Special Article

Reviewing the evidence on breast milk composition and immunological outcomes

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A large number of biologically active components have been found in human milk (HM), and in both human and animal models, studies have provided some evidence suggesting that HM composition can be altered by maternal exposures, subsequently influencing health outcomes for the breastfed child. Evidence varies from the research studies on whether breastfeeding protects the offspring from noncommunicable diseases, including those associated with immunological dysfunction. It has been hypothesized that the conflicting evidence results from HM composition variations, which contain many immune active molecules, oligosaccharides, lactoferrin, and lysozyme in differing concentrations, along with a diverse microbiome. Determining the components that influence infant health outcomes in terms of both short- and long-term sequelae is complicated by a lack of understanding of the environmental factors that modify HM constituents and thereby offspring outcomes. Variations in HM immune and microbial composition (and the differing infantile responses) may in part explain the controversies that are evidenced in studies that aim to evaluate the prevalence of allergy by prolonged and exclusive breastfeeding. HM is a "mixture" of immune active factors, oligosaccharides, and microbes, which all may influence early immunological outcomes. This comprehensive review provides an in-depth overview of existing evidence on the studied relationships between maternal exposures, HM composition, vaccine responses, and immunological outcomes.

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INTRODUCTION

Human milk (HM) is the first source of nutrition available to an infant and is vital to the development of the immune system, affecting a child's health for life. The World Health Organization (WHO) recommends "exclusive breastfeeding for at least 6 months in all infants."[1] According to WHO, exclusive breastfeeding means that the infant receives only HM and no other liquids or solids—not even water—with the exception of oral rehydration solution or drops/syrups of vitamins, minerals, or medicines.[2] There is strong evidence that breastfeeding reduces rates of neonatal infection; it also has putative health benefits in the long-term by preventing hypertension, diabetes, and even improving intelligence quotient (IQ).[3] Yet, only 19% of infants in Europe and 35% of infants worldwide are exclusively breastfed for the first 6 months.[4]

The impact of WHO's breastfeeding recommendations on the risk of development of noncommunicable diseases, as evaluated in several observational studies, suggests protection against asthma development and, to a lesser extent, against eczema and allergic rhinitis.[5] The strength of the association varies by geographical location, with a more prominent impact seen in low-income countries.[5] This variation may be explained by the considerable heterogeneity in study definitions of breastfeeding and/or its exclusiveness, as well as health outcomes reported. On the other hand, a number of experts and organizations have challenged the WHO recommendations with evidence that early complementary food introduction protects against allergy development later in life.[6,7]

Conceivably, the mixed results on the benefits of breastfeeding generated by scientists worldwide are related to the variation in the constituents of HM itself.[8–10] Further, a large number of the biologically active components in HM[10,11] can be modified by maternal exposures and behaviors, which, when modified, can alter health outcomes in offspring.[12,13] One clear example is HM composition changes observed following the use of antibiotics in lactating mothers. It is also plausible that HM bioactive compounds can influence health outcomes through their interaction with infant exposures or treatments that alter the immune system of the gut. Although incompletely described, HM constituents appear to influence the immunogenicity and efficacy of live oral vaccines.[14,15]

This review provides an overview of current evidence on the relationship between HM composition and infant health outcomes. This review has a particular emphasis on HM microbial composition and human milk oligosaccharides (HMOs), as essential constituents that shape the development of the infant gut microbiome and immunity. Both fields of research and bodies of evidence are developing rapidly and attracting increasing attention. The review also addresses 2 understudied areas: maternal antibiotic treatment and infant vaccine response during lactation. Human milk microbiota interaction with milk immunoglobulin A (IgA) is only mentioned briefly. There are few comprehensive reviews on this constituent of breast milk, which can be found elsewhere.[16–19] Lactoferrin is an important defense protein linked with protection against microbial infection. However, it is not discussed in this review due to a large number of very comprehensive review papers[20–23] and systematic reviews[24,25] published recently.

MICROBIAL COMMUNITIES IN HUMAN MILK AND THEIR POTENTIAL IMPACT ON MATERNAL AND INFANT HEALTH

Historically HM was considered sterile, and bacterial colonization was attributed to milk contamination after expression or mammary gland infection.[26–28] The inclusion of new and more specific culture media, as well as anaerobic tests, enabled the isolation of lactic acid bacteria,[29,30] including several species of *Lactobacillus*, *Lactococcus,* and *Leuconostoc,*[21] *Bifidobacterium,*[31] and many others,[32] from milk samples from healthy mothers. These findings changed the perspective on HM sterility, and the recent development of culture-independent techniques, including next-generation sequencing (NGS), resulted in identification of a broad range of microbiota, from *Veillonella* and *Prevotella*, common to the oral cavity, to the skin bacteria *Propionibacterium* to other Gram-negative bacteria, like *Pseudomonas,* and other lactic acid bacteria, such as *Enterococcus* and *Weissella*.[32–36]

Aside from some commonality with other body site microbiota, HM has a unique microbial ecosystem with a dominant core of *Staphylococcus, Streptococcus,* and *Propionibacterium.*[36,37] These genera are ubiquitously present in the HM of healthy lactating women. Human milk bacterial load has been estimated at approximately 10^6 cells/mL, indicating that "a breastfed infant feeding 800 mL of milk per day would ingest 10^7–10^8 bacterial cells daily."[38] Recently several yeasts and fungi were detected in HM from healthy mothers, suggesting that HM could also participate in shaping the infant mycobiome (the fungal fraction of the microbiome).[39]

Early infant microbial colonization is essential for infant metabolic and immunological development. Alterations in this process may be associated with aberrations leading to a higher risk of developing diseases later in life (such as inflammatory bowel disease, obesity, celiac disease, atopy, etc).[40,41] In this crucial period,

