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Lactoferrin as Nutraceutical Protein from Milk

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Abstract

Lactoferrin is present in milk and has several unique properties as it is used as a nutraceutical. One of the important functions of lactoferrin is its inhibitory effect. Lactoferrin (LF) is known for its anti-bacterial, anti-carcinogenic, anti-inflammatory and anti-oxidant activity. It stops the growth of different harmful bacteria. There is a wide range of pathogens (bacteria, fungi, viruses, and parasites) against which LF is capable of performing the antimicrobial activity. Lactoferrin involves the up and downregulation of immune system. Lactoferrin is a cell mediator and plays crucial role in innate and adaptive immune responses. All the immunomodulatory properties of lactoferrin are proved after many *in vivo* studies. Lactoferrin is obtained from different sources as human lactoferrin and bovine lactoferrin. Bovine lactoferrin is mostly known for its anti-bacterial activity. Lactoferrin is important protein present in milk after casein. Lactoferrin has also anti-viral activity against those viruses that are wrapped in an outermost layer made from plasma membrane. Phagocytic activity of lactoferrin is increased in the presence of some viruses. Lactoferrin has many other activities like it has affinity to bind with different substances and compounds like lipopolysaccharides. Antibiotic consumption in animals can be reduced by using lactoferrin. Lactoferrin has potential to act as natural therapies that can be used in several research fields. Lactoferrin helps in the regulation of apoptosis and helps to limit the reactive oxygen to prevent the destruction of biomembranes.

Keywords: Lactoferrin; Bacteria; Cell; Antibiotic; Nutraceutical

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Abbreviations

BLFampin: Bovine Lactoferrampin; bLF: Bovine Lactoferrin; bLFcin: Bovine Lactoferricin; hLF: Human Lactoferrin; LFcin: Lactoferricin

Lactoferrin as a Nutraceutical Protein from Milk

Lactoferrin is a versatile conjugated protein with iron-containing properties. It contains a red portion of milk and now referred to as nutraceutical. It is a universal compound protein of mammals. It is a result of two consistent lobes that are N and C-17 (33%-41% Similarity) lobe with a weight of 80 kDa joined by the means of alpha helix. It is highly maintained kind containing 1900-2600 base pairs and 700 amino acids depending upon the type [1]. The gene for lactoferrin protein is LTF. In this protein there is post-translational modification (i.e. glycosylation). There are two types of active sites: 1. metal-binding amino acids 2. Carbonate binding amino acids [2].

Mostly lactoferrin is present in milk and colostrum and it has much influence on the immune system and its composition. Lactoferrin is found in milk in 1939, because of its properties and composition it is regarded as safe or protected protein by "GRAS". The function of milk protein is to reduce the prevalence of certain diseases as it acts as an inhibitory agent. Efficient drugs used to reduce the disease sometimes result in increasing their benefits because of their side effects [3].

The word nutraceutical means to involve all the useful ingredients and it provides profit to the seller that is why it is attracting the people because of its nutritional value. It is well known for its activity against viruses, bacteria, microbes, parasites, fungi and certain allergies [1]. LF is abundant in the upper and lower respiratory tissue, and in the digestive and urogenital tissues. LF is produced in many mammals the secretions, including the colostrum, milk, tears, saliva, plasma, bile, pancreatic juice, and neutrophils [4].

Lactoferrin is present mostly in milk and most abundant after the

casein and also in camel milk, bovine and colostrum. Camel milk is also a source of protein in humans. Milk is the main source of protein especially for infants and the percentage of identity in the sequence is similar for humans and chimpanzees that is 94-97%. Besides its food benefits, it also used as a therapeutic agent as well as for animal medicine in the prevention of disorders and also helpful in the inhibition of hepatitis virus entry into the body [5].

