intracellular bacteria survive: surface modifications that promote resistance to host innate immune responses. *Journal of Infectious Diseases,* 1999, vol. 179, no. suppl. 2, p. S326-30.

EZEKOWITZ, Alan and HOFFMANN, Jules. *Innate Immunity*. Humana Press, 2003. 410 p. ISBN 1588290468.

FALES-WILLIAMS, A.J.; GALLUP, J.M.; RAMIREZ-ROMERO, R.; BROGDEN, K.A. and ACKERMAN, M.R. Increased anionic peptide distribution and intensity during progression and resolution of bacterial pneumonia. *Clinical Diagnostic Laboratory Immunology,* 2002, vol. 9, no. 1, p. 28-32.

FEARON, D.T. Innate immunity-beginning to fulfil its promise? *Nature Immunology,* 2000, vol. 2, p. 102 -103.

FIMLAND, G.; EIJSINK, V.G. and NISSEN-MEYER, J. Comparative studies of immunity proteins of pediocin-like bacteriocins. *Microbiology,* 2002, vol. 148, p. 3661 - 3670.

FOGACA, A.C.; DA SILVA, P.I. JR.; MIRANDA, M.T.; BIANCHI, A.G.; MIRANDA, A.; RIBOLLA, P.E.M. and DAFFRE, S. Antimicrobial activity of a bovine hemoglobin fragment in the tick *Boophilus microp. Journal of Biological Chemistry,* 1999, vol. 274, no. 36, p. 25330-25334.

FROHM, M.; GUNNE, H.; BERGMAN, A.C.; AGERBERTH, B.; BERGMAN, T; BOMAN, A.; LIDEN, S; JORNVALL, H. and BOMAN, H.G. Biochemical and antibacterial analysis of human wound and blister fluid. *European Journal of Biochemistry,* 1996, vol. 237, no. 1, p. 86-92.

GANZ, T. The role of hepcidin in iron sequestration during infections and in the pathogenesis of anemia of chronic disease. *Israeli Medical Association Journal*, 2002, vol. 4, no. 11, p. 1043-1045.

GAO, A.G.; HAKIMI, S.M.; MITTANCK, CA.; WU, Y.; WOERNER, B.M.; STARK, D.M.; SHAH, D.M.; LIANG, J. and ROMMENS, C.M. Fungal pathogen protection in potato by expression of a plant defensin peptide. *Nature Biotechnology,* 2000, vol. 18, no. 12, p. 1307-1310.

GARCIA-OLMEDO, F; MOLINA, A. and ALAMILO, J.M. Plant defense peptides. *Biopolymers*, 1998, vol. 47, p. 479-491.

GENNARO, R. and ZANETTI, M. Structural features and biological activities of the cathelicidin-derived antimicrobial peptides. *Biopolymers*, 2000, vol. 55, no. 1, p. 31-49.

HANCOCK, R.E.W. Cationic antimicrobial peptides: towards clinical applications. *Expert Opinion on Investigational Drugs,* 2000, vol. 9, p. 1723-1729.

HANCOCK, R.E.W. and CHAPPLE, D.S. Peptide antibiotics (Minireview). *Antimicrobial Agents Chemotherapy,* 1999, vol. 43, no. 6, p. 1317-1323.

HANCOCK, R.E.W. and LEHRER, R. Cationic peptides: a new source of antibiotics. *Trends in Biotechnology,* 1998, vol. 16, p. 82-88.

HAUGHT, C.; DAVIS, G.D.; SUBRAMANIAN, R.; JACKSON, K.W. and HARRISON, R.G. Recombinant production and purification of novel antimicrobial peptide in *Escherichia coli. Biotechnology and Bioengineering,* 1998, vol. 57, no. 1, p. 55-61.

HIEMSTRA, P.S.; EISENHAUER, P.B.; HARWIG, S.S.; VAN DER BARSELAAR, M.T.; VAN BURTH, R. and LEHRER, R.I. Antimicrobial proteins of murine

macrophages. *Infection and Immunity*, 1993, vol. 61, no. 7, p. 3038-3046.

HIRSH, J. Bactericidal action of histone. *The Journal of Experimental Medicine*, 1958, vol. 108, p. 925-944.

