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The role of receptor binding specificity in interspecies transmission of influenza viruses

Masaki Imai¹ and Yoshihiro Kawaoka^{2,3,4,5,6}

¹Influenza Virus Research Center, National Institute of Infectious Diseases, Musashi-Murayama, Tokyo 208-0011, Japan

²Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI 53711, USA

³Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan

⁴Department of Special Pathogens, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan

⁵ERATO Infection-Induced Host Responses Project, Saitama 332-0012, Japan

⁶Department of Microbiology and Infectious Diseases, Kobe University, Hyogo 650-0017, Japan

Abstract

Influenza A virus infection begins with the binding of the hemagglutinin (HA) glycoprotein to sialic acid-containing receptors on the surface of the target cell. Avian influenza viruses, including avian H5N1, H7, and H9N2 viruses, can occasionally cross the species barrier and infect humans; however, these viruses do not spread efficiently from person to person, perhaps, in part, due to differences in the receptor-binding specificities of human and avian influenza viruses. The HAs of avian influenza viruses must adapt to receptors in humans to acquire efficient human-to-human transmissibility. In this review, we discuss the receptor binding specificity of influenza A viruses and its role in interspecies transmission.

Introduction

Influenza is a widespread zoonotic disease caused by influenza A viruses, which infect various species, including humans, lower mammals, and birds [1,2]. Influenza A viruses are enveloped viruses that contain a segmented genome of eight different negative-strand RNA molecules. The envelope accommodates two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA). HA has at least two functions: it recognizes sialic acid-containing receptors on the cell surface and it mediates the fusion of the viral envelope with the endosomal membrane of the host cell, leading to the release of the nucleocapsid into the

cytoplasm [3]. HA is also the major antigen stimulating the host's protective immunity, specifically the production of neutralizing antibodies.

Influenza A viruses are classified into subtypes based on the antigenicity of their HA and NA. To date, sixteen known HA (H1–H16) and nine NA (N1–N9) subtypes of influenza A viruses have been isolated from aquatic birds, which are the reservoir of influenza A virus in nature [4,5]. Viruses with the HA subtypes H1, H2, and H3, and the NA subtypes N1 and N2 are known to have adapted to humans in the past century, and only two subtypes, H1N1 and H3N2, have been circulating in humans for several decades. Avian influenza viruses generally do not infect and replicate efficiently in humans. However, in some situations, several avian influenza virus subtypes (such as H5N1, H7, or H9N2) have broken through the species barrier and acquired the ability to infect humans [6–10]. When a virus with a new HA subtype is introduced from avian species to humans, the resulting virus may cause widespread infection in the immunologically naïve human population, leading to a pandemic.

Although limited human-to-human transmission of viruses with the H5N1 and H7N7 subtypes has occurred [11–15], these avian influenza viruses do not spread readily from person to person. Therefore, avian influenza viruses must overcome host range restriction to become established in the human population. However, the molecular basis for host range restriction has not yet been clearly defined; the HA glycoprotein is likely a major determinant of host switching primarily because of its role in host cell receptor recognition [16–19]. Here, we review our current understanding of the role of HA in controlling the host range specificity of influenza A viruses. We also discuss the role of receptor binding specificity in the interspecies-transmission of influenza A viruses.

Receptor binding specificity of human and avian influenza A viruses and influenza virus receptor distribution among birds, pigs, and humans

Influenza virus infection is initiated via HA, which binds to sialic acid-containing glycans that are associated with glycoproteins and glycolipids on the surface of epithelial cells. Several different methods for measuring the receptor specificity of HA, including agglutination assays using modified-erythrocytes, solid-phase binding assays, and glycan microarray assays, have demonstrated that the HAs of human influenza virus strains preferentially bind to oligosaccharides that terminate with sialic acid linked to galactose by α 2,6-linkages (Sia α 2,6Gal), whereas the HAs of avian influenza virus strains prefer oligosaccharides that terminate with a sialic acid linked to galactose by α 2,3-linkages (Sia α 2,3Gal) [19–21] (Fig. 1a). Correspondingly, sialic acid-specific lectin staining of tissue sections has revealed that epithelial cells in the human upper respiratory tract express predominantly Sia α 2,6Gal, whereas those in duck intestine, where avian viruses replicate, express predominantly Sia α 2,3Gal [22,23]. In addition, immunohistochemical analysis has revealed specific binding patterns of influenza viruses to different tissues of the human respiratory tract [24–26]. Human influenza viruses strongly attach to the epithelial cells of tissue sections from human trachea (Fig. 1b). In contrast, avian influenza viruses bind poorly to these cells. Thus, the relative lack of Sia α 2,3Gal in the human upper respiratory tract is

thought to restrict the efficient replication of avian influenza viruses. A shift from Sia α 2,3Gal- to Sia α 2,6Gal-binding specificity is likely a critical step in the adaptation of avian influenza viruses to human hosts. Indeed, the pandemic strains of 1918 (H1N1), 1957 (H2N2), and 1968 (H3N2), as well as the more recent pandemic H1N1 2009 virus, exhibited human-type receptor-binding specificity [17,20,21,27], although their HAs originated from non-human species.

