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Lactoferricin: a lactoferrin-derived peptide with antimicrobial, antiviral, antitumor and immunological properties

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Abstract. The peptide lactoferricin (Lfcin) can be released from the multifunctional protein lactoferrin (LF) through proteolysis by pepsin under acidic conditions, a reaction that occurs naturally in the stomach. Lfcin encompasses a large portion of the functional domain of the intact protein, and in many cases it not only retains the activities of LF but is more active. Lfcin possesses strong antimicrobial and weak antiviral activities, and it also has potent antitumor and immunological properties. This review covers the current state of research in this field, focusing on the many beneficial activities of this peptide. Throughout we will discuss the breadth of Lfcin activity as well as the mechanism of action. Many recent studies have drawn attention to the fact that the main site of action for the peptide may be intracellular. In addition the results of structural and dynamic studies of Lfcin are presented, and the relationship between structure and activity is explored.

Key words. Lactoferrin; lactoferricin; antimicrobial; antiviral; antitumor; anti-inflammatory; peptide structure; protein structure.

Introduction

Lactoferrin (LF), a member of the transferrin family, is an ~80-kDa iron-binding glycoprotein. Found predominantly in the secreted fluids of mammals such as milk, tears, saliva, bronchial mucus and seminal plasma, LF is also stored in the secondary granules of polymorphonuclear leukocytes (PMNs). Though the physiological effects of the intact protein have yet to be completely determined, it plays an important and multifunctional role in host defense (for a recent review see [1]). The overall three-dimensional structure of LF is very similar to that of the other members of the transferrin family except for a unique, highly positively charged N-terminal region. Thought to convey upon this protein its unique host defense properties, the importance of this basic region is highlighted by the fact that a peptide released through acidic pepsin hydrolysis containing the N-terminal sequence not only retains many of the activities of the intact protein but in some cases it can even be more potent than the parent protein. This peptide, termed lactoferricin (Lfcin), has received much attention recently due to its broad host defense properties. Here we will cover current research in this field. Topics pertaining to the structure of different Lfcins as well as their antimicrobial (antibacterial, antifungal and antiparasidal), antiviral, antitumor and immunomodulatory activities will be discussed.

Structure of Lfcins

To date the two most-studied Lfcins are those derived from bovine and human LF, LfcinB and LfcinH, respectively. Even though both peptides are highly posi-

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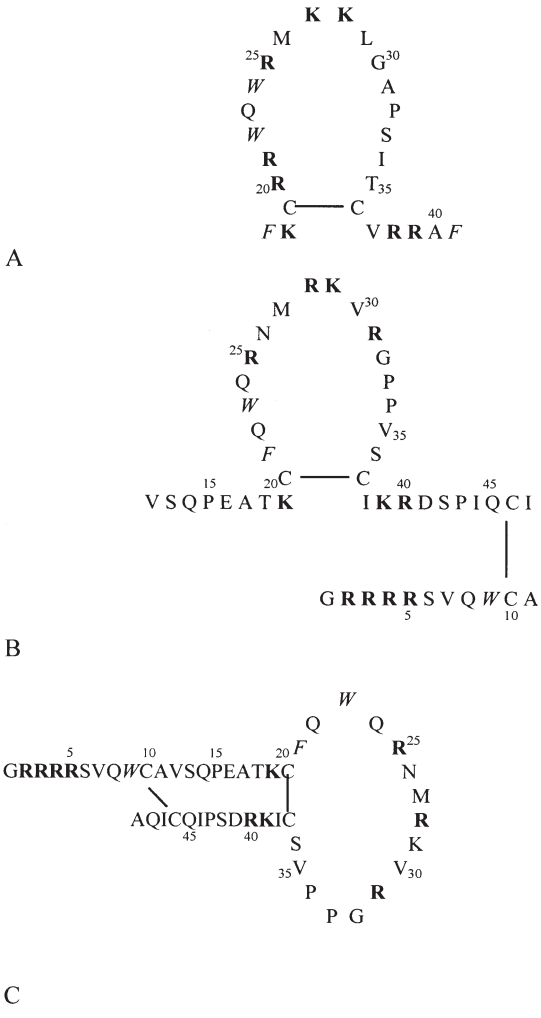

Figure 1. The primary structures of LfcinB and LfcinH. Single-letter codes are used to represent the amino acid sequence of each peptide. The basic residues are indicated in boldface and aromatic residues are indicated in italics. (A) LfcinB and (B) LfcinH from [3]. (C) LfcinH from [4].

tively charged, there is a striking difference in both the length of the peptide and the amino acid sequences of LfcinB and LfcinH. The difference is reflected by the relatively low sequence similarity of 69% between the aligned regions of bovine and human LF (bLF and hLF, respectively) [2]. The primary structure of LfcinB is well established as a 25-residue peptide (residues 17–41 of bLF) that forms into a looped structure through an intramolecular disulfide bond (fig. 1A) [3]. The exact amino acid sequence of LfcinH, however, is more controversial. It was originally thought to contain two disulfide-linked peptide chains encompassing the N-terminal 47 residues of hLF; however, recent mass spectrometry evidence indicates that LfcinH consists of the N-terminal 49 residues in a single continuous chain (figs. 1B, C, respectively) [3, 4]. The original primary structure of LfcinH included a loop of the same length as that seen in LfcinB formed by an intramolecular disulfide bond. The current LfcinH sequence again indicates the presence of this loop, but this time a second disulfide bond extends the overall structure, which is about twice as long as LfcinB.

The difference in primary structure between LfcinB and LfcinH also extends to the three-dimensional structure of these peptides. Nuclear magnetic resonance (NMR) spectroscopy studies indicate that free LfcinB adopts a conformation in aqueous solution that is quite different from that found in the intact protein [5]. In low-salt solutions this peptide loses the α-helix seen in intact bLF and forms a twisted β-sheet (fig. 2A, B). By adopting this structure the released peptide becomes markedly amphipathic as nearly all the hydrophobic residues lie on one face while the positively charged residues lie on the other (fig. 2B). This conformational transformation can be observed in molecular dynamics calculations that indicate that LfcinB can readily undergo a transition from an α-helix to a β-sheet hairpin structure in aqueous solution; this interconversion does not proceed as readily in the presence of 250 mM salt [6]. In contrast, NMR studies of LfcinH in a membrane mimetic solvent indicate that this peptide retains a significant part of the conformation seen in intact hLF and that the α-helix is preserved (fig. 3A, B) [4]. Even in aqueous solution the peptide remains somewhat coiled with the hydrophobic side chains of Trp, Leu, Ile and Val providing centers for the initiation of a preferred conformation. The retention of the α-helical region in LfcinH may be directly related to the additional length of the peptide as compared with LfcinB. This would allow for extra hydrogen bonds that assist in stabilizing the helix, a structure lost in LfcinB. Once liberated from the intact hLF protein and the accompanying restraints, the β-sheet structure of LfcinH is lost most likely due to the presence of two adjacent Pro residues (P33, P34) in the loop of LfcinH. The structure of LfcinH in the membrane mimetic solvent indicates that this peptide, like LfcinB, has a significant amphipathicity (fig. 3B). Interestingly, the hydrophobic surface created by LfcinH is much larger than that in LfcinB; this may account for the fact that LfcinH behaves like a di mer structure in aqueous solution [4].

The ability of both LfcinB and LfcinH to form amphipathic structures with clear hydrophobic and positively charged faces is a trait they share with other peptides that display antimicrobial activity. Almost 900 distinct antimicrobial peptides have been discovered, in organisms from plants to insects and invertebrates to humans, and though little sequence homology exists between the peptides, the vast majority contain a high proportion of hydrophobic and cationic residues which through asymmetric clustering give rise to amphipathic structures (for more information see www.bbcm.units.it/~tossi/amsdb.html and [7]).

