Review Article

Lactoferrin: A General Review

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Received: 23-08-2019; **Revised:** 26-09-2019; **Accepted:** 05-10-2019.

ABSTRACT

This review discusses important aspects of the glycoprotein lactoferrin. Lactoferrin, well known as a minor whey protein, is an 80kDa iron-binding glycoprotein primarily present in milk. It is secreted by epithelial cells and present in almost all mucosal secretions of the body. After degranulation, neutrophils become the main source of lactoferrin in blood plasma. Now-a-days, it has got considerable importance due to its broad range of biological activities, including roles in iron metabolism, immunomodulatory, antioxidant, antimicrobial, anti-inflammatory, anticarcinogenic and metal chelating properties. Many of these functions do not appear to be connected with its iron binding ability. More research is necessary however to obtain clarity with regard to the exact mechanism of action of lactoferrin.

Keywords: Lactoferrin; glycoprotein; milk; neutrophils; iron binding ability; antimicrobial.

INTRODUCTION

Lactoferrin (Lf) was isolated for the first time by Sorensen and Sorensen in 1939 from cow milk.[115] Later it was isolated by Groves (1960), Montreuil *et al.* (1960) and Derechin and Johanson (1962) from human milk, and was recognized as "red protein from milk". Lactoferrin (formerly known as lactotransferrin), a globular glycoprotein with molecular weight of 80kDa, is a unique member of the transferrin family and had iron (Fe^{3+} ion) scavenging ability.[87] Lactoferrin levels in all the biological fluids of the body were increased whenever there is an inflammatory response.[19] Lactoferrin is capable of binding to other macromolecules like DNA, IgA, casein, secretory component, albumin, lysozyme, β-lactoglobulin, glycosaminoglycans, lipopolysaccharides, heparin and metal ions such as Mn^{3+}, Cu^{3+}, Zn^{3+}, Al^{3+}, Ga^{3+}, Co^{3+} etc.[103] The degranulated neutrophils were the main source of Lf (~15µg/10^6 neutrophils) in the blood plasma.[59] Most specific molecular feature accounting for Lf properties in host defence is its very high affinity for iron. It binds two ferric (Fe^{3+}) ions along with two synergistically bound carbonate (CO_3^{2-}) ions very tightly, making the protein a powerful iron scavenger.[12] Lactoferrin concentrations and its messenger RNA expression increase during the mammary gland development, colostrum formation and the gland involution, while its concentration decrease during the lactation, as opposed to the increasing levels of casein.[105] Since the levels were elevated during inflammation responses, and immunosuppressive and latent viral infections, Lf is categorized as an acute phase protein.[62] Recently Lf has shot into utmost prominence due to wide array of biological roles including: antimicrobial activity (against bacteria, viruses, parasites and fungi); anti-inflammatory and anti-oxidant property, contributing to its tissue regeneration capacity; immunomodulatory property by bridging the innate and adaptive immune responses; and anticancer capacity, by direct effect on the distorted cells or by indirectly acting through strengthening the natural immune system.[25] Lactoferrin is secreted by epithelial cells and hence present in all mucosal secretions of the body including milk or colostrum, saliva, nasal secretion, bronchial secretion, tears, digestive fluids, bile, vaginal fluids, semen, and urine in various mammalian species.[6] Among these, the highest concentration is reported in colostrum (~7 g/L) and milk, enabling it to be the second most plentiful milk protein after caseins.

