Sky Journal of Food Science Vol. 6(2), pp. 014 - 020, March, 2017
Available online http://www.skyjournals.org/SJFS

Full Length Research Paper

Review study on lactoferrin: A multifunctional protein

Saima Nazir[1], Muhammad Nasir[2], Ammara Yasmeen[1*] and Shumaila Usman[1]

[1]Food and Biotechnology Research Centre, PCSIR Labs Complex, Ferozpur Road Lahore.
[2]Departement of Food Science & Human Nutrition, University of Veterinary and Animal Sciences, Lahore.

Accepted 19 December, 2016

Lactoferrin is a glycoprotein of 703 amino acids originally isolated from milk. Lactoferrin exists in body fluids and can be found in the form of free iron, monoferric and diferic form. Scientist also found lactoferrin in the secretions of mammal's pancreas, and in secondary neutrophil granules. Lactoferrin has wide range of functions which may range from binding of Lactoferrin with iron, in control of the availability of an enhanced immune system and as a strong bacteriostatic agent, but the exact mechanism of lactoferrin is still not clear.

Key words: Antimicrobial, lactoferrin, iron-binding protein, iron metabolism.

INTRODUCTION

Lactoferrin is an iron –binding protein which was first isolated in 1939 from bovine milk (Sørensen et al., 1939) and from human milk in 1960 (Johansen, 1960). Lactoferrin is found in high concentrations in breast milk and to some extend in smaller amounts in exocrine fluids such as mucosal secretions, bile, intestinal secretions, pancreatic juice (Ashida et al., 2004). Lactoferrin belongs to a family of proteins known as transferrin (Metz-Boutigue et al., 1984). Its most important characteristic is the development of an intense red colour when incubated in the presence of Fe^{3+} ions, which proves that LF is an analog of serum iron binding protein. Traditionally, it was considered as iron transporting protein of milk having bacteriostatic characters. The purpose of this paper is to review the structure and functions of Lactoferrin focusing on its antimicrobial properties.

Structure of lactoferrin

Human lactoferrin contains about 703 amino acid which can be resolved by chemical method (Metz-Boutigue et al., 1984) and cDNA cloning (Powell et al., 1990; Rey et al., 1990). HoloLactoferrin is a single polypeptide chain folded into two globular lobes, the N- and C- terminal each with one iron-binding site (Querinjean et al., 1971).

*Corresponding author. E-mail: ammara_ft@yahoo.com.

The structure of hLF has been determined by X-ray diffraction at 2.2A (Baker et al., 1998). There is internal homology between the N- and C- lobes at 1-338 and 339-703 which demonstrate 125 (or 37%) identical amino acid residues in the corresponding portions (Peter et al., 1995). This give rise to the theory of gene duplication which results in the formation of two lobes (domains) and giving rise to a family of proteins having molecular masses in the range of 80 KDa (Bullen et al., 1987). The isoelectric point of lactoferrin is 8.7A and is a glycoprotein (Furmanski et al., 1989). Lactoferrin exist in various isoforms three such isoforms, two with RNase activity (termed Lactoferrin–β and Lactoferrrin –γ) and one which is without RNase activity (termed Lactoferrin-$\propto$) have been isolated, all these forms can be isolated from human breast milk and in granulocytes. These isoforms have same chemical, physical and antigenic characteristics, But different greatly in functional properties.

Similarities between LF and other transferrin

All members of transferrin family have same polypeptide folding structure (Baker et al., 1993). All members of transferring family have iron binding and transport properties. Lactoferrin and transferrin are closely related to each other by amino acid composition with 59% and 49% homology between the domains of these molecules

(Bluard et al., 1974). The secondary and tertiary structures are also similar. Crystallographic studies reveal that upon iron-binding conformational changes occur in all members of transferrin family (Ward et al., 2005).

Transferrin can exist in any of four molecular forms (Makey et al., 1976; Makino et al., 1991) apotransferrin, monoferric transferring, either in the A- or B- forms, and diferric transferrin. The molecular mass of transferring decrease as the degree of iron saturation increases which proves that the binding of iron to transferring induces a conformational change which leads to closed iron-binding domain.

Some differences may exist between the lactoferrin and transferring. The inter lobe connecting peptide is helical in lactoferrin but in transferring it is irregular. Some key properties also differentiate lactoferrin from other transferring. Transferrin is primarily present in bloodstream and basically its function is to deliver iron to cells (Octave *et al.*, 1983), While LF is found in exocrine secretions. Lactoferrin and transferring differs in surface properties.

