Review

J Vet Intern Med 2008;▮▮:1–19

Innate Immunity and Host Defense Peptides in Veterinary Medicine

A. Linde, C.R. Ross, E.G. Davis, L. Dib, F. Blecha, and T. Melgarejo

Recent years have witnessed a surge in interest directed at innate immune mechanisms. Proper conceptualization of the key elements of innate immunity, however, is still a work in progress, because most research in immunology traditionally has been focused on components of the acquired immune response. The question of why an animal stays healthy in a world filled with many dangers is perhaps as interesting as why it sometimes surrenders to disease. Consequently, studies with an increased focus on inborn mechanisms of animal host defense may help further the development of appropriate preventative and therapeutic measures in veterinary medicine. Host defense peptides (HDPs) are central effector molecules of innate immunity, and are produced by virtually all living species throughout the plant and animal kingdoms. These gene-encoded peptides play a central role in multiple, clinically relevant disease processes. Imbalances in the expression of HDPs can lead to overt pathology in different organ systems and cell types in all species studied. In addition, HDPs are an ancient group of innate chemical protectors, which are now evaluated as model molecules for the development of novel natural antibiotics and immunoregulatory compounds. This review provides an overview of HDPs and is aimed at veterinary practitioners as well as basic researchers with an interest in comparative immunology involving small and large animal species.

Key words: Antimicrobial peptides; Danger-associated molecular patterns; Natural antibiotics; Pathogen-associated molecular patterns; Pattern recognition receptors; Toll-like receptors.

In a world filled with microorganisms, survival without the inherent protection of innate immunity would be virtually unattainable. The clear success of survival based on innate defense mechanisms alone is solidly evident in plants, fungi, and invertebrates – all of which completely lack acquired immune mechanisms.[1] Innate immunity as such constitutes an evolutionarily ancient scheme founded on a relatively generic, but nevertheless quite effective defense strategy. In addition to the immediate anatomical barriers of the organism, this intrinsic resistance system relies primarily on pattern recognition receptors and associated signaling pathways, specialized chemical mediators (cytokines), the complement cascade, leukocytes, and importantly host defense peptides (HDPs).[2] The list of natural compounds with antimicrobial activities is extensive, but largely includes 3 functional groups: (1) digestive enzymes targeting microbial structures (eg, lysozyme), (2) peptides that bind essential elements such as zinc or iron (calprotectin and lactoferrin, respectively), and (3) peptides that disrupt the microbial membrane (eg, defensins and cathelicidins, as discussed below).[3] At the end of the 1920s, Alexander Fleming identified lysozyme as the 1st peptide with antimicrobial activity. It is, however, only in the past 2 decades that developments in molecular biology techniques have allowed isolation and identification of individual peptides, and the establishment of their structural and functional features.[4,5] This review is aimed at providing an overview of the current understanding of HDPs, with special emphasis on defensins and cathelicidins and their role in immunological defense in companion and production animals. Defensins and cathelicidins are highlighted because these currently are the most studied vertebrate HDPs. In addition, the potential application of natural antimicrobial compounds as templates directed at the development of novel antibiotics and immunoregulatory drugs for use in veterinary medicine is discussed.

From the Departments of Anatomy & Physiology (Linde, Ross, Blecha), and Clinical Sciences (Davis), College of Veterinary Medicine; and Department of Human Nutrition (Dib, Melgarejo); and College of Human Ecology, Kansas State University, Manhattan, KS.

Corresponding Author: Dr T. Melgarejo, 143B Justin Hall, Kansas State University, Manhattan, KS 66506-1407; e-mail: tmelgare@ksu.edu

Submitted May 7, 2007; Revised June 27, 2007; Accepted September 6, 2007.

10.1111/j.1939-1676.2007.0038.x

The Importance of an Innate Host Defense

An immediate nonspecific defense system aimed at controlling potential infectious as well as noninfectious dangers efficaciously is vital to ensure animal health. The term "danger" is used here in reference to the "Danger-Model" concept,[6] which entails activation of an immune response not only in response to microorganisms (nonself), but also as a reaction toward all other types of insults (or "danger signals"), including physical trauma, ionizing radiation, oxidative stress, ischemia, and extreme temperatures. Innate immunity thus ensures an immediate mode of defense in virtually all living organisms. A group of multifunctional antimicrobial peptides (ie, the HDPs) comprise the core of this innate immune response.[7]

In animals higher on the evolutionary ladder (eg, mammals being more "evolved" than insects), the initial interaction between microbial intruders and their prospective host takes place on the cutaneous surface or on the epithelial lining of the gastrointestinal, reproductive, respiratory, or urinary tract.[8,9] Thus, it is not surprising that epithelial cells of vertebrates produce HDPs as components of this 1st line of defense. Because inflammation comprises an initial reaction in the innate immune cascade, it is reasonable that HDPs are also produced by inflammatory cells such as neutrophils, and tissue phagocytes, including macrophages.[3,4,10] Perhaps not immediately logical, however, is that HDPs are expressed by less typical cell types (at least from a purely immunological point of view) such as endotheliocytes and myocytes, thus suggesting a universal function of innate immune mechanisms.[11–13] This would further expand the common view of the immune system as

an entity of professional immune cells surveying the body for potential intruders to that of an integrated and inclusive entity of cells communicating and collaborating to ensure maintenance of homeostasis.[14]

Broadly defined, HDPs have the capability of targeting any organism with a cholesterol-free, negatively charged membrane. The functional capacity of different HDPs thus includes broad-spectrum antimicrobial activities against Gram-positive and Gram-negative bacteria, mycobacteria, fungi, intracellular parasites, and enveloped viruses.[4,15] Importantly, HDPs are able to kill transformed or cancerous cells, a cytotoxicity that tends to be neither species-specific nor selective.[2,16,17] A linkage to initiation of an adaptive immune response has been observed for defensins, which act as direct chemoattractants for immature dendritic cells.[2,17,18] Some defensins are opsonic (ie, they enhance phagocytosis) and also have the capability to modify hormonal reactions.[16] Thus, HDPs are far more than "simple natural antibiotics." As such, HDPs seem to play a central role in a number of clinically relevant disease processes, including low grade inflammation, obesity, diabetes, and hyperlipidemia.[19–22] Table 1 outlines clinically relevant disease processes (and associated pathogens) in which HDPs most likely play a role. The physiological properties and regulation of these molecules therefore may hold a key to explaining many complexities in veterinary medicine.

Structural Characteristics of Defensins and Cathelicidins

Natural antimicrobial substances are numerous and, as a group, rather heterogeneous, varying in size from relatively large protein complexes (eg, the complement cascade) to small inorganic molecules (eg, hydrogen peroxide).[8] To date, approximately 900 different HDP sequences have been identified (cataloged in the Italian Trieste Database at http://www.bbcm.univ.trieste.it/). The conventional definition of "antimicrobial peptides" (synonymous with HDPs), however, includes only gene-encoded, ribosomally synthesized polypeptide antimicrobial substances 100 amino acid residues in length.[8] Because the majority of fungal and bacterially derived peptide antibiotics are nonribosomally synthesized peptides incorporating atypical amino acids, the above definition separates HDPs from this category.[8] Two major classes of conventional HDPs are the defensins and cathelicidins. A large number of other HDP families are present in invertebrates, most notably a wide variety of different insects, yet these peptides do not fall within the scope of this paper.

Four broad structural groups of folded HDPs have been described, including α-helical peptides (eg, cathelicidins), β-sheet peptides with 2–4 disulfide bonds (eg, α- and β-defensins), loop peptides with 1 disulfide bond (eg, bactenecin), and extended peptide structures rich in arginine, glycine, histidine, praline, tryptophan, or some combination hereof (eg, indolicidin).[23] The biological effect of these cationic peptides is primarily dependent on their (tertiary) structure, and thus their structural characteristics are of direct interest.[3]

Classical defensin molecules encompass a family of small amphipathic[a] variably arginine-rich cationic peptides (typically 30–40 amino acid residues in length) characterized by 6 disulfide-paired cysteines (linked Cys [1–6], Cys [2–4], and Cys [3–5], for α-defensins, and Cys [1–5], Cys [2–4], and Cys [3–6], for β-defensins—see descriptions below).[2,4,11,16] Some defensins are particularly abundant in mammalian phagocytes, where they can comprise up to 50% of total protein in azurophil[b] granules.[4,16] Defensins have, however, also been identified in other cell types, including tissue macrophages, small intestinal epithelial cells, and cardiomyocytes.[13,24,25] The overall structure of the defensin peptides has been compared with a bent paperclip, because of the characteristic chemical composition consisting of a triple-stranded β-sheet structure and a connecting loop that creates a base from which a β-hairpin hydrophobic structure extends almost perpendicularly[4] (Fig 1). To date, 3 different categories of vertebrate defensins have been described (in addition to the insect and plant defensins) based on size and structural differences in the cysteine linkage (secondary structure).[7,26]

α-defensins are the classical "neutrophil defensins," which were first described in the mid-1980s, whereas the slightly larger β-defensins were reported initially in the early 1990s.[9] The Trieste Database[c] contains 90 β-defensins and 55 α-defensins. More recently, θ-defensins[d] have been described. α- and β-defensins are widely distributed across species, but θ-defensins are expressed only in granulocytes of the rhesus macaque and some other primates, including other Old World monkeys and orangutans.[27] Other great apes (including humans) and New World monkeys do not express θ-defensins.[28–30] θ-defensins are double-stranded small circular molecules, in contrast to α- and β-defensins, which are flat triple-stranded β sheets.[7]

A unifying feature of the cathelicidin peptides is a marked homology termed the cathelin[e] domain at the 5′ region, and a variable C-terminal antimicrobial domain.[5,31,32] Cathelicidins are found in varying numbers in numerous different species, including domestic animals.[15,33] They are stored as inactive propeptides and processed only upon stimulation, thus resulting in the release of active HDPs into the extracellular fluid.[15] Cathelicidins typically are expressed by myeloid precursor cells, but expression also has been reported in mature circulating neutrophils and neonatal lymphoid tissue in some animal species.[15,34] Moreover, the number of cathelicidin antimicrobial peptides varies among species, which most likely leaves different animals with varying levels of resistance toward specific types of infections.[35] Interestingly, cathelicidins and defensins exhibit synergism,[36] implying their combined role in the orchestration of the innate host defense, as further discussed below.

Host Defense Peptides – Synthesis, Expression, and Mechanism of Action

HDPs can be either constitutively expressed or induced in response to specific stressors such as

Table 1. Host defense peptides in veterinary medicine.

Species	Peptide	In vitro Antimicrobial Activity	Clinical Disease (examples)
Dogs	K9CATH cBDs	*Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Candida albicans*	Urinary tract infections
		Salmonella enteritidis, S. typhimurium, Escherichia coli	Gastroenteritis
		Listeria monocytogenes	Meningitis, abortion
		Staphylococcus aureus	Dermatitis
		Candida albicans	Stomatitis, spondylitis, dermatitis
Horses	eNAPs	*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Streptococcus zooepidemicus*	Endometritis
	eCATHs	*Streptococcus equines, Escherichia coli, Klebsiella pneumoniae, Serratia marcescens*	Inflammatory airway disease
	eBD	(*Corynebacterium* sp. and *Staphylococcus intermedius*)[a]	Otitis
Cattle	Epithelial BDs	*Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus, Candida* spp.	Mastitis
	BNBDs		
	LAP	*Mannheimia hemolytica, Mycobacterium paratuberculosis*	Shipping fever, paratuberculosis
	TAP	*Aspergillus* and *Candida* spp.	Systemic mycosis
	Bactenecins	*Escherichia coli, Klebsiella pneumoniae, Salmonella typhimurium, Enterobacter cloacae, Leptospira interrogans* and *L. biflexa*	Mastitis, enterocolitis, meningitis, leptospirosis
	BMAPs	*Staphylococcus aureus, Pasteurella multocida*	Mastitis, pasteurellosis
Sheep	sBDs	*Mannheimia hemolytica*	Shipping fever
	SMAPs OaBac5α	*Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus, Staph epidermitis, Candida albicans*	Mastitis, enterocolitis
Goats	ChBac5	*Escherichia coli, Pseudomonas aeruginosa, Listeria monocytogenes*	Mastitis, listeriosis
Pigs	pBDs	*Escherichia coli, (Salmonella typhimurium), Listeria monocytogenes, Candida albicans*	Gastroenteritis, listeriosis
	PR-39	*Escherichia coli, Salmonella typhimurium, Listeria monocytogenes, Actinobacillus pleuropneumoniae*	Gastroenteritis, listeriosis, wound healing, pleuropneumonia
	PMAPs	*Escherichia coli, Salmonella typhimurium, Pseudomonas aeruginosa, Staphylococcus aureus, Candida albicans*	Gastroenteritis, wound infections, systemic mycosis
	Protegrins	*Staphylococcus aureus, Pseudomonas aeruginosa*	Wound infections
Poultry	Gallinacins THPs	*Haemophilus/Avibacterium paragallinarium, Salmonella Spp., Escherichia coli, Staphylococcus aureus*	Infectious coryza, enteritis, septicemia, dermatitis
	Fowlicidins	*Escherichia coli, Klebsiella pneumoniae, Listeria monocytogenes, Staphyloccocus aureus*	Enteritis, airsacculitis, septicemia, cellulites, tracheitis, encephalitis, gangrenous dermatitis

The table provides an overview of clinically relevant diseases in which HDPs are likely to play a role. It should be noted that this is a non-comprehensive list, because a dysfunctional host defense peptide response in all probability contributes to infectious and inflammatory disorders in general. See text for references and abbreviations.

