MECHANISMS OF THE ANTIBACTERIAL ACTIVITY OF LACTOFERRIN AND LACTOFERRIN-DERIVED PEPTIDES

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Lactoferrin (Lf), a member of the transferrin family of iron-binding proteins, is now known to have a number of properties, including antibacterial activity towards a broad spectrum of Gram-negative and Gram-positive bacteria. The mechanism of the antibacterial activity of Lf is complex and involves beside iron-chelation, direct action on bacteria and/or the activation of the immune system. Lactoferricin (Lfcin) and other peptides derived from Lf or Lfcin are more potent antibacterial agents, a property exhibited by interaction with and penetration of bacterial membrane. This article summarizes the current data on the mechanisms through which Lf and Lf-derived peptides exhibit their antibacterial activity.

Key words: lactoferrin, antibacterial, lactoferricin, lactoferrampin.

INTRODUCTION

Lactoferrin (Lf) is an iron-binding multifunctional glycoprotein present in most biological secretions and in neutrophils. One of the earliest and very well documented functions of Lf is the antibacterial effect against a broad spectrum of Gram-negative and Gram-positive bacteria, such as *Streptococcus* spp., *Enterococcus* spp., *Staphylococcus* spp., *E. coli* spp., *Haemophilus influenzae* etc (1–4). However, Lf can also promote the growth of bacterial species like *Lactobacillus and Bifidobacteria* (5). Based on the *in vitro* and *in vivo* studies it becomes clear that Lf exerts the antibacterial effects through different mechanisms, concisely described in this review.

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IRON-CHELATION

Iron binding was initially considered to be the major mechanism responsible for the bacteriostatic activity of Lf. Its high affinity for iron, together with its presence in an iron-free form in body secretions, allows Lf to produce an iron-deficient environment that limits bacterial growth (6–8). The effect was reported only for apo-Lf and is reduced or completely restored upon iron-saturation. Moreover, most pathogenic bacteria have developed strategies to overcome iron-depletion induced by Lf. One mechanism involves the synthesis and secretion by bacteria of iron-chelators, siderophores, with a higher affinity for iron than Lf. The siderophore-iron complex is then taken up into bacteria by specific receptors for siderophores (FhuA, FepA, and FecA) (9). Another system is the direct acquisition of iron from host transferrin and Lf following binding to specific transferrin and Lf bacterial receptors. Lf receptors are expressed on the surface of highly host-adapted bacterial species, such as *S. penumoniae, H. pylori, H. influenzae, N. meningitis* and *N. gonorrheae*. The Lf receptor comprises an outer-membrane protein, LbpA, and a surface lipoprotein, LbpB. LbpA mediates bacterial growth by using Lf as a source of iron while LbpB has a facilitating role (10). As a mechanism, it has been proposed that the interaction of Lf with LpbA induces conformational changes in the C-lobe of protein which result in iron release. Since the Lf receptor seems to be essential for bacterial survival, it has been considered as a target for vaccine production (11).

Lf has a bacteriostatic action on *P. gingivalis* by binding to the haem receptor protein (HbR), removing it from the cell surface and thus disturbing the iron uptake from hemoglobin (12).

INTERACTION WITH BACTERIA

Iron deprivation induced by Lf may only delay bacterial growth and further studies have demonstrated that Lf exhibits bactericidal activity distinct from its iron-withholding capacity. The molecular mechanism is similar for Gram-negative and Gram-positive bacteria and involves a direct interaction with the bacterial cell membrane (13).

The cationic molecule Lf interacts through its positively charged cluster in the N-terminal region of N-lobe with the anionic part (lipid A) of lipopoly-saccharide (LPS), a component of the outer membrane of Gram-negative bacteria. This interaction damages the bacterial membrane, alters its permeability and results in LPS release. Interestingly, it has been suggested that by the ability to bind Ca^{2+}, Lf could influence the release of LPS by either displacing or chelating the divalent cations from LPS (14). A direct interaction of Lf with the negatively charged region of OmpC and PhoE porins on *E. coli* surface was shown by Sallman *et al.*

(15). In the case of Gram-positive bacteria, Lf acts by binding through electrostatic interactions to the negatively charged lipid matrix of the bacterial membrane.

Other effects of Lf are the modification of the motility and aggregation of bacteria, limitation of biofilm formation and inhibition of bacterial adhesion on abiotic surfaces. These properties of Lf are potentially very important for clinical applications of Lf. For example, recent clinical studies have indicated that oral administration of physiological concentrations of bovine Lf to patients with chronic periodontitis induced by periodontophathogenic bacteria such as *P. gingivalis* or *P. intermedia* inhibited biofilm formation and reduced the established biofilm of these bacteria (16). In chronic wounds, treatment with a combination of Lf and xylitol resulted in structural disruption of the biofilm and permeabilization of the bacterial membrane.