HOFFMANN, J.A. and REICHHART, J.M. *Drosophila* innate immunity: an evolutionary perspective. *Nature Immunology*, 2002, vol. 3, no. 2, p. 121-126.

HOFFMANN, J.A.; KAFATOS F.C.; JANEWAY C.A. and EZEKOWITZ R.A. Phylogenetic perspectives in innate immunity. *Science*, 1999, vol. 284, no. 5418, p. 1313-1318.

HONG, R.W.; SHCHEPETOV, M.; WEISER, J.N.; AXELSEN, P.H. Transcriptional profile of the *Escherichia coli* response to the antimicrobial insect peptide cecropin A. *Antimicrobial Agents and Chemotherapy*, 2003, vol. 47, no. 1, p. 1-6.

HUBERT, F.; NEL, T. and ROCH, P. A member of the arthropod defensin family from edible mediterranean mussels (Mytilus galloprovincialis). *European Journal of Biochemistry,* 1996, vol. 240, no. 1, p. 302-306.

HULTMARK, D.; STEINER, H.; RASMUSON, T. and BOMAN, H.G. Insect immunity. Purification and properties of three inducible bactericidal proteins from hemolymph of immunized pupae of *Hyalophora cecropia*. *European Journal of Biochemistry,* 1980, vol. 106, no. 1, p. 7-16.

IWANAGA, S.; MUTA, T.; SHIGENAGA, T.; MIURA, Y.; SEKI, N.; SAITO, T. and KAWABATA, S. Role of hemocyte-derived granular components in invertebrate defense. *Annals of the New York Academy of Sciences*, 1994, vol. 712, p. 102-116.

JACK, R.W.; TAGG, J.R. and RAY, B. Bacteriocins of gram-positive bacteria. *Microbiology Review*, 1995, vol. 59, no. 2, p. 171-200.

JAMES C.; ROVERSI, P. and TAWFIK, D.S. Antibody multispecificity mediated by conformational diversity. *Science*, 2003, vol. 299, no. 5611, p.1362-1367.

JONES, E.M.; SMART, A.; BLOOMBERG, G.; BURGESS, L. and MILLER, M.R. Lactoferricin, a new antimicrobial peptide. *The Journal of Applied Bacteriology*, 1994, vol. 77, no. 2, p. 208-214.

KOLTER, R. and MORENO F. Genetics of ribosomally synthesized peptide antibiotics. *Annual Review of Microbiology*, 1992, vol. 46, p. 141-163.

KRAGOL, G.; LOVAS, S.; VARADI, G.; CONDIE B.A.; HOFFMANN R. and OTVOS L. Jr. The antibacterial pyrrhocoricin inhibits the ATPase action of DnaK and prevents chaperone-assisted protein folding. *Biochemistry*, 2001, vol. 40, no. 10, p. 3016-3026.

KRIJGSVELD, J.; ZAAT, S.A.J.; MEELDIJK, J.; VAN VEELEN, P.A.; FANG, G.; POOLMAN, B.; BRANDT, E.; EHLERT, J.E.; KUIJPERS, A.J.; ENGBERS, G.H.M.; FEIJEN, J.; and DANKERT, J. Thrombocidins, microbicidal proteins from human blood platelets are C-terminal deletion products of CXC chemokines. *Journal of Biological Chemistry,* 2000, vol. 275, no. 27, p. 20374-20381.

LAI, R.; LIU, H.; HUI LEE, W. and ZHANG, Y. An anionic antimicrobial peptide from toad *Bombina maxima. Biochemical and Biophysical Research Communications*, 2002, vol. 295, no. 4, p. 796-799.

LAMBERTY, M.; ZACHARY, D.; LANOT, R.; BORDEREAU, C.; ROBERT, A.; HOFFMANN, J.A. and BULET, P. Insect immunity. constitutive expression of a cysteine-rich antifungal and a linear antibacterial peptide in termite insect. *Journal of Biological Chemistry,* 2001, vol. 276, no. 6, p. 4085-4092.

LAUTH, X.; SHIKE, H.; BURNS, J.C.; WESTERMAN, M.E.; OSTLAND, V.E.; CARLBERG, J,M.; VAN OLST, J.C.; NIZET, V.; TAYLOR, S.W.; SHIMIZU, C.H. and BULET, P. Discovery and characterization of two isoforms of moronecidins, a novel antimicrobial peptide from hybrid striped bass. *Journal of Biological Chemistry,* 2002, vol. 277, no. 7, p. 5030-5039.

