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REVIEW ARTICLE

Protection of the Neonate by the Innate Immune System of Developing Gut and of Human Milk

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ABSTRACT: The neonatal adaptive immune system, relatively naïve to foreign antigens, requires synergy with the innate immune system to protect the intestine. Goblet cells provide mucins, Paneth cells produce antimicrobial peptides, and dendritic cells (DCs) present luminal antigens. Intracellular signaling by Toll-like receptors (TLRs) elicits chemokines and cytokines that modulate inflammation. Enteric neurons and lymphocytes provide paracrine and endocrine signaling. However, full protection requires human milk. Breast-feeding reduces enteric infection and may reduce chronic disease in later life. Although human milk contains significant secretory immunoglobulin A (sIgA), most of its protective factors are constitutively expressed. Multifunctional milk components are nutrients whose partial digestion products inhibit pathogens. Cytokines, cytokine receptors, TLR agonists and antagonists, hormones, anti-inflammatory agents, and nucleotides in milk modulate inflammation. Human milk is rich in glycans (complex carbohydrates): As prebiotics, indigestible glycans stimulate colonization by probiotic organisms, modulating mucosal immunity and protecting against pathogens. Through structural homology to intestinal cell surface receptors, glycans inhibit pathogen binding, the essential first step of pathogenesis. Bioactive milk components comprise an innate immune system of human milk whereby the mother protects her nursing infant. Interactions between human milk glycans, intestinal microflora, and intestinal mucosa surface glycans underlie ontogeny of innate mucosal immunity, pathobiology of enteric infection, and inflammatory bowel diseases. **(*Pediatr Res* 61: 2–8, 2007)**

At parturition, the mother-infant dyad switches from aseptic transfer of nutrients through the umbilicus to dependence on milk and the neonatal intestine to transfer nutrients and protect against enteric pathogens. A rich cornucopia of protective agents in the infant gut and in human milk may compensate for the naïve state of adaptive immunity in the neonate (1,2) and the immaturity of other gut systems. Many components contribute to a potent innate immune system of neonatal gut and of human milk. The adaptive immune system is characterized by exquisite antigen specificity and delayed reaction that leads to memory, whereas the innate immune system, which is expressed constitutively, provides rapid or ongoing protection against broad groups of molecules without generating memory. The components of these innate immune systems of gut and milk are reviewed below to consider the degree to which they are complementary or synergistic. Their absence or malfunction may allow enteric disease, including inflammatory bowel diseases.

INTESTINAL MUCOSA

The intestinal mucosa is in intimate contact with microbiota, the symbiotic ecosystem of more than 450 species of mutualist and commensal microorganisms, often including opportunistic and obligate pathogens (3,4). The gut actively restrains these symbionts, especially the pathogens, constitutively with some elements of the innate immune system, reactively with other elements, and with some delay *via* the adaptive immune system. The most widely recognized element of the adaptive immune system is sIgA (5). The innate immune system is the sum of physical barriers (6), chemical barriers (including secretions), and reactive elements of local cells and cells recruited to a threatened site (7). The gut epithelium creates a tight barrier that separates luminal antigens and gut microbiota from invading the host (6) while activating underlying lymphoid elements (7). Activation of reactive cellular elements can also stimulate responses by the adaptive immune system. The epithelium is a first responder of the mucosal immune system (8).

The strong acid, proteases, and peptides secreted into stomachs of adults are major components of this barrier. In the infant, in whom these secretions are not as well developed (9), lingual and gastric lipases digest human milk triglyceride into free fatty acid (FFA) and monoglyceride (10), which, at concentrations found in stomachs of breast-fed infants, are known to be highly toxic to many human pathogens, especially enveloped viruses and some parasites (11).

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Abbreviations: DCs, dendritic cells; **NEC,** necrotizing enterocolitis; **PMNs,** polymorphonuclear leukocytes; **sIgA,** secretory immunoglobulin A; **TLR,** Toll-like receptor

A major physical barrier of the gut is the thick mucin-rich glycocalyx that lines the entire alimentary canal (12). In the intestine and colon, the epithelial monolayer is 93%–95% absorptive enterocytes (13), whose surface area is further increased by microvilli on apical surfaces. Because highly glycosylated proteins and lipids saturate microvilli, their electron micrographs resemble thick, dark, brushlike structures reminiscent of furry tails named glycocalyx (14). Within and extending beyond the glycocalyx is a thick layer of mucus secreted by goblet cells (3%–5% of mucosal epithelium) (15) that forms a physical barrier embedded with antimicrobial peptides (16) and Paneth cells (17,18). Where this mucus layer meets the luminal contents of the gut is a loosely formed biofilm of symbiotic microbiota. The composition and glycosylation of intestinal glycocalyx and mucins differ between neonates and adults. These differences may be a primary determinant of the distinct differences in microbial composition of the intestine of neonates and adults (19,20) and of their differing susceptibility to enteric pathogens.

