Annu. Rev. Nutr. 1995. 15:93–110

LACTOFERRIN: MOLECULAR STRUCTURE AND BIOLOGICAL FUNCTION

B. Lönnerdal and S. Iyer
Department of Nutrition, University of California, Davis, California 95616

KEY WORDS: iron, iron absorption, bacteriostasis, immune function, growth factor

CONTENTS

0199-0885/95/0715-0093$05.00

ABSTRACT

Lactoferrin is an 80-kDa, iron-binding glycoprotein present in milk and, to a lesser extent, in exocrine fluids such as bile and tears. It consists of a single-chain polypeptide with two gobular lobes and is relatively resistant to proteolysis. The complete cDNAs for lactoferrin from human milk, neutrophils, and bovine milk have been reported, and recombinant proteins have been produced. Owing to its iron-binding properties, lactoferrin has been proposed to play a role in iron uptake by the intestinal mucosa and to act as a bacteriostatic agent by withholding iron from iron-requiring bacteria. Its presence in neutrophils and its release during inflammation suggest that lactoferrin is also involved in phagocytic killing and immune responses. Additionally, lactoferrin may function in ways not related to iron-binding, e.g. as a growth factor and as a bactericidal agent. This review attempts to evaluate these proposed functions and their biological significance in more detail.

INTRODUCTION

Iron-binding proteins exert many physiological functions in biological systems. Several of these proteins are involved in the transport of iron within the body and its storage in certain compartments. At the same time, they minimize the prooxidant effect of iron. Other iron-binding proteins are enzymes that require iron as a cofactor for optimal activity. Finally, some iron-binding proteins have been shown to regulate gene transcription and mRNA stability (63).

Although lactoferrin was first isolated and characterized in the late 1950s, evidence for a significant biological function(s) for this protein in vivo remains limited. Lactoferrin has been suggested to be involved in several physiological events. For example, investigators have variously defined lactoferrin as a cause of bacteriostasis and/or bactericidal effects, a component of the immune system, a growth factor, and/or an enhancer of iron absorption. However, most of these activities have been demonstrated only in vitro. In this review, we present the known characteristics of lactoferrin and attempt to disseminate the information available on the possible biological functions of this protein.

PROPERTIES OF LACTOFERRIN

History, Discovery, and Presence in Biological Fluids

As implied by its name, lactoferrin was first isolated from milk (55). This red or salmon-pink protein was soon recognized as an iron-binding protein with characteristics similar to but not identical to those of transferrin. It was subsequently found to be present in most exocrine fluids, such as saliva, bile,

pancreatic fluid, and tears. Plasma also contains lactoferrin, but at a concentration several orders of magnitude lower than that of milk (98). During inflammatory reactions, certain cell types (e.g. neutrophils) accumulate lactoferrin, most likely from the plasma pool (62, 102).

Concentrations and Species Differences

The concentration of lactoferrin in milk varies greatly among species. For example, human milk and milk from other primates, pigs, and mice are high in lactoferrin, whereas milk from species such as the cow and other ruminants is very low in lactoferrin. Still other species, e.g. the rat, have no lactoferrin in their milk . Species that have low concentrations of lactoferrin in their milk usually have higher levels of transferrin in their milk, whereas species like the human have very little transferrin in their milk (70). Thus, most species appear to secrete either a transferrin- or a lactoferrin-like protein in the milk.

The concentration of lactoferrin in human milk does not seem to be dependent on maternal iron status. Iron-deficient women appear to have normal concentrations of lactoferrin in their milk (86), and iron supplementation seems to have no effect on the concentration (110, 111). Malnutrition, however, may affect milk lactoferrin concentration (49, 50).

Molecular Weight and Glycans

Lactoferrin is a single-chain protein comprised of 692 amino acids. The sequence has been determined by both amino acid (76) and nucleotide (84, 91) sequencing. The protein contains intramolecular disulfide bonds and no free sulfhydryl groups. Lactoferrin is glycosylated at two distinct sites, and the N-linked glycans have been characterized with regard to both monosaccharide structure and conformation (104). Human milk lactoferrin contains poly-*N*-acetyllactosaminic glycans. The glycans of lactoferrin isolated from polymorphonuclear leukocytes seem to be identical in structure. Although these glycans have distinct spatial features, their role in any biological activity related to lactoferrin remains to be defined. Bovine lactoferrin is also glycosylated but is characterized by α-1,3-linked galactose residues in the terminal nonreducing position. Moreover, it contains additional glycans of the oligomannosidic type (104).

PHYSICAL CHARACTERISTICS OF LACTOFERRIN

Tertiary Structure

The lactoferrin polypeptide chain consists of two globular lobes linked by an extended α-helix that is sensitive to proteolytic attack (3). The two domains are similar in amino acid sequence, probably as a result of an early duplication

of an ancestral gene. Each lobe contains one iron-binding site and one glycan. However, the conformations of the N-lobe and the C-lobe are different, and their affinity for iron is slightly different as well (4). In its iron-free "apo form," the conformation of the lobes changes and lactoferrin becomes a more "open" molecule, which may explain the difference in susceptibility to proteases (see below). The tertiary structure of both human and bovine lactoferrin has been well characterized, with a resolution of 2.8 Å (4).

Metal and Anion-Binding Properties

The most common metal ion associated with lactoferrin in vivo is iron in its ferric (+3) form (4). However, Harrington showed that lactoferrin can also bind other metal ions, such as copper, manganese, and aluminum, in biological systems (47). The proportion of lactoferrin molecules occupied by these other cations may be quite small; lactoferrin isolated from human milk was found to contain 2000 times more iron than manganese (68). Other metal ions (e.g. zinc) can be found associated with lactoferrin in vitro under very specific conditions (17), but whether these ions are specifically incorporated into the lactoferrin molecule or nonspecifically associated with the negatively charged lactoferrin remains to be determined.

For each cation bound to lactoferrin, one atom of bicarbonate (or carbonate) is incorporated into the iron-binding crevice (4). Such an anion is essential for metal binding, and its presence greatly facilitates iron saturation.

