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REVIEW

Diversity in Rotavirus–Host Glycan Interactions: A "Sweet" Spectrum

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SUMMARY

Rotaviruses exploit host glycans as receptors for cell attachment. The discovery that human rotaviruses bind a spectrum of host glycans provides new insights into virus pathogenesis. Glycan expression is determined genetically and regulated developmentally, which may affect susceptibility to infection and vaccination.

Interaction with cellular glycans is a critical initial step in the pathogenesis of many infectious agents. Technological advances in glycobiology have expanded the repertoire of studies delineating host glycan–pathogen interactions. For rotavirus, the VP8* domain of the outer capsid spike protein VP4 is known to interact with cellular glycans. Sialic acid was considered the key cellular attachment factor for rotaviruses for decades. Although this is true for many rotavirus strains causing infections in animals, glycan array screens show that many human rotavirus strains bind nonsialylated glycoconjugates, called *histo-blood group antigens*, in a strain-specific manner. The expression of histo-blood group antigens is determined genetically and is regulated developmentally. Variations in glycan binding between different rotavirus strains are biologically relevant and provide new insights into multiple aspects of virus pathogenesis such as interspecies transmission, host range restriction, and tissue tropism. The genetics of glycan expression may affect susceptibility to different rotavirus strains and vaccine viruses, and impact the efficacy of rotavirus vaccination in different populations. A multidisciplinary approach to understanding rotavirus–host glycan interactions provides molecular insights into the interaction between microbial pathogens and glycans, and opens up new avenues to translate findings from the bench to the human population. *(Cell Mol Gastroenterol Hepatol 2016;2:263–273; http://dx.doi.org/10.1016/j.jcmgh.2016.03.002)*

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The surfaces of cells are decorated heavily with glycans or glycoconjugates, with structures ranging from simple monosaccharides to complex sugars with many different branches, linkages, and orientations.[1] Interaction with host glycans is an essential and critical step in the infectivity of most, if not all, microbial pathogens. Many pathogens exploit these glycans for initial cell recognition and attachment, and for enteric viruses such as rotaviruses and noroviruses, these interactions are frequently the first critical step for initiation of infections. Key fundamental, clinical, and epidemiologic questions on rotavirus disease have been answered through studies on the interactions of rotavirus with host glycans. The approaches exemplify multidisciplinary translational science and involve virologists, structural biologists, glycobiologists, physicians, and epidemiologists (Figure 1). For example, screens using transformative new technologies in glycobiology such as glycan arrays identified histo-blood group antigens (HBGAs) as new glycan partners for human rotaviruses. These interactions were confirmed by enzyme-linked immunosorbent assays (ELISA) using synthetic glycans, and the basis of these interactions was elucidated using x-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy. The biological relevance of these findings has been addressed using hemagglutination, saliva binding, and in vitro infectivity assays. Preliminary findings from cell lines now can be confirmed using physiologically relevant models of the human gut such as intestinal enteroids. Translational studies in people test if findings from the bench hold true at the bedside or at the population level. The findings from such team-science approaches not only have direct implications for our understanding of the biology of the virus, but also influence vaccine strategies, the development of therapeutics, and provide a new foundation for understanding other enteric infections. The diversity of glycans recognized by animal and human rotaviruses, insights gained into various aspects of viral pathogenesis such as interspecies transmission, host restriction, and tissue tropism, and the effect of genetic differences in glycan expression on susceptibility to infection and vaccination are reviewed here.

Abbreviations used in this paper: **GlcNAc, N-acetylglucosamine; HBGA, histo-blood group antigen; HIE, human intestinal enteroid; LacNAc, *N*-acetyllactosamine; Le, Lewis; LNnT, lacto-*N*-neotetraose; LNT, lacto-*N*-tetraose; Neu5Ac, N-acetylneuraminic acid; Neu5Gc, N-glycolylneuraminic acid; NMR, nuclear magnetic resonance; RBC, red blood cell; Sia, sialic acid; VP, viral protein.**