Surface properties and metabolism of lactoferrin

The major functions of LF depend on the ability of LF to bind to other macromolecules such as proteins and DNA. These functions in turn depend on the surface properties of LF. LF has cationic nature with high isoelectric point and this property differentiates LF from other members of transferring family. There are three notable concentrations of positive charge (a) – on N- terminus (residues 1-7), (b)-along the outside of the first helix (residues 13-30) and (c) in the inter lobe region, close to the connecting helix, this distribution of charge in LF is highly uneven. In human lactoferrin the first helix (residues 12-13) forms the basic and major part of the bactericidal domain (Bellamy *et al.*, 1992), identified as the lactoferricin domain (Gifford et al., 2005). The Lfcn peptide, when released by proteolysis of the intact proteins, is a potent bactericidal agent (Senkovich et al., 2007), probably because it's able to form amphipathic structures that disrupt cell membranes (Gifford et al., 2005). This region is a key factor in the antibacterial activity of LF by disruption of cell membrane. This bactericidal domain can also act as binding surface.

According to Lyer 1993 lactoferrin is produced in neutrophils and as well in iron depleted state. It proved that the steroid thyroid receptor super family works in concert to modulate lactoferrin gene expression. This led to formulation of hypothesis that levels of lactoferrin in cells are hormone dependent. After production lactoferrin transfer to its storage granules is dependent on acidification mechanism and occur through Golgi apparatus (Olsson et al., 1988). The neutrophil lactoferrin can be secreted in two way it can either be secreted into the surrounding tissues or blood (Van et al., 1974) or the granules can fuse with phagosomes (Maher et al., 1993). Degranulation factor affects the amount of LF secretion from the polymorph nuclear cells which in turn depends on the activation of guanylate cyclase, cGMP and protein kinase C (calcium dependent). It can be stimulated by interleukin – 8 and surface bound IgG and occurs in both aerobic and anaerobic conditions (Kahler et al., 1988). Usually lactoferrin plasma levels increases during infectious diseases, tumor progression, iron overload and inflammation (Kolb et al., 1989).

After the release of lactoferrin, that connects the metal ions, extracting lactoferrin occurs in one of two ways. Lactoferrin can be removed first from the areas of intraregional traffic, through what appears to be a receptor-mediated endocytosis in Phagocytic cells, with subsequent transfer of ferritin iron (Aolovson et al., 1977).

Degradation of lactoferrin

Lactoferrin can be removed by receptor – mediated endocytosis into phagocytic cells such as macrophages, monocytes and other cells of the reticuloendothelial system (Olofsson et al., 1977; Van et al., 1974; Van et al, 1976). Another way of lactoferrin removal is direct uptake by the liver, involving liver endothelial cells, hepatocytes and kupffer cell (Hu et al., 1993).

Lactoferrin levels in various organs

Lactoferrin is present in plasma in relatively low concentration with higher level being found in colostrum's, human breast milk, and seminal plasma. Plasma lactoferrin is derived from neutrophil (Bullen et al., 1987). In granules its presence can be used to identify these granules. The number of neutrophils did not affect the plasma concentration of LF indirectly depending on how granularity and contribution of other organs such as the bone marrow and lining of the uterus and placenta, and plasma lactoferrin content (Baynes et al., 1986; Scott 1989; Mason et al., 1968).

Lactoferrin has been found in human milk and colostrum at concentration of 1 and 7 mg/ml, respectively, and in bovine milk mid lactation at levels of 0.1 mg/ml (Martur et al., 1990), 0.5 mg/ml in pooled pulmonary secretions and more than 14 mg/ml in infected parotid fluid (Brogan et al., 1975; Tabak et al., 1978). Lactoferrin plasma levels change during pregnancy as maternal plasma lactoferrin levels manifest as a progressive rise in concentration, with stabilization at week 29 of pregnancy (Sykes et al., 1982).

Pregnancy is associated with increased number of leukocytes (Andrews et al., 1951) Lactoferrin production during pregnancy may be affected by hormones (Mason and Taylor, 1979).

Lactoferrin is present in the milk of all mammalian species except dog and rat (Masson and Heremans, 1971). Human milk contains highest levels of lactoferrin as compared to bovine milk. Approximately 30% of the iron in human milk is bound to lactoferrin (Goldsmith et al., 1982). Iron body status in human milk does not dependent on body iron status, but on the general state of maternal nourishment. In malnourished mothers levels of lactoferrin are lower. LF levels in breast milk are not affected by the continuous (Houghton et al., 1985). LF preterm colostrum at first and then increased concentration of colostrum production period (Hirai's et al., 1990) decreases over.

Lactoferrin levels in amniotic fluid were found to be undetectable before the 20^{th} week of pregnancy (Masson et al., 1968). A significant increase occur around week 30, and then it remains high untill term. Lactoferrin of amniotic fluid is of decidual origin (Masson et al., 1968). Amniotic lactoferrin concentrations have highest reported levels after those of colostrums, milk, tears and seminal plasma.

Lactoferrin production in the fetus depends on gestational age and was found by immunohisto-chemical detection, from 13^{th} weeks onwards (Reitamo et al., 1981). Some of the fetal lactoferrin comes from amniotic fluid, which has significantly higher lactoferrin levels than either fetal or maternal sera. Lactoferrin cannot cross the placenta. This is strongly demonstrated by the back of correlation between maternal and neonatal lactoferrin concentrations (Guttenberg et al., 1986).

