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Lactoferrin: Role in iron homeostasis and host defense against microbial infection

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Abstract

The transferrin family of non-heme iron binding glycoproteins are believed to play a central role in iron metabolism and have been implicated in iron transport, cellular iron delivery and control of the level of free iron in external secretions. Lactoferrin (LF) is a member of this family that is widely localized in external fluids including milk and mucosal secretions, in addition to being a prominent component of the secondary granules of neutrophils. Although structurally related to transferrin, LF appears to have a broader functional role mediated by both iron dependent and iron independent mechanisms. In this review, we will focus on our current understanding on the role of LF in regulating iron homeostasis and its role in host protection against microbial infection at the mucosal surface. In addition, recent insights obtained from analyzing the phenotypic consequences of LF ablation in lactoferrin knockout mice (LFKO), which challenge the long held dogma that LF is required for intestinal iron absorption in the neonate, are summarized.

Introduction

LF is an ∼ 80 kDa member of the transferrin family of iron binding glycoproteins (Aisen *et al.* 1980, Metz-Boutigue *et al.* 1984). LF is expressed and secreted by glandular epithelial cells with highest levels (∼ 7 grams/liter) found in human colostrum where it is one of the most abundant whey associated proteins (Masson *et al.* 1971, Hennart *et al.* 1991). LF is also present at lower levels in mature milk and most exocrine secretions in addition to the secondary granules of mature neutrophils (Masson 1966, Masson *et al.* 1969). The precise three-dimensional structure of LF has been revealed by X-ray crystallographic analysis (Anderson *et al.* 1987, Anderson *et al.* 1989). The single polypeptide chain is folded into two homologous globular lobes, each containing a deep cleft that has the capacity to bind reversibly one ferric ion with the synergistic binding of a carbonate anion. Specifically, the iron atom in each lobe is coordinated with six ligands, four of which are provided by the polypeptide chain (one aspartic acid, two tyrosine and one histidine residues), with two additional coordination sites provided by the carbonate anion (Baker *et al.* 2003).

Several diverse physiological functions have been ascribed to LF including regulation of cellular growth and differentiation, intestinal iron homeostasis, host defense against microbial infection and inflammation, regulation of myelopoiesis and protection against cancer [Reviewed in (Iyer *et al.* 1993, Levay *et al.* 1995, Baveye *et al.* 1999, Brock 2002, Ward *et al.* 2002)]. While some of these functions are clearly dependent on the iron binding properties of the protein, others appear to be independent of metal binding and may be mediated in part by a cationic domain located in the N-terminus of the molecule that does not overlap with the iron-binding sites (Bellamy *et al.* 1992). In this paper, we will focus on two of the functions that have long been proposed for LF, regulation of intestinal iron homeostasis and host protection against microbial infection, providing an overview of our current understanding on the role of LF in these two areas.