HM plays an important role, supplying infants with nutrients and microbes during breastfeeding that help shape gut microbiota, which may explain some of the differences between exclusively breastfed and formula-fed infants during the first months of life.[42]

Recent studies have shown that HM may have several functions in the infant health. Human milk seeds the first colonizers to the infant gut, contributes to infant digestion, has a protective role competing with pathogens, and enhances mucine production, which reduces intestinal permeability, thus improving intestinal barrier functions.[32,43] Other HM molecular components likely help to educate the infant's immune system, modulating both natural and acquired immunity.[43,44] Although it is conceivable that relationships between milk microorganisms and other components, such as HMOs and macronutrients, may exist, information is scarce. Hunt et al demonstrated in vitro evidence that HMOs promoted growth of *Staphylococcus* strains,[45] and further research should aim to assess if this effect may occur in the mammary gland.

Several studies have addressed the relationships between HM components (HMOs, fatty acids, immune components, etc) and infant allergy development,[46–48] as well as differences in the gut microbiome between allergic and nonallergic children. However, the potential role of HM bacteria in allergic diseases has not been assessed in depth. Evidence suggests that children who drink unpasteurized cow's milk, which contains live microorganisms, are less likely to develop allergic diseases and asthma.[49] Therefore, it can be hypothesized that HM bacterial communities could act as a natural prebiotic offering protection against allergy development later in life. However, it is premature to make definitive conclusions, and more prospective studies are needed to confirm whether this protective effect is related solely to milk microorganisms rather than other HM compounds destroyed during the pasteurization process. Indeed, some *Lactobacillus* and *Bifidobacterium* strains have shown some effectiveness in eczema prevention,[50] and it is plausible that their transference from HM to the infant could offer immunological protection.

Furthermore, in exclusively breastfed children that developed allergies later in life, differences in the IgA response towards gut microbiota could be detected as early as 1 month of age, meaning that altered antibodies/microbiota transmitted through HM could detrimentally affect the infant's immune development.[51] More studies are urgently required to provide evidence of this potential link. See recent papers from Rogier et al[17] and Pabst et al[16] for a comprehensive review on the role of milk IgA and its interplay with milk microbiota.

POTENTIAL SOURCES OF BACTERIA IN HUMAN MILK

The detection of live bacteria and bacterial DNA from aseptically collected milk samples, including anaerobic endogenous gut species that cannot survive in aerobic conditions,[52,53] together with the finding that bacteria are present in the breast tissue of nonlactating women,[54,55] triggered a debate on the origin of HM bacteria.[34,56]

Maternal skin

Maternal skin, together with the infant's oral cavity, have classically been considered the main source of HM bacteria.[26,57] Microbes residing on maternal skin, especially the nipple, areola, and Montgomery glands, could be transferred to the milk and into the infant's mouth during breastfeeding. Some common skin bacterial isolates, such as *Staphylococcus*, *Corynebacterium,* and *Propionibacterium*,[58,59] are frequently detected in HM. However, studies comparing bacterial communities encountered in HM with those of mammary skin indicate that, although some phylotypes are shared between the 2 communities, major differences in composition and relative abundance exist.[36] This is further complicated by human skin bacteria, such as staphylococci, corynebacterial, and propionibacteria, being common to other human body niches, especially the intestinal and genitourinary tract mucosa. Moreover, HM hosts strictly anaerobic genera, such as *Bifidobacterium*, and skin would be a very unlikely source.[53]

Infant's oral cavity

Ultrasound imaging studies have shown that substantial retrograde flow occurs during the second half of milk ejection,[60] which could be a plausible route for infant oral bacteria to enter the mammary ducts, as well as a potential pathway for exchange between the mammary gland and the infant's oral cavity, suggesting that one could shape the other. Despite scant information about infant oral microbiota development, it is known that species from the *Streptococcus* genus are prevalent in adult saliva[61–63] and in edentulous infants.[64,65] It is also one of the most common genera detected in HM.[36,38,66] Within 48 hours after birth, typical oral bacteria can be detected in colostrum, including *Veionella*, *Prevotella,* and *Streptococcus*.[33] After delivery, the first bacteria to colonize the infant are *Staphylococcus* and *Streptococcus,*[67] supporting the hypothesis that HM could be seeding the first colonizers to the infant and shaping the infant's oral microbiome or/and vice-versa.[68]

Maternal gastrointestinal tract

Although the infant oral cavity and maternal skin are candidate sources of HM microorganisms, major differences were detected between these sources in HM bacterial composition.[33,36] An alternative theory that could fill in the knowledge gap was proposed: possible selective translocation of maternal gastrointestinal tract bacteria to the mammary gland within mononuclear cells.[32,69,70] With a proposed mechanism that is somewhat controversial, research findings suggest that dendrites from dendritic cells (DCs) could cross the gut epithelium, uptake gut lumen bacteria, and transport the bacteria to the mammary gland through the lymphoid system.[71,72] This theory is supported by a single experimental study in which pregnant mice were fed a labeled *Enterococcus* strain that was detected in the animal's milk after delivery.[73]

Summary

According to accumulating evidence, maternal skin and the infant's oral cavity are the most likely sources for microbiota in HM.[32,73] Others not discussed in full include microbes found in amniotic fluid and the placenta,[74,75] neonatal umbilical cords,[76] and breast tissue.[55] Because the human microbiome is a dynamic network of microbial communities that interact with one another, it is entirely possible that several maternal body sites are sources of HM bacteria in conjunction with maternal skin or the infant's oral cavity. The existence of >1 mother–infant communication route offers several opportunities for modulating HM microbiota and decreasing disease risk, as well as preventing and treating mammary infections. Further research is needed to completely elucidate underlying mechanisms.

