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ANTIMICROBIAL PEPTIDES OF VERTEBRATES

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SUMMARY

Antimicrobial peptides have been found in animals as diverse as insects and man. Interest in this class of antibiotics has greatly increased recently as bacterial resistance to existing antibiotics has become a serious clinical problem. These peptides are membrane active agents with a broad spectrum of microbicidal activity. Their hallmark physical features are a highly positive net charge and the ability to adopt amphipathic conformations. Their broad spectrum of antimicrobial activity, unique mechanism of action, and ability in many cases to kill organisms that are resistant to traditional antibiotics makes them attractive for pharmaceutical development. One such peptide, an analogue of a frog magainin, is in phase III clinical trials for infected diabetic foot ulcers. Increasingly the search for these peptides in Nature is focused on higher vertebrates, including humans. Recent developments highlight the importance of vertebrate epithelial cells as sites of antibiotic peptide production and suggest a critical role for this system in human health and disease.

INTRODUCTION

Antibiotics have historically been derived from lower organisms such as bacteria or fungi. These creatures presumably produce such substances to gain competitive advantage in the microbial ecology. However, higher eucaryotes also produce antibiotic substances as part of their immune defences. Gene-encoded antimicrobial peptides are found widely in the animal kingdom and salient examples include the cecropins from insects (*Boman*, 1995), magainins from amphibians (*Zasloff*, 1987), and defensins from mammals (*Ganz* and *Lehrer*, 1995). These molecules tend to exhibit intrinsic specificity for microbial invaders and are relatively much less toxic for the metazoan host's cells. This specificity endows the animal with an "innate" immunity, in contrast to the better studied acquired immunity conferred by the clonal expansion of B and T cells. The possible importance of this system as a check on infection is evident when one considers that most bacteria have generation times of 20-30 minutes whereas the mounting of a specific immune response, dependent on the growth of mammalian cells, may take days or weeks.

STRUCTURE AND MECHANISM OF ACTION

Known antibiotic peptides derived from animals fall into several distinct structural categories. One major structural class of peptides is linear, α-helical, linear, and contains no disulphide bonds. Included in this group are the magainins, insect cecropins, and mammalian cecropins. A second important class are the defensins. In contrast to the α-helical molecules just described defensins have a conserved pattern of disulphide pairing that holds them in a β-sheet conformation. Other structural motifs have been observed for vertebrate antimicrobial peptides but they are in general less well studied.

Despite their structural diversity the activity and specificity for microbes of antimicrobial peptides is likely due to shared features. These characteristics include a highly positive net charge and the ability to adopt ordered amphipathic conformations. Antimicrobial peptides are typically surface and membrane active agents that kill by inserting into the membrane and forming pores, thereby disrupting the structural integrity and homeostatic mechanisms of the target cell (*Cruciani* et al., 1992; *White* et al. 1995). The positive charges serve to attract the antibiotic to the surface of the target microbe. The remarkable selectivity of these peptides is thought to be due to the relative abundance of negative charges in target cell membranes compared to those of the metazoan host (*Matsuzaki* et al., 1995; *White* et al., 1995). These membrane charges are due primarily to anionic phospholipids (*Matsuzaki* et al., 1995). Such charges act as the "receptors" for the highly cationic peptides. The amphipathic nature of the antibiotics is also a critical feature and allows them to insert into the membrane and form pores, holes, or ion channels. Formation of these holes is believed to involve the assembly of higher order structures containing multiple peptide molecules, but the exact nature of these complexes is unclear (*Vaz Gomes* et al., 1993; *White* et al., 1995). The presence of cholesterol in host cell membranes may serve to protect them from the disruptive effects of the peptides (*Tytler* et al., 1995). Since microbes lack cholesterol in their membranes this phenomenon probably further increases the selectivity of these peptides.

In many instances cells with a higher transmembrane potential are more susceptible to the killing effects of cationic antimicrobial peptides (*Matsuzaki* et al. 1995; *White* et al., 1995). Such a potential, being negative on the inside of the cell, may provide an electromotive force that aids in driving the integration of the positively charged peptide into the target membrane (*White* et al., 1995). The fact that synthetic magainins consisting of all D amino acids are as biologically active as their L amino acid containing counterparts indicates that their interaction with the target does not involve chiral interactions (*Wade* et al., 1990). This observation underscores the uniqueness of the mechanism of action of antimicrobial peptides as compared to traditional antibiotics which, like most pharmaceuticals, are direct inhibitors of enzymes with chiral active sites.

