

Lactoferrin: a review

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ABSTRACT: This review discusses the biological properties of the glycoprotein lactoferrin. Lactoferrin has been identified in secretions from exocrine glands and in specific granules of neutrophils. After degranulation, neutrophils become the main source of lactoferrin in blood plasma. Lactoferrin possesses various biological functions, including roles in iron metabolism, cell proliferation and differentiation, and antibacterial, antiviral, and antiparasitic activity. Many of these functions do not appear to be connected with its iron binding ability. Of late, lactoferrin concentrations have been measured mostly in humans but also in some other species. However, the relationship between its concentration and physiological or pathological effects on body functions is not yet well characterised.

Keywords: protein; glycoprotein; transferrin; milk; neutrophils; leukocytes; host defense; antimicrobial

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1. Introduction

Lactoferrin (formerly known as lactotransferrin) is a glycoprotein, and a member of a transferrin family, thus belonging to those proteins capable of binding and transferring Fe³⁺ ions (Metz-Boutique et al., 1984).

Lactoferrin was first isolated by Sorensen and Sorensen from bovine milk in 1939. In 1960 it

was concurrently determined to be the main iron binding protein in human milk by three independent laboratories (Groves, 1960; Johanson, 1960; Montreuil et al., 1960).

Subsequent research identified lactoferrin in secretions from exocrine glands and in specific granules of neutrophils. Neutrophils after degranulation were observed to be the main source of

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lactoferrin in blood plasma (Iyer and Lonnerdal, 1993).

Due to the increase in its concentration during most inflammatory reactions and some viral infections, several authors classify lactoferrin as an acute-phase protein (Kanyshkova et al., 2001). Its concentration increases in all biological fluids, but the highest levels have been detected in the nidus of inflammation (Birgens, 1985).

Thus, lactoferrin has a wide variety of biological functions, many of which do not appear to be connected with its iron binding ability (Brock, 2002).

2. Structure and properties of lactoferrin

Lactoferrin 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 lactoferrin were discovered in 1984. Lactoferrin was then classified as a member of the transferrin family, due to its 60% sequence identity with serum transferrin (Metz-Boutique et al., 1984).

Three different isoforms of lactoferrin 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 (Furmanski et al., 1989).

Lactoferrin 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 a 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. Lactoferrin 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. The degree of glycosylation varies and determines the rate of resistance to proteases or to very low pH.

Lactoferrin's capability of binding iron is two times higher than that of transferrin, which can serve in some cases as donor of Fe³⁺ ions for lactoferrin. Two ferric ions can be bound by one lactoferrin molecule. One carbonate ion is always bound by lactoferrin concurrently with each ferric ion (Aisen and Liebman, 1972; Metz-Boutique et al., 1984; Baker, 1994). Although this bond is very

strong and can resist pH values of as low as 4, its saturation does not exceed 10% in total (Mazurier and Spik, 1980). There are three forms of lactoferrin according to its iron saturation: apolactoferrin (iron free), monoferric form (one ferric ion), and hololactoferrin (binds two Fe³⁺ ions). The tertiary structure in hololactoferrin and apolactoferrin is different (Jameson et al., 1998).

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 (Baker, 1994; Ward et al., 1996).

Besides iron lactoferrin is capable of binding a large amount of other compounds and substances such as lipopolysaccharides, heparin, glycosaminoglycans, DNA, or other metal ions like Al³⁺, Ga³⁺, Mn³⁺, Co³⁺, Cu²⁺, Zn²⁺ etc. However, its affinity for these other ions is much lower. Apart from CO₃²⁻, lactoferrin can bind a variety of other anions like oxalates, carboxylates, and others. In this way it is possible for lactoferrin to affect the metabolism and distribution of various substances (Baker, 1994).

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 lactoferrin also binds iron released from transferrin, which prevents its further usage for bacterial proliferation (Valenti and Antonini, 2005).

Lactoferrin has demonstrated remarkable resistance to proteolytic degradation by trypsin and trypsin-like enzymes. The level of resistance is proportional to the degree of iron saturation (Brock et al., 1976; Brines and Brock, 1983; Iyer and Lonnerdal, 1993).

3. Sources of lactoferrin in the organism

Lactoferrin expression can first be detected in two- and four-cell embryos during embryonic development, then throughout the blastocyst stage up to implantation. Lactoferrin 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 (Ward et al., 1999).

The predominant cell types involved in lactoferrin synthesis are of the myeloid series and secretory

epithelia (Baynes and Bezwoda, 1994). In adults, higher levels of lactoferrin are present in milk and colostrum (Masson and Heremans, 1971; Brock, 1980). 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 (Masson et al., 1966; Baker, 1994; Levay and Viljoen, 1995; Lonnerdal and Iyer, 1995; Kikuchi et al., 2003; Baker and Baker, 2005).

The production of lactoferrin by human kidneys was described by Abrink et al. (2000). Lactoferrin 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 lactoferrin in a highly ordered manner and that only a minor fraction of this protein is secreted into the urine. Therefore, lactoferrin is thought to have important functions in both the immune defense of the urinary tract and in general iron metabolism.

Neutrophils are an important source of lactoferrin in adults. Indeed, most plasma lactoferrin originates from neutrophils (Iyer and Lonnerdal, 1993). Lactoferrin is predominantly stored in specific (secondary) granules (Baggiolini et al., 1970). However, it can also be found in tertiary granules albeit in significantly lower concentrations (Saito et al., 1993).

Lactoferrin is present in blood, plasma or serum in relatively low concentrations (Rumke et al., 1971; Boxer et al., 1982; Brown et al., 1983; Broxmeyer et al., 1983; Otnaess et al., 1983; Chung et al., 1985; Scott, 1989). The quite remarkable differences between the results (varying from 0.02 µg/ml to 1.52 µg/ml in blood) published by these authors are probably caused by the use of different analytical methods, the type of anticoagulant, variations in iron saturation of lactoferrin, spontaneous polymerization, and by the interval between sample collection and analysis or by storage (Levay and Viljoen, 1995).