[a]Pathogens associated with the clinical disease. However, the antimicrobial activity of the listed HDP(s) remains to be tested.

infection and inflammation.[3,31,37–39] α-defensins tend to be produced constitutively, whereas the majority of β-defensins are inducible.[7,15] Moreover, α-defensins have evolved to operate mainly from within phagosomes, whereas β-defensins are produced primarily by epithelial cells.[7]

Lipopolysaccharide (LPS) and the proinflammatory cytokines IL-1β and TNF-α promote HDP synthesis.[3] Their production resembles that of peptide hormones, involving sizable precursor molecules and tissue-specific sequential proteolytic processing.[4] After removal of the signal sequence, the proregion is disposed of, yielding the mature HDP.[15] Defensin molecules are produced as neutral preprodefensins (approximately 95 amino acids), which are not cytotoxic to the cell.[16] The antimicrobial and cytotoxic functional properties of the mature defensins (and other HDPs) generally are thought to be associated with their pore-forming activities as multimers in biological membranes leading to self-promoted uptake,[15,16] a mechanism that has been further described by the Shia-Matsuzaki-Huang model[40–42] (Fig 2). The HDPs target the "Achilles heel" of the microbial membrane (ie, the absence of cholesterol and negatively

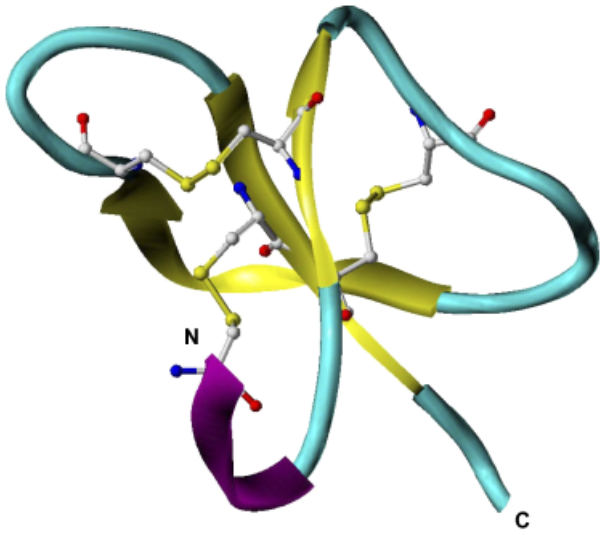

Fig 1. Model of a β-defensin molecule from dog. The structure displays the characteristic 3 disulfide bonds (ball and sticks), an α-helix (purple) and three β-sheets (yellow).

charged phospholipids on the outer leaflet of the cytoplasmic membrane).[43] The positive net charge (+2 to + 7 because of an excess of basic over acidic amino acids)[15] facilitates binding of an increasing number (1–10 billion molecules) of HDPs to the phospholipids on the bacterial surface until the bacterial membrane collapses completely.[7,44] Cholesterol prevents membrane damage, and because this lipid is an essential part of eukaryotic membranes, it explains why normal concentrations of HDPs do not cause host-damage.[7] The membrane potential of eukaryotic cells (−15 mV) also is low compared with the bacterial transmembrane potential (−140 mV), which also minimizes interaction.[15] Resistance to HDPs is rare, because it is exceedingly difficult for any microorganism to change its structural organization of surface phospholipids.[26] Some HDPs target intracellular sites in addition to the bacterial membrane.[45] Also, defensins have been implicated as a link between the innate and adaptive immune responses (Fig 3).

Various tissues and cell types in the body contain gene-encoded pattern recognition receptors (PRRs) and can mandate a number of different signaling pathways in

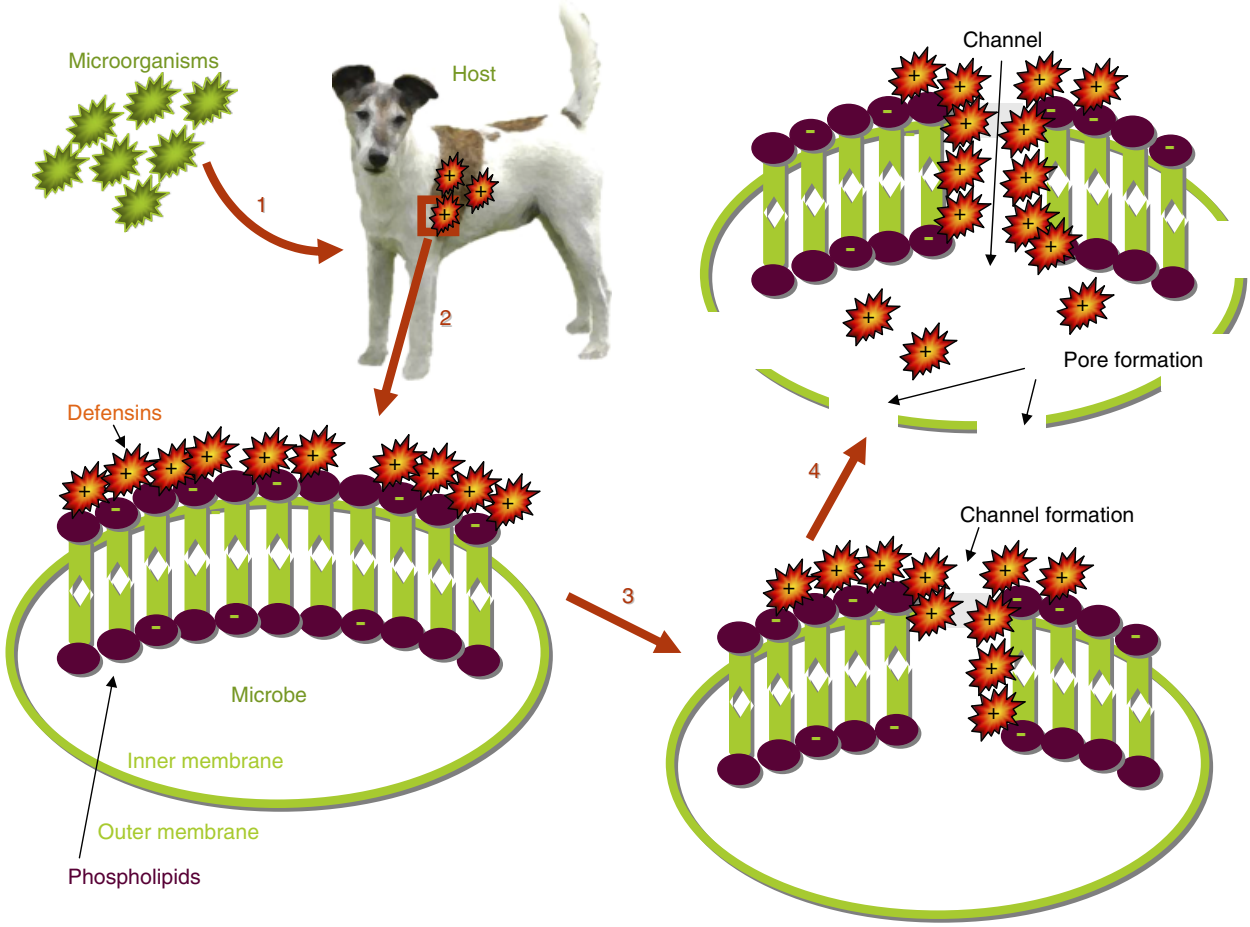

Fig 2. Shia-Matsuzaki-Huang model. The model displays the general consensus for HDPs' antimicrobial mode of action (other possible theories for membrane disruption by AMPs have been published also).[40–42] 1: Host is initially exposed to microorganisms. 2: The innate immune response involves recruitment of cationic HDPs, which are immediately attracted toward the anionic microbial membrane. 3: The HDPs form a carpet-like structure on the microbial membrane, instituting channel formations. 4: The channels lead to pore-formation membrane destabilization and microbial demise. HDP, host defense peptides.

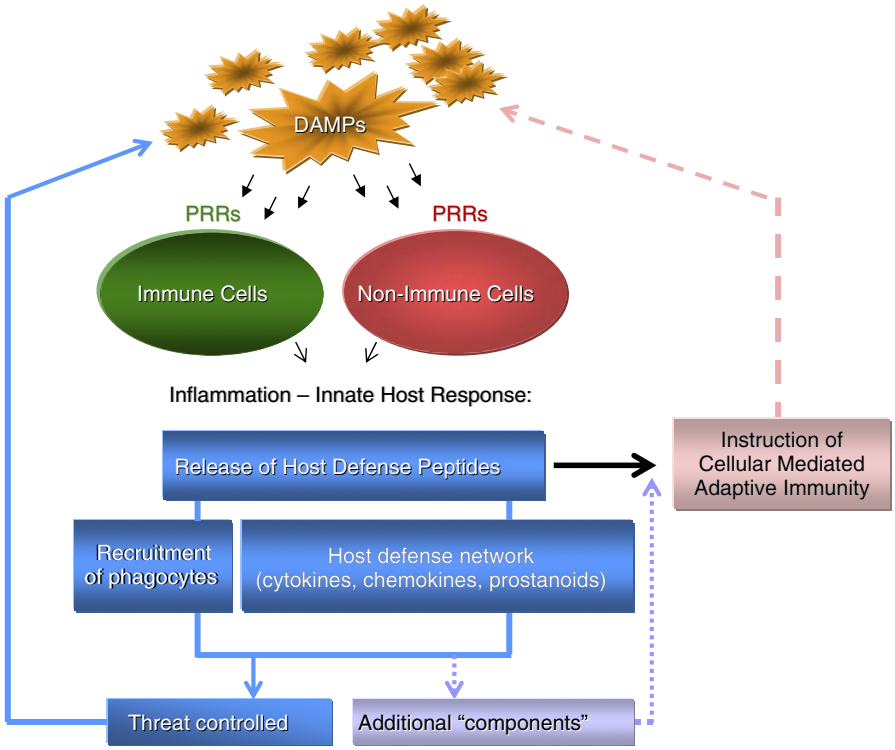

Fig 3. Innate defense mechanisms and host defense peptides (HDPs) linkage to adaptive immunity. The schematic displays the key components of an innate immune response induced by danger-associated molecular patterns' (DAMPs) interaction with pattern recognition receptors (PRRs) on professional as well as nonprofessional immune cells (in the context of the figure "non-immune cell" indicates a nonprofessional immune cell type such as epithelium/endothelium or myocytes). In addition to their immediate actions within the frame work of an inborn immune response, HDPs also create a biological link between innate and acquired immunity, thus orchestrating an appropriate overall host defense.

response to stress, ultimately ensuring production of all necessary signaling and effector molecules required for an appropriate and immediate host defense. Host PRRs generally are surface proteins that immediately identify conserved molecular structures associated with microbial pathogens or other impending dangers. The repertoire of PRRs capable of regulating gene expression encompasses the Toll-like receptors (TLRs) and the virus-sensing RIG-I and Mda5 helicases.[f,47] Other non-TLR recognition molecules, however, also have been described. The structures identified by a given PRR are classified either as pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs). Classical PAMPs include LPS and lipoteichoic acid (LTA) from Gram-negative and Gram-positive bacteria, respectively, viral double-stranded RNA (dsRNA), and fungal β-glucans.[48] The term DAMPs is used here as a common name referring to PAMPs as well as endogenous alarm signals released by dying or injured cells.[14,49] Matzinger's Danger Model defines "dangers" as anything (exogenous or endogenous) that has the potential to cause tissue stress or destruction[6,14] (Fig 4).

Also in the category of innate sensors are the intracellular Nod-like receptors (NLRs), which present a powerful combined defense at the plasma membrane (ie, TLRs) as well as from within the cell (ie, NLRs).[48] Both TLRs and Nod[g] proteins can trigger the nuclear factor κB (NF-κB) transcription factor, thus activating a highly stereotypical signaling pathway responsible for a range of different cellular responses[48] including production of HDPs. The NLRs have been linked to recognition of bacterial components as well as endogenous danger signals.[48] TLRs initially received considerable research interest, and consequently this group of PRRs is most well-described. More than a dozen different members have been reported in 6 major families, with each member recognizing different PAMPs. LPS is the classical ligand for TLR4, whereas LTA and CpG oligodeoxynucleotides are recognized by TLR2 and TLR9, respectively.[50]

NF-κB signaling is one of the main down-stream pathways responsible for HDP production, although other signaling routes (including MAPK[h] and JAK/STAT[i] signaling) have been implicated in their synthesis.[51] NF-κB is a transcription factor involved in the integration of numerous parallel signaling pathways and a variety of cellular responses central to an immediate and functional immune response, including the production of cytokines and cell adhesion molecules.[15] Signaling through these pathways leads to transcriptional activation and subsequent production of HDPs. The TLRs and NLRs also result in activation of the inflammatory caspases,[j] which

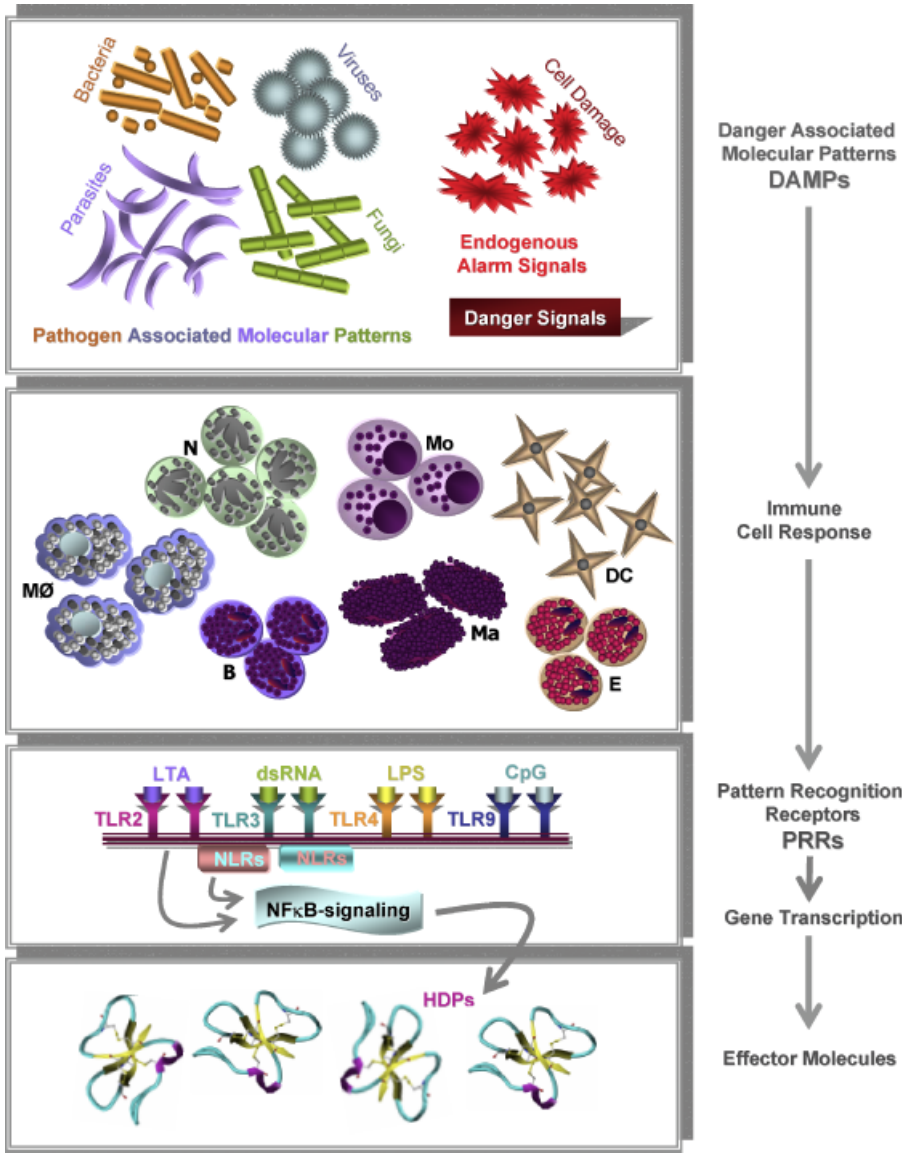

Fig 4. Danger Model of Innate Immunity. Different infectious and noninfectious molecular structures (PAMPs and endogenous alarm signals, respectively) constitute indicators known as danger associated molecular patterns (DAMPs). The DAMPs activate the innate immune system through pattern recognition receptors (PRRs) and NFκB-signaling, leading to production of host defense effector molecules (HDPs). N, neutrophils; E, eosinophils; B, basophils; Mo, monocytes; DC, dendritic cells; Ma, mast cells; MØ, macrophages; LPS, lipopolysaccharide; LTA, lipoteichoic acid; CpG, DNA with cytosine and guanine separated by a phosphate; TLR, toll-like receptor; NLR, nod-like receptor; NF-κB, nuclear factor κB; HDPs, host defense peptides; PAMP, pathogen-associated molecular patterns.