Structure and Properties of Lf

Lactoferrin is a cationic protein with an isoelectric pH of 8.7.[5] The degree of resistance exerted by Lf against hydrolytic degradation caused by proteolytic enzymes like pepsin, trypsin, pronase etc. under acidic conditions is dependent upon the amount of iron saturation.[23,21,59] The molecular structure and amino acid sequence of human Lf. Being an iron scavenging glycoprotein with molecular weight 80kDa and a member of transferrin family, the amino acid sequence was almost 60 per cent identical with the transferrin protein present in serum.[87] The three different isoforms of Lf were α, β and γ. While Lf- β and Lf-γ isoforms possessed ribonuclease property, α-Lf bind two Fe^{3+} ions with very high affinity but lacked ribonuclease capacity.[44] Polymeric forms of Lf were detected in the studies conducted by Bagby and Bennet (1982) and Mantel *et al.* (1994). At high calcium concentration of ~ 10^{10} m*M*, Lf molecules combine together to produce oligomers particularly tetramers. When protein concentration is more than 10^{-5}*M*, Lf form oligomers and monomer to tetramer ratio will be 1:4. Lactoferrin protein occurred as a single polypeptide chain with approximately 690 amino acids arranged as two symmetrical globular portions (C and N terminal regions), which in turn were linked to each other by an α- helix. Both C and N terminal regions is

further composed of two domains (known as C_1, C_2, N_1 and N_2), each of which contained one Fe^{3+} ion binding site and hence two Fe^{3+} ions could be bound by one Lf molecule.[105] Four amino acid residues (namely Asp-60, Tyr-92, Tyr-192 and His -253) are involved in forming Fe^{3+} binding site in each domain while Arg-121 is responsible for binding CO_3^{2-} ion. This enabled Lf to bind one CO_3^{2-} ion concurrently with each Fe^{3+} ion.[87,12] Glycosylation is an important factor that contributes to the stability of Lf protein. N-acetyllactosamine, N-acetylglucosamine, mannose, fucose, galactose, and neuraminic acid are the main sugar moieties present in bovine Lf.[116] Studies by Siebert and Huang in 1997 revealed a truncated isoform of Lf known as delta Lf (δ- Lf). It is secreted intracellularly, has a molar mass of 73kDa and is mainly involved in cell death regulation. Its expression is induced by alternate promoter (exon1-β) activation of Lf gene in DNA and translation occurs without the leader sequence.[110] According to iron saturation, Lf is said to exist in three forms: the form that lack Fe^{3+} binding ability is called apo-lactoferrin (apo-Lf); the form that bind one Fe^{3+} ion is monoferric; and the form that bind two Fe^{3+} ions is called holo-lactoferrin (holo-Lf). The C-lobe and N- terminal lobe of apo-Lf had closed conformation and open conformation respectively, while holo-Lf existed as a closed molecule and showed greater resistance to proteolytic degradation.[60] Lactoferrin had about 300 times higher affinity to iron than transferrin. It could bind iron especially at low pH sites where infection and inflammation persist, and where pH might fall below 4.5 and Lf could bind iron released from transferrin thereby depriving iron needed for bacterial proliferation.[70] Le Parc *et al.* identified The novel glycans in goat milk Lf and revealed that glycosylation pattern of Lf in goat milk is similar to that of human and bovine Lf in 2014. Zainab *et al.* characterised Lf from colostrum whey of Iraq goats in 2015 and found iron saturation of 8.7 per cent and the iron content was 123 ppm respectively.

Sources of Lf

In adults, Lf is present in most mucosal secretions while highest levels occurred in milk and colostrum.[84,12,74] Most plasma Lf is present in the secondary storage granules as well as tertiary granules of neutrophils.[59] Secretory epithelia and myeloid series of cells were the most important cell groups associated with the Lf synthesis.[15] Studies done by Ward *et al.* in 1999 revealed that Lf was first expressed in two- and four- cell embryos at the time of embryonic development, after which it could be seen throughout the blastocyst stage and up to implantation. Afterwards Lf cannot be detected until halfway of gestation. Later, Lf was again detected in the neutrophilic storage granules as well as in the epithelial cells of developing digestive and reproductive systems.[133] Abrink *et al.* reported in the year 2000 that the expression, synthesis and secretion of Lf occurred throughout the collecting tubules while its reabsorption was limited to the distal convoluting tubules.[1] Hence, synthesis of Lf occurred in human kidneys as well.

