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REVIEW ARTICLE

Lactoferrin: Major Physiological Functions and Applications

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Abstract: Lactoferrin (lactotransferrin; Lf) is an iron-binding glycoprotein and one of the most important bioactivators in milk and other external secretions. It has numerous biological roles, including the regulation of iron absorption and modulation of immune responses, and has anti-microbial, anti-viral, antioxidant, anti-cancer, and anti-inflammatory activities. Lf regulates the quantity of iron absorbed in the intestine via its role in iron transport and can also chelate iron, directly or indirectly. Notably, it has been used as an adjuvant therapy for some intestinal diseases. It is now used in nutraceutical-supplemented infant formula and other food products. This article reviews the content, distribution, physiologic functions and current applications of Lf, and aims to shed light on future prospects for additional applications of Lf.

Keywords: Lactoferrin (Lf), iron-binding activity, immunomodulatory function, anti-microbial effect, anti-viral effect, antioxidant effect, anti-cancer effect, application.

1. INTRODUCTION

Lactoferrin (lactotransferrin; Lf) is a globular glycoprotein with high affinity for metal ions, such as copper, zinc and manganese ions, but especially for iron ions [1-3]. It is one of the most important bioactivators in milk and various other external secretions [4]. Lf was first identified as an iron-binding protein in 1939, but it has only been since 1960, when it was purified from bovine milk, that its functions and properties have been gradually characterized [5, 6]. It plays important roles in the regulation of iron absorption, and the modulation of immune responses, and has anti-microbial, anti-viral, antioxidant, anti-cancer, and anti-inflammatory activities (Fig. **1**).

Early investigations focused on its iron-binding ability, which is involved in iron transportation and metabolism. However, since its anti-microbial properties were identified, recent studies have demonstrated that it can limit pathophysiological events associated with oxidative stress, inflammation and carcinogenesis [7]. To date, Lf has displayed a broad range of preventive, therapeutic and biological activities [3]. The purpose of this article is to review the major physiological functions of the natural cationic host defense protein Lf, and to identify potential future nutritional and clinical application.

2. DISTRIBUTION, CONTENT AND STRUCTURE

Available evidence shows that Lf is abundant in the upper and lower respiratory tissues, and in the digestive and urogenital tissues. Lf is produced in many of the secretions of mammals, including the colostrum, milk, tears, saliva, plasma, bile, pancreatic juice, and neutrophils [3]. Mayeur *et al*. [7] also demonstrated the presence of Lf in other mucosal secretions, including intestinal mucus, seminal fluid, and genital secretions. However, the concentration of Lf is highest in human colostrum (6-8 mg/ml), while it is present at 1-2 mg/ml in bovine colostrum and human milk, and at 0.02-0.35 mg/ml in cow milk, although its milk content fluctuates during lactation periods. By contrast, there is only a trace amount present in the milk of goats, horses, dogs, and some rodents [8].

The molecular weight of Lf is 80 kDa and it consists of 792 amino acid residues arranged into a polypeptide chain, which is combined with two chitosan side chains (N and C lobes). Each lobe of Lf contains Asp, Tyr and His residues, which are involved in iron-binding. Lf is typically produced in its iron-free form (apo-Lf), which has the capability to bind ferric irons (Fe^{3+}) until saturation (holo-Lf) [9]. The binding of ferric ions by Lf is reversible, and induces the formation of a 'closed' form (holo-Lf) from the 'open' form (apo-Lf). Thus, naturally synthesized Lf has a low level of iron saturation and a high binding affinity for iron; therefore, it binds iron in bodily secretions [3]. Lf is a positively charged protein with an isoelectric point of 8.0-8.5 [10, 11].