comprise a field of research beyond the scope of this paper.[52,53]

Biological Activity of HDPs

HDPs are the frontiers of inborn immunity in virtually all living species (Fig 3), and the central importance of these peptides is evident by their abundance in circulating neutrophils.[15] HDPs participate in the inflammatory response by acting as chemoattractants for immune cells (including neutrophil recruitment by induction of IL-8 production and mobilization of immunocompetent T-cells[54,55]) as well as enhancers of cellular adhesion and the subsequent cellular transepithelial migration. Furthermore, studies suggest that defensins can enhance the cytotoxicity of NK-cells.[15] The versatile nature of HDPs also includes roles in wound healing (possibly by induction of syndecan[k] synthesis[56]) as well as modulation of the inflammatory response by inhibiting the activation of the classical complement pathway through C1q.[57]

Given the ubiquitous production of HDPs in the organism, it is not surprising that many of these peptides can be found in various types of body fluids and secretions.[3] Plasma α-defensin concentrations of

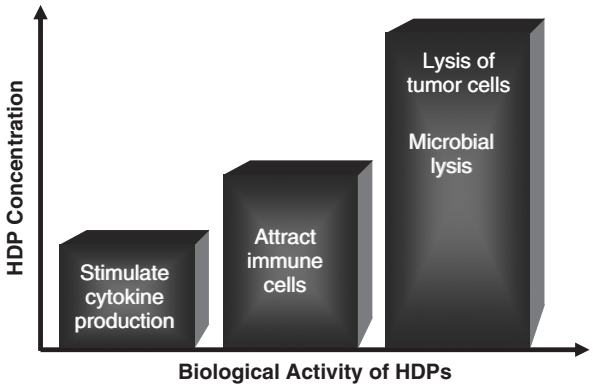

Fig 5. Biological activity of host defense peptides (HDPs) The graph shows that the breadth of activity for HDPs is dependent upon peptide concentration.

40 ng/mL have been measured in normal human subjects, increasing in concentration to $>1\,\mu g/mL$ during infections.[4] Also, plasma concentrations of 170 μg/mL have been measured in sepsis,[58] as have concentrations of $>1600\,\mu g/mL$ in sputum from cystic fibrosis patients.[59] The antimicrobial activity of α-defensins in vitro generally relies on peptide concentrations between 10 and 100 μg/mL, although their contribution to tumor cell lysis occurs at higher concentrations[4] (Fig 5). HDPs are most likely secreted at higher concentrations in infected or otherwise diseased tissue, but local peptide concentrations have yet to be investigated.[4] Certain HDPs act as anti-inflammatory compounds in sepsis because of their LPS- and LTA-binding capacity,[15] and, in addition to neutralizing endotoxin, some cathelicidins act directly to decrease the release of TNF-α.[60]

A number of HDPs are known to be inactivated by salt, and some have decreased antimicrobial activity even at physiological salt concentrations (approximately 150 mM NaCl).[7,17] Current research suggests that extracellular release of certain defensins yields inactive peptides, whereas concomitant release of cathelicidins ensures active HDPs working synergistically.[36] Synergy has also been described between lysozyme and other HDPs.[15] Some HDPs promote angiogenesis and epithelial growth, and some act as chemokines attracting circulatory or migrating cells.[26,61–63] Defensins possess chemotactic features toward monocytes, and can act as "corticostatins" by reversibly interacting with the receptor for adrenocorticotropic hormone (ACTH).[4] Defensins can modify a number of signaling pathways and cellular functions in the body by potent inhibition of protein kinase C.[64] A role of β-defensins in sperm maturation also has been suggested.[65]

Versatile Host Defense Peptides in Companion and Production Animals

Host defense peptides are produced throughout the animal kingdom. Many HDPs have been identified in domestic animals, but a striking interspecies variation exists with regard to the expression of these peptides.[1,10] A given species may have a dozen or more different HDPs, presumably with some overlap in their antimicrobial and immunomodulatory activities, although some peptides tend to function preferentially in only 1 of the these 2 biological roles.[23] The importance of HDPs as microbicidal compounds versus their role as immunomodulators is somewhat controversial.

Owing to the ease of access to material from production animals, cattle, sheep, goats, and pigs have been used widely in the field of HDP research. However, information on HDPs in companion animals is sparse. Studies in horses have focused on defensins and cathelicidins[66,67] and a few reports on canine HDPs are available.[68,69] The need for in vivo experiments in the area initially led to an increased focus on the mouse as an animal model. The mouse possesses a single cathelicidin and a number of enteric α-defensins (cryptidins).[7] Mouse granulocytes, however, lack α-defensins completely, making its usefulness as an animal model questionable.[7] Most species contain a wide range of HDPs with varying expression levels in different tissues, which ensures a broad range of antimicrobial coverage and immunomodulatory regulation throughout the organism.[15] The following section provides an overview of the data available on HDPs in different species of relevance to veterinary medicine, including companion and production animals (Fig 6) and selected other species (Table 2).

Companion Animal Species

Dogs and Cats. The literature on innate immune mechanisms of the dog and cat is limited. Thus far, 3 β-defensins (cBD-1, cBD-2, and cBD-3) and 1 cathelicidin (K9CATH) have been identified in the dog[68,70] whereas none has been reported in the cat. By means of computational analysis only, sequences for 43 β-defensin genes and pseudogenes have been identified in the canine genome.[69] Recently, canine hepcidin (an acute phase protein with antimicrobial and iron regulatory capacity) was extracted from canine liver, which is of interest because hepcidin is thought to be a key mediator in chronic anemia.[71]

Most studies on the immunophysiology of cats have focused on the acquired immune response to infectious disease.[72,73] Although specific HDPs have not yet been identified in the cat, 1 study focused on feline TLR expression.[74] Normal cat lymphoid tissue expresses TLR1–TLR9, an expression that is altered by feline immunodeficiency virus (FIV).[74] Because TLRs are involved in the synthesis of HDPs, these findings suggest that the cat, like virtually all other species, also has a range of natural antimicrobial peptides. Select TLR expression (TLR2, TLR4, and TLR9) has also been reported in different tissues and cells from the dog,[75–79] which would similarly indicate wide spread expression of different canine HDPs. Predicted sequences for canine TLR5 and TLR7 have in addition been generated by automated computational analysis (GeneID: 488605 and 491743, respectively). Functional studies on canine TLRs are lacking, but 1 suggestion has been that dysregulation of TLR2 and TLR4 in intestinal epithelium may contribute to the pathogenesis of canine inflammatory bowel disease.[80]

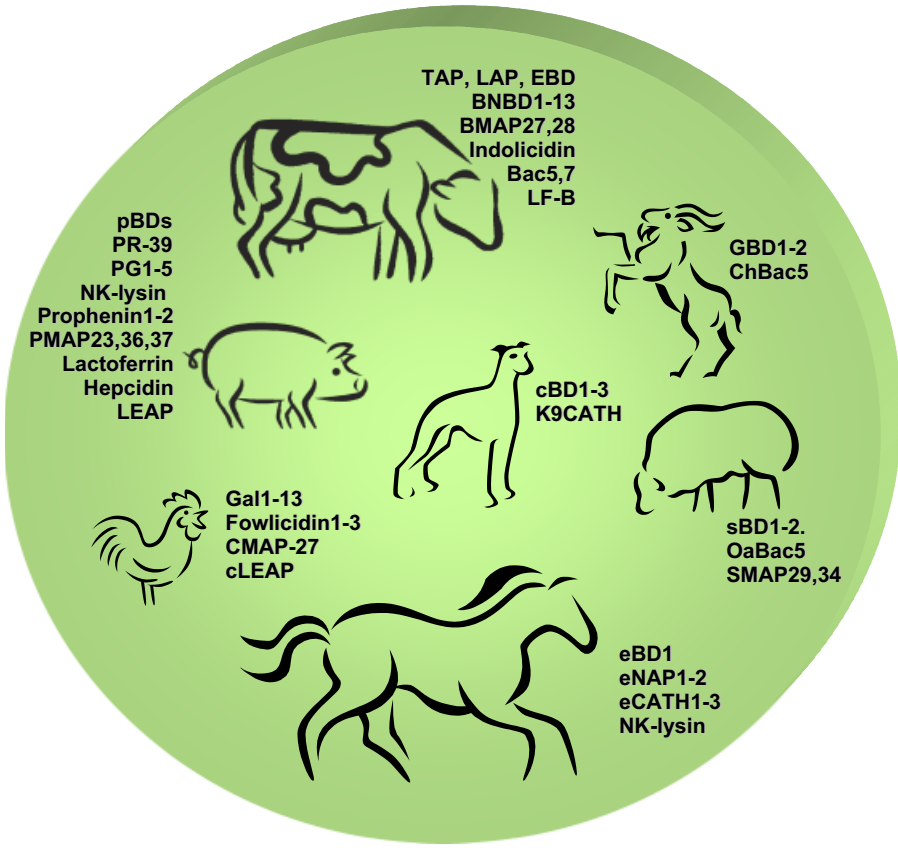

Fig 6. Overview of host defense peptides in veterinary medicine. Cow – TAP and LAP, tracheal and lingual antimicrobial peptide, respectively; EBD, enteric β-defensin; BNBD, bovine neutrophil β-defensin; BMAP, bovine myeloid antimicrobial peptide; Bac, bactenecins; LF-B, bovine lactoferricin B. Goat – GBD, goat β-defensin; ChBac, caprine analog to bovine bactenecin. Sheep – sBD, sheep β-defensin; OaBac, ovine analog to bovine bactenecins; SMAP, sheep myeloid antimicrobial peptide. Pig – pBD, porcine β-defensin; PR, proline rich; PG, protegrin; PMAP, porcine myeloid antimicrobial peptide; LEAP, liver-expressed antimicrobial peptide. Horse – eBD, equine β-defensin; eNAP, equine neutrophil antimicrobial peptide; eCATH, equine cathelicidin. Dog – cBD, canine β-defensin; K9CATH, canine cathelicidin. Chicken – Gal, gallinacin; CMAP, chicken myeloid antimicrobial peptide; cLEAP, chicken liver-expressed antimicrobial peptide.[q]

Table 2. Host defense peptides in special species.

Species	Host Defense Peptide		Place of Expression	Reference
Chinchilla	cBD1	β-defensin	Epithelia	236
Guinea Pig	GPNP1	α-defensin	Neutrophils, bone marrow	237
	GPCSIII	α-defensin	Neutrophils, bone marrow	238
	CAP11	cathelicidin	Neutrophils, bone marrow	239
Hamster	HaNP1-4	α-defensin	Neutrophils	240
	Crp1-6	α-defensin	Paneth cells	23
Mouse	mBD1-15	β-defensin	Epithelia	241
	mBD34-40	β-defensin	Epithelia	241
	CRAMP	cathelicidin	Neutrophils, bone marrow	242
Rabbit	NP1-3a	α-defensin	Neutrophils	243
	NP3b-5	α-defensin	Neutrophils	243
	CAP18	cathelicidin	Granulocytes	244
	rNP1-2,4	α-defensin	Neutrophils, tissue	245
Rat	42 rBDs	β-defensin	Epithelia	69, 246
	rCRAMP	cathelicidin	Granulocytes, tissue	247

BD, β-defensin; NP, neutrophil peptide; Crp, cryptdins; CRAMP, cathelin-related antimicrobial peptide; CAP, cationic antibacterial polypeptide. The data provided in the table are intended as an overview only of some of the defensin and cathelicidin HDPs, which have been reported in small mammals, including examples of their tissue/cellular expression. The majority of the 42 rat β-defensins is based on information from the Rat Genome Database (http://rgd.mcw.edu/), and a recent paper on cross-species analysis of mammalian β-defensins.[69]

Our group recently identified 3 β-defensins in dog testes, with selective expression of the 3 isoforms among different testicular cell types.[68] The most active and longest of the isoforms, canine β-defensin-1 (cBD-1), is expressed more ubiquitously, whereas the relatively shorter peptides (cBD-2 and -3) appear to be testes-specific storage HDPs.[68] The antimicrobial effect of canine β-defensin includes activity against a wide spectrum of Gram-positive (*Listeria monocytogenes* and *Staphylococcus aureus*) and Gram-negative (*Escherischia coli, Klebsiella pneumoniae*, and *Neisseria gonorrhoeae*) bacteria, yeast (*Candida albicans*), and *Ureaplasma* in a salt-dependent fashion.[68] We have also identified a more potent canine HDP, K9CATH (canine cathelicidin), in myeloid bone marrow cells and circulating neutrophils.[70] This peptide has broad-spectrum activity and also exhibits unprecedented antimicrobial potency against *N. gonorrhoeae* and *Ureaplasma* in a salt-independent manner. Because this peptide is expressed in circulatory cells, it has the inherent capability to act not only as a potent antimicrobial compound but also as a potential immunomodulator. These findings may explain why dogs apparently are resilient to sexually transmitted disease pathogens. Consequently, synthetic forms of these canine-derived peptides may provide novel therapeutic options for treating sexually transmitted disease in humans as well as urinary tract infections in dogs. The use of synthetic peptides derived from heterospecifics has proven successful previously (eg, use of the moth-derived synthetic cecropin to treat naturally acquired canine leishmaniasis).[81]

Horses. The existence of antimicrobial compounds in equine neutrophils was first reported nearly 20 years ago.[82] Later, these peptides were characterized as equine neutrophil antimicrobial peptides (eNAP-1 and -2),[83,84] followed by the identification of 3 equine cathelicidins (eCATH-1, -2, and -3).[67,85] Another antimicrobial compound, equine NK-lysin, produced by lymphocytes was recently found in the horse.[86] α-defensins have thus far not been reported in the horse, but expression of 1 β-defensin (eBD-1) was described recently.[66] Eight potentially functional β-defensin genes and an α-defensin-like sequence have been reported in the horse based on computational sequence analysis.[87] Equine β-defensin 1 (eBD-1) appears to be constitutively expressed in several different tissue types and organs, including liver, kidney, spleen, heart, and the intestine.[66] Functional characteristics of eBD-1 have yet to be established, but examination of the peptide sequence indicates similarities to other known β-defensins.[66] A recent study reported on β-defensin production in cerumen, where it most likely acts as a natural antimicrobial to safeguard the equine auditory canal.[88]

Cathelicidins from the horse are stored in the classical unprocessed form (pro-eCATHs) in secretory granules, and released only upon neutrophil activation.[67] Of the 3 cathelicidin genes identified in the horse, only 2 (eCATH-2 and -3) seem to be able to encode a protein.[67] eCATH-1 is expressed at fairly low levels, and the gene is present in only half of the examined horses. The mature eCATH-1 protein has yet to be detected.[85] Common equine neutrophil-dominated inflammatory disorders such as acute bronchiolitis and recurrent airway obstruction result in measurable concentrations of mature eCATH-2 and -3 as well as their respective propeptides in tracheobronchial lavage, findings that are consistent with active processing of these HDPs in equine inflammatory processes.[85] The antimicrobial activity and potency of eCATH-1, -2, and -3 generally is broad, intermediate, and low, respectively.[85] The eCATH-1 peptide (synthetic form) has the strongest antimicrobial capacity and exhibits virtually no hemolytic activity in vitro, whereas eCATH-3 has fairly modest antimicrobial activity in low-salt medium only.[85] It is therefore possible that the eCATH-1 peptide is induced only under specific and different conditions than what has been investigated so far, and based on studies involving a modified version of eCATH-3, the amphipathicity and biological activity of this peptide seem to be highly interdependent.[85] The known versatility of HDPs also leaves open the question of what additional role the eCATHs may play in vivo.