LEEM, J.Y.; JEONG, I.L.; PARK, K.T. and PARK, H.Y. Isolation of p-hydroxycinnamaldehyde as an antibacterial substance from the saw fly, *Acantholyda parki S. FEBS Letters*, 1999, vol. 442, no. 1, p. 53-56.

LEEM, J.Y. Purification and characterization of N--alanyl-5-*S*-gluthathionyl-3,4-dihydroxyphenylalanine, a novel antibacterial substance of *Sarcophaga peregrina* (Flesh fly). *Journal of Biological Chemistry,* 1996, vol. 271, no. 23, p. 13573-13577.

LEHRER, R.I. and GANZ, T. Defensin of vertebrate animals. *Current Opinion in Immunology*, 2002, vol. 14, no. 1, p. 96-102.

LEMAITRE, C.; ORANGE, N.; SAGLIO, P.; SAINT, N.; GAGNON, J. and MOLLE, G. Characterization and ion channel activities of novel antibacterial proteins from the skin mucosa of carp (*Cyprinus carpio). European Journal of Biochemistry,* 1996, vol. 240, no. 1, p. 143-149.

LI ,S.S.; GULLBO, J.; LINDHOLM, P.; LARSSON, R.; THUNBERG, E.; SAMUELSSON, G.; BOHLIN, L. and CLAESON, P. Ligatoxin B, a new cytotoxic protein with a novel helix-turn-helix DNA-binding domain from the mistletoe *Phoradendron liga. Biochemistry Journal*, 2002, vol. 366, no. 2, 405-413.

LIU, L.; ROBERTS, A.A. and GANZ, T. By IL-1 Signaling, monocyte-derived cells dramatically enhance the

epidermal antimicrobial response to lipopolysaccharide. *Journal of Immunology*, 2003, vol. 170, no. 1, p. 575-580.

LIU, Y.; LUO, J.; XU, C.; REN, F.; PENG, C.; WU, G. and ZHAO, J. Purification, characterization, and molecular cloning of the gene of a seed-specific antimicrobial protein from pokeweed. *Plant Physiology*, 2000, vol. 122, no. 4, p. 1015-1024.

LÜDERS, T.; BIRKEMO, G.A.; FIMLAND, G.; NISSEN-MEYER, J. and NES, I.F. Strong synergy between a eukaryotic antimicrobial peptide and bacteriocins from lactic acid bacteria. *Applied and Environmental Microbiology*, 2003, vol. 69, no. 3, p. 1797-1799.

MAINOUS III, Arch and POMEROY, Claire. *Management of Antimicrobials in Infectious Diseases.* Humana Press, 2001. 349 p. ISBN 0-89603-821-1.

MANDARD, N.; BULET, P.; CAILLE, A.; DAFFRE, S. and VOVELLE, F. The solution structure of gomesin an antimicrobial cysteine-rich peptide from spider. *European Journal of Biochemistry,* 2002, vol. 269, no. 4, p. 1190-1198.

MANDARD, N.; SY, D.; MAUFRAIS, C.; BONMATIN J.M.; BULET, P.; HETRU, C. and VOVELLE, F. Androctonin, a novel antimicrobial peptide from scorpion *Androctonus australis*: solution structure and molecular dynamics simulations in the presence of a lipid monolayer. *Journal of Biomolecular Structure and. Dynamics*, 1999, vol. 17, no. 2, p. 367-380.

MARCUS, J.P.; GOULTER, K.C.; GREEN, J.L.; HARRISON, S.J. and MANNERS, J.M. Purification, characterisation and cDNA cloning of an antimicrobial peptide from *Macadamia integrifolia. European Journal of Biochemistry,* 1997, vol. 244, no. 3, p. 743-749.

MARTEMYANOV, K.A.; SHIROKOV, V.A.; KURNASOV, O.V.; GUDKOV, A.T. and SPIRIN, A.S. Cell-free production of biologically active polypeptides: Application to the synthesis of antibacterial peptide cecropin. *Protein Expression and Purification,* 2001, vol. 21, no. 3, p. 456-461.

