CellPress

Trends in Immunology

Review

Human milk oligosaccharides: potential therapeutic aids for allergic diseases

Isabel Tarrant[1,2] and B. Brett Finlay[1,2,3,*]

Childhood allergy, including asthma, eczema, and food allergies, is a major global health burden, with prevalence increasing dramatically and novel interventions needed. Emerging research suggests that human milk oligosaccharides (HMOs), complex glycans found in breastmilk, have allergy-protective properties, indicating exciting therapeutic potential. This review evaluates current literature on the role of HMOs in allergy, assesses underlying immunological mechanisms, and discusses future research needed to translate findings into clinical implications. HMOs may mediate allergy risk through multiple structure-specific mechanisms, including microbiome modification, intestinal barrier maturation, immunomodulation, and gene regulation. Findings emphasize the importance of breastfeeding encouragement and HMO-supplemented formula milk for high allergy-risk infants. Although further investigation is necessary to determine the most efficacious structures against varying allergy phenotypes and their long-term efficacy, HMOs may represent a promising complementary tool for childhood allergy prevention.

Addressing childhood allergy with HMOs

HMOs, glycans found in breastmilk, have powerful immunomodulatory properties and represent an exciting means to aid in the prevention of childhood allergy. Atopy, an exaggerated IgE immune response to an external antigen, affects one in five individuals worldwide [1]. The prevalence of childhood allergy, including asthma, atopic dermatitis, and food allergy, has drastically increased in recent years, with 20% of children worldwide reported to present with atopic dermatitis [2] and 8–10% with asthma [3], significantly impacting quality of life. Pediatric food allergy alone costs the USA $25 billion annually [4], representing a major economic and health burden. Unfortunately, preventive strategies are lacking, with most of the emphasis on treating symptoms. While acknowledging the multifactorial nature of allergic diseases and that there is no singular intervention to effectively tackle them universally, determining the efficacy of novel allergy-preventive measures is vital to help reduce allergy prevalence worldwide.

Early-life is a crucial phase in allergy development as the infant immune system and intestinal microbiota undergo marked development, with the first 1000 days of life thought to be a critical window in which external factors, including nutrition, are most influential on long-term immune programming [5,6]. Accordingly, emerging research suggests that HMOs found in breastmilk may have a potential therapeutic role against infant allergy [7,8]. HMOs are complex glycans comprising glucose, galactose, fucose, sialic acid, and *N*-acetylglucosamine, collectively making up the third most abundant component of breastmilk [9]. Over 200 structurally distinct HMOs have been identified to date [10], broadly categorized into three structural groups (Table 1). Notably, the HMO profile of breastmilk varies significantly between mothers, reflecting numerous maternal factors, including **secretor status** (see Glossary), genetics, geographical location, diet, and body mass index [11,12]. In particular, maternal secretor status, **Lewis blood type**, as well as whether blood group antigens are secreted into bodily fluids, are determined by two

Highlights

Recent findings from infant cohort, animal, and *in vitro* studies suggest that human milk oligosaccharides (HMOs) found in breastmilk exhibit allergy-protective effects.

Maternal HMO composition varies widely; thus, the allergy-protective efficacy of HMOs depends on complex interactions between multiple factors, including maternal secretor status, infant risk status, geographical location, and allergy phenotype.

Individual HMOs have structure-specific immunological actions in the prevention of various allergies.

Aside from the prebiotic effects on the intestinal microbiome, HMOs influence early-life immune programming, including mediating antigen-presenting cell activation and T helper cell balance, contributing to possible allergy-protective benefits.

The presumed allergy-protective properties of HMOs likely reflect multiple direct and indirect mechanisms, including modifications of intestinal microbiomes, epithelial barrier maturation, immunomodulation, and regulation of inflammation-associated genes.

Significance

Emerging research suggests that human milk oligosaccharides (HMOs) found in breastmilk can reduce allergy susceptibility in early life through modification of the intestinal microbiome, strengthening intestinal barrier integrity, and immunomodulation. This has significant implications in the development of HMO-based and microbiota-targeted interventions as putative novel treatments for childhood allergy prevention.

Table 1. Structural classification of HMOs [9][a]

HMO structure	Examples	Proportion of known HMOs
Neutral non-fucosylated (N-containing)	LNT, LNnT	40–55%
Neutral fucosylated	2′FL, 3′FL	35–50%
Acidic sialylated	3′SL, 6′SL	10–14%

[a]Abbreviations: 2′FL, 2′-fucosyllactose; 3′FL, 3′-fucosyllactose; LNnT, lacto-*N*-neotetraose; LNT, lacto-*N*-tetraose; 3′SL, 3′-sialyllactose; 6′SL, 6′-sialyllactose.

gene loci: the secretor gene encoding α1-2-fucosyltransferase (FUT2) and the Lewis gene encoding α1-3/4-fucosyltransferase (FUT3), which largely influence HMO composition [13] (Table 2). Moreover, both the concentration and composition of HMOs fluctuate throughout lactation, with an average HMO concentration in **colostrum** of 20–25 g/l, decreasing to 10–13 g/l in mature milk [14,15]. Such diversity in HMO composition is noteworthy as certain HMO profiles correlate with reduced allergy risk [8,16,17].

Accordingly, an increasing body of research, including observational studies [7], animal models [8], and *in vitro* data [18], suggests that HMOs may provide allergy-protective effects. However, many current findings are varied, unorganized, and lack robustness, making it difficult to draw conclusions on the roles of HMOs in allergic conditions. Given the increasing global allergy prevalence [19,20], the need for effective interventions is considerable. Thus, harnessing HMOs as putative and complementary therapeutic tools has valuable clinical significance as well as industry implications for the development of specialized formula milk, with anticipated economic benefits in reducing the financial burden of allergy worldwide. Therefore, this review examines the current literature, evaluates possible underlying mechanisms of HMOs in allergic diseases, assesses limitations in the field, and discusses future research directions to ultimately enable HMOs as potential therapeutics for reducing childhood allergy prevalence.

Epidemiological data

HMO profiles correlate with infant allergy

To date, several epidemiological studies have reported associations between HMO profile (both composition and concentration) and offspring allergy risk. For instance, in a cohort of 70 infants aged 0–6 months, high concentrations of lacto-*N*-fucopentaose III (LNFP-III), compared with low, correlated with reduced risk of cow's milk allergy (CMA) at 6 months (manifested as vomiting, diarrhea, wheezing, cough, urticaria, or atopic dermatitis following milk consumption). This suggested that Lewis-dependent HMOs might play a protective role against CMA [21]. In contrast, in 421 mother-child dyads from the Canadian Healthy Infant Longitudinal Development study (https://childstudy.ca), breastmilk concentrations of fucosyl-disialyllacto-*N*-hexose (FDSLNH), lacto-*N*-fucopentaose I (LNFP-I), lacto-*N*-neotetraose (LNnT), lacto-*N*-fucopentaose II (LNFP-II),

Table 2. HMO profiles based on maternal secretor and Lewis status, and average percentage of women expressing each genotype worldwide [14][a]

Gene	Lewis positive (le+) FUT3 functional	Lewis negative (le–) FUT3 inactive
Secretor positive (se+) FUT2 functional	All HMOs (70% of population)	2′FL, LNFP-I, 3′FL, LNFP-II, LNFP-III (9% of population)
Secretor negative (se–) FUT2 inactive	3′FL, LNFP-II, LNFP-III (20% of population)	3′FL, LNFP-III, LNFP-V (1% of population)

[a]Abbreviations: 2′FL, 2′-fucosyllactose; 3′FL, 3′-fucosyllactose; FUT2, α1-2-fucosyltransferase; FUT3, α1-3/4-fucosyltransferase; LNFP-I, lacto-*N*-fucopentaose I; LNFP-II, lacto-*N*-fucopentaose II; LNFP-III, lacto-*N*-fucopentaose III; LNFP-V, lacto-*N*-fucopentaose V.

[1]Department of Microbiology and Immunology, University of British Columbia, Vancouver, BC, Canada
[2]Michael Smith Laboratories, University of British Columbia, Vancouver, BC, Canada
[3]Department of Biochemistry, University of British Columbia, Vancouver, BC, Canada

*Correspondence:
bfinlay@msl.ubc.ca (B.B. Finlay).

sialyllacto-*N*-tetraose c (LSTc), and fucosyllacto-*N*-hexaose (FLNH) negatively correlated with risk of **food sensitization** at 1 year; by contrast, higher concentrations of lacto-*N*-hexaose (LNH), lacto-*N*-tetraose (LNT), 2′-fucosyllactose (2′FL), and disialyl-lacto-*N*-hexaose (DSLNH) were associated with an increased risk of food sensitization [8]. However, opposing the aforementioned study, no individual HMOs alone correlated with food sensitization. This suggests that the overall combination of HMOs rather than individual structures may be important for protection against food allergy, reflecting the existence of over 200 HMOs in breastmilk. Thus, supplementing infant formula with a synergistic mixture of HMOs may prove to be more effective against allergy than single HMOs alone. However, future studies are needed to fully validate this conclusion.

Noteworthy, a recent study of high-risk infants followed to age 18 years demonstrated that acidic Lewis-dependent HMOs were associated with higher eczema, wheeze, and asthma risk throughout childhood [16]. However, risk of food sensitization negatively correlated with acidic HMOs, suggesting that the protective effects of various HMO groups may differ depending on atopic disease. Additionally, one group [21] reported that infants (aged 0–6 months) with **delayed-onset CMA** had mothers who were secretors; thus, infants consumed FUT2-dependent HMOs, whereas mothers of infants with immediate IgE-mediated CMA were all nonsecretors. These findings suggest that maternal secretor status may influence the endotype of allergy developed, emphasizing the complex relationship between HMO composition and allergy. Hence, future research should identify distinct HMOs that reduce susceptibility to varying allergy endotypes, rather than assuming they provide protection against all atopic diseases simultaneously.

Moreover, the protective efficacy of HMOs may depend on infant risk status. One group reported that infants born via cesarean section had higher allergy susceptibility than those vaginally delivered; yet, when the former were exposed to FUT2-dependent HMOs, their risk of developing IgE-mediated eczema at 2 years, but not 5 years, was reduced [17]. Notably, this association was only present for cesarean-delivered infants (who suffer microbiome perturbations [22]), suggesting the allergy-protective effects of HMOs may only apply to high-risk infants and depend on complex interactions between several factors. Despite this, further research is necessary to validate these findings in a larger cohort, particularly as HMOs' protective associations were only found in a limited subset of infants.

In contrast, other studies have not found such promising associations between HMO consumption and allergy. In an observational study of Swedish infants, breastmilk HMO composition showed no association with infant allergy risk up to 18 months of age [23], questioning the HMO allergy-protective potential. However, this study was limited by sample size (n = 20) and only nine neutral HMOs were assessed, reducing the validity of these findings.