Proteolytic Resistance of the Lactoferrin Molecule

Lactoferrin was shown early on to be resistant to proteolytic degradation in vitro (20, 22). Trypsin and chymotrypsin were remarkably ineffective in digesting lactoferrin, particularly in its iron-saturated form. Some large fragments of lactoferrin were formed, but proteolysis was clearly limited. Studies in breast-fed and formula-fed infants have subsequently shown that intact lactoferrin (or larger fragments thereof) can be found in significant quantities in the stool (31, 44, 85, 96, 103). Thus, lactoferrin can survive digestion by pepsin and pancreatic enzymes in the infant gut and possibly perform biological functions in the gastrointestinal tract. Although only a small proportion of the total milk lactoferrin may survive intact, this quantity is more than adequate to carry all the iron originally present in human milk (the potential role of lactoferrin in iron absorption is discussed below).

An even higher proportion of lactoferrin is found in the feces of premature infants (35). Intact lactoferrin has also been found in the urine of such infants, indicating that not only do some lactoferrin molecules survive digestion, but they also may be absorbed and excreted in intact form (43, 53). The extent to which this process occurs and its biological significance remain to be explored.

MOLECULAR BIOLOGY OF LACTOFERRIN

The Lactoferrin Gene

The complete cDNA for human lactoferrin has been isolated from a mammary gland cDNA library, and the amino acid sequence has been deduced from the nucleotide sequence (84, 91). The cDNA encodes a protein with a signal peptide of 19 amino acids followed by a mature protein of 692 residues. There is 99.7% agreement between the partial cDNA sequence for neutrophil human lactoferrin reported by Rado et al (87) and the overlapping cDNA sequence from human mammary gland reported by Rey et al (91).

Mead & Tweedie (75) reported the cDNA and amino acid sequence of bovine lactoferrin using a combination of cDNA and protein-sequencing techniques. The mRNA sequence of bovine lactoferrin has also been reported (45). The mRNA codes for a 708–amino acid protein with a 19–amino acid signal peptide immediately preceding a sequence identical to the N-terminal 40 amino acids reported for bovine lactoferrin (90). The nucleic acid sequence and the deduced amino acid sequence of the mature protein of bovine lactoferrin are homologous with published sequences for human lactoferrin (77 and 68%, respectively) and, to a lesser degree, with mouse lactoferrin (72 and 64%), human transferrin (68 and 60%), and porcine transferrin (67 and 61%).

Regulation of lactoferrin synthesis is tissue specific; mammary gland lactoferrin expression has been shown to be dependent on prolactin in organ culture (46) and unaffected by estradiol, whereas uterine lactoferrin synthesis is stimulated by 17β-estradiol treatment in the immature mouse (83, 106). McMaster et al (73) have shown by Northern blot analysis that this stimulation occurs at the transcription level in a time- and dose-dependant manner and can be antagonized by increasing levels of progesterone. Thus, a balance between estrogen and progesterone levels regulates lactoferrin gene expression in mouse uterine tissue. A DNA sequence has subsequently been identified on the 5′-flanking region of the mouse lactoferrin gene corresponding to the estrogen-responsive element (ERE) that overlaps a chicken ovalbumin upstream promoter (COUP) element (65).

Johnston et al (56) have reported the cloning of the human genomic lactoferrin gene using a cDNA from a chronic myelogenous leukemia library. They also report mapping studies of the transcription start site and a preliminary characterization of *cis*-acting 5′-promoter sequences. Striking homology of size and sequence is observed between the first two lactoferrin exons and their counterparts on the transferrin gene. This finding supports the hypothesis that lactoferrin is a byproduct of a series of gene duplications originating with an ancient iron-binding protein (76, 82, 107).

Recombinant Human Lactoferrin

Recombinant human lactoferrin has been expressed in baby hamster kidney cells (105) using a cDNA synthesized by reverse transcriptase from total RNA from human bone marrow. The expressed protein was shown to be virtually identical to that isolated from human milk when migration patterns on sodium dodecyl sulfate (SDS)-polyacrylamide gels and the presence of glycan chains were compared. Interestingly, all the recombinant lactoferrin purified from the cell-culture medium was in a fully iron-saturated form, and yields ranged from 20 to 30 mg/liter. Large-scale production of human lactoferrin for clinical trials is now feasible using transgenic animals (61). Studies are well under way to produce transgenic cows that carry the human lactoferrin gene and that can therefore synthesize and secrete human lactoferrin in their milk. Recombinant human lactoferrin can also be produced on a large scale using microorganisms such as *Saccharomyces* (64) or *Aspergillus* (109).

PROPOSED FUNCTIONS OF LACTOFERRIN

Because lactoferrin was immediately identified as an iron-binding protein, it is perhaps logical that most initially suggested biological functions for lactoferrin were related to this property. Lactoferrin was found to specifically bind to intestinal biopsies and was proposed to play a role in the regulation of iron uptake by the mucosa. Because of its high concentration in the milk of some species, lactoferrin was also thought to be involved in the delivery of iron into milk. The low degree of iron saturation of lactoferrin in human milk and the exceptionally high affinity constant of lactoferrin for iron also led investigators to suggest that lactoferrin was a bacteriostatic agent. This hypothesis was supported by in vitro experiments in which addition of iron to human milk or lactoferrin eliminated the bacteriostatic effect. Since only some bacterial strains are affected by lactoferrin, it was suggested that the presence of lactoferrin in the diet could affect the fecal bacterial flora. Lactoferrin may also aid in phagocytic killing in the macrophage, where activated neutrophils secrete high concentrations of lactoferrin.

Although some of the proposed biological functions for lactoferrin still hinge on its iron-binding capacity, other suggested functions appear to be unrelated to iron. For example, lactoferrin has been shown in some test systems to have a growth-stimulatory effect. This effect was observed for both iron-saturated and apo-lactoferrin in an experiment carefully designed so as not to alter these states. Furthermore, the recently described bactericidal effect of lactoferrin has been ascribed to a region of the molecule that is not involved in iron binding. Several of the proposed functions of lactoferrin in the immune system may not be dependent on the iron saturation of lactoferrin either. Below, we attempt

to evaluate some of these proposed physiological functions of lactoferrin in more detail.

LACTOFERRIN AND IMMUNE FUNCTION

Inflammation

During an inflammatory response, lactoferrin is released into circulation by activated neutrophils. Van Snick & Masson proposed that this increased level of circulating lactoferrin is partially responsible for hyposideremia of inflammation because it removes iron from transferrin and incorporates it into the reticuloendothelial system (108). However, whether the rate of iron transfer from transferrin to lactoferrin is sufficiently high at physiological pH to mediate hyposideremia is unknown. In addition, mice exhibited IL-1-induced hyposideremia even in the presence of neutropenia (a deficiency of granulocytes). Although these findings indicate that lactoferrin may be unimportant for iron scavenging during inflammation, the biological importance of lactoferrin in host defense is emphasized by the observed susceptibility of subjects with congenital or acquired lactoferrin deficiency to recurrent infections (18, 19).

Machnicki et al suggested that lactoferrin plays a regulatory role during cytokine responses (69). At concentrations lower than 10^{-8} M, lactoferrin has been reported to be an inhibitor of cytokine responses in vitro, suppressing the release of IL-1, IL-2, and tumor necrosis factor (TNF) from mixed lymphocyte cultures (29). The biological action of IL-1, IL-2, or TNF was not blocked, thus indicating a regulatory role. An argument against a physiological regulatory function for lactoferrin is the concentration of 10^{-9} M reported in normal plasma (12, 81), as this level is well within the range at which lactoferrin is shown to have an inhibitory effect.

Bacteriostasis and Bactericidal Effects

Investigators hypothesized that lactoferrin would impede iron utilization by bacteria and cause bacteriostasis as a result of its iron-sequestering properties. Bovine lactoferrin in the apo form has been shown to have bacteriostatic activity against mastitic *Escherichia coli* (88). However, a few strains were resistant or unaffected, indicating that mechanisms other than simple iron withholding may be involved in the antimicrobial action of lactoferrin. For example, lactoferrin has been shown to cause release of lipopolysaccharide (LPS) from cell walls of gram-negative bacteria (37). In addition, lactoferrin reportedly inhibits LPS priming of neutrophils (26), presumably by binding to the lipid A part of LPS derived from clinically relevant bacterial species (5). More recently, Erdei et al (38) showed that lactoferrin binds to porins, a group of molecules common in *E. coli,* thus causing permeability changes. Infants

fed human milk are more resistant to intestinal infections than those fed formula (23), presumably because of the presence of considerable amounts of lactoferrin. Bacteriostatic effects of lactoferrin and human milk were demonstrated that could be abolished by the addition of iron. Moreover, gut microflora of the breast-fed infant differs considerably from that of the formula-fed infant in that the former is predominantly comprised of bifidobacteria, lactobacilli, and staphylococci, whereas the latter contains enterococci, coliforms, and bacteroides (7). Supplementation of infant formula with bovine lactoferrin, however, did not influence gut microflora (7, 92), which indicates that lactoferrin may act in conjunction with other factors in breast milk, e.g. secretory IgA, lysozyme, citrate, and bicarbonate.

A lactoferrin domain with bactericidal activity was recently isolated and described in bovine and human lactoferrin (8). This peptide region showed a marked growth-inhibitory effect on *E. coli* 0-111 (95) and is distinct from the iron-binding region (8).

Autoimmune Diseases

Antibodies to lactoferrin have been detected in patients with autoimmune disorders such as rheumatoid arthritis with vasculitis (27), systemic lupus (100), primary sclerosing cholangitis, and other inflammatory diseases (101). The clinical significance of these findings remains to be elucidated.

LACTOFERRIN AS A GROWTH FACTOR

Milk, and particularly colostrum, has been shown to stimulate proliferation of the small intestine (11, 48). Lactoferrin is a major whey protein present in the milk of most mammals and was first identified as a possible growth factor for the intestinal mucosa when Nichols et al (78) reported that thymidine incorporation into DNA of rat crypt cells was enhanced in the presence of human lactoferrin. This stimulation does not appear to be dependent on the presence of bound iron in human lactoferrin (79). Most of the lactoferrin in human milk is present in the apo form, which indirectly supports the above theory (80).

Various cell lines have also been used to study the growth stimulation of lactoferrin. However, no consensus has been reached. In a nonhuman cell line (BALB/c 3T3 mouse embryo) (6) and in neonatal rat hepatocytes (60), only iron-saturated lactoferrin, both human and bovine, was shown to be growth stimulatory (6). Amouric et al (2) reported in their studies with a human adenocarcinoma cell line (HT-29) in a defined serum-free medium that lactoferrin could not substitute for transferrin or allow cell proliferation. In studies on MAC-T bovine mammary epithelial cells, lactoferrin has been shown to be growth inhibitory (89). More recently, our studies in Caco-2 cells have shown little or no effect of lactoferrin present in the medium, as measured by cell

number and DNA synthesis (M Yuen, S Iyer & B Lönnerdal, unpublished data). However, the above studies have considered growth as a parameter and not differentiation, which could very well be more significant when studying cells of intestinal origin. Further studies are clearly needed to separate the two phenomena and to define the effects of lactoferrin on each.

LACTOFERRIN AND IRON ABSORPTION

Clinical Studies

The hypothesis that lactoferrin is involved in the absorption of iron from breast milk was supported early on by two observations. First, breast milk contains an unusually high concentration of lactoferrin, and a major proportion of iron in human milk is bound to lactoferrin (41). Second, despite a relatively low concentration of iron in human milk, exclusively breast-fed infants maintain adequate iron stores up to at least six months of age (36, 67, 99), suggesting a very high bioavailability of breast-milk iron. Radioisotope experiments in infants showed that iron absorption is higher from breast milk than from infant formula (94). However, the technique used to label the breast milk with ^{59}Fe (extrinsic labeling) may not be accurate (66), and the reference dose concept was not used to compensate for individual differences in iron absorption. These methodological concerns led some investigators to question the validity of the observations. Indirect support for a higher bioavailability of iron from human milk than from formula comes from several studies showing lower iron status of infants fed formula that had not been fortified with iron compared with breast-fed infants (93), although the iron concentration of such formula is higher than that in breast milk. Evidence that lactoferrin is the factor in breast milk responsible for this higher bioavailability is still inadequate. Studies in adult humans have implied a higher absorption of iron from lactoferrin-fortified formula (74); however, questions can again be raised regarding the methodology used in those studies.

Studies in nonhuman primate models (infant rhesus monkeys) have failed to demonstrate a pronounced positive effect of human or bovine lactoferrin on iron absorption (30), even if great care was taken to validate the methods used for labeling, to compensate for individual differences in absorption, etc. The infant rhesus monkey is considered an excellent model for the human infant for three reasons: (*a*) its gastrointestinal physiology is similar to that of the human infant; (*b*) monkey milk contains a high concentration of lactoferrin; and (*c*) the rhesus monkey can be reared on regular infant formula without adaptations in nutrient or energy content. In this study, iron absorption was relatively high from both infant formula and breast milk, which may explain why no further increase was observed. Recent modifications of infant formula

composition, including the use of high levels of ascorbic acid, may have optimized iron absorption. It is also possible that neither bovine nor human lactoferrin could play the same role as species-specific monkey lactoferrin, even if their characteristics were similar. Studies in other animal models (mouse, rat) also suggest a positive effect of lactoferrin on iron absorption and status, although the validity of these models may be questionable (40, 58).

