The Role of Lactoferrin in Gastrointestinal and Immune Development and Function: A Preclinical Perspective

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The early postnatal period is a critical time for gastrointestinal (GI) and immune development. Neonates fed mother's milk have more rapid GI and immune development than fed-formula infants. In addition, clinical and epidemiologic data provide strong evidence that breastfeeding reduces the incidence and/or severity of infectious diseases. Lactoferrin is a 77 kDa, iron-binding glycoprotein that is present at high concentration in human milk compared with bovine milk and infant formula. It is a multifunctional protein that mediates many of the physiological processes in which breastfed infants have advantages over their formula-fed peers, including promoting GI and immune development, protection from infections, and improved cognitive development. Feeding bovine lactoferrin or recombinant human lactoferrin was well tolerated and stimulated intestinal cell proliferation and increased villus length and crypt depth in piglets. Lactoferrin also influenced both systemic and GI immune development by stimulating a balanced T-helper-1/T-helper-2 cytokine immune response. Further, there was a tendency for immune cells to secrete more anti-inflammatory cytokines in an unstimulated state, while being primed for a robust pro-inflammatory response when presented with a bacterial trigger in piglets fed lactoferrin. These findings support clinical studies demonstrating benefits of dietary lactoferrin in the prevention of infections, late onset sepsis, and necrotizing enterocolitis. *(J Pediatr 2016;173S:S16-28)*.

The early postnatal period constitutes a key phase of development, as the infant transitions from the relatively protected intrauterine environment to a world rich in antigenic and pathogenic challenges.[1,2] The gastrointestinal (GI) tract undergoes marked structural and functional adaptation to enable digestion and absorption of nutrients. This has been observed in term[3] and preterm infants[4-6] but has been best demonstrated in the pig, where ingestion of colostrum and milk stimulate visceral organ growth and mucosal mass,[7-10] DNA and protein synthesis,[11] morphologic changes,[9] immunoglobulin uptake,[12] and digestive enzyme activity.[13] In addition, the GI tract is home to the largest reservoir of immune and microbial cells in the body, and early nutrition is key to promoting GI growth,[1] colonization with a healthy microbiota,[2] and education of the immune system.[2,14,15] Recent work has highlighted the importance of bidirectional communication between the gut microbiota and the host immune system, in which the microbiota stimulates maturation and specificity of the neonatal mucosal and systemic immune systems.[16,17] In turn, the immune system ensures that the commensal gut microbes are tolerated, while remaining responsive to pathogenic infections.[16,18] In addition, the immune system[19] and microbiota[20,21] are essential for normal GI development. Although often not appreciated, early nutrition is a key regulator of these interactions by directly influencing intestinal growth and barrier function, microbial composition, and the function of the immune system[16,22] (**Figure**).

Breastfeeding and human milk represent the gold standard for infant feeding and nutrition.[23] Clinical and epidemiologic evidence demonstrate both short- and long-term benefits of breastfeeding, particularly exclusive breastfeeding for 6 months or more, relative to formula-feeding.[23] In the short term, breastfeeding decreases the incidence and/or severity of infectious diseases.[23] Most diseases with lower incidence in breastfed vs formula-fed infants have infectious and immune components in their etiology, including diarrhea, respiratory and urinary tract infections, otitis media, bacteremia, and necrotizing enterocolitis (NEC).[23,24] Breastfeeding also has been implicated in long-term reduction of disease risk,[25-27] including those involving the immune system, such as inflammatory bowel disease, celiac disease, asthma, allergy, type 1 diabetes, and acute lymphoblastic and myeloblastic leukemias.[23,28] These benefits may be mediated, in part, through effects of breastfeeding on the microbiome.[29] Systematic reviews of the literature have demonstrated that breastfeeding reduces the risk of noncommunicable diseases, including obesity and type 2 diabetes[30] and improves cognitive development.[31] The American Academy of Pediatrics recommends exclusive breastfeeding for 6 months, followed by continued breastfeeding as complementary foods are introduced, with continuation of breastfeeding for 1 year or longer as mutually desired by mother

Asn	Asparagine	mRNA	Messenger RNA
bLF	Bovine lactoferrin	NEC	Necrotizing enterocolitis
BSA	Bovine serum albumin	NK	Natural killer
ERK	Extracellular signal-regulated kinase	rbLF	Recombinant bovine lactoferrin
GI	Gastrointestinal	rhLF	Recombinant human lactoferrin
hLF	Human lactoferrin	SR	Sow-reared
IFN	Interferon	Th	T-helper
IL	Interleukin	TNF	Tumor necrosis factor
LFR	Lactoferrin receptor	WT	Wild type
MLN	Mesenteric lymph node		

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Please see the author disclosures at the end of the article.

