Clinical Benefits of Lactoferrin for Infants and Children

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To discuss the potential clinical benefits of lactoferrin in preterm and term infants, as well as in young children and to review information on the burden of neonatal sepsis. Current evidence on the mechanisms that explain the role of human milk in the neonatal and infant anti-infective responses will be briefly reviewed and preclinical research data on the potential mechanisms of action by which lactoferrin may impact infant gut health, gut immune development and functions, including the lactoferrin effects on the neonatal microbiome, will be examined. Finally, updated translational research on lactoferrin will be presented and discussed and the current evidence from prospective randomized controlled trials in neonates, infants, and toddlers will be analyzed. These randomized controlled trials demonstrate that lactoferrin has a clinically significant impact on feeding, the microbiome, and clinical outcomes in neonates and infants. *(J Pediatr 2016;173S:S43-52)*.

Neonatal infections and sepsis occur frequently in neonatal intensive care unit (NICUs) with substantial morbidity in preterm very low birth weight (VLBW <1500 g) neonates. Incidence varies from center to center; however, the burden of morbidity related to neonatal infections is generally high and results in prolonged stays in the NICU, increased attributable mortality, as well as poor short- and long-term outcomes, including late neurodevelopmental impairment in survivors. A wide range of causative pathogens may be involved. However, infections are generally acquired horizontally and/or nosocomially and typically occur after 72-96 hours of life (so called late-onset sepsis [LOS]).[1]

Empiric treatment is often instituted when the necessity of treatment has not been clearly established because of the high burden of neonatal infections and sepsis, the difficult diagnosis, and the lack of specific, early, and sensitive biomarkers. It is of particular concern that even when affected infants are treated appropriately and timely, many show poor neurodevelopmental performance after 12-18 months of age, suggesting that current strategies to manage infections are not reliable.[2] Prevention of neonatal infections in the NICU is critical and is a far better strategy than treatment.

A comprehensive outline of all possible preventative strategies to avoid or limit infections in the NICU includes bundles of neonatal management (human fresh milk feeding, use of specific nutrients and bioactive substances with putative anti-infective actions, use of probiotics to enhance the enteric microbiota composition, restriction in the use of H2-blockers [H2B], stringent hygiene measures, and cautious central venous catheter management), as well as prophylactic pharmacologic interventions (mainly, specific antifungal prophylaxis with fluconazole or specific antirespiratory syncytial virus prophylaxis with palivizumab).[3]

Human milk is of paramount importance in preventing infections and other morbidities in neonates. The following is a brief review of the current evidence on the mechanisms that explain its multiple roles in the context of neonatal and infant anti-infective responses. According to published evidence, feeding human fresh milk to infants prevents bronchopulmonary dysplasia/chronic lung disease,[4] retinopathy of prematurity (ROP),[5,6] necrotizing enterocolitis (NEC),[7,8] and other infections.[7,9]

The beneficial effects of human fresh milk for preventing infections in neonates are linearly associated with the volume of intake. Only mean daily intakes of human fresh milk higher than a certain threshold may deliver a preventive effect on infections. However, that threshold has not yet been identified. It should be noted that according to a randomized, blinded trial in premature infants, pasteurized donor human milk used as a substitute for mother's own milk, offers no observed short-term advantage over preterm formula in terms of LOS or NEC incidence rates.[7]

The question, therefore, is: "Why is donor milk not fully protective against sepsis?" It is likely that the processing of human fresh milk decreases the availability and function of a number of bioactive substances that are putatively responsible for its anti-infective properties. Pasteurization at 62.5°C for 30 seconds (Holder methods) produces a decrease in IgA, lactoferrin,

bLF	Bovine lactoferrin
H2B	H2-blockers
ICU	Intensive care unit
LGG	*Lactobacillus rhamnosus* GG
LOS	Late-onset sepsis
NEC	Necrotizing enterocolitis
NICU	Neonatal intensive care unit
RCT	Randomized controlled trial
ROP	Retinopathy of prematurity
VLBW	Very low birth weight

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lysozyme, and lymphocytes by 60%-75%, whereas freezing at +4°C for 24 hours produces a 40% decrease of many of the same bioactive substances.[10-12]

Is Lactoferrin Responsible for the Anti-infective Actions of Human Milk?

Among all of the bioactive substances found in human milk, lactoferrin may be the one most comprehensively involved in milk's well known anti-infective actions. Lactoferrin is, in fact, the major whey protein in all mammalian milk. It is worth noting that there is a very high (77%) structural homology between bovine lactoferrin (bLF) (extracted and purified from cow's milk) and human lactoferrin (either extracted from colostrum or produced via recombinant engineering [talactoferrin]). bLF is added to commercial formulas in some countries and has been granted Generally Recognized As Safe status by the Food and Drug Administration.