Plasma lactoferrin concentrations may or may not correlate with the neutrophil count (Hansen et al., 1975; Olofsson et al., 1977; Baynes et al., 1986). This depends on the extent of degranulation and perhaps on the contribution of other organs, such as bone marrow, endometrium (Masson et al., 1968) and placenta (Niemela et al., 1989). Lactoferrin plasma levels change during pregnancy, and vary also with the menstrual cycle (Sykes et al., 1982; Levay and Viljoen, 1995). The concentration of lactoferrin in

the blood increases during infection, inflammation (Birgens, 1985), excessive intake of iron, or tumor growth (Levay and Viljoen, 1995).

4. Regulation of lactoferrin synthesis

The regulation of lactoferrin synthesis depends on the type of cells producing this protein. The amount of lactoferrin synthesized in the mammary gland is controlled by prolactin (Green and Pastewka, 1978), whereas its production in reproductive tissues is determined by estrogens (Pentecost and Teng, 1987; Walmer et al., 1992; Teng et al., 2002). The synthesis of lactoferrin in endometrium is influenced by not only estrogens but also epidermal growth factor (Nelson et al., 1991). Exocrine glands produce and secrete lactoferrin in a continuous manner. In neutrophils, lactoferrin is synthesized during their differentiation (when promyelocytes develop into myelocytes) and is afterwards stored in specific granules. Mature neutrophils cease to produce lactoferrin (Masson et al., 1969).

Lactoferrin levels might vary with gender and age although the results from different studies are inconsistent (Bennett and Mohla, 1976; Bezwoda et al., 1985; Antonsen et al., 1993).

Lactoferrin 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 (Sykes et al., 1982). There are several factors which may cause this increase: leukocytosis associated with pregnancy, the selective increase of lactoferrin in neutrophil granules (Oberg et al., 1983), or other organs like endometrium, decidua, and mammary glands may all contribute (Levay and Viljoen, 1995).

Serum lactoferrin concentrations have been detected as being higher in the proliferative phase of a menstrual cycle than in the secretory phase (Kelder et al., 1996).

5. Lactoferrin receptors

The biological properties of lactoferrin 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,

trombocytes, fibroblasts, and on some bacteria such as *Staphylococcus aureus* or *Pseudomonas hydrophila* (Levay and Viljoen, 1995; Suzuki and Lonnerdal, 2002; Suzuki et al., 2005). Some cells have also “main receptors”, which enable them to bind not only lactoferrin, but also transferrin or lactoferrins of other species. Besides “classic” receptors, there are also nuclear receptors that bind leukocyte cmDNA (Kanyshkova et al., 2001)

6. Lactoferrin metabolism

There are two ways in which lactoferrin 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 contributes to lactoferrin removal (Levay and Viljoen, 1995). Kidneys seem to be involved in the removal of lactoferrin from the circulation since lactoferrin and its fragments, mainly of maternal origin, have been found in the urine of breast-fed infants (Hutchens et al., 1991).

7. Biological functions of lactoferrin

7.1. Lactoferrin and iron metabolism

Although the influence of lactoferrin on iron distribution in an organism is implied by its resemblance to transferrin, it has thus far not been unequivocally proven that lactoferrin plays an important role in iron transport. This may be due to the fact that lactoferrin plasma concentrations are very low under normal conditions. On the other hand, the lactoferrin level increases when inflammation occurs. In such an environment iron exchange from transferrin is easier due to the lower pH suggesting that lactoferrin may contribute to local iron accumulation at sites of inflammation (Brock, 2002). Lactoferrin has long been known to be responsible for hypoferraemia through binding free iron and shuttling it back to macrophages (Van Snick et al., 1974).

The relationship between biliary lactoferrin concentration and the iron status of the body has been described in rabbits. A significant increase of

lactoferrin in bile was registered in anemic rabbits after acute blood loss, an observation which may be explained by the mobilization of iron stored in liver. In contrast, rabbits to who iron was administered, even in low doses, showed inhibition of lactoferrin secretion in the bile. Thus, lactoferrin might have a control function in situations when increased amounts of iron are released from its depots (Van Vugt et al., 1975). A similar relationship between lactoferrin from the duodenal secretion and iron metabolism was found in humans (De Vet and Van Gool, 1974).

Lactoferrin 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 lactoferrin 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 lactoferrin-bound iron (Suzuki et al., 2005). Breast-fed infants have demonstrated better iron accessibility than babies on formula (Fairweather-Tait et al., 1987). Counter to this, some research fails to identify a positive effect of lactoferrin on iron absorption in the intestines. Indeed, a possible suppressive effect of lactoferrin on absorption is described because higher iron absorption has been reported in infants fed lactoferrin-free human milk (Davidsson et al., 1994).

Even though lactoferrin 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.

7.2. Antimicrobial activity

Lactoferrin is considered to be a part of the innate immune system. At the same time, lactoferrin also takes part in specific immune reactions, but in an indirect way (Legrand et al., 2005). Due to its strategic position on the mucosal surface lactoferrin represents one of the first defense systems against microbial agents invading the organism mostly via mucosal tissues. Lactoferrin affects the growth and proliferation of a variety of infectious agents including both Gram-positive and negative bacteria, viruses, protozoa, or fungi (Kirkpatrick et al., 1971).

7.3. Antibacterial activity

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 lactoferrin (Arnold et al., 1980). A lack of iron inhibits the growth of iron-dependent bacteria such as *E. coli* (Brock, 1980). In contrast, lactoferrin may serve as iron donor, and in this manner support the growth of some bacteria with lower iron demands such as *Lactobacillus* sp. or *Bifidobacterium* sp., generally considered as beneficial (Petschow et al., 1999; Sherman et al., 2004).

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 (Crosa, 1989; Ratledge and Dover, 2000). Some other types of bacteria, including Neisseriaceae family, adapt to new conditions by expressing specific receptors capable of binding lactoferrin, and to cause changes in the tertiary structure of the lactoferrin molecule leading to iron dissociation (Schryvers et al., 1998; Ekins et al., 2004).