Differentiated villus cells are replaced every 5 d in humans and every 3 d in rodents by proliferative cells descended from stem cells at the base of the crypts of Lieberkühn (13,15). These stem cells are protected by their recessed physical location (21,22) and by Paneth cells. As proliferative cells differentiate, one lineage is Paneth cells that migrate back toward the bottom of each crypt, where they secrete antimicrobial peptides, lysozyme, and other products that engender an aseptic sterile microdomain (16,18,23). Inhibiting colonization in the crypt minimizes microbial alteration, insult, or damage to stem cells, protecting their pristine nature for generating the lifelong stream of new naïve pleuripotent cells required to maintain functional epithelium of the gut (24). These antibacterial inhibitors from the crypts may also become incorporated into the glycocalyx/mucin layer to help limit or localize colonization by all enteric symbionts: commensals, mutualists, and pathogens (2,4,19). Moreover, the production of antimicrobial peptides may be both temporally transient and spatially specific, which could limit microbial colonization to only the appropriate locations in the gut (25).

Interdigitating the villus structures are Peyer's patches, occasional domelike follicle-associated epithelial structures located above aggregates of lymphocytes (26) containing uniquely differentiated microfold cells on the surface that monitor luminal antigens (27,28). Microbes and large molecules are sampled on the apical (luminal) side of microfold cells, and their antigens presented at the basal side to mucosal lymphoid elements (26,27), including macrophages and DCs. DCs, the primary antigen-presenting cells of the body, present the antigens locally to T lymphocytes or travel through the lymphatic system and present the antigens, principally in the mesenteric lymph node. Intestinal DCs are also found in the lamina propria, where their cytoplasmic extensions protrude across intact tight junction barriers through the villi into the lumen (8,29). They sample luminal contents and likewise present luminal antigens locally or in other areas of the lymphatic system, including mesentery, where they present processed antigens on appropriate MHC class molecules to generate specific primed T cells (8,27,28). This ultimately results in production of sIgA in the gut, which can prevent luminal bacteria adherence to enterocyte surfaces (5). Although this system is underdeveloped in the newborn, very high concentrations of sIgA in colostrum and early milk may help to compensate.

The major resident phagocytic lymphoid cell in the gut is the macrophage (5,7,18,30,31), found in Peyer's patches and more diffusely in the lamina propria (28,30). The developing intestinal mucosa also acquires many mast cells that affect defense against intestinal parasites and enteric bacterial pathogens through tumor necrosis factor (TNF)-α release (32). Mast cells also mediate IgE-associated allergic responses (33). Epithelium produces various cytokines (34) and chemokines (35) that summon lymphocytes for mucosal immune response to infection or other breach of the epithelial barrier. Dispersed throughout the intestinal mucosa are endocrine cells and neurons; mucosal neuroendocrine secretions coordinate digestion, motility, gut regeneration and maturation, and immune functions both within the alimentary canal and with the other organ systems. The development and mode of distribution of these cells during intestinal ontogeny are not well understood.

Central to intestinal mucosal immunologic integrity are signals for sensing and reacting to both pathogenic and resident microbiota generated by the TLR family (36). TLR molecules (10 in humans, 11 in mice) bind epitopes characteristic of microbes, pattern recognition molecules (36,37). Each TLR initiates characteristic transcellular signals, activating nuclear transcription of genes whose products stimulate innate immune responses, including recruitment of cellular responders (38,39). The constant presence of microbiota requires selective attenuation of TLR signaling pathways in the intestinal epithelium to prevent chronic inflammation. In contrast, Paneth cells in crypts express TLR whose facile activation mediates release of potent antimicrobial agents (40). Overall, the intestinal mucosa must balance constant immunosurveillance, signaling to the periphery, and inflammatory homeostasis. The TLR expressed by intestinal epithelial cells (41) and intestinal macrophages (42) before birth are highly sensitive to stimulation, but after birth, TLR proteins, although still expressed, are much less active (43,44).