http://dx.doi.org/10.1016/j.jpeds.2016.02.072

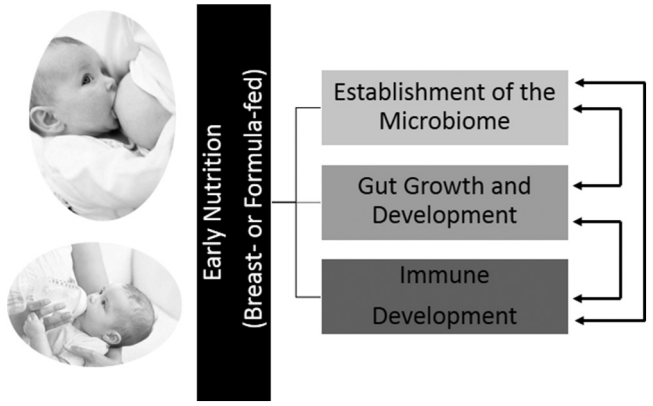

Figure. Early life nutrition regulates GI and immune development and establishment of the microbiota. The intestinal ecosystem represents a complex, interactive environment in which nutrition directly influences intestinal development, establishment of gut microbiota, and maturation of the gut mucosal and systemic immune system. In turn, signals from the microbiota stimulate maturation and specificity of the mucosal and systemic immune systems. In addition, the immune system and microbiota promote intestinal development. Human milk contains several bioactive nutrients that modulate these processes, and lactoferrin is a key component.

and infant.[23] However, based on the Centers for Disease Control and Prevention 2014 Breastfeeding Scorecard, the current breastfeeding rates in the US fall short of the American Academy of Pediatrics and Surgeon General's recommendations for initiation and duration of breastfeeding, with only 79.2% of infants in the US being ever breastfed, and only 49.4% and 26.7% of infants being breastfed at 6 and 12 months of age, respectively.[32]

Human milk contains bioactive proteins, lipids, and carbohydrates that stimulate intestinal development, actively protect the infant from pathogenic infection, and facilitate the establishment of the microbiota.[1,17,33] Therefore, approaches to narrow the compositional and functional gaps between human milk and infant formula continue to be an active area of preclinical and clinical research. Over the past 30 years, the composition of infant formula has been modified by the adjustment of the whey-to-casein ratio, taurine and nucleotide fortification, and the addition of docosahexaenoic acid, among others. However, health disparities between breastfed and formula-fed infants persist.[34,35] Therefore, additional ingredients, including human milk oligosaccharides[36-38] and bioactive proteins, including lactoferrin,[38-40] and osteopontin,[41,42] have been a focus of preclinical and clinical research.

Lactoferrin is of particularly interest because it has been shown to mediate many of the physiological processes in which breastfed infants have advantages over their formula-fed peers, namely GI[40] and immune development; protection from viral,[43] fungal,[44] and microbial[45,46] infections; and NEC[38,47]; as well as improved cognitive development.[48] Several of these topics will be covered by other articles in this supplement, therefore, the goal of this article is to review the evidence for a role of lactoferrin in GI immune development from a preclinical piglet model.

The Piglet as a Preclinical Biomedical Model

Along with the nonhuman primate, the neonatal piglet is considered one of the best preclinical models for the human infant. There are many practical and biological benefits of the piglet model. In terms of nutrition, the pig is omnivorous, easy to artificially rear from birth,[49,50] and can be subjected to under- or overnutrition to investigate protein-energy malnutrition[51] or obesity[52], respectively. Piglets have an accelerated growth rate relative to human infants, and this facilitates shorter-term studies to model infant development, enabling rapid detection of adverse effects of nutritional deficiencies.[49,50] Piglets also can be obtained preterm[53] or at term but small-for-gestational-age,[54-56] allowing for investigation of immaturity or intrauterine growth restriction. The piglet is sufficiently large at birth (~1.2 kg) to facilitate surgical models, such as short bowel syndrome,[56,57] venous and arterial catheterization, and parenteral support.[58,59] The nearly identical digestive anatomy and physiology to humans supports studies on normal nutrition as well as intestinal disease including models of NEC,[56] total parenteral nutrition-induced mucosal atrophy,[59] and viral[37,51] and bacterial[60] infections, among others. The high degree of similarity in immune variables between humans and pig (~80%) compared with humans and rodents (~10%)[61,62] enables studies to focus on immune development[39] or immune responses to infection[37,51] and inflammation such as NEC.[56] Lastly, humans and piglets have gyrencephalic brains and exhibit similar growth trends across brain regions[63,64] enabling screening for effects of milk bioactive components on brain structural development, cognitive behavior, learning, and memory.

Lactoferrin Structure and Lactoferrin Receptors

Lactoferrin is a 77 kDa, iron-binding glycoprotein of the transferrin family.[65,66] It is a multifunctional protein that is present at high concentration in human milk relative to cow milk and infant formulas.[65,67] Lactoferrin is the most abundant protein in human milk whey, with concentrations ranging from 6 g/L in early milk (<28 days lactation) to 2 g/L in mature milk.[68] Lactoferrin concentrations in bovine and porcine colostrum and mature milk are 0.8 and 0.1 g/L[69] and 1.6 and 0.4 g/L,[70,71] respectively. Rat milk is devoid of lactoferrin, and transferrin serves as the milk iron-binding protein.[72]

Bovine lactoferrin (bLF) and human lactoferrin (hLF) consist of 689 and 691 amino acids, respectively, with an overall shared amino sequence identity of 69%.[73] Porcine