IRON BINDING AND RECEPTOR: LACTOFERRIN AND TONB RECEPTORS

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ABSTRACT

In this communication, we discuss about biological, structural and other properties of the lactoferrin (LF). It includes role in iron metabolism, cell proliferation and differentiation. LTF also works as an antibacterial, antiviral and antiparasitic molecule. LF is an iron-binding protein that has been identified in secretions from exocrine glands and in specific granules of neutrophils. On the other hand, TonB-dependent receptors is a family of beta-barrel proteins from the outer membrane of Gram-negative bacteria and work as transporters of iron binding protein like LF across the outer membrane at the expense of energy, which is provided by the inner-membrane anchored TonB complex. LF concentrations have been measured mostly in humans but also in other species like camel, cow etc.

Keywords: LTF, neutrophils, TonB-dependent receptors, beta-barrel proteins, antibacterial, antiviral, antiparasitic

1. Introduction

Lactoferrin (LF) also known as lactotransferrin (LTF), is an iron-binding protein from milk, structurally similar to the transferrins. It is a globular, multifunctional protein with antimicrobial activity (bacteriocide, fungicide) and is part of the innate defense, mainly at mucoses[87]. LF is also present in secondary granules of PMN and also is secreted by some acinar cells. LF can be purified from milk or produced recombinantly. Human colostrum has the highest concentration, followed by human milk, then cow milk. TonB-dependent receptors are a family of beta-barrel proteins from the outer membrane of Gram-negative bacteria. The TonB complex senses signals from outside the bacterial cell and transmits them via two membranes into the cytoplasm, leading to transcriptional activation of target genes. LF is an iron-binding glycoprotein present in various secretions (*e.g.* milk, tears, saliva, pancreatic juice, etc.). It is also stored in specific granules of polymorph nuclear granulocytes from which it is released following activation. LF exerts an antibactericidal activity by damaging the outer membrane of Gram-negative bacteria, immuno-regulatory functions by decreasing the release of interleukin-1 (IL-1), IL-2 and tumor necrosis factor- (TNF-) and enhancing monocyte and natural killer cell cytotoxicity. LF binds with high affinity to lipid A, the toxic moiety of the lipopolysaccharide or endotoxin from Gram-negative bacteria. Lipopolysaccharide interaction with monocytes/macrophages results in the production and release of TNF- that plays an important role in inducing septic shock. In this respect, it has been recently demonstrated that LF inhibits the lipopolysaccharide interaction with CD14 on monocytes/macrophages by competition with the lipopolysaccharide binding protein. Therefore, besides its bactericidal activity, LF may also act by neutralizing the toxic effects of lipopolysaccharide and this protective role against endotoxin lethal shock has been demonstrated in animal models. Moreover, *in vitro* and *in vivo* neutralization of endotoxin by a human LF-derived peptide was also reported and LF or LF-derived peptides could represent useful tools for the treatment of endotoxin-induced septic shock. The recent production and characterization of monoclonal antibodies against different epitopes of human LF, including monoclonal antibodies selectively neutralizing LF binding to lipid A, may allow a better elucidation of the consequence of lactoferrin-lipopolysaccharide interaction. LF is also present in secondary granules of PMN and also is secreted by some acinar cells. LF can be purified from milk or produced recombinantly. Human colostrum ("first milk") has the highest concentration, followed by human milk, then cow's milk[14]. LTF is a glycoprotein, and a member of a transferrin family, thus belonging to those proteins capable of binding and

transferring Fe^{3+} ions[55]. LF was first isolated by Sorensen and Sorensen from bovine milk in 1939 [75]. In 1960, it was concurrently determined to be the main iron binding protein in human milk by three independent laboratories [58]. Subsequent research identified LF in secretions from exocrine glands and in specific granules of neutrophils. Neutrophils after degranulation were observed to be the main source of LF in blood plasma[38]. Due to the increase in its concentration during most inflammatory reactions and some viral infections, several authors classify LF as an acute-phase protein [40]. Its concentration increases in all biological fluids, but the highest levels have been detected in the nidus of inflammation[14]. Thus, LF has a wide variety of biological functions, many of which do not appear to be connected with its iron binding ability[17].