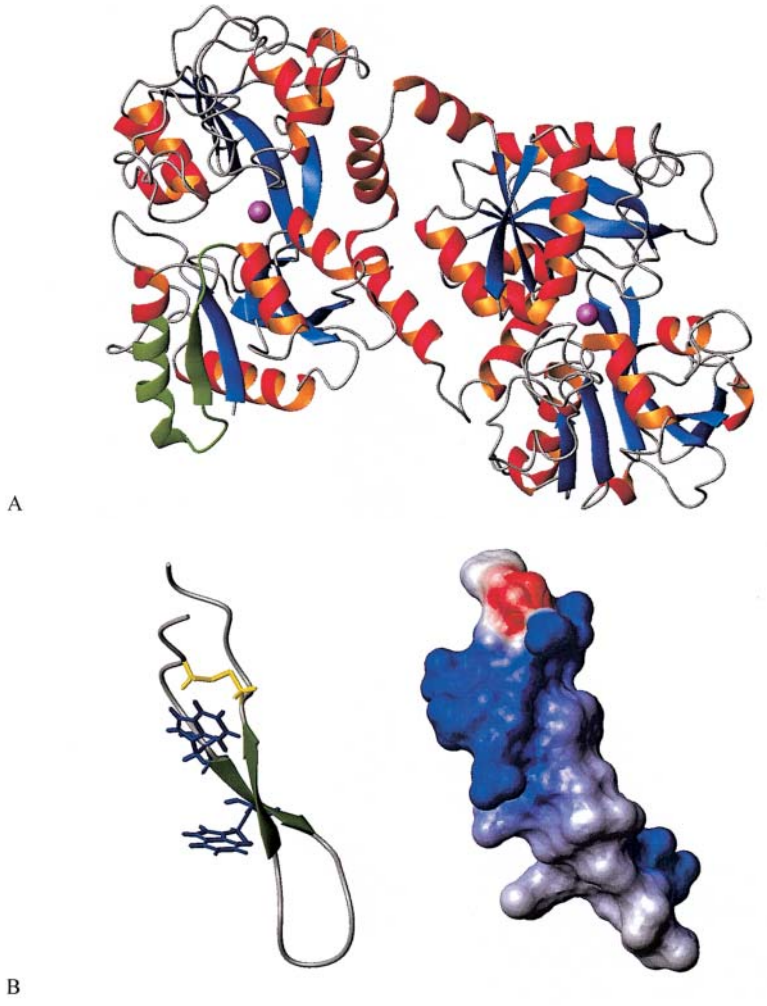

Figure 2. The three-dimensional structure of LfcinB. (A) Crystal structure of bLF. The secondary structure is highlighted with blue representing β-sheets and red and orange representing the α-helices. The region contained in LfcinB is shown in green. PDB file 1BLF. (B) Solution structures of LfcinB. A ribbon diagram representation of the secondary structure of LfcinB is indicated on the left, with the disulfide bond highlighted in yellow and Trp residues indicated in blue. On the right is the peptide shown to illustrate the charged distributions that are colored blue, red and white for positive, negative and neutral charges, respectively. The amphipathic faces can clearly be seen. Both diagrams are in approximately the same orientation. PDB file 1LFC. These figures were produced using Molmol.

Antibacterial activity

Previous research has provided substantial evidence for the in vitro antibacterial activity of both LfcinB and LfcinH. LfcinB is bactericidal and LfcinH, like bLF and hLF, is bacteriostatic against a wide variety of Gram-negative and Gram-positive bacteria (table 1) [8–12]. This lack of species specificity combined with the observation that both the L and D enantiomers of LfcinB are active suggests that the bacterial target of this peptide is a structure of a generalized nature, a prime candidate being the phospholipids present in membranes [13]. As both the lipopolysaccharide (LPS)-containing outer membrane of Gram-negative bacteria and the teichoic acid layer that surrounds the cytoplasmic membrane of Gram-positive bacteria are negatively charged surfaces, it is thought that an electrostatic attraction first binds the cationic peptide to the outside of the bacterial cell. In vitro experiments further support these proposed initial binding sites, as both LfcinB and LfcinH, like LF, have the ability to bind and release LPS from the outer membrane of Gram-negative bacteria [8, 14] and LfcinB can bind teichoic acid originating from Gram-positive bacteria [15]. In contrast, both cationic peptides would have a low affinity for membranes surrounding healthy eukaryotic cells, as these mostly have the neutral zwitterionic phosphatidylcholine headgroup exposed on their outer leaflet.

Following its binding to the outer lipid layer of bacteria, Lfcin then crosses this barrier to interact with the bacterial cytoplasmic membrane. Little is known about this process, but it appears that Lfcin crosses the outer membrane of Gram-negative bacteria through a mechanism consistent with the semi ‘self-promoted uptake’ pathway [16]. Experiments performed with a peptide representing the N-terminal half of the loop in LfcinH indicate that in the presence of LPS the peptide can self-associate in an ordered manner to form an array [17]. The peptides act as a point of nucleation, organizing themselves to give both a hydrophobic and a hydrophilic face to the array. When the polycationic surface of this array interacts with

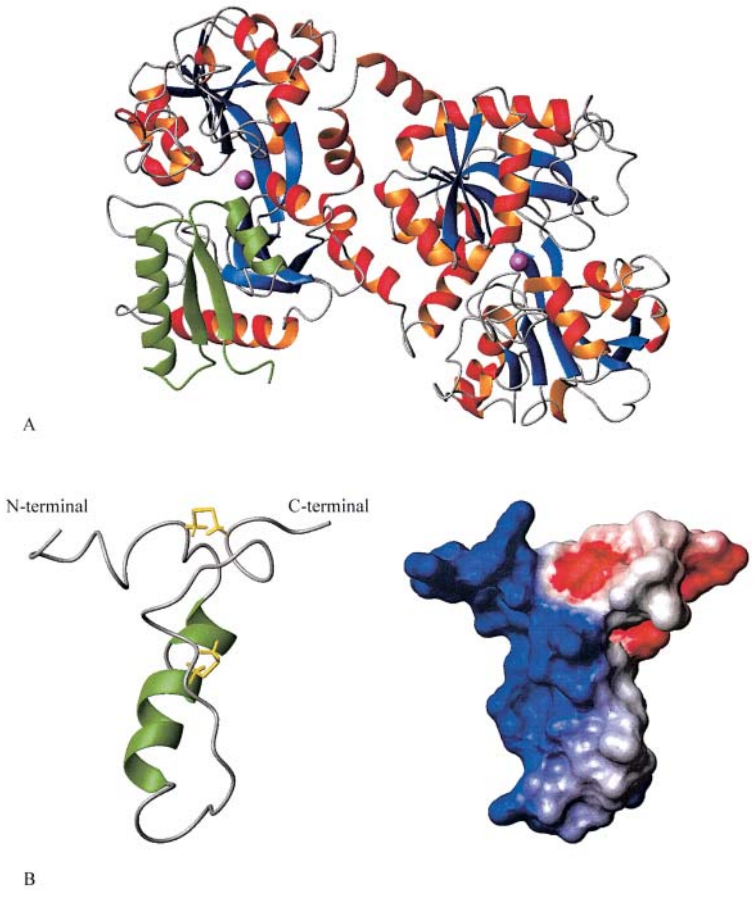

Figure 3. The three dimensional structure of LfcinH. (A) Crystal structure of hLF. The secondary structure is highlighted with blue representing β-sheets and red and orange representing the α-helices. The region contained in LfcinH is shown in green. PDB file 1B0L. (B) Solution structures of LfcinH in a membrane mimetic solvent. A ribbon diagram representation of the secondary structure of LfcinH is indicated on the left, with the disulfide bonds highlighted in yellow. On the right is the peptide shown to illustrate the charged distributions that are colored blue, red and white for positive, negative and neutral charges, respectively. The amphipathic faces can clearly be seen. Both diagrams are in approximately the same orientation. These figures were produced using Molmol.

the polyanionic surface of the bacterial outer membrane, the fluidity of the membrane is disrupted, allowing the peptides to translocate across the cytoplasmic membrane. In this fashion the peptide can enter the bacterial cell and act on intracellular targets. This mechanism is supported not only by the lag time observed in the bactericidal effects but also by the observation of blebs on the surface of certain bacterial cells treated with LfcinB, blebs resulting from expansion in the outer membrane to accommodate the insertion of peptide-organized lipid rafts [8, 10]. Gram-positive bacteria are also more susceptible to Lfcin [18], a susceptibility most likely due to the absence of an outer membrane but possibly due to the mechanism through which Lfcin crosses the teichoic acid layer, a mechanism not yet deduced.

