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Investigating virus–glycan interactions using glycan microarrays

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While all viruses must transit the plasma membrane of mammalian cells to initiate infection, we know little about the complex processes involved in viral attachment, which commonly involve recognition of glycans by viral proteins. Glycan microarrays derived from both synthetic glycans and natural glycans isolated through shotgun glycomics approaches provide novel platforms for interrogating diverse glycans as potential viral receptors. Recent studies with influenza and rotaviruses using such glycan microarrays provide examples of their utility in exploring the challenging questions raised in efforts to define the complex mechanistic protein–glycan interactions that regulate virus attachment to host cells.

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Introduction

The initial step in viral infections of living cells typically involves the interaction of a virus with a cell surface receptor in order to be eventually transported through the plasma membrane into the cell interior. The plasma membrane and exterior of all living cells is comprised of a thick wrapping of complex cell surface glycans on glycoproteins and glycolipids, sometimes referred to as the glycocalyx [1]. The surface of cells has evolved in an environment of constant exposure to pathogens that bind to specific glycans on cells, thus evolutionarily helping to drive the creation of a hugely diverse set of glycan structures, as cells balance their glycan functions and structures with pressures to evade pathogen recognition. At the same time pathogens evolve by changing their glycan coat to appear more host-like, and in addition pathogens exploit host glycans for initial interaction by constantly modifying their glycan recognition molecules in response to glycan structural changes at the host cell surface. This complex interplay is a driving force for molecular evolution at the glycan and protein levels [2–4], reflecting the literal war between viruses and animal cells that is fought on a battlefield of cell surface glycans.

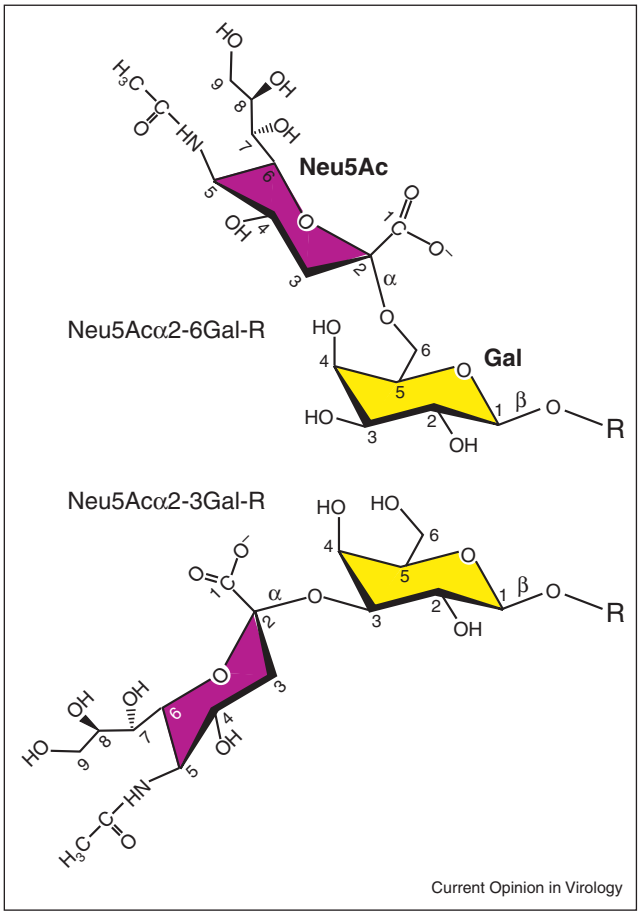
Observations in the 1930s and 1940s on many viruses including influenza viruses indicated that viruses could agglutinate vertebrate erythrocytes, and it was subsequently shown for influenza virus that the receptors and hemagglutinating potential of the erythrocytes were eventually lost upon long exposure to virus [5,6]. The erythrocyte receptors recognized by influenza virus were eventually identified as N-acetylneuraminic acid (sialic acid), and the 'receptor-destroying enzyme' was discovered to be a neuraminidase (NA or sialidase), which was independent of the hemagglutinin (HA) [7,8]. Remarkably and despite these early observations and many years of research on virus–host interactions, it is still not clear exactly how these processes operate in the mechanisms of viral infection, but an optimal balance between the HA and the NA is probably key [9].