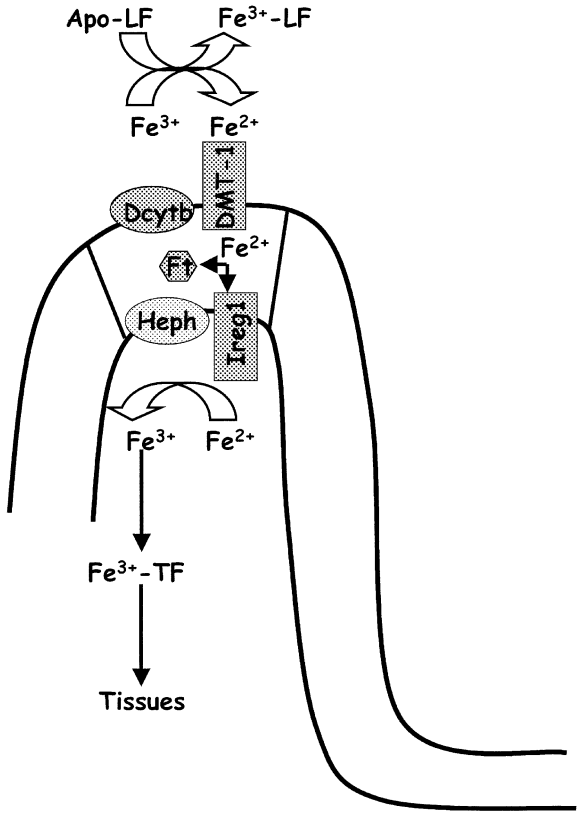

Fig. 1. Intestinal Iron Absorption by the DMT-1 Pathway. Dietary non-heme iron is reduced to the ferrous form (Fe^{2+}) in the lumen of the proximal small intestine, most likely by the ferrireductase, Dcytb. Fe^{2+} is then transported across the apical membrane of absorbtive enterocytes by the divalent metal ion transporter, DMT-1. Once inside the cell, iron can be stored in the form of ferritin (Ft) or transported across the basolateral membrane by Ireg1. A membrane bound hephaestin (Heph) has been proposed to convert Fe^{2+} to the Fe^{3+} form which then allows the iron to bind to apo transferrin for iron transport and delivery to the body. LF may act at the level of the intestinal lumen to limit the amount of iron available for uptake by DMT-1 or other iron uptake pathways.

Iron homeostasis and LF

Tight regulation of iron homeostasis is essential to protect against free radical induced cellular damage and promotion of microbial growth while concurrently providing adequate iron for essential metabolic functions in the body. Dietary iron absorption occurs in the proximal small intestine (duodenum). In mammals, a major non-heme iron uptake pathway involves the divalent metal iron transporter, DMT-1 (also known as NRAMP-2 or DCT1) (Fleming *et al.* 1997, Gunshin *et al.* 1997, Andrews 2000). Ferric iron is first believed to be reduced in the intestinal lumen by a ferrireductase, duodenal cytochrome B (DcytB) followed by transport into the cell by DMT-1 (Figure 1). The iron is either stored in the cell in the form of ferritin, or is thought to exit the enterocyte through a basolateral iron transporter ferroportin 1 (IREG1, MTP1) and is reoxidized to the ferric form, most likely by a membrane associated oxidase, hephaestin. Once across the intestinal barrier, the ferric iron binds to serum transferrin for transport to tissues in the body (Andrews 2000; Fleming *et al.* 2002, Frazer *et al.* 2003). The essential role of the DMT-1 mediated iron uptake pathway is highlighted by rodent loss-of-function mutations of this protein, which result in anemia (Fleming *et al.* 1997, Fleming *et al.* 1998).

Because mammals lack a regulated pathway for iron excretion, iron homeostasis is regulated primarily at the site of iron absorption in the intestine in response to body iron requirements (Andrews 2000, Wessling-Resnick 2000, Frazer *et al.* 2003). Several genes have been implicated in the feedback regulation of intestinal iron absorption including the product of the hemachromatosis gene, HFE , transferrin receptor 1 and 2 (Fleming *et al.* 2002, Morgan *et al.* 2002, Frazer *et al.* 2003). Recent developments in this field also support a key role for the hepatic antimicrobial peptide, hepcidin, in the negative feedback regulation of intestinal iron absorption (Fleming *et al.* 2001, Frazer *et al.* 2003, Ganz 2003). Hepcidin expression is strongly upregulated in response to iron loading (Pigeon *et al.* 2001) and inappropriate expression of this gene has been linked to iron disorders in mice and humans (Nicolas *et al.* 2001, Ahmad *et al.* 2002, Nicolas *et al.* 2002., Weinstein *et al.* 2002, Muckenthaler *et al.* 2003, Nicolas *et al.* 2003). Furthermore, mutation of the human hepcidin gene was recently shown to be a causative agent of juvenile hemochromatosis (Roetto *et al.* 2003). Although the exact mechanism by which hepcidin regulates intestinal iron absorption remains to be elucidated, it has been suggested that this peptide is secreted by the liver and may directly downregulate the expression of gene(s) involved in the DMT-1 iron uptake pathway in the mature enterocyte (Frazer *et al.* 2003).