MATSUZAKI, K. Why and how peptide-lipid interaction utilized for self defense? Magainins and tachyplesins as archetypes. *Biochimica et Biophysca Acta*, 1999, vol. 1462, no. 1-2, p. 1-10.

MITTA, G.; VANDENBULCKE, F.; HUBERT, F. and ROCH, P. Mussel defensins are synthesised and processed in granulocytes then released into the plasma after bacterial challenge. *Journal of Cell Sciences,* 1999, vol. 112, no. 23, p. 4233-4242.

MORASSUTTI, C.; DE AMICIS, F.; SKERLAVAJ, B.; ZANETTI, M. and MARCHETTI, S. Production of recombinant antimicrobial peptide in transgenic plants using a modified VMA intein expression system. *FEBS Letters,* 2002, vol. 519, no. 1-3, p. 141-146.

MUÑOZ, M.; VANDENBULCKE, F.; GUEGUEN, Y. and BACHÈRE, E. Expression of penaeidin antimicrobial peptides in early larval stages of the shrimp *Penaeus vannamei*. *Developmental and Comparative Immuno*logy, 2003, vol. 27, no. 4, p. 283-289.

MUÑOZ, M.; VANDENBULCKE, F.; SAULNIER, D.; and BACHÈRE, E. Expression and distribution of penaeidin antimicrobial peptides are regulated by haemocyte reactions in microbial challenged shrimp. *European Journal of Biochemistry,* 2002, vol. 269, no. 45, p. 2678-2689.

NAKAJIMA Y.; VAN DER GOES VAN NATERS-YASUI, A.; TAYLOR, D. and YAMAKAWA M. Two isoforms of a member of the arthropod defensin family from the soft tick, *Ornithodoros moubata* (Acari: Argasidae*). Insect Biochemistry and Molecular Biology*, 2001, vol. 31, no. 8, p. 747-751.

NATHAN, C. Catalytic antibody bridges innate and adaptive immunity. *Science,* 2002, vol. 298, no. 5601, p. 2143-2144.

NOVAK, R.; HENRIQUES, B.; CHARPENTIER, E.; NORMARK, S. and TUOMANEN, E. Emergence of vancomycin tolerance in *Streptococcus pneumoniae. Nature,* 1999, vol. 399, no. 6736, p. 590-591.

OSCÁRIZ, J.C. and PISABARRO, A.G. Classification and mode of action of membrane-active bacteriocins produced by gram-positive bacteria. *International Microbiol*ogy, 2001, vol. 4, no. 1, p. 13-19.

OSUKY, M.; ZHOU, G.; OSUSKA, L.; HANCOCK, R.E.W.; KAY, W.W. and MISRA, S. Transgenic plants expressing cationic peptide chimeras exibit broad-spectrum resistance to phytopathogens. *Nature Biotechnology,* 2000, vol. 18, no. 11, p. 1162-1166.

OTVOS, L. Jr. Antibacterial peptides isolated from insects. *Journal of Peptide Sciences,* 2000, vol. 6, no. 10, p. 497-511.

PARK, C.J.; PARK, C.B.; HONG S.S.; LEE, H.S.; LEE, S.Y. and KIM, S.C. Characterization and cDNA cloning of two glycine-and histidin-rich antimicrobial peptides from the roots of shepherd´s purse, *Capsella* bursa-pastor. *Plant Molecular Biology*, 2000, vol. 44, no. 2, p. 187-197.

PARK, I.Y. Parasin I, an antimicrobial peptide derived from histone H2A in the catfish, *Parasilurus asotus. FEBS Letters,* 1998, vol. 437, no.3, p. 258-262.

PARK, C.B.; KIM, H.S. and KIM, S.C. Mechanism of action of the antimicrobial peptide buforin II: buforin II kills microorganisms by penetrating the cell membrane and

inhibiting cellular functions. *Biochemical Biophysical Research Communication,* 1998, vol. 244, no. 1, p. 253-257.

PARK, C.B.; KIM MS. and KIM S.C. A novel antimicrobial peptide from *Bufo bufo gargarizans*. *Biochemical Biophysical Research Communications,* 1996, vol. 218, no. 1, p. 408-413.