The antimicrobial peptides eNAP-1 and -2 are structurally unrelated to the family of defensins found in neutrophil granules of other species, and substantial internal differences exist between the 2 peptides.[84] The antimicrobial activity of both eNAPs has been tested against pathogens commonly involved in clinical endometritis in mares, including *E. coli, K. pneumoniae, Pseudomonas aeruginosa*, and *Streptococcus zooepidemicus*.[83,84] The content of eNAP-1 in neutrophils is fairly low compared with eNAP-2,[l] but the peptides appear to have comparable antimicrobial activities against typical uterine pathogens in the horse.[84] The bactericidal activity of eNAP-1 and -2 (after 2 hours and 100 μg/mL concentration for both) against *S. zooepidemicus* seems most pronounced (>99.8% and 94% decrease in colony forming units [CFU]/mL, respectively), with a relatively lower efficacy against *E. coli* and *P. aeruginosa* (mean decrease of 87% in CFU/mL for eNAP-1, and 90% and 78% decrease, respectively, for eNAP-2 after 2 hours, and 200 μg/mL concentration for both). eNAP-2 also exhibits bacteriostatic activity against *K. pneumoniae* at 200 μg/mL.[83,84] In addition to direct antibacterial activity, a selective microbial serine protease inhibition[m] has been reported for eNAP-2.[89] It is thus likely that eNAPs play a central role in the innate uterine defense mechanisms of the horse.

Large Animal Species

Cattle. In the mid-1980s, a group of researchers from University of Trieste initially reported the presence of broad-spectrum antibiotic polypeptides in bovine granulocytes.[90] In the following years, different bovine neutrophil antimicrobial peptides have been isolated, including members of the defensin and cathelicidin families. Cattle possess at least 38 HDPs, including different defensins and cathelicidins (BMAPs [bovine myeloid antimicrobial peptides], bactenecins [loop peptides], and indolicidin [extended peptide]).[15] Bovine oligosaccharide-binding protein (bOBP) is a peptidoglycan recognition protein found in bovine neutrophils and

eosinophils, suggesting that this peptide may contribute to antiparasitic activity.[91] Furthermore, antimicrobial compounds from milk (eg, lactoferricin, LF) have received considerable research attention.[92,93]

Epithelial β-defensins have been isolated from the bovine trachea (tracheal antimicrobial peptide, TAP),[94] tongue (lingual antimicrobial peptide, LAP),[9] intestine (enteric β-defensin, EBD),[95] and mammary gland (bovine β-defensin-1, bBD-1, and others).[96,97] Bovine neutrophil dense granules[n] also contain β-defensins (bovine neutrophil β-defensins, BNBD-1 to -13),[o] some of which also are expressed in alveolar macrophages (predominantly BNBD-4 and -5, in addition to the 2 epithelial β-defensins, TAP and EBD).[98–100] Cattle, on the other hand, do not have α-defensins in neutrophils and the intestinal epithelium.[23] The bovine epithelial and neutrophil β-defensins are different gene products, but share a high degree of structural similarity.[98,101] mRNA expression of some BNBDs can be observed in cells of different tissues, including trachea, lung, spleen, and intestine.[98,101]

Bovine β-defensins possess antimicrobial activity against *E. coli, K. pneumoniae, P. aeruginosa, Staph. aureus* and *Candida* spp.[94] TAP is expressed throughout the bovine airway,[102,103] and is an example of a β-defensin that is inducible by various infectious agents and proinflammatory mediators, including TNF-α, IL-1β, and LTA.[38,39] Incubation of primary cultures with *E. coli* LPS results in a substantial increase in mRNA levels encoding TAP.[37] Synthetic TAP has a rapid and potent bactericidal effect as well as antifungal activity against *Aspergillus* and *Candida* spp.[104] Contrary to TAP, LAP expression is more widespread, involving epithelium of the alimentary tract as well as the respiratory system, mammary glands, and cornea.[101,105] Induction of LAP expression is observed in acute infection with *Mannheimia* (*Pasteurella*) *hemolytica* as well as in chronic paratuberculosis-infected (*Mycobacterium paratuberculosis*) tissue.[101] It also has been suggested that LAP plays a role in the innate immune response against bovine mastitis pathogens, because LAP expression is increased in infections of the udder.[105] Similarly, local expression of BNBD5 as well as that of PRRs TLR2 and TLR4 is upregulated in mastitis.[106,107] Importantly, a recent study reported that steroid-treated cattle have lower expression levels of LAP and TAP, which suggests that stress and exogenous corticosteroid administration can lead to an impaired innate immune response in the lung.[108]

The bovine alimentary tract expresses low levels of a number of different HDPs (including LAP, TAP and BNBD-3, -4, and -9), but the main enteric β-defensin in the gut is EBD.[95] mRNA levels of EBD are increased in experimental cryptosporidiosis in calves, suggesting that this HDP plays an active role in the host response to parasitic infection.[95] The broad spectrum of antimicrobial activity and inducible expression in inflammation strongly support a central role for β-defensins in bovine mucosal host defense.[9,37,95]

Cathelicidins were first reported in cattle myeloid bone marrow cells, and include the bactenecins Bac 5 and Bac 7,[p] which are bactericidal against *E. coli, Salmonella typhimurium*, and *K. pneumoniae*, and bacteriostatic toward *Enterobacter cloacae*.[109–112] Selected antiviral activity also has been noted,[109] as well as killing of spirochetes (*Leptospira interrogans* and *Leptospira biflexa*).[113] A 3rd bactenecin, Bac2S, shows activity against *P. aeruginosa* and some Gram-positive bacteria.[114] Although traditionally associated with myeloid precursor cells, neutrophils also are capable of de novo synthesis of cathelicidin peptides at sites of inflammation.[115] BMAP-27 and BMAP-28 are synthetic bovine cathelicidins with broad-spectrum activity against bacteria, including methicillin-resistant *Staph. aureus*, and fungi,[116] yet exhibiting some cytotoxicity. The synthetic BMAP-34 peptide, however, exhibits a similar breadth of antimicrobial activity, but without any adverse effects on eukaryotic cells. At a sufficiently high dose almost all AMPs may exhibit toxicity toward eukaryotic cells.[117,118] BMAP-28 also has activity against tumor cells in vitro.[119] Moreover, BMAP-27 (as well as Bac7) can effectively bind LPS, and thus may have potential use in treatment of endotoxin-induced septic shock.[120,121] BMAP-28 also exhibits broad-spectrum activity against *Pasteurella multocida* isolates.[122] The role of bovine HDPs as immunomodulatory molecules has received some attention.[123] Indolicidin is one of the shortest cathelicidin peptides (13 amino acids), exhibiting potent and wide antimicrobial activity against Gram-negative (*E. coli*) and Gram-positive (*Staph. aureus*) bacteria[92] as well as fungi,[124] and it also has antiendotoxic and chemokine-inducing properties.[93,123,125,126] Moreover, modified synthetic versions of indolicidin (eg CP-11C) have improved in vitro antibacterial and antifungal activity combined with less cytotoxicity.[125,127]

A range of bioactive peptides has been identified in bovine milk, such as synergistically acting probiotics and antimicrobial compounds, including casecidin and lactoferrin.[128–132] Lactoferrin is also present in other body secretions such as saliva, tears, and bronchoalveolar lavage (BAL) fluid and in leukocyte granules.[133] The peptide LF-B is generated by gastric pepsin degradation of lactoferrin and has a wide range of antimicrobial and antifungal activity as well as immunomodulatory properties.[128,134–138] Lactoferrin is a much larger molecule (80 kDa) than free LF (3 kDa), possibly explaining the peptide fragment's higher degree of activity because of the ease with which it can penetrate the bacterial membrane.[133] Partial synergism with penicillin G against *Staph. aureus* also has been reported.[130] Lactoferrin has in addition been suggested as a good candidate for a novel natural antiviral compound.[139,140] Importantly, it is economically feasible to obtain protein fractions from milk and generate active peptides for use as nutraceuticals and as templates for development of new pharmaceutical compounds.[128,141,142] Oral administration of LF produces a host-protective effect in a number of different animals (including humans), and a pepsin hydrolysate of lactoferrin is already used in infant formulas.[143]

The vast majority of naturally occurring HDPs are cationic peptides. A unique finding, however, is a group

of anionic antimicrobial peptides in the bovine lung that are constitutively expressed and distinctly different from most HDPs with regard to size and polarity.[144] These small peptides (unlike the majority of HDPs) have increased their activity at higher NaCl concentrations.[144] The anionic peptides are not inducible by pathogens or microbial byproducts, but their expression in the lung suggests a role in innate host defense of the bovine respiratory system.[144]

Sheep and Goats. Smaller ruminants have attracted some research attention because of their potential use as animal models to study HDP regulation in epithelial tissue and importantly the impact of pharmaceutical intervention on peptide expression patterns.[145] Two sheep β-defensins (sBD-1 and sBD-2) with differential expression have been identified in the ovine gastrointestinal and respiratory tract epithelium.[145,146] However, unlike cattle, sBDs are not found in neutrophils.[145] In goats, 1 study has reported the expression of β-defensin precursors (preproGBD-1 and preproGBD-2), principally in the caprine respiratory and gastrointestinal tracts.[147] Goat milk also contains lactoferrin, which exhibits antimicrobial properties.[148] Proline-rich antimicrobial peptides are highly conserved HDPs in ruminants, and caprine (ChBac5) and ovine (OaBac5α) analogs to the bovine Bac5 have been described, both exhibiting potent antimicrobial activity.[149] Sequence analysis has determined that there potentially are 8 ovine cathelicidins,[150] but only 2 peptides have actually been isolated from ovine neutrophils.[151,152] The sheep myeloid antimicrobial peptides (SMAP29 and SMAP34) are cathelicidins with broad-spectrum antimicrobial activity against Gram-positive and Gram-negative bacteria, and fungi.[122,153–156] SMAP29 binds LPS with high affinity,[157] and maintains its potent activity under high-salt conditions.[153] This peptide (including synthetic derivatives) may find clinical application in the treatment of respiratory infections.[158,159]

Pigs. Porcine HDPs include more than a dozen different peptides, primarily with representatives from the cathelicidin family (including the prophenins, protegrins, PR-39, and the PMAPs).[160,161] Thus far, no α-defensins have been isolated from the pig, but at least 12 different porcine β-defensins (pBD-1 to pBD-12) have been reported.[162,163] Other HDPs, including NK-lysin and porcine LF, also have been reported in this species.[164–166] Recently, another porcine AMP (ie liver-expressed antimicrobial peptide, LEAP) was described, along with porcine hepcidin (an iron-regulating hormone with antimicrobial effects).[167]

Thirty-nine residue proline-arginine–rich peptide (PR-39) was originally isolated from pig intestine,[168] and later identified in porcine bone marrow cells[169] and neutrophils.[170] In addition to its antimicrobial activity, PR-39 has been implicated in tissue repair[171] and as a chemoattractant of neutrophils[172] as well as an inhibitor of apoptosis.[173] Expression of PR-39 is constitutive in myeloid cells and present in pigs of all ages.[34] The PR-39 peptide is upregulated in the presence of bacterial products,[174,175] and its antimicrobial action relies on a non–pore-forming mechanism.[176] It has a potency against Gram-negative bacteria similar to that of tetracycline.[177] A smaller synthetic peptide (PR-26) derived from PR-39 has been shown to have at least as much potency as its parent molecule.[178] Importantly, PR-39 also has been suggested as a novel biomarker of porcine respiratory health.[179]

The protegrin family of HDPs was first identified in porcine leukocytes,[180] and 5 protegrin sequences (PG-1 to -5) have been identified.[181–183] The protegrins are elastase-activated cathelicidin polypeptides with potent microbicidal activities.[180,184–186] PG-1 exhibits a wide spectrum of in vivo activity against Gram-negative and Gram-positive bacteria, and the synthetic peptide thus has potential for use as an antimicrobial agent in the treatment of clinically relevant antibiotic resistant pathogens.[181,187] PG-1 also exhibits in vitro activity against certain spirochetes.[188] In addition, the peptide has attracted interest because of its potent activity against human STD pathogens, including human immunodeficiency virus (HIV),[189–192] periodontal pathogens,[193,194] and *Mycobacterium tuberculosis*.[195] Importantly, PG-1 also exhibits powerful antimicrobial activity against *P. aeruginosa*, and substantially reduces bacterial growth in established porcine wound infections.[196]

Other members of the cathelicidin family include the prophenins (prophenin-1 and -2)[197,198] and the porcine myeloid antimicrobial peptides (PMAP-23, PMAP-36, and PMAP-37).[199–202] Prophenin-1 has been purified from porcine leukocytes and is substantially more active against Gram-negative bacteria,[197] whereas the PMAPs are broad-spectrum highly potent HDPs derived from pig myeloid cells. Their spectrum of activity includes Gram-negative and Gram-positive bacteria[199] as well as fungi and nematodes.[203,204] Novel peptide analogs of PMAP-23 have shown promising effects against fungi (*C. albicans*), and may act as templates for design of novel antifungal pharmaceutical compounds to treat clinical fungal infections.[205]

Porcine β-defensin-1 (pBD-1) is particularly abundant in tongue epithelium and expressed at only low mRNA levels in other epithelial tissues.[162,206] The expression pattern of the peptide appears to be developmentally regulated,[207] and antimicrobial effects include activity against *E. coli, L. monocytogenes, S. typhimurium*, and *C. albicans* under low-salt conditions.[162,206] pBD-1 acts synergistically with some of the porcine cathelicidins, ensuring antimicrobial activity at higher salt-concentrations.[206] The expression of pBD-1 may be regulated by the recently identified porcine peptidoglycan recognition proteins (pPGRP-L1 and -L2 [ie long-isoforms]).[208] Using bioinformatics and expression analysis, an additional 11 porcine β-defensins have been identified.[209] The main gene expression sites for pBD-2 are liver and kidney, and the peptide is the most highly expressed defensin in the ileum.[167,210] The expression of pBD-1 and pBD-2 has been studied with the porcine intestinal cell line IPI-21[163] as well as the porcine small intestinal segment perfusion (SISP) technique.[210] In vitro, *Salmonella enteritidis* and *S. typhimurium* increase pBD-1 and pBD-2 mRNA levels, respectively, whereas neither of the two is affected by *S. typhimurium* exposure

using the SISP model.[163,210] Despite the common notion of pBD-1 as a constitutively expressed AMP, upregulation of the peptide by *S. typhimurium* (ie, enterocolitis) exposure does seem possible under some circumstances.[163,210]

The expression pattern and activity of porcine HDPs also become important in reference to using porcine organs and tissues in xenotransplantation.[162] Finally, porcine antimicrobial peptides can be of interest in the development of novel functional foods, because digestion of protein of porcine origin may lead to the release of latent bioactive peptides with potential impact on human health.[211]

Special Species

Small Mammals. Very little research has focused on HDPs in small mammals with the exception of the mouse. An exhaustive review of murine HDPs is beyond the scope of this paper, and we have presented an overview of currently reported HDPs in special mammalian species in Table 2. A wide range of AMPs has been discovered in other exotic species and food animals, including amphibians, fish, and other aquatic vertebrates.[212] These studies are also beyond the scope of this paper.

