Human Milk Oligosaccharides Important for Infant Defense

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Abstract

Human milk is the “gold standard” nutrition for infants. Human milk contains all necessary nutrients needed for infant growth and developments. Human milk oligosaccharides (HMOs) are important functional ingredients found in human milk, in addition to standard nutrients. They have complex structure and represent third predominant components in human milk. Functions of HMOs are not fully understood but it is thought that they play an important role in the development of immune system, the prevention of pathogenic infection and, in the modulation of infant gut microbiota. HMOs are indigestible to human digestive enzymes and act as prebiotics. Study shows that HMOs favors promoting the colonization of Bifidobacteria, which is found to be beneficial and major microbiome in the gut of breast-fed infants. HMOs show anti pathogenic effects and may protect infants from different pathogenic microorganisms such as, Campylobacter jejuni, Campylobacter pylori, Escherichia coli, Vibrio cholera and Rotavirus. HMOs may provide specific and/or non-specific immune defense to infants via various mechanisms. They may shape the growth of intestinal microflora, may prevent the attachment of pathogenic microorganisms to intestinal cells and may influence inflammatory processes by reducing leukocyte binding to endothelial cells.

In vivo defense functions of HMOs in the human milk-fed infants have not been established yet. Future research may focus on to elucidate and verify the beneficial effects of HMOs for the breast-fed infant, as well as impact of HMOs to the health of the breast-feeding mother and the composition of other milk components.

Keywords: Milk, Human; Oligosaccharides; Infant

Introduction

Human milk is considered as the “gold standard” nutrition for the first few months of human baby. Infants consuming their mother’s milk were found to have a several-fold lower risk of diarrheal disease, and a lower risk of respiratory infections as well as other types of infectious disease [3]. Human milk consists of all necessary nutrients which are needed for grow and development of infants, in addition, it contains some functional ingredients that provide health benefits beyond traditional nutrients. Human milk oligosaccharides (HMOs) are important parts of these functional ingredients [1].

HMOs represents the third largest solid component (after lactose and lipids) in breast milk, with maximum concentration is in the colostrums (about 20–23 g/L) and, after a couple of weeks about 12–14 g/L in mature milk [4]. The linear and branched HMOs vary in size, from 3 to 32 sugars, composed of five building block monosaccharides (Figure 1): D-glucose (Glc), D-galactose (Gal), N-acetylglucosamine (GlcNAc), L-Fucose (Fuc), and Sialic acid. All HMOs have a core structure consisting of lactose at the reducing
Figure 1: Structure of HMOs (adapted from Newburg and Grave 2014 [5])

end [1].
So far ~200 different types of HMOs have been identified, of them 80 have been fully characterized [6]. It is found that not every woman synthesizes the same type of oligosaccharides and the structural diversity of milk oligosaccharides varies from mother to mother, even varies during lactation for each mother. Mainly tri- to penta-saccharides are produced in the first weeks after parturition, whereas the amount of longer structures increases over time [7].

Functions of HMOs are not fully understood but it has been postulated that HMOs play an important role in the development of immune system, the prevention of pathogenic infection and, in the modulation of infant GI tract to bifidogenic microbiota [3, 8, 9]. The potential health benefits of HMOs have been studied over the years with a special emphasis on prebiotic, immune and other protecting effects. In the present review I will briefly discuss some mechanisms how HMOs can protect infants from various pathogens.

Prebiotic Effects of HMOs: After birth, microbial colonization in infant gut undergoes a complex changing process. HMOs exert important prebiotic functions by promoting the colonisation of the intestine by commensal microbiota. With the availability of oxygen, aerobic microbes initially colonize the intestine of the newborn; however, as oxygen is consumed, the microbiota switches to anaerobic species. The main species of anaerobic bacteria is bifidobacteria; other species such as bacteroides and clostridia also found, but in lesser extent [10]. Bifidobacteria, species commonly found in the feces of breastfed infants. Several families of bacteria including bifidobacteria, express glycosidase enzymes that are able to cleave milk oligosaccharides and use the resulting monosaccharides as a carbon source [11]. Evidence suggests that the catabolic capability of HMOs plays a key role in modulating the bacterial population in gut. It is found that, Bifidobacterium infantis ferment purified HMO as a sole carbon source and colonize, which support the hypothesis that HMO selectively amplify bacterial populations in the infant intestine [12]. In another study [13] HMOs were isolated from human milk and used as the sole source of fermentable carbohydrate in growth medium of different strains of bifidobacteria.

