Lactation and Intestinal Microbiota: How Early Diet Shapes the Infant Gut

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Abstract Breast milk is a multifunctional biofluid that provides nutrients along with highly diverse non-nutritive bioactive components such as antibodies, glycans, bacteria, and immunomodulatory proteins. Research over the past decade has confirmed the essential role of breast milk bioactives in the establishment a healthy intestinal microbiota within the infant. The intestinal microbiota of an exclusively breastfed baby is dominated by several species of Bifidobacteria - the most influential member of which is *Bifidobacterium longum* subspecies *infantis* (*B. infantis*) - and is referred to as the milk-oriented microbiome (MOM). MOM is associated with reduced risk of infection in infancy as well as a reduced risk of certain chronic illnesses in adulthood. Establishment and persistence of MOM is dependent on the selective digestion of complex sugar structures in breast milk that are otherwise indigestible to the infant by *B. infantis* and its relatives. This review focuses primarily on the influence of breast milk glycans and glycosylated proteins on the development of the intestinal microbiome, and how maternal phenotype may influence the development of MOM providing a framework to understand how variation in diet shapes a protective intestinal microbiome.

Keywords Breast milk · Bifidobacterium longum subspecies infantis · B.infantis · Glycans · Microbiome · Phenotype

Abbreviations

FUT	Fucosyltransferase gene
GDM	Gestational diabetes mellitus
HMG	Human milk glycans
HMO	Human milk oligosaccharides
MOM	Milk oriented microbiome
NEC	Necrotizing enterocolitis
sIGA	Soluble Immuglobulin A

Introduction

A high antigenic load coupled with a naïve system defines immunity in the neonatal period. The importance of the gut microbiota in the development and activation of the immune system is well established. Lactation drives a milk-oriented microbiota (MOM) that is characterized by the simultaneous promotion of protective bacteria and suppression of pathogenic bacteria [1]. The development of the microbiota is dictated by complex interactions between the mother, the infant, and their environment (Fig. 1). At birth, the intestinal microbiota is extremely variable, and undergoes several transitions before stabilizing during the first few years of life [2, 3]. The initial environment-derived microbial community is succeeded by microbes that are capable of exploiting and colonizing various niches within the intestine and these niches are largely driven by diet [4]. The exclusive breast feeding period constitutes a rare opportunity to manipulate gut microbiota in favor of health. The components of human milk uniquely support the development of specific microbiota with particular functions and health effects. Lactation protects the infant by delivering bioactive molecules such as growth factors, proteins, antibodies, bacteria, and glycans, which collectively help select the composition and function of the intestinal microbiota [5].

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Fig. 1 The mother infant-dyad is a complex system with a variety of inputs and outputs that can affect infant health outcomes. Both genotype and phenotype of the mother influences the composition and potentially the function of the breast milk that is ingested by the infant. How that infant is able to use the various components of the breast milk for both nutritive and non-nutritive functions will affect the health status of the infant. Health is a spectrum with green representing good health and red representing poor health and/or disease
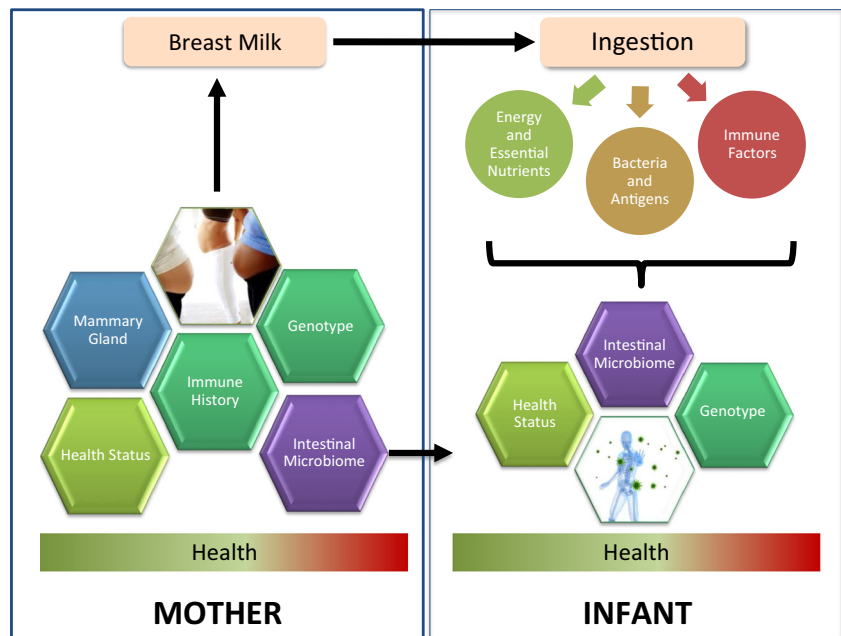
The overall importance of the intestinal microbiota for host physiology and metabolism is well-documented in the scientific literature [6–9]. The intestinal microbiome (the full complement of bacterial genes that are active in the intestinal microbiota) plays an important role in nutrient availability and the absorption of dietary components [10–12] as well as immune function [13–15]. For example, early research with gnotobiotic mice demonstrated the immune and metabolic consequences of a sterile gut such as hypoplastic Peyer's patches, poorly formed lymph nodes, and abnormal quantities of immune cells [16, 17]. Subsequent investigations involving inoculation of germ-free mice with fecal microbes obtained from conventionally raised animals confirmed many of the functional implications of the microbiota at large [6, 8, 18]. Although we know that the intestinal microbiome is essential and key to human health, relatively little is known about what constitutes a "healthy" microbiome and the mechanisms that underlie development.