FACTORS INFLUENCING HUMAN MILK MICROBIOTA AND POTENTIAL FOR MODULATION

The HM microbiome has been found to be influenced by maternal and environmental factors[77] (Figure 1). Despite high intra- and interindividual variation, many studies document transitions in milk microbiota communities from colostrum to mature milk,[33,36,78,79] whereas others have not found this same influence of time since birth.[38,80] There is some evidence, although conflicting as well, on the impact of delivery mode on HM microbiota composition[33,80–83] and glycosylation patterns.[84] Microbial composition also differs between HM of women who deliver term and preterm infants.[81] Milk microbiome shifts have been also linked with maternal health in relation to obesity, allergy, celiac disease, and human immunodeficiency virus (HIV) status.[33,85–87] Finally, recent studies have found an effect of geographic location on milk microbes[82,88] and on breast tissue bacteria.[55] In addition to the aforementioned influences, this geographic variation may be related to maternal macronutrient and micronutrient intake.[78,89]

Potential for breast milk microbiota modulation

Probiotic supplements have the capacity to correct imbalances in the HM microbiome. When they are administered, changes in HM microbiota are observed—namely, increased levels of *Bifidobacterium* and *Lactobacillus* sp. in HM from mothers who delivered vaginally.[90] In clinical trials, oral probiotics such as *Lactobacillus reuteri* ingested by pregnant and lactating women can affect the HM composition and subsequently the infant's gut *Bifidobacteria* colonization as compared with placebo controls.[52,91] However, probiotic treatment may have unexpected effects on HM composition. In a randomized controlled trial of *L. reuteri* administration by Abrahamsson et al,[52] *L. reuteri,* as well as other *Lactobacillus* species, was detected in maternal colostrum. However, the prevalence of *L. reuteri* declined in breast milk after the first week of continuous supplementation. Also, despite being detected in breast milk, gut microbiota levels of *L. reuteri* were lower among breastfed infants compared with formula-fed infants. Authors speculated that immune recognition and reduction of *L. reuteri* was heightened in breastfed infants receiving additional IgA from mother's milk.

Some clinical trials of probiotics have been performed to treat mastitis. In a study, 1 group of mothers was given an oral probiotic consisting of 2 *Lactobacillus* strains (*L. fermentum* and *L. salivarius*) isolated from human milk, whereas another group was treated with antibiotics. Results showed that the probiotic group had better improvement of symptoms as compared with the antibiotic group, and probiotic strains could later be isolated from the mother's milk.[92]

Oral administration to infants of formula supplemented with an HM *Lactobacillus* strain led to lower rates of infection, including gastrointestinal and upper respiratory tract infections.[93]

Certain *Lactobacillus* and *Bifidobacterium* strains can offer protection against eczema and other atopic diseases, although current evidence is not sufficient to use as a general atopic preventer.[50] Interestingly, healthy infant gut often is settled by *Viridans streptococci*, one of the most prevalent groups in HM, whereas atopic infants are not similarly colonized.[94] Current studies are investigating the potential probiotic effects of other strains (*L. rhamnosus*) when administered to pregnant women in order to study their potential to reduce allergy outcomes in breastfed infants.[95]