The effects of Lfcin on the bacterial cytoplasmic membrane are not yet entirely known. Unlike other antimicrobial peptides that form pores or disintegrate the cytoplasmic membrane [7], Lfcin appears to do no significant damage to the integrity of this membrane [19]. However, studies with both LfcinB and LfcinH indicate that this peptide induces a compromise in membrane permeability, allowing the passage of small ions, and resulting in the loss of both the transmembrane electrochemical and pH gradients [14, 20, 21]. As LfcinB has been localized to the cytoplasm of both Gram-negative and Gram-positive bacteria, it is now thought that this peptide exerts its effects intracellularly and that perhaps the membrane depolarization results from perturbations in metabolic pathways [22]. It has been suggested that Lfcin can translocate across the cell membrane in a manner similar to other Arg-rich peptides that are spontaneously internalized, peptides known as 'penetratins' [19]. By inducing the formation of micellar-like structures in the cytoplasmic membrane, these peptides lead to the creation of a hydrophilic cavity that transports them across this barrier [23]. There is some *in vivo* evidence for this hypothesis, as exposure to LfcinB causes curling and fusion of the bacterial cytoplasmic membrane [8, 14]. Interestingly, the penetratin class of peptide can also cross the nuclear envelope, suggesting that nucleic acids may be a potential target of Lfcin. Studies with antimicrobial peptides of varying secondary structures indicate that β-sheet peptides translocate across the cytoplasmic membrane better than α-helical or extended peptides [24], possibly explaining the increased activity of LfcinB compared with LfcinH.

Table 1. Antimicrobial and antiviral activities of LfcinB and LfcinH tested for various organisms

Strain	Reference
Gram-negative bacteria	
Escherichia coli	[3, 8, 10, 11]
Klebsiella pneumoniae	[8, 11]
Proteus vulgaris	[11]
Pseudomonas aeruginosa	[3, 8, 11]
Pseudomonas fluorescens	[3, 11]
Salmonella enteritidis	[11]
montevideo	[9]
salford	[8]
typhimurium	[8, 9]
Yersinia enterocolitica	[11]
Gram-positive bacteria	
Bacillus cereus	[9, 11]
circulans	[11]
natto	[11]
subtilis	[11]
Clostridium paraputrificum	[11]
perfringens	[11]
Corynebacterium ammoniagenes	[11]
diphtheriae	[11]
renal	[11]
Enterococcus faecalis	[11]
Lactobacillus casei	[11]
Listeria monocytogenes	[3, 8, 11, 12]
Staphylococcus aureus	[3, 8, 9, 11]
epidermidis	[11]
haemolyticus	[11]
hominus	[11]
Streptococcus bovis	[11]
cremoris	[11]
lactis	[11]
mutans	[11]
thermophilus	[11]
Yeasts	
Candida albicans	[8, 39–41]
Cryptococcus uniguttulatus	[39]
C. curvatus	[39]
C. albidus	[39]
Trichosporon cutaneum	[39]
Dermatophytes	
Trichophyton mentagrophytes	[39]
T. rubrum	[39]
Nannizzia gypsea	[39]
N. incurvata	[39]
N. otae	[39]
Other filamentous fungi	[39]
Aspergillus fumigatus	[39]
A. niger	[39]
Penicillium pinophilum	[39]
P. vermiculatum	[39]
Rhizopus oryzae	[39]
Parasites	
Eimeria stiedai	[46]
Giardia lamblia	[47]
Toxoplasma gondii	[45, 46]
Viruses	
Adenovirus	[52]
Feline calicivirus	[50]
Herpes simplexvirus-1 and -2	[51]
Human cytomegalovirus	[48]
Human immunodeficiency virus-1	[49]

Table 2. Amino acid sequences for a 15-residue homologous region of various Lfcins

Peptide	Amino acid sequence (single-letter code)
LfcinB	FKCRRWQWRMKKLGA
LfcinH	TKCFQWQRNMRKVRG
LfcinM	EKCLRWQNEMRKVGG
LfcinC	SKCYQWQRRMRKLGA

The linear 15-residue peptide analog for LfcinB has comparable antibacterial activity to the complete LfcinB. For abbreviations see text. Taken from [29].

The cytoplasm contains many polyanionic molecules that are possible interaction sites for Lfcins. Recent studies indicate that LfcinB is capable of inhibiting macromolecular synthesis in both Gram-negative and Gram-positive bacteria [18]. In the case of the Gram-positive bacterium *Bacillus subtilis*, exposure to sublethal concentrations of LfcinB results in the inhibition of DNA, RNA and protein synthesis. The morphological changes that are subsequently observed imply that LfcinB induces a defense response in this bacterium. The effect of LfcinB on Gram-negative bacteria is different than that on Gram-positive bacteria. LfcinB appears to act through a multi-phased mode of action such as that hypothesized for other antimicrobial peptides including apidaecin [25]. The initial interaction between the bacterium and the peptide evokes an SOS-like response as there is an initial decrease in DNA synthesis that is accompanied by an increase in the synthesis of protein and RNA as well as filamentation of the *Escherichia coli* cells. Following this, LfcinB inhibits protein synthesis leading to cell death, possibly by targeting the protein-synthesis machinery. The bactericidal mechanism of LfcinB is not unique to this peptide, as a number of other antimicrobial peptides, such as indolicidin [26] and a short Trp- and Arg-rich peptide derived from human lysozyme [27], have also been shown to translocate across the lipid bilayers and inhibit macromolecular synthesis. However, as an interaction between the peptide and the bacterial cytoplasmic and outer membranes is necessary to ensure antibacterial activity, the permeabilizing effect of this interaction cannot yet be ruled out as part of the cause of the inhibition of macromolecular synthesis. As yet the exact bacteriostatic mechanism of LfcinH has not been deduced.