Increased rates of Cesarean section, exposure to antibiotics, and the increased use of disinfectants in the environments coupled with variable early feeding practices (breast feeding, formula, mixed feeding and complementary feeding) over the last few decades have changed the environment surrounding the newborn infant and are jeopardizing the evolutionarily established MOM. The long-term effects of not achieving MOM in the first 3–4 months of life before complementary feeding is started are underappreciated and understudied. The increased rates of obesity, inflammation-associated diseases such as irritable bowel syndrome and Crohn's disease, and allergies may be attributed to factors that disrupt the development of MOM [19–21]. However, mechanistic studies are lacking and key questions about the early introduction infant formula and/or solid foods that lead to a more diverse adult-like microbiota in early infancy remain unanswered [4]. High-throughput 'omic' technologies are providing an unprecedented opportunity to define the inputs and outputs of the mother-infant dyad, which researchers can use to better understand the impact of diet on health outcomes. To achieve this comprehensive view of lactation and the intestinal microbiome, we must understand the maternal factors that influence milk composition and function, and how these factors, in turn, impact MOM and infant health. This review focuses primarily on the influence of breast milk glycans and glycosylated proteins on the development of the intestinal microbiome, and how maternal genotype and phenotype may influence the development of MOM providing a framework to understand how variation in diet shapes the intestinal microbiome.

What is the Milk-Oriented Microbiota and Why is it Important?

Exclusive breast feeding fosters a *Bifidobacteria*-dominated microbiota that is distinct from those resulting from other infant feeding strategies, and which accounts for a substantial proportion of the benefits ascribed to breast feeding [22]. *Bifidobacteria* can account for 70–80 % of the total bacteria in feces from breast fed babies. *B. longum* and *B. breve* are the most frequently observed species in the breast-fed infant intestine; *B. bifidum*, *B. pseudocatenulatum*, and *B. catenulatum* may also be found [22]. Studies focusing on the genomes of *Bifidobacteria* suggest that specific species have co-evolved with human lactation [12–16]. Several gene clusters have been identified that are responsive to human milk and confer the ability to sequester and breakdown specific linkages in

complex sugar structures that are indigestible to the infant, but are an important food source for these bacteria [23]. Early studies demonstrated that one such microbe is *B. longum* subspecies *infantis* (*B. infantis*) outcompetes other *Bifidobacteria* strains when allowed to grow in cultures where milk glycans are the sole carbon source [24–26]. Sequencing this subspecies uncovered a collection of genes involved in the catabolism of complex carbohydrates, including a 43-kb gene cluster encoding a variety of glycosyl hydrolases as well as transport proteins that were not found in closely-related species [27, 28]. In addition, *B. infantis* is the only known *Bifidobacteria* strain capable of digesting every type of prebiotic glycan (i.e., neutral, fucosylated, and sialylated) present in human milk [29]. Differences such as these help explain why *B. infantis* is capable of out-competing other colonic bacteria when given a steady supply of milk glycans.

