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Antibacterial Activity of Lactoferrin: A Review



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ABSTRACT

Milk is a highest quality source of well balanced nutrients and also displays a range of biological activities that affects digestion, metabolic responses to absorbed nutrients, growth & development of specific organs, and resistance to disease. Bioactive proteins such as lactoferrin (Lf) have been isolated over decades ago and showed their importance in stimulating immune system in the infants through breast milk in addition to immunoglobulin present in the milk. In addition to immune system stimulation, Lf also has antibacterial activity and antioxidant activity in infant and adult of human as well as animal health. In this review paper, antibacterial properties of lactoferrin have been discussed along with its future perspectives.



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1. INTRODUCTION

Milk, the only complete food or nutritious product, provides all the necessary nutrients to all the mammals including human from neonate age to adults due to its diverse content of nutritional compounds such as fats, carbohydrates, proteins, peptides, vitamins, growth factors, etc. Apart from nutritional compounds of milk, bioactive compounds are present in the milk in minor amounts as compared to other nutritional compounds. Research is in progress to extract these bioactive compounds on a large scale at minimum cost globally.

Recent advances in research showed that neonates are protected from various microbial infections and cancer due to presence of such bioactive compounds in the colostrum as well as the milk. As compared to the mature milk, colostrum contains higher amounts of bioactive compounds. These bioactive compounds possess multifunctional activities such as antimicrobial, anti-inflammatory, antioxidative, anticytotoxic, anticancer, immunomodulatory and mineral carrying activities. Bioactive compounds are generally in latent state and are released upon the proteolysis of these compounds either by certain microbial enzymes released from the lactic acid bacteria which are present in the milk or during gastrointestinal or food processing (Gobbetti et. al., 2002).

Among these bioactive compounds, lactoferrin and immunoglobulin G are two important bioactive compounds in research interest which contribute to preservation of milk itself as they possess various microbial infections and cancer fighting properties. Lactoferrin is non heme iron binding glycoprotein with molecular weight 78 – 80 kDa that contains around 690 - 702 amino acids residues. Lactoferrin was first isolated from bovine milk by Sovensson and Sovensson in 1939. In 1960, lactoferrin was isolated from human milk by Johansson in 1960. Lactoferrin is the member of transferrin family (Metz et. al., 1984). Lactoferrin is present in mammalian secretions such as milk, tears, saliva, seminal fluids, vaginal fluids, nasal mucosa, bronchial mucosa as well as in some white blood cells (Birgens, 1985; Iigo et. al., 2009). Lactoferrin is synthesized by glandular epithelial cells (Baynes and Bezwoda, 1994) and by specific granules of polymorphonuclear leukocytes (Lonnerdal et. al., 1995). Rachman et. al. (2015) showed that lactoferrin concentration varies with lactation days i.e. on the 1st day of lactation it was observed that lactoferrin was more than the following lactation days. Thus it can be seen that lactation period, age and other maternal characteristics plays important role in the lactoferrin

concentration. Lactoferrin concentration varies with breeds too. Table 1.1: Reported lactoferrin levels in various human milk forms.

Sr. No.	Milk type	Concentration (mg/mL)
01.	Colostrum	5 – 7
02.	Preterm Colostrum	6.76 ± 1.50
03.	Fullterm Colostrum	6.7 ± 0.7
04.	Transient Milk	3.7 ± 0.1
05.	Mature Milk	1.97 – 3.20

Adapted from Levay et. al. 1995.

The alteration of activity of lactoferrin in milk could have an impact on the shelf life of raw milk and also on the development of neonates (Campanella et. al., 2009). Presence of glycan molecule in the structure of lactoferrin prevents degradation of itself by proteolytic enzymes such as trypsin and trypsin like enzymes which facilitates partial resistance to digestion in the gut. Lactoferrin is considered to be an important host defence molecule and has a diverse range of physiological activities such as antibacterial, antiprotozoan, antifungal, antiviral, anticancer, antioxidant, anti-inflammatory and immunomodulatory (Iigo et. al., 2009; Parhi et. al., 2012).

Lactoferrin, the natural protein, is proving to be a highly promising biodrug in antibacterial therapeutic researches. The use of chemotherapeutic drugs has given rise to drug resistant bacterial infections which can be overcome by the use of lactoferrin powder or tablets as supplementary in addition to chemotherapeutic drugs at optimal concentrations. In this paper, mechanisms of antibacterial property of lactoferrin have been discussed. Advances in the research in the use of lactoferrin as antibacterial agent *in vitro* and *in vivo* has been summarized.