Regulation of Lf synthesis

In 1969, Masson *et al.* found out that Lf after being synthesised during the differentiation phase inside neutrophils (i.e., when promyelocytes were developing into myelocytes) were stored in secondary storage granules.[85] The ability of Lf synthesis cease as the neutrophils mature. Studies carried out by Green and Pastewka in 1978 revealed that regulation of the Lf production varied with the type of cells within which it is being synthesised. For instance, Lf synthesis in the mammary gland was determined by prolactin.[49] Leukocytosis occurring in association with pregnancy and selective Lf level increase in neutrophil granules or other organs like mammary glands, endometrium and decidua are the primary factors responsible for increase in Lf plasma levels during initial stages of pregnancy.[92] Production of Lf in reproductive tissues was decided by oestrogen while the synthesis in endometrium is regulated by both epidermal growth factor (EGF) and oestrogen.[91,131,66] With regard to menstrual cycle, serum Lf concentrations were higher in the proliferative phase than that in the secretory phase.

Lactoferrin Receptors

The existence of Lf receptors in the small intestine was reported by Cox *et al.* in 1979. Studies with Lf fragments done by Rochard *et al.* in 1989 revealed that the receptor binding capacity was attributed by the N- lobe residue from 1-90 amino acids.[101] High isoelectric point (8.7) enables Lf to undergo non-specific binding to the target cells or proteins.[12] Specific regions in both C- and N- lobes of human Lf that had high affinity to to Lf receptors in the bacterial cell membranes were identified in the studies conducted by Yu and Schryvers in 1993.[143] Lactoferrin receptors were present in the T- and B- lymphocytes, polymorphonuclear leukocytes (PMN cells), monocytes, fibroblasts, macrophages, platelets, hepatocytes, mucosal epithelial cells and some bacteria like *Pseudomonas hydrophila* or *Staphylococcus aureus*.[74,117] The "main receptors" present in some cells enabled them to bind Lf and transferrin proteins of other species, besides specific Lf protein.[62] Apart from the "classic" receptors, Lf also possessed specific nuclear receptors with affinity towards leukocyte cell membrane.

Lactoferrin gene (*Lf*) regulation

Lactoferrin (*Lf*) gene present in different tissues had extensive homology among species. The mRNA levels varied with tissue and specific regulation of *Lf* occurred at both cell and tissue level.[86] Study conducted by Liu *et al.* in the year 1993 recognized both constitutive and inducible levels of gene expression for *Lf* gene. Non- oestrogenic compounds such as retinoic acid induced *Lf* gene expression in the embryonic cells.[45] Oestrogen response elements (EREs) were identified by Teng *et al.* in 2002 in the *Lf* promoter region of the mRNA in humans and mice, which overlapped the binding site of other transcription factors like COUP transcription factor (an oestrogen

responsive negative regulator).[120] Size of *Lf* gene ranged from 23kb to 35kb and it was organized in 17 exons, out of which 15 were identical in bovine, swine and mice. Absence of repressor of oestrogen induced receptor activity lead to 100 times increased oestrogen- induced *Lf* expression.[97] After analysing about sixty sequences of *Lf* genes with complete coding regions and after taking into consideration about insertions, deletions, and mutations in the stop codon, Kang *et al.* reported in 2008 that *Lf* gene in different species varied in size from 2055 to 2190 residues.[61] According to Anjusekar *et al.* in 2018, the sequences of coding region of *Lf* gene of both Malabari and Attappady Black goat breeds revealed more than 99 per cent identity with goat lactoferrin mRNA sequences in the database retrieved by nucleotide Basic Local Alignment Search Tool (BLASTn).[8] Malabari showed maximum homology of 99.6 percent with *Capra hircus* while least homology of 81.4 per cent with *Homo sapiens*. Attappady Black showed maximum identity of 99.5 per cent with *Capra hircus* while least identity of 81.3 per cent with *Homo sapiens*.