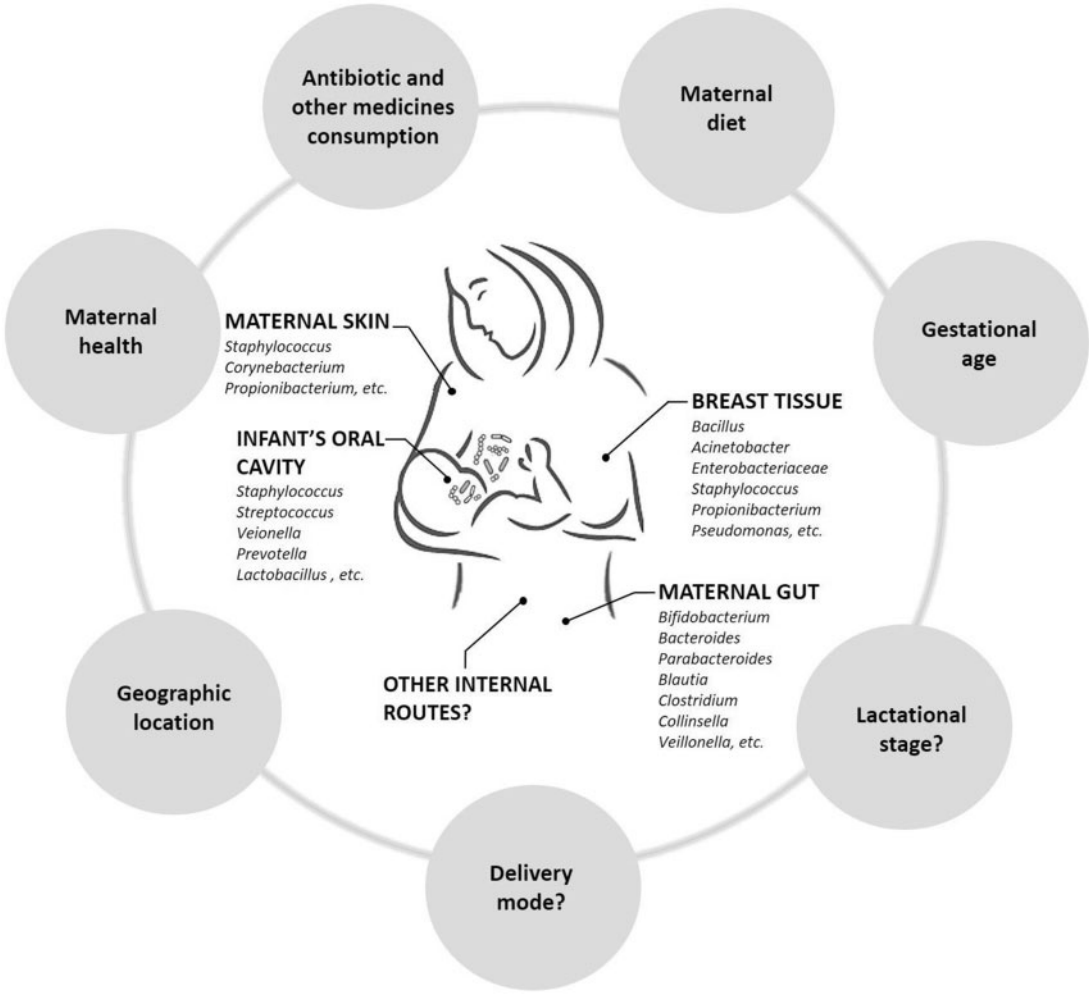

Figure 1 **Factors potentially influencing breast milk microbiota.**

The microbial presence in the mammary gland and HM may have both maternal and infant health implications. Some current results suggest that probiotic treatment could help modulate human milk microbiota and compete against pathogens in the mammary gland. Human milk participates in the bacterial supply to the infant, and therefore its role in microbial settlement may be of importance. If relationships between specific HM microorganisms and infant health/disease status are demonstrated, prebiotic and probiotics could likely be used for modulation of milk and infant microbiota to bring them closer to a healthy microbial composition.

Future research should address the relationships between HM microbiota and mother/infant health and modulating the HM microbiota in preventing noncommunicable diseases in the offspring.

Maternal antibiotic treatment

In North America, at least 40% of infants are exposed to antibiotics by the time they are born from maternal intrapartum administration for Group B streptococcus colonization and cesarean section delivery.[96] Although the full impact of this perinatal exposure on gut microbiota in infants is only beginning to be appreciated,[97] maternal postpartum antibiotics are another understudied source of antibiotic exposure to young infants. Saha et al reported in their review of 14 studies that 33% to almost 100% of women reported taking a medication while breastfeeding.[98] Next to frequent use of vitamin supplements, 14%–38% of women were treated with an antibiotic, most commonly for postpartum endometritis, surgical site infection, and mastitis.[99]

The American Academy of Pediatrics evaluates penicillin-like antibiotics and most antibiotics in general as safe to be prescribed during breastfeeding because benefits of breastfeeding to the infant outweigh the minimal levels of antibiotics detected in HM.[100] However, emerging evidence suggests that the presence of even small quantities of antibiotics in HM has the capacity to alter HM or infant gut microbiota. Soto et al[101] reported a reduced percentage of detectable

bifidobacteria and lactobacilli in HM within 1month of birth in 160 women who received courses of antibiotics (type unknown) during pregnancy, birth, or lactation. Of note, detection of HM microbiota species varied between women, but changes were not different according to whether maternal treatment was during pregnancy or lactation. In the CHILD birth cohort of 176 infants, where postpartum antibiotics were mainly administered to women after an emergency cesarean delivery, a higher infant fecal abundance of genus *Clostridium* was observed at 3 months of age after emergency cesarean in exclusively breastfed infants but not among infants supplemented with formula.[102] The most common antibiotics dispensed postpartum to women in the CHILD cohort were amoxicillin, cephalexin, azithromycin, and cloxacillin.

Two studies from the late 2000s of population-based cohorts point to the potential ramifications of antibiotic exposure of the nursing infant. Kummeling et al found a 3-fold risk of child wheeze with maternal antibiotic therapy during breastfeeding in 10% of 2764 infants in the KOALA cohort,[103,104] whereas this was not evident in 8% of 235 nursing infants in Belgium exposed to maternal antibiotics. In the latter, an elevated but not statistically significant risk of wheeze was observed. Reverse causation—namely, breastfeeding a wheezing infant—cannot be excluded as an explanation for the excess risk of wheeze. No associations between maternal antibiotic use while breastfeeding and offspring atopic disease were found. Discrepant findings in health outcomes and also on the impact of antibiotics on infant gut microbiota are likely attributed to variations in maternal behavior. The breastfed infant's exposure to antibiotics may be less than estimated from reported use because women attempt to limit medication exposure by taking doses immediately after breastfeeding or in some cases by discontinuing treatment or failing to initiate it.[98] The fact that some women opt to formula feed while on antibiotic treatment is strong rational for the need for evidence-based information to avoid this alternative and its potentially greater impact on infant gut microbial development[105] than maternal breastfeeding during antibiotic treatment.