3. REGULATION OF IRON ABSORPTION

Iron is the one of the most important components of hemoglobin, myohemoglobin, iron-sulfur protein, cytochromes, peroxidase, and catalase, which plays essential roles in bio-oxidation and oxygen metabolism. The iron in the body originates from endogenous degradation and the

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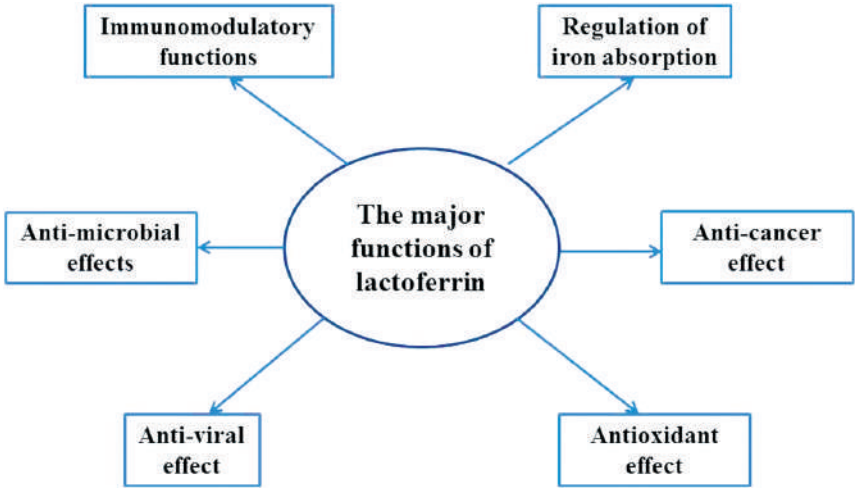

Fig. (1). The major functions of lactoferrin.

ingestion of food and its absorption in the duodenum and the proximal portion of the jejunum. Inorganic Fe^{2+} is more easily absorbed and metabolized than Fe^{3+}. A number of proteins are involved in iron utilization in mammals, including ferritin, which stores iron in cells, uteroferrin, which transports iron in the uterus, transferrin, which transports iron in the blood, and Lf, which transports iron in the intestines. Because transferrin cannot transport iron under acidic conditions, only Lf has the ability to transport iron in the gastrointestinal tract.

Lf regulates the quantity of iron absorbed in the intestine. The iron-binding regions are found at the N- and C-terminals of the molecule, which have high affinity for iron and can bind it reversibly to maintain the absorption and use of iron in the duodenum at a large pH range. Kawakami *et al.* found that the absorption mechanisms for iron differed according to whether it was in the form of iron-saturated Lf or soluble iron salts [12]. Davidsson *et al.* found that the ingestion of milk without Lf promoted iron absorption by infants [13]. However, Lf knockout mice did not show impaired iron absorption [14], and consistent with this, the hemoglobin concentration was not higher in offspring that consumed milk from Lf over-expressing mothers [14].

4. ANTI-MICROBIAL EFFECTS

Lf has both bacteriostatic and bactericidal effects [4] and is broad spectrum, inhibiting Gram-positive bacteria including *Staphylococcus aureus*, *Bacillus subtilis*, *Listeria monocytogenes*, and Gram-negative bacteria, such as *Escherichia coli*, *Klebsiella*, *Helicobacter pylori*, *Salmonella*, and *Shigella* [4, 15]. This is because Lf can chelate iron in pathogenic microorganisms, thus preventing their growth [16]. Lf also scavenges the iron that is an essential nutrient for the development of biofilms in pathogenic microorganisms, thereby reducing bacterial concentration and disease incidence [4]. Additionally, the high density cationic charge on the surface of Lf causes it to non-specifically bind biomolecules [17]. For example, Lf reduces the infectivity of some bacteria by conjugating with proteins on the cell membranes of *Bifidobacteria* and *Pseudomonas* [18].

One of the most important factors involved in the bacteriostatic properties of Lf is disruption of the osmotic function of membranes [11]. Lf has a highly cationic N-terminal region and exerts a potent bactericidal effect by interacting with the negatively-charged elements of bacteria: lipoteichoic acid in Gram-positive bacterial membranes and lipopolysaccharides (LPS) in Gram-negative bacteria membranes [11, 19-21]. This interaction destroys the lipid bilayer of microbial cell membranes, causing greater permeability, loss of cellular contents, and death [22]. However, the growth of some bacteria that have a low requirement for iron is promoted by Lf, which is beneficial for human health [23, 24].