Result shows that not all strains of bifidobacteria can use HMOs as a sole carbon source and HMOs may selectively promote the growth of certain bifidobacteria strains, commonly found in the feces of breastfed infants, which are shown beneficial for them. Although specific bifidobacteria are able to consume HMOs effectively, different species appear to have developed different strategies for using HMOs as a growth substrate [9]. The prebiotic effects of HMOs are probably best known and most referenced. However, about 90% of all HMOs are found intact and not metabolized in the infant’s feces [1]. This suggests that HMOs have additional effects other than just serving as prebiotic “fuel” to establish and maintain a certain desired microbiota composition.
Anti-pathogenic Effects: Most pathogenic microorganisms such as, *Campylobacter jejuni*, *Escherichia coli*, *Vibrio cholera*, *Shigella* and *Salmonella* strains must have to bind their specific receptors in host epithelial cell surface, and this binding is necessary for their pathogenic nature. The receptor that expressed by the virulence pathogens are commonly known as lectins (they are glycan-binding proteins), binds to oligosaccharides found on the epithelial cell surface of host. Some of these glycan-binding determinants are also part of HMOs, suggesting that HMOs may serve as soluble ligand analogs, block pathogen adhesion, and protect the breastfed infant against infections and diarrhea [3]. The most common cause of infant mortality is diarrhea and *Campylobacter jejuni* is the most common cause of bacterial diarrhea which is also the primary cause of motor neuron paralysis. This bacterium binds to intestinal 2′-fucosyllactosamine moiety, which is also present in HMOs. Ruiz-Palacios *et al.* [14] showed that fucosyloligosaccharide in human milk were ample to inhibit *campylobacter* binding in vitro, in human intestine ex vivo, and in mice in vivo. They showed that α-1,2-linked fucosyloligosaccharides, present in HMOs, serve as soluble ligands that compete with intestinal epithelial cell surface receptors thereby inhibiting binding of *campylobacter* to its intestinal receptors, the essential first step of its pathogenesis. In another study, Newburg *et al.* [15] showed that Human milk fucosylated oligosaccharides decrease risk of diarrhea, caused by stable toxin of *E. coli* in breastfed infants. These results strongly suggest that oligosaccharides in human milk contribute to protection of infants against *C. jejuni* and other enteric pathogens. Furthermore, milk oligosaccharides protect against bacterial enterotoxins such as cholera toxin [16] by competing with cell-bound glycan receptors. Also inhibit the adhesion of *Listeria monocytogenes* [17] and *Streptococcus pneumonia* [18] to the intestinal epithelium. The neutralizing effect of milk oligosaccharides is not limited to bacteria, milk oligosaccharides also act as soluble receptors for several viruses. Fucosylated milk oligosaccharides also bind to several strains of norovirus, thereby preventing the docking of the virus to cellular glycan receptors [19] and hence the development of acute gastroenteritis.

According to the UNAIDS there were 2.3 million new HIV infections globally and about 0.26 million children were newly affected in 2012 [44]. Mother-to-child transmission accounts for more than 40% of all HIV-1 infections in children, with breast-feeding being the predominant route of postnatal transmission [20]. Breastfed infants from HIV-1 infected mothers are constantly exposed to the virus over a period of many months. It is currently unknown why a vast majority of these infants does not become HIV-infected. Recent data suggests that HMOs reduce HIV-1 binding to dendritic cell and reduce HIV-1 mother-to-child transmission [21].