Even a bactericidal effect of lactoferrin 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 lactoferrin have been discovered on the surface of some microorganisms. The binding of lactoferrin to these receptors induces cell-death in Gram-negative bacteria due to a disruption in the cell wall. The subsequent release of lipopolysaccharide (LPS) leads to impaired permeability and a higher sensitivity to lysozyme and other antimicrobial agents (Arnold et al., 1977; Yamauchi et al., 1993; Leitch and Willcox, 1998). LPS can be disposed of even without the direct contact of lactoferrin with the cell surface (Rossi et al., 2002). Bactericidal activity affecting Gram-positive bacteria is mediated by electrostatic interactions between the negatively charged lipid layer and the positively charged lactoferrin surface that cause changes in the permeability of the membrane (Valenti and Antonini, 2005).

It has been discovered that lactoferricin, a cationic peptide generated by the pepsin digestion of lactoferrin, has more potent bactericidal activity than the native protein. There are two forms known at present: lactoferricin H (derived from human lactoferrin) and lactoferricin B (of bovine origin) (Bellamy et al., 1992).

As a result of the fusion of secondary granules with phagosomes, lactoferrin becomes an iron provider for the catalysis of free radical production and thereby increases the intracellular bactericidal activity of neutrophils (Sanchez et al., 1992).

In vitro lactoferrin is able to prevent *Pseudomonas aeruginosa* biofilm formation. The lack of iron in the environment forces bacteria to move. Therefore, they cannot adhere to surfaces (Singh et al., 2002).

Lactoferrin may contribute to defense against the invasion of facultative intracellular bacteria into cells by binding both target cell membrane glycoaminoglycans and bacterial invasins, which prevents pathogen adhesion to target cells. This ability was first reported against enteroinvasive *E. coli* HB 101 and later also against *Yersinia enterocolica*, *Yersinia pseudotuberculosis*, *Listeria monocytogenes*, *Streptococcus pyogenes*, and *Staphylococcus aureus* (Valenti and Antonini, 2005).

The proteolytic activity of lactoferrin 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 (Orsi, 2004; Ward et al., 2005)

7.4. Antiviral activity

Lactoferrin is capable of binding certain DNA and RNA viruses (Yi et al., 1997). Nevertheless, its main contribution to antiviral defense consists in its binding to cell membrane glycosaminoglycans. In this manner lactoferrin prevents viruses from entering cells and infection is stopped at an early stage (Ward et al., 2005). Such a mechanism has been demonstrated as being effective against the Herpes simplex virus (Fujihara and Hayashi, 1995; Marchetti et al., 1996), cytomegaloviruses (Andersen et al., 2001), and the human immunodeficiency virus (Harmsen et al., 1995), respectively.

7.5. Antiparasitic activity

Lactoferrin 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 (Omata et al., 2001). The

competition for iron between the parasite and lactoferrin is the basis of its antiparasitic activity against *Pneumocystis carinii* (Cirioni et al., 2000). In contrast, some parasites such as *Tritrichomonas foetus* are able to use lactoferrin as a donor of ferric ions (Tachezy et al., 1996).

7.6. Lactoferrin and host defense

Due to its iron binding properties and interactions with target cells and molecules, lactoferrin can both positively and negatively influence immune system cells and cells involved in the inflammation reaction. In one way, lactoferrin may support the proliferation, differentiation, and activation of immune system cells and strengthen the immune response. On the other hand, lactoferrin acts as an anti-inflammatory factor. Thanks to its antimicrobial activity and capability of binding components of bacterial cell walls (LPS) or their receptors, lactoferrin may prevent the development of inflammation and subsequent tissue damage caused by the release of pro-inflammatory cytokines and reactive oxygen species (Legrand et al., 2005).

The protective effect of lactoferrin is manifested in a reduced production of some pro-inflammatory cytokines such as tumor necrosis factor (TNF α) or interleukins IL-1 β and IL-6 (Machnicki et al., 1993; Haversen et al., 2002). 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, lactoferrin can diminish the harmful influence of reactive oxygen species produced by leukocytes at the sites of inflammation (Ward et al., 2005).

There are conflicting views regarding the influence of lactoferrin on lymphocyte proliferation. While Esaguy et al. (1991) report a stimulatory effect, Ashorn et al. (1986) and Richie et al. (1987) suggest an inhibitory role.

7.7. Lactoferrin and tumor growth

The protective character of lactoferrin has on numerous occasions been demonstrated on chemically induced tumors in laboratory rodents. Lactoferrin has even been reported to inhibit the development of experimental metastases in mice (Bezault et al., 1994; Wang et al., 2000; Wolf et al., 2003).

Lactoferrin 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 (Damiens et al., 1999).

The lactoferrin-dependent, cytokine-mediated stimulation of activity of NK cells and lymphocytes CD4+ and CD8+, represents an important factor in defense against tumor growth. There are an increased number of these cells both in blood and lymphatic tissue after the oral administration of lactoferrin. According to Damiens et al. (1998), smaller concentrations of lactoferrin (10 μ g/ml) stimulate the cytolysis of tumor cells, whereas cytolysis seems to be dependent on the cell phenotype in higher concentrations (100 μ g/ml). Very high doses may reduce the cytotoxic activity of NK cells. The result of lactoferrin influence on tumor cells is equal to the sum of NK cell activation and sensitivity of target cells to lysis.

Lactoferrin-mediated inhibition of tumor growth might be related to apoptosis of these cells induced by the activation of the Fas signaling pathway. Nevertheless, the exact mechanism of this function has not been discovered so far (Fujita et al., 2004).