Role of Lactoferrin as Natural Immune Modulator

Lactoferrin is a valuable glycoprotein and has the iron-binding capacity. It is secreted from the exocrine glands. Lactoferrin plays a vital role in the host defense mechanism [3]. Human milk and bovine milk both are rich sources of lactoferrin. Lactoferrin is crucial protein of all human secretions. Milk contains different important proteins. After casein the second most important protein of milk is lactoferrin [6]. In case of tissue injuries and infections, lactoferrin is considered as the best polyvalent protein that exists in the host defense system. N terminal domain of lactoferrin has propensity to interrelate with many microbes. Besides the anti-microbial properties of lactoferrin, it has role in innate and adaptive immune response [7]. Lactoferrin is also known as "Red Protein" of milk. There are lots of functions of lactoferrin including it makes its interaction with the antigen-presenting cells like dendritic cells, macrophages, B lymphocytes. Macrophages are actually the phagocytic cells that are known for their effective role. They monitored the infection and help to control it in two ways. They act by destruction of intracellular material of the microorganism or they stop their replication process with release of special mediators and cytokines. The damaged tissue is repaired with the release of inflammatory mediators and cytokines. Whenever there is an interaction between lactoferrin and lactoferrin receptors, macrophages are activated. As a result there is synthesis of IL-12, it is a molecule that gives signal to other macrophages towards the inflamed area and stimulates lymphocytes and T CD4 [8]. Human Lactoferrin (hLF) and Bovine Lactoferrin (bLF) both have important roles. They have many similar functions and have high sequence homology. hLF is isolated from the colostrum, inhibition of bacterial growth is prime function of bLF and hLF [3]. bLF is accepted by the food and drug administration. bLF involves the inhibition of growth of many tumor cells. It also induces apoptosis in tumor cells. Apoptosis is induced in two breast cancer cell lines [9]. Lactoferrin obtained from different origins only differs in its structural domain [5]. Lactoferrin is present at the surface of innate immune system like neutrophils. When neutrophils are activated, concentration of lactoferrin rises and it is released into the blood and at the inflamed tissues. Microglial cells are one type of macrophages that are involved in maintenance of health of CNS by deletion of injured neurons. These cells are also involved in the release of lactoferrin as a result of inflammation. The importance of lactoferrin in host defense system indicates the absence of lactoferrin in humans [7]. The antigen is recognized by the dendritic cells, these cells have important role in immune system. Lactoferrin interacts with the dendritic cells with the help of special receptors that are present on their surface. Mostly the activation of dendritic cells is by Talactoferrin. Talactoferrin is recombinant human lactoferrin that is involved in the release of IL-8 and chemokine [8]. Furthermore, lactoferrin is a regular adjuvant that enhanced immunity. Lactoferrin is a natural way to develop protective immunity against pathogens. Lactoferrin has ability to bind with the ferric ions [10]. Secretory IgA also involves the immune protection that is transferred to the infants through the breastfeeding results in a lower risk of illness. Similarly lactoferrin is vital for the babies as it boosts their immune system and protects them from respiratory tract infection in the first year of life. It is found that the concentration of lactoferrin faintly rises in maternal or infant illness [11]. Lactoferrin is involved in the up and down-regulation of the inflammatory response and possesses both pro-anti-inflammatory properties. Cellular mechanisms are responsible for the immunomodulatory properties of lactoferrin. Many *in vivo* and *in vitro* studies tell about the presence of several mechanisms [12]. Lactoferrin can be taken orally to prevent sepsis and NEC. Many studies have reported that exposure to inflammation increases by lacking lactoferrin [13].

Lactoferrin as an Antimicrobial Agent

Lactoferrin (LF), with antimicrobial activity, is present in secretions that recover mucosal spots. These spots are regarded as gateways of attack, for pathogenic agents. In LF of bovine and human, porcine, caprine, camelid, and buffalo origin, antimicrobial activity has been mostly characterized and is isolated from milk [12].

There is a wide range of pathogens (bacteria, fungi, viruses, parasites, etc.) against which LF is capable of performing an antimicrobial activity **(Figure 1)**. Lactoferricin (LFcin) is released when LF is exposed to proteolytic digestion with pepsin (located in the N-terminal region). LFcin is a bioactive peptide that wields a higher antimicrobial activity than LF [14].

Lactoferrin (LF) is a nontoxic nutraceutical protein commercially

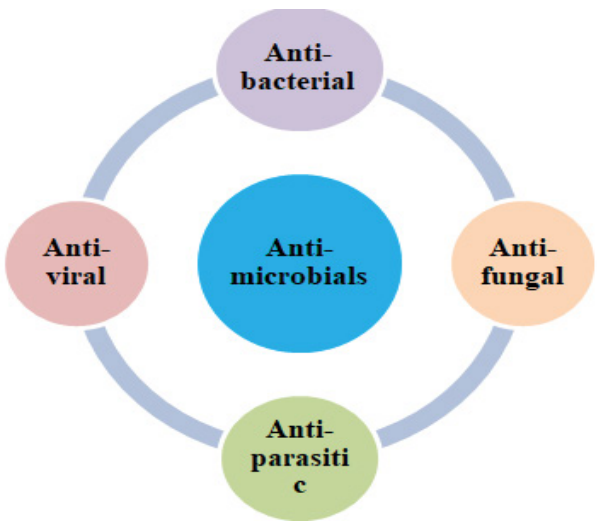

Figure 1 Antimicrobials include antibacterial, antifungal, antiviral, antiparasitic.

