Multidisciplinary Studies on Rotavirus—Human Milk Oligosaccharide Interactions

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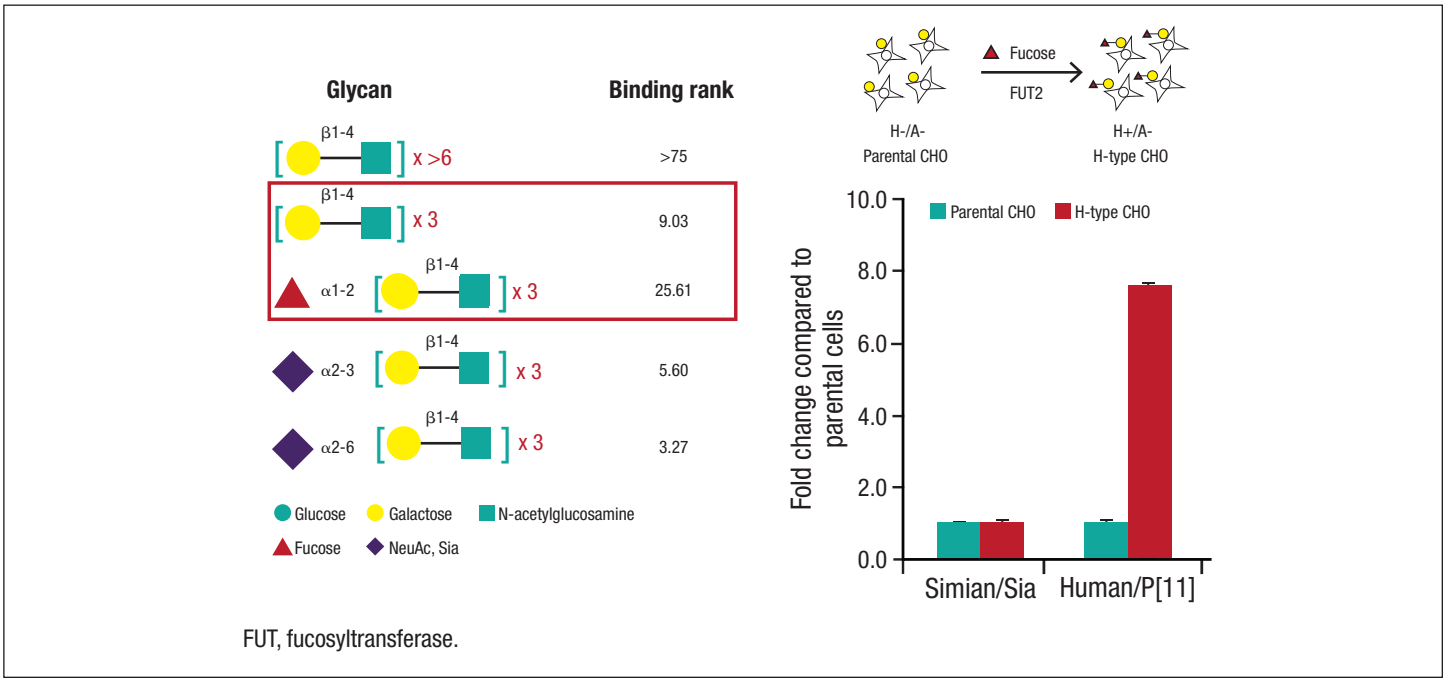
Rotavirus is a leading cause of severe dehydrating gastroenteritis in children younger than 5 years. Despite the introduction of vaccines in 2006, there are still over 200,000 rotavirus-associated deaths in children worldwide, with the majority occurring in sub-Saharan African and southeast Asian countries.[1] In neonates (<4 weeks of age), rotavirus infections are predominantly asymptomatic, although neonatal rotavirus infections associated with severe gastrointestinal presentations (ie, feed intolerance, necrotizing enterocolitis) have been described. Neonatal rotavirus infections are often caused by unusual strains that are geographically restricted. Studies in southern India show that the predominant rotavirus strain infecting neonates is G10P[11].[2,3]