PREBIOTIC OLIGOSACCHARIDES

Within the first few months of life, infants go through a rapid growth phase, receiving all essential nutrients from HM, including HMOs, which are an essential part of its composition.[106] Human milk oligosaccharides, which are exclusive to HM, structurally consist of both short-chain and long-chain oligosaccharide structures in an approximately 9:1 ratio. Together with bacterially fermented metabolites, HMOs are key for microbiome development, creating a basis for healthy and resilient immune system. To date, more than 200 different HM oligosaccharide structures have been identified, which are unique in their structural diversity and are present in proportionally high amounts.[107] Lactose, the largest carbohydrate component of HM, is digested by the infant and serves as a fundamental building block for the larger oligosaccharides. If fucose is coupled to the lactose, this forms fucosyllactose (FL), whereas if lactose is connected to N-acetylneuraminic acid, it generates a sialyllactose (SL). Most HMOs contain fucose; fucosylated oligosaccharides are virtually absent in bovine milk.[108] Human milk oligosaccharide composition varies extensively between women and time of feeding.[107,109–112] Specific HMOs, such as 2'-FL, 3'-sialyllactose (3'-SL), 6'-sialyllactose (6'-SL), and LNnT have been detected within the intestine and in the systemic circulation of infants.[113]

The World Allergy Organization (WAO) guideline panel recommends "using prebiotic supplementation in not-exclusively breastfed infants and not in exclusively breastfed infants."[114] This recommendation is based on the characteristics of infant stool (pH, frequency, consistency, microbiota) observed in 12 of 19 clinical trials of the short-chain galacto-oligosaccharides (scGOS)/long-chain fructo oligosaccharides (lcFOS) (9:1) mixture. Recently, clinical safety studies have found that infant growth and 2'FL uptake following the use of 2'-fucosyllactose (2'FL) and scGOS[115] or the combination of 2 single oligosaccharides 2'FL and LNnT[116] were similar to that of breastfed infants.

Although HM clearly protects against infections, the potential for allergy prevention is more controversial,[117,118] with a recent systematic review highlighting heterogeneity across studies.[119] Conflicting results may also be a function of maternal genetic polymorphisms to the fucosyltransferase 2 (FUT2) secretor gene. When fed an FUT2-dependent mixture of milk oligosaccharides, infants born by cesarean section and with a high hereditary risk for allergies were less likely to develop immunoglobulin E–associated eczema.[48] Yet HMOs do have demonstrated activity on regulatory T-cell responses, as shown by elegant in vitro studies of the addition of specific oligosaccharides during DC development.[120–122] More specific cell interactions of HMOs with the immune system, in particular blockade of DC–pathogen interactions, have been reported by Koning et al.[123] On the other hand, Although some studies are unable to show a modulatory effect of single oligosaccharides on DC marker expression.[125] Others do show the immune modulation potential of isolated diverse mix of HMOs on DC maturation and function.[124] Knowing the interaction between components of HM, including the microbiota and metabolites produced,

further research is required to unravel the direct impact of HMOs on DC differentiation, function and other immune cells. Future studies are needed to confirm the antiallergenic effects of HMOs.

MILK IMMUNE COMPOSITION AND IMMUNOLOGICAL OUTCOMES

Human milk is an immunologically active fluid, which in early life has the capacity to influence immune-related outcomes in infancy and early childhood. It consists of hundreds of proteins (cytokines, inflammatory mediators, signaling molecules, soluble receptors, etc),[8] polyunsaturated fatty acids (PUFAs),[126] and HMOs,[127] and comprises a complex microbiome[36] (see Table 1). Variations in the immune composition of HM (and infant utilization of HM immune constituents) may shed light on conflicting evidence regarding prolonged exclusive breastfeeding as a means of preventing allergic disease (see Table 2[5,47,119,128–143]).[144,145]

The most studied immune marker in HM is TGF-β. In a systematic review by Oddy and Rosales, most of the included studies found an association between higher colostrum TGF-β levels and reduced risk of several immunological outcomes in children.[142] They suggested that this growth factor may affect gut homeostasis, inflammation regulation, and oral tolerance and thus reduce the risk for allergy development. However, this review is now a decade old and combined clinical and immunological outcomes, and of the many observational and interventional randomized controlled studies carried out in the past 5 years,[47,139,141,146,147] only 1 study by Munblit et al found a higher and not lower risk of eczema with higher levels of HM TGFβ2 at 1 month of age.[47] Conflicting results may be explained by heterogeneity in sample collection, processing methodology, as well as outcome definition and assessment. Further, it is well known that levels of immune molecules are much higher in colostrum than in mature milk,[148] and the rate of decline may also be, in part, responsible for the differences in associations with immune health.

Although research has been primarily focused on TGF-β, other HM growth factors, such as hepatocyte growth factor (HGF), epidermal growth factor (EGF), and vascular endothelial growth factor (VEGF) have been less studied. Hepatocyte growth factor suppresses the antigen-presenting capacity of DCs in murine models and inhibits sensitization.[149] It is noteworthy that HGF levels in HM are very high[150]; in fact, they are 20–30 times higher in HM than in maternal serum, pointing to a critical role in infant gut maturation.[150,151] Further, the HGF receptor, which is found on the surface of the intestinal crypt epithelial cells,[152,153] is expressed to a greater extent in infants than adults, indicating a readiness to interact with HM HGF.[154] Despite these intriguing findings, very few studies have evaluated HGF in HM. Epidermal growth factor is involved in cellular proliferation, maturation, migration, and apoptosis,[155] and VEGF is a key regulator of angiogenesis and tissue repair.[156] Concentrations of EGF and VEGF in HM are much higher than in maternal serum, suggesting a mammary gland source of these growth factors.[157] Human milk EGF is believed to increase gut mucosal barrier development[158] and has been associated with reduced risk of necrotizing enterocolitis in infants.[159]

Soluble CD14 (sCD14), a bacterial pattern recognition receptor for cell wall components such as lipopolysaccharide (LPS), has also been a focus of research because it is found in high concentrations in HM and has shown some role in protecting against allergic disease development.[160,161] These findings have not been reproduced,[138,141] highlighting the need for a systematic review of available evidence.