Metabolism of Lf

According to Van-Snick *et al.* in 1974, *Lf* expression is modulated by steroid-thyroid receptor superfamily. Lactoferrin is present in the body in unphosphorylated form and its levels are hormone dependent.[123] Transfer of Lf to storage granules in neutrophils depends upon the acidification mechanisms, which will be occurring through the medial and trans-cisternae of golgi apparatus.[94] Goldman *et al.* identified the presence of low molecular weight fragments of Lf in faeces in 1990. Kidneys were involved in the elimination of Lf from circulation since they could detect maternally derived Lf and its fragments in the urine of breast-fed babies.[57] Lactoferrin, after metabolism, could be removed from the body by endocytosis, which in turn was carried out by hepatocytes, liver endothelial cells and Kupffer cells.[45] Lactoferrin secretion into the circulation from neutrophils depended upon the degranulation factors, which were activated appropriately by calcium dependent protein kinase-C, guanylate cyclase and cGMP.[81] According to the studies undertaken by Levay and Viljoen in 1995, Lf elimination occurred through two ways: a) direct liver uptake by the way of an iron saturation-independent and clathrin-dependent endocytosis process; and b) receptor mediated endocytosis of phagocytic cells with consequent iron delivery to ferritin.

Lactoferrin in different species

Increase in Lf levels by many times, up to 100 mg/mL, have been reported during the involution of mammary gland.[136] In 1995, Levay and Viljoen determined the amount of human Lf in colostrum, milk and venous plasma to be 3.1-6.7 mg/mL, 1.0-3.2 mg/mL and 0.12 µg/mL respectively. Lactoferrin concentration ranged from 1.15 µg/mL to 485.63 µg/mL in the milk of healthy cow.[51] Barton *et al.* measured the level of Lf in the mare colostrum, serum of new-born infants and three-day foals in 2006 and reported them to be 21.7 µg/mL, 0.249 µg/mL and 0.445 µg/mL respectively.[14] In 2007, Berlov *et al.* detected Lf concentration in canine milk to be 40 µg/mL, which is lower than in human milk.[17] Mean milk Lf concentration in camel is 0.229 ± 0.135 mg/mL.[68] Lactoferrin concentration in cow, pig and dog neutrophils were detected by Sinkora *et al.* in 2007 by flow cytometry and by using commercially procured rabbit anti- human polyclonal antisera.[30,118] Cheng *et al.* in 2008 found out the Lf exhibited significant relation with the stage of lactation (r = 0.557) and day-to-day milk production (r = -0.472).

Lactoferrin derived peptides

Bellamy *et al.* in the year 1992 reported the synthesis of lactoferricin (Lfcin) by pepsin mediated hydrolysis of Lf under acidic conditions, which is abundant in both basic amino acids like lysine and arginine, and hydrophobic amino acids namely tryptophan and phenylalanine.[16] They also found that lactoferricin H from humans and lactoferricin B from bovines are the two forms of lactoferricin. Increase in interleukin-8 secretion by the Lf-derived peptides from polymorphonuclear leukocytes suggested their immunomodulatory function.[109] The more potent antimicrobial capacity of Lfcin was because its peptides were connected by N- acylation to hydrophobic chains.[129] Lactoferrampin (Lfampin), comprising 268-284 residues in the N1 domain of Lf and located in close proximity to Lfcin, had a significant role in regulating the membrane mediated activities of Lf.[124] Upon pepsin digestion, amphipathic α- helix in Lf gets transformed into amphipathic β- sheet hairpin in Lfcin. Lactoferricin derived from different mammalian species was rich in hydrophobic and positively charged amino acid residues, among which cysteine residues were linked by a disulfide bridge.[125] Lfcin and Lfampin exhibited *in vitro* synergistic action with antibiotics to help break down the vicious cycle of antibiotic resistance against the strains of *Staphylococcus aureus* bacteria.[41] Lactoferricin had higher biological properties than Lf. Bovine mastitis, piglet gastro-intestinal infection and canine dermatitis were the its most important *in vivo* therapeutic applications in the practical medicine. Lactoferricin exhibited potent antimicrobial activity and a minimum concentration of 64 µg/mL exhibited hemolytic activity.[78] The synthesis of Lf peptides (Lf (1-11), lactoferricin (Lfcin) and lactoferrampin (Lfampin)) occurred in the intestinal lumen after oral ingestion by means of proteolytic enzyme degradation. Cationic charge, hydrophobicity and helical conformation were the three key features that render them amphiphilic molecules.[118] The Lf derived peptides such as Lfcin exhibited more potentiated antimicrobial activity than the native Lf protein, characterized by lower minimum inhibitory concentration and broader host defence properties.[20,27] The highly cationic nature of Lf(1-11) oligopeptide interacted with bacterial membranes by means of eleven hydrophobic and hydrophilic amino acid residues, which were derived from native protein.