Evidence suggests that *B. infantis* provides for a substantial portion of the benefits normally ascribed to breast feeding [22]. The relative abundance of *B. infantis* measured from the stool of infants 15 weeks of age was found to correlate with their vaccination responses to 4 vaccines: oral polio virus (OPV), bacille Calmette-Guérin (BCG), tetanus toxoid (TT), and hepatitis B virus (HBV). Vaccination responses were measured by vaccine specific T-cell proliferation and skin tests or vaccine specific immunoglobulin G levels for each of the vaccines. Positive correlations were observed between abundance of fecal *B. infantis* and CD4 stimulation responses to OPV, PPD, and TT. Furthermore, fecal *B. infantis* was positively associated with thymus index which was independent of length-for-age, a typical marker of nutrition status [30]. While the aforementioned correlation does not guarantee causation, it does support the notion that the composition of the infant microbiota is a crucial factor for immune system development. More evidence supporting this hypothesis is the fact that probiotics containing *B. infantis* decreased the relative risk of developing necrotizing enterocolitis (NEC) in premature infants [31]. In fact, five of six published randomized clinical trials of *B. infantis* alone or in combination when administered to premature infants showed decreased incidence of NEC compared to control [32–37]. In contrast, administration of *B. lactis* to premature infants did not affect the incidence of NEC [38].

The Early Microbial Environment: From Birth to MOM

Two key questions regarding the role of high levels of *B infantis* in MOM remain unanswered: 1) where does the *B. infantis* inoculum come from; and 2) how do *Bifidobacteria* interact with other bacteria in a background of a strictly human milk diet? There is some evidence to suggest that the early colonizers prepare the infant intestine by reducing the pH of the environment to make way for strict anaerobes like *Bifidobacteria* spp. [29]. Therefore, it is imperative that we begin to focus research

on the mechanisms by which the perinatal environment influences the development of the infant's intestinal microbiome.

Babies acquire bacteria from their environment so initial seeding of their intestine is strongly influenced by gestational age (pre-term vs. term) [39–42], mode of delivery (vaginal vs. Cesarean section) [43] and, potentially, delivery environment (hospital vs. hospital; hospital vs. home) [40, 44]. Vaginally delivered infants acquire bacterial communities resembling their mother's vaginal microbiota, which is dominated by *Lactobacillus*, *Prevotella*, or *Sneathia* spp., while infants born by Cesarean section harbor bacterial communities similar to those found on the skin surface, predominantly composed of *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* spp. [43]. Additionally, vaginal delivery invariably leads to contact with maternal fecal matter, thereby exposing the infant to maternal gut bacteria [45]. However, maternal fecal transfer of anaerobic bacteria to the infant is not well understood. In a study comparing the prevalence of ten key species of *Bifidobacteria*, babies born by Cesarean section did not have *Bifidobacteria*, while the guts of vaginally delivered neonates were dominated by *Bifidobacteria* spp. such as *B. longum* and *B. catenulatum* [46]. Premature infant microbiomes are less diverse than full term infants, possibly due to the hospital environment, high rate of C-section and routine use of antibiotics [47, 48]. As a result, premature infants follow a specific gut microbial trajectory that is characterized by abrupt and unpredictable changes in microbial composition [49]. Birth introduces a number of different organisms to the infant as a bolus, and is a distinct, trackable event in the history of that baby providing a piece of the puzzle of how we acquire microbial communities.

Consuming breast milk provides a small, but continuous source, of viable bacteria (10^3 colony forming units/mL) as measured in aseptically collected milk [50–53]. With this in mind one could postulate that milk delivers specific organisms to preferentially seed the infant intestine. Martin et al. (2003) suggested that breast milk was a source of endogenous lactic acid bacteria not resulting from skin contamination [54]. The hypothesis that breast milk is in part responsible for seeding the infant gut further emphasizes the need to understand the mother's health status as it relates to both the exogenous and endogenous microbial environments. One example of a known perturbation to milk microbiota occurs with infectious mastitis. *Staphylococcus epidermidis* is the major microorganism present in breast milk collected from mothers suffering with mastitis [55]. The fate of these organisms and their ability to interfere with the development of a healthy infant microbiota requires additional research particularly since it was also demonstrated that the strains of staphylococcus isolated from mothers with mastitis were antibiotic resistant compared to strains isolated from healthy mothers [56]. The dynamic nature of the mother-infant pair requires careful monitoring and interpretation of the data to fully understand the relationship between maternal intestine, mammary gland and the infant intestinal microbiome.