LF is a glycoprotein with a molecular weight of about 80 kDa, which shows high affinity for iron. The molecular structure and amino acid sequence of human LF were discovered in 1984. LF was then classified as a member of the transferrin family, due to its 60% sequence identity with serum transferrin[55]. Three different isoforms of LF have been isolated. Lactoferrin-α is the iron binding form, but has no ribonuclease activity. On the other hand, lactoferrin-β and lactoferrin-γ demonstrate ribonuclease activity but they are not able to bind iron[28]. LF is comprised of a single polypeptide chain containing 703 amino acids folded into two globular lobes. These lobes, also called C – (carboxy) and N – (amino) terminal regions are connected with α-helix. Each lobe consists of two domains known as C1, C2, N1, and N2. The domains create one iron binding site on each lobe. LF molecules contain (according to the species and protein) varying numbers of sites for potential glycosylation, mostly on the surface of the molecule. The most common saccharide is mannose; around 3% are hexoses, and 1% hexosamines. LF's capability of binding iron is two times higher than that of transferrin, which can serve in some cases as donor of Fe^{3+} ions for LF. Two ferric ions can be bound by one LF molecule. One carbonate ion is always bound by LF concurrently with each ferric ion[7,55]. Although this bond is very strong and can resist pH values of as low as 4, its saturation does not exceed 10% in total[54]. There are three forms of LF according to its iron saturation: apolactoferrin (iron free), monoferric form (one ferric ion), and hololactoferrin (binds two Fe^{3+} ions). The tertiary structure in hololactoferrin and apolactoferrin is different [39]. Four amino acid residues are most important for iron binding (histidine, twice tyrosine, and aspartic acid), while an arginine chain is responsible for binding the carbonate ion[84]. Besides iron, LF is capable of binding a large amount of other compounds and substances such as lipopolysacharides, heparin, glycosaminoglycans, DNA, or other metal ions like Al^{3+}, Ga^{3+}, Mn^{3+}, Co^{3+}, Cu^{2+}, Zn^{2+} etc. However, its affinity for these other ions is much lower. Apart from $CO3^{2-}$, LF can bind to a variety of other anions like oxalates, carboxylates and others. In this way it is possible for LF to affect the metabolism and distribution of various substances. The ability to keep iron bound even at low pH is important, especially at sites of infection and inflammation where, due to the metabolic activity of bacteria, the pH may fall under 4.5. In such a situation, LF also binds iron released from transferrin, which prevents its further usage for bacterial proliferation [81].LF expression can first be detected in two- and four-cell embryos during embryonic development, then throughout the blastocyst stage up to implantation. LF cannot be detected from the time of implantation until halfway through gestation. Later, it is found in neutrophils and epithelial cells of forming reproductive and digestive systems[85]. The predominant cell types involved in LF synthesis are of the myeloid series and secretory epithelia[10]. In adults, higher levels of LF are present in milk and colostrum [17]. It is also found in most mucosal secretions such as uterine fluid, vaginal secretion, seminal fluid, saliva, bile, pancreatic juice, small intestine secretions, nasal secretion, and tears[8]. The production of LF by human kidneys was described first by Abrink *et al.* [1]. LF is expressed and secreted throughout the collecting tubules, and in the distal part of the tubules it may be reabsorbed. These results show that the kidney produces LF in a highly ordered manner and that only a minor fraction of this protein is secreted into the urine. Therefore, LF is thought to have important functions in both the immune defence of the urinary tract and in general iron metabolism. Neutrophils are an important source of LF in adults. Indeed, most plasma LF originates from neutrophils[38]. LF is predominantly stored in specific (secondary) granules[6]. However, it can also be found in tertiary granules albeit in significantly lower concentrations[67]. LF is present in blood, plasma or serum in relatively low concentrations[20,71]. Plasma LF concentrations may or may not

correlate with the neutrophil count[10]. This depends on the extent of degranulation and perhaps on the contribution of other organs, such as bone marrow, endometrium[52] and placenta[60]. LF plasma levels change during pregnancy, and vary also with the menstrual cycle[48]. The concentration of LF in the blood increases during infection, inflammation[14], excessive intake of iron, or tumour growth[48].