Immunologic monitoring of the gut lumen links local mucosal events to the peripheral immune system (8,27,28). Antigen-presenting cells, primarily DCs, continuously sample the luminal environment (29) and can mount a rapid cytokine response typical of innate immune responses and also activate the delayed antigen-specific lymphocyte response typical of the adaptive immune system (8,30). These two arms of the mucosal immune system initiate a self-limited inflammatory response in mature gut by recruiting activated neutrophils and monocytes into the lamina propria from blood vessels, normally mounting a localized immune response rather than a chronic systemic inflammatory response. This complex attenuation of the mucosal response in mature gut is incompletely developed in many premature infants (34,41). This could, in part, account for the excessive inflammatory response in premature infant gut in inflammatory bowel diseases such as necrotizing enterocolitis (NEC). Moreover, discrimination between pathogens and nonpathogenic symbionts involves im-

munomodulation that develops in the infant perinatally. Accordingly, for term infants, whose mucosal immune system is not fully mature, and especially for premature infants, whose mucosal immune system is immature, an exogenous source of supplementary immunosuppression, were it also to provide potent protection from pathogens, would be beneficial, if not essential. Human milk is the richest known source of such immunomodulation and protection.

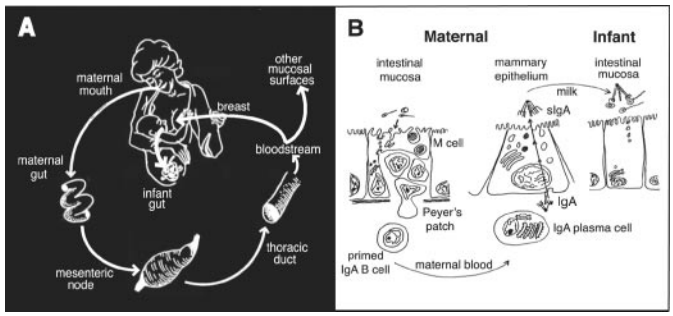

Figure 1. Enteromammary circulation allows the maternal mucosal immune system to protect the intestine of the infant *via* human milk. When an oral inoculum of a pathogen enters the maternal gut, it is sampled from the lumen by Peyer's patches, and its antigens are presented to underlying lymphatic cells. IgA production is induced at the basolateral side of the mammary cell, and the IgA traverses the mammary cell to enter the milk as sIgA. The sIgA enters the gut of breast-fed infants, where it protects the infant by binding to the pathogen. This is illustrated at the organ level in panel A *(adapted from Kleinman RE and Walker WA 1979 Digest Dis Sci 24:876–882, © 1979 Springer Science+Business Media, Inc. 2006, with permission),* and at the cellular level in panel B *(adapted from Kraenhenbuhl J-P et al. 1988 In: Hanson LA (ed) Biology of Human Milk. © 1988 Raven Press Ltd., with permission).*

HUMAN MILK

A relationship between breast-feeding and infant health had been recorded periodically for thousands of years across many disparate civilizations (45). In 1934, a report on 20,000 mother-infant dyads in the United States found that morbidity or mortality due to enteric disease was several times higher for nonbreast-fed infants than for breast-fed infants (46). With improved nutritional content of artificial infant formulas in the 1950s, better hygiene, greater weight gain in artificially fed infants, and the simultaneous dramatic reduction in infant mortality, many thought that this relationship was no longer relevant, and many medical settings actively discouraged breast-feeding. However, subsequent epidemiologic studies (47–49) in heavily populated areas of the world found that artificially fed infants were at three- to 10-fold higher risk of disease, especially enteric infections leading to diarrhea. When of careful design and adequate sample size, studies in developed nations also indicated a significantly lower frequency and/or severity of disease in breast-fed infants relative to those fed artificially (50,51). These differences suggest that human milk has protective and immunomodulatory activities that are lacking in even the best artificial formulas.