Taken together, epidemiological studies (Table 3) suggest that HMOs play a protective role against early-life allergy, yet this depends on HMO structure, infant risk status, maternal secretor status, and allergy type. Despite these promising findings, which are only correlational, many factors are at play that impact the complex relationship between HMOs and allergy.

Mechanisms of HMOs in allergy

Intestinal mechanisms

Microbiome modification

One major mechanism by which HMOs may influence infant allergy is by shaping early-life microbiome colonization. It is well established that HMOs have prebiotic effects in promoting the growth of beneficial intestinal strains and acting as a key substrate for bacterial fermentation

Glossary

β-Hexosaminidase: a lysosomal enzyme released from mast cells during an allergic response, commonly used as a marker for mast cell degranulation.
Colostrum: first type of milk produced by mother's mammary glands during late pregnancy and immediately after birth; it is particularly rich in antibodies, growth factors, lactoferrin, and protein.
Delayed-onset cow's milk allergy (CMA): cow's milk protein allergy subtype, defined as the onset of allergy symptoms 48 hours or later than consuming cow's milk.
Food sensitization: production of allergen-specific IgE antibodies in response to initial exposure to a food antigen, which is remembered for future antigen exposure, predisposing the individual to allergy development.
Forkhead box protein P3 (FoxP3): transcription factor that acts as a key regulator of Treg gene expression, plays a vital role in Treg development, and has immunosuppressive properties.
G protein-coupled receptors 41 and 43 (GPR41/GPR43): mammalian receptors expressed on human colonic epithelial cells, adipocytes, and peripheral blood mononuclear cells; regulate a range of cellular functions; activated by short-chain fatty acids to trigger anti-inflammatory responses.
Gap junction alpha 3: acts as a major structural protein component of lens fiber gap junctions.
High-performance liquid chromatography: analytical technique used to identify and quantify compounds in liquid samples through a chromatographic column. Unlike LC-MS, this technique does not involve mass spectrometry.
ILC2 cells: type 2 innate lymphoid cells are a subset of innate lymphocytes that produce IL-4, IL-5, and IL-15 cytokines and are involved in type 2 inflammation, including in allergic responses.
Lewis blood type: classification of human blood types depending on varying expression of Lewis antigens (Le^a and Le^b) on the surface of red blood cells. Lewis blood group is determined by the fucosyltransferase-3 (*FUT3*) and fucosyltransferase-2 (*FUT2*) genes.
Liquid chromatography-mass spectrometry (LC-MS): analytical technique to identify and quantify compounds in liquid samples by physically separating target compounds and measuring their mass-to-charge ratio.

[24]. As only 1–5% of HMOs are absorbed [25], the large majority go undigested in the colon where they are fermented by intestinal bacteria, predominately *Bifidobacterium* species, which degrade HMOs into several beneficial metabolites, including short-chain fatty acids (SCFAs) and lactate [26]. Accordingly, a randomized controlled trial (RCT) of healthy infants aged 0–3 months (n = 176), demonstrated that HMO-supplemented formula promoted beneficial shifts in infant microbiome composition towards that of breastfed infants, compared with formula milk that was devoid of HMOs (primary outcome: child growth) (NCT01715246)[i] [27].

The infant microbiome plays a prominent role in early-life immune programming and subsequent allergy development [28]. Perturbations to the microbiome through antibiotic exposure [29], caesarean section [30], and formula feeding [31] are associated with increased allergy susceptibility. Notably, early-life microbiome colonization can influence T helper cell balance [32], a key component of allergic immune responses (Figure 1, Key figure). Type 2 allergies are characterized by an exaggerated T helper 2 (Th2) cell response to an antigen, triggering a cascade of proinflammatory cytokines, IgE production, and activation of mast cells and eosinophils [33,34]. However, the **Th1/Th2 paradigm** of allergy is somewhat basic and outdated. Although Th2 cells are a key component of the allergic response, it is now clear that complex interactions exist between multiple heterogeneous T cell subsets, including Th1, Th17, Th22, Tfh, Th9, and regulatory T cells (Tregs) [35,36]. As such, allergies can be categorized into multiple endotypes involving different T cell responses [37]. Tregs, in particular, play a pivotal immunoregulatory role in allergy by suppressing excessive Th2 responses and promoting immune tolerance against antigens [38]. During the development of adaptive immunity in early-life, dysregulation of T cell balance by multiple genetic and environmental factors can result in Th2/Th17 polarization and reduced Treg activity [39–41], increasing allergy susceptibility. The early-life microbiome is one such factor that influences T cell balance during immune development [32]. For instance, oral and intragastric administration of *Clostridium*, *Bifidobacterium*, and *Lactobacillus* spp. reduces Th2 skewing and stimulates intestinal Treg production in mice, inducing an anti-inflammatory response [42–44]. In support, in murine models of asthma [house dust mite (HDM) and ovalbumin (OVA)-induced] [45,46], or food allergy (β-lactoglobulin- and peanut allergen-induced) [47,48], *Lactobacillus* sp. supplementation reduces airway inflammation [measured by airway hyper-responsivity to methacholine and quantity of eosinophils, lymphocytes, monocytes, IL-5, IL-13, and IL-17 in bronchoalveolar (BAL) fluid]. Consequently, HMOs may indirectly influence allergy susceptibility by modifying the intestinal microbiome towards a more 'protective' microbial composition, including promoting the colonization of bacteria that stimulate anti-inflammatory Tregs and discourage Th2 cell skewing, a hallmark of allergy. Accordingly, a metagenomic study of infants followed from birth to 3 years demonstrated that reduced intestinal abundance of *Bifidobacterium* species, and depletion of HMO-utilization genes such as blon_2361 and blon_2331, was associated with intestinal inflammation and immune dysregulation driven by Th2 and Th17 (as assessed by plasma and fecal cytokine concentrations) [49]. Moreover, supplementation of *Bifidobacterium infantis*, a strain possessing all HMO-utilization genes, reduced Th2 and Th17 fecal concentrations and increased IFNβ, implying decreased intestinal inflammation [49]. When assessed *in vitro*, fecal water from *B. infantis*-supplemented infants polarized naive T cells towards a Th1 response, compared with fecal water from non-*B. infantis*-colonized infants, which induced Th2 and Th17 skewing. Taken together, the findings suggest a functional mechanism in which particular *Bifidobacterium* species possess HMO-utilization genes, enabling effective glycosidic degradation and regulating early-life inflammatory responses, particularly a Th1/Th2/Th17 balance: a key component of allergy susceptibility [35].

Additionally, emerging research suggests that the prebiotic effects of HMOs on the intestinal microbiome may elicit anti-inflammatory responses via the regulation of inflammation-

Mast cell mucosal protease-1 (mMCP-1): protease predominately expressed by mucosal mast cells and released during an allergic response, increasing mucosal permeability. Often used as a marker of mast cell degranulation in allergy.
Mechanistic target of rapamycin-6K (mTOR-S6K) pathway: central regulator of cell metabolism, involved in immune cell differentiation, cytokine production, and neutrophil recruitment.
Mucin 2 (MUC2): expressed by goblet cells, the major gel-forming mucin that makes up the intestinal mucosa.
p38 mitogen-activated protein kinase (p38 MAPK): plays an essential role in regulating cellular processes, particularly inflammation; involved in the production of proinflammatory cytokines, including TNF-α, IL-β, and IL-6.
Secretor status: the ability/inability for an individuals' ABO blood group antigens to be secreted into their bodily fluids, including saliva, breastmilk, and tears. Secretor status is determined by the fucosyltransferase-2 (*FUT2*) gene.
Th1/Th2 paradigm: a somewhat reductionist longstanding view that diseases are either cell-mediated, controlled by Th1 cells (and their cytokines), or humorally-mediated by Th2 cells. The Th1/Th2 paradigm posits that the imbalance between Th1 and Th2 cells determines immune responses towards autoimmunity or immunopathology, with overactive Th2 responses responsible for allergic disease and Th1 overactivity responsible for autoimmune disease.
Toll-like receptor 4 (TLR4): pattern recognition receptor recognizing pathogenic microbes, commonly LPS on Gram-negative bacteria; triggers innate immune signaling to release cytokines and immune mediators to defend against infection.
Trefoil factor 3 (*TFF3*): secreted in intestinal goblet cells, lung epithelial cells, and cervical mucosa; involved in mucosal maintenance and repair.

Table 3. Summary of studies herein relating to the potential allergy-protective and immunomodulatory effects of HMOs[a]