Birds. Avian heterophil antimicrobial peptides of the β-defensin family initially were reported in the chicken (CHP-1 and -2/aka Gal-1 and -2) and turkey (THP-1 and -2).[213–216] A total of 13 different β-defensins (gallinacin-1 to -13) and 3 cathelicidins (fowlicidin-1 to -3) are encoded by the chicken genome according to computational analysis.[217,218] The chicken genome does not, however, code for any α-defensins.[217] Furthermore, TLR expression has been reported in chicken heterophils.[219] Based on tissue expression analysis, gallinacin-1 to -7 are found primarily in the respiratory tract and bone marrow, whereas the remaining genes are restricted to the urogenital tract and liver.[217] Gallopavin (GPV-1) and gallinacin-3 are epithelial β-defensins from the turkey and chicken, respectively, and the latter is inducible by experimental infection with *Haemophilus paragallinarium*.[220] Mature fowlicidin peptides exhibit potent LPS-binding and broad antimicrobial activity in a salt-independent manner, features that make them attractive as candidates for novel antimicrobial and antisepsis compounds.[218,221] Another cathelicidin, chicken myeloid antimicrobial peptide (CMAP-27), has been identified in chicken bone marrow cells,[222] and a liver-expressed epithelial antimicrobial peptide (cLEAP-2) also has been reported in the chicken with activity against different *Salmonella* strains.[223,224] In addition to chicken and turkey, avian HDPs have thus far been isolated from ostrich circulatory cells, and from king penguin stomach content, where the peptides are believed to ensure long-term preservation of stored food.[225,226] Description of the avian antimicrobial profile is of interest to identify novel compounds aimed at fighting infectious diseases in avian species, but also because birds act as asymptomatic carriers and thus major reservoirs for bacteria that are known human enteropathogens.[223,227]

Therapeutic Potential in Veterinary Medicine

One of the major problems in modern medicine is an alarming increase in antibiotic resistance to conventional antibiotics, which has created an obvious need to search for novel compounds to maintain a functional armamentarium aimed at fighting pathogens. From an evolutionary viewpoint, HDPs are ancient yet widely successful endogenous biochemical weapons.[43] Contrary to classical antibiotics, which are made in a sequential fashion involving different enzymatic steps, HDPs are all gene-encoded peptides originating from an RNA template.[228] Their consistency in efficacy throughout evolution would furthermore speak against the common belief that microorganisms inevitably will develop resistance against any imaginable antimicrobial compound over time.[43] The structure of naturally occurring antimicrobial compounds from higher eukaryotes is distinctly different from conventional bacterial and fungal types of antibiotics,[8] which makes them highly attractive as potential templates for new therapeutic agents in the continuous search for novel antimicrobials to fight progressively more resistant microbial pathogens.[8] Interestingly, natural HDPs can act synergistically with certain conventional antibiotics targeted at Gram-negative as well as Gram-positive bacteria.[45,229,230] Figure 7 summarizes the main features that warrant consideration of HDPs as a desirable new class of antibiotics. It is furthermore of interest that certain HDPs adopt amphipathic structures only on contact with biological membranes or when exposed to a membrane-mimicking environment.[23]

As a group, HDPs also are of medical interest as possible future model molecules for novel immunoregulating drugs because of their natural capacity to act as immune response modifiers. Pharmaceutical compounds of tomorrow therefore may be designed as immunomodifiers, aimed at optimizing HDP synthesis in a chosen organ or tissue type, which is of the utmost importance because the regulation of innate immunity is organ-specific.[7,231] Isoleucine is an example of 1 such compound, which can induce synthesis of β-defensin production in enteric cells using TLR2 and NF-κB-signaling.[7,43,232,233] Also the use of corticosteroids in certain inflammatory diseases may lead to iatrogenic complications, because these are synthetic compounds that suppress endogenous HDP synthesis.[26]

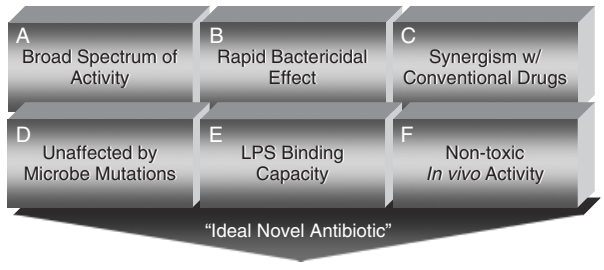

Fig 7. Elemental features of an ideal novel antimicrobial agent. **A.** Broad spectrum of antimicrobial activity (against bacteria, viruses, fungi, parasites). **B.** Rapid bactericidal effect. **C.** Synergy with conventional antibiotics. **D.** Activity unaffected by classical microbial mutations.[r] **E.** Endotoxin neutralizing effect. **F.** Nontoxic activity maintained in animal models.

Four cationic peptides have progressed to Phase 3 trials, of which half have demonstrated clinical efficacy.[23] Pexiganan (a frog HDP derivative) has been used to treat diabetic foot ulcers and Omiganan (a cattle HDP variant) has been used for the prevention of catheter-associated infections). Despite a 90% efficacy, Pexiganan has not obtained FDA approval for clinical use, but Omiganan currently is in Phase 3b trials to confirm initial findings of its clinical usefulness.[23] More than a dozen other peptides and peptidomimetics are currently in commercial development, but the 1st clinically approved cationic peptide aimed primarily at catheter-associated infections is likely to be available within the next few years.[23] Thus, cationic antimicrobial peptides have important potential as model molecules for design of urgently needed novel pharmaceutical compounds. Much research is, however, still warranted to assess the practicality and clinical usefulness of selected compounds from a plethora of naturally occurring HDPs.

Concluding Remarks

The vast majority of species (invertebrates and plants in particular) rely on innate immunity exclusively to effectively fight off potentially lethal pathogens and maintain overall health, whereas immune memory is somewhat of a biological luxury granted only to species higher in the evolutionary hierarchy.[234] The last 10 years in particular have placed innate immunity at the forefront of immunological research as a rapidly developing field, continuously leading to novel ideas together with new discoveries. Increasing antibiotic resistance is a well-known phenomenon in modern medicine, and novel natural antibiotics therefore become immediately attractive. The importance of a well-balanced immunologic response has become evident in a variety of different disease processes (including cardiovascular disease and cancer),[19,235] and the immediate need of novel immunomodifying compounds is consequently obvious. Hopefully much of the ongoing research will translate into original therapeutics for different immunological disease processes, potentially opening up exciting new avenues for immune intervention in veterinary medicine.

The past decade has produced a remarkable amount of new knowledge on the tissue expression and in vitro activity of animal HDPs. Still, these are the formative years for the investigation of innate immune defense mechanisms as they pertain to animal disease with the ultimate goal of elucidating the intricate roles of these versatile peptides in naturally occurring disease affecting small and large animal species.

Footnotes

[a] Amphipathic: Molecules that have both hydrophilic and hydrophobic parts

[b] Azurophil: Primary lysosomal granule found in neutrophil granulocytes. Contains a wide range of hydrolytic enzymes and is released into the extracellular fluid

[c] http://www.bbcm.univ.trieste.it/

[d] A schematic of the molecular motif of these defensins resembles the Greek letter "theta" (θ)

[e] Cathelin domain: so called because it is also present in cathelin, a porcine cysteine protease inhibitor

[f] RIG-I: retinoic acid inducible gene I. Mda5: Melanoma differentiation associated gene 5. The RNA helicases play an essential role in double-stranded RNA-induced innate antiviral responses[46]

[g] Nod: nucleotide-binding oligomerization domain

[h] MAPK: mitogen-activated protein kinases

[i] JAK/STAT: Janus kinase/signal transducer and activator of transcription signaling pathway

[j] Caspases are cysteinyl aspartate-specific proteinases, known for their role in cytokine maturation and apoptosis[52]

[k] Syndecans: cell surface heparan sulfate proteoglycans[56]

[l] The concentration of eNAP-2 in equine neutrophil granulocytes is approximately 4.5–9.0 mg/mL[84]

[m] Microbial exoproteases have the potential of acting as virulence factors, and select anti-proteinase activity may therefore benefit the host[89]

[n] The dense granules distinguish ruminant neutrophils from leukocytes of nonruminant mammals[98]

[o] The BNBDs were initially numbered from 1 to 13 based on their increasing retention time on reversed-phase high performance liquid chromatography (RP-HPLC)[99]

[p] The two bactenecins (from the Latin words "bacterium" and "necare" [to kill]) have molecular masses of approximately 5 and 7 kDa, respectively[109,110]

[q] Animal drawings are clip art images (http://office.microsoft.com/en-us/clipart/default.aspx?lc=en-us)

[r] Relatively invariant bacterial structures have a low frequency of mutations, which may explain why resistance to HDPs (which recognize PAMPs, highly conserved structures) is rare[3,248]

References

1. Ganz T, Weiss J. Antimicrobial peptides of phagocytes and epithelia. Semin Hematol 1997;34:343–354.

2. Oppenheim JJ, Biragyn A, Kwak LW, et al. Roles of antimicrobial peptides such as defensins in innate and adaptive immunity. Ann Rheum Dis 2003;62(Suppl 2):ii17–ii21.

3. Sima P, Trebichavsky I, Sigler K. Mammalian antibiotic peptides. Folia Microbiol (Praha) 2003;48:123–137.

4. Ganz T, Lehrer RI. Defensins. Curr Opin Immunol 1994;6:584–589.

5. Zanetti M, Gennaro R, Romeo D. Cathelicidins: A novel protein family with a common proregion and a variable C-terminal antimicrobial domain. FEBS Lett 1995;374:1–5.

6. Matzinger P. Tolerance, danger, and the extended family. Annu Rev Immunol 1994;12:991–1045.

7. Boman HG. Antibacterial peptides: Basic facts and emerging concepts. J Intern Med 2003;254:197–215.

8. Ganz T, Lehrer RI. Antibiotic peptides from higher eukaryotes: Biology and applications. Mol Med Today 1999;5:292–297.

9. Schonwetter BS, Stolzenberg ED, Zasloff MA. Epithelial antibiotics induced at sites of inflammation. Science 1995;267:1645–1648.

10. Brogden KA, Ackermann M, McCray PB Jr, et al. Antimicrobial peptides in animals and their role in host defences. Int J Antimicrob Agents 2003;22:465–478.

11. Ganz T. Defensins and other antimicrobial peptides: A historical perspective and an update. Comb Chem High Throughput Screen 2005;8:209–217.

12. Lee TD, Gonzalez ML, Kumar P, et al. CAP37, a novel inflammatory mediator: Its expression in endothelial cells and

localization to atherosclerotic lesions. Am J Pathol 2002;160: 841–848.

13. Linde A, Mosier D, Blecha F, et al. Innate immunity and inflammation—New frontiers in comparative cardiovascular pathology. Cardiovasc Res 2007;73:26–36.

14. Matzinger P. Friendly and dangerous signals: Is the tissue in control? Nat Immunol 2007;8:11–13.

15. Scott MG, Hancock RE. Cationic antimicrobial peptides and their multifunctional role in the immune system. Crit Rev Immunol 2000;20:407–431.

16. Ganz T, Selsted ME, Lehrer RI. Defensins. Eur J Haematol 1990;44:1–8.

17. Yang D, Biragyn A, Kwak LW, et al. Mammalian defensins in immunity: More than just microbicidal. Trends Immunol 2002;23:291–296.

18. Yang D, Biragyn A, Hoover DM, et al. Multiple roles of antimicrobial defensins, cathelicidins, and eosinophil-derived neurotoxin in host defense. Annu Rev Immunol 2004;22: 181–215.

19. Kougias P, Chai H, Lin PH, et al. Defensins and cathelicidins: Neutrophil peptides with roles in inflammation, hyperlipidemia and atherosclerosis. J Cell Mol Med 2005;9:3–10.

20. Nassar H, Lavi E, Akkawi S, et al. Alpha-defensin: Link between inflammation and atherosclerosis. Atherosclerosis 2006; 192(2):452–457.

21. Froy O, Hananel A, Chapnik N, et al. Differential effect of insulin treatment on decreased levels of beta-defensins and Toll-like receptors in diabetic rats. Mol Immunol 2007;44:796–802.

22. Bekri S, Gual P, Anty R, et al. Increased adipose tissue expression of hepcidin in severe obesity is independent from diabetes and NASH. Gastroenterology 2006;131:788–796.

23. Hancock RE, Sahl HG. Antimicrobial and host-defense peptides as new anti-infective therapeutic strategies. Nat Biotechnol 2006;24:1551–1557.

24. Ouellette AJ, Selsted ME. Paneth cell defensins: Endogenous peptide components of intestinal host defense. FASEB J 1996;10:1280–1289.