Biological Functions of Lf

Role in Iron Metabolism

Van Snick *et al.* reported in 1974 that the release of iron from its depots was controlled by Lf and the free iron bound could be shuttled back to macrophages, thus leading to hypoferraemia.[126] The regulation of iron metabolism in humans by Lf protein present in the duodenal secretion was elucidated by De Vet and Van Gool in 1974. Van Vugt *et al.* demonstrated the significant increase of Lf concentration after an acute blood loss and in anemic conditions due to the mobilization of stored iron from liver, and hence regulated the iron metabolism.[127] Brock in 1980 determined the significance of Lf in iron absorption and exhibiting defensive action against gastrointestinal infection in newborn infants, and found that Lf acted as a critical component in the modulation of immune responses.[24] Lactoferrin is indirectly involved in physiological functions like synthesis of DNA, RNA, proteins and immunoglobulins through its effect on iron metabolism.[74] Depending upon the organism's requirement for iron, specific receptors present on enterocytes bind Lf and degrade almost 90 per cent of it to release Fe^{3+} ions.[117]

Antibacterial Activity

The affinity of Lf towards iron and its iron scavenging ability resulted in deprivation of iron availability for bacteria and hence formed the basis of antimicrobial property.[9] When iron binding ability of Lf prevented growth of iron dependent bacteria like *Escherichia coli,* it enhanced the growth of beneficial bacteria like *Lactobacterium* spp. and *Bifidobacterium* spp., with lower iron demands.[24,98] The ability of Lf to bind to, liberate and damage the lipid A of lipopolysaccharide of cell membrane facilitated Lf action against gram negative bacteria.[41] The sugar moieties of Lf like oligomannoside sugar chains prevented the binding of bacterial adhesins with host cell receptors and thus enhanced host defence properties.[103] The efficacy of Lf against Gram positive bacteria is explained by its ability to bind to negatively charged molecules such as lipoteichoic acid present on the cell membrane and it also enhanced the bactericidal capability of antibacterial compounds secreted from mucosa, like lysozyme.[73] Lactoferrin could prevent *in vitro* biofilm formation *P. aeruginosa*.[112] The proteins necessary for degradation in bacteria like enteropathogenic *E. coli* and *Shigella flexneri* could be degraded by proteolytic activity of Lf.[134] Sinchu compared the antimicrobial potential of lactoferrin of Attappady and Malabari goats to that of crossbred goats in 2017 and concluded that the native goat lactoferrin is superior to that of crossbreds with respect to anti-bacterial activity.[111]

Antiviral Activity

Lactoferrin protected the host by inhibiting a broad range of DNA and RNA viruses from binding to target cells and thus inhibited consequent intracellular replication, apart from modulating the systemic immune responses.[142] Antiviral activity of Lf against human immunodeficiency virus, herpes simplex virus (types I and II) and cytomegalo virus is reported by Viani *et al.* in 1999[128] Lactoferrin binds to glycosaminoglycan portion of viral receptor especially heparin sulphate and so prevent the virus internalization into host cells.[48] Antiviral ability of Lf occurred by stimulating natural killer (NK) cells and by activating the T helper lymphocytes to release cytokines.[130]

Antifungal Activity

Studies done by Kirkpatrick *et al.* in 1971 reported that the antifungal activity of Lf against *Candida* spp. is attributed to its iron sequestering ability from the micro-environment.[67] Both Lf and Lfcin directly bind to fungal cell surface and alter its permeability to cause fungicidal action.[16] Addition of low level of bovine Lfcin inhibited the growth of fungal hyphae in azole resistant strains.[129] They also found that Lf possess potential antifungal action against body tineas such as *Trichophyton mentagrophytes*. Ferric (Fe^{3+}) ion sequestration by apo- Lf within neutrophilic granules is the basis of antifungal action against *Aspergillus fumigatus*.[145]