Functions of lactoferrin

The exact role and mechanism of action of Lactoferrin has not clearly known. Lactoferrin play a role in the host defense mechanism as well as in iron metabolism. In host defense mechanism it acts as a bacteriostatic agent. LF and it also has a bactericidal effect against fungi and viruses can spread. It also improves immunity. Lactoferrin is known to have a tendency to bind to a number of other molecules or silent receptors. Other features as possible in a normal cell growth regulation function, coagulation, including modulation of cell adhesion.

Role in iron metabolism

As iron absorption from breast milk is high, iron status of breast fed infants is usually satisfactory upto at least 6 months and a major part of iron in human milk is bound to LF, it was earlier suggested that LF facilitates iron absorption in breast fed infants (Fairweather et al., 1987). Lactoferrin from maternal milk is known to be absorbed in the intact form from the gut of infants (Hutchens et al., 1991). A maximum concentration of lactoferrin and bovine milk in humans than in the observation of the greater availability of iron lactoferrin in breast-fed infants can promote the absorption of iron that led to the hypothesis. However, infants fed formula supplemented with bovine lactoferrin ferrous sulfate formula of several studies regarding the iron status showed no benefit. In several reports, among others, support this hypothesis.

i.) The ability of human enterocytes to extract iron from LF (Masson *et al.*, 1971).
ii.) The high Lactoferrin uptake by enterocytes (Masson *et al.*, 1971).
iii.) The correlation between neonatal urinary iron excretion with milk Lactoferrin content as well as with breast milk uptake (Masson and Heremans 1971).
iv.) The transport of iron across the intestinal brush border by lactoferrin.
v.) The accumulation of iron from Lactoferrin in brush border membrane vesicles (Davidson et al., 1998).

The major role of lactoferrin in iron metabolism would appear to be in the control of iron availability. LF may perhaps affect cellular mechanism through its influence on iron availability. Iron is known to affect a host of cell functions such as DNA, and to a lesser extent RNA and protein synthesis, the expression of lymphocyte surface markers, immunoglobulin secretion, interleukin-2 receptor expression and many others (Machnicki 1991).

Antimicrobial effects

Lactoferrin was earlier shown to have bacteriostatic activity against pathogens such as Escherichia coli (Bullen et al., 1972). The exceptionally, strong iron binding activity ($K_{ass}^{=} 10^{24}$) of LF allows it to compete with bacteria for iron, thereby causing inhibition of their growth. Recently growth of enterobacter sakazakii, a food borne pathogen which is known to cause diarrhea in infants, was shown to be inhibited by iron unsaturated lactoferrin (apolactoferrin), but not by hololactoferrin showing that the iron sequestering capacity of LF was responsible for the activity (Wakabayashi et al., 2008). It has also been reported that by direct bactericidal activity, LF can kill effectively a wide variety of pathogens such as vibrio cholera (Amold et al., 1980). Two cationic peptides, called Lactoferrin molecule (Wakabayashi et al., 2003) and lactoferrampin (Haney et al., 2009), respectively, have been shown to have strong antimicrobial activity in cell and animal models. Lactoferrin also show strong antiviral activity against several viruses such as cytomegalovirus (CMV), Hepatitis C Virus (HCV), Herpes simplex virus (HSV), rotavirus, adenovirus and HIV (Valenti et al., 2005). The technique behind this is not yet understood but a hypothesis exist that LF binds to bacterial receptors or mammalians cells, and blocking adhesion of the pathogens to host cells. In a recent study (Wakabayashi et al., 2003) on young children

hospitalized with acute diarrhea, oral rehydration solution with recombinant human Lactoferrin and lysozyme significantly reduced diarrhea duration, diarrhea volume, and recurrence of diarrhea. Three recent clinical studies support that Lactoferrin may prevent infections in children. A study (Haney et al., 2009) on Japanese children showed that daily supplementation with 100mg bovine Lactoferrin resulted in significantly lower frequency and duration of vomiting and diarrhea as compared with the placebo group, although no difference in rotavirus gastroenteritis was detected.

Lactoferrin has also been shown to prevent biofilm formation. This biological function relates to the ability of inhibiting microbes from adhering colonizing and forming biofilm on host cells. Which is a crucial step in the development and persistence of infection (Singh et al., 2002) showed that iron sequestration by LF – inhibited biofilm formation by pseudomonas aeruginosa in continuously cultured mammalian cells by stimulating a bacterial motion called twiching. This motion prevents bacteria from attaching to the surface of mammalian cells and ultimately forming biofilms. This activity of LF was observed even at a very low LF concentration (20 µg/ml), which is much less than the concentration required for bacteriostatic activity.

Lactoferrin exerts its antibacterial effects by means of different mechanisms which are as:

i.) It is an iron-binding protein from iron available which limits the amount of free iron available. Iron is an essential growth factor for microorganisms (Otto *et al.*, 1992).
ii.) It is capable of destabilizing the other membrane of gram negative bacteria (Ellison et al., 1988).
iii.) Liberation of bactericidal peptides.
iv.) Glycans of bovine LF inhibits the binding of gram-negatgive bacteria to cells (Teraguchi *et al.*, 1996).