7.8. Lactoferrin and cell proliferation and differentiation

In the past, lactoferrin was thought to support cell proliferation thanks to its ability to transport iron into cells. However, lactoferrin was later proven to act as a growth factor activator. The effect of lactoferrin alone on small intestine epithelial cells is more potent than that of the epidermal growth factor (Hagiwara et al., 1995). Lactoferrin alone (without the presence of any other cytokines and factors) is able to stimulate the proliferation of endometrium stroma cells (Yanaihara et al., 2000). Lactoferrin has also been identified as a transcription factor. It is able to penetrate a cell and activate the transcription of specific DNA sequences (He and Furmanski, 1995).

7.9. Lactoferrin and bones

Lactoferrin has been identified as a potent anabolic factor affecting osteocytes. Lactoferrin stimulates osteoblast proliferation, enhances thymidine incorporation into osteocytes, and reduces apoptosis of

osteoblasts by 50–70%. A similar effect was also recorded in chondrocytes (Cornish et al., 2004).

Lactoferrin reduces or even inhibits osteoclastogenesis in a concentration-dependent fashion. On the other hand, lactoferrin shows no influence on the bone resorption performed by mature osteoclasts (Lorget et al., 2002).

Besides direct influence, lactoferrin may affect bone cells through the inhibition of osteolytic cytokines such as TNF α or IL-1 β , whose levels rise during inflammation. Thus, lactoferrin contributes to the stabilization of the osseous tissue.

Because of these aforementioned properties, lactoferrin might be potentially useful in the treatment of diseases such as osteoporosis in the future (Cornish et al., 2004).

7.10. Enzymatic activity of ribonuclease A

A remarkable similarity in some motifs between lactoferrin and ribonuclease A has been revealed and lactoferrin is, indeed, capable of RNA hydrolysis. The ribonuclease activity varies depending on the type of RNA. mRNA is the most sensitive to lactoferrin, whereas tRNA is the least. The non-iron-binding isoforms of lactoferrin seem to be responsible for RNA degradation (Furmanski et al., 1989; Devi et al., 1994).

8. Lactoferrin in different species

As mentioned, lactoferrin was discovered first in bovine and later in human milk. Most research has been carried out in the human field, followed by work on bovine milk. In other animal species, information regarding lactoferrin levels is very scarce. Different methods have been used to either detect or even measure lactoferrin. The relationships between lactoferrin concentrations and gender, age or inflammatory processes have been examined with contradictory results. Lactoferrin concentrations in adult human blood were reported to be in the range of 0.02–1.52 $\mu\text{g/ml}$ depending on the method used. Human lactoferrin venous plasma, colostrum and milk concentrations were determined to be 0.12 $\mu\text{g/ml}$, 3.1–6.7 mg/ml , and 1.0–3.2 mg/ml , respectively (Levay and Viljoen, 1995).

A quite wide range of lactoferrin concentrations has been determined in healthy bovine milk. The

values vary from 1.15 $\mu\text{g/ml}$ (Hagiwara et al., 2003), to 485.63 $\mu\text{g/ml}$ in milk from healthy animals. Lactoferrin was shown to be significantly associated with the stage of lactation ($r = 0.557$) and daily milk production ($r = -0.472$) (Cheng et al., 2008). Its concentration increased many times (even to 100 mg/ml) during mammary gland involution (Welty et al., 1976).

Lactoferrin levels in mare colostrum, in the serum of newborns, and in three day old foals were also measured. The obtained results were 21.7 $\mu\text{g/ml}$, 0.249 $\mu\text{g/ml}$, and 0.445 $\mu\text{g/ml}$, respectively (Barton et al., 2006). The mean milk lactoferrin concentration was reported to be $0.229 \pm 0.135 \text{ mg/ml}$ in the camel (Konuspayeva et al., 2007).

Previously, it had been thought that canine milk did not contain any lactoferrin (Masson and Heremans, 1971). However, in 2007, Berlov et al. succeeded in detecting lactoferrin in canine milk. The concentration was lower (40 $\mu\text{g/ml}$) than in human milk. Coincidentally Sinkora et al. (2007) were able to detect lactoferrin in canine, swine and bovine neutrophils using flow cytometry and commercially available rabbit anti-human polyclonal antisera.

9. Conclusions

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 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.

10. REFERENCES

- Abrink M., Larsson E., Gobl A., Hellman L. (2000): Expression of lactoferrin in the kidney: implications for innate immunity and iron metabolism. *Kidney International*, 57, 2004–2010.
- Aisen P., Liebman A. (1972): Lactoferrin and transferrin: a comparative study. *Biochimica et Biophysica Acta*, 257, 314–323.
- Andersen J.H., Osbakk S.A., Vorland L.H., Traavik T., Gutteberg T.J. (2001): Lactoferrin and cyclic lactoferricin inhibit the entry of human fibroblasts. *Antiviral Research*, 51, 141–149.