MAGAININS

The magainins are linear, cationic, amphipathic peptides of 21-27 amino acids originally isolated from the skin of the African clawed frog, *Xenopus laevis*

(*Zasloff*, 1987). Zasloff isolated two peptides, magainin 1 and 2, from *Xenopus* skin based on their antimicrobial activity after noting the remarkable resistance of the frogs to infection despite their lack of an advanced cellular immune system. The magainins are structurally similar to the insect cecropins in their α-helical secondary structure and lack of cysteine residues or disulphide bridges. As such they were the first known representatives of this class of antimicrobial peptides observed in vertebrates.

A broad range of pathogens are susceptible to magainins, including Gram-positive and Gram-negative bacteria, fungi, protozoa, and viruses (*Zasloff*, 1987; *Aboudy* et al., 1994). Magainins are stored in specialised secretory granule containing skin cells called granular glands. Subsequent studies showed that magainins are also produced by granular glands in the frog's digestive tract including stomach, small intestine, and colon (*Reilly* et al., 1994). They are produced as multimers in pro-proteins and the active form is liberated, after secretion, by a unique protease that recognises secondary rather than primary structure (*Resnick* et al., 1991; *Jacob* and *Zasloff*, 1994). Magainins exhibit a near random coil conformation in aqueous solution but adopt an amphipathic, α-helical structure in hydrophobic solvents and also upon interaction with target cell membranes (*Jacob* and *Zasloff*, 1994; *Maloy* and *Kari*, 1995).

Many other antibiotic peptides, distinct from the magainins, are also produced by *Xenopus*. These include PGLa, CPF (10 species), LPF, XPF and PGQ (*Soravia* et al., 1988, *Maloy* and *Kari*, 1995). Interestingly, magainin 2 and PGLa act synergistically against microbes (*Westerhoff* et al., 1995). This diversity in a single animal presages the even greater diversity across frog species. Other examples of antibiotic peptides isolated from frogs include ranalexin from the American bullfrog (*Clark* et al., 1995) and dermaseptin from *P. sauvagii* (*Mor* and *Nicolas*, 1994). These compounds differ from the magainins in sequence and structure and further reflect what is apparently enormous genetic diversity in amphibian antibiotic peptides.

CLASSICAL OR α-DEFENSINS OF GRANULOCYTES

Mammalian granulocytes kill ingested pathogens via two distinct mechanisms. One involves an "oxidative burst" that produces reactive oxygen species that react with and kill the microbe. The other mechanism is non-oxidative and requires antimicrobial peptides known as defensins (*Ganz* and *Lehrer*, 1995). Defensins were first discovered in the macrophages and neutrophils of rabbits and subsequently have been found in a variety of mammalian neutrophils. They are cationic, amphipathic peptides of 29-45 amino acids and contain a highly conserved pattern of six cysteine residues. These cysteines form three disulphide bridges which hold the peptide in a β-sheet conformation (*Martin* et al., 1995). The crystal structure of one human granulocyte defensin has been determined (*Hill* et al., 1991).

Defensins are stored in the azurophil granules of neutrophils and represent a significant proportion of the granule and total cell protein content. Upon phagocytosis a vesicle containing the engulfed microbe is fused with the granules, delivering a local high concentration of antibiotic peptide. Defensins kill a wide

spectrum of pathogens, including Gram-positive and Gram-negative bacteria, fungi, and enveloped viruses (*Ganz* and *Lehrer*, 1995; *Martin* et al., 1995).

Defensins are produced as precursors and are cleaved by proteases to release the active, mature antimicrobial peptide. The pro-piece is typically negatively charged and may serve to neutralise the cationic peptide. This mechanism may help to prevent cytotoxicity to the host cell (*Valore* et al., 1996).

Many defensins have been characterised from a variety species. Tissue and cell type expression vary surprisingly amongst species. Defensins are present in rabbit pulmonary macrophages and circulating neutrophils. However, they are absent from rabbit peritoneal macrophages and monocytes. Humans produce four α-defensins in their neutrophils; HNP-1, 2, 3, and 4. These molecules have not yet been observed in human monocytes. Rats produce neutrophil α-defensins but interestingly they are completely absent in mouse granulocytes (*Eisenhauer* and *Lehrer*, 1992; *Ganz* and *Lehrer*, 1995; *Martin* et al., 1995).