It is known that Lactoferrin and as well the peptide which is derived from bovine and lactoferrin B inhibit the growth of fungi (Bellamy et al., 1993; Vorland et al., 1998; Wakabayashi et al., 1996). The active components of Lactoferrin are assumed to be Lactoferricin (Wakabayashi et al., 1996). The mechanism of action of the lactoferrin related substances has not been fully elucidated. It has been shown that Lactoferricin B directly binds to Candida cells (Bellamy et al., 1993), and is highly effective in disrupting the cell membrane of Candida (Wakabayashi et al., 1996) showed that the anti-Candida activity of Lactoferrin or lactoferricin B in combination with clot rimazole had a synergistic effect.

Lactoferrin and its peptides have an effect against protozoa (turchany et al., 1995; Isamida et al., 1998), though the mode of action is unclear. The effect against toxoplasma gondii may be the same as for bacteria. The cell surface of *T. gondii* tackyzoites is known to have a strong negative charge and binds cationic substances (Cintra and de Souza, 1985). It is hypothesized that lactoferricin has the capacity to bind to the surface of the parasite in the intestinal tract, and this interaction results in loss of infectivity, resulting in disruption of the biological function of the parasite membrane (Heird et al., 1984).

Lactoferrin and cellular proliferation

Lactoferrin played a significant role in cellular proliferation. This suggested better gastrointestinal development in new born animals fed maternal milk as compared to newborn animals fed commercial formulas (Heird et al., 1984; Berseth et al., 1983), increased thymidine incorporation with lactoferrin supplementation of milk formulas (Berseth et al., 1983) and *in vitro* augmentation of thymidine incorporation into rat crypt cell DNA by Lactoferrin (Nichols et al., 1987). It was proved by the fourfold higher DNA synthesis in a mouse embryo cell line under the influence of hololactoferrin than in the same line under the influence of apolactoferrin (Zuma et al., 1989). The effect of LF on cancerous cells would appear to be inhibitory rather than stimulatory (Amouric et al., 1984). Most researchers suggest that lactoferrin can acts as a negative feedback regulatory of myelopoiesis (Garre et al., 1992).The mechanism involves the suppression of the release of cytokines such as interleukin-1, tumor necrosis factor and interleukin-2[87], Interleukin 6 and TNF in response to LPS moncytes (Crouch's et al., 1992; Mattsby-Baltzer et al., 1996).

Shinoda et al. (1996) described that lactoferrin has the ability to stimulate the release of neutrophil activating polypeptide interleukin-8 from human polymorph nuclear leukocytes (Shinoda et al., 1996).

Influence of lactoferrin on immune cells and autoimmune diseases

Lactoferrin is likely to favour the rapid recruitment of polymorphonuclear monocytes from blood to the inflammatory sites (Boxer et al., 1982; Kurose et al., 1994), Iron-saturated lactoferrin inhibits myelopoiesis. Perhaps the dynamic impact factor granulocyte monocyte (GM-CSF) production (Zucali et al., 1989), which reduces the production of interleukin-1, is by suppression. It has been reported that LF can increase the cytotoxicity of natural killer cells in vitro (Damiens et al., 1998). Antibodies to lactoferrin have been found in patients with autoimmune diseases such as systemic lupus erythematosus (Sinico et al., 1993), rheumatoid arthritis with vasculitis (Coremans et al., 1993), primary sclerosing cholangitis, and many other inflammatory diseases.

Other clinical applications

Reported a number of clinical applications and (leukemia,

myeloid chronique98, granulocytic leukemia as a tool in the diagnosis of blood neutrophils or neutrophil lactoferrin plasma dynamics as an index of the total pool is included in determining Olofsson et al., 1977), chronic pancreatitis and calciflying (Figarella and Srles, 1975; Multigner et al., 1980), cystic fibrosis (Rayner et al., 1991), schizophrenia (Hallgren et al., 1982), and rheumatoid arthritis (Bennet et al., 1977). Lactoferrin antibodies in patients with Felty's syndrome have been reported, and the detection of antibodies may be useful in diagnosis (Coremas et al., 1993).

Conclusion

Several recent studies have supported that lactoferrin have a wide spectrum of functions. Although lactoferrin may exert most of its functions through its effect on iron availability, but the exact mechanism of its action is not yet clear. The inhibitory effect of Lactoferrin on carcinogenesis also holds promise, but further trials are needed in all these areas before the therapeutic potential can be clearly established.

REFERENCES

Andrews WC, RW Bonsnes (1951). The leucocytes during pregnancy. Am J. Obstet Gynecol. 61: 1129-35.

Amouric M., Marvaldi J., Pichon J., Bellot F., and Figarella C. (1984). Effect of lactoferrin on the growth of a human colon adenocarcinoma cell line - comparison with transferrin. In Vitro. 20,543-548.

