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Review Article

The Science of Human Milk Oligosaccharides (HMO) for Developing Immunity and Healthy Digestive System in Newborn Infants Throughout Life

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Abstract

Human milk oligosaccharides (HMO) are the third most significant solid component in human milk, working in conjunction with other bioactive components. Numerous factors, including secretor status, race, geography, climate, season, maternal nutrition and weight, gestational age, and delivery method, significantly affect the individual HMO levels and distribution among mothers. In addition to strengthening the epithelial barrier, producing immunomodulatory metabolites, and promoting a gut microbiota rich in Bifidobacterium, HMO also help to enhance the gastrointestinal barrier. HMO perform a range of physiological roles, such as possible immune system support, brain growth, and cognitive function. HMO supplementation to infant formula is safe and supports the newborn's healthy growth, with benefits for infection prevention and the makeup of the microbiota. Through a thorough and methodical evaluation of relevant literature, this study investigated the complex interactions between gut microbiota, the immune system, and HMO in neonates. A sizable corpus of recently released original research publications and thorough review papers were examined in the review. SCOPUS, PubMed, and Google Scholar were reliable and strong sources of information. In addition to these, a few more trustworthy sources were consulted. By reading this article, readers will have a clear understanding of how HMO play a crucial role in influencing the dynamics of the gut microbiota and supporting the development of the immune system in newborns. The knowledge gained from these exchanges may help direct measures meant to improve the health of newborns. However, further investigation is necessary to identify certain underlying processes and possible treatment paths. It is unknown if HMO provide an extra clinical advantage over non-human oligosaccharides due to a lack of research comparing the effects of the two. Better study of the variables controlling HMO composition and their functions will assist to comprehend their short- and long-term advantages for Immunity and Healthy Digestive System in Newborn Infants Throughout Life.

Keywords

Human Milk Oligosaccharide, HMO, Human Milk, Breastfeeding, Microbiota, Immunity, Digestive System

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1. Introduction

Breastfeeding is recommended by the World Health Organization (WHO) and pediatric societies within the first hour of life. Additionally, throughout the first six months of life and for a maximum of two years beyond that, breastfeeding should be the only method utilized [1-3]. Human milk is the sole suggested food source for newborns since it is unique, happens naturally, and is best suited to support vital growing processes in infancy. In addition to providing essential nutrients, human milk is rich in bioactive compounds that promote healthy growth and development, preserve a balanced microbiota, and bolster an infant's immune system [4-6]. There are various health advantages associated with breastfeeding and human milk, for both moms (reduced risks of hypertension, type 2 diabetes, breast and ovarian cancer) and their babies (short- and long-term). Short-term benefits include fewer cases of diarrhea, pneumonia, otitis media, atopic dermatitis, and sudden infant death syndrome; long-term benefits include fewer cases of type 2 diabetes, leukemia, autistic spectrum disorders, and obesity; and beneficial effects on IQ and social behavior [5, 7-13].

Human milk and nursing provide several health benefits for both mothers (lower risks of hypertension, type 2 diabetes, breast and ovarian cancer) and their offspring (short- and long-term). Reductions in diarrhea, pneumonia, otitis media, atopic dermatitis, and sudden infant death syndrome are among the short-term advantages; reductions in type 2 diabetes, leukemia, obesity, and autism spectrum disorders are among the long-term advantages; and improvements in IQ and social behavior are among the other advantages [5, 7–13].

Among the physiological functions that HMO carry out in babies are the formation of a healthy gut microbiota, strengthening of the gastrointestinal barrier, prevention of infections, and potential support for the development of the immune system, brain, and cognitive capacities [4-6, 14-19]. This review aims to give an overview of recent studies on the functional effects of HMO, such as how they promote the development of a balanced immune system, inhibit pathogen adhesion, support the growth of a healthy gut microbiome, and impact brain development and cognitive function over the course of a person's life.