ENTERIC DEFENSINS

In addition to being present in mammalian phagocytic cells, where they were first discovered, classical or α-defensins are also produced by Paneth cells in the small intestines of mice and humans (*Selsted*, 1992, *Jones* and *Bevins*, 1992). Paneth cells are specialised epithelial cells residing in the base of structures called crypts in the intestinal mucosa. Consequently the murine molecules have been termed cryptdins. Although they are epithelial cells, Paneth cells differ in important ways from the cells lining the rest of the intestine. Perhaps most strikingly they contain secretory granules reminiscent of those found in neutrophils. They are known to be active secretory cells and are thought to play a defensive role since they produce lysozyme as well as defensins (*Jones* and *Bevins*, 1992).

The existence of an extensive family of sixteen mouse cryptdins has been reported (*Huttner* et al., 1994). In contrast, human Paneth cells are known to produce only two defensins, HD-5 and HD-6 (*Jones* and *Bevins*, 1992; *Jones* and *Bevins*, 1993). The production of these molecules is developmentally regulated and coincides temporally with the appearance of Paneth cells in the gut (*Mallow* et al., 1996). In humans, expression of HD-5 is first detectable at 13.5 weeks of gestation and HD-6 at 13.5-17 weeks. Levels of mRNA measured by northern blotting at 19-24 weeks ranged from 40-250 fold less than those seen in adult small intestine. Mallow et al. (1966) hypothesise that low levels of Paneth cell defensin expression in the immature gut of pre-term infants may in part be responsible for the prevalence of necrotising enterocolitis in these children.

Cryptdins have been detected in the contents of the small intestine, suggesting that they are actively secreted by Paneth cells into the lumen (*Selsted* et al., 1992). This is in contrast to the situation in myeloid granulocytes where defensins are thought to contact microbes inside the cell after fusion of phagocytic vacuoles with the granules. Detection of constitutive defensin mRNA production in Paneth cells by *in situ* hybridisation (*Jones* and *Bevins*, 1992) indicates a further important difference compared to the regulation of circulating granulocyte defensin production wherein the protein is stored

and transcription ceases in the mature neutrophil (*Martin* et al., 1995).

The secretory nature of the Paneth cell and constitutive nature of defensin synthesis suggests that this system may play a role in maintaining the near sterile environment of the small intestine rather than strictly responding to acute challenges. Experiments in gnotobiotic mice demonstrate that intestinal defensin production is normal and is therefore not dependent on colonisation of the gut by bacteria (*Lehrer* et al. 1993). However, the presence of consensus binding sequences for transcription factors AP2 and NF-IL-6 in the 5' flanking region of the HD-5 and HD-6 (*Mallow* et al., 1996) genes implies that production of these defensins may be inducible in response to inflammatory stimuli. In fact, increased defensin expression in human necrotising colitis was recently reported (*Salzmann* et al., 1996).

β-DEFENSINS

The discovery of the magainins in frog skin suggested the epithelium of other vertebrates, including mammals, as a logical place to search for antibiotic peptides. Previous to that discovery antibiotic peptides had been found only in more traditional immune tissues such as the haemolymph of invertebrates, blood (granulocytes) of vertebrates and the defensively specialised Paneth cell. However, epithelial cell layers represent an important barrier between the animal and the microbial flora in the environment and are increasingly recognised as active immune tissues. These observations led to a search for antibiotic expression in the bovine airway and the discovery of tracheal antimicrobial peptide or TAP (*Diamond* et al. 1991).

TAP was the first of a new, structurally distinct family of mammalian defensins and the first found to be broadly expressed in the epithelial sheet. A search for antibiotic peptides in bovine neutrophils led to the discovery of thirteen peptides homologous to TAP (*Selsted* et al., 1993). Comparison of the amino acid sequences of these peptides and TAP resulted in a consensus sequence and showed clearly that the group represented a family. This observation led to the term β-defensins (*Selsted* et al., 1993).

β-defensins contain six conserved cysteines which form three disulphide bonds forming a molecule that is dominated by β-sheet just like their classical counterparts, but the pattern of these residues and bridges is substantially different (*Selsted* et al., 1993). The three-dimensional structure of one β-defensin, bovine neutrophil-defensin 12, has been determined by NMR and it is similar to that established for α-defensins (*Zimmerman* et al., 1995). They are also cationic, amphipathic, and lysine rich.

