Human Milk Oligosaccharides support immune protection through the early life microbiome
Clinical observational studies with breastfed infants and interventional trials with formula fed infants in combination with basic research position HMOs as multifunctional innate breastmilk component.

HMOs shape the establishing early life gut microbiota and supposedly help the development of appropriate immune competence. This is particular evident in situations of an early life dysbiotic microbiota such as seen upon C-section birth.
The early-life gut microbiome establishes and matures sequentially during infancy and early childhood from an aerobic to an anaerobic milk-oriented early life microbiota to a more diverse adult like microbiota. Different phases of this maturation process can be characterized by a progression of microbiota communities, starting from Enterobacteriaceae, Streptococcaceae dominated communities over Bifidobacteriaceae dominated communities to increasingly diverse Bifidobacteriaceae and Lachnospiraceae dominated communities. The age-appropriate microbiota maturation is considered important for normal digestive, immune competence and metabolic development (1,2).

Most important determinants for the microbiota development are the initial seeding at birth, mode of delivery, antibiotic use and nutrition.

Human Milk Oligosaccharides (HMOs) are elongations of the milk sugar lactose by galactose, N-acetyl-glucosamine, fucose and sialic acid. The resulting structures resemble mucosal surface glycans and generally, the same enzymes are involved in mucosal glycosylation and HMO formation. The most prominent example to this end is the Fucosyltransferase Fut2 encoded by the Secretor gene.
Sequential maturation of the early-life microbiota

At birth (Initial colonization) vs Introduction to oral feeding (Increasing diversity)

Microbiota taxa over the first years of life

- Bifidobacteriaceae
- Lachnospiraceae
- Ruminococcaceae
- Prevotellaceae
- Enterobacteriaceae
- Veillonellaceae
- Bacteroidaceae
- Clostridiaceae
- Lactobacillaceae

Adapted from Arrieta et al. Frontiers Immunol. 2014

Bacterial variability

Interindividual variability

Early life gut microbiota communities (~enterotypes)

Dominant taxa:
- Enterobacteriaceae
- Streptococcaceae
- Bifidobacteriaceae 80%
- Bifidobacteriaceae 92%
- Bifidobacteriaceae 46%
- Lachnospiraceae

Transitions:
- No change
- Progression
- Regression

Fecal microbiota community types

Arrieta et al. Frontiers Immun. 2014; Berger, NRC, unpublished re-analysis of Dogra et al. 2015, mBIO
Clinical observational studies with breastfed infant-mother dyads associate specific HMO or HMO groups, like those containing α-1,2-linked fucose, with gut microbiota (3-5), infections (6-8) and allergies (9-11). These observations backed by basic research data, suggest that HMOs contribute to protection from infections, and in certain conditions of altered microbiota maturation, like after C-section birth, possibly also to atopic eczema onset.

In a Finnish cohort of 266 mother-infant pairs with hereditary risk for allergies, we observed a weak association in C-section born infants to have a higher risk to IgE mediated allergy onset at 2 years of age, when breastfed by mothers having low or no 2’Fucosyl-HMOs such as 2’FL (9). From a subset of 91 infants of the same cohort, stool samples were available from infants at 3 months of age. In C-section born infants, we observed a partial restoration of the dysbiotic gut microbiota compared to vaginal born infants, when fed breastmilk with 2’Fucosyl-HMOs (12).

The alleviation of the effect of C-section birth on the gut microbiota by the 2’Fucosyl-HMOs might thus in part explain the observed effect on the reduced risk to manifest IgE mediated allergies at 2 years of age.
HMOs resemble mucosal glysans at the host-microbe interface
Clinical intervention trials with infant formula supplemented with 2 individual HMOs, demonstrate that they allow for age appropriate growth and are well tolerated (13). A priori defined exploratory outcomes relate feeding an infant formula with 2-HMO to fewer reported lower respiratory tract infections and need for antibiotics during the first year of life, compared to feeding a control formula. Concomitant to these clinical findings, we observed a shift of the early life microbiota composition towards that of breastfed infants (14,15). Briefly, at 3 months, the microbiota composition in 2-HMO formula fed infants appeared closer to the microbiota of breastfed infants than the control formula fed infants both by microbiota alpha (within group) and beta (between groups) diversity analyses, and also by distribution of microbiota community types (A, B, or C). Specifically, 2-HMO formula feeding decreased the number of infants with a control formula specific C-community and increased those with a breastfed infant specific B-community. We found an association among the formula fed infants between their gut microbiota community at 3 months and the likelihood to require antibiotics through the first year of life. Formula fed infants with a C-community required antibiotics through the first year of life at 2 times higher rate (Hazard ratio 2 (95% CI: 1.1–3.9; p=0.02)). Thus, the observed shift of the microbiota community in infants fed the 2-HMO formula towards that of breastfed infants may partly explain the observed reduced risk to require antibiotics in the 2-HMO formula fed infants compared to the control formula fed infants.

However, we did not observe an association of the microbiota community types with the observed reduced risk of lower respiratory tract infections. Yet, when we stratified by mode of delivery, both randomized formula fed groups control and 2-HMO formula had had 36% C-section birth, we observed the following: 2-HMO formula feeding was particularly effective in reducing lower respiratory tract infections in the C-section born infants. Interestingly, the effect of the 2-HMO formula feeding on the stool microbiota at 3 months of age was also particularly effective in the C-section born infants, strongly increasing their bifidobacteria population and decreasing the abundance of Escherichia and Peptostreptococcaceae. Thus, the clinical benefits observed with the 2-HMO formula might in part be mediated by the effect of the 2 HMOs on the early life gut microbiota establishment.
2′Fucosyl-HMOs in relation to risk for IgE-allergy manifestation in C-section born infants with hereditary risk

At 2 years, but not at 5 years of age, presumed lower incidence for IgE-associated allergies in C-section-born infants (n=51) who were fed breast milk containing FUT2-dependent HMOs like 2′FL.

At 3 months of age, FUT2-dependent HMOs like 2′FL alleviate the observed effects of C-section-birth on the infant gut microbiota

Formula fed infants with a control formula typical gut microbiota community at 3 months have a 2 times higher risk to require antibiotics during the first year of life

FCT: Fecal community type

Korpela et al. 2018 Scientific Reports 8:13757; Clinicaltrial.gov NCT002198337

Berger et al. NRC, Abstracts WCPGHAN 2016; Berger et al. 2019, in preparation for resubmission

Kaplan-Meier plot showing time to first antibiotic use by FCT [Without BF]

Hazard ratio 2 for FCT-C (95% CI: 1.1–4.9, p=0.02)
To further substantiate this observation, we used a machine learning approach with input parameters several early life microbiota features (taxonomic and metabolic), and infant stool biomarkers. We asked the question which features predict that an infant experiences or not at least one lower respiratory tract infection or not during the first year of life. We identified as one of the common features from the different models the short chain fatty acid acetate together with other metabolites like succinate, bifidobacterium and stool markers like calprotectin. Using in vitro studies, we then explored a possible contribution of HMO fermentation by bifidobacteria to the observed protection from infections by the 2-HMO formula feeding.

**Benefits of HMOs to reduce lower respiratory tract morbidity are especially seen in C-section born infants together with changes in microbiota composition**

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Together, HMO likely contribute to immune protection in part through their effect on the early life gut microbiota, findings that are currently further investigated in clinical and basic research to appreciate our understanding of the HMO biology and clinical significance for infant nutrition.
References

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