Furthermore, in developed countries, the increased number of premature infants in neonatal intensive care units has been accompanied by an epidemic of inflammatory bowel diseases, especially NEC. Ninety percent of NEC patients had been fed formula without human milk (52,53), and inclusion of human milk in the diet of the premature infant is associated with a lower risk of NEC. This is most often attributed to direct immunomodulation by milk, the prebiotic effect of its indigestible complex carbohydrates (glycans), the ability of glycans to inhibit colonization by pathogens, or combinations of these activities (2,31).

Recent reports associate artificial feeding of neonates with subsequent chronic diseases of later life, especially those with an autoimmune component (54). Notwithstanding unresolved questions regarding the mechanism underlying such associations and seemingly contradictory data, these associations could be attributed to three major phenomena. Neonatal mucosa allows potentially immunogenic macromolecules of the diet to cross the gut. Human milk accelerates maturation of the gut barrier function, but formula does not (55–57). Second, the neonate may be protected from pathogenic insults during this vulnerable period by bioactive components of human milk, including products of the adaptive immune system of the mother, primarily sIgA, and products of an innate immune system of human milk (58). Third, human milk components could actively attenuate early inappropriate inflammatory reactions.

The first bioactive components recognized in human milk were antibodies. Transfer of immunity from mother to infant through milk was reported in 1892, and this was attributed to milk antibodies in 1903 (2,59). In 1905, intestinal microbiota of breast-fed infants was recognized as different from that of adults or of precociously weaned infants: Breast-fed infants had a predominance of lactobacilli, especially *Lactobacillus bifidus* (now *Bifidobacterium bifidum*), thought to acidify the gut and inhibit enteric pathogens from infecting breast-fed infants. Bioactive bifidus factors were identified as human milk glycans that stimulated colonization by *L. bifidus*. However, when 1 g/L sIgA was measured in human milk and up to 12 g/L in colostrum, concurrent with increased recognition that antibodies provide specific and robust protection, research on bioactive materials of human milk shifted to sIgA inhibition of infection by enteric pathogens (60). Enteromammary circulation of sIgA (Fig. 1 *A*) was elucidated: When the mother is exposed to a novel enteric pathogen, the pathogen is presented to the DC, the primary antigen-presenting cell, either indirectly by way of transcytosis through the M cell, or directly through endocytosis by the DC. T lymphocytes are activated, which can stimulate B lymphocytes either locally in the Peyer's patch or after migration to the mesenteric or other lymph node. Plasma cells ultimately produce IgA on the basolateral side of the mammary epithelial cell, the IgA attaches to the polyimmunoglobulin receptor, the complex traverses the mammary epithelial cell, and, with the exception of the secretory component that remains on the sIgA, the polyimmunoglobulin receptor is cleaved by protease on the apical side as dimeric sIgA is secreted from the apex of the acinar cell into the milk. When the infant consumes this milk, the sIgA is resistant to digestion, accumulates in the intestine, and binds to antigens on the pathogen to render it

less infective, thereby protecting the infant from the pathogen (Fig. 1 *B*). However, the many days that elapse between exposure of a mother (and infant) to a novel antigen and protection of the infant by sIgA in the milk make this mechanism of protection incomplete at best. Furthermore, genetically modified mice in which production of sIgA is knocked out are still protected against reinfection with *Salmonella typhimurium* or *Citrobacter rodentium*, indicating that mechanisms independent of sIgA protect the mucosa (61). Clearly, other mechanisms of protection contribute to the highly effective protection of breast-feeding, such as those afforded by milk components that we classify as the innate protective agents of human milk, including multifunctional agents, immunomodulators, and glycans.

Human milk components that serve as a major source of nutrients, but whose native form or partial digestion products also function to protect the infant, are multifunctional agents that we classify as part of an innate immune system of human milk. For example, lactoferrin, present at 1–3 g/L, is a major protein of human milk that chelates free iron, potentially assisting iron absorption by the infant. Unbound iron is an essential nutrient for many bacteria, and by making it unavailable, lactoferrin would also have a broad bacteriostatic effect. Lactoferrin also inhibits pathobiology of several bacteria (62–64), stimulates phagocytosis of pathogens by macrophages (65), and inhibits human immunodeficiency virus, cytomegalovirus, and herpesvirus (66,67). Partial digestion of lactoferrin produces lactoferricin B, a positively charged peptide loop of 18 amino acids with potent broad antibacterial activity for both Gram-positive and Gram-negative bacterial pathogens. Another protective milk protein is lysozyme, an enzyme that breaks β1,4 bonds between *N*-acetylmuramic acid and *N*-acetyl glucosamine, a critical linkage in the peptidoglycans of bacterial cell walls. The amount of lysozyme in human milk varies, but is often approximately 100 μg/L and is found in the feces of breast-fed infants (68), indicating that it survives intestinal digestion sufficiently to potentially break down the more vulnerable cell walls of Gram-negative bacteria. Haptocorrin, a human milk protein that chelates vitamin B_{12}, is resistant to digestion and inhibits enterotoxigenic *E. coli*.