- Antonsen S., Wiggers P., Dalhoj J., Blaabjerg O. (1993): An enzyme linked-immunosorbent assay for plasma-lactoferrin. Concentrations in 362 healthy, adult blood donors. *Scandinavian Journal of Clinical and Laboratory Investigation*, 53, 133–144.
- Arnold R.R., Cole M.F., McGhee J.R. (1977): A bactericidal effect for human lactoferrin. *Science*, 197, 263–265.
- Arnold R.R., Brewer M., Gauthier J.J. (1980): Bactericidal activity of human lactoferrin: sensitivity of a variety of microorganisms. *Infection and Immunity*, 28, 893–898.
- Ashorn R.G., Eskola J., Tuohimaa P.J., Krohn K.J. (1986): Effect of the progesterone-inducible proteins human lactoferrin and chicken avidin on lymphocyte proliferation. *Human Reproduction*, 1, 149–151.
- Baggiolini M., De Duve C., Masson P.L., Heremans J.F. (1970): Association of lactoferrin with specific granules in rabbit heterophil leukocytes. *The Journal of Experimental Medicine*, 131, 559–570.
- Baker E.N. (1994): Structure and reactivity of transferins. *Advances in Inorganic Chemistry*, 41, 389–463.
- Baker E.N., Baker H.M. (2005): Molecular structure, binding properties and dynamics of lactoferrin. *Cellular and Molecular Life Sciences*, 62, 2531–2539.
- Barton M.H., Hurley D., Norton N., Heusner G., Costa L., Jones S., Byars D., Watanabe K. (2006): Serum lactoferrin and immunoglobulin G concentrations in healthy or ill neonatal foals and healthy adult horses. *Journal of Veterinary Internal Medicine*, 20, 1457–1462.
- Baynes R.D., Bezwoda W.R. (1994): Lactoferrin and the inflammatory response. *Advances in Experimental Medicine and Biology*, 357, 133–141.
- Baynes R., Bezwoda W., Bothwell T., Khan Q., Mansoor N. (1986): The non-immune inflammatory response: serial changes in plasma iron, iron-binding capacity, lactoferrin, ferritin and C reactive protein. *Scandinavian Journal of Clinical and Laboratory Investigation*, 46, 695–704.
- Bellamy W., Takase M., Yamauchi K., Wakabayashi H., Kawase K., Tomita M. (1992): Identification of the bactericidal domain of lactoferrin. *Biochimica et Biophysica Acta*, 1121, 130–136.
- Bennett R.M., Mohla C. (1976): A solid-phase radio-immunoassay for the measurement of lactoferrin in human plasma: variations with age, sex and disease. *The Journal of Laboratory and Clinical Medicine*, 88, 156–166.
- Berlov M.N., Korobleva E.S., Andreeva Y.V., Ovchinnikova T.V., Korkyakov V.N. (2007): Lactoferrin from canine neutrophils: isolation and physicochemical and antimicrobial properties. *Biochemistry (Moscow)*, 72, 445–451.
- Bezault J., Bhimani R., Wiprovnick J., Furmanski P. (1994): Human lactoferrin inhibits growth of solid tumors and development of experimental metastases in mice. *Cancer Research*, 54, 2310–2312.
- Bezwoda W.R., Baynes R.D., Khan Q., Mansoor N. (1985): Enzyme linked immunosorbent assay for lactoferrin. Plasma and tissue measurements. *Clinica Chimica Acta; International Journal of Clinical Chemistry*, 151, 61–69.
- Birgens H.S. (1985): Lactoferrin in plasma measured by an ELISA technique: evidence that plasma lactoferrin is an indicator of neutrophil turnover and bone marrow activity in acute leukaemia. *Scandinavian Journal of Haematology*, 34, 326–331.
- Boxer L.A., Coates T.D., Haak R.A., Wolach J.B., Hoffstein S., Baehner R.L. (1982): Lactoferrin deficiency associated with altered granulocyte function. *The New England Journal of Medicine*, 307, 404–410.
- Brines R.D., Brock J.H. (1983): The effect of trypsin and chymotrypsin on the *in vitro* antibacterial and iron-binding properties of lactoferrin in human milk and bovine colostrum. Unusual resistance of human apolactoferrin to proteolytic digestion. *Biochimica et Biophysica Acta*, 759, 229–235.
- Brock J.H. (1980): Lactoferrin in human milk: its role in iron absorption and protection against enteric infection in the newborn infant. *Archives of Disease in Childhood*, 55, 417–421.
- Brock J.H. (2002): The physiology of lactoferrin. *Biochemistry and Cell Biology*, 80, 1–6.
- Brock J.H., Arzabe F., Lampreave F., Pineiro A. (1976): The effect of trypsin on bovine transferrin and lactoferrin. *Biochimica et Biophysica Acta*, 446, 214–225.
- Brown R.D., Rickard K.A., Kronenberg H. (1983): Immunoradiometric assay of plasma lactoferrin. *Pathology*, 15, 27–31.
- Broxmeyer H.E., Gentile P., Bognacki J., Ralph P. (1983): Lactoferrin, transferrin and acidic isoferritins: regulatory molecules with potential therapeutic value in leukemia. *Blood Cells*, 9, 83–105.
- Cheng J.B., Wang J.Q., Bu D.P., Liu G.L., Zhang C.G., Wei H.Y., Zhou L.Y., Wang J.Z. (2008): Factors affecting the lactoferrin concentration in bovine milk. *Journal of Dairy Science*, 91, 970–976.
- Chung S., Hayward C., Brock D.J.H., Van Heyningen V. (1985): A monoclonal antibody-based immunoassay for human lactoferrin. *Journal of Immunological Methods*, 84, 135–141.
- Cirioni O., Giacometti A., Barchiesi F., Scalise G. (2000): Inhibition of growth of *Pneumocystis carinii* by lactoferrins alone and in combination with pyrimethamine, clarithromycin and minocycline. *The Journal of Antimicrobial Chemotherapy*, 46, 577–582.