Members of the transferrin family have also been implicated in regulating iron homeostasis in mammals. The iron regulatory role of transferrin (TF) in iron transport and cellular iron delivery via the transferrin receptor mediated uptake pathway is well established (van Renswoude *et al.* 1982, Bernstein 1987, Levy *et al.* 1999). Human (atransferrinaemia) (Goya *et al.* 1972) and mice (hypotransferrinemic mice) (Bernstein 1987) models of transferrin deficiency result in an-

emia highlighting the essential role of transferrin in iron delivery to erythroid precursor cells. Interestingly, transferrin deficiency is also associated with tissue iron overload due, at least in part, to increased intestinal iron absorption. Thus while TF is essential for iron delivery to erythroid cells, other iron uptake systems must exist in non erythroid cells (Wessling-Resnick 2000). A long-standing controversy existed as to the role of LF in iron homeostasis. The strong iron binding properties of LF, together with the high iron bioavailability and abundant concentrations of LF in breast milk suggested that this protein may play a role in intestinal iron absorption in the neonate (Iyer *et al.* 1993, Levay *et al.* 1995). In addition, specific receptors for LF have been identified on the small intestinal mucosal surface from many species including human, rhesus monkey, pig and mouse (Iyer *et al.* 1993). Recently, a human LF enterocyte receptor (hLfR) has been cloned [also cloned as intelectin (Tsuji *et al.* 2001) and HL-1 (Lee *et al.* 2001)]. While *In vitro* experiments have shown that Caco-2 intestinal cells transfected with hLfR have increased [59Fe]hLF uptake versus mock-transfected cells (Suzuki *et al.* 2001), the physiological role of this receptor *in vivo* remains to be established. Further, tissue culture, animal studies and human clinical trials have shown conflicting results supporting a role for LF in both the enhancement or inhibition of intestinal iron delivery [Reviewed in (Brock 1980, Sanchez *et al.* 1992, Iyer *et al.* 1993, Levay *et al.* 1995)]. However, many of these studies must be viewed with caution where non-homologous LF preparations were used as, at least in the case of the human, binding of LF to the enterocyte receptor has been reported to be species specific (Kawakami *et al.* 1991).

To further clarify the role of LF in iron homeostasis, we analyzed the effect of LF ablation on iron homeostasis using LFKO mice (Ward *et al.* 2003). We have shown that ablation of LF does not result in iron deficiency anemia, demonstrating that LF is not required for intestinal iron delivery to the neonate (Ward *et al.* 2003). To the contrary, comparison of postnatal offspring derived from LFKO to WT intercrosses, respectively, disclosed a mild iron overload phenotype in the LFKO pups. This was reflected by elevated serum iron, transferrin saturation and hepatic iron in LFKO versus WT pups, with the latter two parameters reaching statistical significance. As it is known that iron is sensitive to genetic background and because our analysis was carried out in a mixed background strain, we performed similar analysis in an isogenic 129/Sv strain. Comparison of postnatal offspring derived from isogenic LFKO intercrosses to WT intercrosses showed that ablation of LF also resulted in higher hepatic iron stores, although these increases did not reach significance in the 129/Sv strain (Ward *et al.* 2003). Nonetheless, these results suggest that while genetic background may influence the postnatal iron overload phenotype observed in LFKO mice in the mixed background strain, the increased iron levels observed during the suckling period may also be due, in part, to the lack of LF in the milk. These results are intriguing given the low free iron concentrations in milk and mucosal secretions and the fact that mild iron overloading was observed even in the absence of dietary iron stress.

We further investigated whether LF influenced iron homeostasis in the adult (Ward *et al.* 2003). We reasoned that if LF played a major role in intestinal iron homeostasis in the post weaning period, it would likely be expressed at the site of iron absorption in the duodenum. *In situ* hybridization analysis showed, however, that LF is not endogenously expressed in the duodenum. Consistent with this lack of expression, iron parameters were similar between adult LFKO and WT mice that had been maintained on either a basal or high iron diet (Ward *et al.* 2003). Taken together, these results indicate that LF is not required for intestinal iron delivery in the mouse. Moreover, the mild postnatal iron overload observed in LFKO pups suggests a role for LF in iron sequestration and inhibition of excessive iron uptake during the suckling period. Interestingly, studies in human infants fed on breast milk versus LF depleted breast milk found that iron absorption was higher in the LF-free breast milk also supporting a role for this protein in limiting rather than facilitating intestinal iron uptake in humans (Davidsson *et al.* 1994).

