Immuneological Effects of Human Milk Oligosaccharides

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Human milk oligosaccharides (HMOs) comprise a group of structurally complex, unconjugated glycans that are highly abundant in human milk. HMOs are minimally digested in the gastrointestinal tract and reach the colon intact, where they shape the microbiota. A small fraction of HMOs is absorbed, reaches the systemic circulation, and is excreted in urine. HMOs can bind to cell surface receptors expressed on epithelial cells and cells of the immune system and thus modulate neonatal immunity in the infant gut, and possibly also sites throughout the body. In addition, they have been shown to act as soluble decoy receptors to block the attachment of various microbial pathogens to cells. This review summarizes the current knowledge of the effects HMOs can have on infections, allergies, auto-immune diseases and inflammation, and will focus on the role of HMOs in altering immune responses through binding to immune-related receptors.

Keywords: human milk oligosaccharides, HMO, infection, immunity, infant, allergy, benefit, health

INTRODUCTION

Based on its richness in immune-related components like human milk oligosaccharides (HMOs), milk proteins and lipids, breastmilk can be seen as the first functional food humans encounter during their life (1). HMOs comprise a group of structurally complex, unconjugated glycans found in human breastmilk (see Figure 1). Although the amount and precise composition of HMOs varies depending on time of lactation and the genetic makeup of each woman as well as potential environmental exposures, human breast milk contains an average of 5–15 g of oligosaccharides per liter, making HMOs the third most abundant solid component of breast milk after lactose and lipids (2). Each oligosaccharide is built on a lactose backbone expanded by the addition of galactose, N-acetylglucosamine, fucose or sialic acid, branched and elongated in different ways, generating approximately 200 different structures identified to-date (3). As they are only minimally digested in the gastrointestinal tract, HMOs reach the colon intact or are absorbed in small quantities, reach the systemic circulation and are excreted in urine (4). In this way, they may exert a plethora of functions at multiple sites throughout the body and beyond the intestinal lumen and intestinal mucosal surfaces, including the urinary tract or the immune system. HMOs were first described as prebiotic substrates for the infant gut microbiota, promoting the establishment of bifidobacteria and lactobacilli, based on striking differences in microbiota composition between breastfed and bottle fed infants (5).

However, HMOs are now recognized to have various additional benefits for the developing neonate. HMOs may modulate neonatal immunity by altering host epithelial and immune cell responses in the infant gut (6), modify immune responses systemically or act as soluble decoy receptors to block the attachment of various microbial pathogens to cell surface receptors (7), not only in the intestine but also in other sites such as the urinary tract (8). The benefits of HMOs can...
extend to health outcomes beyond infancy such as allergies (9) or cognitive functions (10), making HMOs the focus of intense current scientific research with increasing number of studies unraveling their role in human physiology.

This review summarizes recent findings, discusses the proposed modes of action, and identifies future prospects and scientific challenges, with a focus on immunity and infection.

**HMO ABSORPTION**

HMOs are resistant to digestion in the infant GI tract (11). Both neutral and acidic HMOs can cross the epithelial barrier, but active transport over intestinal epithelial monolayers has only been demonstrated for neutral HMOs (12). These findings suggest that HMOs may be taken up into the human body. Indeed, HMOs have been detected in feces and urine of breastfed infants (13–17), but also directly in the peripheral blood (18–21). However, lower concentrations of HMOs are detected in blood compared to urine, which may be a reflection of accumulation in urine from a larger volume of blood. For example, concentrations of 2'-fucosyllactose (2'-FL), were around 1.5 mg/l in peripheral blood and 100 mg/l in urine (20).

Absorption of orally administrated single HMOs was also shown in an adult rat model showing indeed that the intestinal epithelium is permeable to HMOs although to a different extent in infancy and adulthood (22). Therefore, these publications indicate that HMOs may, in addition to effects in the GI tract, have effects throughout the human body. Such effects can be conveyed directly through binding to receptors for HMOs, or indirectly via induction of short chain fatty acids and other metabolites produced by the microbiota.

**POTENTIAL HMO RECEPTORS, THEIR EXPRESSION AND FUNCTION**

**Potential HMO Receptors**

Several classes of lectins (glycan-binding proteins) have been described in the literature that have different functions and ligand specificities, namely galectins, siglecs, c-type lectins, and selectins. Different HMOs can bind to these different types of receptors on human cells, primarily expressed on cells of the immune system.

Galectins are lectins that bind N-acetyllactosamine or lactose containing sugars (23–25). Galectins can also bind sulfated, sialylated or fucosylated galactose moieties (25). The work of Hirabayashi et al elegantly shows the oligosaccharide specificity of galectins for several HMO structures (23, 25, 26). More recently, Prudden et al. confirmed binding of HMOs with a terminal type 1 and 2 LacNAc to galectin 9 with a preference for type 1 structures on a solid surface (27). Similar findings were reported for HMO binding specificity for galectins in solution, corroborating these initial studies (28, 29).

Another family of lectins involved in HMO binding are the sialic acid binding immunoglobulin-like lectins (Siglecs). Siglecs have been shown to bind sialylated HMOs (30). Sialyllactose has been shown to bind to sialoadhesin (Siglec-1) (31), but also to Siglec-5 and Siglec-10 (32), Siglec-7 (33), and Siglec-9 (34). However, the affinity of sialyllactoses for Siglecs are relatively low.

In addition to galectins and siglecs, HMOs also interfere with another family of lectins involved in cell adhesion, the selectins (2, 35). Selectins bind to glycans that carry sialylated Le bloodgroup epitopes (36), which are sialylated and fucosylated lacto-N-bioses (Galβ1-3GlcNAc) or N-acetyllactosamines (Galβ1-4GlcNAc)—very similar to HMOs. In fact, HMOs contain Le blood group antigens (37) and are able to reduce selectin-mediated cell–cell interactions (38, 39). In addition, HMOs have been shown to interact with selectins (40), and integrins (39).

Finally, HMOs can bind to C-type lectins like DC-SIGN and Dectin-1. C-type lectins containing an EPN-motif (Glu-Pro-Asn) have high specificity for mannos- and fucose terminating glycans, whereas the presence of a QPD-motif (Gln-Pro-Asp) is important for galactose-or N-acetylgalactosamine(GalNAc) terminating glycans (41). HMOs were shown to bind specifically to DC-SIGN expressed by DCs (42). Although HMO binding to DC-SIGN seems to be weaker than binding to galectins, it was shown that structures containing α-linked fucose could bind to DC-SIGN (34). The results were also confirmed by binding of DC-SIGN to beads derivatized with 2'-FL or 3-FL, but not with LNT.

A limited number of reports have also discussed the possibility of binding of HMOs to other receptors belonging to the Toll like receptor (TLR) family that typically bind to pathogen-related molecules. TLR-4 dependent effects of HMOs have been described in two papers in which HMOs tested in vivo required the expression of TLR-4 for their effect (43, 44). However, formal demonstration of the binding of the HMOs (3’SL and LNFPIII) to TLR-4 in direct binding assays was not provided. In addition, in relation to TLR-signaling of HMOs, a recent paper highlighted the effect that low level LPS contamination of the commercially available HMO 3’SL can have in these studies, indicating that caution is warranted when studying TLR-mediated effects (45).

An overview of putative receptors for HMO is shown in Table 1.

**Expression Profiles and Functions of Potential HMO Receptors**

Galectins are mainly expressed on T cells, and can regulate T cell function (46), but are also present on intestinal epithelial cells (47–49), and on antigen presenting cells and granulocytes (25). Galectins can convert signals into the cell after binding to their ligands directly, but galectins can also be secreted, after which they bind to glycoproteins or receptors at cell surfaces and hence can regulate cell functions (50–52). Binding of HMOs or lactose can thus have direct effects or inhibit the interaction of galectins with their ligands on other cells.

Siglecs are involved in the immune system in multiple ways (53). Siglecs 1–16 are expressed on a variety of blood cells, including monocytes, macrophages, dendritic cells, neutrophils, eosinophils, basophils, and NK cells (53, 54). In contrast to galectins and Dectin-1, Siglecs are not expressed by intestinal epithelial cells. Many of the Siglecs have an intracellular
immunoreceptor tyrosine-based inhibitory motif (ITIM), and are thus known as regulators of immune responses.

