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New insights into influenza A specificity: an evolution of paradigms

Ye Ji, Yohanna JB White, Jodi A Hadden[1], Oliver C Grant and Robert J Woods

Understanding the molecular origin of influenza receptor specificity is complicated by the paucity of quantitative affinity measurements, and the qualitative and variable nature of glycan array data. Further obstacles arise from the varied impact of viral glycosylation and the relatively narrow spectrum of biologically relevant receptors present on glycan arrays. A survey of receptor conformational properties is presented, leading to the conclusion that conformational entropy plays a key role in defining specificity, as does the newly reported ability of biantennary receptors that terminate in Siaα2-6Gal sequences to form bidentate interactions to two binding sites in a hemagglutinin trimer. Bidentate binding provides a functional explanation for the observation that Siaα2-6 receptors adopt an open-umbrella topology when bound to hemagglutinins from human-infective viruses, and calls for a reassessment of virus avidity and tissue tropism.

Address
Complex Carbohydrate Research Center, University of Georgia, 315 Riverbend Rd, Athens, GA 30602, United States

Corresponding author: Woods, Robert J (rwoods@ccrc.uga.edu)
[1] Current address: Beckman Institute for Advanced Science and Technology, University of Illinois at Urbana-Champaign, Urbana, IL, United States.

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Introduction

Wild birds are the primary natural reservoir for influenza A viruses [1], and the 1918 Spanish Flu pandemic that killed an estimated 50 million people [2] is believed to have originated from spontaneous mutations in an avian influenza virus that conferred human-to-human transmissibility [3,4]. While zoonotic influenza can infect humans [5], close contact with infected animals is required [6]. Subsequent human-to-human transmission, leading to pandemics, requires that the virus undergo additional genetic alterations [5,6]. As noted by Reperant *et al.* [5], in order for a zoonotic virus to become human-infective, it must overcome three sets of barriers: animal-to-human transmission, virus-cell interaction, and human-to-human transmission. Seasonal influenza epidemics arise from human-to-human transmission of circulating strains that have undergone sufficient mutation (antigenic drift) to circumvent established immunity within the population [7].

In contrast to the Spanish Flu, the Swine Flu pandemic of 2009 was relatively mild [8]. Nevertheless it raised concerns within the World Health Organization because of the rapidity with which it spread [9]; within 6 weeks of the first case, Swine Flu had spread to over 70 countries [10] and required the development of a new vaccine. Human adaptation is of particular concern in the case of highly pathogenic avian influenza (HPAI) subtypes, such as H5N1. Although infrequent, human infection by avian H5N1 has been reported in 16 countries, resulting in approximately 60% mortality [11]. Preparedness for pandemics therefore necessitates anticipation of the virulence of emerging strains, providing motivation for developing a deeper understanding of the basis for influenza specificity. Here, we reassess the relationship between host glycan structure and influenza specificity in light of recent data that indicates critical roles for glycan sub-structure and dynamics.

Influenza A classification is based on the antigenic properties of the hemagglutinin (HA) and neuraminidase (NA) envelope proteins. Influenza HA is a homotrimeric glycoprotein whose protomers each comprise a globular head domain (HA1) and stalk region (HA2) [12]. Each HA1 domain contains a receptor binding site (RBS), through which the virus adheres to the host cell via binding to host glycans that contain sialic acid (Sia, neuraminic acid, Neu5Ac). There are currently 18 hemagglutinin subtypes, which are classified into two groups based on their antigenic properties: group 1 consists of H1-2, H5-6, H8-H9 H11-13, and H16; group 2 contains H3-4, H7, H10, H14, and H15. The most extensively studied HAs include H1, H3 and H5 [13•,14]. The NA protein mediates the cleaving of Sia from the host receptor glycan post cellular infection, enabling progeny virus to escape from the host cell surface [15]. Cryo-electron tomography indicates that there are approximately 300 HA proteins in the viral envelope [16], with the ratio of HA to NA varying between different strains from 4 to 6:1 [16,17]. Compound factors affect the ability of a particular strain of influenza to infect humans, including

the level of exposure, the replication rate in newly infected individuals, the glycan binding preferences of the viral surface HA, and the activity of the viral surface NA [15,18–23]. Further, the enzyme activity of the NA must balance with the affinity of the HA [15,22]. If the NA is too active, relative to the affinity of the HA, it will attenuate the ability of the virus to infect the host cell. Conversely, a relatively weak NA will impair shedding of the progeny virus.