Several *in vitro* investigations have revealed that Lf suppresses the adhesion of pathogens to epithelial cells and intestinal mucosa [25]. Yen *et al.* [4, 26] demonstrated that Lf is an effective natural host defense protein in the prevention and treatment of infections with pathogens and antibiotic-resistant bacterial strains. Furthermore, a recent *in vivo* study conducted by Rybarczyk *et al.* demonstrated that rectal administration of Lf has the dual effect of stopping *E. coli* O157:H7 colonization and improving the local immune response to infection of 6-month-old Holstein-Friesian calves with this organism [27].

5. IMMUNOMODULATORY FUNCTIONS

Many investigations have shown that Lf has both immunostimulatory and immunomodulatory activities [28, 29], which play roles in the innate and acquired immune systems. Lf regulates antibody formation, T and B cell maturation, and increases the percentage of natural killer cells in the lymphocyte population [30]. Most studies have indicated an effect of Lf on $CD4^+$ T cells, altering the Th1/Th2 cytokine balance towards a Th1-type immune response [3, 31]. Therefore, it has the ability to inhibit allergic reactions, which are predominantly Th2-type immune responses. This altered Th1/Th2 balance can be defined by measurement of the Th1-type cytokine interferon (IFN)-γ and the Th2-type cytokine interleukin (IL)-4 [3].

Oral administration of Lf stimulates the production of IL-18 by epithelial cells in the digestive tract [32], while ingestion of Lf by chronically viremic Hepatitis C virus (HCV) patients potentiates the secretion of IL-18 in the peripheral blood [33]. In addition, administration of recombinant por-

cine Lf stimulated the production of serum IgG, IgA and infectious bursal disease (IBD)-specific antibodies in chickens vaccinated against IBD [34]. Furthermore, the oral administration of porcine Lf has been reported to induce T cells to differentiate into Th1 cells rather than Th2 cells, resulting in higher expression of Th1-type cytokines, including IFN-γ and IL-12 [35].

The ability of Lf to regulate the activity of the immune response may be because it can bind endotoxin (LPS) [36, 37]. LPS in the bacterial outer membrane triggers an assortment of immune responses in leukocytes and platelets when Gram-negative bacteria invade the host [38-40]. If Lf binds to LPS in the membrane of these bacteria, then overstimulation of the immune system can be reduced [41].

Serum Lf is mainly produced by neutrophils in response to the presence of pathogenic bacteria. Neutrophils play essential roles in the innate immune system by generating neutrophil extracellular traps (NETs). Lf is one of the components of NETs, which are involved in the development of autoimmune and inflammatory diseases [42], but recent data suggest that Lf also controls NETs release under these circumstances. Indeed, intravenous Lf injection has been shown to reduce NET formation and release into the circulation in an *in vivo* model of immune complex-induced NET formation [42].

Available evidence has shown that oral administration of Lf stimulated the secretion of IgA and IgG in intestine and spleen in mice [36]. Debbabi *et al.* demonstrated that Lf is the one of the stimulatory factors in the mucosal immune system that functions only through adherence to mucosal cells [43].

6. ANTIOXIDANT EFFECT

Energy is generated during normal cellular respiration, but reactive oxygen species (ROS) are also produced [44]. However, excessive production and accumulation of ROS predisposes human or animals to metabolic diseases [45]. Antioxidant defense systems are responsible for the removal of ROS to maintain normal physiological status (redox homeostasis). However, if the production of ROS overwhelms the capacity of the antioxidant defense systems, oxidative stress develops. The antioxidant effect of Lf was originally attributed to its ability to bind iron, resulting in the suppression of ROS production. At the appropriate pH, Lf binds to iron selectively and reversibly. Lf promotes the production of ROS by granulocytes as part of their lethal effect on pathogens [46], but by contrast, at normal extracellular pH, Lf inhibits the generation of ROS by activating the binding of iron by monocytes, which prevents the lipid peroxidative effects of neutrophils, thereby suppressing the tissue oxidative damage caused by ROS [41]. It has been reported that Lf inhibits the production of thiobarbituric acid and malondialdehyde, which are products of lipid peroxidation. However, its direct hydroxyl· and oxygen scavenging capacity appears to be independent of its iron-binding capacity [47] and Ogasawara *et al*. [48] have shown that Lf acts as a scavenger of ROS and protects DNA from direct oxidative damage.

