

Annotation

Lactoferrin in human milk: its role in iron absorption and protection against enteric infection in the newborn infant

The existence of lactoferrin was first reported by Sørensen and Sørensen,¹ who observed a red protein fraction in human milk. The protein was first isolated by Groves² from bovine and by Johansson³ from human milk, and subsequent studies showed that this protein (which has been variously called red milk protein, ekkriosiderophilin, and lactotransferrin) is strikingly similar in many respects to the serum iron-binding protein transferrin. Human milk is unusually rich in lactoferrin, the normal concentration ranging from about 7 mg/ml in colostrum to not less than 1 mg/ml in mature milk.⁴ Although bovine colostrum also contains up to 5 mg/ml lactoferrin, levels drop very rapidly as lactation proceeds, so that mature bovine milk normally contains only 20-200 µg/ml lactoferrin.⁵ Lactoferrin is now known to be present also in various other exocrine secretions (vaginal, nasal, bronchial, and intestinal), in pancreatic juice, and in the secondary granules of neutrophils.⁶⁻⁸ Although there has been a great deal written about the structure, iron-binding properties, and *in vitro* biological activities of lactoferrin, no *in vivo* biological function has as yet been unequivocally established for it. The possible role of milk lactoferrin in iron absorption and in the control of the intestinal microflora of the newborn is discussed, as is the ability of lactoferrin to remain undegraded in the gastrointestinal tract of the infant.

Structure and biochemical properties of lactoferrin

Although lactoferrin has been identified in a wide variety of species⁴ almost all studies on the structure and iron-binding properties have been carried out with proteins of human or bovine origin. Lactoferrin closely resembles transferrin in that it consists of a single polypeptide chain of molecular weight 75 000-90 000,⁹⁻¹⁰ and that it binds two ferric ions, together with the synergistic binding of two bicarbonate ions.¹¹ It differs in being considerably more basic—the pI of transferrin is 5.9, that of lactoferrin ~ 9.0 ¹²—and in having an even higher association constant for iron-binding.¹³ As a consequence

lactoferrin, in the presence of a relatively low-affinity iron chelator such as citrate, retains its iron below pH 4.0, whereas that of transferrin is lost as the pH is lowered from 6 to 5.³ Further details of some of the physicochemical properties of lactoferrin are given by Parry and Brown.¹⁴

Survival of lactoferrin in the gastrointestinal tract

Discussion of the function of lactoferrin ingested by the breast-fed baby must take into account whether or not the protein reaches and can remain in the intestinal tract without being destroyed by gastric acidity or proteolytic activity. This aspect has been the subject of little discussion and of even less experimental work, and an attempt to assess the possible influence of these factors seems pertinent.

Although data vary somewhat, there seems to be general agreement that both acid and pepsin production in the newborn infant are low in relation to body weight compared with adult levels, and increase slowly with age.¹⁵⁻¹⁶ Certainly there is very little gastric proteolytic activity in newborn infants,¹⁷ but these infants can nevertheless digest milk proteins, presumably due to pancreatic protease production, which appears to be comparable with that of adults.¹⁸⁻¹⁹ This digestive ability increases with age, so that while newborn infants cannot completely digest milks containing >1.3% protein, those of 4 months can digest milk containing 2.5% protein.²⁰ This increase in overall digestive capacity during early life presumably occurs as a result of the increase in gastric proteolytic activity, given that pancreatic protease activity remains fairly constant. In this connection it should be pointed out that the discovery by Laskowski and Laskowski²¹ of a trypsin inhibitor in bovine colostrum has been quite unjustifiably extrapolated, even by the American Academy of Pediatrics,²² as indicating that an analogous inhibitor in human milk or colostrum would protect lactoferrin from digestion in the infant's gastrointestinal tract. In fact, no such inhibitor of this type, which is acid resistant,²³ exists in human milk, which contains only low levels of an

acid-labile (denatured below pH 6.0) inhibitor analogous to serum α -1-antitrypsin.²⁴ It is unlikely that this small amount of labile trypsin inhibitor could significantly affect trypsin activity in the intestine of the human infant.