Ashida KH, Sasaki YA, Suzuki, Lonnerdal B (2004). Cellular internalization of lactoferrin in intestinal epithelial cells. *Biometals*. 17: 311-315.

Baker ED, Lindely PF (1993). New perspectives on the structure and function of transferrins. J. Inorgan. Biochem. 47: 147-60.

Baker EN, Anderson BF, Baker HM, MacGillivray RTA, Moore SA, Peterson NA (1998). Three dimensional structures of lactoferrin implications for function, including comparisons with transferring. *Adv. Exp. Med. Biol*. 443: 1-14.

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. *Scan. J. Clin. Lab. Invest*. 46: 695-704.

Bellamy W, Takase M, Yamauchi K, Wakabayashi H, Kawase K, Tomita M (1992). Identification of the bactericidal domain of Lactoferrin. *Biochim. Biophys. Acta*. 1121: 130-136.

Bellamy, W, H Wakabayashi, M Takase, K Kawase, S Shimamura and M Tomita (1993). Killing of *Candida albicans* by Lactoferricin B, a potent antimicrobial peptide derived from the N-terminal region of bovine lactoferrin. *Med. Microbiol. Immunol*.182: 97-105.

Bennet, RM and JL Skosey (1977). Lactoferrin and lysozyme levels in synovial fluid: differential indices of articular inflammation and degradation. *Arthritis Rheum*. 20: 84-90.

Berseth C.L., Lichtenberger L.M., Morris F.F., 1983. Comparison of the gastrointestinal growthpromoting effects of rat colostrum and mature milk in newborn rat in vivo. Amer. J. Clin. Nutr. 37, 52-60.

Bluard-Deconinck JM, Masson PL, Osinski PA, Heremans JF (1974).
Amino Acid Sequence of cystic peptides of Lactoferrin and
demonstration of similarities between Lactoferrin and transferring.
Biochim. Biophys. Acta 365: 311-317.

Boxer, LA, RA Haak, HH Yang, JB Wolach, JA Whitcomband CJ Butterick (1982). Membrane bound Lactoferrin alters the surface properties of polymorphonuclear leukocytes. *J. Clin. Invest*. 70: 1049-57.

Brines RD, Brock JH (1983). The effect of trypsin and chymotrypsin on the in vitro antimicrobial and iron-binding properties of lactoferrin in human milk and bovin colostrums: unusual resistance of human lactoferrin to proteolytic digestion. Biochem. Biophys Acta., 759: 229-35.

Brock JH, Arzabe F, Lampreave F, Pineira A (1976). The effect of trypsin on bovine transferring and lactorerrin. Biochim Biophys Acta. 446: 214-25.

Brogan TD, Ryley HC, Neale L, Yassa J (1975). Soluble proteins of bronchopulmonary secretions from patients with cystic fibrosis, asthma, and bronchitis. *Thorax*. 30: 72-9.

Bullen JJ, Griffiths E (1987). Iron and infection: molecular physiological and clinical aspects. *Great Britain: Wiley Inter-science*: 1.

Bullen JJ, Griffiths EE (1987). Iron and Infection: molecular physiological and clinical aspects. *Great Britain: Wiley Interscience*, 1.

Bullen, JJ, Rogers HJ, Leigh L (1972). Iron-binding proteins in milk and resistance to Escherichia coli infection in infants. *Br. Med. J.* 1: 69-75.

Cintra M, Silva-Filho FC, W De Souza (1986). The surface charge of Toxoplasma gondii: acyto-chemical and electrophoretic study. *J. Submicrose Cytol*. 18: 773-81.

Cintra, W M, W de Souza (1985). Distribution of intra-membranous particles and filipin-sterol complexes in the cell membranes of Toxoplasma gondii. *Eur. J. Cell Biol*. 37: 63-9.

Coremans IE, EC Hagen and FJ Van der Woude (1993). Anti-Lactoferrin antibodies in patients with rheumatoid arthritis with vasculitis. *Adv. Exp. Med. Biol*. 336: 357-62.

Coremans, IE, EC Hagen, EA van der Voort, FJ van der Woude, MR Daha and FC Breed Veld (1993). Auto-antibodies to neutrophil cytoplasmic enzymes in felty's syndrome. Clin. Exp. Rheumatol. 11: 255-62.

Coremans IEM, Hagen EC, Van FJ Der Woude MR Daha and EAM Van Der Voost.(1993). In: Gross WL, editor. ANCA-associated vasculitides: Immunological and clinical aspects. *New York; Ple+num*. 357-60.

Crouch, SP, KJ Slater and J Fletcher (1992). Regulation of cytokine release from mononuclear cells by the iron-binding protein Lactoferrin. *Blood*. 80: 235-40.