Role of LF in host protection against microbial infection

LF possesses several antimicrobial activities that likely contribute to the innate immune response at the mucosal surface. Most microbial pathogens are dependent on iron for growth. LF, by virtue of its strong iron-binding properties and relatively iron free state in mucosal secretions, can bind and sequester this essential nutrient thus limiting the growth of iron requiring microbes including enteropathogenic *Escherichia coli* (Brock 1980). This antimicrobial activity may have an

added benefit by favoring the growth of bacteria with low iron requirements such as lactic acid producing bacteria, which are generally believed to be beneficial to the host and there is some evidence that LF can also directly support the growth of these bacterial species (Petschow *et al.* 1999). However, in some cases bacteriostasis is temporary as bacteria adapt to their iron deprived conditions, for example, by producing their own high affinity iron chelators called siderophores which compete with LF for iron (Crosa 1989). In addition, certain bacteria including the *Neisseriaceae* species have adapted to the LF imposed iron restrictive conditions by synthesizing specific LF receptors which have been shown to bind and extract iron from LF (Schryvers *et al.* 1998).

Importantly, a recent seminal finding by Singh et al demonstrated that LF could prevent the formation of *Pseudomonas aeruginosa* biofilms *in vitro* (Singh *et al.* 2002). Although dependent on the iron sequestration function of LF, the anti-biofilm activity was observed using concentrations of LF far less than that required to inhibit the growth of the bacteria by bacteriostatic mechanisms. In this case, iron sequestration stimulated a specialized motion called twitching which caused the bacteria to wander randomly around the surface, rather that stay stationary and divide *in situ* to form microcolonies that ultimately give rise to biofilm formation (Singh *et al.* 2002). This elegant study suggests that the ability of LF to maintain an environment devoid of free iron may play a crucial role in preventing against chronic lung infections by *Pseudomonas aeruginosa in vivo*. Further, a recent study by Schaible *et al.* suggests that iron sequestration by LF can protect against the increased suceptibility of β-2 Microglobulin Knockout mice, a genetic mouse model of iron overload, to *Mycobacterium tuberculosis* infection (Schaible *et al.* 2002). Taken together, these results suggest that control of extracellular iron levels by LF may have profound impact on the outcome of microbial pathogenesis.

Antimicrobial activities have also been described for LF which are independent of the iron status of the protein. In this regard, a direct bactericidal activity has been described for LF which is due to a cationic domain located in the N-terminus of the molecule (Bellamy *et al.* 1992). *In vitro* experiments have shown that the isolated bactericidal peptide, lactoferricin, has more potent activity than the intact protein, exerting broad spectrum antimicrobial activity against a broad range of Gram negative and Gram positive bacteria, yeast, fungi and protozoa (Wakabayashi *et al.* 2003). However, how this peptide functions in the context of the intact protein in physiological secretions and/or whether substantial amounts of this peptide are released at sites of infection *in vivo* remain largely unknown. Several reports suggest that LF may also contribute to the innate host defense system by interfering with other aspects of microbial virulence. For example, LF was shown to prevent the adhesin and/or internalization of bacteria and viruses to tissue culture cells (Longhi *et al.* 1993, Valenti *et al.* 1998, van der Strate *et al.* 2001, Ajello *et al.* 2002, Gomez *et al.* 2003) and a serine protease activity has been described for LF that specifically inactivates *Haemophilus influenza* IgA1 protease and Hap adhesion putative colonization proteins (Qiu *et al.* 1998).

In addition to the contribution from local expression and secretion from glandular epithelial cells, a second source of LF in external fluids is provided by neutrophils (Masson *et al.* 1969). These immune cells are rapidly recruited to sites of microbial challenge during the initial acute phase of infection where activation and degranulation of LF rich secondary granules provides an additional source of this protein to aid in the innate immune response against microbial infection at the mucosal surface (Masson *et al.* 1969). LF has also been proposed to enhance the intracellular bactericidal activity of neutrophils whereby fusion of the LF containing secondary granules with the phagosomes provides an environment whereby LF can donate the iron required for catalysis of free radical production (Sanchez *et al.* 1992). Finally, colocalization and synergy of LF with other components of the innate immune response, including lysozyme and defensins, likely further augment the host's ability to robustly respond to and protect against microbial challenge (Ellison *et al.* 1991, Leitch *et al.* 1998, Singh *et al.* 2000).