A further antioxidant mechanism may be counteractivity against the neutrophil oxidative burst [49]. In support of this contention, Baveye *et al.* found that binding of LPS to L-selectin is inhibited by Lf, resulting in decreases in the production of ROS by neutrophils and consequent reductions in tissue damage caused by the excessive release of oxygen radicals [50].

7. ANTI-CANCER EFFECT

The balance between iron and other trace elements is an important factor in the development of clinical diseases induced by iron deficiency or excess. Recently, consumption of Lf has been confirmed to reduce the risk of numerous cancers [51-53]. Lf was shown to have preventive effects against gastrointestinal cancers, such as cancer of the colon, stomach, liver, and pancreas, and also against metastasis of such neoplasms [51, 54]. Xu *et al*. (2010) demonstrated that bovine Lf induces stomach cancer apoptosis, thereby suppressing stomach cancer [52]. Oral administration of Lf decreased the occurrence of colon cancer by 83%, while the quantity of adenocarcinoma cells was reduced in the gut of rats after ingestion of Lf, ameliorating tongue cancer in particular. Finally, Habib *et al*. [55] showed that camel milk Lf has the ability to inhibit the proliferation of a colon cancer cell line *in vitro* through its antioxidant activity [55]. In humans, an exogenous supply of Lf efficiently inhibits cancer growth both *in vitro* and *in vivo* [56, 57]. Bovine Lf efficiently inhibits the growth of breast cancer cells, suggesting that it has potential as an anti-cancer agent for the therapy of breast cancer [53, 57].

8. ANTI-VIRAL EFFECT

Lf is also a broad spectrum anti-viral substance which is efficacious against naked and enveloped DNA/RNA viruses that infect humans and animals [3, 10] including HCV, herpes simplex virus (HSV), feline immunodeficiency virus (FIV), human immunodeficiency virus (HIV), Mayaro virus (MAYV), human cytomegalovirus, hepatitis virus, and influenza virus [3]. However, the mechanism involved is not clear. It has been hypothesized that this wide range of antiviral activities may be attributed to the iron-binding ability of Lf and consequent interference in the binding of pathogen proteins to host cell receptors [3]. González-chávez *et al*. suggested that Lf could bind to either virus or host cells, thereby preventing the adhesion of virus to host cells [11]. It has also been proposed that Lf can alleviate hepatocyte injury by impeding viral entry, and that Lf can interfere with influenza virus infection because of the presence of salivary acid in its carbohydrate residues [58].

Recently, Carvalho *et al*. [59] demonstrated that bovine Lf prevents infection of the host cell, rather than hindering viral replication after the target cell is infected, as has been described by other investigators [10, 60]. When they monitored the effect of bovine Lf on MAYV infection, Carvalho *et al.* found that it has strong anti-viral activity, involving prevention of viral entry into the cell, a finding which may contribute to the development of an effective strategy against MAYV infection in the tropics [59]. However, *in vitro* investigations show that the efficacy of Lf against HIV may be due to its inhibition of viral replication in the host cell [11, 61]. Thus, three anti-viral mechanisms of Lf action have been identified. Firstly, it can block the cellular internaliza-

tion of viruses (including poliovirus type 1); secondly, it can suppress viral replication in the host cell (including HCV); and thirdly it can block the glycosaminoglycan viral receptor heparin sulfate, thereby preventing the interaction between virus and host cell [10, 11, 62, 63].