The regulation of LF synthesis depends on the type of cells producing this protein. The amount of LF synthesized in the mammary gland is controlled by prolactin[30]. Its production in reproductive tissues is determined by estrogens [80]. The synthesis of LF in endometrium is influenced by not only estrogens but also epidermal growth factor[59]. Exocrine glands produce and secrete LF in a continuous manner. In neutrophils, LF is synthesized during their differentiation (when promyelocytes develop into myelocytes) and is afterwards stored in specific granules. Mature neutrophils cease to produce LF [52]. LF levels might vary with gender and age although the results from different studies are inconsistent[3]. LF plasma levels change from the very beginning of pregnancy. There is a progressive rise in its concentration up to the 29th week, after which it settles at a constant level that is higher than the average [78].

The biological properties of LF are mediated by specific receptors on the surface of target cells. These receptors are typical for each cell type and can be found, for example, on mucosal epithelial cells, hepatocytes, monocytes, macrophages, polymorphonuclear leukocytes, lymphocytes, thrombocytes, fibroblasts, and on some bacteria such as *Staphylococcus aureus* or *Pseudomonas hydrophila*[77]. Some cells have also "main receptors", which enable them to bind not only LF, but also transferrin or LF of other species. Besides "classic" receptors, there are also nuclear receptors that bind leukocyte cmDNA [40]. There are two ways in which LF can be eliminated from the organism; either through receptor-mediated endocytosis of phagocytic cells (macrophages, monocytes and other cells belonging to the reticuloendothelial system) with subsequent iron transfer to ferritin or through direct uptake by the liver. Endocytosis performed by Kupffer cells, liver endothelial cells and hepatocytes contribute to LF removal [48]. Kidneys seem to be involved in the removal of LF from the circulation since LF and its fragments, mainly of maternal origin, have been found in the urine of breast-fed infants[37].

In humans, the lactoferrin gene (LTF) is located on chromosome 3; location: 3q21-q23. LF concentration is quite high in milk (up to 10 6 mg/ml), other epithelial secretions, neutrophil granules and blood plasma. It is also a major protein of other barrier liquids such as tears, saliva, and nasal-gland secretions. Lower LF concentrations were found in blood plasma[14]. LF is stored in neutrophil granules, and pathogenic microorganisms stimulate its release [33]. Regulation of LF production depends on the type of LF producing cell. For example, estrogen, regulating LF expression in reproductive tract tissues does not influence LF production by mammary gland cells; prolactin stimulates LF secretion during lactation [30]. The level of these proteins significantly increases during almost all inflammatory processes and some viral diseases; this increase obviously reflects the activation of the body resistance system against these diseases. Increase of LF concentration in blood, tears, and saliva may be used in clinical practice for evaluation of the dynamics of inflammatory processes and the effectiveness of medical treatment. LF level increases when inflammation occurs. In such an environment, iron exchange from transferrin is easier due to the lower pH suggesting that LF may contribute to local iron accumulation at sites of inflammation[18]. LF has long been known to be responsible for hypoferraemia through binding free iron and shuttling it back to macrophages[83].

LF from human milk seems to affect intestinal iron absorption in infants, but it depends on the organisms need for iron. Specific receptors (SI-LfR), present on enterocytes, mediate binding of lactoferrin. After LF is bound to the enterocyte, 90% of it is degraded and Fe^{3+} ions are released. The remaining intact 10% is transported through the cell membrane. A lack of intracellular iron may evoke increased expression of specific receptors on the surface of enterocytes and thereby elevated absorption of LF-bound iron [77]. Breast-fed infants have demonstrated better iron accessibility than babies on formula[27]. Indeed, a possible suppressive effect of LF on absorption is described because higher iron absorption has been reported in infants fed with LF-free human milk. Even though LF does not play the most important role in iron metabolism, its capability of binding Fe^{3+} ions has a significant influence on many of its other biological properties.