Cabrera-Rubio et al. (2012) discovered lactic acid bacteria from the order Lactobacillales and genera *Staphylococcus* were dominant in both colostrum, and mature milk (mature milk samples at 1 and 6 months postpartum). In another prospective study, Hunt et al. 2011 [57] discovered that nine core bacterial genera (*Staphylococcus, Streptococcus, Serratia, Pseudomonas, Corynebacterium, Ralstonia, Propionibacterium, Sphingomonas,* and *Bradyrhizobiaceae*) represented approximately 50 % of total bacteria in breast milk. The most abundant genera found (*Streptococcus* and *Staphylococcus)* were also discovered in the oral cavity; skin and breast tissue, respectively [43, 58]. There are several culture-based studies that have isolated obligate anaerobes from breast milk typical of the lower gastrointestinal tract such as *Bacteroides, Clostridia, Bifidobacterium, Veillonella* [51, 53, 59]. Using PCR denaturing gradient gel electrophoresis, Martin et al. (2009), identified *Bifidobacteria* spp. across the majority of early milk samples from 23 mothers. However, this genera was only a minor member of the milk microbiota, averaging 2.8 % of total bacterial abundance and ranging from 0 to 16 % across samples [51]. Using both culture-independent and dependent methods, Jost et al. (2013) identified that the highest potential for vertical mother–infant transfer via breast milk was observed for the genus *Bifidobacterium* which was consistently detected in all samples at mean relative abundances of 8.4, 1.3 and 51.8 % in maternal feces, breast milk, and infant feces, respectively. However, the presence of identical isolates must be confirmed on the subspecies or strain level within mother–infant pairs to support the hypothesis of vertical transfer via breast milk. Only the strain *B. breve* was shared within one mother–infant dyad, therefore no conclusions can be made about whether this transfer is a common event during lactation. Similarly with vaginal microbial environment, the dominance of lactic acid bacteria in breast milk may function to maintain an acidic environment in the intestine that supports the growth of obligate anaerobes.

There are a number of explanations for the origin of milk microbes; for example, it is reasonable to suggest a proportion arise from skin on and around the nipple [60] or through handling breast pump supplies [61]. Another possibility is the transfer of bacteria from the infant oral cavity to the milk ducts, likely reaching steady state equilibrium between the infant and its mother [62]. This is consistent with the fact that retrograde flow back into the mammary ducts can occur during suckling, and the frequent appearance of typical inhabitants of the oral cavity such as *Streptococcus, Veillonella, Leptotrichia,* and *Prevotella* in breast milk [63]. However, the breastmilk microbiota species that have been identified to date include not only skin and oral sources, but also contain organisms typically associated with the intestine. Perez and colleagues (2007) proposed that mothers transfer their intestinal microbiota via an enteromammary pathway mediated by her immune system. The authors discovered that aseptically collected milk contained viable bacteria in human mothers. Maternal peripheral blood mononuclear cells and milk cells contained intact bacterial structures. Furthermore, these cells contained ribosomal DNA of a greater biodiversity reflective of enteric bacteria (Perez 2007). However, only some of the bacterial signatures were common to fecal samples of maternal origin and their matched infants. B. longum was identified as common between mother feces, mother's blood cells and infant feces. It is important to emphasize that only DNA signatures and not viable bacteria were analyzed in these samples. In a second study, it was demonstrated that pregnant and lactating mice had increased bacterial translocation compared to control mice. More research is needed to validate this mechanism of vertical transfer of bacteria from mother to infant.

A conclusive understanding of the origin and importance of microbes in breast milk remains unknown and how their consumption leads to changes in the infant intestinal microbiome. The above studies still represent small sample sizes. There is a need for greater standardization of methods and the creation of large curated databases to understand the composition as well as the contribution of the milk microbiome on infant development. Above methodological issues, differences could also be attributed to the high inter-individual variation for milk microbiota among women. For example, the bacterial communities of breast milk from women who were obese or women who delivered by elective Cesarean section were different from normal weight women or women who delivered by emergent Cesarean or vaginal delivery, respectively [63]. Understanding the composition of, and the role(s) such communities play in maintaining mammary gland health, bacterial colonization of the infant's gastrointestinal tract, and on short- and long-term indices of maternal and infant health are needed.