The 4% fat (triglyceride) in human milk is a major source of calories for the infant, and a multifunctional component. As milk is consumed and mixes with lingual and gastric lipases, triglycerides are digested into FFAs and monoglycerides; at typical concentrations, these strongly inhibit enveloped viruses, some bacteria, and protozoans (11,69). The strongest inhibition is by monoglycerides, which act as detergents on pathogen membranes, and the fatty acids linoleic and lauric acid, which are especially high in human milk. Free oleic acid in conditions typical of the stomach of the breast-fed infant converts human milk α-lactalbumin into an alternate conformation named HAMLET (70), which is reported to induce apoptosis in tumors, leading to remission. The various multifunctional agents of human milk, working in synergy, provide a broad spectrum of inhibitors for immediate defense of breast-fed infants, providing one part of the proposed innate immune system of human milk.

HUMAN MILK COMPONENTS AND ONTOGENY OF INTESTINAL MUCOSAL IMMUNITY

The newborn infant gut, especially that of premature infants, is hypersensitive to proinflammatory stimuli and vulnerable to pathogens. Human milk contains immunomodulatory molecules that quench proinflammatory processes, large numbers of quiescent leukocytes of unknown function, and glycans, some of which promote colonization by symbionts and others that inhibit specific pathogens.

Many immunomodulatory molecules have been identified in human milk (71). Soluble TNF-α receptors and interleukin (IL)-1RA (receptor antagonist) of milk effectively suppress proinflammatory TNF-α and IL-1 activity, respectively (72,73), as does lactoferrin (74). Milk also contains anti-inflammatory cytokines IL-10 and transforming growth factor β (68), and many antioxidants, protease inhibitors, prostaglandins, and other agents that may contribute to immunosuppression. Human milk factors suppress induction of IL-8 expression (inflammatory response) in cultured intestinal epithelial cells; this suppression is greatest in immature cells, whose IL-8 response is more pronounced (71). These suppressive factors are at their highest concentrations in colostrum (75,76). Colostrum is consumed by neonates when priming and maturation of the mucosal immune system are greatest (77), and when the human gut can absorb macromolecules directly (24,55,56). An 80-kD protein from colostrum modulates the response by epithelial TLR-2, -4, and -5 to bacteria (78). Soluble CD14 mediates TLR-4 binding to lipopolysaccharide, the pattern recognition molecule of Gram-negative bacteria (79). Soluble CD14 concentrations are 20-fold higher in human milk than maternal serum (80); human milk-soluble CD14 may sensitize the innate mucosal immune system to Gram-negative bacteria, which include common pathogens of immature gut. Human milk also contains hormones, including epidermal growth factor, IGF, and leptin that can modulate the immune system of the intestinal mucosa *via* regulation of cytokine expression and other signaling pathways (81,82). Recently, adiponectin was found in human milk; adiponectin suppresses TNF-α production in intestinal epithelium and macrophages (83). Human milk suppresses inflammation in rat gut models (72). Thus, the immature human gut may be hyperresponsive to specific stimuli that could result in mucosal damage, but human milk has a cornucopia of factors that can modulate inflammatory responses.

The period when the immune system of the infant is naïve and priming and development are at their peak coincides with the earliest periods of lactation. Consistent with activation and stimulation of the adaptive immune system by breast-feeding, the thymus of breast-fed humans is significantly larger than the thymus of artificially fed infants (84). Human milk contains more than 10^9 leukocytes per liter for the first several months of lactation, with the highest number in the initial days and weeks. By 6 mo, milk cells are 80% epithelial. Polymorphonuclear leukocytes (PMNs) are the cellular responders to proinflammatory molecules of acute inflammation in humans; PMNs of human milk are hypofunctional (72). Normal PMNs from blood are quenched by human milk: they exhibit reduced

adherence, spontaneous shape change, and deformability, their enzyme activity and release of reactive oxygen metabolites are suppressed, and microbial killing decreases (72). The function of milk cellular components in ontogeny of the mucosal immune system of the breast-fed infant remains a compelling area of research.