The most important feature of lactoferrin in regard to its susceptibility to proteolysis is the extreme lability of the iron-free (apo) form. In an *in vitro* study of the effect of trypsin on the apolactoferrin-mediated bacteriostatic action of bovine colostrum, it was found that bacteriostasis was lost more rapidly than was specific antibody activity in the colostrum,²⁵ indicating that apolactoferrin is more susceptible to proteolysis than are immunoglobulins. Biochemical studies have shown that (bovine) apolactoferrin is rapidly degraded by trypsin to small peptides, whereas the iron-saturated form is relatively resistant to prolonged tryptic digestion, and gives rise only to large fragments which retain their iron-binding capacity.²⁶ Pepsin, however, can partly digest the (human) iron-saturated protein, leaving a half-molecule containing one iron atom.²⁷⁻²⁸

In summary therefore, apolactoferrin is likely to be susceptible to digestion by proteolytic activity of either gastric or pancreatic origin, whereas the iron-saturated form may remain largely intact, at least as far as its iron-binding properties are concerned, until appreciable peptic activity is present. In addition, the rapid passage of the whey fraction from the stomach to the duodenum may further reduce the effectiveness of peptic digestion of lactoferrin, even in infants with significant pepsin activity.

Antimicrobial activity of lactoferrin in the gut of the newborn infant

The ability of lactoferrin to inhibit the growth of certain micro-organisms *in vitro* has been well documented. Most interest has focused on enteropathogenic strains of *Escherichia coli*²⁹⁻³¹ although it may also inhibit *Staphylococcus albus*,³² *Staphylococcus aureus*, *Pseudomonas aeruginosa*,³³ *Bacillus stearothermophilus*, *Bacillus subtilis*,³⁴ and *Candida albicans*.³⁵ A bactericidal effect of lactoferrin on *Vibrio cholerae* and *Streptococcus mutans* has also been reported.³⁶ It is generally considered that the *in vitro* inhibition of *E. coli* requires the addition of bicarbonate to the milk or colostrum^{30 37} and the presence of specific antibodies,²⁹ although good bacteriostatic activity in the absence of specific antibodies has also been reported.^{36 38} The need for added bicarbonate is probably due to the labilising effect on lactoferrin-bound iron of citrate³⁹ which is present in milk and colostrum.^{30 37} Additional bicarbonate would enhance iron-binding by

lactoferrin and prevent iron being made available to bacteria via the citrate complex. The role of antibody is more obscure. It may cause the inhibition of synthesis of microbial iron chelators,⁴⁰ although it is not clear how this relates to the observation that the antibody responsible has a specificity directed towards a component sugar of the cell wall O- antigen.⁴¹