Recently, much research in this field has been focused on determining the molecular mechanism through which Lfcin interacts with membranes, especially the membranes of bacteria. As previously mentioned, both LfcinB and LfcinH can form amphipathic structures, which enable the interaction with negatively charged membranes. The positive residues on the polar side of Lfcin interact with the negatively charged polar head groups of the

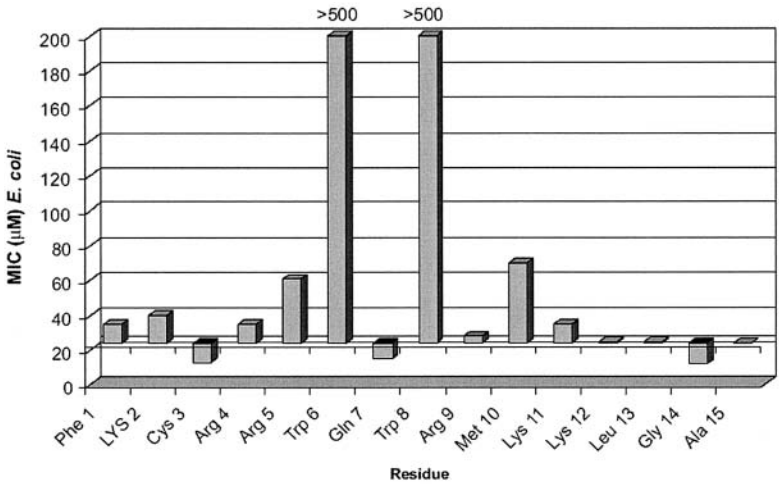

Figure 4. Deviation in the antibacterial activity (μM) against *E. coli* for an alanine-scan of a 15-residue LfcinB derivate with respect to Lfcin. As the baseline is set at the antibacterial activity of LfcinB, a positive deviation indicates a reduction in antibacterial activity, while a negative deviation indicates an increase in antibacterial activity. The results clearly illustrate the important role of the two Trp residues. Taken from [31].

membrane. The importance of the initial electrostatic interaction is highlighted first by the high overall positive charge of the peptides, with a net charge of at least +4 necessary for optimal antibacterial activity [28]; second by the fact that murine Lfcin (LfcinM), which contains two Glu residues, lacks antibacterial activity (table 2) [29]; and third by the increased activity of C-terminally amidated unadecapeptides derived from various Lfcins [13, 28]. As Arg can interact both electrostatically and through multiple hydrogen bonds with the negatively charged surface of the bacteria, it is thought that this amino acid is the most effective for targeting the peptide to the membrane. In addition the guanidinium group adds bulk to the side chain, thereby potentially contributing to membrane disruption [30].

Once the positively charged residues bring Lfcin into contact with the bacterial cell, the hydrophobic residues interact with the lipophilic portion of the membrane, becoming embedded into its surface and destabilizing the packing of the phospholipids. Of the hydrophobic residues present in Lfcin, Trp is clearly the most important. The bactericidal activity of LfcinB appears to be dependent not just on one of these residues but on at least two (see fig. 4). In fact, studies with Lfcin derivatives indicate that the presence of three Trp residues ensures a maximal thinning of the membrane in a certain radius around the peptide [31]. The requirement for more than one Trp seemingly explains the increased in vitro antibacterial activity of LfcinB compared with LfcinH and the other studied Lfcins; unlike LfcinB with two Trps, the same regions of LfcinH, LfcinM and caprine Lfcin (LfcinC) have only one (table 2) [29]. Interestingly studies of peptides with non-natural Trp analogs have shown that neither the hydrogen-bonding ability nor the amphipathicity of the indole system are essential for the effect of Trp. Instead the size, shape and aromatic character of this amino acid seem to be the most important features for the activity. Trp acts as an anchor, with the bulk of this residue helping to bind the peptide to the membrane. Substitution of Lfcin's Trp with bulkier unnatural amino acids such as Tpc (β-[2-(2,2,5,7,8-pentamethylchroman-6-sulphonyl)-indol-3-yl]alanine) leads to an increase in antibacterial activity [32]. The larger size of the Tpc side chain allows this residue to anchor the peptide more deeply in the membrane, thereby more efficiently disrupting the phospholipid packing. The impact of side-chain size on antibacterial activity is more pronounced against *Staphylococcus aureus* than against *Escherichia coli*, indicating that bacterial selectivity can be altered by choosing different Trp analogs [33]. Nevertheless, optimization of the antibacterial activity in this direction has its limits, as the addition of too many aromatic residues or too much hydrophobic bulk renders the peptides not quite as selective, and hemolytic activity increases [28]. The importance of both Arg and Trp for the antibacterial activity of Lfcin highlights the importance of both electrostatic and hydrophobic interactions for the activity of Lfcin. Three-dimensional structures determined for short 6- and 11-residue LfcinB analogs bound to membrane mimetic SDS micelles suggest that the Trp residues intercalate into the interface region of the membrane, while the Arg side chains point further outward [34]. This also applies to a cyclic 11 residue analog that has slightly improved antimicrobial activity [35].

Recently, a computational strategy was used to search for more effective derivatives of Lfcin. Based on a combination of quantitative structure-activity relationship analysis (QSAR) and multivariate data analysis, this approach relies on structural parameters that can be used to design more potent peptides [36]. The structural parameters used in this study include net charge, mean hydrophobic moment, lipophilicity, charge asymmetry and micelle affinity, and it has been applied to the design of unadeca- and pentadecapeptide derivatives of Lfcin [36, 37]. The QSAR and multivariable analysis techniques are also

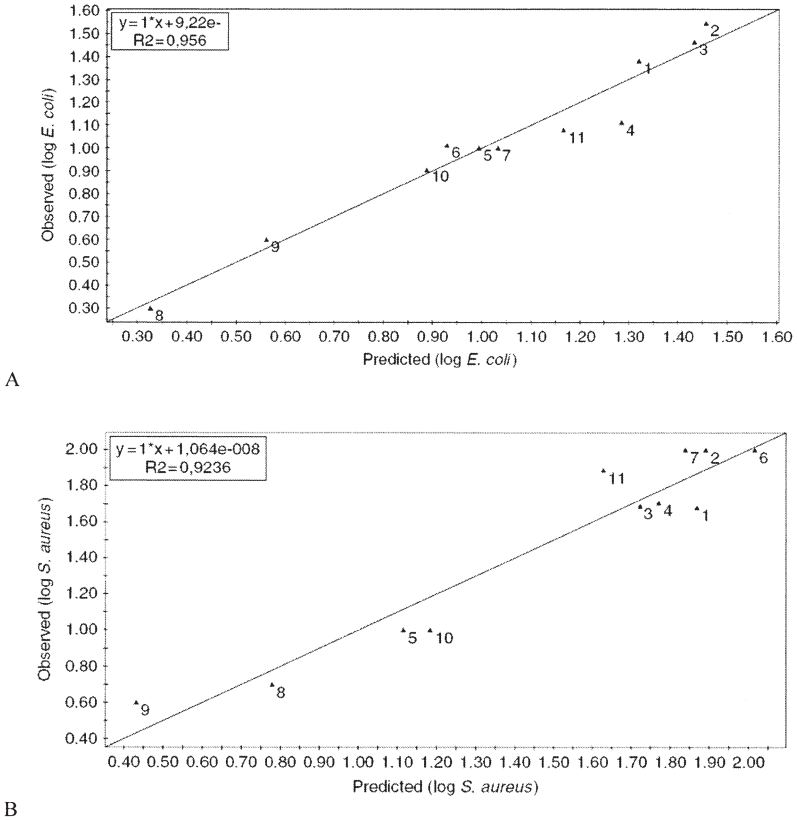

Figure 5. The relationship between observed and QSAR-predicted antibacterial activity (μM) of various Lfcin derivatives. The relationship vis à vis *E. coli* (A) and *S. aureus* (B) is shown. Taken from [38].

used to predict the antibacterial activity of proposed peptides. Based on the assumption that individual molecules can be described by a grouping of physico-chemical variables, models are calculated using macroscopic descriptors such as α-helicity, lipophilicity and high-performance liquid chromatography (HPLC)-retention times as well as theoretical properties such as different measures for charge localization and Eisenberg α-helix propensities. As the calculated models reveal a good correlation between the observed and predicted activities, it is now possible to fairly accurately predict the activity of new Trp- and Arg-rich antimicrobial peptides (fig. 5) [38].