Large amounts and a number of complex carbohydrate structures, glycans, and especially oligosaccharides are unique to human milk and essentially indigestible by mammalian gut. That approximately 10% of the maternal caloric input for milk production would be expended synthesizing nonnutrient glycans seems counterintuitive, but indigestible dietary glycans often influence the composition of the intestinal microbiota. Most of these glycans arrive intact in the distal gut, where, as substrates for fermentation, they stimulate colonization by microbes that have a positive effect on the health of the mammalian host; that is, they are prebiotics (85). The importance of a fully colonized gut may be of increasing importance in societies where exposure to microflora is reduced by high, perhaps excessive, levels of hygiene (86,87). Increased colonization by bifidobacteria and lactobacillae in breast-fed infants may enhance subsequent long-term formation of a stable microbial ecosystem by favoring symbiotic (mutualist) anaerobes (88,89) and inhibiting colonization by enteric pathogens, protecting the infant from disease (85).

Specific human milk glycans inhibit binding by specific pathogens to their cell surface receptors on the intestinal epithelium (Fig. 2 *A* and *B*) based on structural homology between the milk glycan and the glycan moiety of the host cell receptor. In humans, many of these glycans are fucosylated and contain the Lewis histo-blood group antigens. The Lewis epitopes are expressed constitutively and vary in expression due to genetic variation in the population. A strong direct association exists between concentrations of these glycans in milk and protection from specific pathogens in breast-fed infants, and this association can account for much of the protection by human milk against many human enteropathogens (90,91). Although specific glycans inhibit only specific pathogens, the large numbers of human milk glycans in aggregate seem to defend the infant from many bacterial, viral, fungal, and other pathogens. We define these glycans as the major contributors to the innate immune system of human milk whereby the mother protects her infant from a wide array of pathogens.

Expression of inhibitory glycans varies in milk according to the genotype of the mother (91), and expression of glycans in the intestinal mucosa of the infant varies by genotype of the infant. Glycan expression in milk and gut also vary developmentally (92,93). Gut expresses more sialylated glycans from birth to weaning (3 wk in rats and mice), shifting to a predominance of fucosylated glycans (94). The expression of sialylated glycans longitudinally in human milk is not known, but expression of different patterns of fucosylated glycans in human milk seems to undergo a transition at around 6 to 9 mo (92). The relationship between expression of fucosylated glycans in intestine, expression in milk, inhibition of specific pathogens, and regulation of colonization (95) may have significant clinical ramifications, especially in high-risk and premature infants.

SPECULATIONS

Expression of components of the innate immune system of human milk change over the course of lactation, while simultaneously the ontogeny of the infant gut, and of its mucosal

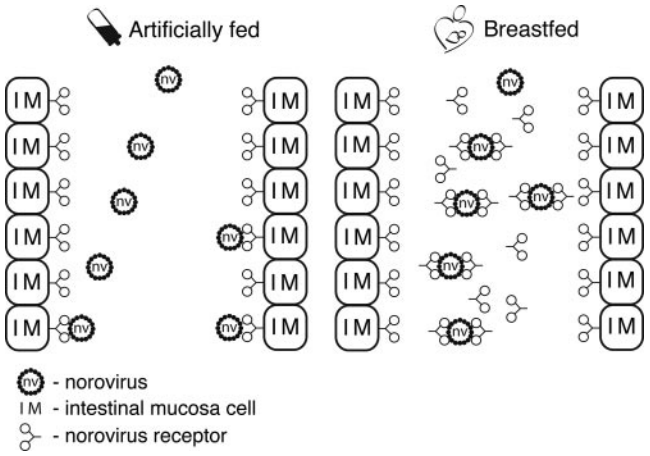

Figure 2. Protection of infant gut by human milk glycans. (*A*) In infants fed artificial formula or milk lacking protective glycans, pathogens such as the noroviruses bind to their glycan receptors on the mucosal surface, which is the essential first step in their pathogenesis. (*B*) For infants consuming human milk that contains glycans homologous to the infant gut receptors, when pathogens such as noroviruses enter the gut, they are bound to the soluble glycans of milk, rendering them less likely to bind to mucosal receptors, thereby protecting the infant. *(Adapted from Newburg DS and Street JM 1997 Nutr Today 32:191–201, © 1997 Lippincott Williams & Wilkins, with permission).*