PATRZYKAT, A.; ZHANG, L.; MENDOZA, V.; IWAMA, G .K. and HANCOCK, R.E.W. Synergy of histone-derived peptides of coho salmon with lysozyme and flounder pleurodicin. *Antimicrobial Agents Chemother*apy, 2001, vol. 45, no. 5, p. 1337-1342.

PESCHEL, A. How do bacteria resist human antimicrobial peptides? *Trends in Microbiol*ogy, 2002, vol. 10, no. 4, p. 179-196.

POLLOCK, J.J.; DENPITIYA, L.; MACKAY, B.J. and IACONO, V.J. Fungistatic and fungicidal activity of human parotid salivary histidine-rich polypeptides on *Candida albicans*. *Infection and Immunity,* 1984, vol. 40, no. 3, p. 702-707.

PONTI, D.; MANGONI, M.L.; MIGNOGNA, G.; SIMMACO M. and BARRA D. An amphibian antimicrobial peptide variant expressed in *Nicotiana tabacum* confers resistance to phytopathogens. *Biochemical Journal*, 2003, vol. 15, no. 370, p.121-127.

PUTSEP, K.; BRANDEN, C.I.; BOMAN, H.G. and NORMARK, S. Antibacterial peptide from *Helicobacter pylori*. *Nature,* 1999, vol. 398, no. 6729, p. 671-672.

RODER, B.L.; WANDALL, D.A.; FRIMODT-MOLLER, N.; ESPERSEN, F.; SKINHOJ, P. and ROSDAHL, V.T. Clinical features of *Staphylococcus aureus* endocarditis: a 10-year experience in Denmark. *Archives Internal Medicine,* 1999, vol. 159, no. 5, p. 462-469.

RECIO, I.; SLANGEN, C.J. and VISSER, S. Method for the production of antibacterial peptides from biological fluids at an ionic membrane. Application to the isolation of nisin and caprine lactoferricin. Internal Report, 2003, Department of Product Technology, NIZO food research, P.O. Box 20, 6710 BA eds., The Netherlands.

RUISSEN, A.L.A.; GROENINCK, J.; HELMERHORST, E.J.; WALGREEN-WETERINGS, E.; VAN'T HOF, W.; VEERMAN, E.C.I. and NIEUW AMERONGEN, A.V. Effects of Histatin 5 and derived peptides on *Candida albicans*. *Biochemical Journal,* 2001, vol. 356, no. 2, p. 361-368.

SAITOH, H.; KIBA, A.; NISHIHARA, M.; YAMAMURA, S.; SUZUKI, K. and TERAUCHI, R. Production of antimicrobial defensin in *Nicotiana benthamiana* with a potato virus X vector. *Molecular Plant and Microbe Interactions,* 2001, vol. 2, no. 1, p. 111-115.

SALZET, M. Vertebrate innate immunity resembles a mosaic of invertebrate immune responses. *Trends in Immunology,* 2001, vol. 22, no. 6, p. 285-288.

SALZET, M. and TASIEMSKI, A. Involvement of pro-enkephalin-derived peptides in immunity. *Developmental and Comparative Immunology,* 2001, vol. 25, no. 3, p. 177-185.

SCHIBLI, D.J.; EPAND, R.F.; VOGEL, A.J. and EPAND, R.M. Tryptophan-rich antimicrobial peptides: comparative properties and membrane interactions. *Biochemistry and Cellular Biology,* 2002*,* vol. 80, no. 5, p. 667-677.

SCHILLINGER, U.; GEISEN, R. and HOLZAPFEL, W.H. Potential of antagonistic microorganisms and bacteriocins for the biological preservation of food. *Trends in Food Sciences and Technology*, 1996, vol. 7, no. 5, p. 158-164.

SCHITTEK, B.; HIPFEL, R.; SAUER, B.; BAUER, J; KALBACHER, H.; STEVANOVIC, S.; SCHIRLE, M.; SCHROEDER, K.; BLIN, N.; MEIER, F.; RASSNER, G. and GARBE, C. Dermcidin: a novel human antibiotic peptide secreted by sweat glands. *Nature Immunology,* 2001, vol. 2, no. 12, p. 1133-1137.