TAP was originally isolated from bovine tracheal mucosa based on its antibiotic activity. It is translated as a 63-amino acid propeptide. The mature peptide consists of 38 amino acids and is active against Gram-negative bacteria, Gram-positive bacteria, and fungi. Southern blotting of bovine genomic DNA with a TAP probe resulted in multiple hybridising bands implying the existence of a family of related genes. *In situ* hybridisation studies showed that TAP is expressed throughout the airway but not at other epithelial surfaces of the body (*Diamond* et al., 1993).

The TAP gene has been cloned and analysis of the 5' flanking sequence revealed the presence of a binding site for the transcription factor NF-KB. This

factor has been associated with numerous genes that are induced in immune response and inflammation (*Nolan* and *Baltimore*, 1992). *Diamond* et al. have reported that exposure of primary bovine tracheal epithelial cells to LPS in culture results in induction of TAP RNA (*Diamond* and *Bevins*, 1994; *Diamond* et al, 1996). The induction was a robust thirteen fold at sixteen hours post exposure. In these cells the LPS effect appears to be mediated, at least in part, by the CD14 antigen on the surface of the epithelial cells (*Diamond* and *Bevins*, 1994; *Diamond* et al., 1996).

The digestive tract represents another system that, like the airway, is exposed to the outside environment and is protected by an epithelial barrier. The tongue is covered by perhaps the most exposed and vulnerable epithelium in this system yet is rarely observed to become infected. This epithelium therefore seemed likely to be protected by antibiotic peptides as well. Prompted by these observations *Zasloff* and colleagues (1987) isolated another β-defensin from the mucosa of the bovine tongue. Reflecting its site of expression, this molecule was designated lingual antimicrobial peptide or LAP. A cDNA for LAP was also isolated which predicted a 64 amino acid pro-peptide (*Schonwetter* et al., 1995).

In situ hybridisation of the LAP cDNA with bovine tongue tissue demonstrated that LAP is expressed in the middle layers of the stratified epithelium of the normal tongue. Interestingly, similar experiments using tissue from cows with tongue lesions showed a dramatic induction of LAP mRNA synthesis associated with the wounds and accompanying areas of inflammation. This result served to generalise observations made with TAP in the airway to other exposed epithelia in mammals and most importantly showed that epithelial antibiotics are induced in response to insult. Induction may be the result of exposure to microbial products such as LPS, to inflammatory cytokines, or both.

A survey of bovine tissues by northern blotting showed expression of LAP or closely related molecules in many epithelial sites throughout the body, including conjuctiva, airway, and the digestive and urinary tracts (*Schonwetter* et al., 1995). Interestingly, expression in the tongue also appears to be developmentally regulated, being absent in the foetus and expressed robustly postnatally. Whether this is genetically programmed or due to induction after exposure to environmental microbes remains to be determined.

Antimicrobial peptides homologous to the β-defensins have been isolated from chicken leukocytes (*Harwig* et al., 1994). These three peptides were named gallinacins to reflect their source, *Gallus gallus.* Of the 36-39 residues in the gallinacins, 9, including 6 cysteines, are invariant amongst the gallinacins and known bovine β-defensins. The presence of β-defensins in birds suggests an ancient origin for these molecules, before the evolutionary divergence of birds and mammals.

Recently the amino acid sequence for a human peptide with homology to TAP was reported (*Bensch* et al., 1995). This molecule was isolated from human haemodialysis fluid in a general search for peptides. A computer homology search revealed a similarity to TAP. The activities, sites of expression, and regulation of this molecule remain to be addressed.

OTHER ANTIMICROBIAL PEPTIDES OF VERTEBRATES

The classes of vertebrate antimicrobial peptides discussed above represent the best studied and best understood to date, but the family of known peptides is considerably more diverse. A search for antibiotic activities in pig small intestine by Hans Boman's group at the University of Stockholm led to the discovery of cecropin P1, a molecule with surprising similarity to the insect cecropins (*Lee* et al., 1989). Cecropin P1 is 31 amino acids long and contains several amino acids which are conserved amongst the insect cecropins. It is linear, cationic, amphipathic and α-helical like the magainins and cecropins and as such represents the first of this structural class of antibiotic to be described in a mammal.

Boman's group also isolated PR39 from pig intestine. PR39 is a proline rich, 39 amino acid peptide which lacks cysteine. Subsequent cDNA cloning demonstrated that this peptide is produced in bone marrow rather than intestine, suggesting the source in intestine may be resident lymphocytes (*Boman*, 1995). FALL-39 is a peptide derived from a human bone marrow cDNA coding for a larger putative precursor. One 39 residue portion is similar to PR-39, and synthesis of this peptide yielded a compound with antibiotic activity (*Agerberth* et al., 1995). If this peptide indeed exists physiologically it represents the first human cysteine free peptide antibiotic, distinguishing it from the human defensins.