Rotaviruses are triple-layer particles; the outer capsid consists of the glycoprotein VP7 and the spike protein VP4.[4,5] During infection, the VP4 spike is cleaved into 2 fragments, VP5* and VP8*. The VP8* domain interacts with cellular glycans and mediates initial attachment to target cells. Sialic acid has traditionally been considered the key mediator of interactions for VP8*; however, recent data indicate the VP8* of many human rotavirus strains can bind nonsialylated glycoconjugates called histo blood group antigens (HBGAs).[6,7] Human neonatal G10P[11] viruses are naturally occurring bovine–human reassortant strains and have a bovine VP8*spike that binds previously uncharacterized glycan partners. Potential binding partners for the G10P[11] VP8* were identified using a glycan array screen comprising >600 cellular glycans.[8] G10P[11] VP8*was found to specifically bind glycans with the Galβ1-4GlcNAc (LacNAc) motif, a precursor for type II HBGA **(Figure 1)**.[8] Expression of H-type HBGA in Chinese hamster ovary (CHO) cells significantly enhanced G10P[11] infectivity, providing biological relevance to the glycan array results. The binding of G10P[11] to these precursor glycans that are developmentally regulated may explain the predilection of this strain for neonates.

Figure 1. The GP10[11]VP8* domain binds to glycans with the LacNAc motif.[8]



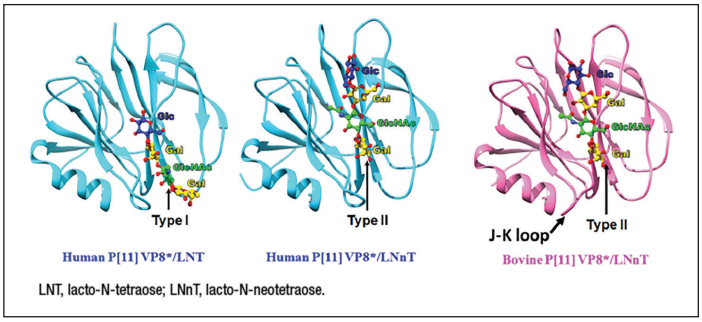
FUT, fucosyltransferase.

Similar glycan structures are present in human milk as human milk oligosaccharides (HMOs). Binding of P[11]VP8* to HMOs was tested using a shotgun milk glycan array developed using pooled donor milk samples. Although the human G10P[11] spike protein is of bovine origin, the human and bovine G10P[11] VP8* domains bind to different glycans in human milk. The human G10P[11] VP8* binds both type I and type II HMOs, while the bovine P[11] VP8* binds only to type II HMOs **(Table 1)**.[9] Crystallographic studies demonstrate that this difference in binding is due to small variations in protein structure that prevent interaction with type I glycans **(Figure 2)**.[7] Since bovine milk contains predominantly type II glycans and human milk contains type 1 and type II glycans, it is possible the bovine G10P[11] strain evolved to recognize both types of glycans and infect human neonatal hosts.

Table 1. Differences in milk glycan binding.[7,9]

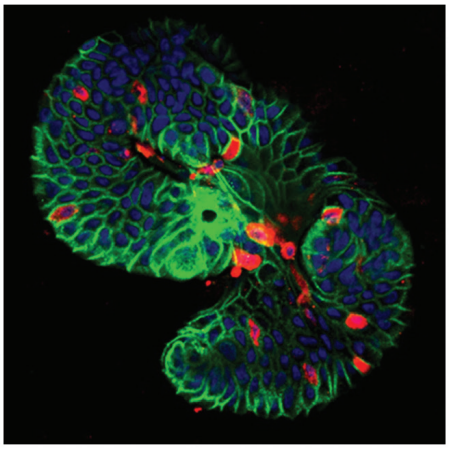
Species	P[11] VP8* binding	Predominant glycans in milk
Bovine	Type II	Type II
Human	Type I and type II	Type I, some type II

Figure 2. Structural basis for the differences in HMO binding.[7]



Specific HMOs have been shown to reduce infectivity of 2 clinically important rotavirus strains that affect older infants.[10] Current studies using the G10P[11] strain are testing the hypothesis that complex interactions between intestinal glycans, HMOs, and the breast milk microbiome affect neonatal susceptibility to rotavirus infections. The development of non-transformed human intestinal epithelial cultures, called human intestinal enteroids **(Figure 3)**, provides a new tool to study the molecular basis of rotavirus–HMO interactions and a new preclinical model for enteric pathogens.[11]

Figure 3. Human intestinal enteroid.


Rotavirus (red), E-cadherin (green), nuclei (DAPI).

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