SEGURA, A.; MORENO, M.; MOLINA, A. and GARCÍA-OLMEDO, F. Novel defensin subfamily from spinach (*Spinacia oleracea*). *FEBS Letters,* 1998, vol. 435, no. 13, p. 159-162.

SELITRENNIKOFF, C.P. Antifungal proteins. *Applied and Environmental Microbiology,* 2001, vol. 67, no.7, p. 2883-2894.

SILVA, Jr.P.I.; DAFFRE, S. and BULET, P. Isolation and characterization of gomesin, an 18-residue cysteine-rich defense peptide from the spider *Acanthoscurria gomesiana* hemocytes with sequence similarities to horseshoe crab antimicrobial peptides of the tachyplesin family. *Journal of Biological Chemistry,* 2000, vol. 275, no. 43, p. 33464-33470.

SIMMACO, M.; MIGNOGNA, G. and BARRA, D. Antimicrobial peptide from amphibian skin: What do they tell us? *Biopolymers*, 1998, vol. 47, no. 6, p. 435-450.

SIMON, L.; FREMAUX, C.; CENATIEMPO, Y. and BERJEAUD, J.M. Sakacin G, a new type of antilisterial bacteriocin. *Applied and Environmental Microbiology,* 2002, vol. 68, no. 12, p. 6416-6420.

SINGH, P.K.; PARSEK, M.R.; GREENBERG, E.P. and WELSH, M.J. A component of innate immunity prevents bacterial biofilm development. *Nature, 2002,* vol. 417, no. 6888, p. 552-555.

TAILOR, R.H.; ACLAND, D.P.; ATTENBOROUGH, S.; CAMMUE, B.P.; EVANS, I.J.; OSBORN, R.W.; RAY, J.A.; REES, S.B. and BROEKAERT, W.F. A novel family

of small cysteine-rich antimicrobial peptides from seed of *Impatiens balsamina* is derived from a single precursor protein. *Journal of Biological Chemistry,* 1997, vol. 272, no. 39, p. 24480-24487.

TANAKA, K. P-I3 - kinase p85 is a target molecule of proline-rich antimicrobial peptide to suppress proliferation of ras - transformed cells. *Japanese Journal of Cancer Research,* 2001, vol. 92, no. 9, p. 959-967.

TANG, Y.Q.; YUANG, J.; OSAPAY, G.D.; OSAPAY, K.; TRAN, D.; MILLER, C.J.; OUELLETTE, A.J. and SELSTED, M.E. A cyclic antimicrobial peptide produces in primate leukocytes by the ligation of two truncated alpha-defensins. *Science,* 1999, vol. 286, no. 5439, p. 498-502.

THEVISSEN, K.; CAMMUE, B.; LEMAIRE, K.; WINDERICKX, J.; DICKSON, R.C.; LESTER, R.L.; FERKET, K.K.; VAN EVEN, F.; PARRET, A.H. and BROEKAERT, W. A gene encoding a sphingolipid biosynthesis enzyme determines the sensitivity of *Saccharomyces cerevisiae* to an antifungal plant defensin from dahlia (*Dahlia merckii*). *Proceedings of the National Academy of Science USA,* 2000, vol. 97, no. 8, p. 9531-9536.

THEVISSEN, K.; TERRAS, F.R.G. and BROEKAERT, W.F. Permeabilization of fungal membranes by plant defensins inhibits fungal. *Applied and Environmental Microbiology*, 1999, vol. 65, no. 12, p. 5451-5458.

TOSSI, A. and SANDRI, L. Molecular diversity in gene-encoded, cationic antimicrobials polypeptides. *Current Pharmaceutical Design*, 2002, vol. 8, no. 9, p. 743-761.

TSAI, H. and BOBEK, L.A. Human salivary histatins: promising anti-fungal therapeutic agents. *Critical Reviews in Oral Biology and Medicine,* 1998, vol. 9, no. 4, p. 480-497.

TZOU, P.; REICHART, J.M. and LEMAITRE, B. Constitutive expression of a single antimicrobial peptide can restore wild-type resistance to infection in immunodeficient Drosophila mutants. *Proceedings of the National Academy of Science USA,* 2002, vol. 99, no. 4, p. 2152-2157.