At least two other proline rich antibiotics have been isolated from mammals. These are the bactenecins Bac 5 and Bac 7 which were isolated from bovine neutrophils (*Frank* et al., 1990). Also from bovine neutrophils comes a tryptophan rich molecule known as indolicidin (*Boman*, 1995). Pig immune cells contain molecules called protegrins. Protegrins have two disulphide bridges rather than the three seen in defensins. They are also substantially smaller, consisting of only 16-18 amino acids (*Boman*, 1995). In addition to the variety described here from mammals is the great diversity in sequence and structure mentioned earlier for the antibiotic peptides of frogs. It appears that the surface has only been scratched in the search for vertebrate antimicrobial peptides.

PEPTIDE ANTIBIOTICS IN HUMAN HEALTH AND DISEASE

Most of the data discussed above are derived from *in vitro* or cell culture experiments. However, several observations support a critical role for antimicrobial peptides in the *in vivo* immune response. For example, a human genetic disease known as specific granule deficiency results in an almost total absence of defensin containing granules in neutrophils. Patients with this disease produce little or no defensin in their leukocytes and are prone to repeated and severe infection (*Ganz* et al. 1988). This unfortunate natural experiment lends support to the important role that the classical defensins play as antibiotics used to kill pathogens phagocytised by PMN. The genetic lesion causing this deficiency has not been elucidated, but decreased defensin mRNA levels in some cases suggest that the defect may be due to a transcriptional defect (*Tamura* et al. 1994).

It has also been observed that the level of circulating defensins in the blood increases dramatically in patients

suffering from septicaemia (*Panyutich* et al., 1993). Since most defensins are found as inactive complexes with proteins such as a_2-macroglobulin in the blood (*Panyutich* and *Ganz*, 1991) this probably represents a marker for increased neutrophil activity rather than a systemic antimicrobial host response.

It has recently come to light that a defect in epithelial defensin activity may be responsible for the devastating lung infections suffered by cystic fibrosis (CF) patients. Although the defective gene leading to CF has been identified and characterised the genesis of these often fatal airway infections, with organisms such as *Pseudomonas aeruginosa*, has remained a mystery. Numerous hypotheses have been forwarded including poor clearance of microbes due to abnormal mucous (*Engelhardt*, 1992), defective phagocytosis by epithelial cells (*Pier* et al., 1996), as well as altered antimicrobial peptide metabolism or activity (*Zasloff*,1987).

Smith et al. (1996) have now reported the presence of an antibiotic activity on the surface of human airway epithelial cells in culture. They found that lung epithelial cells from healthy individuals were able to directly kill *Pseudomonas aeruginosa* and *Staphylococcus aureus* placed on their apical surfaces but that similar cells from CF patients could not. One obvious explanation for this result would be that the antibiotic activity is not produced or is otherwise absent from the CF cell's surface. However, antibiotic activity was recoverable from the fluid on the surface of both normal and CF epithelial cells. This activity is heat stable and less than 10 kD in size, consistent with it being due to a defensin.

It is known that the Na^+ and Cl^- concentrations in CF lung secretions are increased (*Joris* et al., 1993) and that the activity of defensins is inhibited in higher salt (*Martin* et al., 1995). Together these facts may offer an explanation for the lack of natural antibiotic activity observed on CF cells. Indeed, a series of experiments by *Smith* et al. (1996) demonstrated that lowering the salt concentration revealed antibiotic activity produced by CF cells and raising the concentration masked the activity described in normal lung epithelial cells. The identity of the antibiotic as a defensin has yet to be conclusively proven, but these results may represent the first evidence that epithelial defensins are critical for human health and may lead to new therapeutic approaches to CF lung pathology.