Model	HMO treatment	Outcome	Refs
Murine model			
Murine model of necrotizing enterocolitis Caco-2 and LS174T intestinal epithelial cells	HMOs isolated from pooled breastmilk	HMO treatment upregulated MUC2 and TFF3 expression in Caco-2 cells, suggesting increased mucus production. HMO supplementation reduced intestinal permeability and increased MUC2 in a murine model of necrotizing enterocolitis.	[89]
OVA-induced murine model of food allergy. Bone marrow-derived mast cells (BMMCs) from sensitized mice	2′FL, 6′SL	Both 2′FL and 6′SL reduced food allergy symptoms (diarrhea and hypothermia), increased Treg numbers in Peyer's patches, and suppressed intestinal MMCP-1 and mast cell numbers in the murine model. *In vitro*, 6′SL inhibited degranulation of sensitized murine BMMCs.	[8]
β-Lactoglobulin-induced murine model of cow's milk allergy (CMA). β-Lactoglobulin-sensitized murine macrophage RAW 264.7 cells	2′FL and HMOs isolated from pooled breastmilk	Both 2′FL and pooled HMO treatment reduced β-lactoglobulin-IgE, mast cell degranulation, TNF-α, IL-4, and IL-6; increased miR-146a expression *in vivo*. *In vitro*, 2′FL and pooled HMOs decreased ROS, nitric oxide release, and proinflammatory cytokines from sensitized RAW 264.7 murine macrophage cells. 2′FL and pooled HMO inhibited activation of the TLR4/NFκB inflammatory pathway and upregulated miR-146a expression in RAW 264.7 cells.	[111]
In vitro			
Caco-2 intestinal epithelial cells	HMOs isolated from pooled breastmilk	*Bifidobacterium breve* and *Bifidobacterium infantis* grown on HMO-supplemented media significantly downregulated chemokine-associated genes in Caco-2 cells, including *CXCL1*, *CXCL2*, and *CXCL3*, compared with *Bifidobacterium* grown on lactose and glucose.	[50]
Caco-2 intestinal epithelial cells	Pooled galacto-oligosaccharides (GOS) from lactose	GOS treatment strengthened tight junctions in deoxynivalenol-damaged Caco-2 cells and prevented loss of epithelial barrier function, measured by transepithelial electrical resistance (TEER) and markers of intestinal permeability.	[90]
Caco-2 intestinal epithelial cells	3′-Galactosyllactose (3′GL)	3′GL with a β1–3 glycosidic linkage protected Caco-2 cells from deoxynivalenol-induced damage, whereas 3′GL with an α1–3 glycosidic linkage did not, as measured by TEER.	[91]
Human monocyte-derived dendritic cells CD4+ naive T cells	HMOs isolated from pooled breastmilk	HMO treatment increased production of IL-10 and IL-27, but not TNF-α or IL-6 from dendritic cells. During co-culture, HMO-treated DCs stimulated the production of Tregs and IL-10 from naive T cells, while decreasing Th1 and IFN-γ.	[96]
Electrospray ionization mass spectrometry (ESI-MS)	32 pure HMOs	Using ESI-MS, 25 of 32 HMOs bound to all four human galectin proteins assessed: hGal-1, hGal-3, hGal-7, hGal-9.	[97]
Shotgun glycan microarray (SGM) analysis	Pre-existing database of 247 HMOs in a SGM library	DC-SIGN lectin (found on dendritic cells) showed robust binding to most HMOs, including 2′FL and 3′FL, as measured by flow cytometry. Siglec-5 and Siglec-9 lectins showed weak, but present, binding to sialylated HMO structures.	[98]
Cord blood mononuclear cells (CBMCs) from healthy neonates	Pooled HMOs grouped into either acidic or neutral HMOs	Acidic HMO treatment increased production of IFN- γ and IL-10 and decreased IL-13 from human CBMCs	[101]
T-84 and HT-29 intestinal epithelial cells	2′FL and 6′SL	2′FL and 6′SL treatment inhibited the release of CCL20 and IL-8 chemokines from T-84 and HT-29 intestinal epithelial cells stimulated with an antigen–antibody complex (model of food allergy). 2′FL suppressed chemokine release through NFκB inhibition; 6′SL acted through inhibition of AP-1 signaling.	[18]
HT-29 colonic epithelial cells	Pooled HMOs, bovine colostrum oligosaccharides (BCOs) and 3′GL	HMOs and BCOs influenced the expression of several immune-associated glycogenes, including cytokine, chemokine, and cell surface receptor genes such as those encoding CXCL3, CXCL1, CXCL2, CXCL6, IL-17C, GM-CSF2, IL-8, IL-1β. HMOs upregulated IL-17c and IL-1β expression.	[116]
Human observational studies			
70 Mother–child dyads Infants aged 0–6 months	HMOs present in human breastmilk	Low breastmilk concentrations of LNFP-II were associated with increased risk of CMA at 6 months, compared with higher LNFP-II concentrations. The mothers of infants who developed delayed-onset CMA were all secretors (FUT2-dependent), whereas those who developed IgE-mediated CMA had nonsecretor mothers.	[21]
421 Mother–child dyads from CHILD Cohort study. Assessed at 1 year	HMOs present in human breastmilk	High concentration of FDSLNH, LNFP-I, LNnT, LNFP-II, LSTc, and FLNH was associated with a decreased risk of food sensitization at 1 year of age, whereas high concentrations of LNH, LNT, 2′FL, and DSLNH were associated with an increased risk.	[7]

Table 3. (continued)

Model	HMO treatment	Outcome	Refs
285 Mother–child dyads of high-risk infants. Followed till 18 years old	HMOs present in human breastmilk	Neutral and acidic Lewis HMOs were associated with increased risk of allergy and asthma throughout childhood, whereas acidic HMOs were associated with decreased risk of food sensitization.	[16]
226 Mother–child dyads of high-risk infants. Assessed at 2 and 5 years old	HMOs present in human breastmilk	FUT2-dependent HMOs were associated with increased risk of allergy in infants born via caesarean section. Infants born via caesarean-section had a higher allergy risk, yet FUT2-dependent HMOs reduced eczema risk at 2 years, but not 5 years.	[17]
20 Mother–child dyads, from 6 to 18 months old	HMOs present in human breastmilk	No association found between breastmilk concentration of nine neutral HMOs and allergy risk up to 18 months old.	[23]
33 Infants Breastfed *n* = 17 Formula-fed *n* = 16	HMOs present in human breastmilk	HMOs in plasma and urine of breastfed infants correlated with concentrations in milk, indicating HMO absorption. Plasma and urine HMO concentrations were low: 0.1% milk in plasma and 4% milk in urine.	[25]
56 Mother–child dyads. Followed from day 6 to 6 months of age	HMOs present in human breastmilk	HMO composition correlated with infant fecal microbiome: concentration of fucosylated HMOs correlated with fecal *Bifidobacterium* abundance and *N*-glycans correlated with *Lactobacillus* abundance. Infants with secretor mothers showed increased intestinal expression of genes encoding glycosyl transferases and ATP-binding cassette transporters, compared with those with nonsecretor mothers, indicating functional differences in microbial genomes based on secretor status.	[61]
10 Mother–child dyads Followed from day 2 to 4 months of age	HMOs present in human breastmilk	Infant oral and fecal microbiome correlated with HMO composition of breastmilk. Higher intake of LNT, LNnT, LNFP-I, LNFP-II, LNFP-III, LSTb, DFLNT, FLNH, LNH, DFLNH positively associated with *Enterococcus* sp., *Parabacteroides* sp., and *Bifidobacterium* sp. abundance. Both breastmilk HMO composition and infant microbiome, and their correlations, varied over time.	[62]
93 Mother–child dyads Followed from 0 to 2 years	HMOs present in human breastmilk	Higher LNF-I and 2′FL to LNF-II and 3′FL ratio was associated with decreased risk of diarrhea in infants.	[103]

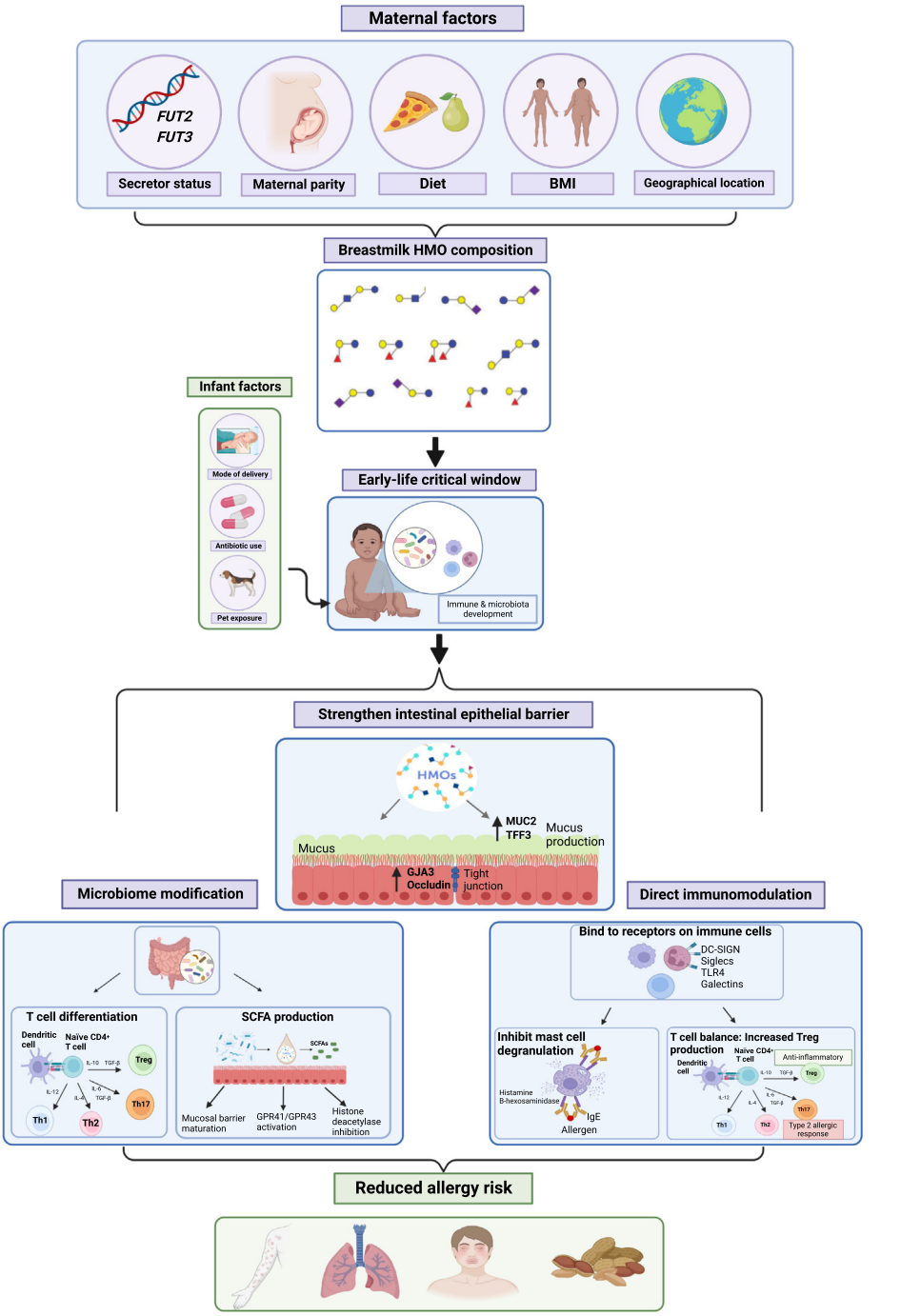
[a]Abbreviations: CXCL1, CXCL2, and CXCL3, C-X-C motif chemokine ligands 1, 2, and 3; DC, dendritic cells; DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; DFLNH, difucosyllacto-*N*-hexaose; DFLNT, difucosyllacto-*N*-tetrose; DSLNH, disialyl-lacto-*N*-hexaose; FDSLNH, fucosyl-disialyllacto-*N*-hexose; 2′FL, 2′-fucosyllactose; FLNH, fucosyllacto-*N*-hexaose; FUT2, α1-2-fucosyltransferase; LNFP-I, lacto-*N*-fucopentaose I; LNFP-II, lacto-*N*-fucopentaose II; LNFP III, lacto-*N*-fucopentaose III; LNH, lacto-*N*-hexaose; LNnT, lacto-*N*-neotetraose; LNT, lacto-*N*-tetraose; LSTb, sialyl-lacto-*N*-tetraose b; LSTc, sialyllacto-*N*-tetraose c; miR-146a, miRNA 146a; MMCP-1, mucosal mast cell protease-1; MUC2, mucin 2; OVA, ovalbumin allergen; ROS, reactive oxygen species; TFF3, Trefoil factor.

associated genes. Specifically, *B. infantis* and *Bifidobacterium breve* supplemented with HMOs in culture, were reported to bind to Caco-2 intestinal epithelial cells (IECs) and inhibit the expression of chemokine-related genes encoding C-X-C motif chemokine ligands 1, 2, and 3 (CXCL1, CXCL2, CXCL3), demonstrating anti-inflammatory effects [50]. In contrast, *B. infantis* and *B. breve* grown with lactose or glucose were unable to influence gene expression in Caco-2 IECs, emphasizing an exciting immunomodulatory capacity of HMOs via microbiome modification, which remains to be robustly demonstrated *in vivo*. Notably, these chemokine genes are repeatedly implicated in allergy; the proteins CXCL1, CXCL2, and CXCL3 are neutrophil recruiters involved in type 2 inflammation and upregulated in both asthma [51] and atopic dermatitis [52]. Collectively, these findings suggest that HMOs may provide allergy protection via prebiotic effects on intestinal bacteria, subsequently influencing the expression of inflammation-associated genes in IECs. However, findings are preliminary and greater research, both *in vitro* and *in vivo*, is necessary to confirm the complex interaction between HMOs, the intestinal microbiome, gene regulation, and allergy.