Damiens E, Mazurier E, Yazidi EL , Masson M, IDuthille I, Spik G (1998). Effects of human Lactoferrin on NK cell cytotoxicity against

Davidson LA, Lönnerdal B (1989). Fe Saturation and proteolysis of human Lactoferrin effect on brush border receptor mediated uptake of Fe^{2+} and Mn^{2+}. *Am. J. Physiol*. 257:930-934.
development. *Nature*. 417: 552-555.

Ellison, RTIII, TJ Giehl and FM LaForce (1988). Damage of the outer membrane of enteric Gram-negative bacteria by lactoferrin and transferring. *Infect Immun*. 64: 2774-81.

Fairweather Tait, S, Balmer SE, Scott PH, Ninski MJ (1987). Lactoferrin and iron absorption in newborn infants. *Pediar. Res*. 22: 651-4.

Figarella C, Srles H (1975). Lactoferrin, a protein of human pancreatic externalsecretion. *Scand J. Gastroenterol*. 10: 449-51.

FurmanskiP ZP, Li MB, Fortuna CVB Swamyu, Das MR (1989). Multiple molecular forms of human lactoferrin. J. Exp. Med. 170: 415-29.

Gifford JL, Hunter HN, Vogel HJ (2005). Lactoferricin: a lactoferrin derived peptide with anti-microbial, antiviral, antitumor and immunological properties. Cell Mol. Life. Sci. 62: 2588-2598.

Goldsmith SJ, Eitenmiller RR, Barnhart HM, Toledo RT, Rao VN (1982). Unsaturated iron binding capacity of human milk. J. Fd. Sci., 18: 512-5.

Guttenberg TJ, Askvik K, Jorgensen T (1986). Serum lactoferrin and C reactive proteinin mother and newborn after preterm rupture of membranes. *Acta Obstet.Gynecol. Scand*. 65: 203-5.

haematopoietic and epithelial tumour cells. *Biochem. Biophys. Acta*. 1402: 277-87.

Hallgren R, Venge P, Wistdedt B (1982). Elevated serum levels of Lactoferrin and cosinophil cationic protein in schizophrenic patients. *Br. J. Psychiatr*. 140: 55-60.

Haney EF, Nazmi K, Kau F (2009). Novel lactoferrampin antimicrobial peptides derived from human lactoferrin. *Biochimie*. 91: 141-154.

Hansen NE, Malmquist J, Thorell J (1975). Plasma myeloperoxidase and lactoferrin measured by radioimmunoassay: relations to neutrophil kinetics. *Acta Med. Scand*. 198: 437-43.