9. APPLICATIONS OF LACTOFERRIN

9.1. Infant Formula

Following the development of ultrafiltration and freeze-drying in the dairy industry, highly bioactive and pure Lf can be produced, allowing the inclusion of Lf in infant formula [8, 64]. Although the concentration of iron in human milk is low and it decreases during the course of lactation [65, 66], no infants who are exclusively breast-fed show iron deficiency during their first 6 months of life. This is likely because Lf serves as a promoter of iron absorption in human milk [67]. Sherman *et al.* reported that Lf is beneficial for late-onset sepsis and necrotizing enterocolitis in infants with a very low birth weight [32]. Because bovine and human Lf possesses similar structures, biochemistry, and bioactivity, bovine Lf has been widely used for its putative health benefits. Chen *et al.* showed that ingestion of bovine Lf-fortified milk is associated with higher total body iron and more efficient intestinal iron absorption in exclusively breast-fed infants [64]. Importantly, non-iron-saturated Lf in milk always scavenges iron from bacteria, which suppresses the growth of the bacteria and reduces the occurrence of diarrhea in infants. Because of this, Lf has been added to infant formula in a number of countries in recent years. In China, the amount of Lf added ranges from 300-1000 mg/kg. Five hundred milligrams per kilogram Lf is recommended as the level of supplementation in powdered infant formula by the FAO and WHO.

9.2. Adjuvant Therapy of Metabolic Diseases

Lf can have multiple beneficial effects besides its intrinsic iron-binding ability [41]. Due to its anti-microbial, anti-cancer, anti-viral, and immunomodulating functions, Lf has been used in the pharmaceutical industry, food industry and in the production of feed additives. Bovine Lf has been produced as a nutraceutical that does not pose any apparent risk to health [68].

It has been reported that Lf may affect glucose metabolism [7]. Moreno-Navarrrrete *et al.* found a negative correlation between blood Lf and fasting glucose concentrations but a positive correlation between blood Lf and insulin sensitivity in patients suffering from type 2 diabetes with altered glucose metabolism [69-72]. Furthermore, Lf regulates intestinal glucose absorption; thus it might be useful in the restoration of glucose transport, especially under inflammatory conditions [73]. Blood Lf concentration has been reported to correlate with body-mass index and waist-to-hip ratio in overweight people [69, 70, 74]. Ono *et al*. found that oral administration of enteric-coated Lf in humans for 8 weeks reduced total adiposity, specifically visceral fat accumulation [75].

Recent studies suggest that Lf may be used as an adjuvant therapy for intestinal diseases. As described above, the ingestion of Lf may have positive effects on diseases of the gastrointestinal tract, by modulating the intestinal flora and preventing diarrhea [76]. Some clinical studies have proved that Lf attenuates vomiting and diarrhea and reduces *Giardia lamblia* colonization in children infected by rotavirus [77, 78]. Importantly, Lf has been found to restore the humoral immune response in immunocompromised mice [79]. Based on its immunomodulatory functions, Lf can be used as an adjuvant therapy in immunocompromised patients to restore T and B cells [4]. Lf may prevent the birth of preterm human infants because it reduces the production of proinflammatory factors and inhibits premature cervical maturation [17]. Additionally, Lf helps to promote the development of the brain and cognition. Bovine Lf has been shown to protect the brain from neuronal loss and reduce inflammation during antenatal and perinatal stress [17].

Future uses of Lf may be focused on the clinical therapy of diseases in certain tissues and organs. For example, Lf may be exploited as organ-targeting ligand for drug delivery. Since Huang *et al*. [80] first used Lf as a brain-targeting ligand, many Lf-drug carrier models have been designed [81]. Targeted therapies for liver and lung diseases are likely to be particular future aims for the use of Lf.

9.3. Food Additives

With the rapid development of transgenic techniques, Lf can now be produced in cows, goats and rice [82]. Bovine Lf has been used as a health promoting additive in commercial food products for decades in Japan [83], but recently, it has also been approved as an ingredient in food products by the European Food Safety Authority [84]. In China, Lf has also been produced as a food additive, certified by the Chinese Food and Drug Administration (CFDA) [85]. As the ingestion of Lf increases with the consumption of milk, the mechanisms underlying Lf synthesis, regulation and action need to be better understood, and this will help comprehension of the key roles of Lf in metabolism.

CONSENT FOR PUBLICATION

Not applicable.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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The review was mainly conceived and designed by PS. Literature was collected and analyzed by LYH. The manuscript was mainly written by LYH, and edited by QS, JYW, FTM and PS. PS resourced the project. All the authors contributed to, read and approved the final manuscript.

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