There is also growing interest in extracellular membrane vesicles, particularly the exosomes, which are released by a variety of mammalian cells to function as intercellular communication agents.[162] Exosomes have been detected in HM[163] and may have a role in allergy prevention by presenting allergen-derived peptides and inducing T-cell proliferation and Th2 cytokine production.[140,164,165] Human cohort data suggests that maternal sensitization may influence exosomes in HM.[140] Authors reported significantly ($P = 0.02$) higher MUC1 expression on CD63-enriched exosomes from HM of nonsensitized women, compared with sensitized. They also found higher levels of HLA-ABC on exosomes selected for anti-CD63 from women whose children subsequently developed allergic sensitization at 2 years of age. Exosomes in HM may also play a role in protection against virus transmission, such as HIV-1, during breastfeeding.[166] Although there are some studies assessing proteomics and micronutrient analysis of exosomes in HM, it is still a largely unexplored area,[162] and further research is needed to improve the overall understanding of breastfeeding/HM composition association with infant health outcomes.

In summary, many earlier studies failed to find consistent links between cytokines and other HM immune active molecules and risk for allergy.[141,161,167,168] Among more recent papers, Jepsen et al reported that HM with high levels of interleukin 1β (IL-1β) is associated with reduced incidence of eczema by 3 years,[137] and Munblit et al found interleukin 13 (IL-13) presence in HM to be associated with less eczema and food allergy,[47] whereas Sato-Ramirez et al reported associations between high levels of IL-13 or interleukin

Table 1 **Selected components of human breast milk**

Bioactive compounds		Target
Microorganisms	Predominant Staphylococcus, Streptococcus groups Lactic acid bacteria as Lactobacillus, Enterococcus, Weissella Presence of Bifidobacterium, Typical oral bacteria: Prevotella, Veillonella Typical skin bacteria: Propionibacterium, Corynebacterium Other organisms (such as Malassezia or Saccharomyces yeasts)	Probiotics: Support neonatal oral and gut microbiota colonization Stimulation of immune system: immune modulation and epithelial receptors Protection against infections: competitive exclusion of pathogens Metabolisms: productions of SCFA and some vitamins
HMO	>200 HMOs detected in HM up to date, consisting of short-chain and long-chain structures in an ≈9:1 ratio. 2-Fucosyllactose (2FL) Lacto-N-neotetraose (LNnT) Sialyllactose (SL). Other oligosaccharides used in infant formula: Galacto-oligosaccharides (scGOS); Fructo oligosaccharides (lcFOS)	Prebiotic effect: favor the beneficial bacteria, such as *Bifidobacterium* and *Bacteroides* spp. growth in the neonatal gut Stimulation of immune system Protection against infection: Antiadhesive and antimicrobial activities Stimulation of immune system: epithelial receptors and immune modulation
Bioactive proteins	Cytokines: IL-1β, IL-5, IL-13 Growth Factors: transforming growth factor-β (TGF-β), hepatocyte growth factor (HGF), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF) Immunoglobulins: sIgA, sIgG, sIgM C-type lectins sCD14 Caseins (alpha, beta, kappa), Whey proteins: (α-lactalbumin, β-lactoglobulin), lactoferrin and lactoperoxidase	Protection against infections Maturation and development of the immune system
Polyunsaturated fatty acids	omega-6 omega-3	Membrane structure Maturation of the immune system Precursor for immunological mediators
Other compounds	Minerals: Mg, Zn, Fe, Se, Vitamins: A, C, E Nucleotides Hormones: leptin, adinopectine Cells: lymphocytes, macrophages, granulocytes	Co-enzyme, antioxidant Satiety, control of appetite Active protection against infections

Abbreviations: Fe, iron; HM, human milk; HMO, human milk oligosaccharides; IL-1β, interleukin 1β; IL-5, interleukin 5; IL-13, interleukin 13; Mg, magnesium; SCFA, short-chain fatty acid; Se, selenium; sIgA, immunoglobulin A; sIgG, immunoglobulin G; sIgM, immunoglobulin M; Zn, zinc.

5 (IL-5) and risk of asthma-like symptoms in infants.[169] These results contradict earlier reports of null associations between levels of HM interleukins and allergy development.[167] Human milk cytokines are present in very low quantities, and many studies included samples with undetectable levels.[137,170,171] This may, in part, explain inconclusive data on HM cytokines and even more recent reports of positive associations with immune-related outcomes.

Effectiveness of therapies that target human milk immune factors

The maternal immunity modifier hypothesis proposes that the maternal diet, such as probiotic or fish intake, can alter HM composition and infant immune responses, leading to reduced risk of allergy development.[160,172–176] The impact of maternal diet on HM immune composition has been assessed in several observational and interventional studies,[173,176,177] with inconclusive results. Most studies have tested probiotics[168,173,174,178] or fish oil/whole fish,[176,179,180] but other options have included mixtures of pro- and prebiotics[181] and even blackcurrant seed oil,[177] which is rich in omega-3 and omega-6 fatty acids. Future research should focus on standardization of methodology and investigation of new perspective intervention options that have the capacity to modify HM components. These may include HGF stimulants or HGF receptor agents, which are also attractive therapeutic options for airway remodeling in chronic asthma.[182,183]

ORAL VACCINES

It is clear that HM is an immunologically active source of infant nutrition. Although not well studied, it appears that breastfeeding influences infant antibody responses to vaccination, with some vaccines enhancing immune

Table 2 **Outline of the studies on breastfeeding/breast milk composition association with immunological outcomes and infections**