Furthermore, HMO-induced microbiome modifications may also mediate allergy through microbe–mast cell interactions. Indeed, mast cells play a crucial role in allergic immune responses by releasing inflammatory mediators, including histamine, leukotrienes, and prostaglandins,

Key figure

The link between maternal and infant factors, human milk oligosaccharide (HMO) composition, and childhood allergy risk



(See figure legend at the bottom of the next page.)

contributing to atopic manifestations [53]. Mast cells are present at mucosal sites, including the intestinal epithelium, and are thus in close contact with intestinal microbes. Commensal bacteria mediate mast cell responses, including activation, tissue recruitment, and survival [54]. For instance, *Lactobacillus rhamnosus*, *Escherichia coli*, and *Enterococcus faecalis* suppress mast cell activation, as evidenced by **β-hexosaminidase**, histamine, and serotonin release *in vitro* [55–57]. Furthermore, in an OVA-induced food allergy model, germ-free mice did not develop clinical manifestations of food allergy (diarrhea and anaphylactic hypothermia) and showed impaired mast cell functionality, including reduced mast cell maturation, expression of tissue-homing markers such as CXCL1 and CXCL2, edema formation, and **mast cell mucosal protease-1 (mMCP-1)** production, compared with mice with an established microbiome [58]. Accordingly, co-housing germ-free mice with colonized mice restored their susceptibility to food allergy symptoms and improved mast cell functionality. Thus, these findings suggest that the intestinal microbiome is necessary for the maturation of mast cell function. There are several mechanistic pathways by which intestinal bacteria influence mast cell activity; one group demonstrated that blocking **toll-like receptor 4 (TLR4)** in murine mast cells attenuated microbiota-induced mast cell degranulation and mediated histamine release [59], suggesting that intestinal microbes can mediate mast cell activation through TLR4-activated pathways. Additionally, *L. rhamnosus* can downregulate the expression of allergy-associated genes, including the IgE receptor *FCER1* and the histamine H4 receptor *HRH4*, along with genes encoding proinflammatory IL-8 and TNF-α in human mast cells [55]. These findings suggest that intestinal bacteria, including *Lactobacillus* spp., may mediate mast cell activity in allergy via suppression of histamine and IgE receptor genes. Accordingly, HMO consumption increases fecal *Lactobacillus* spp. abundance in infants [60,61]. Similarly, *E. faecalis* inhibits antigen-induced mast cell degranulation *in vitro* [57] and infant fecal abundance of this species positively correlates with HMO consumption [62]. Therefore, HMO consumption may promote the colonization of intestinal bacteria that modulate mast cell activity, ultimately reducing allergic inflammation.

An additional mechanism by which HMO-induced microbiome modification may influence allergy risk is via SCFA production. *Bifidobacterium*, *Lactobacillus*, and *Bacteroides* spp., in particular, breakdown HMOs to produce microbial-derived metabolites SCFAs, including butyrate, acetate, and propionate [26,63]. SCFAs are documented to have multiple beneficial effects, including strengthening intestinal barrier integrity, neuroprotection, and reducing inflammation [64]. SCFAs are repeatedly linked to allergy protection, with high fecal butyrate and propionate concentrations associated with decreased asthma and food allergy at 6 years of age [65]. Moreover, compared with healthy controls, allergic infants show reduced genetic potential for intestinal butyrate production, due to an absence of genes encoding enzymes that ferment carbohydrates into butyrate [66]. One key pathway by which SCFAs may influence allergy development is via their ability to bind to **G protein-coupled receptors 41 and 43** (**GPR41/GPR43**) found on immune cells, IECs, and lung epithelium [67]. Activation of these receptors induces anti-

Figure 1. A range of maternal factors, including secretor status, body mass index (BMI), diet, geographical location, and parity influence the HMO composition of breastmilk [11,12]. HMOs are then consumed during the early-life critical window, influencing both microbiota and immune development [6]. In addition, infant factors such as antibiotic use [29], mode of delivery [30], and pet exposure [120] also alter early-life immune programming and subsequent allergy risk. Collectively, HMO consumption mediates infant allergy susceptibility through several direct and indirect coexisting mechanisms, including modification of the intestinal microbiome [27] [subsequently altering T cell differentiation [49], short-chain fatty acid (SCFA)-induced G protein-coupled receptors 41 and 43 (GPR41/43) activation [64,67,69], and histone deacetylase inhibition [84]], strengthening intestinal barrier integrity, and directly immunomodulating the niche (including mediating T cell balance [96] and mast cell degranulation) [8]. Collectively, various maternal factors influence breastmilk HMO composition, which can differentially influence infant allergy outcomes through several structure-specific immunological mechanisms, depending on complex interactions with infant risk status and early-life factors. Figure created using BioRender.com.

inflammatory effects via **p38 mitogen-activated protein kinase (p38 MAPK)** pathways, as demonstrated in human renal cortical epithelial cells, whereby SCFA treatment inhibited TNFα-induced MCP-1 expression by suppressing p38 MAPK signaling (assessed by using SB203580, a p38 MAPK inhibitor) [68]. Accordingly, SCFAs exhibited decreased airway inflammation, as demonstrated by reduced immune cell infiltration and Th2 cytokine concentrations (in BAL fluid) via GPR41 activation in a HDM-induced murine model of asthma [69]. Thus, HMOs may reduce allergy risk by increasing the production of microbial-derived SCFAs, particularly butyrate and acetate [26,70] which suppress inflammation via GPR41/43 activation. Notably, if HMOs' allergy-protective properties result from SCFA production in circumstances in which obtaining rare HMOs is difficult, SCFA supplementation may prove to be an effective alternative to reducing allergy risk, although this warrants further investigation.

In addition, HMO-induced microbial SCFA production may influence allergy development through histone deacetylase (HDAC) inhibition. HDACs (enzymes involved in the deacetylation of histone proteins) are expressed in almost all mammalian cells and are key regulators of T cell differentiation [71], playing crucial roles in allergic immune responses. Allergen exposure upregulates the expression of several HDACs, including HDAC 1, 5, 6, 9, and 11, in individuals with allergic rhinitis and asthma [72–74], mediating immune responses via **mechanistic target of rapamycin-6K (mTOR-S6K)** signaling, and resulting in increased IL-6 and IL-4 production, as well as Th2 and Th17 cell differentiation [75]. Additionally, HDAC expression is reported to suppress **forkhead box protein P3 (FoxP3)** transcription in murine $CD4^+$ T cells, leading to decreased Treg generation [76,77], contributing to T cell imbalances seen in allergy. Accordingly, individuals with allergic rhinitis [78] and asthma [79] show increased HDAC1 expression compared with healthy controls. Notably, SCFAs, particularly butyrate and propionate, are powerful HDAC inhibitors [80]. Microbial-derived SCFAs cross the intestinal barrier and inhibit HDACs in intestinal epithelial and immune cells, thus increasing *Foxp3* transcription, suppressing IL-4 and IL-6 and ultimately promoting the generation of anti-inflammatory Tregs [81,82]. Moreover, butyrate supplementation, through a mechanism of inhibiting HDAC expression, has reduced airway inflammation in a murine model of innate lymphoid cell type 2 (**ILC2**)-driven asthma (measured by airway hyper-responsivity to methacholine and quantity of eosinophils, ILC2 cells, IL-5, and IL-13 in BAL fluid) [83]. Notably, this effect was independent of GPR41/43 activation [83]. In addition, the authors reported that supplementation of butyrate-producing *Clostridium* spp. significantly increased pulmonary butyrate and propionate and alleviated asthma in mice. Furthermore, another group demonstrated that acetate supplementation promoted Treg generation and alleviated murine asthma via HDAC inhibition and FoxP3 acetylation [84]. Taken together, these findings suggest at least one microbiota-dependent pathway by which HMOs may mediate allergy risk: namely, via SCFA-induced HDAC inhibition, regulating T cell balance away from Th2 skewing seen in allergy. However, despite supportive findings on the potential mechanistic roles of the microbiome in allergy, further research involving HMO supplementation in animal and *in vitro* models is needed to validate HMO-induced microbiota-dependent mechanisms in allergy prevention.

Collectively, the current literature suggests that HMOs modify early-life microbiome composition and subsequently influence allergy risk via microbial mediation of Th1/Th2 cell balance, mast cell activation, and gene regulation, but also indirectly through microbially-derived SCFAs. Further research should identify specific microbial communities that are altered by HMOs and promote immune tolerance, which could inform HMO and probiotic interventions.

Intestinal barrier maturation

A further mechanism by which HMOs may alter allergy susceptibility is by strengthening the intestinal barrier epithelium. During allergic sensitization, an otherwise harmless antigen crosses the

mucosal barrier and is presented by antigen-presenting cells (APCs), triggering a Th2 immune response, the production of Th2 cytokines, IgE, eosinophils, and ultimately, allergy development [33]. Accordingly, epithelial barriers protect against external antigens, allowing only certain compounds to enter systemic circulation. Notably, epithelial barrier dysfunction is repeatedly associated with allergy [85,86], and increased intestinal permeability is a key characteristic of food allergy [87]. Research suggests that HMOs can strengthen intestinal barrier integrity by influencing **mucin 2 (MUC2)** expression, resulting in increased mucus production [88]. Accordingly, treatment of IECs with pooled HMOs *in vitro* stimulates the expression of mucosal regeneration genes *MUC2* and **Trefoil factor 3 (*TFF3*)**, resulting in increased mucus production [89]. Moreover, HMO-like galacto-oligosaccharides can reverse pollution-induced epithelial barrier degradation in Caco-2 cells [90], highlighting the powerful role of HMOs in maintaining epithelial barrier integrity.

However, it is worth noting that the beneficial effects of HMOs on epithelial barrier function are structure-specific; one group [91] reported that 3′-galactosyllactose with a β1–3 glycosidic linkage effectively protected IECs from pollution-induced damage, whereas 3′-galactosyllactose with an α1–3 glycosidic linkage did not. This suggests that different HMOs may act via distinct immunological pathways to modulate allergy risk.