Antifungal and antiparasitic activity

In addition to a number of bacteria, Lfcin, and particularly LfcinB, is also effective at inhibiting the growth of a number of yeasts, molds and filamentous fungi, including the pathogen *Candida albicans*, as well as dermatophytes (table 1) [8, 39–41]. Recent evidence suggests that Lfcin has two antifungal mechanisms, with the first involving a direct fungicidal activity. Both LfcinB and LfcinH appear to interact with the plasma membrane, as treatment of *Candida albicans* results in the dissipation of the proton gradient across the cell membrane [39, 41]. Both Lfcins also appear to affect the cytoplasm of fungal cells. Aggregation of cytoplasmic material has been observed with LfcinB-exposed cells, and treatment of the blastoconidia life stage of *C. albicans* with a LfcinH-derived peptide results in synthesis and secretion of ATP from the mitochondria. Interestingly, this ATP is released extracellularly, where it can interact with extracellular ATP binding sites on the plasma membrane, giving rise to pore formation and cell death [41, 42].

The second antifungal action of Lfcin appears to involve upregulating host defense. A decapeptide representing the N-terminal portion of LfcinB upregulates the *Candida* cell-killing activity of PMN leukocytes, a cell type that plays an important role in the inhibition of *Candida* growth and invasion [43]. Exposure of PMNs to this derivative of LfcinB induces generation of the reactive oxygen species superoxide through upregulation of protein-Tyr kinase activity and activation of the NADPH oxidase complex [42]. Both the phagocytic and candidacidal activities of these cells are correlated with the creation of reactive oxygen species such as superoxide. This peptide also increases signaling from both mitogen-activated protein kinase (MAPK) and protein kinase C cascades, upregulates inducible nitric oxide synthase (iNOS) ex-

pression and the production of NO, and increases the generation of LF and defensins from PMNs [42, 44].
Though Lfcin is parasiticidal against a range of protozoans (table 1), not much is known about how Lfcin exerts this effect [45-47]. The cell surface of *Toxoplasma gondii* tachyzoites is negatively charged, and there is evidence that Lfcin peptides are attracted to this surface, resulting in the disruption of the membrane. It is also possible that this interaction releases parasitic structural components that subsequently activate host defense systems.

Antiviral activity

Unlike the antimicrobial effects of Lfcin, the relative antiviral activity of this peptide is less than that of the mature protein. Though Lfcin moderately inhibits in vitro multiplication of a number of viruses (table 1), the activity of intact LF against these same viruses is as much as seven times higher, suggesting that either the size of the molecule is important or that other regions of LF contribute to the antiviral activity [48–52]. This activity of LF is attributed to the affinity of this protein for heparin sulfate (HS) and glycosaminoglycans (GAGs), carbohydrates that are the typical viral binding sites on the cell membrane [48]. By binding these molecules, LF prevents viral entry into the cell. As the binding site for both of these carbohydrates is located in Lfcin, it is possible that this peptide also interacts with HS or GAGs, thereby blocking viral cellular entry [52,53]. However, there is evidence that LF and Lfcin act via different mechanisms [54]. Perhaps Lfcin directly inactivates the virus particles, or as the antiviral activity is independent of the presence of this peptide at the cell surface, it is possible that Lfcin exerts its effects inside the host cell. As it has been shown that LF can bind nuclear DNA and act as a transcription factor [55], perhaps Lfcin, which contains the DNA binding region of LF [56], acts in the same manner and upregulates host cell defense in response to viral attack.
As noted for the antibacterial activity of Lfcin, both a high net positive charge and the position of the cationic residues appear to be important for antiviral activity [54]. A stabilized secondary structure is also important for antiviral activity, as the absence of the disulfide bond in both LfcinB and LfcinH and the resultant loss of the cyclic form cause a loss of antiviral activity. In addition the type of secondary structure may be important for the antiviral activity of this peptide. As recent studies have indicated that the antiviral activity of β-sheet cationic antimicrobial peptides is higher than their α-helical compatriots [57] the increased potency of LfcinB compared with LfcinH against some viruses [54] may be due to the β-sheet conformation of LfcinB in solution compared with the helical structures of LfcinH. Structure-activity relationship studies indicate that features such as hydrophobicity, molecular size and spatial positioning between the charged and hydrophobic amino acids in the secondary structure also appear to be important for the antiviral activity [54].

Antitumor activity

Lfcin, like LF, has been shown to have antitumor effects against a number of cell lines including leukemic [58, 59], fibrosarcomas, melanomas and coloncarcinomas [60], at a concentration that does not affect normal fibroblasts and erythrocytes. Lfcin is targeted to tumor cells by the changes that occur in their cell membrane, such as the exposure of phosphatidylserine, a negatively charged headgroup, due to the loss of phospholipid asymmetry in diseased cells [61]. Once bound to the tumor cell, Lfcin is thought to disrupt the cell membrane and trigger a Ca^{2+}/Mg^{2+} endonuclease and oxidant-dependent apoptotic pathway. The exact mechanism through which Lfcin triggers apoptosis is not known, but interestingly cationic liposomes, compounds with similar properties to these peptides, also induce apoptosis through an oxidant-dependent pathway [62].
Interestingly, the structural parameters that describe the antitumor effects of Lfcin are very similar to those that describe the antibacterial activity. The lack of antitumor activity of the Glu-containing LfcinM homolog (table 2) indicates that this activity of Lfcin, like the antibacterial activity, requires a high net positive charge [63]. However, for maximum antitumor activity a net positive charge of +7 is needed, a much higher charge compared with the +4 required for antibacterial activity. Again, it appears that an amphipathic structure is required, as peptide derivatives with clear cationic and lipophilic sectors have increased activity [64]. As was the case for bacterial membranes, the cationic residues would target the peptide to the tumor cell with the hydrophobic residues subsequently inserting into the membrane, thereby stably anchoring the peptide in the membrane interface. The importance of these hydrophobic residues is again highlighted by the increased activity of LfcinB derivatives containing large and rigid Trp analogs, in this case Tbt [β-(2,5,7-Tri-*tert*-butyl-indole-3-yl)alanine], or an N-terminus modified by an acyl group or another bulky moiety [32]. In addition there appears to be a requirement for a stabilized secondary structure, for the antimicrobial activity, as the cyclic peptide is more active than the linear [60]. Interestingly, this is not a property required for the antibacterial effects but it is also required for the antiviral activity, perhaps indicating a constraint for the peptide to traverse mammalian membranes. Since the same structural parameters of lipophilicity, volume, placement of cationic residues, bulkiness and hydrophobicity are identified by QSAR analysis of Lfcin derivatives as important, the future development of rationally designed potent Lfcin-based antitumor peptides seems highly possible [64].

Immunomodulatory role

Another possible role of Lfcin is as an immunomodulatory agent. Several molecules that are released from bacteria when they divide or are killed, including endotoxin (LPS) and unmethylated CpG-containing oligonucleotides, trigger the immune system and lead to an inflammatory response [65]. Lfcin appears to have an anti-inflammatory effect, as both LfcinB and LfcinH have an affinity for endotoxin [8,14] and LfcinB binds unmethylated CpG-containing oligonucleotides [66]. By binding and neutralizing these molecules, Lfcin prevents both activation of mononuclear cells and the resulting secretion of cytokines such as interleukins and tumor necrosis factor alpha, which can lead to inflammation and possibly to septic shock and death [67]. Recently it was shown that an acylated peptide encompassing the N-terminal 11 amino acids of LfcinH also has a potent endotoxin-neutralizing activity [68]. There is also evidence that LfcinB has the ability to further inhibit the inflammatory response by suppressing the effects of already released cytokines such as interleukin-6 [69].