Antiparasitic Activity

Competition between parasite and Lf with regard to sequestering environmental iron is the basis of the antiparasitic action of Lf.[135] Parasites like *Tritrichomonas foetus* and *Trichomonas vaginalis* possessed the ability to utilise Lf as an iron (Fe^{3+}) donor.[119] Lactoferricin deteriorated the parasitic membrane integrity in order to induce changes in host-parasite interactions.[95] Increase in T CD4+ lymphocytes by Lf and Lfcin is responsible for their antiparasitic action against parasites like *Toxoplasma Gondii*.[90]

Antineoplastic Activity

In 1994, Bezault *et al.* reported that experimental metastases developed in mice is inhibited by Lf. When Lf was administered in smaller concentrations (10 µg/mL), it induced tumour cell cytolysis, which in turn depend upon the cell phenotype.[18] Lactoferrin at concentrations such as 100 µg/mL augmented the cytotoxic activity of NK cells. The growth of human mammary gland carcinoma cells was halted by Lf, specifically between G_1 and S stage of the cell cycle.[35] *In vivo* studies by Wolf *et al.* in 2003 confirmed that Lf upon oral administration repressed squamous cell carcinoma in neck and T- cell dependent tumour in head. Tumour growth inhibition by Lf occurred due to induction of apoptosis or programmed cell death, as a result of triggering of Fas signaling pathway.[43] Anticarcinogenic activity of Lf occurred by stimulating its immuno-expression in the human kidney cell carcinomas and adjacent normal healthy tissues.[46] Lactoferrin upon oral administration stimulates the activity of NK cells as well as CD4+ and CD8+ cells.[3] Chelating activity of Lf by which it sequestered iron from the body tissues and resulted in the mitigation of oxidative stress induced apoptosis is significant for its anticancer activity.[102] Different action mechanisms responsible for anticancer activity of Lf are activation of caspases causing induction of apoptosis, cell

immunomodulation, cell cycle arrest, cell membrane modification, cell necrosis, anti-angiogenic action and inhibition of metastasis.[147] Bovine apo- Lf induced apoptosis of HeLa cells by oxygen radical burst and decreased glutathione level.[80]

Enzymatic Activity

Multiple isoforms, degree of glycosylation, tertiary structure (holo- or apo- Lf) and degree of oligomerisation were the main factors that determined that nature of Lf protein and regulated its enzymatic activities .[44] Remarkable similarity existed between some motifs of Lf and ribonuclease A.[44,38] The ribonuclease activity of Lf-α causing RNA hydrolysis varied with the type of RNA (mRNA was the most sensitive while tRNA was the least) whereas Lf- β and Lf- γ isoforms could potentially cause RNA degradation. Among the many milk proteins, Lf had maximum ATPase, DNAse, RNAse and amylase enzyme activities.[63]

Immunostimulatory and Anti-Inflammatory Properties of Lf

Studies done by Bullen and Armstrong in 1979 and Lima and Kierszenbaum in 1987 help to understand the bactericidal activity caused by Lf within the neutrophilic granules occurred through two opposing mechanisms.[28,75] When apo-Lf prevented the growth of phagocytosed bacteria through iron scavenging activity, iron-Lf acted as iron donor to produce antimicrobial action within the phagolysosome of phagocytic cells. The phagocytic cell function was significantly enhanced by Lf.[75] Iron chelating activity of Lf enabled it to cause reduction in the formation of extracellular hydroxyl radicals by activated human polymorphonuclear (PMN) cells and hence facilitate mitigation of oxidation stress.[22] When human milk Lf (at the dose rate of 1-10 µg/mL) was injected into mice, five-fold elevation in humoral immune response (expressed as number of plaque-forming cells) to ovine red blood cells *in vivo* occurred.[149] Even though apo-Lf could overcome the lymphocyte proliferation inhibition caused by 'free' iron, it itself inhibited the proliferation in an iron-bound transferrin form.[103] Lactoferrin was able to significantly enhance the lymphocyte-activated killer (LAK) cell activity, which denoted the cytotoxic activity of NK cells, which in turn was mediated by the lymphocyte fraction of peripheral blood mononuclear cell.[108] Lactoferrin activated the classical pathway of complement by substituting for antibodies. Lactoferrin uptake protected mononuclear phagocytes from free-radical injury of the cell membranes by inhibiting the formation of reactive oxygen species especially hydroxyl radicals.[88] CD4 antigen expression was stimulated by Lf in the Jurkat-lymphoblastic T-cells. This occurred due to the triggering of a transduction pathway by Lf, thereby inducing a cascade of phosphorylation reactions on tyrosine residues of several cellular proteins.[39]

