

Human milk oligosaccharides and infant health

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ABSTRACT

Human milk oligosaccharides (HMOs) have several biological activities beneficial for health maintenance and disease avoidance in human infants. They function as prebiotics and promote the growth of beneficial bacteria such as bifidobacteria, over pathogens which cannot metabolize HMOs. They act as soluble decoy receptors for pathogens or toxins. The HMOs bind either to the host cell receptor or to the pathogen, blocking the adhesion process and thereby protecting infants from infection. This antiadhesive function has been demonstrated in a large number of pathogens, both bacteria and viruses and this is particularly important in avoiding and limiting gastrointestinal diseases. As cellular response modifiers they impact upon brain development and food allergies.

INTRODUCTION

Mature human milk contains 5–20 g/L of a mixture of oligosaccharides known as human milk oligosaccharide (HMOs). The HMOs are the third most abundant solid component of human milk after lactose and lipids, yet have no nutritional value, since they are not digestible. No other mammal has comparable concentration or diversity of oligosaccharides in milk compared to human milk. However, numerous studies have pointed out that despite having no nutritional value, HMOs play a unique and important role in the development of the human infant.

It is recognised that breastfed infants experience decreased instances of diarrhoea, respiratory infection, urinary tract infection, ear infection, necrotizing enterocolitis and sudden infant death syndrome, compared to their formula-fed counterparts. Furthermore, the microbiome in breastfed neonates tends to be colonized to a lesser extent by infectious species such as *Escherichia coli*, *Clostridium difficile*, and *Campylobacter jejuni*. Many of these protective properties have been attributed to HMOs (1). The HMOs not only provide for protection against pathogens but also have a long-range effect in influencing the cognitive functions of the new-born. They appear in the bloodstream of the infant and may supply sialic acid that plays an important role in brain development (2).

The HMOs have an extremely wide spectrum of biological activities which are beneficial for health maintenance and disease avoidance in the human infant. They act as prebiotics, antiadhesives and as cellular response modulators. These biological activities also help explain the evolutionary significance of the production of such large quantities of oligosaccharides in the milk of human mothers (3).

HUMAN MILK OLIGOSACCHARIDES AS PREBIOTICS

The HMOs are not digested in the small intestine and a large proportion of them reach the colon where they are selectively metabolized by health-promoting bacteria such as bifidobacteria. This gives these species a competitive advantage over pathogens which cannot metabolize them. For example, none out of 10 Enterobacteriaceae strains tested, including several *E. coli* strains and one *Shigella dysenteriae* strain, were able to grow on the HMOs 2'-fucosyllactose (2'-FL), 6'-sialyllactose (6'-SL), and lacto-N-neotetraose (LNnT) (4). As a result of this selective metabolism, beneficial bacteria can grow and outcompete harmful pathogens.

Another advantage from the prebiotic effect of HMOs is the production of short-chain fatty acids. These compounds lower the pH of the gastrointestinal tract which further stunts the growth of many pathogenic species.

HUMAN MILK OLIGOSACCHARIDES AS ANTIADHESIVES

Human milk oligosaccharides act as antiadhesives by serving as soluble decoy receptors for pathogens or toxins. This activity is due to the resemblance of HMOs to various cell surface glycan receptors on the cells of the gastrointestinal tract or on the surface of pathogens. Consequently, pathogens bind to HMOs rather than to cell surface glycans thereby avoiding the binding of pathogenic species to epithelial cells which is often the first step of infection (3).

Confirmation of this concept is provided by results with *Campylobacter jejuni* where α -1,2 fucosylated HMOs were able to inhibit adherence of the pathogen to host cell receptors (5). In another study, human epithelial cells, were infected with a virulent strain of *C. jejuni* (6). Infected cells treated with an HMO attenuated 80% of *C. jejuni* invasion (6).

The invasive pathogen, uropathogenic *E. coli* (UPEC), causes urinary tract infections and remains a serious global health concern in infants (7). The bacterium adheres to and invades bladder epithelial cells. Treatment of epithelial cells with HMOs led to a rapid decrease in host cell cytotoxicity. This indicates that HMOs protect bladder epithelial cells from deleterious cytotoxic and proinflammatory effects of UPEC infection and may be one contributing mechanism underlying the epidemiological evidence of reduced incidence of urinary tract infections in breastfed infants.

Group B *Streptococcus* (GBS) are Gram-positive bacteria associated with pneumonia, septicaemia, and meningitis in the neonate. Human milk oligosaccharides were able to directly inhibit the growth of GBS (8). This inhibitory response

was bacteriostatic and not bactericidal because they did not kill GBS even at very high concentration.