Selectins are cell adhesion molecules that mediate the earliest stages of leukocyte trafficking. At sites of inflammation, leukocytes need to migrate from the blood stream through the endothelium into sub endothelial regions of inflammation (55, 56). Induced by pro-inflammatory cytokines, endothelial cells express P- and E-selectin, which bind to glyco-conjugates on leukocytes passing by with the blood stream. This initial contact decelerates the leukocytes and makes them roll over the endothelial cell layer. Subsequently, additional adhesion molecules bring leukocytes to a complete stop and facilitate their transmigration into sub endothelial regions. Initial selectin-mediated rolling is essential for leukocyte extravasation and mucosal infiltration.

Sialylated HMOs have been shown to interact with selectins (40), and integrins (39), and affect leukocyte-endothelial cell and leukocyte-platelet interactions (39, 57–59). Similarly, sialylated HMOs reduce PNC formation and subsequent neutrophil activation in an ex vivo model with whole human blood (38). In both cases, non-sialylated HMOs are ineffective and pooled HMOs are more effective than monovalent sialyl-Le X, indicating the importance of Sia and suggesting potential multivalent interactions with higher molecular HMOs that carry more than one sialylated blood group epitope.

C-type lectins are primarily expressed by antigen presenting cells (monocytes, macrophages, dendritic cells) and are of crucial importance for regulating immune responses to pathogens. The can be divided into four subgroups, the sialo-glycoprotein receptor family (e.g., DC-SIGN), the dectin-1 subfamily of asialo-glycoprotein receptors (e.g., Dectin-1), the DCIR subfamily (e.g., DCIR), and the Mannose receptor family (e.g., CD206) for a review see Geijtenbeek and Gringhuis (60).

In general, c-type lectins are primarily expressed on dendritic cells and macrophages, and play a role in the internalization of saccharide-containing antigens, resulting in antigen presentation (41). However, dectin-1 can also be detected on intestinal epithelial and on M cells, and play a role in IgA transcytosis.
Apart from promoting antigen presentation, some c-type lectins like DCIR may—just like Siglecs—contain an immunoreceptor tyrosine-based inhibitory motif (ITIM) motif in their intracellular domains, that inhibit immune activation.

DC-SIGN interacts with a variety of pathogens, including HIV-1, and binding of HMOs inhibited the transfer of HIV-1 to CD4+ T lymphocytes. These data may suggest that oligosaccharides act systemically and are thereby modulating the immune response in a microbiota-independent manner. In addition, recent publications have also demonstrated that the c-type lectin Dectin-1 can modulate innate immune function, possibly explaining the cross-protection against other pathogens seen after vaccination.

The functions of these receptors thus indicates that binding of HMOs to these structures may result in regulation of adaptive and innate immune protection against infection and inflammation.

### EFFECTS OF HMOS ON INFECTION, ALLERGY AND IMMUNE PARAMETERS IN HUMAN STUDIES

As can be seen in Table 2, there are currently only a few infant studies on effects of HMOs on infection and immune function. Most of these studies are observational studies on breastfeeding infants, correlating HMOs in breastmilk with these outcomes. Placebo controlled studies with HMOs have been performed but have to date focused on safety rather than on anti-infective and immunomodulatory effects.

Four of these studies showed an effect of HMOs on prevention of diarrhea, respiratory tract infections, and severe outcomes like sepsis and death. Morrow et al. showed in another study that HIV exposed, non-infected children receiving breastmilk of secretor+ mothers have a reduced risk of early mortality compared to secretor− breastfeeding.

Also, in relation to cow's milk allergy, the level of Lacto-N-fucopentaose (LNFP) III in breast milk correlated with the prevalence of cow's milk allergy. Similarly, Sprenger et al. reported that FUT2-dependent breast milk oligosaccharides, with the levels of 2′FL as proxy for secretor status, were associated with lower levels of IgE-mediated allergies and eczema.

Finally, Biesbroek et al. reported recently that 6 week old breastfed children have a different nasopharyngeal microbiota, suggesting that milk components like HMOs may influence the nasopharyngeal microbiota composition—which may contribute to the protective effect of breastfeeding on decreased respiratory infections.

It should be stressed that none of these studies have formally demonstrated direct effects of HMOs, and that other breastfeeding components may be associated with the effects described. Only in a recent study the administration of 2′-FL in combination with LNnT could reversely correlate with parental reported episodes of bronchitis, lower respiratory tract infections, and use of antipyretics or antibiotics at different ages.

However, as reviewed in detail in section Effects of HMOs on Infection, Allergy and Immune Parameters in Human Studies and in several recent reviews, quite some information is available of effects of HMOs on microbiota composition, pathogens, and pathogen adhesion in vivo, as well as on infection in vivo. In addition, effects on intestinal epithelium and barrier function, as well as immune function have been described in these reviews and the underlying literature.

In infants another study showed that the secretor or non-secretor genotype of mothers of infants that were breastfed correlated with enterocolitis (low secretor) and sepsis (non-secretor).

It has been shown as well that the amount of 2-linked fucosylated oligosaccharides in breast milk inversely correlates with the incidence of diarrhea in infants, and similarly the amount of fucosyl oligosaccharides in breast milk inversely correlates with the severity of infection with E coli that has a stable. Similarly, the amount of 2FL inversely correlated with Campylobacter diarrhea.

Bode et al demonstrated that the risk of HIV transmission in breastfeeding children of HIV infected mothers inversely correlates with HMO concentration. In another study the amount of LDFH-1 in breast milk inversely correlated with norovirus diarrhea. In addition to effects of HMOs on...
### Table 2 | Human studies with HMOs and measured outcomes.

| Title of study                                                                 | Health-related effects | HMO used                   | Target group | Study setup                  | Outcome of effect HMO (Short)                                                                 | Reference |
|--------------------------------------------------------------------------------|------------------------|----------------------------|--------------|------------------------------|---------------------------------------------------------------------------------------------|-----------|
| Fucosyltransferase 2 non-secretor and low secretor status predicts severe outcomes in premature infants. | Mortality, necrotizing enterocolitis (NEC), sepsis | Breast milk             | Infants (n = 410) | Observational study | Mortality, NEC and gram - sepsis increased in infants receiving low secretor status breast milk | (66)      |
| Human milk oligosaccharides are associated with protection against diarrhea in breast-fed infants. | Diarrhea              | Breast milk             | Infants (n = 93) | Observational study | (1) High levels of 2-FL in breast milk protective against Campylobacter diarrhea (2) High levels of lacto-N-difucohexaose (LDFH-I), also a 2-linked fucosyloligosaccharide, protective against calicivirus diarrhea | (67)      |
| Innate protection conferred by fucosylated oligosaccharides of human milk against diarrhea in breastfed infants | Diarrhea              | Breast milk             | Infants (n = 93) | Observational study | Breast milk with higher 2-linked to non-2-linked fucosyloligosaccharide ratios affords greater protection against infant diarrhea | (68)      |
| Early consumption of human milk oligosaccharides is inversely related to subsequent risk of respiratory and enteric disease in infants. | Diarrhea and respiratory infection | Breast milk             | Infants (n = 49) | Observational pilot study | LNF-II levels in breast milk and in infant feces at 2 weeks of age (as representative of total HMO) associated with fewer infant respiratory problems and gastrointestinal problems by week 6 and week 12 | (69)      |
| FUT2-dependent breast milk oligosaccharides and allergy at 2 and 5 years of age in infants with high hereditary allergy risk | Eczema                | Breast milk             | Infants at risk for allergy (n = 266) | Observational study (in placebo arm of controlled study) | At 2 years, but not at 5 years, FUT2-dependent oligosaccharides associated with lower IgE-associated eczema manifestations. Only in C-section-born infants with high allergy risk | (70)      |
| Human milk oligosaccharides and development of cow’s milk allergy in infants | CMA                    | Breast milk             | Infants with (n = 35) and children without CMA (n = 39) | Observational study | Infants receiving breast milk with low LNFP III levels more likely to become affected with CMA than infants receiving higher levels of LNFP III | (71)      |
| Effects of infant formula with human milk oligosaccharides on growth and morbidity: A randomized multicenter trial | Respiratory infection (bronchitis) and antibiotic use | Formula containing 2′fucosyllactose (2′FL) + lacto-N-neotetraose (LNnT) | Infants receiving cow’s milk-based infant formula (n = 87) vs. the same formula with 2′FL and LNnT (n = 88) | Multicenter, randomized, double-blind trial | Infant formula supplemented with 2′FL and LNnT associated with lower parent-reported morbidity (particularly bronchitis) and medication use (antipyretics and antibiotics) | (72)      |
| Infants fed a lower calorie formula with 2′-fucosyllactose (2′FL) Show Growth and 2′FL Uptake Like Breast-Fed Infants | Growth                | Formula supplemented with 2-Fucosyllactose (2′FL) and galactooligosaccharides (GOS) | Infants exclusively formula-fed in 3 groups: (1; n = 101 control formula GOS 2; n = 104 formula high GOS and low 2′FL 3; n = 109 medium GOS and medium 2′FL) or breast fed (n = 106) from enrollment to 4 mo of age | A prospective, randomized, controlled, multicenter growth and tolerance study | Growth and 2′FL uptake similar to breast milk | (73)      |