In summary, the antimicrobial activities of LF highlight the many possible modes by which LF may contribute to host protection against microbial infections at the mucosal surface, both by iron dependent and iron independent mechanisms.

Future perspectives

It is becoming increasingly evident that the iron binding properties of LF likely contribute, in large part, to the physiological role of this protein *in vivo*. Studies carried out in LFKO mice have shown that ablation of LF results in mild iron overload during the post-

natal period, suggesting that the major iron regulatory role of LF during the suckling period is iron sequestration, preventing against excessive iron uptake and likely contributing to an environment refractory to microbial pathogenesis and cellular oxidative damage. Finally, the fact that mild iron overload was observed in LFKO pups even in the absence of dietary iron challenge is intriguing, especially in light of the inherent complexity and likely functional redundancy present in milk. Future studies examining the phenotypic consequences of LF ablation during the postnatal period under conditions of dietary iron stress and in response to microbial challenge will undoubtedly reveal new insights into the physiological role of this iron binding protein at the mucosal surface.

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References

Ahmad KA, Ahmann JR, Migas MC *et al.* 2002 Decreased liver hepcidin expression in the hfe knockout mouse. *Blood Cells Mol Dis* **29**, 361–366.

Aisen P, Listowsky I. 1980 Iron transport and storage proteins. *Annu Rev Biochem* **49**, 357–393.

Ajello M, Greco R, Giansanti F *et al.* 2002 Anti-invasive activity of bovine lactoferrin towards group A streptococci. *Biochem Cell Biol* **80**, 119–124.

Anderson BF, Baker HM, Dodson EJ *et al.* 1987 Structure of human lactoferrin at 3.2-A resolution. *Proc Natl Acad Sci USA* **84**, 1769–1773.

Anderson BF, Baker HM, Norris GE, Rice DW, Baker EN. 1989 Structure of human lactoferrin: crystallographic structure analysis and refinement at 2.8 A resolution. *J Mol Biol* **209**, 711–734.

Andrews NC. 2000 Iron homeostasis: insights from genetics and animal models. *Nat Rev Genet* **1**, 208–217.

Baker HM, Anderson BF, Baker EN. 2003 Dealing with iron: common structural principles in proteins that transport iron and heme. *Proc Natl Acad Sci USA* **100**, 3579–3583.

Baveye S, Elass E, Mazurier J, Spik G, Legrand D. 1999 Lactoferrin: a multifunctional glycoprotein involved in the modulation of the inflammatory process. *Clin Chem Lab Med* **37**, 281–286.

Bellamy W, Takase M, Yamauchi K *et al.* 1992 Identification of the bactericidal domain of lactoferrin. *Biochim Biophys Acta* **1121**, 130–136.

Bernstein SE. 1987 Hereditary hypotransferrinemia with hemosiderosis, a murine disorder resembling human atransferrinemia. *J Lab Clin Med* **110**, 690–705.

Brock JH. 1980 Lactoferrin in human milk: its role in iron absorption and protection against enteric infection in the newborn infant. *Arch Dis Child* **55**, 417–421.

Brock JH. 2002 The physiology of lactoferrin. *Biochem Cell Biol* **80**, 1–6.

Crosa JH. 1989 Genetics and molecular biology of siderophore-mediated iron transport in bacteria. *Microbiol Rev* **53**, 517–530.

Davidsson L, Kastenmayer P, Yuen M, Lonnerdal B, Hurrell RF. 1994 Influence of lactoferrin on iron absorption from human milk in infants. *Pediatr Res* **35**, 117–124.

Ellison RT 3rd, Giehl TJ. 1991 Killing of gram-negative bacteria by lactoferrin and lysozyme. *J Clin Invest* **88**, 1080–1091.