UTENG, M.; HAUGE, H.H.; BRONDZ, I.; NISSEN-MEYER, J. and FIMLAND, G. Rapid two-step procedure for large-scale purification of Pediocin-like bacteriocins and other cationic antimicrobial peptides from complex culture medium. *Applied and Environmental Microbiology,* 2002, vol. 68, no. 2, p. 952-956.

VAN DER BIESEN, E.A. Quest for antimicrobial genes to engineer disease-resistant crops. *Trends in Plant Sciences,* 2001, vol. 6, no. 3, p. 89-91.

VIZIOLI, J. and SALZET, M. Antimicrobial peptides: new weapons to control parasitic infections? *Trends in Parasitology,* 2003, vol. 19, In press.

VIZIOLI, J., and SALZET, M. Antimicrobial peptides from animals: focus on invertebrates. *Trends in Pharmacological Sciences,* 2002, vol. 23, no. 11, p. 494-496.

VIZIOLI, J.; BULET, P.; HOFFMANN, J.A.; KAFATOS, F.C.; MÜLLER H.M. and DIMOPOULOS, G. Gambicin: a novel immune responsive antimicrobial peptide from the malaria vector *Anopheles gambiae. Proceedings of the National Academy of Science USA,* 2001, vol. 98, no. 22, p. 12630-12635.

WANG, Y.; GRIFFITHS W.J.; CURSTEDT, T. and JOHANSSON, J. Porcine pulmonary surfactant preparations contain the antibacterial peptide prophenin and a C-terminal 18-residue fragment thereof. *FEBS Letters,* 1999, vol . 460, no. 2, p. 257-262.

WELLING, M.M.; PAULUSMA-ANNEMA, A.; BALTER, H.S.; PAUWELS, E.K. and NIBBERING, P.H. Tenehtium-99m labeled antimicrobial peptide discriminate between bacterial infection and sterile inflammations. *European Journal of Nuclear Medicine,* 2000, vol. 27, no. 3, p. 292-301.

YAMAUCHI, K. Biologically functional proteins of milk and peptides derived from milk proteins. *Bulletin IDF*, 1992, vol. 272, no. 1, p. 51-58.

ZASLOFF, M. Antimicrobial peptides of multicellular organisms. *Nature,* 2002, vol. 415, no. 6870, p. 389-395.

ZASLOFF, M. Magainins, a class of antimicrobial peptides from Xenopus skin: Isolation characterization of two active forms, and partial cDNA sequence of a precursor. *Proceeding of the National Academy of Sciences USA*, 1987, vol. 84, no. 9, p. 5449-5453.

ZHENG, X.L. and ZHENG, A.L. Genomic organization and regulation of three cecropin genes in *Anopheles gambiae*. *Insect Molecular Biology,* 2002, vol. 11, no. 6, p. 517-525.

ZHOU, Q.J.; SHAO, J.Z. and XIANG, L.X. Progress in fish antibacterial peptide research. *Progress in Biochemistry and Biophysics,* 2002, vol. 29, no. 5, p.682-685.

APPENDIX

Tables

Table 1. Cationic antimicrobial peptides (Modified with permission from Vizioli and Salzet, 2002).