(Continued)
| Title of study | Health-related effects | HMO used | Target group | Study setup | Outcome of effect HMO (Short) | Reference |
|---------------|------------------------|----------|--------------|-------------|-----------------------------|-----------|
| Similar to those who are breastfed, infants fed a formula containing 2′-fucosyllactose have lower inflammatory cytokines in a randomized controlled trial | Immune parameters | Formula supplemented with 2-FL and GOS | Infants exclusively formula-fed in 3 groups: (1; n = 75 control formula GOS 2; n = 76 formula high GOS and low 2′FL 3; n = 78 medium GOS and medium 2′FL) or breastfed (n = 86) from enrollment to 4 mo of age | Observational substudy nested within a randomized, double-blind, controlled study | Infants fed formula supplemented with 2′-FL exhibit lower plasma and ex vivo inflammatory cytokine profiles, similar to those of a breastfed reference group | (72) |
| Human milk oligosaccharide concentration and risk of postnatal transmission of HIV through breastfeeding. | HIV transmission | Breastmilk | Breast milk of HIV-infected women who did not transmit HIV despite breastfeeding (n = 86), and uninfected women (n = 36) | Nested case-control study was conducted within a larger cohort study | (1) Higher concentrations of non-3′-SL HMOs were associated with protection against postnatal HIV transmission (2) A trend toward higher concentrations of lacto-N-neotetraose (LNnT) being associated with reduced transmission | (73) |
| Human milk oligosaccharides differ between HIV-infected and HIV-uninfected mothers and are related to necrotizing enterocolitis incidence in their preterm very-low-birth-weight infants | NEC, HIV infection (secretor/nonsecretor) | Breastmilk | HIV infected mothers (n = 41 of which 22 secretor, 19 non-secretor) and non-infected mothers (n = 41 of which 20 secretor, 21 non-secretor) | Substudy of a larger clinical trial on HIV-infected and HIV-uninfected mothers and their preterm infants | (1) HIV-infected mothers have higher relative abundances of 3′-SL in breastmilk (2) Low concentrations of DSLNT in breastmilk increased infant’s risk of NEC | (74) |
| Growth and Morbidity of Gambian Infants are Influenced by Maternal Milk Oligosaccharides and Infant Gut Microbiota. | Morbidity | Breastmilk | Mother/infant pairs (n = 33, of which 21 secretors and 22 non-secretors) | Sub-study embedded within a randomized trial | (1) Higher breast milk levels of lacto-N-fucopentaose I (secretor) associated with decreased infant morbidity (2) Higher breast milk levels of LNT (non-secretor) associated with higher infant morbidity 3) Breast milk levels of 3′-sialyllactose indicator of infant weight-for-age | (5) |
| Oligosaccharide composition of breast milk influences survival of uninfected children born to HIV-infected mothers in Lusaka, Zambia | HIV infection, mortality | Breastmilk | HIV-infected children (n = 103) and HIV exposed uninfected children (n = 143). | Nested case-cohort study | High levels of fucosylated HMOs in breastmilk of mothers of HEU children protective against mortality | (75) |
| The impact of breastfeeding on nasopharyngeal microbial communities in infants. | Respiratory infection | Breastmilk | Infants receiving exclusive breastfeeding (n = 101) vs. and exclusive formula feeding (n = 101) | Case-cohort analysis | (1) Association between breastfeeding and microbial community composition in the upper respiratory tract (2) Possible link to protective effect of breastfeeding on respiratory infections and wheezing in early infancy | (76) |
intestinal infections, HMOs have been linked to other infections such as infections of the urogenital tract (8, 90), and airway infection (69, 91, 92).

Such results were expanded lately with measuring inflammatory cytokines in systemic circulation if infants receiving infant formula supplemented with 2′-FL (72). Much more is known about the direct effects of HMOs on pathogens, on adhesion and infection in in vitro models, and in animal models.

**EFFECTS OF HMOS ON IMMUNE FUNCTION AND INFECTION IN IN VITRO AND ANIMAL STUDIES**

**Effects on Bacterial Adhesion and Infection**

HMOs have been shown to prevent adhesion of several potential pathogens to epithelial surfaces in the intestine and other organs by acting as decoy receptors for bacterial pathogens like Campylobacter or *E. coli* (86, 93–95).

Several recent manuscripts report specific effects of isolated HMOs. For example, Weichert et al. showed that 2′FL, and to a lesser extent 3FL, reduce the adhesion of Campylobacter, EPEC, Salmonella and Pseudomonas, although the inhibitory effects were very small (96). Sialylated oligosaccharides were shown to reduce adhesion of EPEC (97, 98). In addition to reducing the adhesion of entire bacteria, HMOs may also compete with binding of bacterial toxins and mitigate their diarrheal activity (99, 100).

However, not always is the beneficial effect of HMOs to bacterial infection arising from preventing association or invasion of the pathogen. For example, HMOs can alter gene expression in intestinal cells that can block infection of *Listeria monocytogenes* (101), or have a direct effect on the growth of pathogens as was shown for neutral HMOs and especially LNT and LNFP I against group B *Streptococcus* (102). Similarly, HMOs were shown to modulate hyphal induction in *Candida albicans*, which is necessary for invasion of the intestinal epithelium (103). Another mechanism could be attenuation of pathogenic virulence through metabolites from fermentation of HMOs from the intestinal microflora. That seems to be at least partially the case for *Escherichia coli* O157:H7 and *Salmonella typhimurium* (104). When bifidobacteria of human and bovine origin were grown on medium containing 3′-SL, they could produce metabolites that could block expression of virulence genes in both pathogens. In addition, HMOs may have an indirect effect on bacterial infection by reducing epithelial inflammatory responses as it has been shown for 2′-FL and Campylobacter-induced inflammation (105).

While 2′-FL protected against adherent-invasive *E. coli*-induced pathology in mice (106), it failed to improve *E. coli*-induced diarrhea in piglets (107). These contradicting results could be explained by the difference in model, virulence of different *E. coli* strains, dosage and timing of administration of pathogens and HMOs, etc.

**Effects on Intestinal Viruses**

Shang et al. demonstrated that different HMOs can bind to norovirus (LNFPII and 2′FL) and Norwalk virus (LNFP I and LNDFHII), indicating that several potential Noro- and Norwalk virus-binding glycans are present in HMOs that can play a role in viral infection (108). Notably, they also showed that LNFP III-HSA and 2′-FL-BSA – but not their monovalent forms (LNFP III-Gly and 2′-FL-Gly) bound to VA287 capsids. This suggests that polyvalent oligosaccharides on a carrier protein may be more potent in anti-adhesion effects than their monovalent sugars themselves. However, recently it was also shown that 2′-FL can block both the GI.1 and GI.17 noroviruses from binding to HBGs (109).

In addition to effects on gut bacteria, HMOs can also have effects on viral pathogens as rotavirus, norovirus, and HIV [reviewed in (85)].

In Rotavirus infected piglets, HMO-supplemented piglets had a shorter duration of diarrhea compared to the control Group (110, 111). There have been several HMO structures identified that bind the glycan rotavirus receptor VP8*. The sialic acid containing HMOs inhibited rotavirus infection in vitro, but in vivo both neutral HMOs and sialic acid containing HMOs decreased replication during acute RV infection in situ. These data are confirmed by recent in vitro findings where 2′-FL, 3′-SL, and 6′-SL could block infectivity of human rotaviral strains in cells (7). Apparently simple HMO structures can act as decoy receptors for viruses. However, since there are differences in the infectious mechanism of porcine and human rotaviral strains extrapolation from porcine to human models can be treacherous and more research is needed to clarify the role of HMOs in rotaviral infections.