obtainable from milk whey and it has found no resistance to it. It is a therapeutic substitute against pathogens. It does not disturb the microbiota. LF could be used meritoriously as a substitute or adjunct therapy to antimicrobials in the handling of infectious diseases in veterinary medicines [5].

1. The anti-bacterial activity of LF is due to two different mechanisms. Its primary role is to sequester free iron, thus removing an essential substrate required for bacterial growth and exerting a bacteriostatic effect
2. The other mechanism involves the direct interaction of LF with the infectious agent. It can be bactericidal due to its interaction with LPS and porins in Gram-negative bacteria, or with teichoic acids in Gram-positive bacteria

These interactions lead to membrane damage and bacterial death [15]. LF damages bacteria by binding to the lipopolysaccharide of bacterial cell walls. It may also destroy the bacteria via the formation of peroxides catalyzed by LF-bound iron (III) ions which affects membrane permeability and results in bacterial cell lysis [11].

There is a strong antibacterial effect of lactoferrin on Gram-positive bacteria (*B. cereus*) as well as on Gram-negative (*Salmonella*) bacteria. Because of its capability to bind iron, LF shows *in-vitro* antibacterial activity. In this way, iron will become unapproachable to the bacteria. The existence of iron in the environment is necessary for bacterial growth. Lactoferrin binds to iron to make it out-of-reach for the bacteria. Lactoferrin also causes apoptosis in cells [16].

LF shows antiviral activity against common viral infections. It induces type I interferon (α/β) with antiviral action as a result of which, it blocks the cellular attachment or viral replication. Thus, LF from a variety of mammals presents a potent activity against replication of HIV (Human Immunodeficiency Virus), CMV (*Cytomegalovirus*), and HCV (Hepatitis C Virus) [15]. LF presents an effective antiviral action against enveloped as well as naked viruses, like CMV (Cytomegalovirus), HSV (Herpes Simplex Virus), HIV (Human Immunodeficiency Virus), HCV (Human hepatitis C) and HBV (Human hepatitis B virus).

An antiparasitic activity seems to involve the interference of LF with the iron acquisition in some parasites (*Pneumocystis carinii*). Contrary to it, LF appears to act as a specific iron donor in other parasites (such as *Tritrichomonas foetus*) [12].

LF is able to inhibit the growth of *Plasmodium berghei* (protozoan parasite). LF binds directly to the plasmodial CS protein. Less information exists about the microbicide action of LF against fungi and protozoa [15]. In forthcoming years, the lactoferrin as an antifungal and will be used in the different antifungal drug development [17].

Colostrum also has antiviral, antifungal and antibacterial properties that enable it to kill different pathogens like *Escherichia coli*, *Rotavirus* and *Cryptosporidium*. The strong antiviral activity is reported by immunoglobulins, lactoferrins, and cytokines in colostrum [18]. LF is playing a significant role in elevating the production of industrial antibacterial and chemical drugs. For this purpose, we can isolate milk lactoferrin gene from different sources and expressed it in the developed organisms. LF is mainly, a natural antibacterial agent and can be expressed in higher plants or animals [16].

The use of LF and LFcin against microorganisms has not been restricted to human pathogens. Fukuta expressed lactoferricin in transgenic tobacco plants [14]. In the inhibition and treatment of infections with pathogens and antibiotic-resistant bacterial strains, LF is an effective natural host defense protein [4]. In public health drug-resistant strains of bacteria have become a serious problem. The risks related to the resistance and up-to-the-minute cost of antimicrobial drugs has stimulated researchers to search for efficient and economically suitable broad-spectrum pharmaceuticals. The development of new effective antimicrobial compounds is critical. Silver ions have attended people in the fight against microorganisms for hundreds of years. Nanocomplexes of silver is widely used as antimicrobial, antifungal and antiviral agents. They show a lesser amount of cytotoxicity than silver ions. The synthesis of silver nano-complexes with lactoferrin represents an unconventional/innovative utilization in the field of medicine. Although LF has medicinal applications, bioactive lactoferrin nano-complexes with silver have the gigantic potential for nutraceutical and pharmaceutical applications [19].