NON-ANTIBIOTIC ACTIVITIES OF DEFENSIVE PEPTIDES

In addition to being antibiotic, other activities have been ascribed to defensive peptides. For example, *Territo* et al. (1989) have demonstrated that two human defensins are chemotactic for monocytes. HNP-1 and HNP-2 were active at nanomolar concentrations. The activity was specific for monocytes as no effect was seen with any defensin on human neutrophils. This activity is of obvious utility in a wound or infection setting where defensins are released and attracting immune cells is desirable. These authors point out that patients with specific granule deficiency, who are deficient in neutrophil defensins, fail to recruit monocytes to wounds as immunologically normal patients do. Similarly, *Chertov* et al. (1996) have shown that HNP-1 and HNP-2 are chemotactic for T-cells. These observations, coupled with the impressive potency of the chemotactic activity, suggest an important physiological role for defensins in

potentiating the cellular immune response at appropriate sites.

Kudryashov et al. (1990) reported that defensins promoted wound healing. *Murphy* et al. (1993) pursued this observation by testing the ability of rabbit and human α-defensins to stimulate the growth of cultured epithelial cells and fibroblasts. They demonstrated that rabbit NP-1 and NP-5 as well as HNP-1, 2, and 3 stimulated DNA synthesis at physiologic concentrations. Additionally, each of these defensins acted synergistically with insulin. HNP-1 was also tested against fibroblasts and found to promote DNA synthesis. These workers suggest that the effects observed are receptor independent or due to co-operative allosteric interactions based on sharp dose response curves. The physiologic relevance of these findings has not yet been proven but seems an interesting area for further work. Such activities, in the setting of a wound, could obviously be of great importance.

Some defensins have also been called corticostatins because they potently inhibit corticosteroid production by cultured adrenal cells (*Zhu* et al., 1988). This activity occurs in the concentration range of 5-500 nanomolar and is thought to be due to competitive inhibition of ACTH binding to its receptor. One study extended these studies *in vivo* and showed that rabbit defensins could indeed reduce ACTH and stress induced corticosterone production in mice and rats (*Shamova* et al., 1993). Since glucocorticoids are known to be immunosuppressive, this activity may serve to globally bolster the immune response.

Other unknown functions may reside in the diverse classes of vertebrate antimicrobial peptides. Methods such as yeast two hybrid systems may in the future aid in revealing them.

CLINICAL APPLICATIONS

Antibiotic peptides of animal origin are currently under development as pharmaceuticals. The most advanced is MSI-78 produced by Magainin Pharmaceuticals. It is in phase III clinical trials as a topical treatment for infected diabetic foot ulcers. MSI-78 is a modified version of magainin 2, one of the original magainins discovered in *Xenopus* skin. It contains 22 amino acids and differs from the natural product only by 6 substitutions and one deletion. These changes result in a molecule that is substantially more potent than magainin 2. MSI-78 exhibits a number of additional attractive features, including the broad spectrum of microbicidal activity typical of defensive peptides and the ability to kill drug resistant bacteria such as methicillin resistant *Staphylococcus aureus* (*Jacob* and *Zasloff*, 1994). An interim analysis of an ongoing phase III trial showed that a 1 % cream of MSI-78 was equivalent to oral ofloxacin treatment for the clinical endpoints measured.

Another peptide antibiotic, MSI-843, is not derived from a naturally occurring peptide but was designed based on principles learned from antimicrobials observed in Nature. It is α-helical, cationic, and amphipathic. MSI-843 is active against many multiple drug resistant strains of *Pseudomonas aeruginosa* isolated from the lungs of cystic fibrosis patients. It may therefore be useful as an inhaled therapeutic in CF patients. MSI-843 is in pre-clinical development for this indication. *In vitro* studies suggest that the potency of this compound is less sensitive to fluctuations in ionic strength than known defensins.

Other possibilities include the use of magainins or similar compounds in cancer treatment. Many cancer cells exhibit membrane differences relative to non-transformed cells that make them significantly more susceptible to these peptides. Magainins and their derivatives have been used successfully in treating tumours in animal models of leukaemia, ovarian cancer, and malignant melanoma (*Baker* et al., 1993, *Soballe* et al., 1995). These compounds are currently in pre-clinical development for oncological applications.

CONCLUSIONS

Understanding of the role that antimicrobial peptides play in vertebrates, and especially humans, is in its infancy. Only recently have the β-defensins been discovered and shown to be expressed and induced in mammalian epithelial cells. The number and types of these peptides, their tissue specific expression and genetic regulation all remain to be addressed. Understanding these aspects may ultimately allow manipulation of this arm of the immune system for the benefit of human health, analogous to stimulation of the cellular and humoral immune system by vaccination. Derivatives of vertebrate antimicrobial peptides are already in clinical trials and observation suggests that many more remain undiscovered. This class of compounds may soon represent a new weapon against human pathogens.

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