The distribution of these two types of sialyloligosaccharides at replication sites likely varies among avian species. In aquatic birds, including ducks and geese, avian-type receptors (Sia α 2,3Gal) dominate in tracheal epithelial cells [28–30]. On the other hand, in terrestrial birds, including chickens, turkeys and quails, both avian-type (Sia α 2,3Gal) and human-type (Sia α 2,6Gal) receptors are detected in their tracheal epithelial cells [28–32], suggesting that these species can support the replication of both avian and human influenza viruses and act as adaptation hosts for receptor switching of avian strains. Notably, the receptor specificity of H9N2 viruses isolated from terrestrial birds, but not aquatic birds, resembles that of human isolates [33]. Moreover, a recent study reported that a duck influenza virus that had adapted to quails but not the original duck virus replicated in human respiratory epithelial cells [32]. These findings indicate that some land-based poultry species can serve as potential intermediate hosts for avian viruses to be transmitted to humans.

Traditionally, pigs have been considered as intermediate hosts or mixing vessels for the reassortment of avian and human influenza viruses due to their susceptibility to infection with both avian and human isolates [34]. Initially, tracheal epithelial cells from pigs were reported to express substantial amounts of both types of receptors [22]. However, subsequent studies have shown that human-type receptors are more abundant than avian-type receptors on the tracheal epithelia of pigs [35,36]. In addition, mass spectrometry analysis has shown that α 2,6-sialylated glycans are expressed predominantly on primary swine respiratory epithelial cells [37]. Thus, the receptor distribution in the respiratory tract of swine is likely similar to that in humans.

The integration of data from HA-glycan conformational analysis and glycan binding assays has led to the proposal that the size and shape of glycan receptors, rather than the specific linkage type, are important determinants for human adaptation of influenza A viruses [38]. These data suggest that long α 2,6-linked glycans with specific structural topology are recognized by human-adapted H1 and H3 HAs. Although the structural diversity of the oligosaccharides on the surface of the epithelial cells of the human upper respiratory tract is still not well understood, a number of sialylated oligosaccharides with differing branching patterns and chain lengths are believed to be present on these cells [39]. The interaction of HA with specific sialylated receptors possessing characteristic structures or lengths may be associated with the ability of human-adapted influenza viruses to replicate and transmit efficiently in humans.

Amino acid changes in HA that confer human-type receptor recognition to avian influenza viruses

The receptor-binding domain (RBD) of HA is formed by the 190-helix at the top of HA, the 220-loop at the edge of the globular head, and the 130-loop at the other edge of the globular head (Fig. 2). Amino acid changes in and around the RBD dramatically alter the receptor binding preference of influenza viruses. However, the specific amino acids that determine receptor binding specificity vary among the different HA subtypes. For H2 and H3 HAs, the substitutions of Q226L and G228S (all amino acid positions listed herein refer to H3 numbering) could confer a complete switch from α 2,3- to α 2,6-glycan binding [20,40–42]. In the case of H1 HAs, the E190D and D225G mutations are critical for the shift from α 2,3- to α 2,6-glycan recognition [16,21]. These amino acid changes (E190D, D225G, Q226L, and G228S) have not been observed among avian H5N1 viruses isolated from humans.

The human-type amino acids at positions 226 and 228 increase binding to human-type receptors when tested together in experimental settings [43,44]; by contrast, the individual human-type amino acids at positions 226, 190, and 225 do not confer human-type receptor specificity [44]. A small number of avian H5N1 viruses isolated from humans exhibit increased binding to human-type receptors (although to a limited extent), a property conferred by several amino acid changes, including S125N, L133V/A138V, 133deletion/I155T, G143R, S159N, N186K, K193R, Q196R, Q196H, N197K, V214I, S227N, or S239P [43,45–49]. However, viruses with these mutations retain their receptor binding preference for α 2,3-glycans. A recent reverse genetic study demonstrated that Q196R/Q226L/G228S mutations in an H5 HA resulted in a shift from α 2,3- to α 2,6-glycan recognition [50]. Since this study did not evaluate the binding of the mutant HA to human respiratory tract tissues, it is not clear whether the three amino acid changes (Q196R/Q226L/G228S) can create an H5 HA with receptor-binding capability akin to that of seasonal influenza virus HA.

Recent avian H7N2 viruses isolated from humans in North America have been reported to bind to both α 2,3- and α 2,6-glycans [51]. The HAs of these viruses are characterized by an eight amino acid deletion in the 220-loop of the RBD. Although amino acid residues in an H7 HA that confer increased recognition of human-type receptors have not yet been identified, the loss of the 220-loop seems to facilitate enhanced α 2,6-glycan binding [52].

Avian H9N2 influenza viruses circulating throughout South East Asia have occasionally transmitted to humans and pigs. Numerous recent H9N2 isolates contain a human-like amino acid residue at position 226 (i.e., 226L) in their HAs and show preferential binding to α 2,6-glycan receptors [33]. Importantly, 226L-containing H9N2 viruses have been shown to replicate efficiently in differentiated human airway epithelial cells [53].