Heird, WC, SM Schwarz, I H Hasen (1984). Colostrum-induced enteric mucosal growth in beagle puppies. *Pediatr Res*. 18: 512-5.
Hirai, Y, N Kawakata, K Satoh (1990). Concentration of lactoferrin and iron in human milk at different stages of lactation. *J. Nutr. Sci Vita*. 36: 531-44.
Houghton MR, Gracey M, Burke V, Botrell C, Spargo RM (1985). Breast milk lactoferrin levels in relation of maternal nutritional status. *J. Pediatr. Gastroenterol Nutr.*4:230-3.
Hu WL, Regoeczi E, Chindemi PA, Bolyos M (1993). Lactoferrin interferes with uptake of iron from transferring and asialotransferrin by the rat liver. An. J. Physiol., 264: G112-7.
Hutchens, T W, J F Henry, T T Yip (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. *Pediatr. Res*. 29: 243-50.
Isamida, T, T Tanaka, Y Omata, K Yamauchi, K shimazaki, A Saiko. (1998). Protective effect of lactoferricin against *Toxoplasma gondii* infection in mice. *J. Vet.Med. Sci*. 60: 241-4.
Iyer S, and B Lonnerdal (1993). Lactoferrin, lactoferrin receptors and iron metabolism. Eur. J Clin. Nutr. 47: 232-41.
Iyer S, Lonnerdal B (1993). Lactoferrin, lactoferrin receptors and iron metabolism. Eur. J. Clin. Nutr., 47: 232-41.
Johansen BG (1960). Isolation of an iron containing red protein from human milk. *Acta. Chem. Scand*. 14: 510-2.
Kahler S, Christophers E, Schroder JM (1988). Plasma lactoferrin reflects neutrophil activation in psoriasis. Br. J. Dermatol. 119: 289-93.
Kolb E (1989). Recent knowledge of the structure and function of lactoferrin and ferritin. *Zeitschrift fur Die Gesamte Innere Medizin Und Ihre Grenzgebiete*. 44: 345-50.
Kurose, I, T Yamada, R Wolf and DN Granger (1994). P-selectin-dependent leukocyte recruitment and intestinal mucosal injury induced by Lactoferrin. *J. Keukoe Biol*. 55: 771-7.
Kuwata H, Yip T, Tomita M, Hutchens TW (1998). Direct evidence of the generation in human stomach of an antimicrobial peptide domain (lactoferricin) from ingested Lactoferrin. Biochim. Biophys. Acta., 1429: 129-141.
Machnicki, M (1991). Biological properties of lactoferrin. *Folia Biologica*. 37: 65-76.
Maher RJ, Cao D, Boxer LA, Petty HR (1993). Simultaneous calcium dependent delivery of neutrophil lactoferrin and reactive oxygen metabolites to erythrocyte targets: evidence supporting granule dependent triggering of superoxide deposition. J. Cell Physiol., 156: 226-34.
Makey DG, Seal US (1976). The detection of four molecular forms of human transferring during the iron binding process. Biochem. Biophys. Acta. 453: 250-6.
Makino Y, Kwaanishi E (1991). High performance liquid chromatographic separation of human apotransferrin and monferric and diferric transferins. J. Chromatogar. 567: 248-53.
Martur NB , Dwarkadas AM, Sharma VK, Saha K, Jain N (1990). Anti-infective factors in preterm human colostrums. *Acta Paediatr Scand*. 79: 1039-44.
Masson DY, Taylor CR (1978). Distribution of transferring, ferritin, and lactoferrin in human tissues. *J Clin Pathol*. 31: 316-27.
Masson PL, Heremans JF (1971). Lactoferrin in milk from different species. *Comp.* Biochem. Physiol., 39: 119-29.
Masson PL, Heremans JF, Ferin F (1968). Presence of an iron binding protein (Lactoferrin) in the genital tract of the female. I. Its immunohistochemical localization in the endometrium. *Fertil Steril*. 19: 679-89.
Masson PL, Heremans JF (1971). Lactoferrin in milk from different species. Comp. *Biochem. Physiol*. 39B: 119-29.
Mattsby-Baltzer I, Roseanu A, Roseanu A, Motas C, Elverfors J, Engberg I, Hanson LA (1996). Lactoferrin or a fragment thereof inhibits the endotoxin-induced interleukin-6 response in human moncytic cells. *Pediatr Res*. 40: 257-62.
Metz-Boutigue MH, J Jolles and J Mazurier (1984). Human lactotransferrin amino acid sequence and structural comparisons with other transferrins. Eur. J. Biochem., 145: 659-676.
Multgner L, Figarella CL, Sakel J, Sarles H (1980). Lactoferrin and albumin in human pancreatic juice: a valuable test for diagnosis of pancreatic disease. *Digest Dis. Sci*. 25: 173-8.
Nichols BL, McKee KS, Henry JF, Putman M (1987). Human lactoferrin stimulates thymidine incorporation into DNA of rat crypt cell. *Pediatr Res*. 21: 563-7.
Oberg G, Lindmark G, Moberg L, Venge P (1983). Peroxide activity and cellular content of granule protein in PMN during pregnancy. Br. J. Haematol., 55: 701-6.
Octave JN, Schneider YJ, Trouet A, Crichton RR (1983). Iron uptake and utilization by mammalian cells: cellular uptake of transferring and iron. Trands Biochem. Sci. 8: 217-220.
Olofsson T, Olsson I, Venge P (1977). Myeloperoxidaseand Lactoferrin of blood neutrophils and plasma in chronic granulocytic leukaemia. Scand J. Haematol. 18: 113-20.
Olsson I, Lantz M, Persson AM, Arnljots K (1988). Biosynthesis and processing oflactoferrin in bone marrow cells, a comparison with processing of myeloperoxidase. Blood. 71: 441-7.
Olga S, William JC, Shaper Mirza, Susan KH, Irina I. Protasevich, David EB, and Debasish Chattopadhyay (2007). Structure of a Complex of Human Lactoferrin N-lobe with Pneumococcal Surface Protein A Provides Insight into Microbial Defense Mechanism. J Mol Biol. 2007 Jul 20; 370(4): 701–713.
Otto BR, Verweij-van Vught AMJJ, MacLaren DM (1992). Transferrins and heme-compounds as iron sources for pathogenic bacteria. *Crit. Rev. Microbiol*. 18: 217-33.
Peter FL, Margaretha V (1995). Lactoferrin :A General Review. *Haematologica.* 80: 252-267.
Powell MJ, Ogden JE (1990). Nucleotide sequence of human lactoferrin cDNA. *NucleicAcids Res.* 18: 4013.
Querinjean P, Masson PL, Heremans JF (1971). Molecular weight, signle chain structure and amino acid composition of human lactoferrin. Eur J. Biochem., 20: 420-5.
Rayner, RJ, MS Wiseman, SM Cordon, D Norman, EJ Hiller and DJ Shale (1991). Inflammatory markers in cystic fibrosis. *Repair Med*. 85: 139-45.
Reitamo, S, Konttinen YT, Dodd S, Adinoli M (1981). Distribution of lactoferrin in human fetal tissues. *Acta Paediatr.Scand.* 70: 395-8.
Rey MW, Woloshuk SL, deBoer MA, Pieper FR (1990). Complete nucleotide sequence of human mammary gland lactoferrin. *Nucleic Acids Res*. 18: 5288.
Saito N, Takemori N, Hirai K, Onoder R, Watanabe S, Naiki M (1993). Ultra structural localization of lactoferrin in the granules other than typical secondary granules of human neutrophils. Human cell. 6: 42-8.
Scott PH (1989). Enzyme immunoassay of lactoferrin in newborn term infants: reference values and influence of diet. *Ann Clin. Biochem*. 26: 407-11.
Shinoda, I, Takase M, Fukuwatari Y, Shimamura S, Koller M, Konig W (1996). Effects of Lactoferrin and lactoferricin on the release of interleukin 8 from human polymorphonuclear leukocytes. *Biosci. Biotech. Biochem*. 60: 521-3.
Singh PK, Parsek MR, Greenberg EP, Welsh MJ (2002). A component of innate immunity prevents bacterial biofilm
Sinico RA, Pozzi C, Radice A , Tincani A (1993). ANCA with specificity for Lactoferrin in systemic lupus erythematosus (SLE), In: Gross WL, editor. ANCA-associated vasculitides: Immunological and clinical aspects. *New York; Plenum*. 385-7.
Sorensen M, Sorensen SPL (1939). The protein in whey CR*Trav. Lab. Carlsberg*. 23: 59-99.
Sykes JAC, Thomas MJ, Goldie DJ, Turner GM (1982). Plasma lactoferrin levels in pregnancy and cystic fibrosis. Clin Chem Acta., 122: 385-93.
Tabak L, Mandel ID, Herrera M, Baurmash H (1978). Changes in lactoferrin and other proteins in a case of chronic parotitis. J. Oral Pathol. 7: 91-9.
Teraguchi S, Shin K, Fukuwatari Y, Shimamura S (1996). Glycans of bovine lactoferrin function as receptors for the type I fimbrial lectin of *Escherichia coli*. *Infect Immun*. 64: 1075-7.
Turchany JM, Aley SB, Gillin FD (1995). Giardicidal activity of lactoferrin and N-terminalpeptides. *Infect Immun*. 63: 4550-2.
Valenti P, Antonini G (2005). Lactoferrin: an important host defence against microbial and viral attack. *Cell Mol. Life Sci*. 62: 2576-2587.
Van-Snick JL, Masson PL (1976). The binding of human lactoferrin to