Alongside the direct effects of HMOs on intestinal barrier integrity, HMOs might also act on the intestinal epithelial barrier via their metabolites; as mentioned, intestinal bacteria ferment HMOs to produce SCFAs [26]. SCFAs are also able to strengthen intestinal barrier integrity by upregulating MUC2 expression, thus stimulating mucus secretion [92,93]. Additionally, SCFAs maintain tight junctions by increasing the expression of tight junction protein-related genes, **Gap junction alpha 3** and Occludin [94]. Therefore, not only might HMOs directly strengthen intestinal barrier integrity, but also indirectly via their resulting metabolites.

Taken together, these findings suggest that HMOs may offer allergy protection by promoting intestinal barrier maturation and mucosal homeostasis, presumably leading to reduced allergenic load and protecting against sensitization in the gut. Nevertheless, the importance of epithelial barrier integrity in allergy remains to be validated.

Systemic mechanisms

Direct immunomodulation

HMOs bind to receptors on immune cells

Aside from the well-established prebiotic properties of HMOs, emerging research indicates that HMOs also have direct immunomodulatory effects, which might further explain their potential role in allergy prevention. Although 95–99% of HMOs are fermented by intestinal bacteria, 1–5% are absorbed, cross the intestinal epithelium, and enter circulation [25,95]. These HMOs can interact directly with immune cells via several glycan receptors, including DC-SIGN [96], galectin [97], siglecs 5 and 9 [98], and TLR2, TLR4, TLR5, TLR7, TLR8 [96,99,100] (Table 4). For instance, 2′FL and 3′-fucosyllactose (3′FL) can bind to DC-SIGN, a glycan-binding protein expressed on the surface of APCs including dendritic cells and macrophages [98]. Thus, HMOs are thought to directly influence immune responses via glycan receptors on immune cells.

HMOs influence mast cell degranulation

Regarding direct immunomodulation by HMOs in allergy, in an OVA-induced murine model of food allergy, 6′SL significantly inhibited IgE-mediated degranulation of mast cells (measured via β-hexosaminidase release) from OVA-sensitized murine bone marrow-derived mast cells (BMMCs) *ex vivo* and increased intestinal Treg numbers *in vivo* [8]. Moreover, when BMMC activation was assessed in the presence of $CD4^+CD25^+$ T cells from HMO-treated and control mice,

Table 4. Summary of immune-associated receptors known to bind HMOs[a]

Receptor	Expression	Receptor function	Established HMO ligands	Refs
TLR2 TLR4 TLR5 TLR7 TLR8	Antigen-presenting cells Lymphocytes Mast cells	Pathogen recognition	6′SL, 3′SL, 2′FL, LNFP-I, LNFP-III	[96,99,100]
DC-SIGN	Antigen-presenting cells	Antigen presentation	3′FL, 2′FL, LNFP-III, LNFP-IV	[96,98]
Siglec-5, Siglec-9	Dendritic cells Neutrophils Monocytes	Immune signaling	Sialylated HMOs (e.g., 3′SL, 6′SL)	[96]
Galectins	Intestinal epithelial cells Lymphocytes Antigen-presenting cells	Immune signaling	LNT, LNnT, LNFP-II	[97]

[a]Abbreviations: DC-SIGN, dendritic cell-specific intercellular adhesion molecule-3-grabbing non-integrin; 2′FL, 2′-fucosyllactose; 3′FL, 3′-fucosyllactose; LNFP-III, lacto-*N*-fucopentaose III; LNFP-IV, lacto-*N*-fucopentaose IV; LNnT, lacto-*N*-neotetraose; LNT, lacto-*N*-tetraose; 3′SL, 3′-sialyllactose; 6′SL, 6′-sialyllactose; TLR, Toll-like receptor.

T cells from 2′FL and 6′SL-treated mice inhibited mast cell degranulation, whereas those from control mice did not, suggesting that HMO-induced Treg production reduces inflammation by suppressing mast cell activation. These findings indicate that HMOs might directly influence key immune cells involved in allergy, further contributing to their protective effects.

However, in this study, 6′SL inhibited mast cell degranulation only at high concentrations (1 mg/ml), which mast cells beyond the gastrointestinal tract are unlikely to be exposed to, thus reducing the plausibility that direct inhibition of mast cell degranulation is the sole mechanism by which HMOs might alleviate food allergy. Nevertheless, mast cell stabilization might be one of the multiple pathways by which HMOs modulate allergic responses. Moreover, only 6′SL, not 2′FL, inhibited mast cell degranulation *ex vivo*, although both HMOs alleviated food allergy *in vivo*, further supporting the notion that individual HMOs may act via distinct immunological pathways. Therefore, although this study suggests that 2′FL and 6′FL alleviate food allergy via Treg production and mast cell stabilization, there are likely multiple structure-specific mechanisms at play.

HMOs influence T cell balance

Additionally, *in vitro* findings suggest that HMOs directly alter Th1/Th2 balance. Specifically, acidic HMOs can increase the production of anti-inflammatory IL-10 and IFNγ and decrease IL-13 from human neonatal cord blood mononuclear cells *in vitro*, altering the T cell balance towards a regulatory Th1 response [101], rather than the Th2 skewing seen in allergy [33,34]. Moreover, HMO-treated dendritic cells that were co-cultured with naive T cells showed increased Treg production and decreased lipopolysaccharide (LPS)-induced IL-6 and TNF-α production, suggesting that HMOs can modulate T cell balance away from an allergy-associated Th2 response and towards an anti-inflammatory Treg/Th1 response [96].

HMOs alter inflammatory signaling

HMOs may influence allergic responses via inhibition of nuclear factor kappa B (NFκB) and activator protein-1 (AP-1) inflammatory signaling pathways. Accordingly, 6′SL and 2′FL inhibited the release of chemokines CCL20 and IL-8 from T-84 and HT-29 IECs stimulated with an antigen–antibody complex as a model of food allergy, demonstrating anti-inflammatory effects on allergic immune responses [18]. Notably, 2′FL and 6′SL inhibited chemokine production via different immunological pathways, specifically, 2′FL inhibited antigen-induced NFκB binding activity

of T-84 and HT-29 cells yet had no effect on the AP-1 pathway. In contrast, 6′SL showed significant dose-dependent effects on the AP-1 pathway (transcriptional activity), while only its high concentrations inhibited NFκB binding activity. Additionally, the inhibitory effects of 6′SL on AP-1 and NFκB were dependent on peroxisome proliferator-activated receptor gamma (PPARγ), a ligand-activated receptor that mediates intestinal inflammation [102], whereas the anti-inflammatory effects of 2′FL were not PPARγ-dependent [18]. Overall, these data suggest that varying HMOs may alter allergic responses through inhibition of NFκB and AP-1 pathways, resulting in suppression of inflammatory mediator release from allergen-stimulated IECs.

Harboring immunomodulatory capabilities, HMOs might protect against various allergies by mediating APC activation, T cell balance, mast cell activity, and NFκB signaling. However, further investigation in allergy models is needed to determine and validate the distinct immunological pathways by which individual HMOs might act.

Limitations to overcome

Although murine and *in vitro* models provide valuable insights into underlying mechanisms of HMOs in allergic responses, they do not truly represent human allergy. Accordingly, RCTs are optimal for determining the efficacy of HMOs against allergy. Unfortunately, RCTs are severely lacking, with none assessing the therapeutic efficacy of HMOs on allergy outcomes conducted to date, due to ethical issues in randomizing infants to breastfeeding/supplementation. Further, RCTs should be conducted, where ethically possible (e.g., HMO supplementation in formula-fed infants), alongside well-controlled longitudinal observational studies to complement the external validity of *in vitro* and murine/animal findings.

Moreover, while numerous studies point towards the allergy-protective effects of HMOs, others demonstrate limited benefits [23], questioning their therapeutic value. Such inconsistencies may reflect the diversity between HMO structures assessed. For instance, one study [17] demonstrated that only FUT2-dependent HMOs were associated with reduced infant allergy, while other structures showed no benefits. However, in an observational study of CMA [21], LNFP-III concentrations negatively correlated with infant CMA, suggesting that the allergy-protective effects of HMOs were structure-specific. Notably, while both 2′FL and 6′SL significantly reduced anaphylactic responses, $CD4^+CD25^+IL\text{-}10^+$ cell populations, and serum mMCP-1 concentrations in a murine model of food allergy, only 6′SL mediated IL-10 and TNF-α production and inhibited mast cell granulation *in vitro* [8], again highlighting the relevance of HMO structure-specific immunological pathways. Such inconsistencies between studies also likely reflect the varying HMO groups and immune markers assessed. With over 200 distinct structures identified to date [10], it is important to acknowledge the large diversity between HMOs and their biological actions, rather than simply classifying HMOs as a single entity as either allergy-protective or not. Consequently, future research should discriminate distinct immunological pathways targeted by individual HMOs in allergy. Research thus far has largely focused on the predominant HMOs, such as 2′FL and 6′SL; however, exploration of more diverse structures may uncover unique biological actions. Such research can ultimately aid the development of an optimized HMO consortium as a potential therapeutic approach for high allergy-risk infants.

Furthermore, contrasting findings regarding the allergy-protective potential of HMOs may reflect several other factors. Stage of lactation in which HMOs are measured is often not controlled in observational studies and can have confounding effects because HMO concentrations and compositions vary significantly throughout lactation [14,15]. Similarly, variation in breastfeeding duration and quantity of breastmilk consumed may influence the findings. Moreover, as the proportion of FUT2-positive women varies depending on ethnicity and geographical location, homogenous

study populations might reduce the generalizability of findings. For instance, one group reported that HMO composition correlated with decreased risk of diarrheal disease at 2 years of age [103]; however, this study was conducted in a Mexican population of which all mothers expressed a functional *FUT2* gene, thus reducing the variation in HMO profiles. These findings are not generalizable to European populations, where a higher proportion (~25%) of individuals are nonsecretors compared with Central American populations [104]. Additionally, variance in study populations (high-risk vs. general population) and the allergy outcomes assessed (any IgE sensitization vs. diagnosed CMA) may further contribute to contrasting findings, making it difficult to draw conclusions. Accordingly, future large-scale studies should control for these variables. Such limitations of cohort studies highlight the importance of animal and *in vitro* mechanistic research to further validate the role of HMOs in allergy.