Antimicrobial activity: LF also takes part in specific immune reactions, but in an indirect way [46]. Due to its strategic position on the

mucosal surface, LF represents one of the first defence systems against microbial agents invading the organism mostly via mucosal tissues. LF affects the growth and proliferation of a variety of infectious agents including both Gram-positive and negative bacteria, viruses, protozoa, or fungi [42]. Its ability to bind free iron, which is one of the elements essential for the growth of bacteria, is responsible for the bacteriostatic effect of LF[4]. Lack of iron inhibits the growth of iron-dependent bacteria such as *E. coli* [17]. In contrast, LF may serve as iron donor and in this manner support the growth of some bacteria with lower iron demands such as *Lactobacillus* sp. or *Bifid bacterium* sp.[72]. Nevertheless, some bacteria are able to adapt to the new conditions and release siderophores (iron chelating compounds of bacterial origin) that compete with lactoferrin for Fe^{3+} ions [63]. Some other types of bacteria, including *Neisseriaceae* family adapt to new conditions by expressing specific receptors capable of binding LF and to cause changes in the tertiary structure of the LF molecule leading to iron dissociation [25]. Even a bactericidal effect of LF has been described. This bactericidal activity is not iron-dependent and may be mediated through more than one pathway. Receptors for the N-terminal region of LF have been discovered on the surface of some microorganisms. The binding of LF to these receptors induces cell-death in Gram-negative bacteria due to disruption in the cell wall. The subsequent release of lipopolysacharide (LPS) leads to impaired permeability and a higher sensitivity to lysozyme and other antimicrobial agents [47]. LPS can be disposed of even without the direct contact of LF with the cell surface [65]. Bactericidal activity affecting Gram-positive bacteria is mediated by electrostatic interactions between the negatively charged lipid layer and the positively charged LF surface that cause changes in the permeability of the membrane[81].

It has been discovered that lactoferricin, a cationic peptide generated by the pepsin digestion of LF, has more potent bactericidal activity than the native protein. There are two forms known at present: lactoferricin H (derived from human LF) and lactoferricin B (of bovine origin) [12]. As a result of the fusion of secondary granules with phagosomes, LF becomes an iron provider for the catalysis of free radical production and thereby increases the intracellular bactericidal activity of neutrophils [66]. *In vitro* LF is able to prevent *Pseudomonas aeruginosa* biofilm formation. Lack of iron in the environment forces bacteria to move. Therefore, they cannot adhere to surfaces[73]. LF may contribute to defence against the invasion of facultative intracellular bacteria into cells by binding both target cell membrane glycoaminoglycans and bacterial invasions, which prevents pathogen adhesion to target cells. This ability was first reported against entero invasive *E. coli HB 101* and later against *Yersinia enterocolica*, *Yersinia pseudo-tuberculosis*, *Listeria monocytogenes*, *Streptococcus pyogenes*, and *Staphylococcus aureus* [81]. The proteolytic activity of LF is considered to inhibit the growth of some bacteria such as *Shigella flexneri* or enteropathogenic *E.coli* through degrading proteins necessary for colonization. However, this can be disabled by serine protease inhibitors[62,86].

LF is capable of binding to certain DNA and RNA viruses [91]. Nevertheless, its main contribution to antiviral defence consists in its binding to cell membrane glycosaminoglycans. In this manner, LF prevents viruses from entering cells and infection is stopped at an early stage[86]. Such a mechanism has been demonstrated as being effective against the *Herpes simplex* virus[29,51], cytomegaloviruses [2], and the human immunodeficiency virus[34], respectively. LF acts against parasites in various ways. For example, the infectivity of *Toxoplasma gondii* and *Eimeria stiedai* sporozoites is reduced after their incubation with lactoferricin B. It is thought that lactoferricin breaches parasitic membrane integrity causing subsequent changes in interactions between the host and the parasite [61]. The competition for iron between the parasite and LF is the basis of its antiparasitic activity against *Pneumocystis carinii* [21]. In contrast, some parasites such as *Tritrichomonas foetus* are able to use LF as a donor of ferric ions[79].