Intestinal epithelial cell surface glycans (known as “glycocalyx”), which are the attachment sites for most pathogens and commensal bacteria, needed for their interaction with cell. Angeloni *et al.* [22] carried out an in-vitro study and showed that HMOs may also play an important role in modified expression of these glycocalyx. They showed for the very first time that Caco-2 cells change their cell surface glycan profile upon exposure to 3′-sialyllactose, one of the major oligosaccharides in human milk. Upon exposure to 3′-sialyllactose, cell surface expression of α-2-3 and α-2-6-linked sialic acid residues was significantly reduced. Fucose and galactose residues were also diminished. To evaluate the significance of these cell surface glycome changes in the context of bacteria-host interactions, they also assessed whether adherence of enteropathogenic *Escherichia coli* (EPEC) is altered or not. EPEC adheres to epithelial cell surface glycans in the host’s intestine. Indeed, 3′-sialyllactose-induced changes in the epithelial cell surface glycome led to a 90% reduction in EPEC adhesion compared to control cells. These results suggest a novel mechanism by which milk oligosaccharides, such as 3′-sialyllactose, regulate bacteria host interactions.

Systemic and Innate Immune Effects: HMOs has been found in the urine of milk-fed infants which implies that an intestinal absorption of HMOs may occur [23]. These data were furthermore supported by in vitro studies on Caco-2 cells demonstrating that
neutral HMOs are transported across the intestinal epithelium by receptor-mediated transcytosis and paracellular pathways, whereas acidic HMOs cross the intestinal lining via paracellular routes [24]. Severe tissue damage occurs in a variety of inflammatory diseases and one of the major causes of such damage is excessive leukocyte infiltration [25]. Leukocyte infiltration occurs by selectins (act as a receptor) on activated endothelium and their oligosaccharide ligands on leukocytes. HMO also contains binding determinants for selectins and competes with leukocytes to adhere to endothelial cells and exert the potential to affect leukocyte rolling. Bode et al. [26] have shown that the adhesion of monocytes, lymphocytes, or neutrophils isolated from human peripheral blood passing over TNF-α-activated endothelial cells was reduced by up to 50% using sialylated HMO. These results indicate that specific oligosaccharides in human milk may serve as anti-inflammatory components and might therefore contribute to the lower incidence of inflammatory diseases in human milk-fed infants.

In a study Terrazas et al. [27] HMOs leads to increase the percentage of IFN-γ producing immune cells and the IL-13 production. Therefore, HMOs may influence lymphocyte maturation in breast-fed newborns.

Necrotizing enterocolitis (NEC) is one of the most common and often fatal disorders in preterm infants [28]. About 5-10% infants born with very low weight (<1500 g) develop NEC and more than a quarter of them die, however, who survives face severe neurological complications [29]. The etiology and pathogenesis of NEC are poorly understood and there are currently no biomarkers to identify neonates at risk to develop NEC. Treatment options also very limited with surgical resection of the necrotic intestine often the last remaining option [30]. Studies found that, breast-fed infants had lower incidence of NEC (about 6–10-fold lower risk) to develop NEC than formula-fed infants [31, 32]. As infant formula devoid of complex HMOs, researcher hypothesized that HMOs may contribute to the protective effects of breast-feeding against NEC. Test this hypothesis is not feasible in human, so Jantscher-Krenn et al. [33] developed a rat model of NEC and showed that HMOs indeed protect from NEC. Survival rates and pathology scores significantly improve when HMOs are added to the orally gavaged formula. They showed that disialyllacto-N-tetraose, an oligosaccharide found in human milk, responsible for the protection, which suggests a highly structure-specific and potentially host receptor-mediated effect.

Enteropathogenic E. coli is one of the major causes of diarrhea in infants and its pathogenesis is associated with two toxins, known as, labile toxin and stable toxin (ST). Labile toxin is homologous with cholera toxin and ST, which is stable to heat and organic solvents, inhibits chloride transport from the gut to the intestinal epithelial cell, thereby inhibiting electrolyte and water resorption from the gut, resulting in secretory diarrhea in susceptible hosts [34]. Newburg et al. [35] showed that, fucosylated oligosaccharides of human milk protect suckling mice from heat-stable enterotoxin of E. coli. They used suckling mice, as an animal model for ST pathology, which is sensitive to this toxin between 2 and 4 d of life. Mice fed with the toxin develop secretory diarrhea, which is lethal within several hours. Mice fed ST along with HMOs have a significantly reduced rate of death, providing evidence that HMOs has a potent ability to protect infants against lethal pathogens.