In a recent study, iron absorption was determined in term human infants using two stable isotopes of iron (34). In a crossover design, breast-fed infants were fed either intact human milk or human milk from which lactoferrin had been specifically removed. Iron absorption, estimated from erythrocyte iron incorporation, was slightly higher from lactoferrin-free human milk than from intact human milk. This finding indicates that lactoferrin does not promote iron absorption from breast milk and supports an earlier hypothesis that lactoferrin inhibits iron absorption at an age when the need for iron is questionable (21). However, the age of the infant may be an important factor when evaluating the role of lactoferrin in iron absorption. Most infants in our study were four months or older, as we needed to give a certain quantity of stable isotopes to allow detection of differences in iron incorporation. At this age, digestion has become much more efficient than it is at a younger age, and very small quantities of lactoferrin are found in the stool (31). Although we cannot draw any conclusions based on only one infant, it is noteworthy that iron absorption was considerably higher from lactoferrin-containing breast milk than from lactoferrin-free milk in the only infant less than three months of age. Further studies are obviously needed to evaluate the effect of human lactoferrin on iron absorption in infants. Such studies may be facilitated by the availability of recombinant human lactoferrin (see below).

The effect of bovine lactoferrin on iron absorption has also been evaluated in human infants. Results to date do not support a role for this protein in iron absorption in formula-fed infants. Three studies showed no significant difference in iron status between infants fed formula supplemented with bovine lactoferrin and those fed formula supplemented with ferrous sulfate (25, 39, 67). In one study, iron status was marginally better in infants fed a high level of bovine lactoferrin than in those fed a lower level of bovine lactoferrin or ferrous sulfate (97). However, we cannot draw any conclusions about the role of lactoferrin in iron absorption, as the iron level was also higher in the formula containing a higher level of lactoferrin. Another possible, less specific function of lactoferrin is to help keep iron in solution at the neutral pH of the small intestine (57).

Studies in Cells and Biological Membranes

Specific binding of human lactoferrin to duodenal biopsies from adults was demonstrated by Cox et al (28). This finding suggested that lactoferrin may

bind to certain sites in the small intestine and is therefore directly or indirectly involved in the acquisition of iron by the enterocyte. Studies on lactoferrin binding to brush-border membrane preparations from mice (51, 52), piglets (42), rhesus monkeys (32, 33), and human infants (59) supported this hypothesis. More recently, human lactoferrin was shown to bind to two human cell lines, HT-29 and Caco-2, in a saturable and specific manner (54, 77). These cell lines are colon carcinoma cells that in culture spontaneously differentiate into small intestinal cells with features characteristic of the enterocyte, including a brush-border membrane. They have both been used in numerous studies on nutrient metabolism and are believed to be good models of the human small intestinal epithelial cell. Thus, lactoferrin has been documented to bind specifically to intestinal cells and to the brush-border membrane.

Uptake and Intracellular Processing of Lactoferrin and Iron

Dual isotope studies on human intestinal cells in culture have shown that both lactoferrin and iron are taken up by the enterocyte (54, 77). These studies show that lactoferrin is completely degraded intracellularly (54) or, when monolayers are used to follow vectorial transport, that only a very small proportion of lactoferrin is transferred to the serosal side (77). Iron is therefore released within the cell and rapidly complexed to another protein, most likely ferritin (54). Thus, lactoferrin brings iron into the intestinal cell, but the ultimate fate of the iron will be determined by other factors, such as the individual's need for iron. When iron status is low, the internalized iron will likely be mobilized and transferred into the body, whereas when iron status is satisfactory, this iron may be lost in desquamated cells.

LACTOFERRIN RECEPTORS

Lactoferrin Receptors in the Small Intestine

Lactoferrin receptors in the small intestinal mucosa were first reported by Mazurier (72) in rabbit brush-border membranes, using ligand blotting. This observation was in accordance with the earlier finding that human lactoferrin could deliver iron to mucosal cells of small intestinal biopsy tissues (28), whereas bovine lactoferrin, human transferrin, and chick ovotransferrin could not. Studies in the infant rhesus monkey showed that rhesus lactoferrin and human lactoferrin bound to a receptor in the rhesus brush-border membrane in a specific and saturable manner (32). Bovine lactoferrin and transferrin exhibited no such binding. The binding affinity of iron-saturated lactoferrin for the receptor was higher than that of apo-lactoferrin (33). Recent studies in the piglet (42) have documented a specific receptor on the brush-border membrane with a K_d of approximately 3×10^{-6} M that was shown to be present in

all segments of the small intestine. Human lactoferrin, bovine lactoferrin, and pig transferrin did not bind to the receptor. This degree of species specificity is noteworthy because sow milk is known to contain transferrin as an iron carrier and because rat pup intestine has been reported to contain transferrin receptors but no lactoferrin receptors in the brush-border membrane. Kawakami & Lönnerdal (59) have found lactoferrin receptors in the brush-border membranes of both fetal and infant human small intestine. Binding was pH dependent, with optimum binding occurring at pH 6.5–7; the apparent K_d was ~1 μM. Enzymatic deglycosylation of lactoferrin did not inhibit binding, indicating that although the glycan chains may not have been structurally involved in receptor binding, they nonetheless may contribute to the structural integrity of lactoferrin during digestion.

Lactoferrin Receptors in the Monocyte/Macrophage System

Lactoferrin exerts several effects on the inflammatory and immune responses of an animal. During this process, circulating levels of lactoferrin increase significantly. In most cases, the target cell is a member of the monocyte/macrophage system. This finding implies that lactoferrin interacts with the monocytic cells through a receptor-like mechanism. Human monocytes were shown to bind lactoferrin with high affinity (4.5×10^{-9} M) (14, 16) virtually independent of temperature (ranging from 0 to 37° C) but to some extent dependent on the presence of Ca^{2+} (10, 15, 23). Lactoferrin binding to other cells of the monocyte/macrophage line, namely adherent mononuclear cells (9, 14) and alveolar macrophages (24), occurs at a lower affinity. The apparent K_d is 2.7×10^{-6} M for adherent cells and 1.7×10^{-6} M for mouse peritoneal cells. The specificity of lactoferrin binding to these cells has been demonstrated in competitive binding experiments with human transferrin, monomeric and aggregated IgG (9, 14, 108), bovine albumin (24), and cytochrome c (14). None of the above proteins was shown to be competitive.

Characteristics of the Lactoferrin Receptor

The human intestinal receptor was isolated and partially characterized (59). Gel electrophoresis indicated a molecular weight of ~115,000 under nonreducing conditions and ~38,000 under reducing conditions. The receptor was glycosylated, and the molecular weight of the glycan was ~4000. The purified receptor maintained its ability to bind human lactoferrin, as shown by ligand blotting. Mazurier et al (71) isolated a putative lactoferrin receptor from a Triton X–100 extract of phytohemagglutinin-stimulated human lymphocytes and reported the presence of two proteins with molecular weights of 100,000 and 110,000. In addition, Birgens (13) proposed the presence of a high-affinity receptor in addition to the previously documented receptor.

Whether the protein described in the cells outlined above represents a

common lactoferrin receptor is unknown. Although the protein has been documented and only partially characterized in terms of physical parameters of binding, its full biological significance remains to be determined. We cannot define the precise function(s) of lactoferrin on the basis of our present knowledge about the receptor. Studies on postbinding events, differences in behavior during development, and changes in physiological status will provide valuable insight into the biological role of lactoferrin.

IMPLICATIONS AND SIGNIFICANCE

Considerable data indicate several physiological roles for lactoferrin, although firm evidence in vivo is still lacking, particularly in humans. Studies in humans have been severely limited owing to a lack of adequate quantities of lactoferrin for long-term clinical trials. Although some studies have been performed with bovine lactoferrin, species-specific lactoferrin may be needed for these functions. The production of recombinant human lactoferrin will make it possible to evaluate several of the above-mentioned biological activities of lactoferrin. However, the recombinant forms of lactoferrin will always differ in glycan composition. If this feature of lactoferrin is involved in cellular recognition or in stability and turnover of the molecule, such studies still may not reveal the full range of activities exerted by lactoferrin. However, results to date suggest that at least cellular recognition of the lactoferrin molecule is not affected by the presence or absence of the glycans. The next few years will likely provide much needed and interesting information on lactoferrin and its biological functions. Future studies will give direction for the possible applications of lactoferrin in therapy.

Literature Cited

1. Ainscough EW, Brodie AM, Plowman JE. 1979. The chromium, manganese, cobalt and copper complexes of human lactoferrin. *Inorg. Chim. Acta* 33:145–53
2. Amouric M, Marvaldi J, Pichon J, Bellot F, Figarella C. 1984. Effect of lactoferrin on the growth of a human colon adenocarcinoma cell line—comparison with transferrin. *In Vitro* 20:543–48
3. Anderson BF, Baker HM, Dodson EJ, Norris GE, Rumball SV, et al. 1987. Structure of human lactoferrin at 3.2 Å resolution. *Proc. Natl. Acad. Sci. USA* 84:1769–73
4. Anderson BF, Baker HM, Norris GE, Rice DW, Baker EN. 1989. Structure of human lactoferrin: crystallographic structure analysis and refinement at 2.8 Å resolution. *J. Mol. Biol.* 209:711–34
5. Appelmelts BJ, An Y, Geerts M, Thijs BG, de Boer MA, et al. 1994. Lactoferrin is a lipid A–binding protein. *Infect. Immun.* 62:2628–32

6. Azuma N, Mori H, Kaminogawa S, Yamauchi K. 1989. Stimulatory effect of human lactoferrin on DNA synthesis in BALB/c 3T3 cells. *Agric. Biol. Chem.* 53:31–35
7. Balmer SE, Scott PH, Wharton BA. 1989. Diet and fecal flora in the newborn: lactoferrin. *Arch. Dis. Child.* 64: 1685–90
8. Bellamy W, Takase M, Yamauchi K, Wakabayashi H, Kawase K, Tomita M. 1992. Identification of the bactericidal domain of lactoferrin. *Biochim. Biophys. Acta* 1121:130–36
9. Bennett RM, Davis J. 1981. Lactoferrin binding to human peripheral blood cells: an interaction with B-enriched population of lymphocytes and a subpopulation of adherent mononuclear cells. *J. Immunol.* 127:1211–16
10. Bennett RM, Davis J, Campbell S, Portnoff S. 1983. Lactoferrin binds to cell membrane DNA: association of surface DNA with an enriched population of B cells and monocytes. *J. Clin. Invest.* 71:611–18
11. Berseth CL, Lichtenberger LM, Morriss FH. 1983. Comparison of the gastrointestinal growth-promoting effects of rat colostrum and mature milk in newborn rats in vivo. *Am. J. Clin. Nutr.* 37:52–60
12. Birgens HS. 1985. Lactoferrin measured by an ELISA technique: evidence that plasma lactoferrin is an indicator of neutrophil turnover and bone marrow activity in acute leukemia. *Scand. J. Haematol.* 34:326–31
13. Birgens HS. 1991. The interaction of lactoferrin with human monocytes. *Dan. Med. Bull.* 38:244–52
14. Birgens HS, Hansen NE, Karle H, Kristensen LO. 1983. Receptor binding of lactoferrin by human monocytes. *Br. J. Haematol.* 54:383–91
15. Birgens HS, Karle H, Hansen NE, Kristensen LO. 1984. Lactoferrin binding to peripheral human leukocytes. *Prot. Biol. Fluids* 31:145–48
16. Birgens HS, Kristensen LO, Borregaard N, Karle H, Hansen NE. 1988. Lactoferrin-mediated transfer of iron to intracellular ferritin in human monocytes. *Eur. J. Haematol.* 41:52–57
17. Blakeborough P, Salter DN, Gurr MI. 1983. Zinc binding in cow's milk and human milk. *Biochem. J.* 209: 505–12
18. Boxer LA, Coates TD, Haak RA, Wolach JB, Hoffstein S, Baehner RL. 1982. Lactoferrin deficiency associated with altered granulocyte function. *New Engl. J. Med.* 307:404–10
19. Breton-Gorius J, Mason D, Buriot D, Vilde J, Griscelli C. 1980. Lactoferrin deficiency as a consequence of a lack of specific granules in neutrophils from a patient with recurrent infections. *Am. J. Pathol.* 99:413–19
20. 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 bovine colostrum: unusual resistance of human lactoferrin to proteolytic digestion. *Biochim. Biophys. Acta* 759: 229–35
21. Brock JH. 1985. Lactoferrin in human milk: its role in iron absorption and protection against enteric infection in the newborn infant. *Arch. Dis. Child.* 55:417–21
22. Brock JH, Arzabe F, Lampreave F, Pineira A. 1976. The effect of trypsin on bovine transferrin and lactoferrin. *Biochim. Biophys. Acta* 446:214–25
23. 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
24. Campbell EJ. 1982. Human leukocyte elastase, cathepsin G and lactoferrin: family of neutrophil granule glycoproteins that bind to an alveolar macrophage receptor. *Proc. Natl. Acad. Sci. USA* 79:6941–45
25. Chierici R, Sawatzki G, Tamisari L, Volpato S, Vigi V. 1992. Supplementation of an adapted formula with bovine lactoferrin. 2. Effects on serum iron, ferritin and zinc levels. *Acta Paediatr.* 81:475–79
26. Cohen MS, Mao J, Rasmussen GT, Serody JS, Britigan BE. 1992. Interaction of lactoferrin and lipopolysaccharide (LPS): effects on the antioxidant-property of lactoferrin and the ability of LPS to prime human neutrophils for enhanced superoxide formation. *J. Infect. Dis.* 166:1375–78
27. Coremans IEM, Hagen EC, van der Woude FJ, Daha MR, van der Voost EAM, et al. See Ref. 46a, pp. 357–60
28. Cox TM, Mazurier J, Spik G, Montreuil J, Peters TJ. 1979. Iron binding proteins and influx of iron across the duodenal brush-border. Evidence for specific lactotransferrin receptors in the human intestine. *Biochim. Biophys. Acta.* 558: 129–41
29. Crouch SPM, Slater KJ, Fletcher J. 1992. Regulation of cytokine release from mononuclear cells by the iron-binding protein lactoferrin. *Blood* 80: 235–40
30. Davidson LA, Litov RE, Lönnerdal B. 1990. Iron retention from lactoferrin-

supplemented formulas in infant rhesus monkeys. *Pediatr. Res.* 27:176–80

31. Davidson LA, Lönnerdal B. 1987. Lactoferrin and secretory IgA in the feces of exclusively breast-fed infants. *Am. J. Clin. Nutr.* 41:852–61
32. Davidson LA, Lönnerdal B. 1988. Specific binding of lactoferrin to brush-border membrane: ontogeny and effect of glycan chain. *Am. J. Physiol.* 254:G580–85
33. Davidson LA, Lönnerdal B. 1989. Fe-saturation and proteolysis of human lactoferrin: effect on brush-border receptor-mediated uptake of Fe and Mn. *Am J. Physiol.* 257:G930–34
34. Davidsson L, Kastenmayer P, Yuen M, Lönnerdal B, Hurrell RF. 1994. Influence of lactoferrin on iron absorption from human milk in infants. *Pediatr. Res.* 35:117–24
35. Donovan S, Atkinson S, Whyte R, Lönnerdal B. 1989. Partition of nitrogen intake and excretion in low-birth-weight infants. *Am. J. Dis. Child.* 143:1485–91
36. Duncan B, Schifman RB, Corrigan JJ, Schaefer C. 1985. Iron and the exclusively breast-fed infant from birth to 6 months. *J. Pediatr. Gastroenterol. Nutr.* 4:421–25
37. Ellison RT, Giehl TJ, Laforce FM. 1988. Damage of the outer membrane of enteric gram-negative bacteria by lactoferrin and transferrin. *Infect. Immun.* 56: 2774–81
38. Erdei J, Forsgren A, Naidu AS. 1994. Lactoferrin binds to porins Omp F and Omp C in *Escherichia coli*. *Infect. Immun.* 62:1236–40
39. Fairweather-Tait SJ, Balmer SE, Scott PH, Ninski MJ. 1987. Lactoferrin and iron absorption in newborn infants. *Pediatr. Res.* 22:651–54
40. Fransson G-B, Keen CL, Lönnerdal B. 1983. Supplementation of milk with iron bound to lactoferrin using weanling mice. I. Effects on hematology and tissue iron. *J. Pediatr. Gastroenterol. Nutr.* 2:693–700
41. Fransson G-B, Lönnerdal B. 1980. Iron in human milk. *J. Pediatr.* 2:693–701
42. Gislason J, Iyer S, Hutchens TW, Lönnerdal B. 1993. Lactoferrin receptors in piglet small intestine: binding kinetics, specificity, ontogeny and regional distribution. *J. Nutr. Biochem.* 4:528–33
43. Goldblum RM, Schanler RJ, Garza C, Goldman AS. 1989. Human milk feeding enhances the urinary excretion of immunologic factors in low birth weight infants. *Pediatr. Res.* 25:184–88
44. Goldman AS, Garza C, Schanler RJ, Goldblum RM. 1990. Molecular forms of lactoferrin in the stool and urine from infants fed human milk. *Pediatr. Res.* 27:252–55
45. Goodman RE, Schanbacher FL. 1991. Bovine lactoferrin mRNA: sequence analysis and expression in the mammary gland. *Biochem. Biophys. Res. Commun.* 180:75–84
46. Green MR, Pastewka JV. 1978. Lactoferrin is a marker for prolactin response in mouse mammary explants. *Endocrinology* 103:1510–13

46a. Gross WL, ed. 1993. *ANCA-Associated Vasculitides: Immunological and Clinical Aspects.* New York: Plenum