Understanding the Impact of Human Milk on the Infant Intestine

Overall, the composition and development of infant gut microbiota are influenced by body mass index, weight, and maternal gestational weight gain. For example, greater maternal weights and BMIs were correlated with higher concentrations of *Bacteroides, Clostridium,* and *Staphylococcus* spp. along with lower concentrations of the *Bifidobacterium* in their infants [64]. Furthermore, infants with older siblings have been found to have slightly higher numbers of *Bifidobacteria*, compared with infants without siblings [4]. Molecular nutrition and technological advances have provided scientists with the tools necessary to investigate the mechanisms that promote MOM. However, natural variation presents several major challenges. Typical phenotyping research explores the impact of individual variation on personal health. Uniquely, variation in human milk will impact the nursing infant. The remainder of this paper will focus on the glycan components of breast milk that help shape the infant gut microbiome as well as factors that contribute to variation in milk composition.

Direct Effects of Glycans on Intestinal Microbiota

Compositionally, human milk contains an appreciable amount of oligosaccharides that is indigestible to the infant. Human milk glycans (HMG) are a large and diverse group of polysaccharides that include free human milk oligosaccharides (HMO), glycoproteins, glycopeptides, and glycolipids capable of enriching populations of *Bifidobacteria*. This enrichment is understood to be driven by the prebiotic effect of HMOs. HMOs are superior substrates for *B. infantis* in additional ways. For example, HMO consumption alters phenotypic expression and subsequent interactions with the host in ways that aid in its colonization and persistence in the infant intestine. Chichlowski et al. (2012) showed that growth of *B. infantis* ATCC15697 on HMOs compared with lactose increased binding to intestinal epithelial cells in vitro [65]. In a separate study, a similar increase in binding was determined using sialyllactose compared with oligofructose [66]. Compared with lactose, when *B. infantis* was incubated with HMOs, the production of inflammatory cytokines was diminished and the release of anti-inflammatory cytokines was increased [67]. When delivered in combination with breast milk, *B. infantis* supplementation led to decreases in γ-Proteobacteria compared with a matched formula fed group [68]. These studies suggest that the specific growth phenotype of milk glycan-enriched *Bifidobacteria* populations promotes persistence in situ and positively modulates the host epithelium.

HMO concentrations decrease from 23 g/L in colostrum to about 7 g/L in mature milk [69, 70]. The functional implications of the structural diversity of HMO are an important area of research as these complex sugars are indigestible by the infant. HMOs are comprised of glucose, galactose, N-acetylglucosamine, fucose, or sialic acid monosaccharides. The biosynthesis of HMOs in the mammary gland begins with the formation of a lactose core from galactose and glucose catalyzed by β-galactosyltransferase in the presence of α-lactalbumin. With few exceptions, all HMO structures consist of a lactose core [71]. Lactose can be elongated enzymatically by β1-3 linkage to lacto-N-biose or by β1-6 linkage to N-acetyllactosamine. The core HMO structure can be further elongated by the addition of lacto-N-biose and N-acetyllactosamine units by β1-3 and β1-6 linkages; terminal positions can be fucose monomers connected by α1-2, α1-3 or α1-4 linkages and/or sialic acid residues attached by α2-3 or α2-6 linkages. The distribution in term breast milk was reported as 35–50 % fucosylated, 12–14 % sialylated and 42–55 % non-fucosylated neutral HMO structures i [72]. Unlike HMO, glycoconjugates are even more complicated due to the respective protein or lipid moieties.

Lewis blood type and secretor status determine the structure and concentration of fucosylated oligosaccharides providing a clear example of how maternal genotype can affect milk composition and, subsequently, the infant gut microbiota [72, 73]. Women who possess a functional FUT2 gene, i.e., who express detectable levels of α-1,2-fucosyltransferase, in body fluids such as tears, saliva, and milk are called secretors. Non-secretors are women who fail to produce measureable levels of this enzyme, resulting in milk glycans with distinct linkages compared to secretors [74]. FUT3 is referred to as the Lewis gene and encodes an α-1,3/4-fucosyltransferase, adding even more complexity to HMO profiles [74]. Infants that are breast fed by secretor mothers are protected from moderate-to-severe diarrhea caused by a variety of enteric pathogens, ostensibly the HMOs act as decoy binding sites for the pathogens thus reducing access to epithelial binding sites [72, 75]. This is possible because secretor mothers produce more free milk oligosaccharides in terms of both structural diversity and total amount produced compared to non-secretor mothers [72, 73]. However, there is a great deal of functional redundancy in lactation. For example, the absence α-1,2-linked fucosylated HMOs in breast milk in non-secretors is offset by increased concentrations of lacto-N-fucopentaose III, lacto-N-fucopentaose II, 3-fucosyllactose, and lacto-N-tetraose [73, 76]. These oligosaccharides are also capable of pathogen deflection in the infant intestine [77], and potent prebiotics for beneficial *Bifidobacteria* strains [78].