Due to its iron binding properties and interactions with target cells and molecules, LF can both positively and negatively influence immune system cells and cells involved in the inflammation reaction. In one way, LF may support the proliferation, differentiation and activation of immune system cells and strengthen the immune response. On the other hand, LF acts as an anti-inflammatory factor. LF may prevent the development of inflammation and subsequent tissue damage caused by the release of pro-inflammatory cytokines and reactive oxygen species[46]. The protective effect of LF is manifested in a reduced production of some pro-inflammatory cytokines such as tumor necrosis factor (TNFα**)** or interleukins IL-1β and

IL-6 [35, 50]. An increased amount of anti-inflammatory interleukin IL-10 has also been reported in several cases. Iron is essential as a catalyst for the production of reactive oxygen species. Therefore, LF can diminish the harmful influence of reactive oxygen species produced by leukocytes at the sites of inflammation [86]. There are conflicting views regarding the influence of LF on lymphocyte proliferation. While Esaguy *et al.,*[26] report a stimulatory effect; Ashorn *et al.,* [5] and Richie *et al.,*[64] suggest an inhibitory role.

LF has even been reported to inhibit the development of experimental metastases in mice [89]. LF is able to halt the growth of human mammary gland carcinoma cells between the G1 and S stage. Such a negative effect on cell proliferation may be ascribed to the altered expression or activity of regulatory proteins [23]. The LF-dependent, cytokine-mediated stimulation of activity of NK cells and lymphocytes CD4+ and CD8+, represents an important factor in defence against tumor growth. There is an increased number of these cells both in blood and lymphatic tissue after the oral administration of LF. Damiens *et al.*[23] opine that smaller concentrations of LF (10 µg/ml) stimulate the cytolysis of tumor cells, whereas cytolysis seems to be dependent on the cell phenotype at 100 µg/ml. Very high doses may reduce the cytotoxic activity of NK cells. The result of LF influence on tumor cells is equal to the sum of NK cell activation and sensitivity of target cells to lysis.

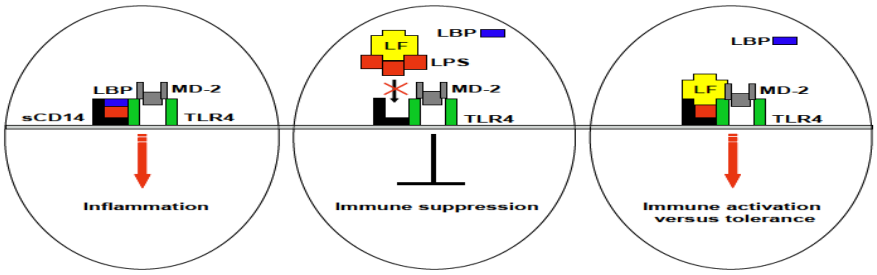
In the past, LF was thought to support cell proliferation due to its ability to transport iron into cells. However, LF was later proven to act as a growth factor activator. The effect of LF alone on small intestine epithelial cells is more potent than that of the epidermal growth factor [31]. LF alone (without the presence of any other cytokines and factors) is able to stimulate the proliferation of endometrium stroma cells [90]. LF has also been identified as a transcription factor. It is able to penetrate a cell and activate the transcription of specific DNA sequences[36].

LF has been identified as a potent anabolic factor affecting osteocytes. LF stimulates osteoblast proliferation, enhances thymidine incorporation into osteocytes and reduces apoptosis of osteoblasts by 50–70%. A similar effect was also recorded in chondrocytes [22]. LF reduces or even inhibits osteoclastogenesis in a concentration-dependent fashion. On the other hand, LF shows no influence on the bone resorption performed by mature osteoclasts [49]. Besides direct influence, LF may affect bone cells through the inhibition of osteolytic cytokines such as TNFα or IL-1β, whose levels rise during inflammation. Thus, LF contributes to the stabilization of the osseous tissue. Because of these aforementioned properties, LF might be potentially useful in the treatment of diseases such as osteoporosis in the future[22].