**Effects on Respiratory Viruses**

In addition to effects on intestinal pathogens, HMOs have been suggested to also play a role in infections from respiratory viruses. For example, 2′FL was shown to decrease RSV viral load, whereas LNnT and 6′SL decreased influenza viral load. Also effects were observed on innate cytokines in response to both viruses (92) suggesting an effect of HMOs on respiratory virus infection. This is supported by an early study by Stepsans-Flanders on the fact that HMO consumption is inversely linked to respiratory infection (69). In this study higher LNFPII levels in breastmilk correlated with decreased respiratory and gastrointestinal infections in early infancy.

Immobilized 3′SL and 6′SL haven been shown to prevent infectivity of influenza viruses as a result of blocking the haemagglutins of influenza viruses (112, 113), and Yu et al. identified a number of additional sialic acid containing HMOs that bind to influenza virus (114). The effects of these HMOs was confirmed in a functional infection assay in vitro, where 6′SL and LNnT were shown to reduce the viral load of influenza in airway epithelial cells, and 2′FL did the same for respiratory syncytial virus (RSV) (92). In one recent in vivo study 2′FL enhanced responses to vaccination in mice (115). The mechanism was postulated to involve also a direct effect of 2′FL on dendritic cells as shown in vitro. However, concentrations used in their experiments were more than 1000-fold higher than what has
been described to be found in circulation (20), warranting further clarification and research on the mechanism of action and relevance to human breast-fed infants.

**Enterocolitis**

In relation to necrotizing enterocolitis, Jantscher-Krenn et al. noted in a rat model that disialylated LNT (DSLNT) increased survival rates and improved pathology scores (116), while low amounts of DSLNT in mother’s milk could be a predicting risk factor for the development of NEC in premature infants (117) corroborating the previous findings. More HMOs could have a beneficial effect in NEC as it was shown also in a rat study where 2′-FL ameliorated the pathology of NEC, however there was no association between 2′FL and NEC risk in the corresponding human cohort (115). Similar observations were made for rats fed sialylated galacto-oligosaccharides (Sia-GOS) (118). Studies in mice have also shown a beneficial effect of 2′-FL in an induced NEC model (119). However such effect could not be seen in a piglet model where piglets born with caesarian section were fed control formula or formula supplemented with 2′-FL and were let to develop NEC spontaneously (120). Differences between induction vs. natural progression to NEC or species differences could account for such outcomes. In infants another study showed that the secretor or non-secretor genotype of mothers of infants that were breastfed correlated with enterocolitis (low secretor) and sepsis (non-secretor) (66).

**Effects of HMOs on Intestinal Epithelium**

In another study immunomodulation by 2′FL in vivo was shown to be dependent on the downregulation of CD14 on intestinal epithelial cells (106). As CD14 is a co-receptor for LPS and is involved in TLR-4 signaling, this may lead to decreased inflammatory responses in the intestine after exposure to LPS.

In a recent study by He et al. the effect of colostrum oligosaccharides on gene expression in fetal immature intestinal mucosa was tested (121). They identified several immune related pathways were induced by HMOs, such as Immune cell communication, homeostasis, and intestinal immune differentiation. HMOs could reduce the response to TLR stimuli, and induced cytokines that are involved in tissue repair. 3′, 4′, and 6′ galactosyllactoses were the most potent oligosaccharides.

Sialyllactose has been described to be able to promote the differentiation and growth of human intestinal epithelial cells as measured by upregulation of expression of alkaline phosphatase (122). Alkaline phosphatase is a molecule that is important in maintaining gut barrier function, possibly through the inactivation of LPS, by cleaving of a phosphate group from LPS. This suggests that on the one hand epithelial cells may yet have a receptor that recognizes Sialyllactose, and that Sialyllactose may be beneficial for promoting a good epithelial barrier in the gut.

In addition, two recent papers suggest that SL or goat milk oligosaccharides containing SL may have an effect on epithelial cells via activating through TLR4 (43, 123).

In contrast, another paper showed that 3′SL had anti-inflammatory activity by reducing the expression of IL-12 and IL-8 in Caco-2 cells, mediated via NFkB, and stimulates the anti-inflammatory nuclear receptor PPARg (124). Especially neutral HMOs have been shown to have an anti-inflammatory effects on the intestinal epithelium in *in vitro* inflammatory models (106, 121).

Lane compared effects of HMOs and BMOs on gene expression in HT-29 cells, noting that “both treatments including a response to stimulus, signaling, locomotion, and multicellular, developmental and immune system processes” (125).

Combined, these studies suggests that milk oligosaccharides contribute to the development and maturation of the intestinal immune response.

**Effects of HMOs on Immune Function**

Acidic HMOs (but not acidic cow’s milk oligosaccharides) were shown to induce IFN-γ and IL-10 in human cord blood T cells, and could decrease IL-4 production in allergen-specific T cells (126). These data suggest that acidic HMOs may downregulate Th2 responses in infants as well.

Such results were expanded lately with measuring inflammatory cytokines in systemic circulation if infants receiving infant formula supplemented with 2′-FL (72). In this study, supplementation of 2′-FL alone could lower levels of TNFα, IL-1α, IL-1β, and IL-6 resembling those found in breast fed infants. In early studies LNFPIII and LNNT were shown to have immunosuppressive effects (127, 128), and LNFPIII can induce IL-10 in macrophages (129, 130). Unexpectedly, a link with helminth infections exists. It is now known that upon infection with helminths confers a protective effect on allergy development (131, 132). Schistosoma eggs, but not the helminth itself, induce potent IL-10 responses that inhibit Th2 responses (133, 134). These effects are at least in part mediated by the oligosaccharides LNFPIII GalNAcβ1-4(Fucα1-2Fucα1-3) GlcNac (LDN-DF) and Lewis-X that is present in the egg shells. These oligosaccharides are also found in breast milk, suggesting a functional anti-allergic/anti-inflammatory role of these HMOs. Likewise, Comstock et al. demonstrated a similar effect of HMOs *in vivo* in piglets, where HMOs induced IL-10 levels and inhibited T cell proliferation (135). The same was noted by Hester et al. that showed enhanced T helper type 1 (interferon-gamma) and anti-inflammatory (interleukin-10) cytokines in the ileum in response to HMO supplementation of piglets in a rotavirus infection model (110).

Interestingly, LNFPIII and Lewis X glycoconjugates can also inhibit TLR signaling in innate immune cells through possible involvement of c-type lectins (132). LNFPIII is a very well-studied HMO that has been linked to many different effects including hepatosteatosis and insulin resistance (136), autoimmunity (137), and transplantation (138). Especially in the case of insulin resistance and autoimmunity, HMOs have been shown to elicit a protective effect in a murine model of Type 1 Diabetes (T1D) (139). The paper shows that supplementation of HMOs can alter microbiota composition and SCFA production in a NOD-mouse model that can prevent spontaneous progression to diabetes. The protective effect of SCFA-producing diets on T1D has been documented beforehand (140, 141). On the other hand, by increasing barrier integrity
HMOs may also reduce gut permeability, which has been argued to contribute to the onset of T1D (142).

**Indirect Effects of HMOs on Intestinal Epithelium and Immune Function via SCFA**

Another important role of HMOs is establishing and maintaining the intestinal microbiota. Breastfed infants have higher numbers of beneficial bifidobacteria and lactobacilli than bottle-fed infants. This is the result of preferential fermentation of HMOs by the microbiota by bifidobacterial and lactobacilli (143–145). Upon fermentation of HMOs these bacteria produce, in addition to lactic acid, the short chain fatty acids (SCFA) butyrate, acetate, and propionate. These SCFA improve intestinal barrier function (146) lower the pH in the colon, and have well established anti-inflammatory properties (147).

The notion that composition and metabolic activity of the intestinal microbiota affects the development of allergies has become clearer over the last years (148–151). Exactly how the microbiota composition influences allergy development is not clear at this point, but data from animal models strongly suggest a protective role for SCFA (152–155). A similar role was shown for the SCFA receptors GPR43 in asthma, arthritis, and colitis models (156), and for GPR41 in allergic airway inflammation.

Taken together, these data suggests that HMOs may also have an indirect effect on allergy.