Fleming MD, Romano MA, Su MA *et al.* 1998 Nramp2 is mutated in the anemic Belgrade (b) rat: evidence of a role for Nramp2 in endosomal iron transport. *Proc Natl Acad Sci USA* **95**, 1148–1153.

Fleming MD, Trenor CC, 3rd, Su MA *et al.* 1997 Microcytic anaemia mice have a mutation in Nramp2, a candidate iron transporter gene. *Nat Genet* **16**, 383–386.

Fleming RE, Sly WS. 2001 Hepcidin: a putative iron-regulatory hormone relevant to hereditary hemochromatosis and the anemia of chronic disease. *Proc Natl Acad Sci USA* **98**, 8160–8162.

Fleming RE, Sly WS. 2002 Mechanisms of iron accumulation in hereditary hemochromatosis. *Annu Rev Physiol* **64**, 663–680.

Frazer DM, Anderson GJ. 2003 The orchestration of body iron intake: how and where do enterocytes receive their cues? *Blood Cells Mol Dis* **30**, 288–297.

Ganz T. 2003 Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. *Blood*.

Gomez HF, Ochoa TJ, Carlin LG, Cleary TG. 2003 Human lactoferrin impairs virulence of *Shigella flexneri. J Infect Dis* **187**, 87–95.

Goya N, Miyazaki S, Kodate S, Ushio B. 1972 A family of congenital atransferrinemia. *Blood* **40**, 239–245.

Gunshin H, Mackenzie B, Berger UV *et al.* 1997 Cloning and characterization of a mammalian proton-coupled metal-ion transporter. *Nature* **388**, 482–488.

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.

Iyer S, Lonnerdal B. 1993 Lactoferrin, lactoferrin receptors and iron metabolism. *Eur J Clin Nutr* **47**, 232–241.

Kawakami H, Lonnerdal B. 1991 Isolation and function of a receptor for human lactoferrin in human fetal intestinal brush-border membranes. *Am J Physiol* **261**, G841–846.

Lee JK, Schnee J, Pang M *et al.* 2001 Human homologs of the Xenopus oocyte cortical granule lectin XL35. *Glycobiology* **11**, 65–73.

Leitch EC, Willcox MD. 1998 Synergic antistaphylococcal properties of lactoferrin and lysozyme. *J Med Microbiol* **47**, 837–842.

Levay PF, Viljoen M. 1995 Lactoferrin: a general review. *Haematologica* **80**, 252–267.

Levy JE, Jin O, Fujiwara Y, Kuo F, Andrews NC. 1999 Transferrin receptor is necessary for development of erythrocytes and the nervous system. *Nat Genet* **21**, 396–399.

Longhi C, Conte MP, Seganti L *et al.* 1993 Influence of lactoferrin on the entry process of *Escherichia coli* HB101 (pRI203) in HeLa cells. *Med Microbiol Immunol (Berl)* **182**, 25–35.

Masson PL, Heremans JF. 1971 Lactoferrin in milk from different species. *Comp Biochem Physiol B* **39**, 119–129.

Masson PL, Heremans JF, Schonne E. 1969 Lactoferrin, an iron-binding protein in neutrophilic leukocytes. *J Exp Med* **130**, 643–658.

Masson PL, Heremans, JF, Dive C. 1966 An iron-binding protein common to many external secretions. *Clinica Chimica Acta* **14**, 735–739.

Metz-Boutigue MH, Jolles J, Mazurier J *et al.* 1984 Human lactotransferrin: amino acid sequence and structural comparisons with other transferrins. *Eur J Biochem* **145**, 659–676.

Morgan EH, Oates PS. 2002 Mechanisms and regulation of intestinal iron absorption. *Blood Cells Mol Dis* **29**, 384–399.

Muckenthaler M, Roy CN, Custodio AO *et al.* 2003 Regulatory defects in liver and intestine implicate abnormal hepcidin and Cybrd1 expression in mouse hemochromatosis. *Nat Genet* **34**, 102–107.

Nicolas G, Bennoun M, Devaux I *et al.* 2001 Lack of hepcidin gene expression and severe tissue iron overload in upstream stimulatory factor 2 (USF2) knockout mice. *Proc Natl Acad Sci USA* **98**, 8780–8785.