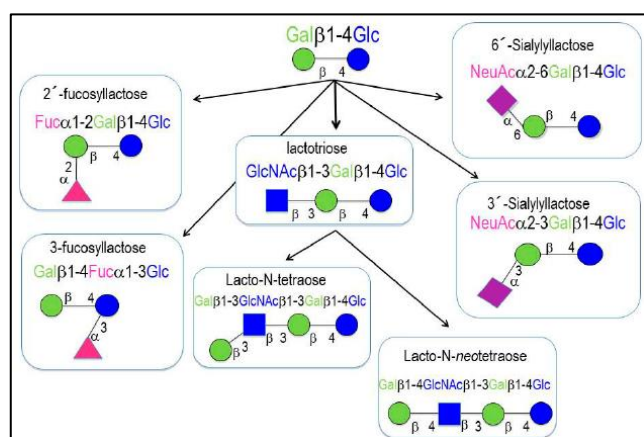# 2. Human Milk Oligosaccharides (HMO): Composition and Associated Elements

Several structurally distinct oligosaccharides—carbohydrates that are indigestible to humans—are found non human milk. Compared to animal milk, human milk has far more oligosaccharides. Despite having little nutritional value for the baby, human milk oligosaccharides (HMO) are the third most significant solid component in human milk after lipids and lactose [4-6, 20, 21]. There are currently more than 200 structurally distinct HMO known [20, 22]. HMO tolerate both heat and cold and remain hence untouched by pasteurization and freeze-drying [23]. HMO are resistant to the low pH of the stomach as well as brush border and pancreatic enzymes. Most HMO are either excreted intact or broken down by the baby's gut bacteria. One to two percent of the HMO that are consumed are absorbed, enter the bloodstream, and are removed via urine [14].

HMO are non-digestible, unconjugated, multifunctional glycans. N-acetylglucosamine, galactose, glucose, fucose, and the sialic acid derivative N-acetyl-neuraminic acid are the five monosaccharide building blocks that make up HMO [14, 15, 24].

To date, over two hundred distinct HMO have been recognized. HMO are made up of the monosaccharides glucose (Glc), galactose (Gal), fucose (Fuc), N-acetylglucosamine (GlcNAc), and sialic acid (Sia). Lactose is present at the reducing end of all HMO, and it can be extended by adding N-acetyllactosamine (Gal_1-4GlcNAc-, type 2 chain) or _1-3- or _1-6-linked lacto-N-biose (Gal_1-3GlcNAc-, type 1 chain) [25]. The chain appears to be broken upon elongation with lacto-N-biose, but N-acetyllactosamine can be further prolonged by adding either of the two disaccharides. Chain branching occurs when two disaccharide units connect together via a _1-6 linkage. Iso-HMO are branched structures, and para-HMO are linear structures without branches. Elongated oligosaccharide chains or lactose can be sialylated with _2-3 or _2-6 connections, or fucosylated with _1-2, _1-3, or _1-4 linkages. Certain HMO, such as lacto-N-fucopentaose and sialyllacto-N-tetraose, exist in several isomeric forms [25]. It has been reported that HMO with more than 15 disaccharide units form intricate structural backbones that can be further altered by adding Fuc or/and Sia [26, 27]. Three criteria (Figure 1) are used to classify HMO [26, 27]:

a) Fucosylated or neutral HMO, such as lactodifucopentaose and 20-fucosyllactose (20-FL), are neutral and contain fucose at the terminal position. They make up between 35% and 50% of the entire HMO composition.

b) Neutral N-containing (nonfucosylated) HMO make up 42% to 55% of the total HMO content. They are neutral and have N-acetylglucosamine at the terminal position (e.g., lacto-N-tetraose). More than 75% of the HMO in human breast milk are neutral HMO.

c) Sialylated acid Acidic in nature, HMO have sialic acid at the terminal position (20-sialyllactose, for example). They account for between 12% and 14% of the entire HMO composition.

Figure 1. Human Milk Oligosaccharides (HMO) Basic Science.

Figure 2. Main lymphocyte populations of the gut-associated lymphoid tissue (GALT).

HMO composition and levels fluctuate during breastfeeding as well. Although the concentration of HMO in colostrum can reach up to 20–25 g/L, once milk production reaches maturity, HMO concentrations drop to 5–20 g/L, which is still higher than the concentration of total milk protein [25]. The HMO concentrations in preterm mothers' milk are higher than those in term milk [25], but the fucosylated HMO levels in preterm milk are lower than those in term milk [28], and the neutral and acidic HMO are the same in both preterm and term milk [29].