A remarkable similarity in some motifs between LF and ribonuclease A has been revealed. LF is indeed, capable of RNA hydrolysis. The ribonuclease activity varies depending on the type of RNA. mRNA is the most sensitive to LF, whereas tRNA is the least. The non-iron-binding isoforms of LF seem to be responsible for RNA degradation[22]. As mentioned, LF was discovered first in bovine and later in human milk. Most research has been carried out in the human field, followed by bovine milk. In other animal species, information regarding LF levels is very scarce. Different methods have been used to either detect or even measure LF. The relationships between LF concentrations and gender, age or inflammatory processes have been examined with contradictory results. LF concentrations in adult human blood were reported to be in the range of 0.02–1.52 µg/ml depending on the method used. Human LF venous plasma, colostrum and milk concentrations were determined to be 0.12 µg/ml, 3.1–6.7 mg/ml, and 1.0–3.2 mg/ml, respectively [48]. A wide range of LF concentrations have been determined in healthy bovine milk. The values vary from 1.15 µg/ml[31] to 485.63 µg/ml in milk from healthy animals. LF was shown to be significantly associated with the stage of lactation (r = 0.557) and daily milk production (r = –0.472) [19]. Its concentration increased many times (even to 100 mg/ml) during mammary gland involution [88]. LF levels in mare colostrum, in the serum of newborns, and in three day old foals were also measured. The obtained results were 21.7 µg/ml, 0.249 µg/ml, and 0.445 µg/ml, respectively [9]. The mean LF concentration was reported to be 0.229 ± 0.135 mg/ml in the camel[43,] Previously, it had been thought that canine milk did not contain any LF [52]. However, Berlov *et al.*[13] succeeded in detecting LF in canine milk. The concentration was lower (40 µg/ml) than in human milk. Coincidently, Sinkora *et al.,*[74] were able to detect LF in canine, swine and bovine neutrophils using flow cytometry and commercially available rabbit anti-human polyclonal antisera. Nevertheless, much 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. According to Latorre *et al.,*[44], LF is a natural defence component of the innate immunity only found in mammals. This exclusive characteristic has suggested that this molecule could be involved in newborn nutrition and protection. These multiple functions rely not only on the capacity to sequester iron, but also on its property to interact with molecular and cellular components of both host and pathogens, including endo-toxins and their receptors. In this respect, the ability of LF to bind LPS or limit its *in vitro* interaction with LBP and sCD14 suggests that LF behaves as a versatile molecule by efficiently suppressing endotoxin-induced excessive immune reaction in sepsis or promoting, in particular conditions, a protective response against pathogen challenge (Figure 1.1).

Figure1.1: Lactoferrin interplay on LPS-induced inflammatory response.



A schematic representation of LF interaction with LPS highlighting the multitasking strategy of LF to maintain immune homeostasis. LF behaves as a versatile molecule by efficiently suppressing endotoxin-induced excessive immune reaction in sepsis or promoting, in particular conditions, a protective response against pathogen challenge.