Nicolas G, Bennoun M, Porteu A *et al.* 2002 Severe iron deficiency anemia in transgenic mice expressing liver hepcidin. *Proc Natl Acad Sci USA* **99**, 4596–4601.

Nicolas G, Viatte L, Lou DQ *et al.* 2003 Constitutive hepcidin expression prevents iron overload in a mouse model of hemochromatosis. *Nat Genet* **34**, 97–101.

Petschow BW, Talbott RD and Batema RP. 1999 Ability of lactoferrin to promote the growth of *Bifidobacterium* spp. *in vitro* is independent of receptor binding capacity and iron saturation level. *J Med Microbiol* **48**, 541–549.

Pigeon C, Ilyin G, Courselaud B *et al.* 2001 A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. *J Biol Chem* **276**, 7811–7819.

Qiu J, Hendrixson DR, Baker EN *et al.* 1998 Human milk lactoferrin inactivates two putative colonization factors expressed by *Haemophilus influenzae*. *Proc Natl Acad Sci USA* **95**, 12641–12646.

Roetto A, Papanikolaou G, Politou M *et al.* 2003 Mutant antimicrobial peptide hepcidin is associated with severe juvenile hemochromatosis. *Nat Genet* **33**, 21–22.

Sanchez L, Calvo M, Brock JH. 1992 Biological role of lactoferrin. *Arch Dis Child* **67**, 657–661.

Schaible UE, Collins HL, Priem F, Kaufmann SH. 2002 Correction of the iron overload defect in beta-2-microglobulin knockout mice by lactoferrin abolishes their increased susceptibility to tuberculosis. *J Exp Med* **196**, 1507–1513.

Schryvers AB, Bonnah R, Yu RH, Wong H, Retzer M. 1998 Bacterial lactoferrin receptors. *Adv Exp Med Biol* **443**, 123–133.

Singh PK, Parsek MR, Greenberg EP, Welsh MJ. 2002 A component of innate immunity prevents bacterial biofilm development. *Nature* **417**, 552–555.

Singh PK, Tack BF, McCray PB Jr., Welsh MJ. 2000 Synergistic and additive killing by antimicrobial factors found in human airway surface liquid. *Am J Physiol Lung Cell Mol Physiol* **279**, L799–805.

Suzuki YA, Shin K, Lonnerdal B. 2001 Molecular cloning and functional expression of a human intestinal lactoferrin receptor. *Biochemistry* **40**, 15771–15779.

Tsuji S, Uehori J, Matsumoto M *et al.* 2001 Human intelectin is a novel soluble lectin that recognizes galactofuranose in carbohydrate chains of bacterial cell wall. *J Biol Chem* **276**, 23456–23463.

Valenti P, Marchetti M, Superti F *et al.* 1998 Antiviral activity of lactoferrin. *Adv Exp Med Biol* **443**, 199–203.

van der Strate BW, Beljaars L, Molema G, Harmsen MC, Meijer DK. 2001 Antiviral activities of lactoferrin. *Antiviral Res* **52**, 225–239.

van Renswoude J, Bridges KR, Harford JB, Klausner RD. 1982 Receptor-mediated endocytosis of transferrin and the uptake of fe in K562 cells: identification of a nonlysosomal acidic compartment. *Proc Natl Acad Sci USA* **79**, 6186–6190.

Wakabayashi H, Takase M, Tomita M. 2003 Lactoferricin derived from milk protein lactoferrin. *Curr Pharm Des* **9**, 1277–1287.

Ward PP, Mendoza-Meneses M, Cunningham GA, Conneely OM. 2003 Iron status in mice carrying a targeted disruption of lactoferrin. *Mol Cell Biol* **23**, 178–185.

Ward PP, Uribe-Luna S, Conneely OM. 2002 Lactoferrin and host defense. *Biochem Cell Biol* **80**, 95–102.

Weinstein DA, Roy CN, Fleming MD *et al.* 2002 Inappropriate expression of hepcidin is associated with iron refractory anemia: implications for the anemia of chronic disease. *Blood* **100**, 3776–3781.

Wessling-Resnick M. 2000 Iron transport. *Annu Rev Nutr* **20**, 129–151.