While there is little doubt that human milk does indeed tend to favour a lactobacillus or bifidus flora and suppress coliforms in the neonatal gut, *in vivo* evidence that lactoferrin is a contributory factor to this effect is scarce and certainly insufficient to justify the recent statement by the American Academy of Pediatrics that '... lactoferrin ... has an inhibitory effect on *Escherichia coli* in the intestine'.⁴² The experiments most widely cited as suggesting an *in vivo* antimicrobial effect for lactoferrin are those of Bullen *et al.*,²⁹ using guinea-pigs. Guinea-pig milk has a lactoferrin content similar to that of human milk.⁴ It was found that a challenge dose of *E. coli* administered to suckling guinea-pigs proliferated more rapidly in the intestines of animals dosed with haematin than in undosed animals. While this does indeed suggest that haematin caused enhanced growth of *E. coli* in the intestine, it cannot be assumed that this effect was directly due to saturation of the guinea-pig milk lactoferrin with iron, or indeed to the iron content of haematin at all. Haem iron is not available to nonhaem iron-binding proteins such as lactoferrin, and very little iron is released from haem by digestion.⁴³⁻⁴⁴ Haem is taken up intact by many micro-organisms, including *E. coli*, by pathways independent of non-haem iron uptake.⁴⁵ Furthermore, haem compounds may have effects unrelated to their properties as potential sources of iron for *E. coli*—for example, they alter the metabolism of various lactic acid bacteria, causing the production of cytochromes and, in at least one case, a pronounced reduction in lactic acid production.⁴⁶⁻⁴⁷ This might in itself, be sufficient to cause overgrowth of *E. coli* if intestinal lactobacilli were similarly affected. Haem compounds may also inactivate hydrogen peroxide, and could thus interfere with the peroxide-thiocyanate bactericidal mechanism of milk,⁵ and the bactericidal activity of milk phagocytes.⁴⁸ However, the case for an *in vivo* protective role of lactoferrin has been considerably strengthened by the more recent work of Antonini *et al.*⁴⁹ These workers showed that the number of coliforms in the intestine of newborn guinea-pigs dosed with *E. coli* and fed cows' milk was lower if the milk was supplemented with ovotransferrin, the iron-binding protein of avian egg white which is structurally related to lactoferrin. Mortality of guinea-pigs infected with a high dose (5×10^8 cells) of *E. coli* during the first 24 hours of

life was also lower in the group given ovotransferrin-supplemented milk, and was similar to the mortality among challenged animals allowed to suckle their mothers.

It must be remembered however that human and guinea-pig milks are unusually rich in lactoferrin whereas in other species—such as the cow and pig, where levels are high in colostrum but fall rapidly after the first few days of lactation—the levels in mature milk are insufficient to promote *in vitro* bacteriostasis.³⁰ Dog milk, furthermore contains only traces of lactoferrin and transferrin, yet also contains appreciable amounts (5 µg/ml) of iron.⁴ It thus seems unlikely that lactoferrin could be important in protecting the young of these species from gastrointestinal infections. This is not to deny the potential antimicrobial function of the unusually high levels of lactoferrin in human milk, but does suggest that the principal function of this protein may be of a more subtle nature, and one which can be more easily related to these fundamental differences in iron and iron-binding protein content in milks of different species.

Lactoferrin and iron absorption

In adults, the capacity to absorb iron is closely related to intermediary iron metabolism, particularly erythropoiesis. In neonates however, iron absorption appears to be less dependent on intermediary iron metabolism, and is generally greater, in terms of percentage ingested iron absorbed, than in adults. The relatively efficient absorption of breast-milk iron^{50–51} and the close biochemical relationship between lactoferrin and transferrin would tend to suggest that lactoferrin, like transferrin, possesses an iron-transport role and the possibility that it serves to transport iron to the intestinal mucosa in a readily absorbed form. Such a suggestion is not however supported by the rather limited amount written on the role of lactoferrin in iron absorption. McMillan *et al.*⁵² showed that the addition of lactoferrin, presumably iron-saturated (the iron content given (140 mg/g) is actually 100-fold higher than that of saturated lactoferrin) to a lactoferrin-free simulated human milk administered to adults did not increase iron absorption. De Laey *et al.*⁵³ showed that apolactoferrin actually inhibited iron uptake by rat and guinea-pig everted duodenal sacs, and Fe₂-lactoferrin was without effect. De Vet and Van Gool⁵⁴ demonstrated a negative relationship between duodenal lactoferrin concentration and iron absorption in adults. Both groups suggested that lactoferrin may serve to protect the mucosa against excessive uptake of heavy metal ions, and Van Vugt *et al.*⁵⁵ have proposed that the increased biliary lactoferrin con-