- Cornish J., Callon K.E., Naot D., Palmano K.P., Banovic T., Bava U., Watson M., Lin J.M., Tong P.C., Chen Q., Chan V.A., Reid H.E., Fazzalari N., Baker H.M., Baker E.N., Haggararty N.W., Grey A.B., Reid I.R. (2004): Lactoferrin is a potent regulator of bone cell activity and increases bone formation *in vivo*. *Endocrinology*, 145, 4366–4374.
- Crosa J.H. (1989): Genetics and molecular biology of siderophore-mediated iron transport in bacteria. *Microbiological Reviews*, 53, 517–530.
- Damiens E., Mazurier J., El Yazidi I., Masson M., Duthille I., Spik G., Boilly-Marier Y. (1998): Effects of human lactoferrin on NK cell cytotoxicity against haematopoietic and epithelial tumour cells. *Biochimica et Biophysica Acta*, 1402, 277–287.
- Damiens E., El Yazidi I., Mazurier J., Duthille I., Spik G., Boilly-Marier Y. (1999): Lactoferrin inhibits G1 cyclin-dependent kinases during growth arrest of human breast carcinoma cells. *Journal of Cellular Biochemistry*, 74, 486–498.
- Davidsson L., Kastenmayer P., Yuen M., Lonnerdal B., Hurrell R.F. (1994): Influence of lactoferrin on iron absorption from human milk in infants. *Pediatric Research*, 35, 117–124.
- De Vet B.J., Van Gool J. (1974): Lactoferrin and iron absorption in the small intestine. *Acta Medica Scandinavica*, 196, 393–402.
- Devi A.S., Das M.R., Pandit M.W. (1994): Lactoferrin contains structural motifs of ribonuclease. *Biochimica et Biophysica Acta*, 1205, 275–281.
- Ekins A., Khan A.G., Shouldice S.R., Schryvers A.B. (2004): Lactoferrin receptors in gram-negative bacteria: insights into the iron acquisition process. *Biometals*, 17, 235–243.
- Esaguy N., Aguas A.P., Vilanova M., Silva M.T. (1991): Activation of human neutrophils by phorbol ester decreases the cytoplasm compactness and the lactoferrin content of the granulocytes. *Journal of Leukocyte Biology*, 50, 444–452.
- Fairweather-Tait S.J., Balmer S.E., Scott P.H., Minski M.J. (1987): Lactoferrin and iron absorption in newborn infants. *Pediatric Research*, 22, 651–654.
- Fujihara T., Hayashi K. (1995): Lactoferrin inhibits herpes simplex virus type-1 (HSV-1) infection to mouse cornea. *Archives of Virology*, 140, 1469–1472.
- Fujita K., Matsuda E., Sekine K., Iigo M., Tsuda H. (2004): Lactoferrin enhances Fas expression and apoptosis in the colon mucosa of azoxymethane-treated rats. *Carcinogenesis*, 25, 1961–1966.
- Furmanski P., Li Z.P., Fortuna M.B., Swamy C.V.B., Das M.R. (1989): Multiple molecular forms of human lactoferrin. Identification of a class of lactoferrins that possess ribonuclease activity and lack iron-binding capacity. *The Journal of Experimental Medicine*, 170, 415–429.
- Green M.R., Pastewka J.V. (1978): Lactoferrin is a marker for prolactin response in mouse mammary explants. *Endocrinology*, 103, 1510–1513.
- Groves M.L. (1960): The isolation of a red protein from milk. *Journal of the American Chemical Society*, 82, 3345–3350.
- Hagiwara T., Shinoda I., Fukuwatari Y., Shimamura S. (1995): Effect of lactoferrin and its peptides on proliferation of rat intestinal epithelial cell line, IEC-18, in the presence of epidermal growth factor. *Bioscience, Biotechnology, and Biochemistry*, 59, 1875–1881.
- Hagiwara S., Kawai K., Anri A., Nagahata H. (2003): Lactoferrin concentrations in milk from normal and subclinical mastitic cows. *The Journal of Veterinary Medical Science*, 65, 319–323.
- Hansen N.E., Malmquist J., Thorell J. (1975) Plasma myeloperoxidase and lactoferrin measured by radioimmunoassay: relations to neutrophil kinetics. *Acta Medica Scandinavica*, 198, 437–443.
- Harmsen M.C., Swart P.J., De Bethune M.P., Pauwels R., De Clercq E., The T.H., Meijer D.K.F. (1995): Antiviral effects of plasma and milk proteins: lactoferrin shows potent activity against both human immunodeficiency virus and human cytomegalovirus replication *in vitro*. *The Journal of Infectious Diseases*, 172, 380–388.
- Haversen L., Ohlsson B.G., Hahn-Zoric M., Hanson L.A., Mattsby-Baltzer I. (2002): Lactoferrin down-regulates the LPS-induced cytokine production in monocytic cells via NF-kappa B. *Cellular Immunology*, 220, 83–95.
- He J., Furmanski P. (1995): Sequence specificity and transcriptional activation in the binding of lactoferrin to DNA. *Nature*, 373, 721–724.
- Hutchens T.W., Henry J.F., Yip T.T., Hachey D.L., Schanler R.J., Motil K.J., Garza C. (1991): Origin of intact lactoferrin and its DNA-binding fragments found in the urine of human milk-fed preterm infants. Evaluation by stable isotopic enrichment. *Pediatric Research*, 29, 243–250.
- Iyer S., Lonnerdal B. (1993): Lactoferrin, lactoferrin receptors and iron metabolism. *European Journal of Clinical Nutrition*, 47, 232–241.
- Jameson G.B., Anderson B.F., Norriss G.E., Thomas D.H., Baker E.N. (1998): Structure of human apolactoferrin at 2.0 Å resolution. Refinement and analysis of ligand-induced conformational change. *Acta Crystallographica. Section D, Biological Crystallography*, 54, 1319–1335.