mouse peritoneal cells. *J. Exp. Med*. 144: 1568-80.
Van-Snick JL, Masson PL (1976). The binding of human Lactoferrin to mouse peritionealcells. *J. Exp. Med*. 144: 1568-80.
Van-Snick JL, Masson PL, Heremans JF (1974). The involvement of lacoferrin in the hyposideremia of acute inflammation. J. Exp. Med. 140: 1068-84.
Van-Snick JL, Masson PL, Heremants JF (1974). The involvement of lactoferrin in the hyposideremia of acute inflammation. J. Exp. Med. 140: 1068-84.
Vercellotti G, Stroncek D, Jacob HS (1987). Granulocyte oxygen radical as potential suppessors of hemopoiersis: potentiating roles of lactoferrin and elastasc; inhibitory role of oxygen radiacal scavenger. *Blood cells*. 13: 199-206.
Vorland LH, Ulvatne H, Anderse Jn, Haukland HH, O Rekdal O, Andersen J, Vorland LH, J S Svendsen JS, Gutteberg TJ (1998). Lactoferricin of bovine origin is more active than lactoferricins of human, murine and caprine origin. *Scand J. Infect Dis*. 30: 513-7.
Wakabayashi H, Yamauchi K, Takase M (2008). Inhibitory effects of bovine lactoferrin and lactoferricin B on Enterobacter sakazakii. *Biocontrol. Sci*. 13: 29-32.
Wakabayashi H, Takase M, Tomita M (2003). Lactoferricin derived from milk protein lactoferrin. *Curr. Pharm Des*. 9: 1277-1287.
Wakabayashi, H, S Abe, T Okutomi, S Tansho, K, Kawase and H Yamaguchi (1996). Cooperative anti *Candida* effects of lactoferrin or its peptides in combination with azole antifungal agents. *Microbiol Immunol*. 40: 821-5.
Wakabayashi, H, T Hiratani, K Uchida and H Yamaguchi (1996). Antifungal spectrum and fungicidal mechanism of an N-terminal peptide of bovine lactoferrin. *J. Infect Chemother*. 1: 185-9.
Ward PP, EPaz, Conneely OM (2005). Multifunctional roles of Lactoferrin: a critical overview, cell. *Mol. Life. Sci*. 62: 2540-2548.
Zucali JR., Broxmeyer HE, Levy D, Morse C (1989). Lactoferrin decreases moncyte-induced fibroblast production of myeloid colony-stimulating activity by suppressing moncyte release of interleukin-1. *Blood*. 74: 1531-6.
Zuma AN, Mori H, Kaminogawa S, Yamauchi KY (1989). Stimulatory effect of human lactoferrin on DNA synthesis in BALB/C $3T_3$ cells. *Agri Biol Chem*; 53: 31-5.