3. The Intestinal Immune System-Gut-Associated Lymphoid Tissue (GALT)

The processing of antigens that interact with the intestinal mucosa and the immune response's dissemination are the responsibilities of the intestinal immune system, sometimes referred to as gut-associated lymphoid tissue (GALT) [30]. In the intestine, lymphocytes are found in two main locations: the inductive sites, which are the sites where an antigen stimulates the immune system and triggers the immune response; Peyer patches are the most common examples of these inductive sites; and the effector sites, which are the sites where the immune response is carried out and completed. Additionally, the gut has two primary populations of lymphocytes: the intraepithelial lymphocytes (IELs), which are found among the enterocytes along the villus, and the lymphocytes of the lamina propria (LPL), which are found in the internal portion of the villus. It is important to highlight that, in addition to the Peyer patch lymphocytes (PPLs), the peritoneal lymphocytes, namely the B1 cells, are important progenitors of one population of plasmatic cells found in the lamina propria. Consequently, the two main inductive populations that can be distinguished at the intestinal level are the B1 cells, which are located in the peritoneum, and the B2 cells, which are found in the Peyer patches (Figure 2) [31].
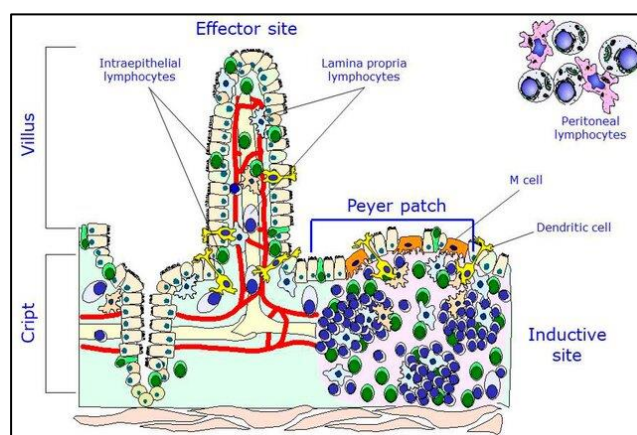
The M cells are located among the enterocytes in the epithelium and are responsible for processing and delivering the antigens found in the intestinal lumen into the Peyer patches. After entering the Peyer patches, the antigens interact with antigen-presenting cells (APCs), which are in charge of presenting the antigens to B and T lymphocytes that are still developing and dwell in the interfollicular regions as well as the germinal centers. Activated by antigens, the immature B and T lymphocytes leak down the lymph nodes and enter the circulation via the thoracic duct. They may circulate for a few days after developing into mature effector cells that move to the lamina propria or memory cells that return to the Peyer patches [30, 31]. It has been shown that the so-called dendritic cells, which are present in the Peyer patches and lamina propria, produce pseudopods and interact directly with antigens in the intestinal lumen. After that, they deliver the antigens to other underlying cell lineages without requiring M cells to digest them [32, 33]. IELs include a distinct subpopulation of effector cells that could have different interactions with antigens that enter the gastrointestinal tract than those mentioned above. The roles of a unique class of cells called innate lymphoid cells (ILCs) have just lately come to light [34]. ILCs are present in the colon and other mucosae and have a role in inflammation, autoimmune diseases, and tissue homeostasis, despite their main function being to build the gut barrier. Due to their capacity to interact with a wide variety of receptors present in intestinal immune cells, HMO may have positive effects [35].

4. Beneficial Effects of HMO in Developing Life-Long Immunity and Healthy Digestive System in Infants

Because humans lack the sialidases and fucosidases needed to break down HMO, these compounds enter the colon undigested, where they are processed by intestinal microbiota bacteria. In this sense, HMO are prebiotics since they promote

the growth of a healthy microbiota. Moreover, it has been shown that HMO provide additional benefits to the host; the three main results are shown below.

4.1. Role of HMO in Inhibition of Microorganism Adhesion to the Intestinal Mucosa

The formation of the gut microbiota ecology is a complex and dynamic process that is shaped by internal and external variables that impact variability. An immediate effect at birth lasts for several years through subsequent developmental stages. Streptococcus and Staphylococcus species are the most often identified bacterial genera in human milk, with Bifidobacterium, Lactobacillus, Propionibacteria, Enterococcus, and Enterobacteriaceae family members following [36, 37]. During the early stages of life, a variety of external variables can affect the composition and form of the microbiota. These factors include the mode of delivery, feeding strategy, ambient factors, antibiotic exposure, and ingestion of functional foods [38]. Immune system coevolution with the microbiota throughout newborn life allows the host and bacteria to coexist in a mutually beneficial relationship [38]. Metabolic diseases are linked to malfunction of the innate and adaptive immune systems. Certain cytokines, such as TNF-_ and IL-1, have been shown to increase insulin resistance, which raises the risk of diabetes [39] and metabolic inflammation [40]. Similarly, LPS-binding proteins and lipoproteins carry Gram lipopolysaccharide (LPS) components [41], which circulate in the blood and exacerbate inflammation [38]. HMO may provide breastfed children with protection against microbial infections because they share structural similarities with the glycoconjugates that microbes employ on their cell surfaces [42-44]. In addition to other protective processes, experimental results show that oligosaccharides can improve the protective gut microbiota, regulate microbial adherence and invasion of the infant intestinal mucosa, and improve cell signaling and cell-to-cell recognition events [45-49]. Most enteric pathogens require cell surface glycans to identify and adhere to their target cells, a crucial first step in the pathogenesis process. Fumylated HMO have been demonstrated to prevent (i) the binding of several pathogens to intestinal cells, such as Helicobacter pylori [52], Campylobacter jejuni [50], and Norwald-like virus [51], and (ii) the heat-stable enterotoxin of Escherichia coli [53]. The addition of HMO was tested in T84 cell membranes to see if it inhibited Escherichia coli, a source of enterotoxins. The application of HMO decreased E. coli guanylate cyclase activity and the generation of cyclic GMP in these cells [54]. Urinary tract infections are caused by uropathogenic E. Coli strains that express P-like (Prs) and P (Pap) fimbriae. These strains caused hemagglutination, which was reduced by HMO, especially the sialylated component [55]. It was determined if fractions of HMO could stop Vibrio cholerae, Salmonella fyris, and E. Coli serotype O119 from sticking to differentiated Caco-2 cells. The evaluated HMO reduced these infections' capacity

to attach to epithelial cells [56]. The human pathogen Pseudomonas aeruginosa's fucose-binding lectin PA-IIL may be inhibited by milk oligosaccharides by competing for the receptor and then binding [57]. More specifically, uropathogenic E. coli internalization into HMO-pretreated epithelial cells was significantly reduced without any interaction to these cells [58]. HMO generated from combined human milk significantly reduced the adherence of enteropathogenic E. Coli strain 2348/69 (serotype O127:H6) to cultivated epithelial cells [59]. Similarly, dose-dependent treatment of HMO reduced the penetration of C. albicans into human preterm intestinal epithelial cells [60]. Trophozoites need to stick to the host's mucosa in order to colonize and invade. Human intestinal epithelial HT-29 cells are protected against E. histolytica-induced destruction in a dose-dependent manner by HMO. Furthermore, HMO reduce E. histolytica attachment and cytotoxicity; in fact, pooled HMO detach E. histolytica by over 80% [61].
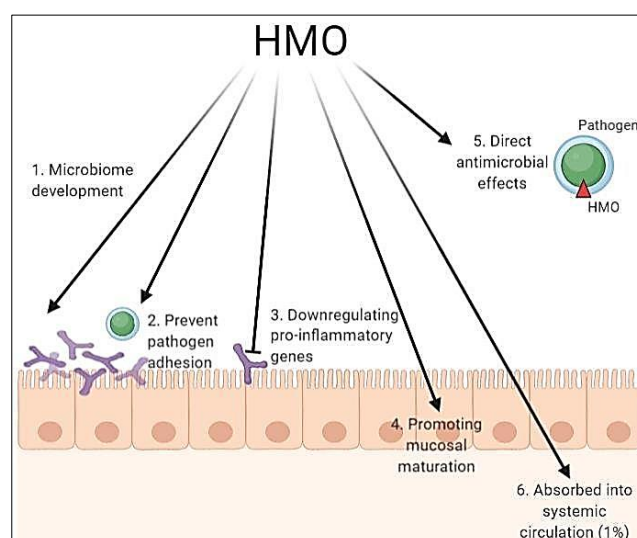
Figure 3. Functions of Human Milk Oligosaccharides (HMO).

4.2. Role of HMO in Gastrointestinal Contractility

An in vitro model of murine colon peristalsis highlights the significant influence of many HMO on gastrointestinal motor contractions. Fucosyllactose (2'-FL) and 3'-fucosyllactose (3'-FL) reduced contractility in a concentration-dependent manner [62]. Within five to ten minutes of administration, fucose and fucosylated molecules had a noticeable influence on colon smooth muscle contractility. The idea that these HMO effects are the result of bifidobacteria activation is unlikely. Rather, they represent a direct impact on neuron-dependent motor complexes [62]. Current clinical research indicates a specific interaction between fucose and/or fucosylated HMO and tissue receptors, which regulates intestinal motility and may have antinociceptive effects. These

results support the hypothesis that fucosylated HMO have beneficial effects on the central nervous system and may be useful in the treatment or prevention of disorders affecting intestinal motility or pain.

4.3. HMO and Its Protective Effect in Allergies

Researchers are starting to look at how HMO could shield the body against allergens. It has been proven that feeding with a milk rich in 2'FL reduces the incidence of allergy and dermatitis associated with IgE at the age of 2 [63-65], even for infants born by Caesarean procedure. Compared to healthy neonates, the bifidobacteria flora of allergic babies is very different. This might potentially be explained by a shortage of certain HMO due to maternal FUT2 polymorphisms [63–66].

It has been shown that HMO influence how human epithelial cells respond to allergic disorders. In particular, 6-sialyl-lactose (6'-SL) may alleviate the symptoms of food allergies by preventing the production of inflammatory chemokines, which may stop inflammatory cells from invading the stomach. In a food allergy murine model, the effects of two HMO, 2' FL and 6'-SL, on anaphylactic symptoms brought on by oral ovalbumine (mice that have grown sensitive to ovalbumine) have been studied. Among the food allergy symptoms that have improved with daily oral treatment with 2'-FL or 6'-SL are diarrhea and hypothermia. According to the findings, 2'-FL and 6'-SL reduce symptoms associated with food allergies by producing TIL-10(+) cells that regulate them and indirectly stabilizing mast cells [67].
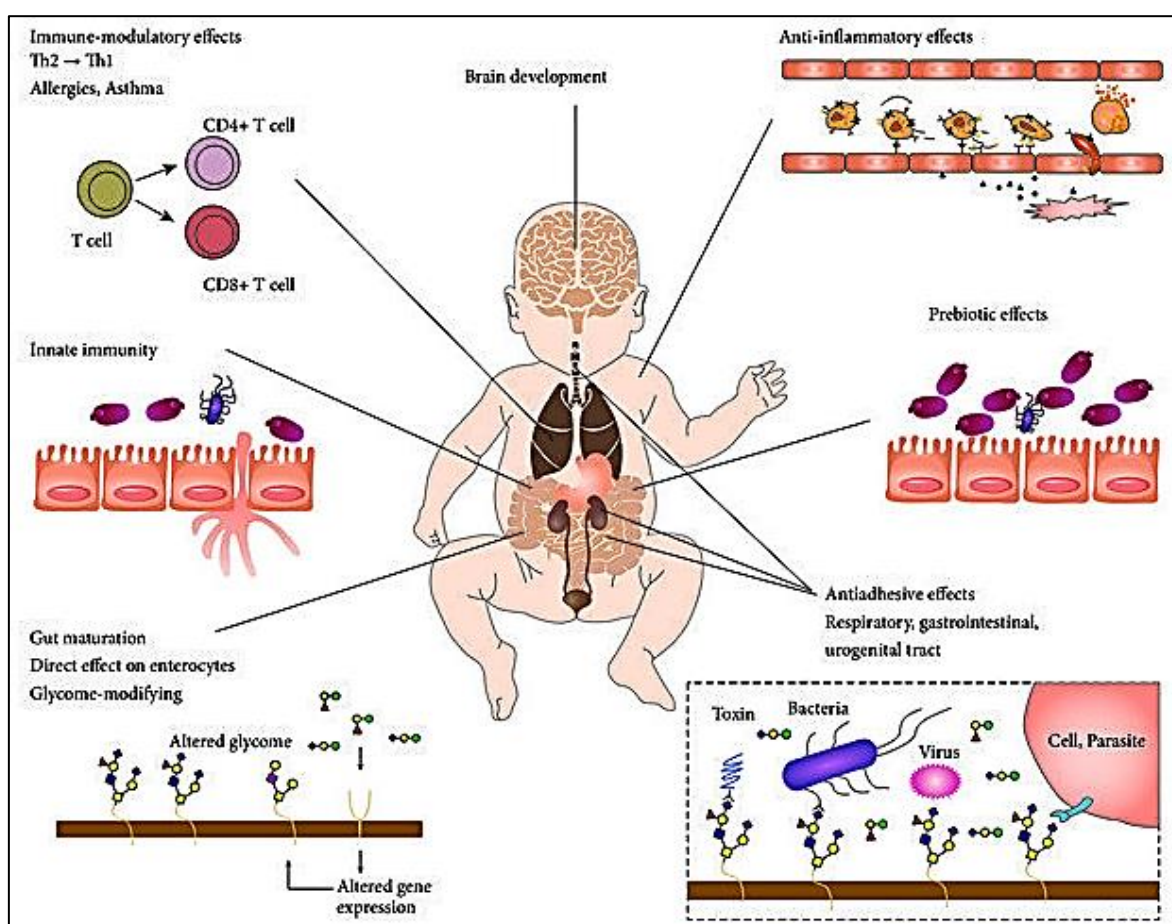
Figure 4. Human Milk Oligosaccharides HMO may serve as prebiotics, immune-modulators, and signaling molecules to enhance the gut immunity of newborns.

4.4. HMO in Prevention of Necrotizing Enterocolitis in Newborns

A very important application sector for HMO is prevention of necrotizing enterocolitis in infants. Studies using rats as animal models are promising [68]. A human cohort investiga-

tion revealed that disialyllacto-N-tetraose, or DSLNT, may be a more pertinent HMO in reversing this severe pathology: [69].

5. Conclusions and Future Perspectives

Neonatal immunity may be modulated by the broad diver-

sity of HMO in both innate and adaptive ways. HMO directly interacts with gastrointestinal epithelial cells, mucosal immune cells, and systemic immune cells to influence immune function, according to data from in vitro investigations and animal models. Additionally, breastfeeding infants' microbiomes are positively shaped by HMO. The addition of 2′FL alone or in conjunction with LNnT to infant formulae has recently occurred due to the growing availability of HMO from commercial sources and the mounting evidence showing that formula supplemented with HMO is safe and may impart benefits for human newborns. HMO may also be helpful for other demographic segments that have weakened immune systems or are at a greater risk of infection because of its positive effects on host defense and immunological function. There are few studies where HMO has been fed to humans or animals. Furthermore, the effects of feeding complex mixes of HMO on the immune response have not been well studied. Therefore, more study is required to identify the processes and completely grasp the potential of HMO to enhance the immune system in babies.

Abbreviation

IQ: Intelligence Quotient
GALT: Gut-Associated Lymphoid Tissue
IELs: Intraepithelial Lymphocytes
LPL: Lymphocytes of the lamina propria
PPLs: Peyer patch lymphocytes
APCs: Antigen-Presenting Cells
ILCs: Innate Lymphoid Cells
LPS: Lipopolysaccharide
FUT2: Fucosyltransferase 2
DSLNT: Disialyllacto-N-tetraose

Conflicts of Interest

The authors declare no conflicts of interest.

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