25. Wah J, Wellek A, Frankenberger M, et al. Antimicrobial peptides are present in immune and host defense cells of the human respiratory and gastroinstestinal tracts. Cell Tissue Res 2006;324:449–456.

26. Zasloff M. Antimicrobial peptides in health and disease. N Engl J Med 2002;347:1199–1200.

27. Selsted ME, Ouellette AJ. Mammalian defensins in the antimicrobial immune response. Nat Immunol 2005;6:551–557.

28. Nguyen TX, Cole AM, Lehrer RI. Evolution of primate theta-defensins: A serpentine path to a sweet tooth. Peptides 2003;24:1647–1654.

29. Selsted ME. Theta-defensins: Cyclic antimicrobial peptides produced by binary ligation of truncated alpha-defensins. Curr Protein Pept Sci 2004;5:365–371.

30. Tang YQ, Yuan J, Osapay G, et al. A cyclic antimicrobial peptide produced in primate leukocytes by the ligation of two truncated alpha-defensins. Science 1999;286:498–502.

31. Ganz T, Lehrer RI. Antimicrobial peptides of vertebrates. Curr Opin Immunol 1998;10:41–44.

32. Tomasinsig L, Zanetti M. The cathelicidins—Structure, function and evolution. Curr Protein Pept Sci 2005;6:23–34.

33. Zanetti M. Cathelicidins, multifunctional peptides of the innate immunity. J Leukoc Biol 2004;75:39–48.

34. Wu H, Zhang G, Ross CR, et al. Cathelicidin gene expression in porcine tissues: Roles in ontogeny and tissue specificity. Infect Immun 1999;67:439–442.

35. Lee PH, Ohtake T, Zaiou M, et al. Expression of an additional cathelicidin antimicrobial peptide protects against bacterial skin infection. Proc Natl Acad Sci USA 2005;102: 3750–3755.

36. Nagaoka I, Hirota S, Yomogida S, et al. Synergistic actions of antibacterial neutrophil defensins and cathelicidins. Inflamm Res 2000;49:73–79.

37. Diamond G, Russell JP, Bevins CL. Inducible expression of an antibiotic peptide gene in lipopolysaccharide-challenged tracheal epithelial cells. Proc Natl Acad Sci USA 1996;93:5156–5160.

38. Russell JP, Diamond G, Tarver AP, et al. Coordinate induction of two antibiotic genes in tracheal epithelial cells exposed to the inflammatory mediators lipopolysaccharide and tumor necrosis factor alpha. Infect Immun 1996;64:1565–1568.

39. Diamond G, Kaiser V, Rhodes J, et al. Transcriptional regulation of beta-defensin gene expression in tracheal epithelial cells. Infect Immun 2000;68:113–119.

40. Shai Y. Mechanism of the binding, insertion and destabilization of phospholipid bilayer membranes by alpha-helical antimicrobial and cell non-selective membrane-lytic peptides. Biochim Biophys Acta 1999;1462:55–70.

41. Matsuzaki K. Why and how are peptide-lipid interactions utilized for self-defense? Magainins and tachyplesins as archetypes. Biochim Biophys Acta 1999;1462:1–10.

42. Yang L, Weiss TM, Lehrer RI, et al. Crystallization of antimicrobial pores in membranes: Magainin and protegrin. Biophys J 2000;79:2002–2009.

43. Zasloff M. Antimicrobial peptides of multicellular organisms. Nature 2002;415:389–395.

44. Steiner H, Andreu D, Merrifield RB. Binding and action of cecropin and cecropin analogues: Antibacterial peptides from insects. Biochim Biophys Acta 1988;939:260–266.

45. Xiong YQ, Yeaman MR, Bayer AS. In vitro antibacterial activities of platelet microbicidal protein and neutrophil defensin against *Staphylococcus aureus* are influenced by antibiotics differing in mechanism of action. Antimicrob Agents Chemother 1999;43:1111–1117.

46. Yoneyama M, Kikuchi M, Matsumoto K, et al. Shared and unique functions of the DExD/H-box helicases RIG-I, MDA5, and LGP2 in antiviral innate immunity. J Immunol 2005;175:2851–2858.

47. Robinson MJ, Sancho D, Slack EC, et al. Myeloid C-type lectins in innate immunity. Nat Immunol 2006;7:1258–1265.

48. Fritz JH, Ferrero RL, Philpott DJ, et al. Nod-like proteins in immunity, inflammation and disease. Nat Immunol 2006;7: 1250–1257.

49. Seong SY, Matzinger P. Hydrophobicity: An ancient damage-associated molecular pattern that initiates innate immune responses. Nat Rev Immunol 2004;4:469–478.

50. Dalpke AH, Lehner MD, Hartung T, et al. Differential effects of CpG-DNA in Toll-like receptor-2/-4/-9 tolerance and cross-tolerance. Immunology 2005;116:203–212.

51. Krisanaprakornkit S, Kimball JR, Dale BA. Regulation of human beta-defensin-2 in gingival epithelial cells: The involvement of mitogen-activated protein kinase pathways, but not the NF-kappaB transcription factor family. J Immunol 2002;168: 316–324.

52. Scott AM, Saleh M. The inflammatory caspases: Guardians against infections and sepsis. Cell Death Differ 2007;14:23–31.

53. Martinon F, Tschopp J. Inflammatory caspases and inflammasomes: Master switches of inflammation. Cell Death Differ 2007;14:10–22.

54. van WS, Mannesse-Lazeroms SP, Van Sterkenburg MA, et al. Effect of defensins on interleukin-8 synthesis in airway epithelial cells. Am J Physiol 1997;272(5 Pt 1):L888–L896.

55. Chertov O, Michiel DF, Xu L, et al. Identification of defensin-1, defensin-2, and CAP37/azurocidin as T-cell chemoattractant proteins released from interleukin-8-stimulated neutrophils. J Biol Chem 1996;271:2935–2940.

56. Gallo RL, Ono M, Povsic T, et al. Syndecans, cell surface heparan sulfate proteoglycans, are induced by a proline-rich

antimicrobial peptide from wounds. Proc Natl Acad Sci USA 1994;91:11035–11039.

57. van den Berg RH, Faber-Krol MC, van WS, et al. Inhibition of activation of the classical pathway of complement by human neutrophil defensins. Blood 1998;92:3898–3903.

58. Panyutich AV, Panyutich EA, Krapivin VA, et al. Plasma defensin concentrations are elevated in patients with septicemia or bacterial meningitis. J Lab Clin Med 1993;122:202–207.

59. Soong LB, Ganz T, Ellison A, et al. Purification and characterization of defensins from cystic fibrosis sputum. Inflamm Res 1997;46:98–102.

60. Bals R, Weiner DJ, Moscioni AD, et al. Augmentation of innate host defense by expression of a cathelicidin antimicrobial peptide. Infect Immun 1999;67:6084–6089.

61. Gennaro R, Zanetti M. Structural features and biological activities of the cathelicidin-derived antimicrobial peptides. Biopolymers 2000;55:31–49.

62. Chertov O, Yang D, Howard OM, et al. Leukocyte granule proteins mobilize innate host defenses and adaptive immune responses. Immunol Rev 2000;177:68–78.

63. De Y, Chen Q, Schmidt AP, et al. LL-37, the neutrophil granule- and epithelial cell-derived cathelicidin, utilizes formyl peptide receptor-like 1 (FPRL1) as a receptor to chemoattract human peripheral blood neutrophils, monocytes, and T cells. J Exp Med 2000;192:1069–1074.

64. Charp PA, Rice WG, Raynor RL, et al. Inhibition of protein kinase C by defensins, antibiotic peptides from human neutrophils. Biochem Pharmacol 1988;37:951–956.

65. Zhou CX, Zhang YL, Xiao L, et al. An epididymis-specific beta-defensin is important for the initiation of sperm maturation. Nat Cell Biol 2004;6:458–464.

66. Davis EG, Sang Y, Blecha F. Equine beta-defensin-1: Full-length cDNA sequence and tissue expression. Vet Immunol Immunopathol 2004;99:127–132.

67. Scocchi M, Bontempo D, Boscolo S, et al. Novel cathelicidins in horse leukocytes (1). FEBS Lett 1999;457:459–464.

68. Sang Y, Ortega MT, Blecha F, et al. Molecular cloning and characterization of three beta-defensins from canine testes. Infect Immun 2005;73:2611–2620.

69. Patil AA, Cai Y, Sang Y, et al. Cross-species analysis of the mammalian beta-defensin gene family: Presence of syntenic gene clusters and preferential expression in the male reproductive tract. Physiol Genomics 2005;23:5–17.

70. Sang Y, Teresa OM, Rune K, et al. Canine cathelicidin (K9CATH): Gene cloning, expression, and biochemical activity of a novel pro-myeloid antimicrobial peptide. Dev Comp Immunol 2007; 31(12):1278–1296.

71. Fry MM, Liggett JL, Baek SJ. Molecular cloning and expression of canine hepcidin. Vet Clin Pathol 2004;33:223–227.

72. Lin DS, Bowman DD, Jacobson RH. Immunological changes in cats with concurrent *Toxoplasma gondii* and feline immunodeficiency virus infections. J Clin Microbiol 1992;30:17–24.

73. Lappin MR, George JW, Pedersen NC, et al. Primary and secondary *Toxoplasma gondii* infection in normal and feline immunodeficiency virus-infected cats. J Parasitol 1996;82:733–742.

74. Ignacio G, Nordone S, Howard KE, et al. Toll-like receptor expression in feline lymphoid tissues. Vet Immunol Immunopathol 2005;106:229–237.

75. Wassef A, Janardhan K, Pearce JW, et al. Toll-like receptor 4 in normal and inflamed lungs and other organs of pig, dog and cattle. Histol Histopathol 2004;19:1201–1208.

76. Ishii M, Hashimoto M, Oguma K, et al. Molecular cloning and tissue expression of canine Toll-like receptor 2 (TLR2). Vet Immunol Immunopathol 2006;110:87–95.

77. Bazzocchi C, Mortarino M, Comazzi S, et al. Expression and function of Toll-like receptor 2 in canine blood phagocytes. Vet Immunol Immunopathol 2005;104:15–19.

78. Hashimoto M, Asahina Y, Sano J, et al. Cloning of canine toll-like receptor 9 and its expression in dog tissues. Vet Immunol Immunopathol 2005;106:159–163.

79. Linde A, Blecha F, Melgarejo T. Toll-like receptor (TLR) 2 and TLR4 gene expression in canine heart. Am J Anim Vet Sci 2007;2:6–10.

80. Swerdlow MP, Kennedy DR, Kennedy JS, et al. Expression and function of TLR2, TLR4, and Nod2 in primary canine colonic epithelial cells. Vet Immunol Immunopathol 2006;114:313–319.

81. Alberola J, Rodriguez A, Francino O, et al. Safety and efficacy of antimicrobial peptides against naturally acquired leishmaniasis. Antimicrob Agents Chemother 2004;48:641–643.

82. Pellegrini A, Hageli G, von FR. Isolation and characterization of two new low-molecular-weight protein proteinase inhibitors from the granule-rich fraction of equine neutrophilic granulocytes. Biochim Biophys Acta 1988;952:309–316.

83. Couto MA, Harwig SS, Cullor JS, et al. eNAP-2, a novel cysteine-rich bactericidal peptide from equine leukocytes. Infect Immun 1992;60:5042–5047.

84. Couto MA, Harwig SS, Cullor JS, et al. Identification of eNAP-1, an antimicrobial peptide from equine neutrophils. Infect Immun 1992;60:3065–3071.

85. Skerlavaj B, Scocchi M, Gennaro R, et al. Structural and functional analysis of horse cathelicidin peptides. Antimicrob Agents Chemother 2001;45:715–722.

86. Davis EG, Sang Y, Rush B, et al. Molecular cloning and characterization of equine NK-lysin. Vet Immunol Immunopathol 2005;105:163–169.

87. Looft C, Paul S, Philipp U, et al. Sequence analysis of a 212 kb defensin gene cluster on ECA 27q17. Gene 2006;376: 192–198.

88. Yasui T, Tsukise A, Fukui K, et al. Aspects of glycoconjugate production and lysozyme-and defensins-expression of the ceruminous glands of the horse (*Equus przewalskii* f.dom.). Eur J Morphol 2005;42:127–134.

89. Couto MA, Harwig SS, Lehrer RI. Selective inhibition of microbial serine proteases by eNAP-2, an antimicrobial peptide from equine neutrophils. Infect Immun 1993;61:2991–2994.

90. Savoini A, Marzari R, Dolzani L, et al. Wide-spectrum antibiotic activity of bovine granulocyte polypeptides. Antimicrob Agents Chemother 1984;26:405–407.

91. Tydell CC, Yount N, Tran D, et al. Isolation, characterization, and antimicrobial properties of bovine oligosaccharide-binding protein. A microbicidal granule protein of eosinophils and neutrophils. J Biol Chem 2002;277:19658–19664.

92. Selsted ME, Novotny MJ, Morris WL, et al. Indolicidin, a novel bactericidal tridecapeptide amide from neutrophils. J Biol Chem 1992;267:4292–4295.

93. Hsu CH, Chen C, Jou ML, et al. Structural and DNA-binding studies on the bovine antimicrobial peptide, indolicidin: Evidence for multiple conformations involved in binding to membranes and DNA. Nucleic Acids Res 2005;33:4053–4064.

94. Diamond G, Zasloff M, Eck H, et al. Tracheal antimicrobial peptide, a cysteine-rich peptide from mammalian tracheal mucosa: Peptide isolation and cloning of a cDNA. Proc Natl Acad Sci USA 1991;88:3952–3956.

95. Tarver AP, Clark DP, Diamond G, et al. Enteric beta-defensin: Molecular cloning and characterization of a gene with inducible intestinal epithelial cell expression associated with *Cryptosporidium parvum* infection. Infect Immun 1998;66:1045–1056.

96. Aono S, Li C, Zhang G, et al. Molecular and functional characterization of bovine beta-defensin-1. Vet Immunol Immunopathol 2006;113:181–190.

97. Roosen S, Exner K, Paul S, et al. Bovine beta-defensins: Identification and characterization of novel bovine beta-defensin genes and their expression in mammary gland tissue. Mamm Genome 2004;15:834–842.

98. Yount NY, Yuan J, Tarver A, et al. Cloning and expression of bovine neutrophil beta-defensins. Biosynthetic profile during neutrophilic maturation and localization of mature peptide to novel cytoplasmic dense granules. J Biol Chem 1999;274:26249–26258.

99. Selsted ME, Tang YQ, Morris WL, et al. Purification, primary structures, and antibacterial activities of beta-defensins, a new family of antimicrobial peptides from bovine neutrophils. J Biol Chem 1993;268:6641–6648.

100. Ryan LK, Rhodes J, Bhat M, et al. Expression of beta-defensin genes in bovine alveolar macrophages. Infect Immun 1998;66:878–881.