Cell surface carbohydrate, however, appears to be a major pathway of entry for influenza as well as other viruses, and the initial virus–host interactions involve recognition among a diverse set of glycan structures; thus, the specificities of viral surface adhesion molecules are thought to play important roles in viral tropism. This concept was demonstrated by the observation in the early 1980s showing that the HA of human influenza viruses prefer α2–6 linked sialic acid while avian viruses prefer the α2–3 linked form (Fig. 1), and that a single amino acid substitution was responsible for the specificity switch [10–12]. The proposed location of these linkages in the intestinal tract of birds and upper respiratory tract of humans is thought to correspond with the specificity of the appropriate HA [13]. Although many viruses including norovirus, polyomavirus, rotavirus, and paramyxovirus exploit surface carbohydrate to facilitate entry into cells [14,15], we will specifically focus on influenza viruses and rotaviruses, which have received significant attention in recent years.

Analysis of influenza A binding specificity on defined glycan microarrays

The Consortium for Functional Glycomics (CFG), a National Institute of General Medical Sciences-funded program, developed a glycan microarray of >600 defined

Figure 1



Depiction of sialic acid linked to galactose in either α2–6 or α2–3 linkages.

glycans including >160 sialylated structures that could be interrogated with fluorescence labeled glycan binding proteins or intact viruses simultaneously generated significant interest in defining the fine specificity of influenza HAs to determine the relationship of the glycan structures underlying the terminal sialic acid to infection and transmission of influenza [16–18] (www.functionalglycomics.org/). This resource was made available to investigators worldwide and was a revolutionary development in the area of protein–glycan interactions, since both the glycan microarray resources as well as the database of results were freely available.

Investigating the binding specificity of different virus strains on a glycan microarray is typically accomplished by analysis of recombinant HA or intact viruses. Since the interaction of the recombinant HA with receptor is relatively weak with dissociation constant in the mM range [19], which is below the detection limit of a GBP on the CFG array, the avidity of this system is increased by precomplexing the His-tagged trimer with fluorescence labeled mouse anti-HisTag-IgG and fluorescence labeled anti-mouse-IgG in approximate molar ratio of 4:2:1 [16,20•]. This protocol has been generally used for analyzing the specificity of recombinant, His-tagged HA in large-scale screening. An alternate approach has been the analysis of fluorescent labeled whole virus [21•,22] that is directly labeled with a fluorescent dye and assayed post labeling with hemagglutination assays to ensure no loss of binding activity during the labeling procedure and to have an estimate of the amount of virus added to the array so that analyses can be carried out at multiple concentrations of virus for detection of strongest binding glycans [19]. Both of these approaches have been used extensively by the CFG Protein–Glycan Interaction Resource at Emory University and by the Centers for Disease Control and Prevention in Atlanta, GA for determining specificities of virus binding to arrays of glycans with comparable results [19].

After nearly a decade of studies on influenza virus recognition of glycans using defined glycan microarrays, the general conclusion is that most, but not all, avian and human virus HA retain their canonical specificity for Siaα2–3Gal- and Siaα2–6Gal-, respectively. However, the underlying glycan structure is increasingly recognized as also being critical to recognition. For example, human virus HAs may prefer the presentation or topology of Siaα2–6 expression on extended poly-N-acetyllactosamine (PL) [-3Galβ1–4GlcNAcβ1-]$_n$ glycans [23,24]. Other studies evaluating the binding of influenza virus to synthetic sialylated PLs noted that while most influenza virus strains demonstrated differences in their preference for the structures presented on glycan arrays, there appears to be no consistent recognition pattern associated with the underlying glycan structures. Such results suggest that the fine specificity of the receptors may drift due to antigenic selective pressure, while binding glycans with a Siaα2–6 determinant might be sufficient for infection and transmission [25]. This observation is in agreement with the results of a systematic investigation of human H3N2 influenza viruses [26••]. In that study the binding properties of all major variants of human H3N2 viruses from their appearance in 1968–2012 were characterized on the large CFG defined glycan microarray. The binding specificity of viruses isolated in the same season was similar, but the specificities showed significant variation from year-to-year [26••]. Since all viruses were representative of epidemic strains, they all could infect and transmit efficiently among humans. The results indicate that the year-to-year variation in binding specificity is due to amino acid sequence changes driven by antigenic drift, and that in spite of having different binding specificity and avidity the viruses were still able to infect and transmit in the population.

Thus, the idea that influenza viruses simply recognize the terminal sialic acid in a specific linkage to galactose