Lfcin also appears to inhibit the inflammatory response through the classical complement pathway [70]. Both LfcinB and LfcinH, like their parent proteins, inhibit this pathway that leads to the creation of pores in the plasma membrane of invading microorganisms [71, 72]. Studies have shown that LF inhibits the classical complement pathway through inhibiting the formation of C3 convertase [73] and Lfcin might act in the same way. Interestingly, mammalian α-defensins can also act directly on this pathway [74].

Finally, Lfcin may act as an immunomodulatory agent through its DNA binding ability. Once in the cytoplasm this peptide may traverse the nuclear membrane and act as a transcription factor activating genes involved in the host defense of infected cells, a mechanism proposed for antiviral activity, or genes involved in the immunological activities of cells such as neutrophils. Of course, this peptide could indirectly influence gene expression by leading to the activation of a transcription factor.

Synergistic effects

Though there are no reported effects with LfcinH, LfcinB has been shown to be an effective synergistic agent when used in combination with antibiotics and antifungal agents. It is thought that this synergism is due to the membrane-disorganizing nature of this peptide, which leads not only to increased permeability through the bacterial cell wall [75] but also to dissipation of the proton-motive force, resulting in decreased activity of ATP-dependent multi-drug efflux pumps [40, 76]. The synergistic nature of Lfcin also extends to a common antiviral drug. By inhibiting the cellular entry of HSV, Lfcin helps to increase the antiviral activity of acyclovir (ACV), a nucleoside analog used for inhibition of virus replication [77]. The ability of LfcinB to increase the effectiveness of common antimicrobial and antiviral agents not only provides a means for treating resistant strains but also suggests a method for decreasing the rapidly growing occurrence of drug resistance by allowing lower amounts of the drugs to be used.

Conclusions

There is little importance in the work done to date on this peptide if it has no application *in vivo*. Fortunately, there is evidence that Lfcin is produced from LF both in the gastrointestinal tract by gastric pepsin [78] as well as at the site of infection by either bacterial or mammalian proteases [3,79]. The release of the peptide would help to curb bacterial populations at both of these locations. Moreover, there is evidence that oral administration of Lfcin peptides not only reduces and prevents both bacterial and parasite infections [80,81] but also inhibits tumor metastasis, suppresses tumor-induced angiogenesis and significantly reduces solid tumor size in mice [58, 60].

At the time of writing much is known about the in vitro effects of this peptide, but more work needs to be done to analyze the *in vivo* effects. Both genomic and proteomic analysis of not only bacterial, fungal and parasitic cells but also virally infected, cancerous and immunological human cells treated with this peptide would help provide insights into the broad effects of this multifunctional peptide.

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1 Caccavo D., Pellegrino N. M., Altamura M., Rigon A., Amati L., Amoroso A. et al. (2002) Antimicrobial and immunoregulatory functions of lactoferrin and its potential therapeutic application. J. Endotoxin Res. **8:** 403–417

2 Baker H. M., Anderson B. F., Kidd R. D., Shewry S. C. and Baker E. N. (2000) Lactoferrin three-dimensional structure: a framework for interpreting function. In: Lactoferrin: Structure, Function and Applications, pp. 3–15, Shimazaki, K. Tsuda H., Tomita M., Kunata T. and Perraudin J. P. (eds.) Elsevier Science, New York

3 Bellamy W., Takase M., Yamauchi K., Wakabayashi H., Kawase K. and Tomita, M. (1992) Identification of the bactericidal domain of lactoferrin. Biochim. Biophys. Acta **1121:** 130-136

4 Hunter H. N., Demcoe A. R., Jenssen H., Gutteberg T. J. and Vogel H. J. (2005) Human lactoferricin is partially folded in aqueous solution and is better stabilized in membrane mimetic solvent. Antimicrob. Agents Chemother **49:** 3387–3895

5 Hwang P. M., Zhou N., Shan X., Arrowsmith C. H. and Vogel H. J. (1998) Three-dimensional solution structure of lactoferricin B, an antimicrobial peptide derived from bovine lactoferrin. Biochemistry **37:** 4288–4298