Lactoferrin has a high rate of survival after oral administration. In the stomach, pepsin digests and releases a potent peptide antibiotic called lactoferricin from native lactoferrin. This occurs with both human lactoferrin and bLF, as they share the same N-terminal, 11-aminoacidic peptide with antimicrobial activity.[13] After stomach passage, lactoferrin has a high intestinal uptake and gut action[14] with poor (10%) intestinal absorption. It is of note that concentrations of lactoferrin decrease in mature human milk vs colostrum, and this decrease typically occurs in all mammalians. In humans, mothers of premature infants may have sustained higher levels in colostrum and in intermediate milk, compared with mothers of term infants.[15]

Lactoferrin May Impact Gut Health and Gut Immune Development and Functioning

There is a body of evidence from preclinical research exploring the potential mechanisms of action by which lactoferrin may impact infant gut health and gut immune development and functioning, including lactoferrin's effects on the neonatal microbiome.

Several mechanisms account for the potent anti-infective activity of lactoferrin. It is intriguing to consider the ability of lactoferrin to modulate the gastrointestinal tract of infants in the very early stages of life, by interfering with its permeability and ultimately exerting a mucosal trophic effect.[16] In this view, lactoferrin may play a specific role in the development of the nascent gut by being a major modulator of gut permeability. It is known that intestinal permeability changes as a function of age and type of feeding. The feeding of human milk may modulate the trophism of the gastrointestinal tract of preterms, enabling a more rapid maturation of intestinal epithelium.[16] Gut permeability and mucosal trophic effect are key factors for the prevention of infections and NEC in infants fed human milk. This is in line with data showing that the feeding of human milk (vs formula) is associated with decreased permeability at 28 days of age.[17] A number of preclinical studies, both in vitro and in an animal (piglet) model, suggest that lactoferrin may play a key role in increased intestinal maturation and decreased permeability.[18,19]

Buccigrossi et al[18] assessed lactoferrin for its trophic effect on enterocytes and on the development of gut function in enteric Caco 2 cells in vitro. A wide range of bLF and human lactoferrin concentrations was tested on the proliferation of rapidly growing enteric Caco-2 cells (measured as number of enterocytes), and on the differentiation of enteric Caco-2 cells (measured as sucrase and lactase activities). bLF was compared with human lactoferrin, and bLF was used in concentration equimolar to human lactoferrin. This experiment showed that lactoferrin had a trophic effect on enterocytes that was related to its concentrations; the higher the lactoferrin concentration, the faster the enterocytes proliferation occurred, whereas the lower the lactoferrin concentration, the faster the enterocytes differentiation occurred. These actions occurred with both bLF and human lactoferrin.

This study possibly reproduced a natural model, in which lactoferrin is highly concentrated in colostrum, and less in mature milk and demonstrates that lactoferrin is a key modulator of intestinal epithelium development, and that bLF and human lactoferrin have similar actions on the nascent gut. It can be speculated that by promoting fast proliferation of the enterocytes in a nascent intestine, lactoferrin may create a less permeable environment as gut wall leaks and gap junctions become tighter, resulting in fewer colonizing pathogens disseminating to the bloodstream through translocation via a leaky gut wall. It appears that infants may require large amounts of lactoferrin in the very early stages of life, to mimic what they would consume naturally via the mother's breast milk. Additional data show that commercial bLF exerts several of the bioactivities of human lactoferrin if added to infant formula.[14]

Recent evidence provides interesting insights on the beneficial effect that lactoferrin exerts on the microbiota of infants and toddlers. Mastromarino et al[20] measured the content of lactoferrin and the microbiota of breast milk and feces of infants of 48 mother–infant pairs (34 full-term and 14 preterm infants) at birth and 30 days after delivery. They found that in the term group, there was a significant decrease of mean lactoferrin concentration between colostrum (7.0 ± 5.1 mg/mL) and mature milk (2.3 ± 0.4 mg/mL). In the preterm group, breast milk lactoferrin levels were similar to those observed in full-term group. When measuring the lactoferrin content of feces, they found that fecal lactoferrin concentration of healthy infants was extremely high both in term and preterm infants (higher than the amount reported in healthy children and adults). In term infants mean fecal lactoferrin levels significantly increased from birth (994 ± 1828 lg/mL) to 1 month of age (3052 ± 4323 lg/mL). The amount of lactoferrin in the feces of 30-day-old term infants was significantly associated with maternal mature milk lactoferrin concentration ($P = .030$), confirming that breast milk represents the main source of lactoferrin found in the gut of infants. In preterm infants, higher mean concentrations of fecal lactoferrin