2. Antibacterial Effect

2.1. Bacteriostatic Activity related to iron

Bacteria utilizes iron for its growth, thus sequestering iron by lactoferrin can cause bacterial growth stasis. Lactoferrin in milk is found in its free iron free apoform (Makino and Nishimura, 1992) but has the ability to bind iron, as Fe^{3+} binds firmly. In fact, lactoferrin binds two Fe^{3+} ions

per molecule, with an affinity and stability much higher than that of transferrin, the iron transport protein in serum (Ward et. al., 1996; Baker and Baker, 2004). Several studies conducted by various researchers during the decades have shown that iron sequestering ability of lactoferrin have facilitated the inhibition of bacterial growth *in vivo* and *in vitro*. In this mechanism, bacterial growth is only delayed by iron deprivation and can be completely restored after iron supplementation. In addition, most pathogenic bacteria can overcome lactoferrin generated iron deprivation, acquiring iron by means of either secreting small iron chelators (siderophores) or acquiring iron directly from host transferrin and lactoferrin (Valenti and Antoni, 2005). Further, antibacterial activity shows in Bovine milk lactoferrin and its hydrolysates prepared with pepsin, chymosin and microbial rennet against foodborne pathogen *Listeria monocytogenes* (Ripolles et.al.,2015) and another foodborne pathogen *Cronobacter sakazakii* (Harouna et.al. 2015) as well as same results were observed of Nisin and Lactoferrin hydrolysates against *Staphylococcus aureus* (Lee. et.al.,2015). Apart from its static activity, Lactoferrin supports growth of certain bacteria such as *Lactobacillus sp.* and *Bifidobacterium sp.* which are beneficial (Petschow et. al., 1999; Sherman et. al., 2004)

2.2. Bactericidal Activity not related to iron

As indicated above, iron sequestering by lactoferrin only delays bacterial growth; however, findings by Arnold et. al. (1977), reported that bactericidal activity can occur in addition to bacteriostatic activity. Ellison et. al. (1988, 1990) reported that in Gram negative bacteria, lactoferrin specifically binds to porins present on the outer membrane and induces the rapid release of lipopolysaccharides which is known to enhance bacterial susceptibility to osmotic shock, to lysozyme and to other antibacterial molecules (Gado et. al., 1991; Leitch and Willcox, 1998). In mediating LPS release, lactoferrin appears to act in two ways. First, it is a polycationic molecule, with the maximal density of surface positive charge located in the N terminal region (Baker et. al., 2002). Most of the iron independent antibacterial activity of lactoferrin is concentrated into a cluster of positively charged residues near the N terminus of the lactoferrin from many mammalian species (Tomita et. al., 1994; Vorland et. al., 1998; Ellass – Rochard et. al., 1998; Nibbering et. al., 2001). This positive cluster binds to the lipid A part of lipopolysaccharide molecules present on the outer membrane of clinically relevant bacterial species (Appelmeik et. al., 1994; Brandenburg et. al., 2001). In particular the binding takes place

to the phosphate group within the lipid A part, inducing a rigidification of the acyl chains of lipopolysaccharide (Brandenburg et. al., 2001). Rissi et. al. (2002) reported that lactoferrin can bind Ca^{2+} releasing significant amounts of lipopolysaccharide from Gram negative bacteria without the need of direct contact with bacteria. Ellass – Rochard et. al. (1998) on performing various experiments using *E. coli* O55B5 LPS found two lipopolysaccharide binding sites and reported that the residues 28 – 34 participated in high affinity lipopolysaccharide binding, in addition to the N terminal basic stretch 1 – 5 which is located in the vicinity of residues 28 – 34. Tomita et. al. (1991) reported that the lactoferricin, a peptide derived from N - terminal region of lactoferrin on gastric pepsin cleavage of lactoferrin have much more effect than lactoferrin on wide range of Gram negative and Gram positive bacteria. It binds to lipopolysaccharide in Gram negative bacteria and to teichoic acid in Gram positive bacteria (Vorland, 1999). Bactericidal activity is mediated through one or more pathways. Receptors for N - terminal region of lactoferrin have been discovered and studied on the surface of some bacteria at which binding of lactoferrin to these receptors induces cell death in Gram negative bacteria due to a disruption in the cell wall. This release of lipopolysaccharide leads to impaired permeability and a higher sensitivity to lysozyme and other antimicrobial agents (Arnold et. al., 1977 and Willcox, 1998). Valenti and Antonini (2005) reported that bactericidal activity affecting Gram positive bacteria is mediated by electrostatic interactions between negatively charged lipid bilayer and the positively charged lactoferrin surface that causes changes in the permeability of membrane. In vitro lactoferrin is able to prevent *Pseudomonas aeruginosa* biofilm formation due to lack of iron in the environment which forces bacteria to move, and hence, they cannot adhere to surfaces (Singh et. al., 2002, Moradia et.al.,2014). Lactoferrin also shows strong antibacterial effect on both Gram positive (*S. epidermidis* and Gram-negative (*C jejuni*, *Salmonella*) bacteria; however, it was more effective on Gram-positive rather than gram –negative bacteria (Jahani et.al., 2015).