PROTEOLYTIC ACTIVITY

It has been suggested that Lf displays serine protease activity (17). As a result of this proteolytic activity, Lf reduces the pathogenic potential of *H. influenzae*, a frequent cause of respiratory tract diseases. The mechanism involves the cleavage of two colonizing factors, IgA1 protease protein and Hap adhesion, at homologous arginine-rich sequences. This activity of Lf was found to be located in the N-terminal lobe of Lf and is also used to degrade other virulence factors, such as IpaA and IpaC from *S. flexneri* and secreted proteins A, B, D from *E. coli* (18, 19).

INTERACTION WITH HOST CELLS

Lf binds to many cells and studies have demonstrated its capacity to inhibit bacterial entry into cells. Such an inhibitory effect was reported for *E. coli* and *S. aureus* and was related to the ability of Lf to bind to cell integrins and GAGs (20, 21). The binding of Lf to host cell GAGs appears to be important for the inhibition of bacterial internalization in target cells. Since Lf can be internalized and localized into the nucleus, it was suggested that the inhibition of bacterial internalization could arise from gene regulation or cytoskeleton rearrangement (3).

STIMULATION OF THE IMMUNE SYSTEM AND OTHER EFFECTS

Lf may protect against bacterial infections indirectly by stimulation of the immune response. For example, the clearance of *S. aureus* in Lf transgenic mice was the result of the upregulation of the T helper type 1 response induced by Lf (22).

Lf modulates the antibacterial activity of lysozyme and antibiotics towards Gram-negative and Gram-positive bacteria. The proposed mechanism in the case of

Gram-positive bacteria is that Lf interacts with bacterial surface lipoteichoic acid leading to a decrease in the negative charge in the membrane (23, 24).

A synergy between Lf and bacteriophages, also known as antibacterial agents, has been demonstrated in an *in vivo* model of mice infected with *E. coli* or *S. aureus* (25).

Although a multitude of studies indicate that Lf plays a critical role in the protection against bacterial infection, in some cases Lf can be rendered inactive by pathogens or can enhance their effect. Thus, the binding of Lf to pneumococcal protein A, present on the surface of *S. pneumoniae,* impaired the killing effect of protein. VvpE metalloproteinase, a key player for surface adhesion and colonization of *Vibrio vulnificus,* is able to inactivate two important components of mucosal immunity, IgA and Lf (27).

ANTIBACTERIAL ACTIVITY OF LACTOFERRICIN AND OTHER PEPTIDES

Human Lfcin (HLfcin, amino acid residues 1–47) and bovine Lfcin (BLfcin, amino acid residues 17–41), both derived from the N-terminal region of the N-lobe of Lf, have greater antibacterial activity than the native proteins. There is substantial evidence for *in vitro* antibacterial activity of the peptides – bactericidal in the case of B Lfcin and bacteriostatic in the case of HLfcin – against a wide variety of Gram-negative and Gram-positive bacteria (28).

Lfcin displays antibacterial activity due to its membrane-disruptive properties resulting in blisters on the outer membrane of Gram-negative bacteria. The 28–34 loop region in HLfcin and its homologous region in BLfcin were found to interact with LPS (29). The results of studies performed with Lfcin and synthetic peptides demonstrated that hydrophobic interactions and basic residues, especially involving tryptophan and arginine residues, are the major determinants of antibacterial activity. The mode of action proposed by computer modelling involves the interaction of positively charged residues of Lfcin with negative charges present in the inner core of LPS. These interactions disorganize the structure of the outer membrane and facilitate the approach of tryptophan residues to lipid A and promote hydrophobic interactions (1, 28).

Lfcin is produced in the gastrointestinal tract by gastric pepsin and at the site of inflammation by the action of proteases (30). This fact is important for its clinical applications, for inhibition of bacterial infections at both locations. It has been shown that oral administration of Lfcin peptides reduced experimental *E. coli* urinary tract infections in mice (31).

The N1-domain of Lf contains, beside Lfcin, a second antibacterial stretch corresponding to residues 268–284 of Lf and located in close proximity to Lfcin within its 3D structure. This novel peptide, named lactoferrampin (LFampin), was

found to be active against *B. subtilis, E. coli, and P. aeruginosa*, but ineffective against *P. gingivalis, S. mutans* and *S. sanguis.* As with Lfcin, LFampin exerts its bactericidal activity after cell membrane penetration and damage (32).

BLfcin (residues 17–30) and LFampin (residues 265–284) were linked into a chimerical construct called LFchimera, containing both peptides and was found to have a stronger bactericidal action than the two separate components. Experiments carried out using confocal and electron microscopy revealed that interaction and membrane disruption are probably the mechanism of the bactericidal effect on the growth of antibiotic-resistant strains of *S. aureus* and *E. coli* (33).

CONCLUSION

The mechanism of the antibacterial activity of Lf is complex and several lines of evidence indicate that beside iron-chelating it involves a direct action on bacteria and/or the activation of the immune system. Lfcin and other peptides derived from Lf or Lfcin are more potent antibacterial agents, a property exhibited by interaction and penetration of bacterial membrane.

All these observations indicate that Lf and Lf-derived peptides are compounds able to protect the host from harmful bacterial infections.

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