Lactoferrin activated the systemic immune responses against skin allergens by accumulating dendritic cells in the lymph nodes as well as by inhibiting migration of Langerhans cells in a dose dependent manner.[33] It acted as an efficient anti-inflammatory factor by significantly reducing or preventing the secretion of many pro-inflammatory cytokines as well as reactive oxygen species, thus minimizing the tissue damage and modulating innate immunity.[54] The immunomodulatory activity of Lf is mediated by its DNA binding ability to activate various signaling pathways.[71] Oral administration of Lf to mice stimulated the proliferation, differentiation and activation of B- lymphocytes, thereby potentiating the immune responses of T lymphocytes.[106] The surface of various cells of immune system had negative charge that facilitated the binding of positively charged Lf molecule and triggering cell signaling pathways to cause cell proliferation, maturation and differentiation.[33] Iron scavenging activity of Lf helped in the mitigation of free radical injury and oxidative stress caused by reactive oxygen species, which in turn were generated by leukocytes at the nidus of inflammation.[133] Lactoferrin is an innate and acquired immune system modulator.[72] It bind to receptors on the surface of macrophages and T CD^{4+} lymphocytes and activate the macrophages to increase their phagocytic activity as well as increase synthesis of IL-12, thereby attracting more macrophages to inflamed area.[2] Lactoferrin also enhanced antigen presenting activity of B-cells and allowed their interaction with the T cells to potentiate antibody mediated humoral immune response. Lactoferrin also modulated dendritic cell function to cause T- cell activation.[48] Lactoferrin modulated the immune response of gut associated lymphoid tissue (GALT) by triggering the intracellular signaling pathways to cause the activation, proliferation and differentiation of small intestine epithelial cells in a dose-dependent manner.[107] In a murine study, Tomita *et al.* in 2009 found that when Lf was bound to its receptors on the surface of immune cells like dendritic cells and lymphocytes, it activated the systemic response by increasing the population of immune cells like NK, CD4+ and CD8+ cells, and by inducing the secretion of cytokines in the lymph nodes and spleen.[121] It interacted with antigen- presenting cells to cause the intracellular destruction or replication inhibition of microorganisms by facilitating the secretion of cytokines and immune mediators like nitric oxide.[99] When piglets were fed with 3.6 g/L of bLf, it produced elevated serum IgG levels and stimulated spleenocytes to increase the release of IL-10 cytokine into the culture media.[104]

In 2012, Kawashima *et al.* demonstrated the therapeutic application of Lf with regard to Dry Eye Syndrome as it decreased the inflammatory cell infiltration in eye and protected from age-related reduction in the secretory capabilities of lacrimal gland.[64] Feeding piglets with transgenic bovine milk containing recombinant human Lf decreased systemic inflammation by the virtue of decrease in neutrophils and increase in lymphocytes.[31] When bLf was administered orally to piglets, natural killer (NK) cells number was elevated in blood along with NK Lf receptor expression, without causing any cytotoxicity.[78] Lactoferrin