Maternal phenotype may also influence the composition of HMO. For example, mothers with a BMI of 14–18 have significantly lower total HMOs compared with mothers with a BMI of 24–28 [79]. Very few studies take into consideration differences related to maternal phenotype and even less are able to link these to their impact on infant microbiome and infant gut function. A more comprehensive and systematic examination of the mother-infant dyad is required to understand the importance of variation in breast milk composition on infant health outcomes.

Breast Milk Proteins and Protection from Pathogens

In order to cultivate a healthy intestinal ecosystem, facilitating the growth of beneficial bacteria is needed but not sufficient. The growth of potentially harmful microorganisms must also be limited. This dichotomy is difficult to tease apart, because reducing the number of detrimental microbes may liberate niches that can be exploited by beneficial microbes may not directly affect the growth of these commensals. Glycosylated breast milk proteins such as lactoferrin, lysozyme, and immunoglobulins have important non-nutritive functions that protect infants from infection and, as a result, affect the development of the intestinal microbiome [80–82]. These bioactive proteins are the first line of defense against potential pathogens within the infant gut, and exert their protective effects via multiple and often overlapping mechanisms. These may have

either direct or indirect effects on the intestinal microbiome by promoting the growth of beneficial bacteria, acting as substrates for microbial metabolism, engaging in pathogen destruction/deflection, in addition to other antibacterial activities such as modulating the infant immune system and stimulating epithelial barrier function.

Lysozyme (also known as muramidase or N-acetylmuramide glycanhydrolase) is an enzyme found in several mammalian secretions, including breast milk, which hydrolyzes cell wall peptidoglycans, resulting in cell lysis. This is a particularly effective defense against gram-positive pathogens such as *Streptococcus* and *Bacillus* [83]. Lysozyme is more highly concentrated in human milk (400 mg/ml) than milk from other species (0.130 mg/ml in cow's milk and 0.250 mg/ml in goat's milk) [84]. McInnis et al. (2012) analyzed the fecal microbiota of piglets fed milk produced by transgenic goats expressing human lysozyme at the same level found in human milk using both 16S rRNA gene sequencing and a Phylochip microarray [85]. Consumption of lysozyme-rich milk decreased levels of *Firmicutes* and increased those of *Bacteroidetes* compared to control-fed animals over time. More specifically, the abundance of families associated with gut health (*Bifidobacteriaceae* and *Lactobacillaceae*) increased, while those associated with disease (*Mycobacteriaceae*, *Streptococcaceae*, *Campylobacterales*) decreased in animals given the lysozyme-rich milk compared to controls.

Lactoferrin is the most abundant glycoprotein in milk [86], and has an extremely high affinity for iron. It's structure and function has been extensively reviewed elsewhere [87–89]. It plays a key role in iron homeostasis in the newborn; sequestering this important nutrient away from potentially harmful bacteria and reserving it for uptake by enterocytes [90]. Lactoferrin may be partially digested in the infant's intestine, resulting in the release of the peptide lactoferricin. Lactoferricin possesses even greater antibacterial activity than its precursor [91]. Lactoferrin also exhibits several immune-modulating properties in addition to those better-known iron-dependent, anti-infective capabilities [92, 93]. Differences in these functions, may be strongly influenced by glycosylation patterns, which vary over the course of lactation [94, 95]. As changes in protein glycosylation can significantly affect structure and stability, this could lead to changes in predominant function attributed to lactoferrin at different times in lactation.

Although all classes of immunoglobulins can be detected in human milk, the most abundant is IgA, specifically a form known as secretory IgA (sIgA). The infant is reliant on sIgA in breast milk since the infant since it possess an immature immune system, and does not produce sufficient amounts of sIgA on its own. Mucosal IgA production and, thus, sIgA levels, does increase with age, but it does not reach adult levels until age 5 [96]. Milk sIgA molecules are derived from maternal IgA antibodies that were developed against pathogens that the mother encountered via her own mucosal membranes, especially her digestive tract. *Camphylobacter*, *Vibrio cholerae*, *Escherichia coli*, *Giardia*, *Salmonella*, and *Shigella* [97, 98]. arecommon bacterial causes of diarrheal disease, which is second leading cause of death among children (<5 years of age) worldwide [99]. For example, milk sIgA antibodies against the above mentioned enteric pathogens helps explain the protective effect that breast feeding has against diarrheal disease [97].