Carbohydrate-lectin interactions also play an important role in viral infections (9). Many viruses, including human immunodeficiency virus (HIV), Ebola, dengue, cytomegalovirus, and hepatitis C, possess glycans that recognize C-type lectins for infection. Other viruses possess lectins on their surfaces that recognize glycan epitopes on human epithelial cells for infection. Human milk oligosaccharides are receptors for various viruses and therefore could block infections.

A curious, but potentially beneficial effect of HMOs, is in the transmission of HIV (10). Breastfeeding is the predominant transmission route for HIV infection in children. However, the majority of breastfed infants do not become HIV-infected, despite continuous exposure to the virus through their mothers' milk over many months. This raises the question: does human milk have properties that protect the infant against HIV transmission?

Human noroviruses are the leading cause of outbreaks of acute gastroenteritis. Norovirus interactions with histo-blood group antigens (HBGAs) are known to be important for an infection. Noroviruses also interact with HMOs, which mimic HBGAs and may function as receptor decoys. An HMO, 2'-fucosyllactose, blocked both the GI.1 and GI.17 noroviruses from binding to HBGAs. The HMO 2'-fucosyllactose functions as an antiviral against multiple norovirus genogroups (11).

The protozoa *Entamoeba histolytica*, is the third leading cause of death by parasitic diseases, surpassed only by malaria and

schistosomiasis. The parasite passes through the stomach and small intestine to reach the colon, where it can colonise and invade the host's mucosa and cause dysentery. Physiological concentrations of HMOs were able to inhibit *E. histolytica* attachment and cytotoxicity to enteric cell layers *in vitro* in a dose-dependent manner (12).

Bacterial toxins also exert their pathogenic effects by binding to the cells of the gastrointestinal tract. Enterotoxins from *Vibrio cholerae* and enterotoxigenic *E. coli* recognize monosialoganglioside 1 on the cell surface as a receptor. The toxins adhere to the intestinal mucosa, and cause diarrhoea. Human milk contains a large amount of sialic acid in oligosaccharides. The sialylated oligosaccharides behave as inhibitors against the enterotoxins produced by these bacteria (13).

In tissue culture assays, invasion of *Candida albicans* on premature intestinal epithelial cells was reduced when treated with physiologic concentrations of pooled HMOs. These results suggest that HMOs could help treat and/or prevent life-threatening candidiasis, particularly among extremely low-birth-weight preterm infants (3).

HUMAN MILK OLIGOSACCHARIDES AS CELLULAR RESPONSES MODULATORS

Human milk oligosaccharides can also act as signalling molecules directly interacting with the host. In tissue culture models, HMOs modulate gastrointestinal cell proliferation, differentiation, and apoptosis via receptor binding and mitogen-activated protein kinases (MAPK)- signalling (14). It is likely that the consistent exposure of an infant's intestinal

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epithelial cells to large amounts of HMOs (infants fed every 3–4 hours) promotes normal development of the gastrointestinal tract (15).

The rapid growth of infant brains places an exceptionally high demand on the supply of nutrients from the diet, particularly for preterm infants. Sialic acid is an essential component of brain gangliosides and sialic acid is a component of HMOs. Therefore, HMOs may provide sialic acids to the infant that are important in brain development. Low levels of HMOs similar in structure to those found in human milk are present in the blood of infants, which would be necessary to supply sialic acid to the developing brain (16, 17).

Food allergy is now extremely prevalent and studies suggest 8% of children may be affected. The foods that account for most allergic reactions are cow's milk, soybeans, wheat, eggs, peanut, tree nuts, finned fish, shellfish, and sesame. The effect of two HMOs, 2'-fucosyllactose (2' FL) and 6'-sialyllactose (6'-SL) on anaphylactic symptoms induced by oral ovalbumin were studied in an ovalbumin sensitized mouse model of food allergy (18). Daily oral treatment with 2'-FL or 6'-SL attenuated food allergy symptoms including diarrhoea and hypothermia.

In a subsequent study the HMOs modulated human epithelial cell responses related to allergic disease (19). 6'-sialyllactose in particular, may have additional benefits by inhibiting chemokine release that would in turn inhibit the influx of inflammatory cells to the intestine, potentially attenuating symptoms of food allergy. These findings encourage further investigation of the therapeutic potential of specific HMOs in food allergy.

CONCLUSIONS

Human milk oligosaccharides have multiple beneficial effects on infant health. With their combined activities as prebiotics, antiadhesion agents and cell modulating compounds they support health maintenance and disease avoidance in infants. Such a programme inevitably requires less antibiotic use and will also establish a good health status which persists through later life.

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