47. Harrington JP. 1992. Spectroscopic analysis of the unfolding of transition metal-ion complexes of human lactoferrin and transferrin. *Int. J. Biochem.* 24: 275–80
48. Heird WC, Schwarz SM, Hansen IH. 1984. Colostrum-induced enteric mucosal growth in beagle puppies. *Pediatr. Res.* 18:512–15
49. Hennart PF, Brasseur DJ, Delogne-Desnoeck JB, Dramaix MM, Robyn CE. 1991. Lysozyme, lactoferrin and secretory immunoglobulin A content in breast milk: influence of duration of lactation, nutrition status, prolactin status and parity of mother. *Am. J. Clin. Nutr.* 53:32–39
50. Houghton MR, Gracey M, Burke V, Botrell C, Spargo RM. 1985. Breast milk lactoferrin levels in relation to maternal nutritional status. *J. Pediatr. Gastroenterol. Nutr.* 4:230–33
51. Hu WL, Mazurier J, Montreuil J, Spik G. 1990. Isolation and partial characterization of a lactotransferrin receptor from mouse intestinal brush border. *Biochemistry* 29:535–41
52. Hu WL, Mazurier J, Sawatzki G, Montreuil J, Spik G. 1988. Lactotransferrin receptor of mouse small intestinal brush border. *Biochem. J.* 248:435–41
53. Hutchens TW, Henry JF, Yip TT, Hachey DI, Schanler RJ, et al. 1991. Origin of intact lactoferrin and its DNA-binding fragments found in the urine of human milk fed preterm infants. Evaluation of stable isotope enrichment. *Pediatr. Res.* 29:243–50
54. Iyer S, Yuen M, Lönnerdal B. 1994. Binding and uptake of human lactoferrin by its intestinal receptor studied in Ca-Co-2 cells. *FASEB J.* 7:A64 (Abstr.)
55. Johansson BG. 1960. Isolation of an iron-containing red protein from human milk. *Acta Chem. Scand.* 14:510–12
56. Johnston JJ, Rintels P, Chung J, Sather J, Benz EJ, Berliner N. 1992. Lactoferrin gene promoter: structural integrity and

non-expression in HL-60 cells. *Blood* 79:2998–3006
57. Kawakami H, Dosako S, Nakajima I. 1993. Effect of lactoferrin on iron solubility under neutral conditions. *Biosci. Biotech. Biochem.* 57:1376–77
58. Kawakami H, Hiratsuka M, Dosako S. 1988. Effects of iron-saturated lactoferrin on iron absorption. *Agric. Biol. Chem.* 52:903–8
59. Kawakami H, Lönnerdal B. 1991. Isolation and function of a receptor for human lactoferrin in human fetal intestinal brush-border membranes. *Am. J. Physiol.* 261:G841–46
60. Kohno Y, Shisaki K, Mura T, Ikawa S. 1993. Iron-saturated lactoferrin as a comitogenic substance for neonatal rat hepatocytes in primary culture. *Acta Paediatr.* 82:650–55
61. Krimpenfort P. 1993. The production of human lactoferrin in the milk of transgenic animals. *Cancer Detect. Prev.* 17: 301–5
62. Leffel MS, Spitznagel JK. 1975. Fate of human lactoferrin and myeloperoxidase in phagocytosing human neutrophils: effects of immunoglobulin G subclasses and immune complexes coated on latex beads. *Infect. Immunol.* 12:813–20
63. Leibold EA, Guo B. 1992. Iron-dependent regulation of ferritin and transferrin receptor expression by the iron-responsive element binding protein. *Annu. Rev. Nutr.* 312:345–68
64. Liang Q, Richardson T. 1993. Expression and characterization of human lactoferrin in yeast *Saccharomyces cerevisiae*. *J. Agric. Food Chem.* 41: 1800–7
65. Liu Y, Teng CT. 1992. Estrogen response module of the mouse lactoferrin gene contains overlapping chicken ovalbumin up-stream promoter transcription factor and estrogen receptor-binding elements. *Endocrinology* 6:355–64
66. Lönnerdal B. 1984. Iron in breast milk. In *Iron Nutrition in Infancy and Childhood*, ed. A Stekel, pp. 95–118. Nestle Nutrition Workshop Series. New York: Vevey/Raven
67. Lönnerdal B, Hernell O. 1994. Iron, zinc, copper and selenium status of breast-fed infants and infants fed trace element fortified milk-based infant formula. *Acta Paediatr.* 83:367–73
68. Lönnerdal B, Keen CL, Hurley LS. 1985. Manganese binding proteins in human and cow's milk. *Am. J. Clin. Nutr.* 41:550–59
69. Machnicki M, Zimecki M, Zaguski T. 1993. Lactoferrin regulates the release of tumor necrosis factor alpha and interleukin-6 in vivo. *Int. J. Exp. Path.* 74:433–39
70. Masson PL, Heremans JF. 1971. Lactoferrin in milk from different species. *Comp. Biochem. Physiol.* 39B:119–29
71. Mazurier J, Legrand D, Hu WL, Montreuil J, Spik G. 1989. Expression of human lactotransferrin receptors in phytohemagglutinin-stimulated human peripheral blood lymphocytes. Isolation of the receptors by antiligand-affinity chromatography. *Eur. J. Biochem.* 179:481–87
72. Mazurier J, Montreuil J, Spik G. 1985. Visualization of lactotransferrin brush-border receptors by ligand blotting. *Biochim. Biophys. Acta* 821:453–60
73. McMaster MT, Teng CT, Dey SK, Andrews GK. 1991. Lactoferrin in the mouse uterus: analyses of the preimplantation period and regulation by ovarian steroids. *Mol. Endocrinol.* 6: 101–11
74. McMillan JA, Landaw SA, Oski FA. 1976. Iron sufficiency in breastfed infants and the availability of iron from human milk. *Pediatrics* 58:686–91
75. Mead PE, Tweedie JW. 1990. cDNA and protein sequence of bovine lactoferrin. *Nucleic Acids Res.* 18:7167
76. Metz-Boutigue M, Jolles H, Mazurier JJ, Schoentgen F, Legrand D, et al. 1984. Human lactotransferrin: amino acid sequence and structural comparisons with other transferrins. *Eur. J. Biochem.* 145: 659–66
77. Mikogami T, Heyman M, Spik G, Desjeux JF. 1994. Apical-to-basolateral transepithelial transport of human lactoferrin in the intestinal cell line HT-29CL.19A. *Am. J. Physiol.* 267:G308–15
78. Nichols BL, McKee KS, Henry JF, Putman M. 1987. Human lactoferrin stimulates thymidine incorporation into DNA of rat crypt cells. *Pediatr. Res.* 21:563–67
79. Nichols BL, McKee KS, Heubers HA. 1990. Iron is not required in the lactoferrin stimulation of thymidine incorporation into the DNA of rat crypt enterocytes. *Pediatr. Res.* 27:525–28
80. Nichols BL, McKee K, Putman M, Henry JF, Nichols VN. 1989. Human lactoferrin supplementation of infant formulas increases thymidine incorporation into the DNA of rat crypt cells. *J. Pediatr. Gastroenterol. Nutr.* 8:102–9
81. Nuijens JH, Abbink JJ, Wachtfogel YT, Colman RW, Eerenberg AJM, et al. 1992. Plasma elastase α1-antitrypsin and lactoferrin in sepsis: evidence for