From another angle, methodological limitations in HMO analysis may further contribute to contrasting findings and make comparison between studies difficult. There is currently no standard analytical method for the identification and quantification of HMOs, with significant variation in the sensitivity and validity of techniques. For instance, **liquid chromatography-mass spectrometry (LC-MS)** is often limited by co-elution of multiple compounds [105], resulting in poor structural selectivity and difficulty in identifying distinct isomers. This is problematic because individual HMOs may fundamentally differ in their immunological actions. Moreover, **high-performance liquid chromatography** relies on the availability of pure standards for HMO identification [106], which is particularly challenging for rare structures such as disialyllacto-*N*-tetraose. As a result, current methods can only isolate ~20 HMOs out of 200 known structures. Additionally, while some studies quantify HMOs using absolute concentrations [21], others assess the relative proportion of HMOs present [103], resulting in inconsistencies between findings. Accordingly, future research should develop a standardized method of HMO analysis that addresses limitations, is robust, reproducible, and has high sensitivity and selectivity to validate the putative therapeutic efficacy of HMOs.

Future directions

Preclinical studies

While the prebiotic effects of HMOs on the infant microbiome are fairly well established, their direct role on immune development and intestinal barrier integrity, specifically in allergy, are unclear. Further development of *in vitro* models of allergic sensitization, including the interaction between IECs, dendritic cells, and eosinophils, would aid in understanding certain immunomodulatory properties. Moreover, how HMOs might directly or indirectly provide allergy protection via the regulation of inflammation-associated genes (Box 1), particularly genes involved in antigen tolerance, remains unclear. Additionally, while TLR4 is the most relevant HMO-binding toll-like receptor (TLR) in the context of allergy, varying HMOs are reported to both activate and inhibit several other TLRs [99,100], warranting further investigation. Moreover, as synthetically produced HMOs become increasingly available, offering value for formula-fed infants, research should investigate not only the therapeutic efficacy of natural HMOs, but also that of synthetically produced products.

Clinical studies

The impact of HMOs on long-term allergy outcomes remains unknown. Early-life is a critical window in which immune programming, allergy development, and microbiome colonization take place, justifying why studies have focused on infancy. However, little is known about the influence of early-life HMO consumption on allergy throughout the lifespan. Interestingly, FUT2-dependent HMOs are associated with reduced eczema risk at 2 years of age, but not 5 years, in caesarean-delivered infants (NCT00298337)[ii] [17], suggesting that HMOs might only provide protection early

Box 1. HMO-induced regulation of inflammation-associated genes

A preliminary mechanism by which HMOs may mediate immune responses is by modulating the expression of inflammation-associated genes. 2′FL can dose-dependently upregulate miRNA-146 (miR-146) expression in β-lactoglobulin-treated murine macrophage (RAW 264.7) cells, as a food allergy-like model [111]. miR-146a expression was increased via HMO-induced inhibition of the TLR4/NFκB inflammatory pathway. Moreover, the addition of miR-146a inhibitors on RAW 264.7 cells eliminated 2′FL's anti-inflammatory effect on TLR4-mediated inflammation, as shown via increased cytokine production (IL-4, IL-6, and TNF-α) and IRAK1 and TRAF6 expression. These findings suggest that HMOs can modulate allergic responses via inhibition of TLR4/NFκB signaling, altering, the expression of inflammation-associated genes, including miR-146a. Indeed, miR-146a is repeatedly implicated in allergic diseases; asthmatic individuals show significantly reduced plasma miR-146a expression compared with healthy counterparts and increased miR-146a expression restores damage in human airway epithelial cells [112]. Additionally, miR-146a mimics alleviate asthma in an OVA-induced murine model [113] and abnormal miR-146a expression is associated with Th1/Th17 cell imbalance in a murine model of CMA [114]. Notably, certain HMOs are TLR4 ligands [96,99] and thus can trigger downstream immune signaling via TLR4-expressing immune cells [111,115]. Therefore, activation of glycan receptors including TLR4 on APCs might represent a key pathway by which HMOs can influence the expression of immune-associated genes, such as miR-146a, contributing to allergy protection.

The treatment of colonic epithelial HT-29 cells with pooled HMOs can significantly alter the expression of numerous immunoregulatory glycogenes, including cytokine, chemokine, and cell surface receptor genes [116]. IL-17C expression, in particular, was upregulated in HT-29 cells exposed to pooled HMOs. IL-17C is a member of the IL-17 proinflammatory cytokine family and its increased expression contradicts the expected anti-inflammatory effects of HMOs. However, during early life, when neonates are exposed to an influx of bacteria, IL-17C induction stimulates the production of cytokines and antimicrobial products to protect the infant from bacterial infection [117,118]. In addition, IL-17C regulates intestinal mucosal homeostasis and alleviates colitis in mice (dextran sulfate sodium-induced model) [119]. Therefore, IL-17C is thought to support intestinal barrier maturation, microbiota establishment, and immune development in early life, paradoxically having a protective role during the neonatal stage. Consequently, HMOs might still elicit anti-inflammatory responses via modulation of immune-associated genes, potentially contributing to their allergy-protective effects.

Despite intriguing findings, only a sparse number of studies have investigated the impact of HMOs on the expression of immune-associated genes and the association to allergy prevention is speculative. Whether modulation of gene expression is a legitimate pathway by which HMOs exert anti-allergy effects warrants further investigation before conclusions can be drawn.

in life. However, further research is needed to validate this conclusion. Accordingly, investigation into the long-term impact of early-life HMO consumption on allergy outcomes throughout childhood, adolescence, and adulthood is needed to understand HMO's lasting therapeutic role. As synthetic HMO-like supplements become available for adults, understanding whether such consumption in adulthood can provide anti-allergy benefits outside the critical window of immune development would be of value.

Additionally, further investigation into various non-genetic factors that influence HMO composition might aid interventions for reducing childhood allergy. Because certain HMO profiles are associated with decreased infant allergy, determining the non-genetic modifiable factors that influence HMO composition, such as diet, might allow these to be targeted by maternal interventions to help reduce offspring allergy.

Of note, infant microbiome composition varies, depending on geographical location and socio-economic status [107,108]. Specifically, *B. infantis*, a key HMO-utilizing species, shows increased abundance in infants from less economically developed countries [109], where allergy prevalence is generally low [110], potentially reflecting enhanced bacterial degradation of HMOs and consequent immunoregulation. Therefore, determining the potential therapeutic value of HMO supplementation across differing socio-economic strata and locations in which HMO profiles and infant microbiome composition vary would be informative.

Lastly, it is unclear whether HMOs have remedial or preventive effects in allergy. Cohort studies suggest that HMOs might play a preventive role against allergy development; however, animal

research also indicates therapeutic effects in existing allergies [8]. Due to the scarcity of RCTs, robust research is necessary to establish the remedial compared with preventive potential of HMOs.

Concluding remarks

Overall, human observational studies, animal models, and *in vitro* data suggest that HMOs have valuable potential as putative treatments for childhood allergy prevention. While allergic diseases are complex and the efficacy of interventions is influenced by many environmental, genetic, and socio-economic factors, HMOs may offer a promising approach to reduce childhood allergy worldwide. Noteworthy, not all HMOs are equal and individual structures may harbor differing immunological actions, providing varying levels of protection against different allergies. Moreover, the allergy-protective efficacy of HMOs depends on complex interactions between numerous maternal factors, infant characteristics, and allergy phenotypes. Findings should be interpreted with caution, particularly regarding limitations of observational studies. Ultimately, the current literature highlights the potential of HMO-supplemented formulas, breastfeeding encouragement, and maternal interventions to reduce childhood allergy risk. Further research, particularly investigating the long-term efficacy of HMOs, their structure-specific mechanisms, and the value of synthetically produced HMOs (see Outstanding questions), is necessary to enable translation into real-world interventions, representing a fruitful and exciting area of investigation.

Acknowledgments

Research at Dr Brett Finlay's lab is supported by a Canadian Institutes for Health Research (CIHR) Foundation Grant. B.B.F. is also a Canadian Institute for Advanced Research (CIFAR) Senior Fellow and a University of British Columbia Peter Wall Distinguished Professor. I.T. is supported by the University of British Columbia John Richard Turner Fellowship in Microbiology award, President's Academic Excellence Initiative Award and International Tuition award.

Declaration of interests

No interests are declared.

Resources

[i]https://clinicaltrials.gov/ct2/show/NCT01715246

[ii]https://clinicaltrials.gov/ct2/show/NCT00298337

References

1. Bantz, S.K. *et al.* (2014) The atopic march: progression from atopic dermatitis to allergic rhinitis and asthma. *J. Clin. Cell. Immunol.* 5, 67–73
2. Nutten, S. (2015) Atopic dermatitis: global epidemiology and risk factors. *Ann. Nutr. Metab.* 66, 8–16
3. Asher, M.I. *et al.* (2021) Worldwide trends in the burden of asthma symptoms in school-aged children: Global Asthma Network Phase I cross-sectional study. *Lancet* 398, 1569–1580
4. Gupta, R. *et al.* (2013) The economic impact of childhood food allergy in the United States. *JAMA Pediatr.* 167, 1026–1031
5. Cukrowska, B. (2018) Microbial and nutritional programming—the importance of the microbiome and early exposure to potential food allergens in the development of allergies. *Nutrients* 10, 1541
6. Romano-Keeler, J. and Sun, J. (2022) The first 1000 days: assembly of the neonatal microbiome and its impact on health outcomes. *Newborn* 1, 219–226
7. Miliku, K. *et al.* (2018) Human milk oligosaccharide profiles and food sensitization among infants in the CHILD Study. *Allergy* 73, 2070–2073
8. Castillo-Courtade, L. *et al.* (2015) Attenuation of food allergy symptoms following treatment with human milk oligosaccharides in a mouse model. *Allergy* 70, 1091–1102
9. Bode, L. (2015) The functional biology of human milk oligosaccharides. *Early Hum. Dev.* 91, 619–622
10. Ruhaak, L.R. and Lebrilla, C.B. (2012) Advances in analysis of human milk oligosaccharides. *Adv. Nutr.* 3, 406S–414S
11. Han, S.M. *et al.* (2021) Maternal and infant factors influencing human milk oligosaccharide composition: beyond maternal genetics. *J. Nutr.* 151, 1383–1393
12. Seferovic, M.D. *et al.* (2020) Maternal diet alters human milk oligosaccharide composition with implications for the milk metagenome. *Sci. Rep.* 10, 1–18
13. Lefebvre, G. *et al.* (2020) Time of lactation and maternal fucosyltransferase genetic polymorphisms determine the variability in human milk oligosaccharides. *Front. Nutr.* 7, 574459
14. Soyyilmaz, B. *et al.* (2021) The mean of milk: a review of human milk oligosaccharide concentrations throughout lactation. *Nutrients* 13, 2737
15. Plows, J.F. (2021) Longitudinal changes in human milk oligosaccharides (HMOs) over the course of 24 months of lactation. *J. Nutr.* 151, 876–882
16. Lodge, C.J. *et al.* (2021) Human milk oligosaccharide profiles and allergic disease up to 18 years. *J. Allergy Clin. Immunol.* 147, 1041–1048
17. Sprenger, N. *et al.* (2017) FUT2-dependent breast milk oligosaccharides and allergy at 2 and 5 years of age in infants with high hereditary allergy risk. *Eur. J. Nutr.* 56, 1293–1301

Outstanding questions

To what extent does early-life HMO consumption influence long-term allergy outcomes throughout lifespan?