centrations observed in anaemic rabbits serve as an additional control of iron absorption in conditions where other controls have been relaxed to permit increased absorption. This mechanism could be of importance in the term newborn infant, who has both a high potential for iron absorption and replete iron stores during the immediate postnatal period, when milk lactoferrin levels are at their highest. Indeed, Cavell and Widdowson⁵⁶ have shown that during the first week of life breast-fed infants excrete 10 times more iron than they absorb. In this study 10 babies aged 5–8 days excreted daily on average 1.12 mg/kg iron. The mean daily milk intake was 160 ml/kg, from which it can be calculated that if the excreted iron were all bound by milk lactoferrin, a concentration of about 5 mg/ml in the milk would be required, which is close to values observed by Masson and Heremans⁴ during the first week of lactation. The origin of this excreted iron is unknown, although the bile seems a likely source, and lactoferrin may thus serve to prevent this iron being reabsorbed. This postnatal unloading of iron may serve to reduce susceptibility to infection⁵⁷ or even, as suggested by Neffgen and Rakušan⁵⁸ to stimulate myocardial growth.

McMillan *et al.*⁵² have shown that the iron in human milk, despite the high lactoferrin content, is more readily absorbed than iron from a simulated human milk of comparable iron content, and furthermore this availability was not affected by boiling the milk, which would denature lactoferrin. However, these studies were performed in adults, whose capacity to digest lactoferrin would be greater than that of infants, and furthermore there were significant differences in mineral content between the simulated and human milks,⁵⁹ which might affect iron availability. Likewise it must be remembered that the infants studied by Saarinen *et al.*,⁵¹ in whom high bioavailability of breast-milk iron was demonstrated using the extrinsic tag method, were all over 6 months old and would also have had a relatively well developed digestive system. Nevertheless, a tracer dose of iron administered after a 3-hour fast was slightly less well absorbed than a similar dose administered during a breast-milk feed,⁵¹ suggesting that breast milk does contain factors which actively promote iron absorption. It is perhaps possible to envisage two opposing effects operating in the breast-fed infant: in the newborn, iron absorption is relatively poorly controlled, but high lactoferrin levels in the milk and low gastrointestinal proteolytic activity tend to prevent iron becoming available for absorption. As the infant matures his need for exogenous iron increases, milk lactoferrin levels decrease and proteolytic activity increases, resulting in progressively more iron being released from

lactoferrin. Once released, absorption of this iron is facilitated by other factors present in human milk, as suggested by Saarinen *et al.*⁵¹ It may be more than coincidental that lactoferrin levels are highest in the milk of man and the guinea-pig, both of whose young grow notably more slowly than those of other species of comparable size, and whose need for exogenous iron will be relatively retarded. In species whose young grow rapidly—for example, rabbit, rat, pig, and cow—lactoferrin levels are, except in colostrum, low or nonexistent⁴ and would not significantly control the absorption of milk iron which would be required to meet the rapidly-increasing blood volume. It is noteworthy in this respect that newborn rats, unlike human infants,⁶⁰ absorb virtually 100% of milk iron. Some basis for the hitherto unexplained erratic distribution of lactoferrin in the milk of different species may thus be provided by this hypothesis. However, further experimental work, preferably using purified lactoferrin in newborn animals or babies will be required to test the hypothesis that lactoferrin in milk serves to control iron absorption in the neonate.

Conclusions

This annotation has attempted to discuss critically the data on the role of milk lactoferrin in antimicrobial activity and iron absorption. Considerable attention has been focused on the role of lactoferrin in protecting the neonate against gastrointestinal infections, but further *in vivo* experimental data are needed before such a function can be considered as unequivocally established. More neglected, but perhaps more deserving of further study, is the role of lactoferrin in iron absorption. In this annotation it is proposed that a major function of lactoferrin may be to control iron absorption, and perhaps even to facilitate excretion of iron in the immediate postnatal period. Although this theory needs to be backed by direct experimental data, it has the attraction of providing a rational explanation of the curious variation in lactoferrin levels in milks of different species.

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