Breastfeeding					
Reference	Study type	Outcome(-s) assessed	Exposure	Reported effect	Effect size OR (95%CI)
Horta et al (2015)[128]	Systematic review	Overweight/obesity	Breastfeeding ever vs never	Reduced risk	0.74 (0.70–0.78)
Horta et al (2015)[128]	Systematic review	Type 2 diabetes	Breastfeeding ever vs never	Reduced risk	0.65 (0.49–0.86)
Lodge et al (2015)[5]	Systematic review	Asthma	Breastfeeding ever vs never	Reduced risk	0.88 (0.82–0.95)
			Breastfeeding >3 mo vs <3–4 mo	No influence	0.89 (0.71–1.11)
Lodge et al (2015)[5]	Systematic review	Eczema	Breastfeeding ever vs never	No influence	1.07 (0.98–1.16)
			Breastfeeding >3 mo vs <3–4 mo	Reduced risk	0.74 (0.57–0.97)
Bowatte et al (2015)[129]	Systematic review	Otitis media	Breastfeeding ever vs never	Reduced risk	0.67 (0.56–0.80)
			Breastfeeding >3 mo vs <3–4 mo	No influence	0.85 (0.70–1.02)
Dogaru et al (2014)[130]	Systematic review	Asthma	Breastfeeding ever vs never	Reduced risk	0.78 (0.74–0.84)
Groome et al (2014)[131]	Randomized controlled trial	Vaccine immunogenicity	No breastfeeding 1 h before and after vaccination vs unrestricted breastfeeding	No influence	No difference in anti-rotavirus immunoglobuliln A reported
Quigley et al (2007)[132]	Prospective cohort	Diarrhea	Breastfeeding ever vs never	Reduced risk	0.37 (0.18–0.78)
Quigley et al (2007)[132]	Prospective cohort	Lower respiratory tract infections	Breastfeeding ever vs never	Reduced risk	0.66 (0.47–0.91)
Silfverdal et al (2007)[133]	Prospective cohort	Vaccine immunogenicity	Breastfeeding duration	Better protection	Exclusive breastfeeding for ≥90 d is associated with higher antipPneumococcal and anti-Hib immunoglobulin G
Kramer et al (2001)[134]	Randomized controlled trial	Gastrointestinal infections	Breastfeeding ever vs never	Reduced risk	0.60 (0.40–0.91)
Kramer et al (2001)[134]	Randomized controlled trial	Respiratory tract infections	Breastfeeding ever vs never	No influence	0.87 (0.59–1.28)
Breast milk composition					
Reference	Study type	Outcome(-s) assessed	Exposure	Reported effect	Effect direction
Doherty et al (2018)[119]	Systematic review	Allergic outcomes	HMOs in human milk	Mixed evidence	1 of 3 studies reported protective effect
Ramani et al (2018)[135]	Prospective cohort	Rotavirus-positive neonates with gastrointestinal symptoms	HMOs in human milk	Higher risk	High levels of LNT, 2′FL and 6′SL were associated with greater risk of symptomatic rotavirus infection

(continued)

Table 2 **Continued**

Breastfeeding

Reference	Study type	Outcome(-s) assessed	Exposure	Reported effect	Effect size OR (95%CI)
Logan et al (2018)[136]	Prospective cohort	Eczema	sCD14 in human milk	No influence	No associations found
Munblit et al (2017)[47]	Prospective cohort	Eczema	TGFβ2 in human milk	Higher risk	Higher levels were associated with eczema
Munblit et al (2017)[47]	Prospective cohort	Wheeze	TGFβ 1 and 2 in human milk	No influence	No associations found
Jepsen et al (2016)[137]	Prospective cohort	Eczema and wheeze	TGFβ 1 in human milk	No influence	No associations found
Savilahti et al (2015)[138]	Prospective cohort	Allergic outcomes	sCD14 in human milk	Higher risk	Higher incidence of allergic sensitization and eczema
Orivuori et al (2014)[139]	Prospective cohort	Allergic outcomes	TGFβ1, IgA in human milk	No influence	No associations found with eczema, asthma, and allergic sensitization
Torregrosa Paredes et al (2014)[140]	Prospective cohort	Allergic sensitization	Exosomes in human milk	Higher risk	Higher levels of HLA-ABC on exosomes selected for anti-CD63
Ismail et al (2013)[141]	Prospective cohort	Eczema and allergic sensitization	TGFβ1, sCD14, IgA in human milk	No influence	No associations found
Oddy and Rosales (2010)[142]	Systematic review	Immunological outcomes	TGFβ in human milk	Reduced risk	8 of 12 studies found TGFβ being protective against immunological outcomes
Stepans 2006[143]	Prospective cohort	Respiratory and gastrointestinal infections	HMOs in human milk	Mixed evidence	Increased LNFP-II reduced the risk at 6 and 12 wk but not at 24 wk

responses in breastfed infants, and other vaccines causing immune interference. In 2010, Moon et al reported on the inhibitory effect of HM on infectivity of live oral rotavirus vaccines, which they attributed to the high titers and neutralizing activity of IgA in HM.[184] Subsequently, the reduced efficacy of rotavirus vaccines in the developing world as a possible consequence of breastfeeding has stimulated considerable debate, with some experts even suggesting avoiding breastfeeding at the time of vaccination.