**HMOs AND ALLERGY**

Several, but not all, studies on the association between breastfeeding and allergy have shown effects on allergic outcomes (157–159). One of the factors that may explain the conflicting findings described above may be the result of differences in breastmilk composition (in relation to milk proteins) (160). None of these studies have correlated their findings with HMO composition.

However, two studies have done just this. In a cohort study of cow’s milk allergy (CMA) (9) it was observed that the concentration of 6SL, DSLNT, LNFPI, and LNFPIII was lower in the breast milk of mothers having infants with CMA. After further corrections, only breast milk levels of LNFPIII associated reversely and significantly with development of CMA. In the same study it was observed that FUT2 status of mothers correlated with a delayed onset of CMA while CMA infants born to non-secretor mothers (FUT2 negative) were prone to acute CMA (IgE-mediated). FUT2 status seemed to play a role also in IgE-mediated eczema developed in infants born with C-section (70). In a study of 266 infants followed for 5 years, they observed that infants born to secretor mothers had lower incidence of IgE-mediated eczema. That effect was evident at 2 years but not at 5 years of age though. 2′-FL (one of the main HMOs produced by secretor mothers) was shown to have a significant association with any allergic disease, acute or delayed in infants born with C-section in the same study. 2′-FL and 6′SL had also a beneficial
effect in a mouse model of OVA-induced allergy (161). Both HMOs could increase numbers of IL10 producing Treg cells and alleviate allergic symptoms but through different mechanisms.

CONCLUSIONS

HMOs contribute to the development of the microbiota and the immune system of newborn infants. The mechanisms by which HMOs contribute have become clearer over the past few years, and our current knowledge is summarized in Figure 2.

However, despite many in vitro- and animal experiments, HMOs have not been tested extensively in placebo controlled infant studies. It is clear that several HMOs will be introduced in the near future into infant nutrition to supplement or replace non-human prebiotics like galactooligosaccharides and/or fructooligosaccharides. Prebiotics have been added to infant nutrition in the early 2000’s as non-digestible oligosaccharides in an attempt to mimic some of the function of HMOs. With these prebiotics a large number of studies have shown effects on intestinal infection, respiratory infection and allergy (162–167). As the selection of prebiotics is based on functional similarities with HMOs, and extrapolating from in vitro and animal experiments with HMOs, it is to be expected that inclusion of HMOs to infant formula will have additional benefits to infant health, and may supplement the functionality of the prebiotics that are already used. Still more research is needed to clarify whether HMOs may also have a therapeutic rather than a protective effect in human immune disorders. Our emerging evidence for the beneficial effects of HMOs once again provide a powerful rationale to encourage women to breastfeed their infants to provide the full scope of benefits that stem from a diverse composition of HMOs that is provided through mother’s milk and could potentially be personalized to match the genetic context and environmental exposures of the mother-infant dyad.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

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REFERENCES

1. Gura T. Nature’s first functional food. Science (2014) 345:747–9. doi: 10.1126/science.345.6198.747
2. Bode L. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology (2012) 22:1147–62. doi: 10.1093/glycob/cws074
3. Ninomura UH, Park Y, Yin H, Zhang J, Ward RE, Flowers BH, et al. A strategy for annotating the human milk glycome. J Agric Food Chem. (2006) 54:7471–80. doi: 10.1021/jf0518510
4. Rudloff S, Pohlentz G, Borsch C, Lentze MJ, Kunz C. Urinary excretion of human milk oligosaccharides in an attempt to mimic some of the functional similarities with HMOs, and extrapolating from in vitro- and animal experiments, it is to be expected that inclusion of HMOs to infant formula will have additional benefits to infant health, and may supplement the functionality of the prebiotics that are already used. Still more research is needed to clarify whether HMOs may also have a therapeutic rather than a protective effect in human immune disorders. Our emerging evidence for the beneficial effects of HMOs once again provide a powerful rationale to encourage women to breastfeed their infants to provide the full scope of benefits that stem from a diverse composition of HMOs that is provided through mother’s milk and could potentially be personalized to match the genetic context and environmental exposures of the mother-infant dyad.

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4. Rudloff S, Pohlentz G, Borsch C, Lentze MJ, Kunz C. Urinary excretion of human milk oligosaccharides in an attempt to mimic some of the functional similarities with HMOs, and extrapolating from in vitro- and animal experiments, it is to be expected that inclusion of HMOs to infant formula will have additional benefits to infant health, and may supplement the functionality of the prebiotics that are already used. Still more research is needed to clarify whether HMOs may also have a therapeutic rather than a protective effect in human immune disorders. Our emerging evidence for the beneficial effects of HMOs once again provide a powerful rationale to encourage women to breastfeed their infants to provide the full scope of benefits that stem from a diverse composition of HMOs that is provided through mother’s milk and could potentially be personalized to match the genetic context and environmental exposures of the mother-infant dyad.

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2. Bode L. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology (2012) 22:1147–62. doi: 10.1093/glycob/cws074
3. Ninomura UH, Park Y, Yin H, Zhang J, Ward RE, Flowers BH, et al. A strategy for annotating the human milk glycome. J Agric Food Chem. (2006) 54:7471–80. doi: 10.1021/jf0518510
4. Rudloff S, Pohlentz G, Borsch C, Lentze MJ, Kunz C. Urinary excretion of human milk oligosaccharides in an attempt to mimic some of the functional similarities with HMOs, and extrapolating from in vitro- and animal experiments, it is to be expected that inclusion of HMOs to infant formula will have additional benefits to infant health, and may supplement the functionality of the prebiotics that are already used. Still more research is needed to clarify whether HMOs may also have a therapeutic rather than a protective effect in human immune disorders. Our emerging evidence for the beneficial effects of HMOs once again provide a powerful rationale to encourage women to breastfeed their infants to provide the full scope of benefits that stem from a diverse composition of HMOs that is provided through mother’s milk and could potentially be personalized to match the genetic context and environmental exposures of the mother-infant dyad.

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REFERENCES

1. Gura T. Nature’s first functional food. Science (2014) 345:747–9. doi: 10.1126/science.345.6198.747
2. Bode L. Human milk oligosaccharides: every baby needs a sugar mama. Glycobiology (2012) 22:1147–62. doi: 10.1093/glycob/cws074
3. Ninomura UH, Park Y, Yin H, Zhang J, Ward RE, Flowers BH, et al. A strategy for annotating the human milk glycome. J Agric Food Chem. (2006) 54:7471–80. doi: 10.1021/jf0518510
4. Rudloff S, Pohlentz G, Borsch C, Lentze MJ, Kunz C. Urinary excretion of human milk oligosaccharides in an attempt to mimic some of the functional similarities with HMOs, and extrapolating from in vitro- and animal experiments, it is to be expected that inclusion of HMOs to infant formula will have additional benefits to infant health, and may supplement the functionality of the prebiotics that are already used. Still more research is needed to clarify whether HMOs may also have a therapeutic rather than a protective effect in human immune disorders. Our emerging evidence for the beneficial effects of HMOs once again provide a powerful rationale to encourage women to breastfeed their infants to provide the full scope of benefits that stem from a diverse composition of HMOs that is provided through mother’s milk and could potentially be personalized to match the genetic context and environmental exposures of the mother-infant dyad.