6 Zhou N., Tieleman D. P. and Vogel H. J. (2004) Molecular dynamics simulations of bovine lactoferricin: turning a helix into a sheet. Biometals **17:** 217–223
7 Epand R. M. and Vogel H. J. (1999) Diversity of antimicrobial peptides and their mechanisms of action. Biochim. Biophy. Acta **1462:** 11–28
8 Yamauchi K., Tomita, M., Giehl T. J., and Ellison R. T. (1993) Antibacterial activity of lactoferrin and a pepsin-derived lactoferrin peptide fragment. Infect. Immun. **61:** 719–728
9 Dionysius D. A. and Milne J. M. (1997) Antibacterial peptides of bovine lactoferrin: purification and characterization. J. Diary Sci. **80:** 667–674
10 Shin K., Yamauchi K., Teraguchi S. Hayasawa H., Tomita M., Otsuka Y. et al. (1998) Antibacterial activity of bovine lactoferrin and its peptides against enterohaemorrhagic *Escherichia coli* O157:H7. Lett. Appl. Microbiol. **26:** 407–411
11 Bellamy W., Takase M., Wakabayashi H., Kawase K. and Tomita M. (1992) Antibacterial spectrum of lactoferricin B, a potent bactericidal peptide derived from the N-terminal region of bovine lactoferrin. J. Appl. Bacteriol. **73:** 472–479
12 Wakabayashi H., Bellamy W., Takase M. and Tomita M. (1992) Inactivation of *Listeria monocytogenes* by lactoferricin, a potent antimicrobial peptide derived from cow's milk. J. Food Prot. **55:** 238–240
13 Wakabayashi H., Matsumoto H., Hashimoto K., Teraguchi S., Takase M. and Hayasawa H. (1999) N-acylated and D-enantiomer derivatives of a nonamer core peptide of lactoferricin B showing improved antimicrobial activity. Antimicrob. Agents Chemother. **43:** 1267–1269
14 Chapple D. S., Mason D. J., Joannou C. L., Odell E. W., Gant V. and Evans R. W. (1998) Structure-function relationship of antibacterial synthetic peptides homologous to a helical surface region on human lactoferrin against *Escherichia coli* serotype O111. Infect. Immun. **66:** 2434–2440
15 Vorland L. H., Ulvatne H., Rekdal Ø. and Svendsen J. S. (1999) Initial binding site of antimicrobial peptides in *Staphylococcus aureus* and *Escherichia coli*. Scand. J. Infect. Dis. **31:** 467–473
16 Hancock R. E. W. and Chapple D. S. (1999) Antibiotic peptides. Antimicrob. Agents Chemother. **43:** 215–220
17 Chapple D. S., Hussain R., Joannou C. L., Hancock R. E. W., Odell E., Evans R. W. et al. (2004) Structure and association of human lactoferrin peptides with *Escherichia coli* lipopolysaccharide. Antimicrob. Agents Chemother. **48:** 2190–2198
18 Ulvatne H., Samuelsen Ø., Haukland H. H., Krämer M. and Vorland L. H. (2004) Lactoferricin B inhibits bacterial macromolecular synthesis in *Escherichia coli* and *Bacillus subtilis*. FEMS Microbiol. Lett. **237:** 337–384
19 Vogel H. J., Schibli D. J., Jing W., Lohmeier-Vogel E. M., Epand R. F. and Epand R. M. (2002) Towards a structure-function analysis of bovine lactoferricin and related tryptophan- and arginine-containing peptides. Biochem. Cell Biol. **80:** 49–63
20 Hoek K. S., Milne J. M., Grieve P. A., Dionysius D. A. and Smith R. (1997) Antibacterial activity of bovine lactoferrin-derived peptides. Antimicrob. Agents Chemother. **41:** 54–59
21 Aguilera O., Ostolaza H., Quiros L. M. and Fierro J. F. (1999) Permeabilizing action of an antimicrobial lactoferricin-derived peptide on bacterial and artificial membranes. FEBS Lett. **462:** 273–277
22 Haukland H. H., Ulvatne H. Sandvik K. and Vorland L. H. (2001) The antimicrobial peptides lactoferricin B and magainin 2 cross over the bacterial cytoplasmic membrane and reside in the cytoplasm. FEBS Lett. **508:** 389–393
23 Prochiantz A. (2000) Messenger proteins: homeoproteins, TAT and others. Curr. Opin. Cell Biol. **12:** 400–406
24 Zhang L., Rozek A. and Hancock R. E. W. (2001) Interaction of cationic antimicrobial peptides with model membranes. J. Biol. Chem. **276:** 35714–35722
25 Castle M., Nazarian A., Yi S. S. and Tempst P. (1999) Lethal effects of apidaecin on *Escherichia coli* involve sequential molecular interactions with diverse targets. J. Biol. Chem. **274:** 32555–32564
26 Subbalakshmi C. and Sitaram N. (1998) Mechanism of antimicrobial action of indolicidin. FEMS Microbiol. Lett. **160:** 91–96
27 Hunter H. N., Jing W., Schibli D. J., Trinh T., Park I. Y., Kim S. C. et al. (2005) The interactions of antimicrobial peptides derived from lysozyme with model membrane systems. Biochim. Biophys. Acta **1668:** 175–189
28 Strøm M. B., Rekdal Ø. and Svendsen J. S. (2002) The effects of charge and lipophilicity on the antibacterial activity of undecapeptides derived from bovine lactoferricin. J. Pept. Sci. **7:** 36–43
29 Vorland L. H., Ulvatne H., Andersen J., Haukland H. H., Rekdal Ø., Svendsen J. S. et al. (1998) Lactoferricin of bovine origin is more active than lactoferricins of human, murine and caprine origin. Scand. J. Infect. Dis. **30:** 513–517
30 Kang J. H., Lee M. K., Kim K. L. and Hahm K. (1996) Structure-biological activity relationships of 11-residue highly basic peptide segment of bovine lactoferrin. Int. J. Pept. Res. **48:** 357–363
31 Strøm M. B., Haug B. E., Rekdal Ø., Skar M. L., Stense W. et al. (2002) Important structural features of 15-residue lactoferrin derivatives and methods for improvement of antimicrobial activity. Biochem. Cell Biol. **80:** 65–74
32 Eliassen L. T., Haug B. E., Berge G. and Rekdal Ø. (2003) Enhanced antitumor activity of 15-residue bovine lactoferricin derivatives containing bulky aromatic amino acids and lipophilic C-terminal modifications. J. Pept. Sci. **9:** 510–517
33 Haug B. E., Skar M. L. and Svendsen J. S. (2001) Bulky aromatic amino acids increase the antibacterial activity of 15-residue bovine lactoferricin derivatives. J. Pept. Sci. **7:** 425–432
34 Schibli D. J., Hwang P. M. and Vogel H. J. (1999) The structure of the antimicrobial active center of lactoferricin B bound to sodium dodecyl sulphate micelles. FEBS Lett. **446**: 213–217
35 Nguyen L. T., Schibli D. J. and Vogel H. J. (2005) Structural studies and model membrane interactions of two peptides derived from bovine lactoferricin. J. Pept. Sci. **11:** 379–389
36 Strøm M. B., Rekdal Ø., Stensen W. and Svendsen J. S. (2001) Increased antibacterial activity of 15-residue murine lactoferricin derivatives. J. Pept. Res. **57:** 127–139
37 Lejon T., Strøm M. B. and Svendsen J. S. (2001) Antibiotic activity of pentadecapeptides modeled from amino acid descriptors. J. Pept. Sci. **7:** 74–81
38 Lejon T., Stiberg T., Strøm M. B. and Svendsen J. S. (2004) Prediction of antibiotic activity and synthesis of new pentadecapeptides based on lactoferricins. J. Pept. Sci. **10:** 329–335
39 Bellamy W., Yamauchi K., Wakabayashi H., Takase M., Takakura N., Simamura S. et al. (1994) Antifungal properties of lactoferricin B, a peptide derived from the N-terminal region of bovine lactoferrin. Lett. Appl. Microbiol. **18:** 230–233
40 Wakabayashi H., Abe S., Teraguchi S., Hayasawa H. and Yamaguchi H. (1998) Inhibition of hyphal growth of azole-resistant strains of *Candida albicans* by triazole antifungal agents in the presence of lactoferrin-related compounds. Antimicrob. Agents Chemother. **42:** 1587–1591
41 Lupetti A., Paulusma-Annema A., Welling M. W., Senesi S., van Dissel J. T. and Nibbering P. (2000) Candidacidal activities of human lactoferrin peptides derived from the N-terminus. Antimicrob. Agents. Chemother. **44:** 3257–3263
42 Ueta E., Tanida T. and Osaki T. (2001) A novel bovine lactoferrin peptide, FKCRRWQWRM, suppresses *Candida* cell growth and activates neutrophils. J. Pept. Res. **57:** 240–249
43 Kullberg B. J., Netea M. G., Vonk A. G. and van der Meer J. W. (1999) Modulation of neutrophil functions in host defense against disseminated *Candida albicans* infection in mice. FEMS Immunol. Med. Microbiol. **26:** 299–307
44 Tanida T., Rao, F., Hamada T., Ueta E. and Osaki T. (2001) Lactoferrin peptide increases the survival of *Candida albicans*-inoculated mice by upregulating neutrophil and macrophage functions, especially in combination with amphotericin B and granulocyte-macrophage colony-stimulating factor. Infect. Immun. **69:** 3883–3890

45 Tanaka T., Omata Y., Saito A., Shimazaki K., Yamauchi K., Takase M. et al. (1995) *Toxoplasma gondii*: parasiticidal effects of bovine lactoferricin against parasites. Exp. Parasitol. **81:** 614–617

46 Omata Y., Satake M., Maeda R., Saito A., Shimazaki K., Yamauchi K. et al. (2001) Reduction of the infectivity of *Toxoplasma gondii* and *Eimeria stiedai* sporozoite by treatment with bovine lactoferricin. J. Vet. Med. Sci. **63:** 187–190

47 Turchany J. M., Aley S.B. and Gillin F. D. (1995) Giardicidal activity of lactoferrin and N-terminal peptides. Infect. Immun. **63:** 4550–4552

48 Andersen J. H., Osbakk S. A., Vorland L. H. Traavik T. and Gutteberg T. J. (2001) Lactoferrin and cyclic lactoferricin inhibit the entry of human cytomegalovirus into human fibroblasts. Antiviral Res. **51:** 141–149

49 Berkhout B., van Wamel J. L. B., Beljaars L., Meijer D. K. F., Visser S. and Floris R. (2002) Characterization of the anti-HIV effects of native lactoferrin and other milk proteins and protein-derived peptides. Antiviral Res. **55:** 341–355

50 McCann K. B., Lee A., Wan J., Roginski H. and Coventry M. J. (2003) The effect of bovine lactoferrin and lactoferricin B on the ability of feline calicivirus (a norovirus surrogate) and poliovirus to infect cell cultures. J. Appl. Microbiol. **95:** 1026–1033

51 Siciliano R., Rega B., Marchetti M., Seganti L., Antonini G. and Valenti P. (1999) Bovine lactoferrin peptidic fragments involved in inhibition of herpes simplex virus type 1 infection. Biochem. Biophys. Res. Commun. **264:** 19–23