- Johanson B. (1960): Isolation of an iron containing red protein from human milk. *Acta Chemica Scandinavica*, 14, 510–512.
- Kanyshkova T.G., Buneva V.N., Nevinsky G.A. (2001): Lactoferrin and its biological functions. *Biochemistry (Moscow)*, 66, 1–7.
- Kelver M.E., Kaul A., Nowicki B., Findley W.E., Hutchens T.W., Nagamami M. (1996): Estrogen regulation of lactoferrin in human endometrium. *American Journal of Reproductive Immunology*, 36, 234–247.
- Kikuchi M., Mizoroki S., Kubo T., Ohiwa Y., Kubota M., Yamada N., Orino K., Ohnami Y., Watanabe K. (2003): Seminal plasma lactoferrin but not transferrin reflects gonadal function in dogs. *The Journal of Veterinary Medical Science*, 65, 679–684.
- Kirkpatrick C.H., Green I., Rich R.R., Schade A.L. (1971): Inhibition of growth of *Candida albicans* by iron-unsaturated lactoferrin: relation to host-defense mechanisms in chronic mucocutaneous candidiasis. *The Journal of Infectious Diseases*, 124, 539–544.
- Konuspayeva G., Faye B., Loiseau G., Levieux D. (2007): Lactoferrin and immunoglobulin contents in camel's milk (*Camelus bactrianus*, *Camelus dromedarius*, and hybrids) from Kazakhstan. *Journal of Dairy Science*, 90, 38–46.
- Legrand D., Ellass E., Carpentier M., Mazurier J. (2005): Lactoferrin: a modulator of immune and inflammatory responses. *Cellular and Molecular Life Sciences*, 62, 2549–2559.
- Leitch E.C., Willcox M.D. (1998): Synergic antistaphylococcal properties of lactoferrin and lysozyme. *Journal of Medical Microbiology*, 47, 837–842.
- Levy P.F., Viljoen M. (1995): Lactoferrin: a general review. *Haematologica*, 80, 252–267.
- Lonnerdal B., Iyer S. (1995): Lactoferrin: molecular structure and biological function. *Annual Review of Nutrition*, 15, 93–110.
- Lorget F., Clough J., Oliveira M., Daury M.C., Sabokbar A., Offord E. (2002): Lactoferrin reduces *in vitro* osteoclast differentiation and resorbing activity. *Biochemical and Biophysical Research Communications*, 296, 261–266.
- Machnicki M., Zimecki M., Zagulski T. (1993): Lactoferrin regulates the release of tumour necrosis factor alpha and interleukin 6 *in vivo*. *International Journal of Experimental Pathology*, 74, 433–439.
- Marchetti M., Longhi C., Conte M.P., Pisani S., Valenti P., Seganti L. (1996): Lactoferrin inhibits herpes simplex virus type 1 adsorption to Vero cells. *Antiviral Research*, 29, 221–231.
- Masson P.L., Heremans J.F. (1971): Lactoferrin in milk from different species. *Comparative Biochemistry and Physiology. B, Comparative Biochemistry*, 39, 119–129.
- Masson P.L., Heremans J.F., Dive C. (1966): An iron-binding protein common to many external secretions. *Clinica Chimica Acta*, 14, 735–739.
- Masson P.L., Heremans J.F., Ferin J. (1968): Presence of an iron-binding protein (lactoferrin) in the genital tract of the human female. I. Its immunohistochemical localization in the endometrium. *Fertility and Sterility*, 19, 679–689.
- Masson P.L., Heremans J.F., Schonke E. (1969): Lactoferrin, an iron-binding protein in neutrophilic leukocytes. *The Journal of Experimental Medicine*, 130, 643–658.
- Mazurier J., Spik G. (1980): Comparative study of the iron-binding properties of human transferrins. I. Complete and sequential iron saturation and desaturation of the lactotransferrin. *Biochimica et Biophysica Acta*, 629, 399–408.
- Metz-Boutique M.H., Jolles J., Mazurier J., Schoentgen F., Legrand D., Spik G., Montreuil J., Jolles P. (1984): Human lactotransferrin: amino acid sequence and structural comparisons with other transferrins. *European Journal of Biochemistry*, 145, 659–676.
- Montreuil J., Tonnelat J., Mullet S. (1960): Preparation and properties of lactosiderophilin (lactotransferrin) of human milk. *Biochimica et Biophysica Acta*, 45, 413–421.
- Nelson K.G., Takahashi T., Bossert N.L., Walmer D.K., McLachlan J.A. (1991): Epidermal growth factor replaces estrogen in the stimulation of female genital-tract growth and differentiation. *Proceedings of the National Academy of Sciences of the United States of America*, 88, 21–25.
- Niemela A., Kulomaa M., Vija P., Tuohimaa P., Saarikoski S. (1989): Lactoferrin in human amniotic fluid. *Human Reproduction*, 4, 99–101.
- Oberg G., Lindmark G., Moberg L., Venge P. (1983): The peroxidase activity and cellular content of granule proteins in PMN during pregnancy. *British Journal of Haematology*, 55, 701–708.
- Olofsson T., Olsson I., Venge P., Elgefors B. (1977): Serum myeloperoxidase and lactoferrin in neutropenia. *Scandinavian Journal of Haematology*, 18, 73–80.
- Omata Y., Satake M., Maeda R., Saito A., Shimazaki K., Yamauchi K., Uzuka Y., Tanabe S., Sarashina T., Mikami T. (2001): Reduction of the infectivity of *Toxoplasma gondii* and *Eimeria stiedai* sporozoites by treatment with bovine lactoferricin. *The Journal of Veterinary Medical Science*, 63, 187–190.
- Orsi N. (2004): The antimicrobial activity of lactoferrin: current status and perspectives. *Biometals*, 17, 189–196.

