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LITERATURE HIGHLIGHTS

A sideways glance: Take it or leave it? The role of lactoferrin in iron sequestration and delivery within the body

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Lactoferrin (Lf) is a cationic iron binding protein of 80 kDa, belonging to the transferrin family, and like transferrin it can bind two Fe^{3+} atoms per molecule. Lf is expressed and secreted by several gland epithelial cells in mammals and, as a result, it is present in different mucosal secretions. It is found at highest levels in human colostrum (about 7 g/l) and at lower level in mature milk (between 2 and 4 g/l); pancreatic juice, bile, sperm vaginal and bronchial secretions also contain Lf, although to a lesser extent. Lfs are expressed in almost all mammalian species (with few exceptions, such as rats and dogs) with marked interspecies conservation in primary sequence and conformation, mainly differing in the glycan moiety [4].

Two main physiological functions have been ascribed to this protein: a key role in iron homeostasis in the newborn, and active participation to pre-immune defence against microbial and viral attacks. In this commentary, I will mainly focus on the involvement of Lf in the regulation of iron metabolism. For an updated review of Lf functions in host defence I suggest reading the paper by Valenti and Antonini [11].

Initial evidence for the importance of Lf came from the observations of Lönnerdal and collaborators who, more then 10 years before, reported that breast fed-infants delivered by women with good iron status benefit themselves from adequate iron status during the first 6 months of neonatal life [3]. Their observations provided a background to the hypothesis that Lf, the major Fe carrier in human milk, was responsible for high bioavailability of iron from this dietary source. The Lf protein is found intact in the stool of breast-fed infants, and a specific Lf receptor (Lf-R), expressed in the intestine of newborn infants, was cloned and characterised by Suzuki et al. [10].

In a recent paper [6], the role of Lf/intestinal Lf-R in iron absorption during early life was further clarified. Using a mouse model, the authors examined intestinal localization of Lf-R, as well as that of divalent metal ion transporter 1 (DMT1), the major Fe^{2+} transporter in adult intestine, by immunohistochemistry during foetal life (gestational days 13.5, 15.5 and 18.5) and in newborn pups [postnatal days (PD) 0, 5, 10 and 20], reporting expression of Lf-R during mid to late gestation. After birth, the immunolocalization pattern of Lf-R was initially diffused (PD 0 and PD 5) becoming more intense by PD 10 and PD 20. However, Western blots of proteins obtained from intestinal homogenates, probed with anti DMT1 antibodies, revealed a major protein band of 120 kDa (corresponding to fully glycosylated, functional DMT1) peaking at PD 20, only in brush border membrane vesicle-enriched fractions, whereas only the immature form of 50 kDa was detected in whole tissue homogenates. The authors concluded that iron uptake through DMT1 is not the prevalent pathway in the time window considered, since this protein appears to be mislocalized during late gestation, expressed at very low level during early neonatal life and predominantly expressed as immature form at PD 20. This expression pattern supports a central role for Lf in receptor mediated iron uptake from milk in the early post-natal period.

This result was somehow in disagreement with the conclusions put forward by Conneely and collaborators on the role of lactoferrin in newborn Fe homeostasis [12]. These authors found that pups from Lf KO mice (LFKO) did not indeed develop iron deficient anemia. On such basis, they ruled out Lf as a determinant factor in iron delivery during early neonatal life in the mouse. However,

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their observations are quite puzzling, in that they also observed mild iron overload during the suckling period in LFKO mice, possibly indicating a pivotal role of Lf in regulating iron distribution within the body. This mechanism would support, in their view, a role of this protein in iron sequestration, rather than in iron delivery.

A clinical trial involving 300 pregnant women affected by iron-deficient anemia, recently performed by Valenti and collaborators, contributes to clarifying this long-standing controversy on the role of Lf in iron homeostasis, at least in adulthood [8]. After 30 days of the oral administration of bovine Lf, the group of treated women showed higher hemoglobin and total serum iron (i.e. mainly transferrin iron) than that observed in both ferrous sulphate-treated and control women (those refusing any kind of treatment). This result appears very promising for the use of Lf in the treatment of iron-deficient anemia, which is, at present, one of the prevalent nutritional deficiencies in western countries. Moreover, significantly high increase of total serum iron in all women supplemented with bovine Lf led the authors to speculate about the involvement of this protein in a more general mechanism of regulation of iron homeostasis within the body.

To add further information to the debate about the most relevant physiological role of Lf, I would also like to mention that nuclear localization of Lf was reported in intestinal Caco-2 cells [1]. In agreement with this localization, as far back as 1995, He and Furmansky reported that Lf binds DNA with sequence specificity, leading to transcriptional activation of CAT-reporter constructs containing copies of the putative consensus Lf DNA-binding sites in transfected K562 myelogenous leukemia cells [2]. Lf-dependent expression of the *IL-1β* gene was later demonstrated by Son et al. [9] in K562 cells treated with both Lf and PMA. In contrast with these results, it was recently shown by Sanderson and collaborators that milk Lf does not regulate transcription by binding to Lf-responsive elements in genomic DNA of K562 and Caco-2 cells and does not localise to the nucleus; moreover, in the same paper they demonstrated that specific binding to DNA only occurs extracellularly to pro-inflammatory bacterial DNA sequences (CpG) motifs. This binding inhibited NF-κB regulated gene transcription induced by CpG-motif in B cells [7].

Rather than coming to unequivocal conclusions, I will end this commentary with what I believe are the most interesting and still open questions:

1. Is there a role for Lf as a transcription factor?
2. Is this protein better designed to take up iron from the environment and hence act as a regulator of iron metabolism by sequestration, rather than devised to deliver iron to cells in a highly bioavailable form?
3. Intestinal Lf-R is expressed in human infants ingesting Lf with maternal milk. What about the adult intestine? Is Lf partially resistant to proteolysis also in the adult gastrointestinal tract? In other words, is there any specific role for milk and/or other Lfs in adult iron metabolism?
4. The answer to this latter question is particularly relevant when considering the potential use of human recombinant Lfs in infant formulas [5]. However, would it be necessary to use homologous Lf for iron supplementation of human adults, when bovine Lf was proved to successfully alleviate iron-deficient anemia?

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