52 Di Biase A. M., Pietrantoni A., Tinari A., Siciliano R., Valenti P., Antonini G. et al. (2003) Heparin-interacting sites of bovine lactoferrin are involved in anti-adenovirus activity. J. Med. Virol. **69:** 495–502

53 Andersen J. H., Jenssen H., Sandvik K. and Gutteberg T. J. (2004) Anti-HSV activity of lactoferrin and lactoferricin is dependent on the presence of heparin sulphate at the cell surface. J. Med. Virol. **74:** 262–271

54 Jenssen H., Andersen J. H., Uhlin-Hansen L., Gutteberg T. J. and Rekdal Ø. (2004) Anti-HSV activity of lactoferricin analogues is only partly related to their affinity for heparan sulfate. Antiviral Res. **61:** 101–109

55 He J. and Furmanski P. (1995) Sequence specificity and transcriptional activation in the binding of lactoferrin to DNA. Nature **373:** 721–724

56 van Berkel P. H. C., Geerts M. E. J., van Veen H. A., Mericskay M., deBoer H. A. and Nuijens J. H. (1997) N-terminal stretch Arg^2, Arg^3, Arg^4 and Arg^5 of human lactoferrin is essential for binding to heparin, bacterial lipopolysaccharide, human lysozyme and DNA. Biochem. J. **328:** 145–151

57 Yasin B., Pang M., Turner J. S., Cho Y., Dinh N. N., Waring A. J. et al. (2000) Evaluation of the inactivation of infectious herpes simplex virus of host-defense peptides. Eur. J. Clin. Microbiol. Infect. Dis. **19:** 187–194

58 Yoo Y, Watanabe S., Watanabe R., Hata K, Shimazaki K. and Azuma I. (1997) Bovine lactoferrin and lactoferricin, a peptide derived from bovine lactoferrin, inhibit tumor metastasis in mice. Jpn. J. Cancer Res. **88:** 184–190

59 Yoo Y. Watanabe R., Koike Y., Mitobe M., Shimazaki K, Watanabe S. et al. (1997) Apoptosis in human leukemic cells induced by lactoferricin, a bovine milk protein-derived peptide: involvement of reactive oxygen species. Biochem. Biophys. Res. Commun. **237:** 624–628

60 Eliassen L. T., Berge G., Sveinbjørnsson B., Svendsen J. S., Vorland L. H. and Rekdal Ø. (2002) Evidence for a direct antitumor mechanism of action of bovine lactoferricin. Anticancer Res. **22:** 2703–2710

61 Fadok V. A., de Cathelineau A., Daleke D. L., Henson P. M. and Bratton D. L. (2001) Loss of phospholipid asymmetry and surface exposure of phosphatidylserine is required for phagocytosis of apoptotic cells by macrophages and fibroblasts. J. Biol. Chem. **276:** 1071–1077

62 Aramaki Y. Takano S., Arima H. and Tsuchiya S. (2000) Induction of apoptosis in WEHI 231 cells by cationic liposomes. Pharm. Res. **17:** 515–520

63 Yang N., Strøm M. B., Mekonnen S. M., Svendsen J. S. and Redal Ø. (2004) The effects of shortening lactoferrin derived peptides against tumour cells, bacteria and normal human cells. J. Pept. Sci. **10:** 37–46

64 Yang N., Lejon T. and Redal Ø. (2003) Antitumour activity and specificity as a function of substitutions in the lipophilic sector of helical lactoferrin-derived peptide. J. Pept. Sci. **9:** 300–311

65 Kreig A. M., Yi A. K., Matson S., Waldschmidt T. J., Bishop G. A., Teasdale R. et al. (1995) CpG motifs in bacterial DNA trigger direct B-cell activation. Nature **374:** 546–549

66 Britigan B. E., Lewis T. S., Waldshcmidt M., McCormick M. L. and Krieg A. M. (2001) Lactoferrin binds CpG-containing oligonucleotides and inhibits their immunostimulatory effects on human B cells. J. Immunol. **167:** 2921–2928

67 Beutler B. and Cerami A. (1988) Tumor necrosis, cachexia, shock and inflammation: a common mediator. Annu. Rev. Biochem. **57:** 505–518

68 Andrä J., Lohner K., Blondelle S. E., Jerala R., Moriyon I., Koch M. H. J. et al. (2005) Enhancement of endotoxin neutralization by coupling of a C12-alkyl chain to a lactoferricin-derived peptide. Biochem. J. **385:** 135–143

69 Mattsby-Baltzer I., Roseanu A., Motas C., Elverfors J., Engberg I. and Hanson L. Å. (1996) Lactoferrin or a fragment thereof inhibits the endotoxin-induced interleukin-6 response in human monocytic cells. Pediatr. Res. **40:** 257–262

70 Samuelsen Ø., Haukland H. H., Ulvatne H. and Vorland L. H. (2004) Anti-complement effects of lactoferrin-derived peptides. FEMS Immunol. Med. Microbiol. **41:** 141–148

71 Walport M. J. (2001) Complement. First of two parts. N. Engl. J. Med. **344:** 1058–1066

72 Walport M. J. (2001) Complement. Second of two parts. N. Engl. J. Med. **344:** 1140–1144

73 Kijlstra A. and Jeurissen S. H. (1982) Modulation of classical C3 convertase of complement by tear lactoferrin. Immunology **47:** 263–270

74 van den Berg R. H., Faber-Krol M. C., van Wetering S., Hiemstra P. S. and Daha M. R. (1998) Inhibition of activation of the classical pathway of complement by human neutrophil defensins. Blood **92:** 3898–3903

75 Vorland L. H., Osbakk S. A., Perstølen T., Ulvatne H., Rekdal Ø., Svendsen J. S. et al. (1999) Interference of the antimicrobial peptide lactoferricin B with the action of various antibiotics against *Escherichia coli* and *Staphylococcus aureus*. Scand. J. Infect. Dis. **31:** 173–177

76 Wakabayahsi H., Teraguchi S. and Tamura Y. (2002) Increased *Staphylococcus*-killing activity of an antimicrobial peptide, lactoferricin B, with minocylcin and monoacylglycerol. Biosci. Biotechnol. Biochem. **66:** 2161–2167

77 Andersen J. H., Jenssen H. and Gutteberg T. J. (2003) Lactoferrin and lactoferricin inhibit Herpes simplex, 1 and 2 infection and exhibit synergy when combined with acyclovir. Antiviral Res. **58:** 209–215

78 Kuwata H., Yip T., Tomita M. and Hutchens T. W. (1998) Direct evidence of the generation in human stomach of an antimicrobial peptide domain (lactoferricin) from ingested lactoferrin. Biochim. Biophys. Acta **1429:** 129–141

79 Britigan B. E., Hayek M. B., Doebbeling B. N. and Fick R. B. (1993) Transferrin and lactoferrin undergo proteolytic cleavage in the *Pseudomonas aeruginosa*- infected lungs of patients with cystic fibrosis. Infect. Immun. **61:** 5049–5055

80 Haversen L. A., Engberg I., Baltzer L., Dolphin G., Hanson L. A. and Mattsby-Baltzer I. (2000) Human lactoferrin and peptides derived from a surface-exposed helical region reduce experimental *Escherichia coli* urinary tract infections in mice. Infect. Immun. **68:** 5816–5823

81 Isamida T., Tanaka T., Omata Y., Yamauchi K., Shimazaki K. and Saito A. (1998) Protective effect of lactoferricin against *Toxoplasma gondii* infection in mice. J. Vet. Med. Sci. **60:** 241–244