By what mechanistic pathways do individual HMO structures have distinct immunological actions in allergy development? Establishing this may allow the identification of the most effective HMO structures needed to ideally prevent allergic inflammation, or to be used in allergy interventions.

How do various HMO groups mediate allergy risk through the regulation of genes involved in inflammation and antigen tolerance?

To what extent does HMO-induced maturation of the intestinal epithelial barrier contribute to allergy susceptibility?

Do synthetically produced HMO-like oligosaccharides provide equal immunological benefits and allergy protection compared with natural HMOs? Establishing the efficacy of synthetically produced HMO-mimicking oligosaccharides might allow economical and practical HMO-based interventions given that HMOs can be expensive and time-consuming to obtain.

Do HMOs offer therapeutic benefits to existing allergy as well as allergy prevention? Determining the remedial benefits of HMOs in existing allergy broadens the clinical implications to putative allergy treatments.

Which HMO structures can be identified as the most effective optimized consortium to be used as an allergy-preventative intervention for high-risk infants?

Do HMOs provide allergy protection in adulthood, outside the critical window of immune and microbiota development? Determining this may enable the use of HMOs as a putative therapeutic strategy for adult, as well as childhood, allergy.

Which modifiable maternal factors can be targeted in maternal interventions to mediate the HMO composition of breastmilk to a more 'allergy protective' profile?

18. Zehra, S. *et al.* (2018) Human milk oligosaccharides attenuate antigen–antibody complex induced chemokine release from human intestinal epithelial cell lines. *J. Food Sci.* 83, 499–508
19. Loh, W. and Tang, M.L. (2018) The epidemiology of food allergy in the global context. *Int. J. Environ. Res. Public Health* 15, 2043
20. Spolidoro, G.C. *et al.* (2023) Frequency of food allergy in Europe: an updated systematic review and meta-analysis. *Allergy* 78, 351–368
21. Seppo, A.E. *et al.* (2017) Human milk oligosaccharides and development of cow's milk allergy in infants. *J. Allergy Clin. Immunol.* 139, 708–711
22. Kim, G. *et al.* (2020) Delayed establishment of gut microbiota in infants delivered by cesarean section. *Front. Microbiol.* 11, 2099
23. Sjögren, Y.M. *et al.* (2007) Neutral oligosaccharides in colostrum in relation to maternal allergy and allergy development in children up to 18 months of age. *Pediatr. Allergy Immunol.* 18, 20–26
24. Thomson, P. *et al.* (2018) Human milk oligosaccharides and infant gut bifidobacteria: molecular strategies for their utilization. *Food Microbiol.* 75, 37–46
25. Goehring, K.C. *et al.* (2014) Direct evidence for the presence of human milk oligosaccharides in the circulation of breastfed infants. *PLoS One* 9, e101692
26. Yu, Z.T. *et al.* (2013) Utilization of major fucosylated and sialylated human milk oligosaccharides by isolated human gut microbes. *Glycobiology* 23, 1281–1292
27. Steenhout, P. *et al.* (2016) Term infant formula supplemented with human milk oligosaccharides (2′ fucosyllactose and lacto-N-neotetraose) shifts stool microbiota and metabolic signatures closer to that of breastfed infants. *FASEB J.* 30, 275–277
28. Gensollen, T. *et al.* (2016) How colonization by microbiota in early life shapes the immune system. *Science* 352, 539–544
29. Ni, J. *et al.* (2019) Early antibiotic exposure and development of asthma and allergic rhinitis in childhood. *BMC Pediatr.* 19, 1–8
30. Huang, L. *et al.* (2015) Is elective cesarean section associated with a higher risk of asthma? A meta-analysis. *J. Asthma* 52, 16–25
31. Hu, Y. *et al.* (2021) Breastfeeding duration modified the effects of neonatal and familial risk factors on childhood asthma and allergy: a population-based study. *Respir. Res.* 22, 1–11
32. Qian, L.J. *et al.* (2017) Early-life gut microbial colonization shapes Th1/Th2 balance in asthma model in BALB/c mice. *BMC Microbiol.* 17, 1–8
33. Caminati, M. *et al.* (2018) Type 2 immunity in asthma. *World Allergy Organ. J.* 11, 1–10
34. Akdis, C.A. *et al.* (2020) Type 2 immunity in the skin and lungs. *Allergy* 75, 1582–1605
35. Berker, M. *et al.* (2017) Allergies–AT cells perspective in the era beyond the TH1/TH2 paradigm. *Clin. Immunol.* 174, 73–83
36. Calzada, D. *et al.* (2018) Immunological mechanisms in allergic diseases and allergen tolerance: the role of Treg cells. *J. Immunol. Res.* 2018, 6012053
37. Hudey, S.N. *et al.* (2020) Mechanisms of non-type 2 asthma. *Curr. Opin. Immunol.* 66, 123–128
38. Boonpiyathad, T. *et al.* (2020) The role of Treg cell subsets in allergic disease. *Asian Pac. J. Allergy Immunol.* 38, 139–149
39. Krusche, J. *et al.* (2019) Role of early life immune regulation in asthma development. *Semin. Immunopathol.* 42, 29–42
40. Martino, D. *et al.* (2018) Epigenetic dysregulation of naive CD4+ T-cell activation genes in childhood food allergy. *Nat. Commun.* 9, 3308
41. Zhang, X. *et al.* (2021) An antibiotic-impacted microbiota compromises the development of colonic regulatory T cells and predisposes to dysregulated immune responses. *mBio* 12, e03335-20
42. Shi, C.W. *et al.* (2020) Probiotic *Lactobacillus rhamnosus* GG promotes mouse gut microbiota diversity and T cell differentiation. *Front. Microbiol.* 11, 607735
43. Atarashi, K. *et al.* (2013) Treg induction by a rationally selected mixture of *Clostridia* strains from the human microbiota. *Nature* 500, 232–236
44. Barletta, B. *et al.* (2013) Probiotic VSL# 3-induced TGF-β ameliorates food allergy inflammation in a mouse model of peanut sensitization through the induction of regulatory T cells in the gut mucosa. *Mol. Nutr. Food Res.* 57, 2233–2244
45. Li, L. *et al.* (2020) *Lactobacillus reuteri* attenuated allergic inflammation induced by HDM in the mouse and modulated gut microbes. *PLoS One* 15, e0231865
46. Wu, C.T. (2016) Effects of immunomodulatory supplementation with *Lactobacillus rhamnosus* on airway inflammation in a mouse asthma model. *J. Microbiol. Immunol. Infect.* 49, 625–635
47. Fu, G. *et al.* (2019) Effect of 3 lactobacilli on immunoregulation and intestinal microbiota in a β-lactoglobulin–induced allergic mouse model. *J. Dairy Sci.* 102, 1943–1958
48. Yang, X. *et al.* (2019) Antiallergic activity of *Lactobacillus plantarum* against peanut allergy in a Balb/c mouse model. *Food Agric. Immunol.* 30, 762–773
49. Henrick, B.M. *et al.* (2021) Bifidobacteria-mediated immune system imprinting early in life. *Cell* 184, 3884–3898
50. Wickramasinghe, S. *et al.* (2015) Bifidobacteria grown on human milk oligosaccharides downregulate the expression of inflammation-related genes in Caco-2 cells. *BMC Microbiol.* 15, 1–12
51. Al-Alwan, L.A. *et al.* (2013) Differential roles of CXCL2 and CXCL3 and their receptors in regulating normal and asthmatic airway smooth muscle cell migration. *J. Immunol.* 191, 2731–2741
52. Cole, C. *et al.* (2014) Filaggrin-stratified transcriptomic analysis of pediatric skin identifies mechanistic pathways in patients with atopic dermatitis. *J. Allergy Clin. Immunol.* 134, 82–91
53. Amin, K. (2012) The role of mast cells in allergic inflammation. *Respir. Med.* 106, 9–14
54. Forsythe, P. (2016) Microbes taming mast cells: implications for allergic inflammation and beyond. *Eur. J. Pharmacol.* 778, 169–175
55. Oksaharju, A. *et al.* (2011) Probiotic *Lactobacillus rhamnosus* downregulates FCER1 and HRH4 expression in human mast cells. *World J. Gastroenterol.* 17, 750
56. Magerl, M. *et al.* (2008) Non-pathogenic commensal *Escherichia coli* bacteria can inhibit degranulation of mast cells. *Exp. Dermatol.* 17, 427–435
57. Kasakura, K. *et al.* (2014) Commensal bacteria directly suppress in vitro degranulation of mast cells in a MyD88-independent manner. *Biosci. Biotechnol. Biochem.* 78, 1669–1676
58. Schwarzer, M. *et al.* (2019) Germ-free mice exhibit mast cells with impaired functionality and gut homing and do not develop food allergy. *Front. Immunol.* 10, 205
59. Shimbori, C. *et al.* (2022) Gut bacteria interact directly with colonic mast cells in a humanized mouse model of IBS. *Gut Microbes* 14, 2105095
60. Rubio-del-Campo, A. *et al.* (2020) Human milk and mucosa-associated disaccharides impact on cultured infant fecal microbiota. *Sci. Rep.* 10, 1–12
61. Bai, Y. *et al.* (2018) Fucosylated human milk oligosaccharides and N-glycans in the milk of Chinese mothers regulate the gut microbiome of their breast-fed infants during different lactation stages. *mSystems* 3, e00206-18
62. Cheema, A.S. *et al.* (2022) Exclusively breastfed infant microbiota develops over time and is associated with human milk oligosaccharide intakes. *Int. J. Mol. Sci.* 23, 2804
63. Šuligoj, T. *et al.* (2020) Effects of human milk oligosaccharides on the adult gut microbiota and barrier function. *Nutrients* 12, 2808
64. Xiong, R.G. *et al.* (2022) Health benefits and side effects of short-chain fatty acids. *Foods* 11, 2863
65. Roduit, C. *et al.* (2019) High levels of butyrate and propionate in early life are associated with protection against atopy. *Allergy* 74, 799–809
66. Cait, A. *et al.* (2019) Reduced genetic potential for butyrate fermentation in the gut microbiome of infants who develop allergic sensitization. *J. Allergy Clin. Immunol.* 144, 1638–1647
67. Li, M. *et al.* (2018) The anti-inflammatory effects of short chain fatty acids on lipopolysaccharide-or tumor necrosis factor α-stimulated endothelial cells via activation of GPR41/43 and inhibition of HDACs. *Front. Pharmacol.* 23, 533

Can short-chain fatty acids and synthetically produced oligosaccharides be used in conjunction/instead of HMOs to prevent allergy in infancy? If so, SCFAs and synthetically produced oligosaccharides might offer a feasible and practical addition to HMOs as a supplement for formula-fed infants at high risk of allergy.

68. Kobayashi, M. *et al.* (2017) Short-chain fatty acids, GPR41 and GPR43 ligands, inhibit TNF-α-induced MCP-1 expression by modulating p38 and JNK signaling pathways in human renal cortical epithelial cells. *Biochem. Biophys. Res. Commun.* 486, 499–505
69. Trompette, A. *et al.* (2014) Gut microbiota metabolism of dietary fiber influences allergic airway disease and hematopoiesis. *Nat. Med.* 20, 159–166
70. Natividad, J.M. *et al.* (2022) Human milk oligosaccharides and lactose differentially affect infant gut microbiota and intestinal barrier in vitro. *Nutrients* 14, 2546
71. Haery, L. *et al.* (2015) Histone acetyltransferases and histone deacetylases in B-and T-cell development, physiology and malignancy. *Genes Cancer* 6, 184
72. Wawrzyniak, P. *et al.* (2017) Regulation of bronchial epithelial barrier integrity by type 2 cytokines and histone deacetylases in asthmatic patients. *J. Allergy Clin. Immunol.* 139, 93–103
73. Shao, J.B. *et al.* (2018) Histone deacetylase 11 inhibits interleukin 10 in B cells of subjects with allergic rhinitis. *Int. Forum Allergy Rhinol.* 8, 1274–1283
74. Steelant, B. *et al.* (2019) Blocking histone deacetylase activity as a novel target for epithelial barrier defects in patients with allergic rhinitis. *J. Allergy Clin. Immunol.* 144, 1242–1253
75. Park, J. *et al.* (2015) Short-chain fatty acids induce both effector and regulatory T cells by suppression of histone deacetylases and regulation of the mTOR–S6K pathway. *Mucosal Immunol.* 8, 80–93
76. Deng, G. *et al.* (2020) FoxP3 in Treg cell biology: a molecular and structural perspective. *Clin. Exp. Immunol.* 199, 255–262
77. Lee, J.H. *et al.* (2022) Histone deacetylase 6 plays an important role in TGF-β-induced murine Treg cell differentiation by regulating cell proliferation. *Sci. Rep.* 12, 22550
78. Wang, Y. *et al.* (2015) Regulation of Trek1 expression in nasal mucosa with allergic rhinitis by specific immunotherapy. *Cell Biochem. Funct.* 33, 23–28
79. Butler, C.A. *et al.* (2012) Glucocorticoid receptor β and histone deacetylase 1 and 2 expression in the airways of severe asthma. *Thorax* 67, 392–398
80. Lin, M.Y. *et al.* (2015) Redirection of epithelial immune responses by short-chain fatty acids through inhibition of histone deacetylases. *Front. Immunol.* 6, 554
81. Furusawa, Y. *et al.* (2013) Commensal microbe-derived butyrate induces the differentiation of colonic regulatory T cells. *Nature* 504, 446–450
82. Arpaia, N. *et al.* (2013) Metabolites produced by commensal bacteria promote peripheral regulatory T-cell generation. *Nature* 504, 451–455
83. Thio, C.L.P. *et al.* (2018) Regulation of type 2 innate lymphoid cell–dependent airway hyperreactivity by butyrate. *J. Allergy Clin. Immunol.* 142, 1867–1883
84. Thorburn, A.N. *et al.* (2015) Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. *Nat. Commun.* 6, 7320
85. Gon, Y. and Hashimoto, S. (2018) Role of airway epithelial barrier dysfunction in pathogenesis of asthma. *Allergol. Int.* 67, 12–17
86. Krohn, I.K. *et al.* (2020) Nasal epithelial barrier dysfunction increases sensitization and mast cell degranulation in the absence of allergic inflammation. *Allergy* 75, 1155–1164
87. Parrish, A. *et al.* (2022) Intestinal mucus barrier: a missing piece of the puzzle in food allergy. *Trends Mol. Med.* 28, 36–50
88. Cheng, L. *et al.* (2020) Human milk oligosaccharides differently modulate goblet cells under homeostatic, proinflammatory conditions and ER stress. *Mol. Nutr. Food Res.* 64, 1900976
89. Wu, R.Y. *et al.* (2019) Human milk oligosaccharides increase mucin expression in experimental necrotizing enterocolitis. *Mol. Nutr. Food Res.* 63, 1800658
90. Akbari, P. *et al.* (2015) Galacto-oligosaccharides protect the intestinal barrier by maintaining the tight junction network and modulating the inflammatory responses after a challenge with the mycotoxin deoxynivalenol in human Caco-2 cell monolayers and B6C3F1 mice. *J. Nutr.* 145, 1604–1613
91. Varasteh, S. *et al.* (2019) Human milk oligosaccharide 3′-galactosyllactose can protect the intestinal barrier to challenges. *J. Pediatr. Gastroenterol. Nutr.* 68, N-P-016:1049
92. Burger-van Paassen, N. *et al.* (2009) The regulation of intestinal mucin MUC2 expression by short-chain fatty acids: implications for epithelial protection. *Biochem. J.* 420, 211–219
93. Giromini, C. *et al.* (2022) Role of short chain fatty acids to counteract inflammatory stress and mucus production in human intestinal HT29-MTX-E12 cells. *Foods* 11, 1983
94. Gao, Y. *et al.* (2021) Short-chain fatty acid butyrate, a breast milk metabolite, enhances immature intestinal barrier function genes in response to inflammation in vitro and in vivo. *Am. J. Physiol. Gastrointest. Liver Physiol.* 320, G521–G530
95. Vazquez, E. *et al.* (2017) Major human milk oligosaccharides are absorbed into the systemic circulation after oral administration in rats. *Br. J. Nutr.* 117, 237–247
96. Xiao, L. *et al.* (2019) Human milk oligosaccharides promote immune tolerance via direct interactions with human dendritic cells. *Eur. J. Immunol.* 49, 1001–1014
97. Shams-Ud-Doha, K. *et al.* (2017) Human milk oligosaccharide specificities of human galectins. Comparison of electrospray ionization mass spectrometry and glycan microarray screening results. *Anal. Chem.* 89, 4914–4921
98. Noll, A.J. *et al.* (2016) Human DC-SIGN binds specific human milk glycans. *Biochem. J.* 473, 1343–1353
99. Cheng, L. *et al.* (2019) Human milk oligosaccharides and its acid hydrolysate LNT2 show immunomodulatory effects via TLRs in a dose and structure-dependent way. *J. Funct. Foods* 59, 174–184
100. Asakuma, S. *et al.* (2010) Effect of human milk oligosaccharides on messenger ribonucleic acid expression of Toll-like receptor 2 and 4, and of MD2 in the intestinal cell line HT-29. *J. Appl. Glycosci.* 57, 177–183
101. Eiwegger, T. *et al.* (2004) Human milk–derived oligosaccharides and plant-derived oligosaccharides stimulate cytokine production of cord blood T-cells in vitro. *Pediatr. Res.* 56, 536–540
102. Speca, S. *et al.* (2014) Peroxisome proliferator–activated receptor gamma in the colon: inflammation and innate antimicrobial immunity. *J. Clin. Gastroenterol.* 48, S23–S27
103. Newburg, D.S. *et al.* (2004) Innate protection conferred by fucosylated oligosaccharides of human milk against diarrhea in breastfed infants. *Glycobiology* 14, 253–263
104. Payne, D.C. *et al.* (2015) Epidemiologic association between FUT2 secretor status and severe rotavirus gastroenteritis in children in the United States. *JAMA Pediatr.* 169, 1040–1045
105. Olivares, M. *et al.* (2015) Human milk composition differs in healthy mothers and mothers with celiac disease. *Eur. J. Nutr.* 54, 119–128
106. van Leeuwen, S.S. (2019) Challenges and pitfalls in human milk oligosaccharide analysis. *Nutrients* 11, 2684
107. Lackey, K.A. *et al.* (2019) What's normal? Microbiomes in human milk and infant feces are related to each other but vary geographically: the INSPIRE study. *Front. Nutr.* 6, 45
108. Lewis, C.R. *et al.* (2021) Family SES is associated with the gut microbiome in infants and children. *Microorganisms* 9, 1608
109. Grzeskowiak, L. *et al.* (2012) Distinct gut microbiota in southeastern African and northern European infants. *J. Pediatr. Gastroenterol. Nutr.* 54, 812–816
110. Li, J. *et al.* (2020) Comparative study of food allergies in children from China, India, and Russia: the EuroPrevall-INCO surveys. *J. Allergy Clin. Immunol. Pract.* 8, 1349–1358
111. Li, A. *et al.* (2021) The human milk oligosaccharide 2′-fucosyllactose attenuates β-lactoglobulin–induced food allergy through the miR-146a–mediated toll-like receptor 4/nuclear factor-κB signaling pathway. *J. Dairy Sci.* 104, 10473–10484
112. Yan, F. *et al.* (2021) MicroRNA miR-146a-5p inhibits the inflammatory response and injury of airway epithelial cells via targeting TNF receptor-associated factor 6. *Bioengineered* 12, 1916–1926
113. Han, S. *et al.* (2018) miR-146a mimics attenuate allergic airway inflammation by impacted group 2 innate lymphoid cells in an ovalbumin-induced asthma mouse model. *Int. Arch. Allergy Immunol.* 177, 302–310

114. Wang, J.J. *et al.* (2018) Effect of *Lactobacillus acidophilus* KLDS 1.0738 on miRNA expression in in vitro and in vivo models of β-lactoglobulin allergy. *Biosci. Biotechnol. Biochem.* 82, 1955–1963
115. Sodhi, C.P. *et al.* (2021) The human milk oligosaccharides 2'-fucosyllactose and 6'-sialyllactose protect against the development of necrotizing enterocolitis by inhibiting toll-like receptor 4 signaling. *Pediatr. Res.* 89, 91–101
116. Lane, J.A. *et al.* (2013) Transcriptional response of HT-29 intestinal epithelial cells to human and bovine milk oligosaccharides. *Br. J. Nutr.* 110, 2127–2137
117. Black, A. *et al.* (2012) Developmental regulation of Th17-cell capacity in human neonates. *Eur. J. Immunol.* 42, 311–319
118. Chen, Y.S. *et al.* (2020) IL-17–producing γδ T cells protect against *Clostridium difficile* infection. *J. Clin. Invest.* 130, 2377–2390
119. Ramirez-Carrozzi, V. *et al.* (2011) IL-17C regulates the innate immune function of epithelial cells in an autocrine manner. *Nat. Immunol.* 12, 1159–1166
120. Hesselmar, B. *et al.* (2018) Pet-keeping in early life reduces the risk of allergy in a dose-dependent fashion. *PLoS One* 13, e0208472