Lactoferrin can contribute to defence against the invasion of facultative intracellular bacteria into cells by binding both target cell membrane glycoaminoglycans and bacterial invasions, which prevents pathogens adhesion to target cells. This ability was first reported against enteroinvasive *E. coli* HB101 and later against *Yersinia enterocolica*, *Yersinia pseudotuberculosis*, *Listeria monocytogenes*, *Streptococcus pyogenes* and *Staphylococcus aureus* (Valenti and Antonini, 2005).

2.3. Proteolytic Activity

In addition to bactericidal activity, lactoferrin inhibits the growth of some bacteria such as *Shigella flexneri* and *E. coli* through degradation of proteins necessary for colonization (Orsi, 2004; Ward et. al., 2005). Degradation of *Haemophilus influenzae* IgA1 protease was observed when *Haemophilus influenzae* was cultured in human milk as the sole source of nutrient (Plaut et. al., 1992). Qiu et. al. (1998) found that human lactoferrin causes the proteolytic degradation of both the IgA1 protease and Hap adhesin and that serine protease like activity is located in the N – lobe of lactoferrin. Lactoferrin treatment of *Shigella flexneri* 5 strain M90T impaired invasiveness by inducing release and degradation of invasion plasmid antigens B (IpaB) and C (IpaC) (Gomez et. al., 2001; 2002; 2003). Lactoferrin blocks enteropathogenic *E. coli* adherence, hemolysis and induction of actin polymerisation in Hep2 cells as a result of lactoferrin mediated degradation of *E. coli* secreted proteins A, B and D (EspABD) (Ochoa et. al., 2003; 2004).

Table 2.1: Biological Activity of Lactoferrin

Activity	Target	Mode of Action
Gram positive bacteria	<i>S. mutans</i>	Iron – independent interaction with bacterial cell surface.
	<i>S. epidermis</i>	Interaction with lipoteichoic acid on bacterial surface.
	<i>S. epidermis</i>	Prevents biofilm formation through iron sequestering.
Gram negative bacteria	<i>E. coli, S. typhimurium</i>	Cation chelators, damaging the bacterial membrane, altering the outer membrane permeability, resulting in the release of LPS.
	<i>H. influenzae</i>	Altering bacterial virulence – degrading IgA1 and Hap.
	<i>S. flexneri</i>	Disrupt bacterial type III secretion system – degrading IpaB and IpaC.
	<i>E. coli</i>	Disrupt bacterial type III secretion

	<p><i>S. typhimurium</i></p> <p><i>P. aeruginosa</i></p> <p><i>B. cepacia</i></p>	<p>system – degrading EspA, EspB and EspC.</p> <p>Interaction with the bacterial surface.</p> <p>Prevents biofilm formation through iron sequestering.</p> <p>Prevents biofilm formation through iron sequestering.</p>
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Adapted from Jensen et. al. (2009).

2.4. Lactoferrin enhances the uptake of pathogens

Anand et. al. (2015) conducted the research which showed that the presence of iron bound lactoferrin plays a vital role in enhancing the uptake of intracellular pathogenic bacteria such as *Mycoplasma*, *Mycobacterium*, *Chlamydia*, *Borrelia* which can be degraded by free radical ions or reactive oxygen species (ROS) in RBCs and macrophages as compared to untreated control (normal cells). Lactoferrin can play important role in eradicating or inhibiting the intracellular pathogen caused diseases by enhancing the production of free radical ions and ROS in addition to the uptake of pathogens as indicated in the studies conducted by group of researchers showed that production of free radical ions increases in iron saturated lactoferrin treated macrophages compared to untreated normal macrophages. In addition to above, low expression of MDR1 was observed when treated with iron saturated lactoferrin which helps in lowering drug resistance of pathogens thus leading to decrease in the drug resistance by increasing the sensitivity of drug resistant pathogens towards drugs by retaining the drug inside the cell which helps in eradication of drug resistant bacteria. Macrophages become activated after treatment with iron saturated lactoferrin and perform various metabolic activities leading to reorganization, binding, engulfment and inhibition of pathogens through phagocytosis. These cellular processes vary with degrees of iron saturation levels of lactoferrin.

3. Future perspectives

All the studies made by researchers so far till date proves that lactoferrin can play important role in eradicating the bacterial infections caused by mentioned Gram positive and Gram negative bacteria above. In addition to chemotherapeutic compounds and other antibacterial agents,

lactoferrin and its derived peptide, lactoferricin, can play vital role in eliminating the bacterial infections thus giving diverse range of immunity to the host and to the patients. Lactoferrin can boost the immune system by increasing the release of cytokines and other immune cells such as dendritic cells, macrophages and so on. Hence, consumption of lactoferrin in the recombinant form or in the tablet form can facilitate the building up of immune system. Numerous bacteria are prone to resistance to various chemotherapeutic drugs due to frequent use of same drug enhancing the chances of infections to the host. Lactoferrin can overcome this limitation of chemotherapeutic drugs by the means of supplementary doses of lactoferrin to eliminate the bacterial infections. Eye infections, gastric infections, urinary tract infections and mouth infections can be eradicated by the use of lactoferrin as a supplementary in addition to the chemotherapeutic drugs.

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