promoted IL-2 and IFN-γ production by TH1 lymphocytes, and inhibited IL-4, IL-5 and IL-13 production by TH2 lymphocytes thereby enabling it to be therapeutically effective against allergic rhinitis.[132] CD4 expression in immature T lymphocytes by Lf was instrumental in the formation of functional CD4+ T lymphocyte subpopulation.[114] A rat model of short bowel syndrome was investigated by Wu *et al.* in 2014 and it was demonstrated that Lf caused up-regulation of sIgA and TJ protein expression in the small intestine. As a result, Lf modulated intestinal barrier integrity and exerted host-protective antibacterial activity.[139] When Lf, fish oil, zinc, vitamin C, lutein, vitamin E, γ-aminobutyric acid (GABA) and *Enterococcus faecium* WB2000 were incorporated to produce a combined dietary supplement, it helped to cure the dry eye syndrome with no side effects.[32] The nutraceutical role of bLf in the distal small intestine as it caused negative feedback of inflammatory response by stimulating B and T cell responses as well as antibodies level in both Peyer's patches and lamina propria.[10] Administration of Lf decreased the expression of pro-inflammatory cytokines like TNF-α and monocyte chemotactic protein-1 (MCP-1) as well as markers for free radical induced oxidative damage.[65]

Cell Growth Promoting Activity

Broxmeyer *et al.* in 1983 reported that Lf lead to down regulation of myelopoiesis.[26] Human Lf possess greater cell growth promoting activity than human transferrin, and it was found to stimulate the proliferation and differentiation of human lymphocytic cell lines.[53] Lactoferrin promoted the synthesis and secretion of nerve growth factor (NGF) in the fibroblast L-M cells of mouse and it also enhanced the proliferation and differentiation of endometrial cell populations.[139,141]

Potential Therapeutic Applications of Lf

Hansen *et al.* in 1975 and Olofsson *et al.* in 1977 reported that the plasma levels of Lf were indicative of neutrophil kinetics or total neutrophil pool in the blood.[52,93] Aguas *et al.* suggested in 1990 that the levels of β and γ isoforms of Lf could be used for the clinical diagnosis of breast cancer.[4] Bovine Lf showed synergy with antibiotic penicillin G against resistant strains of *S. aureus, in vitro*.[40] Being an acute phase protein, the increased levels of lactoferrin in blood plasma might be considered as an indicator of inflammatory Severe Acute Respiratory Syndrome (SARS) or septicemia.[148] Treatment with bovine Lf is highly effective with regard to treatment of recurring *Helicobacter pylori* infections.[122] Lactoferrin could induce delayed hypersensitivity response, by which it enhanced the expression of IL-10 and IL-12 cytokines in the antigen presenting cells (APCs).[137] Hence it augmented the pathology caused by *Mycobacterium tuberculosis* and so was used as an adjuvant for Bacille Calmette- Guerin (BCG) vaccine. Chaneton *et al.* demonstrated in 2008 that *S. aureus* and *E. coli* strains were susceptible to bovine Lf (>2 mg/mL), whereas *S. uberis* strains were resistant.[29] Both Lf and Lfcin, by altering the gene expression, reduces the β-lactamase activity of *S. aureus*.[69] Zhang *et al.* reportedin 2008 that by the way of plasmid-mediated gene transfer technique, bovine lactoferricin prevented mastitis in goats.[147] Lactoferrin promoted the proliferation of lactic acid bowel bacteria (such as *Lactobacillus* and *Bifidobacterium*), and so exerted host defensive role against harmful effects of pathogenic bacteria.[82] Lactoferrin also had the ability to act as a molecular marker and the urinary tract infections could be diagnosed by detection of urinary Lf by using electrochemical sensors.[96] In experimentally tumour induced rats, Xie *et al.* used lactoferrin-conjugated super-paramagnetic iron oxide nanoparticles in 2011 as contrast agent in magnetic resonance imaging (MRI) scanning. By crosslinking tyramine substituted bovine Lf in the presence of horse radish peroxidase (HRP) enzyme and hydrogen peroxide (H_2O_2), bovine Lf could enhance the growth factor production and promoted the proliferation of osteoblasts.[7]

CONCLUSION

Lactoferrin has been the focus of intense research of late. Due to its unique antimicrobial, immunomodulatory, and even antineoplastic properties, lactoferrin seems to have great potential in practical medicine. Nevertheless, much more research and many experiments still need to be carried out in order to obtain a better understanding of its activity and interactions and to enable the full and safe utilization of this glycoprotein.

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Source of Support: Nil, **Conflict of Interest:** None.