101. Stolzenberg ED, Anderson GM, Ackermann MR, et al. Epithelial antibiotic induced in states of disease. Proc Natl Acad Sci USA 1997;94:8686–8690.

102. Diamond G, Jones DE, Bevins CL. Airway epithelial cells are the site of expression of a mammalian antimicrobial peptide gene. Proc Natl Acad Sci USA 1993;90:4596–4600.

103. Caverly JM, Diamond G, Gallup JM, et al. Coordinated expression of tracheal antimicrobial peptide and inflammatory-response elements in the lungs of neonatal calves with acute bacterial pneumonia. Infect Immun 2003;71:2950–2955.

104. Lawyer C, Watabe M, Pai S, et al. A synthetic form of tracheal antimicrobial peptide has both bactericidal and antifungal activities. Drug Des Discov 1996;14:171–178.

105. Swanson K, Gorodetsky S, Good L, et al. Expression of a beta-defensin mRNA, lingual antimicrobial peptide, in bovine mammary epithelial tissue is induced by mastitis. Infect Immun 2004;72:7311–7314.

106. Goldammer T, Zerbe H, Molenaar A, et al. Mastitis increases mammary mRNA abundance of beta-defensin 5, Toll-like-receptor 2 (TLR2), and TLR4 but not TLR9 in cattle. Clin Diagn Lab Immunol 2004;11:174–185.

107. Yang W, Molenaar A, Kurts-Ebert B, et al. NF-kappaB factors are essential, but not the switch, for pathogen-related induction of the bovine beta-defensin 5-encoding gene in mammary epithelial cells. Mol Immunol 2006;43:210–225.

108. Mitchell GB, Al-Haddawi MH, Clark ME, et al. Effect of corticosteroids and neuropeptides on the expression of defensins in bovine tracheal epithelial cells. Infect Immun 2006; 75:1325–1334.

109. Gennaro R, Skerlavaj B, Romeo D. Purification, composition, and activity of two bactenecins, antibacterial peptides of bovine neutrophils. Infect Immun 1989;57:3142–3146.

110. Zanetti M, Litteri L, Gennaro R, et al. Bactenecins, defense polypeptides of bovine neutrophils, are generated from precursor molecules stored in the large granules. J Cell Biol 1990;111:1363–1371.

111. Frank RW, Gennaro R, Schneider K, et al. Amino acid sequences of two proline-rich bactenecins. Antimicrobial peptides of bovine neutrophils. J Biol Chem 1990;265:18871–18874.

112. Scocchi M, Romeo D, Zanetti M. Molecular cloning of Bac7, a proline- and arginine-rich antimicrobial peptide from bovine neutrophils. FEBS Lett 1994;352:197–200.

113. Scocchi M, Romeo D, Cinco M. Antimicrobial activity of two bactenecins against spirochetes. Infect Immun 1993;61:3081–3083.

114. Wu M, Hancock RE. Interaction of the cyclic antimicrobial cationic peptide bactenecin with the outer and cytoplasmic membrane. J Biol Chem 1999;274:29–35.

115. Tomasinsig L, Scocchi M, Di LC, et al. Inducible expression of an antimicrobial peptide of the innate immunity in polymorphonuclear leukocytes. J Leukoc Biol 2002;72:1003–1010.

116. Skerlavaj B, Gennaro R, Bagella L, et al. Biological characterization of two novel cathelicidin-derived peptides and identification of structural requirements for their antimicrobial and cell lytic activities. J Biol Chem 1996;271:28375–28381.

117. Gennaro R, Scocchi M, Merluzzi L, et al. Biological characterization of a novel mammalian antimicrobial peptide. Biochim Biophys Acta 1998;1425:361–368.

118. Scocchi M, Wang S, Zanetti M. Structural organization of the bovine cathelicidin gene family and identification of a novel member. FEBS Lett 1997;417:311–315.

119. Risso A, Braidot E, Sordano MC, et al. BMAP-28, an antibiotic peptide of innate immunity, induces cell death through opening of the mitochondrial permeability transition pore. Mol Cell Biol 2002;22:1926–1935.

120. Ghiselli R, Giacometti A, Cirioni O, et al. Neutralization of endotoxin in vitro and in vivo by Bac7(1–35), a proline-rich antibacterial peptide. Shock 2003;19:577–581.

121. Mookherjee N, Wilson HL, Doria S, et al. Bovine and human cathelicidin cationic host defense peptides similarly suppress transcriptional responses to bacterial lipopolysaccharide. J Leukoc Biol 2006;80:1563–1574.

122. Brogden KA, Nordholm G, Ackermann M. Antimicrobial activity of cathelicidins BMAP28, SMAP28, SMAP29, and PMAP23 against *Pasteurella multocida* is more broad-spectrum than host species specific. Vet Microbiol 2007;119:76–81.

123. Bowdish DM, Davidson DJ, Scott MG, et al. Immunomodulatory activities of small host defense peptides. Antimicrob Agents Chemother 2005;49:1727–1732.

124. Lee DG, Kim HK, Kim SA, et al. Fungicidal effect of indolicidin and its interaction with phospholipid membranes. Biochem Biophys Res Commun 2003;305:305–310.

125. Falla TJ, Hancock RE. Improved activity of a synthetic indolicidin analog. Antimicrob Agents Chemother 1997;41:771–775.

126. Subbalakshmi C, Sitaram N. Mechanism of antimicrobial action of indolicidin. FEMS Microbiol Lett 1998;160:91–96.

127. Ryge TS, Doisy X, Ifrah D, et al. New indolicidin analogues with potent antibacterial activity. J Pept Res 2004;64:171–185.

128. Clare DA, Swaisgood HE. Bioactive milk peptides: A prospectus. J Dairy Sci 2000;83:1187–1195.

129. Kilara A, Panyam D. Peptides from milk proteins and their properties. Crit Rev Food Sci Nutr 2003;43:607–633.

130. Vorland LH. Lactoferrin: A multifunctional glycoprotein. APMIS 1999;107:971–981.

131. Tomita M, Takase M, Bellamy W, et al. A review: The active peptide of lactoferrin. Acta Paediatr Jpn 1994;36:585–591.

132. Tomita M, Takase M, Wakabayashi H, et al. Antimicrobial peptides of lactoferrin. Adv Exp Med Biol 1994;357:209–218.

133. Yamauchi K, Tomita M, Giehl TJ, et al. Antibacterial activity of lactoferrin and a pepsin-derived lactoferrin peptide fragment. Infect Immun 1993;61:719–728.

134. Haukland HH, Vorland LH. Post-antibiotic effect of the antimicrobial peptide lactoferricin on *Escherichia coli* and *Staphylococcus aureus*. J Antimicrob Chemother 2001;48:569–571.

135. Bellamy W, Wakabayashi H, Takase M, et al. Killing of *Candida albicans* by lactoferricin B, a potent antimicrobial peptide derived from the N-terminal region of bovine lactoferrin. Med Microbiol Immunol (Berlin) 1993;182:97–105.

136. Vorland LH, Ulvatne H, Andersen J, et al. Lactoferricin of bovine origin is more active than lactoferricins of human, murine and caprine origin. Scand J Infect Dis 1998;30:513–517.

137. Jones EM, Smart A, Bloomberg G, et al. Lactoferricin, a new antimicrobial peptide. J Appl Bacteriol 1994;77:208–214.

138. Di Biase AM, Tinari A, Pietrantoni A, et al. Effect of bovine lactoferricin on enteropathogenic *Yersinia* adhesion and invasion in HEp-2 cells. J Med Microbiol 2004;53(Pt 5):407–412.

139. van der Strate BW, Beljaars L, Molema G, et al. Antiviral activities of lactoferrin. Antiviral Res 2001;52:225–239.

140. Pietrantoni A, Di Biase AM, Tinari A, et al. Bovine lactoferrin inhibits adenovirus infection by interacting with viral

structural polypeptides. Antimicrob Agents Chemother 2003; 47:2688–2691.

141. Chen PW, Shyu CL, Mao FC. Antibacterial activity of short hydrophobic and basic-rich peptides. Am J Vet Res 2003;64:1088–1092.

142. Meisel H Biochemical properties of peptides encrypted in bovine milk proteins. Curr Med Chem 2005;12:1905–1919.

143. Tomita M, Wakabayashi H, Yamauchi K, et al. Bovine lactoferrin and lactoferricin derived from milk: Production and applications. Biochem Cell Biol 2002;80:109–112.

144. Fales-Williams AJ, Brogden KA, Huffman E, et al. Cellular distribution of anionic antimicrobial peptide in normal lung and during acute pulmonary inflammation. Vet Pathol 2002; 39:706–711.

145. Huttner KM, Brezinski-Caliguri DJ, Mahoney MM, et al. Antimicrobial peptide expression is developmentally regulated in the ovine gastrointestinal tract. J Nutr 1998;128(2 Suppl):297S–299S.

146. Ackermann MR, Gallup JM, Zabner J, et al. Differential expression of sheep beta-defensin-1 and −2 and interleukin 8 during acute *Mannheimia haemolytica* pneumonia. Microb Pathog 2004;37:21–27.

147. Zhao C, Nguyen T, Liu L, et al. Differential expression of caprine beta-defensins in digestive and respiratory tissues. Infect Immun 1999;67:6221–6224.

148. Kimura M, Nam MS, Ohkouchi Y, et al. Antimicrobial peptide of Korean native goat lactoferrin and identification of the part essential for this activity. Biochem Biophys Res Commun 2000;268:333–336.

149. Shamova O, Brogden KA, Zhao C, et al. Purification and properties of proline-rich antimicrobial peptides from sheep and goat leukocytes. Infect Immun 1999;67:4106–4111.

150. Huttner KM, Lambeth MR, Burkin HR, et al. Localization and genomic organization of sheep antimicrobial peptide genes. Gene 1998;206:85–91.

151. Anderson RC, Yu PL. Isolation and characterisation of proline/arginine-rich cathelicidin peptides from ovine neutrophils. Biochem Biophys Res Commun 2003;312:1139–1146.

152. Bagella L, Scocchi M, Zanetti M. cDNA sequences of three sheep myeloid cathelicidins. FEBS Lett 1995;376:225–228.

153. Travis SM, Anderson NN, Forsyth WR, et al. Bactericidal activity of mammalian cathelicidin-derived peptides. Infect Immun 2000;68:2748–2755.

154. Anderson RC, Hancock RE, Yu PL. Antimicrobial activity and bacterial-membrane interaction of ovine-derived cathelicidins. Antimicrob Agents Chemother 2004;48:673–676.

155. Skerlavaj B, Benincasa M, Risso A, et al. SMAP-29: A potent antibacterial and antifungal peptide from sheep leukocytes. FEBS Lett 1999;463:58–62.

156. Lee DG, Kim PI, Park Y, et al. Antifungal mechanism of SMAP-29 (1–18) isolated from sheep myeloid mRNA against *Trichosporon beigelii*. Biochem Biophys Res Commun 2002; 295:591–596.

157. Tack BF, Sawai MV, Kearney WR, et al. SMAP-29 has two LPS-binding sites and a central hinge. Eur J Biochem 2002;269:1181–1189.

158. Brogden KA, Kalfa VC, Ackermann MR, et al. The ovine cathelicidin SMAP29 kills ovine respiratory pathogens in vitro and in an ovine model of pulmonary infection. Antimicrob Agents Chemother 2001;45:331–334.

159. Kalfa VC, Jia HP, Kunkle RA, et al. Congeners of SMAP29 kill ovine pathogens and induce ultrastructural damage in bacterial cells. Antimicrob Agents Chemother 2001;45:3256–3261.

160. Zhang G, Ross CR, Blecha F. Porcine antimicrobial peptides: New prospects for ancient molecules of host defense. Vet Res 2000;31:277–296.

161. Oswald IP. Role of intestinal epithelial cells in the innate immune defence of the pig intestine. Vet Res 2006;37:359–368.

162. Zhang G, Wu H, Shi J, et al. Molecular cloning and tissue expression of porcine beta-defensin-1. FEBS Lett 1998;424:37–40.

163. Veldhuizen EJ, Hendriks HG, Hogenkamp A, et al. Differential regulation of porcine beta-defensins 1 and 2 upon *Salmonella* infection in the intestinal epithelial cell line IPI-2I. Vet Immunol Immunopathol 2006;114:94–102.

164. Lee JY, Boman A, Sun CX, et al. Antibacterial peptides from pig intestine: Isolation of a mammalian cecropin. Proc Natl Acad Sci USA 1989;86:9159–9162.

165. Andersson M, Gunne H, Agerberth B, et al. NK-lysin, a novel effector peptide of cytotoxic T and NK cells. Structure and cDNA cloning of the porcine form, induction by interleukin. 2, antibacterial and antitumour activity. EMBO J 1995;14: 1615–1625.

166. Chen HL, Yen CC, Lu CY, et al. Synthetic porcine lactoferricin with a 20-residue peptide exhibits antimicrobial activity against *Escherichia coli, Staphylococcus aureus*, and *Candida albicans*. J Agric Food Chem 2006;54:3277–3282.

167. Sang Y, Ramanathan B, Minton JE, et al. Porcine liver-expressed antimicrobial peptides, hepcidin and LEAP-2: Cloning and induction by bacterial infection. Dev Comp Immunol 2006;30:357–366.

168. Agerberth B, Lee JY, Bergman T, et al. Amino acid sequence of PR-39. Isolation from pig intestine of a new member of the family of proline-arginine-rich antibacterial peptides. Eur J Biochem 1991;202:849–854.

169. Storici P, Zanetti M. A novel cDNA sequence encoding a pig leukocyte antimicrobial peptide with a cathelin-like prosequence. Biochem Biophys Res Commun 1993;196:1363–1368.

170. Shi J, Ross CR, Chengappa MM, et al. Identification of a proline-arginine-rich antibacterial peptide from neutrophils that is analogous to PR-39, an antibacterial peptide from the small intestine. J Leukoc Biol 1994;56:807–811.

171. Shi J, Ross CR, Leto TL, et al. PR-39, a proline-rich antibacterial peptide that inhibits phagocyte NADPH oxidase activity by binding to Src homology 3 domains of p47 phox. Proc Natl Acad Sci USA 1996;93:6014–6018.

172. Huang HJ, Ross CR, Blecha F. Chemoattractant properties of PR-39, a neutrophil antibacterial peptide. J Leukoc Biol 1997;61:624–629.

173. Ramanathan B, Wu H, Ross CR, et al. PR-39, a porcine antimicrobial peptide, inhibits apoptosis: Involvement of caspase-3. Dev Comp Immunol 2004;28:163–169.

174. Zhang G, Ross CR, Dritz SS, et al. *Salmonella* infection increases porcine antibacterial peptide concentrations in serum. Clin Diagn Lab Immunol 1997;4:774–777.