neutrophils as mediators in fatal sepsis. *J. Lab. Clin. Med.* 119:159–68

82. Park I, Schaeffer E, Sidoli A, Baralle FE, Cohen GN, Zakin MM. 1985. Organization of the human transferrin gene: direct evidence that it originated by gene duplication. *Proc. Natl. Acad. Sci. USA* 82:149–52
83. Pentacost BT, Teng CT. 1987. Lactoferrin is the major estrogen inducible protein of mouse uterine secretions. *J. Biol. Chem.* 262:10134–39
84. Powell MJ, Ogden JE. 1990. Nucleotide sequence of human lactoferrin cDNA. *Nucleic Acids Res.* 18:4013
85. Prentice A, Ewing G, Roberts SB, Lucas A, MacCarthy A, et al. 1987. The nutritional role of breast-milk IgA and lactoferrin. *Acta Pediatr. Scand.* 76: 592–98
86. Prentice A, MacCarthy A, Stifling DM, Vasquez-Velasquez L, Ceesay SM. 1989. Breast-milk IgA and lactoferrin survival in the gastrointestinal tract—a study of rural Gambian children. *Acta Paediatr. Scand.* 78:505–12
87. Rado TA, Wei X, Benz EJ. 1987. Isolation of lactoferrin cDNA from a human myeloid library and expression of mRNA during normal and leukemic myelopoiesis. *Blood* 70:989–94
88. Rainard P. 1986. Bacteriostatic activity of bovine milk lactoferrin against mastitic bacteria. *Vet. Microbiol.* 11: 387–92
89. Rejman JJ, Oliver SP, Muenchen RA, Turner JD. 1992. Proliferation of the MAC-7 bovine mammary epithelial cell line in the presence of mammary secretion whey proteins. *Cell Biol. Int. Rep.* 16:993–1001
90. Rejman JJ, Hegarty HM, Hurley WL. 1989. Purification and characterization of bovine lactoferrin from secretions of involving mammary gland: identification of multiple molecular weight forms. *Comp. Biochem. Physiol.* 93B:929–34
91. Rey MW, Woloshuk SL, deBoer MA, Pieper FR. 1990. Complete nucleotide sequence of human mammary gland lactoferrin. *Nucleic Acids Res.* 18:5288
92. Roberts AK, Chierici R, Sawatzki G, Hill MJ, Volpato S, Vigi V. 1992. Supplementation of an adapted formula with bovine lactoferrin. 1. Effect on the infant faecal flora. *Acta Paediatr. Scand.* 8: 119–24
93. Saarinen UM, Siimes MA. 1977. Iron absorption from infant milk formula and the optimal level of iron supplementation. *Acta Paediatr. Scand.* 66:719–22
94. Saarinen U, Siimes M, Dallman P. 1977. Iron absorption in infants: high bioavailability of breast-milk iron as indicated by the extrinsic tag method of iron absorption and by the concentration of serum ferritin. *J. Pediatr.* 91:36–39
95. Saito H, Miyakawa H, Tamura Y, Shimamura S, Tomita M. 1991. Potent bactericidal activity of bovine lactoferrin hydrolysate produced by heat treatment at acidic pH. *J. Dairy Sci.* 74: 3724–30
96. Schanler R, Goldblum R, Garza C, Goldman AS. 1986. Enhanced fecal excretion of selected immune factors in very low birthweight infants fed fortified human milk. *Pediatr. Res.* 20: 711–15
97. Schulz-Lell G, Dörner K, Oldigs HD, Sievers E, Schaub J. 1991. Iron availability from an infant formula supplemented with bovine lactoferrin. *Acta Paediatr. Scand.* 80:155–58
98. Scott PH. 1989. Plasma lactoferrin levels in newborn preterm infants: effect of infection. *Ann. Clin. Biochem.* 26: 412–15
99. Siimes MA, Salmenperä L, Perheentupa J. 1984. Exclusive breast-feeding for 9 months: risk of iron deficiency. *J. Pediatr.* 104:196–99
100. Sinico RA, Pozzi C, Radice A, Tincani A. 1993. ANCA with specificity for lactoferrin in systemic lupus erythematosus (SLE). See Ref. 46a, pp. 385–87
101. Skogh T, Peen E. 1993. Lactoferrin, anti-lactoferrin antibodies and inflammatory disease. See Ref. 46a, pp. 533–38
102. Slater K, Fletcher J. 1987. Lactoferrin derived from neutrophils inhibits the mixed lymphocyte reaction. *Blood* 69: 1328–33
103. Spik G, Brunet B, Mazunier-Dehaine C, Fontaine G, Montreuil T. 1982. Characterization and properties of the human and bovine lactotransferrins extracted from the feces of newborn infants. *Acta Paediatr. Scand.* 71:979–85
104. Spik G, Coddville B, Montreuil J. 1988. Comparative study of the primary structures of sero- lacto- and ovotransferrin glycans from different species. *Biochimie* 70:1459–69
105. Stowell KM, Rado TA, Funk WD, Tweedie JW. 1991. Expression of cloned human lactoferrin in baby-hamster kidney cells. *Biochem. J.* 276:349–55
106. Teng CT, Pentacost BT, Chen YM, Newbold RR, Eddy EM, McLachlan JA. 1989. Lactoferrin gene expression in the mouse uterus and mammary gland. *Endocrinology* 124:992–99
107. Teng CT, Pentacost BT, Marshall A,

Soloman A, Bowman B, et al. 1987. Assignment of the lactotransferrin gene to human chromosome 3 and to mouse chromosome 9. *Somat. Cell Mol. Genet.* 13:689–93

108. Van Snick JL, Masson PL. 1976. The binding of lactoferrin to mouse peritoneal cells. *J. Exp. Med.* 144:1568–80
109. Ward PP, May GS, Headon DR, Conneely OM. 1992. An inducible expression system for the production of human lactoferrin in *Aspergillus nidulans*. *Gene* 122:219–23
110. Zapata CV, Donangelo CM, Trugo NMF. 1994. Effect of iron supplementation during lactation on human milk composition. *J. Nutr. Biochem.* 5:331–37
111. Zavaleta N, Nombera J, Rojas R, Hambraeus L, Gislason, J, Lönnerdal B. 1995. Iron and lactoferrin in milk of anemic mothers given iron supplements. *Nutr. Res.* In press