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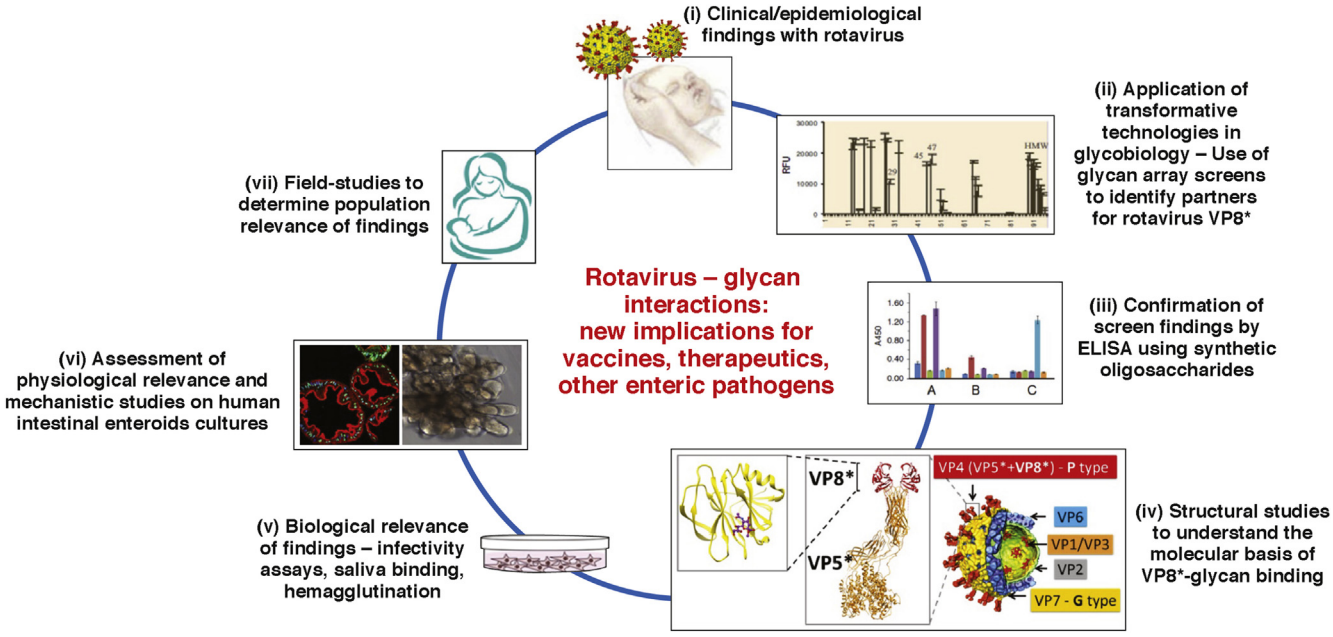

Figure 1. Schematic representation of multidisciplinary studies on rotavirus interaction with host glycans. A combination of glycobiology, structural biology, basic virology, and field studies on infectious diseases have been used to understand rotavirus–host-glycan interactions. (i) Clinical and epidemiologic questions on rotavirus infections in children have been addressed through these approaches. (ii) A representative image of results from a glycan array is shown, with numeric order of glycans in the array listed on the x-*axis* and binding intensity in relative florescence units (RFU) on the *y-axis*. (iii) A representative image of enzyme-linked immunosorbent assay (ELISA) results showing the binding of VP8* from 3 rotavirus strains A, B, and C to synthetic oligosaccharides. Each *colored bar* represents a synthetic oligosaccharide. Binding is measured by optical density value at 450 nm. (iv) A cut-away of a cryo-electron microscopic reconstruction of a rotavirus triple-layered particle. The core layer comprises VP2 and the intermediate layer is made of the protein VP6. The outer capsid is made of the glycoprotein VP7. Sixty spikes made of the protease-sensitive protein VP4 extend from the VP7 layer, and comprise 2 domains, VP5* and VP8* (*inset*). The crystal structure of rotavirus VP8* in complex with a glycan is seen in the second inset. (v) The biological relevance of binding assays were confirmed through infectivity assays on transformed cell lines and through hemagglutination and saliva binding assays. (vi) HIEs provide a novel intestinal culture system to study rotaviruses. Confocal microscopy shows cross-section of an HIE stained with Ki-67 (green) for proliferating cells, actin (red) for highlighting the apical surface of the epithelial cells, and 4′,6-diamidino-2-phenylindole (blue) for the nucleus (*left panel*). A multilobular, differentiated, 3-dimensional HIE is shown in the *right panel*. (vii) Field studies using samples from mother–infant pairs will determine the relevance of these findings at a population level.

Rotavirus Structure, Classification, and Disease Burden

Rotaviruses are the leading cause of severe dehydrating gastroenteritis in children younger than 5 years of age. Globally, rotavirus infections result in approximately 453,000 deaths each year, accounting for approximately 5% of child deaths.[2] The majority of these deaths occur in developing countries in Asia and sub-Saharan Africa. Two live-attenuated oral vaccines against rotavirus (Rotarix, GlaxoSmithKline Biologicals, Belgium and RotaTeq, Merck & Co Inc, Whitehouse Station, NJ) were licensed for use in 2006, and as of October 2015, have been introduced into the national immunization programs of 79 countries worldwide.[3] Large clinical trials have shown that the vaccines are highly efficacious in developed countries,[4,5] and have significantly reduced the burden of rotavirus gastroenteritis in many high- and middle-income countries.[6–12] However, the vaccines remain less efficacious in developing countries, which have the greatest burden of disease.[13–16] A number of factors contribute to the lower efficacy in these populations, including higher rates of malnutrition and tropical enteropathy, early infections, competing enteric infections at the time of vaccination, and interference by maternal antibodies.[13] Efforts are ongoing to improve the efficacy of existing vaccines and to develop more effective next-generation vaccines, and this is critical for decreasing the disease burden in highly affected populations.

Rotavirus is a member of the family *Reoviridae*. The viral genome consists of 11 segments of double-stranded RNA that code for 6 structural viral proteins (VP) and 6 nonstructural proteins.[17] The infectious virion is a triple-layered particle consisting of a core layer made of VP2, an intermediate layer made of VP6, and an outer shell made of glycoprotein VP7. Sixty protein spikes made of a protease-sensitive protein, VP4, extend from the VP7 shell (Figure 1, iv). Similar to the classification of influenza virus into H- and N- types based on the hemagglutinin and neuraminidase proteins, variability in the genes encoding