Fully bio-based edible antimicrobial films were developed in which bovine lactoferrin (bLF) was the central component [20].

Lactoferrin as an Antioxidant

The production rate of ROS (Reactive Oxygen Species) can be controlled by their rate of termination can be supervised by the Lactoferrin. In the direct oxidative cell abrasion, we usually use buffers naturally. Lactoferrin also plays an important role in the upregulation of antioxidant enzymes. During some destruction occurred so lactoferrin saves the damaging effect by the integrity of commandeering Fe ions. Intracellular oxidative stress decreased lactoferrin. When the lactoferrin present under the *in vivo* effects and it depends upon the baseline immune status of individual [10], Mesenchymal Stem Cells (MSCs) enlarged in *ex vivo* system and have some modified capabilities. When these cells venerable and transplanted in the oxidative stress these cells survive imperfectly or some time conduct the apoptosis because these cells have restricted clinical implementation [21-23].

Role of Lactoferrin as an Anticancerous Agent

Lactoferrin is cut off from the bovine milk. They constrained the gastric cancer cell lines AGS (Apoptosis of human gastric adenocarcinoma) have any effect on normal cells. Lactoferrin as anticancer minimizes the risk of proliferation because of some multifunctional qualities including anticancer anti-inflammatory, cellular growth, and differentiation. We minimize the AOM azoxy-methane by bovine lactoferrin [24]. Curcumin used in anticancer and anti-inflammatory drugs. Treatment of cancer through the curcumin is the great dispense in nowadays. Curcumin

conducts the active and passive targeting when we conjugate the curcumin with LF, then reduced the risk of cancer cells by potential drug delivery. We improved the cytotoxicity an HCT 116 cells (Hematocrit) by the conjugated curcumin. Conjugated curcumin has greater production than normal curcumin. After 2h incubation with the help of conjugated curcumin we conduct premature apoptosis which is occurred in HCT 116 cell lines [25].

Many deaths caused by cancer, NPW we use the milk with lactoferrin for the treatment of the cancerous patient. In milk casein and whey protein is present which is supportive of the treatment. In recent year we use this method but in past we use the radiations and chemotherapy for cancer and also these techniques is a high cost for the patients. Sometimes these kill the normal healthy cells as compared to the cancerous cells. The reduction of cancer cells also occurs by peptides. Lactoferrin used for the treatment of lung cancer. This method needs some nano-complex and nanocomposites with Doxorubicin for the treatment of cancer cells. DO for this reason we use the LF-Chondroitin sulfate nano-complex because this noncomplex carrier for drug delivery for the lung cancer patients and we inbreathe them. Nanocrystals increase the folds 33.3 easily entangle onto the hydrophilic matrix and increase the aqueous solidify due to anti precipitating and anti-solvent qualities [26]. LF is used in the treatment of the different cancers now we indicated that the LF functional for the treatment Oral Squamous Cell Carcinoma (OSCC). The LF actively works against cancer due to the bovine Lactoferrin. By the amalgamation of p53 and LF they reduced the risk of cancer and hinder the cell cycle at S, G1, or G_o phase and when they arrest the cell cycle they induced apoptosis. They also manage cell proliferation and in OSCC the cellular growth reduced [27].

BLF also use in the treatment of prostate cancer. For prostate cancer, we use some drugs because different chemotherapies fail. Mostly for cancer treatment we use Doxorubicin which reduces the chemo-resistance to enhance the effectiveness of DOX. We attach with the recent time period that has different anticancer properties. They improve immunity. We also conjugate the iron with bLF DOX they affect the functionality of bLF-DOX. When they bind, both bLF and DOX, they easily enter in cancer cells [27].

Discussion and Conclusion

Due to the vast variety of lactoferrin functions, it creates a wide range of new nutritional products and benefits. Lactoferrin involves cell secretions as well as in maintaining the breakdown system of the cell. There are milk protein formulas for kids are being examined present in bovine lactoferrin. As the bovine lactoferrin is breakdown by enzymatic degradation so it is rarely available and results in oral transportation of lactoferrin. Many aspects are studied and being reported meanwhile but still there is less information on lactoferrin that exists until now [5].

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