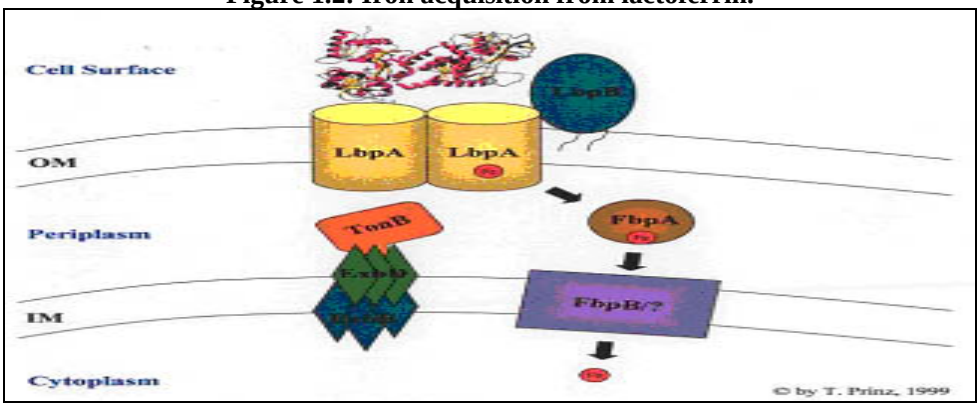
According to Legrand and Mazurier[46], LF possesses pleiotropic roles which turn it into either a weapon or a shield in the host defence system. The beneficial effects of LF administered in prevention or treatment of infectious pathologies have led to many applications for health. These applications have emphasized the importance of LF in the regulation of immunity but most of them poorly account for the actual activities of host-expressed LF in this control. It appears that LF acts as an anti-microbial and antioxidant molecule, not only through direct interactions with microbes or through its iron-binding capability, but also by stimulating the migration and functions of cells of the innate and adaptive immunities. The immuno-modulatory properties of LF are mainly related to its PAMPs (mostly LPS) binding ability which generally turns LF into an anti-inflammatory molecule able to protect the host from harmful immune responses. Conversely, it may be hypothesized that LF also acts as a vector of PAMPs for immune cell activation. Other putative mechanisms, which still need further investigations, would require signalling or nuclear targeting following interactions with multifunctional or specific cell membrane receptors.

Mucosal surfaces are able to use human LF as a source of iron for growth, a presumed adaptation to the high levels of LF and low levels of free iron in these environments[56]. These pathogens were also able to use human transferrin as a source of iron for growth[57] that clearly would be advantageous during invasive infection. The discovery of surface receptor proteins in these species that specifically bound host transferrin or LF[45,68,69] is a critical step in the subsequent characterization of the iron acquisition pathways and ultimately provided avenues for exploring the prevalence of these pathways in other species and their role in survival in the host.

The relatively high pI of LF initially complicated the identification and characterization of the LF receptor proteins due to their propensity for nonspecific interactions. Solid phase binding assays and affinity capture experiments were routinely performed under high pH and high salt conditions to reduce nonspecific interactions and as a consequence some interactions were overlooked. The initial identification of the LF receptor from *N. meningitidis* described a single 100 kDa protein[68]; the integral outer membrane protein is now termed LF binding protein A (LbpA). Similar observations were made when isolating LF receptors from other species[70]. The

lipoprotein component of the receptor, now termed LbpB, was only identified by affinity capture when alternate conditions for affinity isolation were explored[16], but resulted in an increased propensity for nonspecific background. Analogous to most transferrin receptors, genes encoding the lactoferrin receptor proteins *lbpA* and *lbpB*, are organized in an operon with *lbpB* preceding lbpA [92] with a third gene of unknown function included in the operon in *M. catarrhalis*[16]. The ability to use LF as an iron source is abolished by mutation of the gene encoding the energy transducing protein TonB[76], the periplasmic iron binding protein FbpA[41] or the integral membrane protein LbpA but not the surface lipoprotein LbpB[16]. Thus, the pathway for iron acquisition from LF is illustrated in Figure 1.2, and resembles other TonB-dependent pathways for acquiring iron from transferrin, iron siderophore complexes or B12 (conalbumin).

Figure 1.2: Iron acquisition from lactoferrin.



The lactoferrin receptor is composed of two proteins, the integral OMP LbpA and the lipoprotein LbpB. After binding of lactoferrin to the receptor, iron is released from lactoferrin and transported across the outer membrane at the expense of energy, which is provided by the inner-membrane anchored TonB complex. The iron is bound by the periplasmic FbpA protein and further transported across the inner membrane by an ABC transporter. Both LbpA and LbpB are vaccine candidates

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