Campylobacter pylori infection is another major cause of bacterial diarrhea in infants. Study shows that breastfed infants have a reduced rate of diarrhea caused by Campylobacter pylori compared with those non breastfed [36]. To exert its pathogenicity campylobacter need to bind with the H-2 epitope that is expressed by human intestinal epithelium. 2′-fucosyllactose (2′-FL), found in human milk, has a structure which is analogous to H-2 epitope. To investigate whether 2′-FL inhibits campylobacter infection, Ruiz-Palacios et al. [14] developed a transgenic mouse model that expresses an increased H-2 epitope in their milk whereas the milk of wild-type (i.e., nontransfected) dams did not. Suckling mice were infected with campylobacter, and result shows that pups with transgenic mice had significantly lower level of campylobacter colonization than wild type. This demonstrates that, HMOs are capable of inhibiting binding by campylobacter to its host cell glycans, and inhibition of this binding may protect infants from enteric infection by virulent strains of campylobacter.

Rotavirus (RV) infection is the leading cause of gastroenteritis and viral diarrhea in infants and young children which accounts for 5% of all deaths in children aged less than 5 years [37]. Studies showed that breast-fed infants have a lower incidence of RV-induced acute gastroenteritis and diarrheal diseases compared to formula-fed infants [3, 38].

Figure 4: Synergistic effects of HMOs and intestinal microbiota against pathogens (adapted from Newburg and Walker 2007). The intestinal mucosal surface glycans are targets of pathogens, but can also be used as receptors for mutualist microbiota that colonize the gut and form a biofilm. Human milk glycans promote this colonization through their probiotic activity. The human milk glycans also bind to the pathogens, rendering them less able to bind to their receptors on the mucosal cell surface. These processes would provide multiple layers of synergistic defense of the intestine of breast-feeding infants [2].
In vitro and in situ study [39] with animal model shows that isolated HMOs inhibits RV infectivity in vitro and decrease rotavirus infectivity in acutely infected animal. In a very recent study Li et al. [40] showed that, HMOs shorten rotavirus-induced diarrhea, lead to significantly increased IFN-γ and IL-10 expression in the piglet intestine, modulated piglet mucosal immunity and showed prebiotic effects by changing colonic microbiota. Study results shows that HMOs may act both in systemic and/or non-systemic manner and protect infants from pathogens.

Along with lower risks of diarrhea it is found that breastfed infants suffer from less respiratory infections. Stepans et al. [41] tested the relationship between HMOs consumption, oligosaccharide content of feces, and subsequent disease in breastfed infants, to clarify, whether human milk glycans might also contribute to protection of the respiratory tract. The concentration of lacto-N-fucopentaose II (LNF-II) was measured in milk samples from wk 2 of lactation as a representative glycan. Cumulative risk of respiratory disease was measured at wk 6 and 12. Results shows that, high concentrations of lacto-N-fucopentaose II in milk were inversely related to subsequent risk of respiratory and enteric disease in breastfed infants. Thus, the protection afforded by human milk oligosaccharides to the breastfed infant is not limited to diarrhea; protection of the respiratory tract indicates that protection by human milk glycans may extend to other mucosal tissues.

Conclusion: Studies indicate that HMOs provide non-specific and possibly specific defense to infants. HMOs could have influence infants immunity through various mechanisms. They may affect the growth of intestinal microflora and may prevent the attachment of pathogenic microorganisms to intestinal cells by acting as receptor analogues of mucosal adhesion molecules. In addition to this, they might function as ligands for selectins that influence inflammatory processes by reducing leukocyte binding to endothelial cells [23]. They may also have effect on systemic immunity as HMOs found in circulation as well as in the urine of breast-fed infants. Whether these functions occur in vivo in the human milk-fed infant has not been established yet. One major roadblock is limited availability of HMOs which are needed to better understand the underlying mechanisms of action and to confirm that the observed effects translate to measurable health benefits for the neonate [30]. However, future research on HMOs will likely continue to elucidate and verify the beneficial effects of HMOs for the breast-fed infant, in addition to that, it will be intriguing to observe whether and how HMOs impact the health of the breast-feeding mother and the composition of other milk components.

It is well known that human milk contains a variety of immunological and anti-inflammatory components. The recent discoveries regarding the complex interactions of milk oligosaccharides with intestinal microbiota and the mucosal immune system have demonstrated that breast milk provides much more than just nutrients to infants [7]. Breast milk is definitively the gold standard for infant nutrition.

References:


