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 live, attenuated oral vaccines in 2006, there are still >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 association with severe gastrointestinal presentations such as feed intolerance and necrotizing enterocolitis have been described.^{2–4} Neonatal rotavirus infections are often caused by unusual strains that are geographically restricted.⁵ Studies in southern India show that the predominant rotavirus strain infecting neonates is G10P[11].³

Rotaviruses are triple-layer particles; the outer capsid consists of the glycoprotein VP7 and the protease-sensitive spike protein VP4.^{6,7} During infection, the VP4 spike is cleaved into

two fragments, VP5* and VP8*. The VP8* domain interacts with cellular glycans and mediates initial attachment to host cells. Sialic acid has traditionally been considered the key mediator of interactions for VP8*; however, recent data indicate that the VP8* of many human rotavirus strains can bind nonsialylated glycoconjugates called histo-blood group antigens (HBGAs).^{8–14}

Human neonatal G10P[11] viruses are naturally occurring bovine–human reassortant strains and have a bovine VP8* spike. Potential binding partners for the P[11] VP8* were identified using a glycan array screen comprising >600 cellular glycans.¹⁴ P[11] VP8* was found to specifically bind glycans with the Gal β 1-4GlcNAc (LacNAc) motif, a precursor for type II HBGA (Fig. 1A).¹⁴ Expression of H-type HBGA in Chinese hamster ovary cells significantly enhanced G10P[11] infectivity (Fig. 1B), providing biological relevance to the glycan array results. The binding of P[11] VP8* to these

FIG. 1. (A) The G10P[11] VP8* domain binds to glycans with the LacNAc motif.¹⁴ (B) Expression of H-type HBGA enhanced human neonatal G10P[11] infectivity in Chinese Hamster Ovary (CHO) cells.

TABLE 1. DIFFERENCES IN MILK GLYCAN BINDING

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

Differences in milk glycan binding.^{11,19}

precursor glycans that are developmentally regulated may explain the predilection of this strain for neonates.

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.¹⁵ Although the VP8* spike protein of the human neonatal G10P[11] virus is of bovine origin, VP8* from human and bovine G10P[11] strains bind different glycans in human milk. The human G10P[11] VP8* binds both type I and type II HMOs, whereas the bovine P[11] VP8* binds only to type II HMOs (Table 1).¹⁵ Crystallographic studies demonstrate that this difference in binding may be mediated by subtle variations in the glycan binding domain that prevent interaction with type I glycans in the bovine P[11] VP8*.⁹ Since bovine milk contains predominantly type II glycans and human milk contains type I and type II glycans, it is possible that the bovine G10P[11] strain may have evolved to recognize both types of glycans and infect human neonatal hosts.

Current studies using the G10P[11] strain are testing the hypothesis that complex interactions between intestinal glycans, and HMOs, affect neonatal susceptibility to rotavirus infections. Specific HMOs have been shown to reduce infectivity of two clinically important rotavirus strains that affect older infants.¹⁶ The development of nontransformed human intestinal epithelial cultures, called human intestinal enteroids, provides a new physiologically relevant tool to study the molecular basis of rotavirus–HMO interactions.¹⁷

References

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