- Otnaess A.B.K., Meberg A., Sande H.A. (1983): Plasma lactoferrin measured by an enzyme-linked immunosorbent-assay (ELISA). Measurements on adult and infant plasma. *Scandinavian Journal of Haematology*, 31, 235–240.
- Pentecost B.T., Teng C.T. (1987): Lactotransferrin is the major estrogen inducible protein in mouse uterine secretions. *The Journal of Biological Chemistry*, 262, 10134–10139.
- Petschow B.W., Talbott R.D., Batema R.P. (1999): Ability of lactoferrin to promote the growth of *Bifidobacterium* spp. *in vitro* is independent of receptor binding capacity and iron saturation level. *Journal of Medical Microbiology*, 48, 541–549.
- Ratledge C., Dover L.G. (2000): Iron metabolism in pathogenic bacteria. *Annual Review of Microbiology*, 54, 881–941.
- Richie E.R., Hilliard J.K., Gilmore R., Gillespie D.J. (1987): Human milk-derived lactoferrin inhibits mitogen and alloantigen induced human lymphocyte proliferation. *Journal of Reproductive Immunology*, 12, 137–148.
- Rossi P., Giansanti F., Boffi A., Ajello M., Valenti P., Chiancone E., Antonini G. (2002): Ca^{2+} binding to bovine lactoferrin enhances protein stability and influences the release of bacterial lipopolysaccharide. *Biochemistry and Cell Biology*, 80, 41–48.
- Rumke P., Visser D., Kwa H.G., Hart A.A. (1971): Radioimmuno assay of lactoferrin in blood plasma of breast cancer patients, lactating and normal women; prevention of false high levels caused by leakage from neutrophil leucocytes *in vitro*. *Folia Medica Neerlandica*, 14, 156–168.
- Saito N., Takemori N., Hirai K., Onodera R., Watanabe S., Namiki M. (1993): Ultrastructural localization of lactoferrin in the granules other than typical secondary granules of human neutrophils. *Human Cell*, 6, 42–48.
- Sanchez L., Calvo M., Brock J.H. (1992): Biological role of lactoferrin. *Archives of Disease in Childhood*, 67, 657–661.
- Schryvers A.B., Bonnah R., Yu R.H., Wong H., Retzer M. (1998): Bacterial lactoferrin receptors. *Advances in Experimental Medicine and Biology*, 443, 123–133.
- Scott P.H. (1989): Enzyme immunoassay of lactoferrin in newborn term infants: reference values and influence of diet. *Annals of Clinical Biochemistry*, 26, 407–411.
- Sherman M.P., Bennett S.H., Hwang F.F., Yu C. (2004): Neonatal small bowel epithelia: enhancing anti-bacterial defense with lactoferrin and *Lactobacillus* GG. *Biometals*, 17, 285–289.
- Singh P.K., Parsek M.R., Greenberg E.P., Welsh M.J. (2002): A component of innate immunity prevents bacterial biofilm development. *Nature*, 417, 552–555.
- Sinkora J., Samankova P., Kummer V., Leva L., Maskova J., Rehakova Z., Faldyna M. (2007): Commercially available rabbit anti-human polyclonal antisera as a useful tool for immune system studies in veterinary species. *Veterinary Immunology and Immunopathology*, 119, 156–162.
- Sorensen M., Sorensen S.P.L. (1939): The proteins in whey. *Comptes-rendus des Travaux du Laboratoire Carlsberg*, 23, 55–99.
- Suzuki Y.A., Lonnerdal B. (2002): Characterization of mammalian receptors for lactoferrin. *Biochemistry and Cell Biology*, 80, 75–80.
- Suzuki Y.A., Lopez V., Lonnerdal B. (2005): Mammalian lactoferrin receptors: structure and function. *Cellular and Molecular Life Sciences*, 62, 2560–2575.
- Sykes J.A., Thomas M.J., Goldie D.J., Turner G.M. (1982): Plasma lactoferrin levels in pregnancy and cystic fibrosis. *Clinica Chimica Acta*, 122, 385–393.
- Tachezy J., Kulda J., Bahnikova I., Suchan P., Razga J., Schrevel J. (1996): *Tritrichomonas foetus*: iron acquisition from lactoferrin and transferrin. *Experimental Parasitology*, 83, 216–228.
- Teng C.T., Beard C., Gladwell W. (2002): Differential expression and estrogen response of lactoferrin gene in the female reproductive tract of mouse, rat and hamster. *Biology of Reproduction*, 67, 1439–1449.
- Valenti P., Antonini G. (2005): Lactoferrin: an important host defense against microbial and viral attack. *Cellular and Molecular Life Sciences*, 62, 2576–2587.
- Van Snick J.L., Masson P.L., Heremans J.F. (1974): The involvement of lactoferrin in the hyposideremia of acute inflammation. *The Journal of Experimental Medicine*, 140, 1068–1084.
- Van Vugt H., Van Gool J., Ladiges N.C., Boers W. (1975): Lactoferrin in rabbit bile: its relation to iron metabolism. *Quarterly Journal of Experimental Physiology and Cognate Medical Sciences*, 60, 79–88.
- Walmer D.K., Wrona M.A., Hughes C.L., Nelson K.G. (1992): Lactoferrin expression in the mouse reproductive tract during the natural estrous cycle: Correlation with circulating estradiol and progesterone. *Endocrinology*, 131, 1458–1466.
- Wang W.P., Iigo M., Sato J., Sekine K., Adachi I., Tsuda H. (2000): Activation of intestinal mucosal immunity in tumor-bearing mice by lactoferrin. *Japanese Journal of Cancer Research*, 91, 1022–1027.
- Ward P.P., Zhou X., Conneely O.M. (1996): Cooperative interactions between the amino- carboxyl-terminal lobes contribute to the unique iron-binding stability

- of lactoferrin. *The Journal of Biological Chemistry*, 271, 12790–12794.
- Ward P.P., Mendoza-Meneses M., Mulac-Jericevic B., Cunningham G.A., Saucedo-Cardenas O., Teng C.T., Conneely O.M. (1999): Restricted spatiotemporal expression of lactoferrin during murine embryonic development. *Endocrinology*, 140, 1852–1860.
- Ward P.P., Paz E., Conneely O.M. (2005): Multifunctional roles of lactoferrin: a critical overview. *Cellular and Molecular Life Sciences*, 62, 2540–2548.
- Welty F.K., Smith K.L., Schanbacher F.L. (1976): Lactoferrin concentration during involution of the bovine mammary gland. *Journal of Dairy Science*, 59, 224–231.
- Wolf J.S., Li D., Taylor R.J., O'Malley B.W. Jr. (2003): Lactoferrin inhibits growth of malignant tumors of the head and neck. *ORL; Journal for Oto-Rhino-Laryngology and Its Related Specialties*, 65, 245–249.
- Yamauchi K., Tomita M., Giehl T.J., Ellison R.T. 3rd (1993): Antibacterial activity of lactoferrin and pepsin-derived lactoferrin peptide fragment. *Infection and Immunity*, 61, 719–728.
- Yanaihara A., Toma Y., Saito H., Yanaihara T. (2000): Cell proliferation effect of lactoferrin in human endometrial stroma cells. *Molecular Human Reproduction*, 6, 469–473.
- Yi M., Kaneko S., Yu D.Y., Murakami S. (1997): Hepatitis C virus envelope proteins bind lactoferrin. *Journal of Virology*, 71, 5997–6002.

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