Rotavirus

Globally, severe diarrhea in young children is most often caused by rotavirus infection. Rotavirus vaccination in Africa has reduced the incidence of severe diarrhea in infants, with an efficacy of 61.2%,[185] although this efficacy is reportedly lower than has been observed in European and Latin American infants (96.4% and 84.8%, respectively).[186–189] The efficacy of other live oral vaccines has also been found to vary by geographic location,[190,191] with studies often showing reduced immunogenicity of oral vaccines in developing countries compared with industrialized nations. Geographic variations in oral vaccine efficacy have been explained by host characteristics, including poor nutrition and enteric co-infection; co-administration of other oral vaccines, such as the oral polio vaccine; and interference from maternal antibodies.[192] Finally, the presence of anti-rotavirus antibodies in HM, if given during vaccination, may reduce vaccine efficacy.[193,194]

As noted, HM has been shown to inhibit the infectivity of live oral rotavirus vaccination.[184] In this study, milk samples collected from mothers in India, Vietnam, South Korea, and the United States contained rotavirus-specific IgA and exhibited neutralizing activity against 3 rotavirus vaccine strains (RV1, 116E and RV5 G1). The HM of women in India contained the highest concentration of IgA and neutralizing titers against rotavirus strains, followed by the HM from women in Korea and Vietnam; the HM from women in the United States contained the lowest titers. In a study of rural and urban populations in Vietnam, urban mothers had rotavirus-specific IgA antibody titers in HM that were noticeably higher than their rural counterparts.[195] Groome et al undertook a follow-up study to investigate the temporary cessation of breastfeeding during RV vaccination of infants on their immune response to the RV vaccine.[131] Mother–infant pairs in South Africa were randomly assigned to defer breastfeeding by at least 1 h before and after each dose of RV vaccine or to unrestricted breastfeeding. Titers of RV-specific IgA in serum samples, measured before each vaccination and 1 month after the second vaccination, were similar between infants of the feeding deferral and unrestricted feeding groups. Authors concluded that abstaining from breastfeeding at time of vaccination did not significantly ($P = 0.69$) influence the infants' immune response to RV vaccination.[131] In a review of the literature on RV vaccine performance in low- and middle-income countries,[196] Mwila et al concluded that withholding breastfeeding does not affect infant vaccine response. However, 1 factor that appeared to reduce seroconversion in infants was exposure to higher concentrations of transplacental rotavirus-specific immunoglobulin G (IgG).[197,198] Less research has been undertaken on the performance of the oral polio vaccination in relation to breastfeeding or the effect of HM on its immunogenicity. One study from Pakistan demonstrated the high neutralizing capacity of colostrum against the oral polio vaccine that might interfere with its administration at birth.[199]

Injected vaccines

There has also been some limited investigation of the impact of breastfeeding on infants' responses to injected vaccines. The most plentiful evidence is with respect to the haemophilus influenzae type B (Hib) vaccine in infancy, although divergent results have been reported. Studies by Pabst and Spady,[200] Silfverdal et al,[133] and Greenberg et al[201] have all reported enhanced anti-Hib antibody responses following the vaccination of breastfed infants. No associations between breastfeeding status and anti-Hib antibody titers have been documented by others,[202–205] although in the Pickering et al study,[205] there was a trend for higher anti-Hib antibody levels in those babies breastfed for more than 6 months compared with infants breastfed for a shorter time period. There is one published study on the association between breastfeeding and lower plasma antibody concentrations before and after primary Hib vaccination.[206]

Regarding other injected vaccines, Silfverdal and colleagues reported a trend toward higher antibody titers against 5 pneumococcal serotypes (4, 6B, 9 V, 14, and 23F) at age 13 months in infants breastfed for at least 90 days.[133] In a study of a protein derivative of the *Mycobacterium tuberculosis* vaccine, the lymphocyte blast transformation response was noted to be notably higher in breastfed infants versus those never breastfed.[207] Investigating infant response to diphtheria and tetanus (DT) vaccination in relation to breastfeeding status, Hahn-Zoric and colleagues found that breastfed infants have significantly ($P < 0.01$) higher IgG anti-diphtheria toxoid levels 1–2 years after vaccination.[208] Breastfed infants also had higher concentrations of secretory IgA against the DT vaccine than

formula-fed infants. However, in another study, breastfed infants showed no improved antibody response to DT vaccination.[205]

CONCLUSION

The World Health Organization (WHO) recommends exclusive breastfeeding for 6 months in all infants.[1] This review highlights the important role of HM constituents in the development of the infant immune system and presents supporting evidence for their role in reducing the risk of the main allergic phenotypes. Study variation in methodologies, including the stage of HM collection and outcome definitions, challenge the integrity of the meta-analysis of this data. However, it is clear that HM components are potential targets in preventing the development of allergic disease. Indeed, the most promising HM components are HMOs, TGF-β, sCD14, exosomes, and microbiota.

At the same time, new developments have challenged the notion of prolonged exclusive breastfeeding. This is because oral tolerance can be induced by exposure to antigens via breast milk,[117] highlighting the importance of food protein transfer via HM as the first exposure to foods for the infant and of a role for the early introduction of food to promote tolerance rather than sensitization. This theory has been tested in a randomised controlled trial of the early introduction of allergenic foods to breastfed infants[6] and of a dose–response association between egg consumption and ovalbumin levels in HM.[209,210] This opens the door for early intervention via the maternal diet to provide an infant with a high levels of food proteins via HM before solid food introduction.

It is also clearly evident that investigation of a limited range of potentially active constituents in HM can lead to inconclusive results if taken in isolation because HM molecules may act in concert in order to be effective. There is a paucity of studies assessing HM as a whole rather than focusing on a single component. A full understanding of the relationship between HM composition and the development of noncommunicable diseases, and particularly allergy, may lead to effective HM modulation that will optimize infant immune system development and a reduction in allergic manifestation.[211] In the interim, systematic reviews of available evidence are urgently needed to highlight unmet needs and suggest potential routes for future research.

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Abbreviations: Hib, haemophilus influenzae type B; HLA-ABC, human leukocyte antigens; HMO, human milk oligosaccharides; IgA, immunoglobulin A; LNFP-II, lacto-N-fucopentaose II; LNT, lacto-N-tetraose; TGFβ, transforming growth factor β; 2'FL, 2'-fucosyllactose; 6'SL, 6'-sialyllactose.

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