Structure and representative Peptides	Organism	Antimicrobial activity	References
Linear α-helix peptides			
Cecropins	Insects, pig	Bacteria, fungi, virus	Andreu and Rivas, 1998; Putsep et al. 1999; Zasloff, 2002; Vizioli and Salzet, 2003
Clavanin, styelin	Tunicates	Bacteria	Zasloff, 2002
Megainin, dermaseptin	Amphibians	Bacteria, protozoa	Zasloff, 2002, Vizioli and Salzet, 2003
Buforins	Amphibians	Bacteria, fungi	Park et al. 1996, 1998; Zasloff, 2002
Pleurocidin	Fish	Bacteria, fungi	Cole et al. 2000
Moronecidin	Fish	Bacteria	Lauth et al. 2002
Linear peptides amino acid-rich			
Pro-rich:			
Drosocin, metchnikowins	Fruit fly	Bacteria	Bulet et al. 1999
Pyrrhocoricin	Hemipteran	Bacteria, fungi	Bulet et al. 1999
metalnikowin	Hemipteran	Bacteria, fungi	Bulet et al. 1999
Gly-rich:			
Diptericins, attacins	Dipterans	Bacteria	Bulet et al. 1999
shepherin I and shepherin II	Plants	Bacteria G- , fungi	Park et al. 2000
Ac-AMP1- Ac-AMP2	Plants	Bacteria G+, fungi	Broekaert et al. 1992
His-rich			
Histatin	Human	Bacteria, fungi	Andreu and Rivas, 1998; Zasloff, 2002
shepherin I and shepherin II	Plants	Bacteria G- , fungi	Park et al. 2000
Try-rich			
Indolicidin	Cattle	Bacteria	Andreu and Rivas, 1998; Zasloff, 2002
Tritrpticin, lactoferrin B, LfcinB4-9	Eukaryots	Bacteria	Schibli et al. 2002
Single disulfide bridge			
Thanatin	Hemipteran	Bacteria, fungi	Dimarcq et al. 1998; Bulet et al. 1999; Zasloff, 2002
Brevinins	Frog	Bacteria	Andreu and Rivas, 1998; Zasloff, 2002
Lanthionins	Bacteria (G+)	Bacteria	Hancock, 2000
Two disulfide bridges			
Tachyplesin II	Horseshoe crab	Bacteria, fungi, virus	Andreu and Rivas, 1998, Dimarq et al. 1998; Zasloff, 2002
Androctonin	Scorpion	Bacteria, fungi	Dimarq et al. 1998; Zasloff, 2002
Protegrin I	Pig	Bacteria, fungi, virus	Zasloff, 2002
Three disulfide bridges			
α Defensins	Mammals	Bacteria, fungi	Andreu and Rivas, 1998; Zasloff, 2002
β Defensins	Mammals	Bacteria, fungi	Andreu and Rivas, 1998; Zasloff, 2002

Defensis	Insects	Bacteria, fungi, protozoa	Dimarq et al. 1998 ; Bulet et al. 1999; Vizioli and Salzet, 2003
Penaeidins	Shrimp	Bacteria, fungi	Dimarq et al. 1998; Bulet et al. 1999; Vizioli and Salzet, 2003
More than three disulfide bridges			
Tachycitin	Horseshoe crab	Bacteria, fungi	Dimarq et al. 1998
Drosomycin	Fruit fly	Fungi	Dimarq et al. 1998
Gambicin	Mosquito	Bacteria, fungi, protozoa	Vizioli et al. 2001
Heliomicin	Lepidopteran	Bacteria, fungi	Lamberty et al. 2001
Plant defensins			
defensin protein WT1	Plants	Fungi	Saitoh et al. 2001
alfAFP defensin	Plants	Fungi	Gao et al. 2000
cysteine-rich	Plants	Bacteria , fungi	Tailor et al. 1997
So-D1-7	Plants	Bacteria ,fungi	Segura et al.. 1998
DmAMP1	Plants	Fungi	Thevissen et al. 2000

Table 2. Non-Cationic antimicrobial peptides. (Modified with permission from Vizioli and Salzet, 2002).

Structure and representative Peptides	**Organism**	Antimicrobial activity	**Refs**
Anionic peptides			
Neuropeptide Derived:			
Enkelytin	Bovine, human	Bacteria	Salzet 2001; Salzet and Tasiemski, 2001
Peptide B	Bovine, human	Bacteria	Salzet 2001; Salzet and Tasiemski, 2001
	Leech, mussel		
Aspartic acid rich:			
H-GDDDDDD-OH	Ovine	Bacteria	Brogden et al. 1996
Dermcidin	Human	Bacteria	Schittek et al. 2001
Maximin H5	Amphibian	Only Gram+ bacteria	Lai et al. 2002
Aromatic dipeptides			
N-β-alanyl-5-S-glutathionyl-	Fiesh fly	Bacteria fungi	Leem, 1996
3,4-dihydroxyphenylalanine			
p-Hydroxycinnamaldehyde	Saw fly	Bacteria, fungi	Leem et al. 1999
Peptides derived from oxygen-binding			
proteins			
Hemocyanin derived	Shrimp	Bacteria	Destoumieux-Garzon et al. 2001
Hemoglobin derived	Tick	Bacteria	Fogaca et al. 1999
Lactoferrin	Human	Bacteria, virus	Andersen et al. 2001