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REFERENCES
22. Vazquez E, Santos-Fandila A, Buck R, Rueda R, Ramirez M. Major human milk oligosaccharides are absorbed into the systemic circulation after oral administration in rats. Br J Nutr. (2017) 117:237–47. doi: 10.1017/S0007114516004554

23. Hirabayashi J, Hashidate T, Arata Y, Nishi N, Nakamura T, Hiroshima M, et al. Oligosaccharide specificities of galectins: a search by frontonal affinity chromatography. Biochim Biophys Acta (2002) 1572:232–254. doi: 10.1016/S0304-4167(02)00311-2

24. Leffler H, Carlsson S, Hedlund M, Qian Y, Poier F. Introduction to galectins. Glycocon J (2002) 19:433–40. doi: 10.1021/gc010472z

25. Rapoport EM, Kurmyshkina OV, Bovin NV. Mammalian galectins: structure, carbohydrate specificity, and functions. Biochemistry (2008) 73:393–5. doi: 10.1134/S0006297008400322

26. Shams-ud-doha K, Kitova EN, Kitov PL, St-Pierre Y, Klassen JS. Human milk oligosaccharide specificities of human galectins. Comparison of electrospray ionization mass spectrometry and glycan microarray screening results. Anal Chem. (2017) 89:4914–21. doi: 10.1021/acs.analchem.6b05169

27. Prudden AR, Liu L, Capicciotti CJ, Wolfert MA, Wang S, Gao Z, et al. Synthesis of asymmetrical multitandentary human milk oligosaccharides. Proc Natl Acad Sci USA. (2017). 114:6954–59. doi: 10.1073/pnas.1701785114

28. El-Hawiet A, Chen Y, Shams-Ud-Doha K, Kitova E, Kitov P, Bode L, et al. Screening natural libraries of human milk oligosaccharides against lectins using CaR-ESI-MS. Anal Chem. (2018) 90:336–48. doi: 10.1021/acs.analchem.8b00124

29. El-Hawiet A, Chen Y, Shams-Ud-Doha K, Kitova EN, St-Pierre Y, Klassen JS. High-throughput label- and immobilization-free screening of human milk oligosaccharides against lectins. Anal Chem. (2017) 89:8712–22. doi: 10.1021/acs.analchem.7b05042

30. Kolwier-Brandl H, Siegert N, Umnu S, Kelm A, Tolkach A, Kulozuk U, et al. Lectin inhibition assay for the analysis of bioactive milk sialoconjugates. Int Dairy J. (2011) 21:413–20. doi: 10.1016/j.idairyj.2011.01.005

31. Bhunia A, Jayalakshmi V, Benie AJ, Schuster O, Kelm S, Krishnan NR, et al. Saturation transfer difference NMR and computational modeling of a sialoadhesin-sialyl lactose complex. Carbohydr Res. (2004) 339:259–67. doi: 10.1016/j.carres.2004.07.061

32. Li N, Zhang W, Wan T, Zhang J, Chen T, Yu Y, et al. Cloning and characterization of Siglec-10, a novel sialic acid binding member of the Ig superfamily, from human dendritic cells. J Biol Chem. (2001) 276:28106–12. doi: 10.1074/jbc.M100467200

33. Alphrey MS, Attrill H, Crocker PR, van Aalten DMF. High resolution crystal structures of Siglec-7. Insights into ligand specificity in the Siglec family. J Biol Chem. (2003) 278:3372–77. doi: 10.1074/jbc.M210602200

34. Noll AJ, Yu Y, Lasanajak Y, Duksa-McEwen G, Buck RH, Smith DF, et al. Human DC-SIGN binds specific human milk glycans. Biochem J. (2016) 473:1343–53. doi: 10.1042/BCJ20160046

35. Chichlowski M, German JB, Lebrilla CB, Mills D A. The influence of milk oligosaccharides on microbiota of infants: opportunities for formulas. Annu Rev Food Sci Technol. (2011) 2:331–51. doi: 10.1146/annurev-food-022510-133743

36. van Kooiy Y, Rabinovich GA. Protein-glycan interactions in the control of innate and adaptive immune responses. Nat Immunol. (2008) 9:593–601. doi: 10.1038/nri2350

37. Naarding MA, Ludwig IS, Groot F, Berkhout B, Geijtenbeek TBH, Pollakis G, Paxton WA. Lewis X component in human milk binds DC-SIGN and inhibits HIV-1 transfer to CD4 + T lymphocytes. J Clin Invest. (2005) 115:3256–64. doi: 10.1172/JCI25105

38. Perdijk O, van Neerijn RJ, Meijer B, Savelkoul HJF, Brugman S. Reduction of human tolerogenic dendritic cells by 3′-sialyllactose via TLR4 is explained by LPS contamination. Glycobiology (2018) 28:126–30. doi: 10.1093/glycob/cwx106

39. Rabinovich, GA. and Toscano, MA. Turning sweet on immunity: galectin glycans in immune tolerance and inflammation. Nat Rev Immunol. (2009) 9:338–52. doi: 10.1038/nri2536

40. Delacour D, Koch A, Ackermann W, Eude-Le Parco I, Ellasser H-P, Poirier F, et al. Loss of galectin-3 impairs membrane polarization of mouse enterocytes in vivo. J Cell Sci. (2008) 121:458–65. doi: 10.1242/jcs.020800

41. Huflejt ME, Jordan ET, Gitt MA, Barondes SH, Leffler H. Strikingly different localization of galectin-3 and galectin-4 in human colon adenocarcinoma T84 cells. Galectin-4 is localized at sites of cell adhesion. J Biol Chem. (1997) 272:14294–303. doi: 10.1074/jbc.272.22.14294

42. Wasano K, Hirakawa Y. Recombinant galectin-1 recognizes mucin and epithelial cell surface glycolaycans of gastrointestinal tract. J Histochem Cytochem. (1997) 45:275–83. doi: 10.1177/00221554970450012

43. Lahm H, Andre S, Hoechel A, Fischer JR, Sordat B, Kalter H, et al. Comprehensive galectin fingerprinting in a panel of 61 human tumor cell lines by RT-PCR and its implications for diagnostic and therapeutic procedures. J Cancer Res Clin Oncol. (2001) 127:375–86. doi: 10.1007/s004320002007

44. Lippert E, Fulk W, Bataille F, Kaehne T, Naumann M, Goekke M, et al. Soluble galectin-3 is a strong, colonic epithelial-cell-derived, lamin-a propria fibroblast-stimulating factor. Gut (2007) 56:43–51. doi: 10.1136/gut.2005.081646

45. Nio-Kobayashi J, Takahashi-Iwanaga H, Iwanaga T. Immunohistochemical localization of six galectin subtypes in the mouse digestive tract. J Histochem Cytochem. (2007) 55:255–66. doi: 10.1038/nri2056

46. Crocker PR, Paulson JC, Varki A. Siglecs and their roles in the immune system. Nat Rev Immunol. (2007) 7:255–66. doi: 10.1038/nri2056

47. Reilly MKO, Paulson JC, O’Reilly MK. SiglecS as targets for therapy in immune-cell-mediated disease. Trends Pharmacol Sci. (2009) 30:240–48. doi: 10.1016/j.tips.2009.02.005

48. Osborn L. Leukocyte adhesion to endothelium in inflammation. Cell (1999) 92:633–6. doi: 10.1016/S0092-8674(99)03250-C

49. Springer TA. Traffic signals for lymphocyte recirculation and leukocyte emigration: the multistep paradigm. Cell (1994) 76:301–14. doi: 10.1016/0092-8674(94)90337-9

50. Lasky LA. Selectin-carbohydrate interactions and the initiation of the inflammatory response. Annu Rev Biochem. (1995) 64:113–39. doi: 10.1146/annurev.bio.64.070195.000553

51. McEvor RP. Role of selectins in leukocyte adhesion to platelets and endothelium. Ann N Y Acad Sci. (1994) 714:185–89. doi: 10.1111/j.1749-6632.1994.tb10435.x

52. Schwertmann A, Rudloff S, Kunz C. Potential ligands for cell adhesion molecules in human milk. Ann Nutr Metab. (1996) 40:252–62. doi: 10.1159/000177965

53. Geijtenbeek TBH, Gringhuis SI. Signalling through C-type lectin receptors: shaping immune responses. Nat Rev Immunol. (2009) 9:465–79. doi: 10.1038/nri2569
61. Cohen-Kedar S, Baram L, Elad H, Brazowski E, Guzner-Gur H, Dotan I. Human intestinal epithelial cells respond to beta-glucans via Dectin-1 and Syk. Eur J Immunol. (2014) 44:3729–40. doi: 10.1002/eji.201444876

62. Volman M, Mensink RP, Buurman WA, Onning G, Plat J. The absence of functional Dectin-1 on enterocytes may serve to prevent intestinal damage. Eur J Gastroenterol Hepatol. (2010) 22:88–94. doi: 10.1097/MEG.0b013e3283a20dc

63. Rochereau N, Drocourt D, Perouzel E, Pavot V, Redelighsuy P, Brown EA, et al. The impact of breastfeeding on nasopharyngeal microbial communities in infants. J Nutr. (2011) 141:1694–701. doi: 10.3945/jn.111.144957

64. Morrow AL, Meinzen-Derr J, Huang P, Schibler KR, Cahill T, Keddache M, et al. Fucosyltransferase 2 non-secretor and low secretor status predicts severe outcomes in premature infants. Pediatr Res. (2014) 75:336–41. doi: 10.1203/PDR.0b013e3182a7e8c2