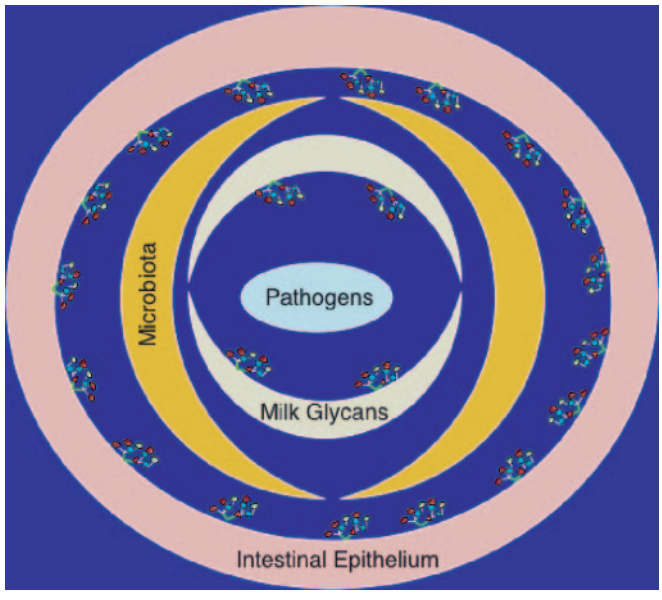

Figure 3. Milk glycans and intestinal microbiota can protect synergistically. The intestinal mucosal surface glycans are targets of pathogens, but can also be used as receptors for mutualist microbiota that colonize the gut and form a biofilm. Human milk glycans promote this colonization through their probiotic activity. The human milk glycans also bind to the pathogens, rendering them less able to bind to their receptors on the mucosal cell surface. These processes would provide multiple layers of synergistic defense of the intestine of breast-feeding infants.

immune system, is unfolding. It is tempting to consider these as coordinated complementary events, but our understanding of both of these systems remains quite incomplete. Notwithstanding, we speculate that human milk could provide exogenous signals and components for the ontogeny of the gut. We further hypothesize that human milk components modulate inflammation induced by microbes, both pathogenic and mutualist symbionts, in the immature intestinal mucosa of infants. During early exposure to some pathogens, this could result in subclinical infections that allow the formation of immunologic memory to pathogens without the negative consequences of hyperimmune responses that otherwise could divert nutrients away from growth and development.

The ontogeny of a complex dynamic microflora may also be intimately linked to changes during ontogeny of the gut and may be influenced by changes in human milk components over the course of lactation. Microbiota differs between breast-fed infants and artificially fed infants of the same age, and in both, the microbiota changes over the course of maturation of the intestine. We have observed changes in glycosylation of the intestinal mucosa during early development, changes in expression of human milk glycans over the course of lactation, and changes in gut microflora coincident with these two. A synergistic relationship among the three is proposed in Figure 3.

SUMMARY

Compelling but incomplete evidence suggests interdependent links among human milk components, ontogeny of intestinal function, development of the mucosal immune system, colonization by intestinal microbiota, and protection against pathogens. Timely coordination of these interactions seems to optimize the health potential of neonates. However, the challenge remains to identify the specific components that coordinate these interactions. Our understanding is confounded by the spatial and temporal specificity for many of the active components of both milk and the intestine, which can have complementary, additive, or synergistic interactions (58). Normal regulation of these systems seems to minimize acute conditions such as diarrhea, otitis media, and respiratory disease, and chronic conditions such as inflammatory bowel disease, allergy, obesity, cancer, and other manifestations of autoimmune dysfunction. The complexity and potential for dysfunction is multiplied in the premature infant in whom the immaturity of these systems may underlie NEC and other inflammatory bowel diseases. Moreover, inappropriate inflammatory responses during early life can divert nutrients from their support of growth (86): The brain is developing rapidly during this period, and any decrement of growth can lead to long-term deficits in learning (96). Better understanding of these interactions should help improve the composition of formulas for premature and term infants and may provide further evidence supporting promotion of breast-feeding for all infants.

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