175. Wu H, Zhang G, Minton JE, et al. Regulation of cathelicidin gene expression: Induction by lipopolysaccharide, interleukin-6, retinoic acid, and *Salmonella enterica* serovar Typhimurium infection. Infect Immun 2000;68:5552–5558.

176. Boman HG, Agerberth B, Boman A. Mechanisms of action on *Escherichia coli* of cecropin P1 and PR-39, two antibacterial peptides from pig intestine. Infect Immun 1993; 61:2978–2984.

177. Gudmundsson GH, Magnusson KP, Chowdhary BP, et al. Structure of the gene for porcine peptide antibiotic PR-39, a cathelin gene family member: Comparative mapping of the locus for the human peptide antibiotic FALL-39. Proc Natl Acad Sci USA 1995;92:7085–7089.

178. Shi J, Ross CR, Chengappa MM, et al. Antibacterial activity of a synthetic peptide (PR-26) derived from PR-39, a proline-arginine-rich neutrophil antimicrobial peptide. Antimicrob Agents Chemother 1996;40:115–121.

179. Hennig-Pauka I, Jacobsen I, Blecha F, et al. Differential proteomic analysis reveals increased cathelicidin expression in

porcine bronchoalveolar lavage fluid after an *Actinobacillus pleuropneumoniae* infection. Vet Res 2006;37:75–87.

180. Kokryakov VN, Harwig SS, Panyutich EA, et al. Protegrins: Leukocyte antimicrobial peptides that combine features of corticostatic defensins and tachyplesins. FEBS Lett 1993;327:231–236.

181. Steinberg DA, Hurst MA, Fujii CA, et al. Protegrin-1: A broad-spectrum, rapidly microbicidal peptide with in vivo activity. Antimicrob Agents Chemother 1997;41:1738–1742.

182. Storici P, Zanetti M. A cDNA derived from pig bone marrow cells predicts a sequence identical to the intestinal antibacterial peptide PR-39. Biochem Biophys Res Commun 1993;196:1058–1065.

183. Zhao C, Liu L, Lehrer RI. Identification of a new member of the protegrin family by cDNA cloning. FEBS Lett 1994;346:285–288.

184. Zhao C, Ganz T, Lehrer RI. The structure of porcine protegrin genes. FEBS Lett 1995;368:197–202.

185. Shi J, Ganz T. The role of protegrins and other elastase-activated polypeptides in the bactericidal properties of porcine inflammatory fluids. Infect Immun 1998;66:3611–3617.

186. Panyutich A, Shi J, Boutz PL, et al. Porcine polymorphonuclear leukocytes generate extracellular microbicidal activity by elastase-mediated activation of secreted proprotegrins. Infect Immun 1997;65:978–985.

187. Aumelas A, Mangoni M, Roumestand C, et al. Synthesis and solution structure of the antimicrobial peptide protegrin-1. Eur J Biochem 1996;237:575–583.

188. Sambri V, Marangoni A, Giacani L, et al. Comparative in vitro activity of five cathelicidin-derived synthetic peptides against *Leptospira*, *Borrelia* and *Treponema pallidum*. J Antimicrob Chemother 2002;50:895–902.

189. Yasin B, Harwig SS, Lehrer RI, et al. Susceptibility of *Chlamydia trachomatis* to protegrins and defensins. Infect Immun 1996;64:709–713.

190. Qu XD, Harwig SS, Oren AM, et al. Susceptibility of *Neisseria gonorrhoeae* to protegrins. Infect Immun 1996;64:1240–1245.

191. Fortney K, Totten PA, Lehrer RI, et al. *Haemophilus ducreyi* is susceptible to protegrin. Antimicrob Agents Chemother 1998;42:2690–2693.

192. Tamamura H, Murakami T, Horiuchi S, et al. Synthesis of protegrin-related peptides and their antibacterial and anti-human immunodeficiency virus activity. Chem Pharm Bull (Tokyo) 1995;43:853–858.

193. Miyasaki KT, Iofel R, Oren A, et al. Killing of *Fusobacterium nucleatum, Porphyromonas gingivalis* and *Prevotella intermedia* by protegrins. J Periodontal Res 1998;33:91–98.

194. Miyasaki KT, Iofel R, Lehrer RI. Sensitivity of periodontal pathogens to the bactericidal activity of synthetic protegrins, antibiotic peptides derived from porcine leukocytes. J Dent Res 1997;76:1453–1459.

195. Miyakawa Y, Ratnakar P, Rao AG, et al. In vitro activity of the antimicrobial peptides human and rabbit defensins and porcine leukocyte protegrin against *Mycobacterium tuberculosis*. Infect Immun 1996;64:926–932.

196. Ceccarelli AV, Cole AM, Park AK, et al. Therapeutic effect of a pig-derived peptide antibiotic on porcine wound infections. Comp Med 2001;51:75–79.

197. Harwig SS, Kokryakov VN, Swiderek KM, et al. Prophenin-1, an exceptionally proline-rich antimicrobial peptide from porcine leukocytes. FEBS Lett 1995;362:65–69.

198. Zhao C, Ganz T, Lehrer RI. Structures of genes for two cathelin-associated antimicrobial peptides: Prophenin-2 and PR-39. FEBS Lett 1995;376:130–134.

199. Zanetti M, Storici P, Tossi A, et al. Molecular cloning and chemical synthesis of a novel antibacterial peptide derived from pig myeloid cells. J Biol Chem 1994;269:7855–7858.

200. Storici P, Scocchi M, Tossi A, et al. Chemical synthesis and biological activity of a novel antibacterial peptide deduced from a pig myeloid cDNA. FEBS Lett 1994;337: 303–307.

201. Tossi A, Scocchi M, Zanetti M, et al. PMAP-37, a novel antibacterial peptide from pig myeloid cells. cDNA cloning, chemical synthesis and activity. Eur J Biochem 1995;228: 941–946.

202. Scocchi M, Zelezetsky I, Benincasa M, et al. Structural aspects and biological properties of the cathelicidin PMAP-36. FEBS J 2005;272: 4398–4406.

203. Lee DG, Kim DH, Park Y, et al. Fungicidal effect of antimicrobial peptide, PMAP-23, isolated from porcine myeloid against *Candida albicans*. Biochem Biophys Res Commun 2001;282:570–574.

204. Park Y, Jang SH, Lee DG, et al. Antinematodal effect of antimicrobial peptide, PMAP-23, isolated from porcine myeloid against *Caenorhabditis elegans*. J Pept Sci 2004;10:304–311.

205. Lee DG, Kim PI, Park Y, et al. Design of novel peptide analogs with potent fungicidal activity, based on PMAP-23 antimicrobial peptide isolated from porcine myeloid. Biochem Biophys Res Commun 2002;293:231–238.

206. Shi J, Zhang G, Wu H, et al. Porcine epithelial beta-defensin 1 is expressed in the dorsal tongue at antimicrobial concentrations. Infect Immun 1999;67:3121–3127.

207. Elahi S, Buchanan RM, Attah-Poku S, et al. The host defense peptide beta-defensin 1 confers protection against *Bordetella pertussis* in newborn piglets. Infect Immun 2006;74:2338–2352.

208. Sang Y, Ramanathan B, Ross CR, et al. Gene silencing and overexpression of porcine peptidoglycan recognition protein long isoforms: Involvement in beta-defensin-1 expression. Infect Immun 2005;73:7133–7141.

209. Sang Y, Patil AA, Zhang G, et al. Bioinformatic and expression analysis of novel porcine beta-defensins. Mamm Genome 2006;17:332–339.

210. Veldhuizen EJ, van Dijk A, Tersteeg MH, et al. Expression of beta-defensins pBD-1 and pBD-2 along the small intestinal tract of the pig: Lack of upregulation in vivo upon *Salmonella* typhimurium infection. Mol Immunol 2007;44:276–283.

211. Rutherfurd-Markwick KJ, Moughan PJ. Bioactive peptides derived from food. J AOAC Int 2005;88:955–966.

212. Hancock RE, Lehrer R. Cationic peptides: A new source of antibiotics. Trends Biotechnol 1998;16:82–88.

213. Evans EW, Beach GG, Wunderlich J, et al. Isolation of antimicrobial peptides from avian heterophils. J Leukoc Biol 1994;56:661–665.

214. Harwig SS, Swiderek KM, Kokryakov VN, et al. Gallinacins: Cysteine-rich antimicrobial peptides of chicken leukocytes. FEBS Lett 1994;342:281–285.

215. Evans EW, Beach FG, Moore KM, et al. Antimicrobial activity of chicken and turkey heterophil peptides CHP1, CHP2, THP1, and THP3. Vet Microbiol 1995;47:295–303.

216. Brockus CW, Jackwood MW, Harmon BG. Characterization of beta-defensin prepropeptide mRNA from chicken and turkey bone marrow. Anim Genet 1998;29:283–289.

217. Xiao Y, Hughes AL, Ando J, et al. A genome-wide screen identifies a single beta-defensin gene cluster in the chicken: Implications for the origin and evolution of mammalian defensins. BMC Genomics 2004;5:56.

218. Xiao Y, Cai Y, Bommineni YR, et al. Identification and functional characterization of three chicken cathelicidins with potent antimicrobial activity. J Biol Chem 2006;281:2858–2867.

219. Kogut MH, Iqbal M, He H, et al. Expression and function of Toll-like receptors in chicken heterophils. Dev Comp Immunol 2005;29:791–807.

220. Zhao C, Nguyen T, Liu L, et al. Gallinacin-3, an inducible epithelial beta-defensin in the chicken. Infect Immun 2001;69:2684–2691.

221. Xiao Y, Dai H, Bommineni YR, et al. Structure-activity relationships of fowlicidin-1, a cathelicidin antimicrobial peptide in chicken. FEBS J 2006;273:2581–2593.

222. van Dijk A, Veldhuizen EJ, van Asten AJ, et al. CMAP27, a novel chicken cathelicidin-like antimicrobial protein. Vet Immunol Immunopathol 2005;106:321–327.

223. Townes CL, Michailidis G, Nile CJ, et al. Induction of cationic chicken liver-expressed antimicrobial peptide 2 in response to *Salmonella enterica* infection. Infect Immun 2004;72:6987–6993.

224. Lynn DJ, Higgs R, Gaines S, et al. Bioinformatic discovery and initial characterisation of nine novel antimicrobial peptide genes in the chicken. Immunogenetics 2004;56:170–177.

225. Sugiarto H, Yu PL. Avian antimicrobial peptides: The defense role of beta-defensins. Biochem Biophys Res Commun 2004;323:721–727.

226. Thouzeau C, Le Maho Y, Froget G, et al. Spheniscins, avian beta-defensins in preserved stomach contents of the king penguin, Aptenodytes patagonicus. J Biol Chem 2003;278:51053–51058.

227. Joseph SW, Hayes JR, English LL, et al. Implications of multiple antimicrobial-resistant enterococci associated with the poultry environment. Food Addit Contam 2001;18:1118–1123.

228. Boman HG. Peptide antibiotics and their role in innate immunity. Annu Rev Immunol 1995;13:61–92.

229. Scott MG, Gold MR, Hancock RE. Interaction of cationic peptides with lipoteichoic acid and Gram-positive bacteria. Infect Immun 1999;67:6445–6453.

230. Scott MG, Yan H, Hancock RE. Biological properties of structurally related alpha-helical cationic antimicrobial peptides. Infect Immun 1999;67:2005–2009.

231. Raz E. Organ-specific regulation of innate immunity. Nat Immunol 2007;8:3–4.

232. Birchler T, Seibl R, Buchner K, et al. Human Toll-like receptor 2 mediates induction of the antimicrobial peptide human beta-defensin 2 in response to bacterial lipoprotein. Eur J Immunol 2001;31:3131–3137.

233. Fehlbaum P, Rao M, Zasloff M, et al. An essential amino acid induces epithelial beta -defensin expression. Proc Natl Acad Sci USA 2000;97:12723–12728.

234. Sansonetti PJ. The innate signaling of dangers and the dangers of innate signaling. Nat Immunol 2006;7:1237–1242.

235. Ma XT, Xu B, An LL, et al. Vaccine with beta-defensin 2-transduced leukemic cells activates innate and adaptive immunity to elicit potent antileukemia responses. Cancer Res 2006;66:1169–1176.

236. Harris RH, Wilk D, Bevins CL, et al. Identification and characterization of a mucosal antimicrobial peptide expressed by the chinchilla (*Chinchilla lanigera*) airway. J Biol Chem 2004;279:20250–20256.

237. Selsted ME, Harwig SS. Purification, primary structure, and antimicrobial activities of a guinea pig neutrophil defensin. Infect Immun 1987;55:2281–2286.

238. Befus AD, Mowat C, Gilchrist M, et al. Neutrophil defensins induce histamine secretion from mast cells: Mechanisms of action. J Immunol 1999;163:947–953.

239. Nagaoka I, Tsutsumi-Ishii Y, Yomogida S, et al. Isolation of cDNA encoding guinea pig neutrophil cationic antibacterial polypeptide of 11 kDa (CAP11) and evaluation of CAP11 mRNA expression during neutrophil maturation. J Biol Chem 1997;272:22742–22750.

240. Mak P, Wojcik K, Thogersen IB, et al. Isolation, antimicrobial activities, and primary structures of hamster neutrophil defensins. Infect Immun 1996;64:4444–4449.

241. Zaballos A, Villares R, Albar JP, et al. Identification on mouse chromosome 8 of new beta-defensin genes with regionally specific expression in the male reproductive organ. J Biol Chem 2004;279:12421–12426.

242. Gallo RL, Kim KJ, Bernfield M, et al. Identification of CRAMP, a cathelin-related antimicrobial peptide expressed in the embryonic and adult mouse. J Biol Chem 1997;272:13088–13093.

243. Selsted ME, Brown DM, DeLange RJ, et al. Primary structures of six antimicrobial peptides of rabbit peritoneal neutrophils. J Biol Chem 1985;260:4579–4584.

244. Zarember KA, Katz SS, Tack BF, et al. Host defense functions of proteolytically processed and parent (unprocessed) cathelicidins of rabbit granulocytes. Infect Immun 2002;70:569–576.

245. Com E, Bourgeon F, Evrard B, et al. Expression of antimicrobial defensins in the male reproductive tract of rats, mice, and humans. Biol Reprod 2003;68:95–104.

246. Page RA, Malik AN. Elevated levels of beta defensin-1 mRNA in diabetic kidneys of GK rats. Biochem Biophys Res Commun 2003;310:513–521.

247. Termen S, Tollin M, Olsson B, et al. Phylogeny, processing and expression of the rat cathelicidin rCRAMP: A model for innate antimicrobial peptides. Cell Mol Life Sci 2003;60:536–549.

248. Risso A. Leukocyte antimicrobial peptides: Multifunctional effector molecules of innate immunity. J Leukoc Biol 2000;68:785–792.