65. Kuhn L, Kim H-Y, Hsiao L, Nissan C, Kankasa C, Mwiya M, et al. Human milk oligosaccharides enhance innate immunity to respiratory syncytial virus and influenza in vitro. Food Nutr Sci. (2014) 5:1384–90. doi: 10.4236/fns.2014.521162

66. Morrow AL, Ruiz-Palacios GM, Altaye M, Jiang X, Lourdes Guerrero M, Newburg DS, Ruiz-Palacios GM, Altaye M, Chaturvedi P, Meinzen-Derr J, Guerrero ML, et al. FUT2-dependent breast milk oligosaccharides and allergy at 2 and 5 years of age in infants with high hereditary allergy risk. (2017) 56:1293–301. doi: 10.1007/s00394-016-1180-6

67. Bode L, Kuhn L, Kim HY, Hsiao L, Nissan C, Sinkala M, et al. Human milk oligosaccharides differ between HIV-infected and HIV-uninfected mothers and are related to necrotizing enterocolitis incidence in uninfected infants born to HIV-infected mothers in Lusaka, Zambia. J Nutr. (2015) 145:66–72. doi: 10.3945/jn.114.197799

68. Bode L, Kuhn L, Kim HY, Hsiao L, Nissan C, Sinkala M, et al. Human milk oligosaccharides differ between HIV-infected and HIV-uninfected mothers and are related to necrotizing enterocolitis incidence in their preterm very-low-birth-weight infants. J Nutr. (2014) 144:1227–33. doi: 10.1093/jn/nju011

69. van Niekerk E, Autran CA, Nel DG, Kirsten GF, Blaauw R, Bode L. Human milk oligosaccharides differ between HIV-infected and HIV-uninfected mothers and are related to necrotizing enterocolitis incidence in their preterm very-low-birth-weight infants. J Nutr. (2014) 144:1227–33. doi: 10.1093/jn/nju011

70. Bode L, Kuhn L, Kim HY, Hsiao L, Nissan C, Sinkala M, et al. Human milk oligosaccharides differ between HIV-infected and HIV-uninfected mothers and are related to necrotizing enterocolitis incidence in their preterm very-low-birth-weight infants. J Nutr. (2014) 144:1227–33. doi: 10.1093/jn/nju011

71. van Niekerk E, Autran CA, Nel DG, Kirsten GF, Blaauw R, Bode L. Human milk oligosaccharides differ between HIV-infected and HIV-uninfected mothers and are related to necrotizing enterocolitis incidence in their preterm very-low-birth-weight infants. J Nutr. (2014) 144:1227–33. doi: 10.1093/jn/nju011

72. van Niekerk E, Autran CA, Nel DG, Kirsten GF, Blaauw R, Bode L. Human milk oligosaccharides differ between HIV-infected and HIV-uninfected mothers and are related to necrotizing enterocolitis incidence in their preterm very-low-birth-weight infants. J Nutr. (2014) 144:1227–33. doi: 10.1093/jn/nju011
Prebiotic oligosaccharides: effects of 2′-fucosyllactose and 6′-sialyllactose on the adhesion of *Escherichia coli* and *Salmonella* fyuA to Caco-2 cells. *J Matern Neonatal Med.* (2018) 21:1–3. doi: 10.1080/14767058.2018.1458064

102. Lin AE, Atruran CA, Smyska A, Escudiello T, Huang M, Godula K, et al. Human milk oligosaccharides inhibit growth of group B *Streptococcus*. *J Biol Chem.* (2017) 292:11223–49. doi: 10.1074/jbc.M117.899974

103. Chen P, Reiter T, Huang B, Kong N, Weimer B. Prebiotic oligosaccharides potentiate host protective responses against *L. Monocytogenes* infection. *Pathogens* (2017) 6:E68. doi: 10.3390/pathogens6040068

104. Bondue P, Crevècoeur S, Boste F, Daube G, Seghaye MC, Griffiths MW, et al. Cell-free spent media obtained from *Bifidobacterium bifidum* and *Bifidobacterium crudulicus* grown in media supplemented with 3′-sialyllactose modulate virulence gene expression in *Escherichia coli* O157:H7 and *Salmonella Typhimurium*. *Front. Microbiol.* (2016). 7:1460. doi: 10.3389/fmicb.2016.01460

105. Yu Z-T, Nanthakumar NN, Newburg DS. The human milk oligosaccharide 2′-fucosyllactose quenches *Campylobacter jejuni*-induced inflammation in human epithelial cells HEp-2 and HT-29 and in mouse intestinal mucosa. *J Nutr.* (2016) 146:1980–90. doi: 10.3945/jn.115.214940

106. He Y, Liu S, Kling DE, Leone S, Lawlor NT, Huang Y, et al. The human milk oligosaccharide 2′-fucosyllactose modulates CD14 expression in human enterocytes, thereby attenuating LPS-induced inflammation. *Gut* (2016) 65:33–46. doi: 10.1136/gutjnl-2014-307544

107. Cilieborg MS, Sangild PT, Jensen ML, Østergaard M, Christensen LB, et al. Prebiotic human milk glycans that inhibit norovirus attachment to peritoneal Gr1(02826)+ macrophages that suppress naive CD4(02826) T cell proliferation via an IFN-gamma and nitric oxide-dependent mechanism. *J Immunol.* (2016) 201:1179–87. doi: 10.1172/jimmunol.2016.010062.x

108. Hester SN, Chen X, Li M, Huang P, Jiang X, Likhosherstov LM, et al. Functional glycomic analysis of human milk glycans reveals the presence of virus receptors and embryonic stem cell biomarkers. *J Biol Chem.* (2012) 287:44784–99. doi: 10.1074/jbc.M112.425819

109. Xiao L, Leusink-Muis T, Kettelarij N, van Ark I, Blijenberg B, Hesen NA, et al. Human milk oligosaccharide 2′-fucosyllactose improves innate and adaptive immunity in an influenza-specific murine vaccination model. *Front Immunol.* (2018) 9:452. doi: 10.3389/fimmu.2018.00452

110. Jantscher-Krenn E, Zherebtsov M, Nissan C, Goth K, Guner YS, Naidu N, et al. The human milk oligosaccharide disialylacto-N-tetrose prevents necrotising enterocolitis in neonatal rats. *Gut* (2012) 61:1417–25.

111. Atruran CA, Kellman BP, Kim JH, Azaltos E, Blood AB, Spence ECH, et al. Human milk oligosaccharide composition predicts risk of necrotising enterocolitis in preterm infants. *Gut* (2018) 67:1064–70. doi: 10.1136/gutjnl-2016-312819

112. Kwon SJ, Na DH, Kwak JH, Douaisi M, Zhang F, Park EJ, et al. Identification of oligosaccharides in human milk bound onto the toxa carbohydrate binding site of clostridium difficile. *J Microbiol Biotechnol* (2018) 126:8:1–11. doi: 10.1007/s42246-018-0025-5

113. El-Hawiet A, Kitova EN, Klassen JS. Recognition of human milk glycans by the human epithelial cell line HT-29 and binding assays to lectins and swine influenza H1N1 virus. *Daitsiotis M. Sialic acid and sialyl-lactose glyco-conjugates: design, synthesis and binding assays to lectins and swine influenza H1N1 virus. *J Pept Sci.* (2012) 18:52–58. doi: 10.1002/psc.1415

114. Yu Z-T, Nanthakumar NN, Newburg DS. The human milk oligosaccharide 2′-fucosyllactose attenuates the severity of experimental necrotising enterocolitis by enhancing mesenteric perfusion in the neonatal intestine. *Br J Nutr.* (2016) 116:1175–87. doi: 10.1017/S0007114516002944

115. Cilieborg MS, Bering SB, Østergaard MV, Jensen ML, Krych L, Newburg DS, et al. Minimal short-term effect of dietary 2′-fucosyllactose on bacterial colonisation, intestinal function and necrotising enterocolitis in preterm pigs. *Br J Nutr.* (2016) 116:834–41. doi: 10.1017/S0007114516002646

116. He Y, Liu S, Leone S, Newburg DS. Human colostrum oligosaccharides modulate major immunologic pathways of immature human intestine. *Mucosa.* (2014) 17:1326–39. doi: 10.1002/muc.204104