In addition to receptor specificity, zoonotic infection is also sensitive to differences in the susceptibility of the HA to pH-mediated endosomal fusion [24], and differences in the efficiency of translocation of the viral ribonucleoprotein complex to the host nucleus [25] (host adaptation). Moreover, ease of transmission and replication appears to be dependent on the distribution and composition of the receptors on host tissue. Viral attachment studies have shown that human influenza viruses adhere more strongly to human trachea and bronchi than avian viruses, and attach to different cell types [26]. Thus, the lack of a suitable receptor has been invoked as being responsible for the inefficient transmission [27] and replication of avian viruses in humans [28,29]. Much work has been done to elucidate the molecular basis for the observed tissue tropism [28,30,31,32••].

Defining receptor specificity

The canonical view of the relationship between HA receptor specificity and species infectivity is that the HA in human-infective viruses prefers to bind to glycans present on the cell surface that terminate with the Siaα2-6Gal (α2-6) sequence; in avian-infective viruses, the HA prefers to bind to glycans that terminate in Siaα2-3Gal (α2-3). Some species, such as swine, can be co-infected by viruses that prefer either α2-3 or α2-6 structures, leading to the potential for genetic reassortment (antigenic shift) in swine that results in the introduction of α2-6 binding preference (enhanced human infectivity) into a zoonotic framework [5].

The discovery of the α2-6/α2-3 infectivity relationship originated not from quantitative biophysical studies, but from more qualitative, yet robust, hemagglutination assays [33]. Rogers and Paulson's [34••] seminal work on enzymatically modified red blood cells (RBCs) established that influenza receptor specificity depends, to an extent, on the species from which the virus was isolated. They reported that isolates of human subtype H3N2 agglutinated RBCs whose modified surface glycans terminated in the α2-6 sequence, but that these isolates did not agglutinate RBCs with α2-3 glycans. Conversely, avian isolates preferentially agglutinated RBCs containing the α2-3 linkage. While hemagglutination by influenza is a general phenomenon not limited to chicken RBCs [35], not all virus strains agglutinate all types of RBCs equally well [35,36]. Unmodified chicken RBCs contain a diversity of multiantennary glycans, roughly in an equal ratio of α2-3:α2-6 [36], but these represent only a limited subset of the glycans found on human epithelial tissue, which also include multiple lactosamine repeats in the antennae. The observation that the necessary human-type receptors are not present provides an explanation of the inability of certain human-adapted influenza strains to agglutinate chicken RBCs [36]. As noted by Ovsyannikova *et al.* [35], species selection of red blood cells (RBCs) is critical to determine antibody titers to influenza viruses reliably, however, further glycomics analyses are required to elucidate the origin of the differences in RBC agglutination behavior.

Affinity versus avidity

Monomeric binding affinities for HA-glycan interactions confirm the canonical view of HA specificity, but show remarkably modest differences between α2-3 and α2-6 receptors (Table 1). Avidity arising from interactions between multiple host glycans and multiple trimeric HAs on the viral surface has been invoked to explain the difference between the weak (mM) monomeric affinities for HA-glycan interactions and the sub-μM binding for whole virus [37,38•,39,40••]. Indeed, models of binding kinetics [38•,39] have shown that avidity can exponentially amplify the subtle differences in monomeric affinities, resulting in agreement with experimental virus binding kinetics.

In 2012, Lin *et al.* [44••] reported that the avidity of H3N2 viruses for an α2-6 trisaccharide receptor decreased approximately fourfold between 1968 and 2001, then progressively decreased a further 200-fold from 2001 to 2010, to such an extent that higher virus concentrations were required to observe any binding for the 2010 strains. This decrease in binding avidity was shown to be the result of mutations (antigenic drift) that weakened specific interactions between the RBS and the glycan receptors [44••]. Recently, Peng *et al.* [45••] screened the HAs from a number of H3N2 viruses against a custom glycan array that included multiantennary glycans of the type found in the human respiratory tract [46••], and confirmed that binding to short, or linear, glycans had steadily decreased, consistent with the observations of Lin *et al.* [44••]. However, strong binding to long biantennary sialoglycans was observed that was relatively insensitive to the effects of antigenic drift.

Recently, Peng *et al.* [45••], and de Vries *et al.* [47•] have raised the intriguing possibility that both branches in a biantennary glycan could bind simultaneously to two RBSs in an HA trimer, provided the branches were sufficiently long to reach two RBSs (Figure 1). Such bidentate binding would amplify the affinity of the glycan, potentially resulting in an apparent affinity of as much as the square of the monovalent K_D ($K_{D,mono}^2$) [40••], although this would likely be reduced by entropic