One mechanism that explains immunoglobulin function in the intestinal lumen is immune exclusion, which involves preventing an antigen from entering into the systemic circulation through mucosal membranes. sIgA accomplishes this through agglutination, which precipitates antigens by mucus entrapment followed by mucociliary clearance [100]. sIgA may also bind to bacterial adhesins, which are glycosylated cell surface proteins that facilitate adherence to other cells or surfaces. This blocks attachment of ingested bacteria to receptors on host epithelial cells, preventing infection [101]. Perrier et al. (2006) showed that the secretory component of IgA isolated from human colostrum could bind to enteropathogenic *E. coli*, thereby protecting epithelial cells from bacterial invasion [102]. Another study demonstrated that secretory component isolated from human colostrum inhibited adhesion of *Helicobacter pylori* to human gastric mucous cells [103]. Rogier et al. (2014) developed a polymeric immunoglobulin receptor knock-out mouse model to assess the benefits of maternally derived sIgA on gut microbiome communities [104]. PhyloChip microarray hybridization of fecal DNA was used to identify roughly equivalent bacterial species at the phylogenetic level. Weanling mice that received maternal sIgA in breast milk had a significantly different gut microbial compositions compared to mice that did not receive maternal sIgA, and these differences persisted into adulthood. Specifically, Gram-negative *Comamonadaceae* was present only in weanling offspring of homozygote knock-out dams and Gram-negative *Pasteurellaceae* and Gram-positive *Lachnospiraceae* were also up-regulated in the absence of sIgA. They also found that maternal sIgA was needed to prevent the translocation of aerobic bacteria including, but not limited to, *Ochrobacterium anthropi,* a common opportunistic pathogen in the gut of preterm infants and mesenteric lymph nodes of immunocompromised individuals.

Maternal phenotype has been shown to influence levels and glycan composition of immunoglobulins. For example, colostrum from women diagnosed with type 2 diabetes has 30 % lower concentrations of IgA, IgG, and complement C3 protein compared to colostrum obtained from normoglycemic women [105, 106]. Glycomic profiling using nano-HPLC chip/TOF MS + multivariate modeling by Smilowitz et al. (2013) showed that total abundances for HMO and HMO composition did not vary in breast milk between women with and without gestational diabetes mellitus

(GDM), but that the total protein and composition of oligo-saccharides N-linked to sIgA and lactoferrin were significantly different [107]. Specifically, the percent of mannose, fucose, and sialic acid residues per total oligosaccharide residues linked to sIgA were lower by up to 43 % in milk from women with GDM. On the other hand, the percent of fucose and sialic acid per total oligosaccharide residues linked to lactoferrin were higher up by up to 72 % in milk from women with GDM. The effects of altered glycosylation patterns of human milk glycoproteins by gestational diabetes mellitus on infant gut microbiota have not been investigated.

Conclusion

The development of the infant intestinal ecosystem is a highly coordinated process with profound implications for both short- and long-term health. The pre-weaning period, i.e., the first 3–4 months of life, is a critical period in the development of the intestinal microbiome as early microbial colonization events provide important stimuli that guide the maturation of the immune system and help establish proper gut barrier function. Diet and other environmental factors such as mode of delivery and exposure to antibiotics play a significant role in determining the number and diversity of bacteria that the infant is exposed to. Breast milk is the gold standard for infant nutrition, because it delivers prebiotic glycans, live bacteria, and several different types of immune factors that provide passive protection from pathogens while simultaneously seeding the gut with wholesome bacterial species.

Recent data suggests that maternal genotype and phenotype may affect the quantity, composition, and functionality of milk components as well. The impact of maternal phenotypic variation on the infant intestinal microbiome and the implications for future infant health warrants further investigation. A better understanding of the role of milk in the metabolic and immunological programming of the neonate and the effects of maternal variation on the components responsible would provide insight into novel means of improving infant health. High resolution, comprehensive datasets on a large number mother-infant pairs that link milk composition, intestinal microbiome to health status of mother and infant will provide the insights necessary to understand the role of the intestinal microbiome on the developing metabolic and immune function.

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