117. Kunz S, Rudloff S, Kunz C. Oligosaccharides from human milk influence growth-related characteristics of intestinally transformed and non-transformed intestinal cells. *Br J Nutr.* (2008) 99:462–71. doi: 10.1017/S0007114507007820860

118. Ortega-González M, Ocon B, Romero-Calvo I, Anzola A, Guadix E, Zarruzo A, et al. Nondigestible oligosaccharides exert nonprobiotic effects on intestinal epithelial cells enhancing the immune response via activation ofTLR4-NFκB. *Mol Nutr Food Res.* (2014) 58:384–93. doi: 10.1002/mnr.20030296

119. Zenhom, M. Hyder A, de Vrese M, Heller KJ, Roeder T, Schrezenmeir J. Prebiotic oligosaccharides reduce proinflammatory cytokines in intestinal caco-2 cells via activation of PPARα and pep2tidoglycan recognition protein. *J. Nutr.* (2011) 141:971–7. doi: 10.3945/jn.110.136176

120. Lane JA, O’Callaghan J, Carrington SD, Hickey RM. Transcriptional response of HT-29 intestinal epithelial cells to human and bovine milk oligosaccharides: effects on intestinal epithelial cells enhancing the immune response *in vitro* evidence for gastrointestinal epithelial transfer and immunomodulatory properties. *Pediatr Allergy Immunol.* (2010) 21:1179–88. doi: 10.1111/j.1399-3038.2010.01062.x

121. Atochina O, Harn D. LNFPIII/LeX-stimulated macrophages activate natural killer cells from schistosome-infected mice: a mechanism for prebiotic oligosaccharides: *in vivo* evidence for gastrointestinal epithelial transfer and immunomodulatory properties. *Pediatr Allergy Immunol.* (2011) 22:1250–302. doi: 10.1111/j.1399-3038.2010.01062.x

122. Atochina O, Daly-Engel T, Piskorska D, McGuire E, Harn DA. A chitososome-impresed immunomodulatory glycoconjugate expands peritoneal Gr1(+)-macrophages that suppress naive CD4(+)- T cell proliferation via an IFN-gamma and nitric oxide-dependent mechanism. *J Immunol.* (2001) 167:4293–302. doi: 10.4099/jimmunol.167.8.4293

123. Atochina O, Harn D. LNFPIII/LeX-stimulated macrophages activate natural killer cells via CD40-CD40L interaction. *Clin Diag Lab Immunol.* (2005) 12:1041–49. doi: 10.1128/CDLI.12.1041-1049.2005

124. Velupillai P, Harn DA. Oligosaccharide-specific induction of interleukin 10 production by B20+ cells from schistosome-infected mice: a mechanism for regulation of CD4(+) T-cell subsets. *Proc Natl Acad Sci USA.* (1994) 91:18–22. doi: 10.1073/pnas.91.1.18

125. Yazdanbakhsh M, Kremsner PG, Van Ree R. Immunity: allergy, parasitology, and the hygiene hypothesis. *Science* (2002) 296:490–4. DOI: 10.1126/science.296.5567.490

126. Harn DA., McDonald J, Atochina O, Drdara A. Modulation of host immune responses by helminth glycans. *Immunol Rev.* (2009) 230:247–57. doi: 10.1111/j.1600-065X.2009.00799.x
133. Velupillai P, dos Reis EA, dos Reis MG, Harn DA. Lewis(x)-containing oligosaccharides attenuates schistosome egg antigen-induced immune depression in human schistosomiasis. *Hum Immunol.* (2000) 61:225–32. doi: 10.1016/S0198-8839(99)00136-6

134. Van der Kleij D, Van Remoortere A, Schuitemaker JHN, Kapsenberg ML, Deelder AM, Tiudens AGM, et al. Triggering of innate immune responses by schistosome egg glycolipids and their carbohydrate epitope GalNAc beta 1-4(Fuc alpha 1-2Fuc alpha 1-3)GlcNAc. *J Infect Dis.* (2002) 185:531–9. doi: 10.1086/338574

135. Comstock SS, Wang M, Hester SN, Li M, Donovan SM. Select human milk oligosaccharides directly modulate peripheral blood mononuclear cells isolated from 10-d-old pigs. *Br J Nutr.* (2014) 111:819–28. doi: 10.1017/S0007114513003267

136. Bhargava P, Li C, Stanyk JJ, Jacobs D, Dai L, Liu S, et al. Immunomodulatory glycan LNFPII alleviates hepatosteatosis and insulin resistance through direct and indirect control of metabolic pathways. *Nat Med.* (2012) 18:1665–72. doi: 10.1038/nm.2962

137. Zhu B, Trikudanathan S, Zouyula A, Sandoval-Garcia C, Kennedy J, Atouchina O, et al. Immune modulation by Lacto-N-fucopentaose III in experimental autoimmune encephalomyelitis. *Clin Immunol.* (2012) 142:351–61. doi: 10.1016/j.clim.2011.12.006

138. Dutta P, Hullet DA, Roenneburg DA, Torrealba JR, Sollinger DH, et al. Bifidobacteria can protect from enteropathogenic infection through direct and indirect control of metabolic pathways. *Nat Med.* (2012) 18:1665–72. doi: 10.1038/nm.2962

139. Xiao L, Land BV, Engen PA, Nasib A, Green SJ, Nato A et al. Human milk oligosaccharides protect against the development of autoimmune diabetes in NOD-mice. *Sci Rep.* (2018) 8:3829. doi: 10.1038/s41598-018-22052-y

140. Marino E, Richards JL, McLeod KH, Stanley D, Yap YA, Knight J, et al. Gut microbiota composition and development of atopic inflammation: a review of the evidence. *Clin Exp Allergy.* (2013) 43:948–55. doi: 10.1111/j.1365-2222.2013.03925.x

141. Van Oudijk IV, Kull I, Borres MP, Brandtzaeg P, Edberg I, Kuitunen M, et al. Breastfeeding and allergic disease: a multidisciplinary review of the literature (1966-2001) on the mode of early feeding in infancy and its impact on later atopic manifestations. *Allergy* (2003) 58:833–43. doi: 10.1034/j.1398-9995.2003.00264.x

142. Lodge C, Tan D, Lau M, Dai X, Tham R, Lowe A, et al. Breastfeeding and asthma and allergies: a systematic review and meta-analysis. *Acta Paediatr.* (2015) 104:53–58. doi: 10.1111/apa.13132

143. Munblit D, Boyle RJ, Warner JO. Factors affecting breast milk composition and potential consequences for development of the allergic phenotype. *Clin Exp Allergy* (2014) 44:535–60. doi: 10.1111/cex.12381

144. Castillo-Courtade I, Han S, Lee S, Mian FM, Buck R, Forsythe P. Attenuation of food allergy symptoms following treatment with human milk oligosaccharides in a mouse model. *Allergy* (2015) 70:1091–102. doi: 10.1111/all.12659

145. Eigenmann PA. Evidence of preventive effect of probiotics and prebiotics for infantile eczema. *Curr Opin Allergy Clin Immunol.* (2013) 13:426–31. doi: 10.1097/ACI.0b013e3283630bad

146. Bruzzese E, Volpicielli M, Squaglia V, Bruzzese D, Salvini F, Bisceglia M, et al. A formula containing galacto- and fructo-oligosaccharides prevents intestinal and extra-intestinal infections: an observational study. *Clin Nutr.* (2009) 28:156–61. doi: 10.1016/j.clnu.2009.01.008

147. Bruzzese E, Volpicielli M, Squaglia M, Tartaglione A, Guarino A. Impact of probiotics on human health. *Dig Liver Dis.* (2006) 38:S283–87. doi: 10.1016/j.dld.2006.01.001

148. Arslanoglu S, Moro GE, Boehm G. Early supplementation of prebiotic oligosaccharides protects formula-fed infants against infections during the first 6 months of life. *J Nutr.* (2007) 137:2420–4. doi: 10.1093/jn/137.11.2420

149. Osborn DA, Sinn JKH. Prebiotics in infants for prevention of allergy. *Cochrane database Syst Rev.* (2013) 1:CD006474. doi: 10.1002/14651858.CD006474.pub3

150. Lomax AR, Calder PC. Prebiotics, immune function, infection and inflammation: a review of the evidence. *Br J Nutr.* (2009) 101:633–58. doi: 10.1017/S00071145080055608

**Conflict of Interest Statement:** VT and RVN are employees of FrieslandCampina.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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