Studies with H5N1, H7N2, and H9N2 viruses all highlight the fact that amino acid substitutions in the 220-loop may be critical for avian HAs to acquire human-type receptor specificity. Interestingly, crystal structure analysis has revealed that, compared with human H3 HA, the 220-loop in avian H5 HA is closer to the opposing 130-loop, suggesting that the wider receptor-binding pocket of human H3 HA, compared to that of avian H5 HA, may be required to optimize contacts with the larger α 2,6-glycan receptors [54]. Mutations in the

220-loop may alter the orientation of the 220-loop and thus optimize the contacts between the amino acids located at the 220-loop and the human-type receptors, thereby increasing the preference for α 2,6-linkages.

Several subtypes of avian influenza A viruses possessing mutations in their HAs can transmit in a ferret model via respiratory droplets

Efficient and sustained human-to-human transmission is a critical feature of seasonal and pandemic influenza viruses. The ferret model has been used widely to study the transmission of H5, H7, and H9 subtypes of avian influenza viruses, as well as the 1918 H1N1 and 1957 H2N2 viruses. Ferrets are susceptible to infection with human influenza viruses and develop some symptoms of influenza that closely resemble those of humans. More importantly, the respiratory tract of ferrets expresses predominantly human-type receptors, and is thus very similar to human respiratory epithelia [26,55].

The HA Q226L mutation in a prototypic pandemic H2N2 strain allowed human-type receptor recognition and improved the efficiency of respiratory droplet transmission in a ferret model [56]. In addition, two amino acid changes (D190E and G225D) that cause a shift from human-type to avian-type receptor recognition in the HA of recombinant 1918 H1N1 viruses abolished transmission via respiratory droplets in ferrets [57]. These studies suggest that amino acid changes in HA that confer binding specificity for human-type receptors are a prerequisite for cross-species transmission and human adaptation of avian influenza viruses.

Although an H5N1 virus that recognizes both human- and avian-type receptors has been isolated from humans [46], this virus did not transmit via respiratory droplets between ferrets [58] (Table 1). In addition, H5N1 viruses with three or five mutations in the RBD of HA were not transmitted via respiratory droplet in ferrets, despite recognizing only human-type receptors [50,59]. Similarly, no respiratory droplet transmission was detected with North American H7 viruses that exhibited enhanced α 2,6-glycan binding and decreased binding to α 2,3-glycan [51]. In addition, H9N2 viruses that exhibit an α 2,6-glycan binding preference did not transmit efficiently via respiratory droplets among ferrets [60]. Collectively, these studies indicate that human-type receptor recognition by avian influenza viruses is probably necessary but not sufficient for their transmission via respiratory droplet in a ferret model.

A transmissible strain of avian influenza viruses may emerge either by genetic mutation or by reassortment of human and avian influenza viruses. In fact, the 1957 and 1968 pandemics originated from avian-human reassortant viruses that had acquired human-type receptor binding specificity [17,20]. Avian H5N1/human H3N2 reassortant influenza viruses, which express the HA and NA proteins from an H5N1 virus, exhibited no respiratory droplet transmission in ferrets [58]. However, a recent study reported that a reassortant bearing an H5N1 virus HA protein with human-type receptor binding specificity and a human H3N2 virus NA protein on the framework of an avian H5N1 virus could be transmitted by respiratory droplet in ferrets because viral shedding was detected in the nasal washes of one of two contact animals [50]. For H9N2 viruses, reassortants with six human H3N2 virus

internal genes failed to transmit via respiratory droplets in ferrets, despite their binding preference for α 2,6-glycans [60]. However, the same group reported that after adaptation by serial passage, these avian H9N2/human H3N2 reassortants displayed efficient respiratory droplet transmission in ferrets [61]. They also showed that avian-human reassortants with HA and NA proteins from an avian H9N2 virus in a pandemic 2009 H1N1 virus background transmitted efficiently by respiratory droplet without prior adaptation [62]. Taken together, these studies show that although efficient transmission of avian influenza viruses in a ferret model requires adaptation of HA from avian-type to human-type receptor specificity, other viral factors are also important determinants of virus transmission. For example, amino acid changes in the PB2 protein are associated with mammalian adaptation, efficient transmission via respiratory droplets between ferrets, and replication in human cells [63–67]. The NA protein is also likely to contribute to viral transmissibility as shown by Chen et al. [50]. During the budding process, the NA protein cleaves sialic acids from cellular receptors to facilitate the release of virus particles from the infected cell surface. An optimal interplay between the activities of HA and NA is required for efficient virus replication [68]. In addition, a recent study reported that the balance between HA and NA activities is critical for efficient respiratory droplet transmission of a pandemic 2009 H1N1 virus in ferrets [69].

Conclusions

Highly pathogenic avian H5N1 influenza A viruses continue to cause outbreaks in poultry with human cases in Indonesia, Vietnam, Egypt, and elsewhere. A significant proportion of H5N1 field isolates circulating in Europe, the Middle East, and Africa have already acquired the ability to recognize human-type receptors to some extent. In addition, avian H9N2 influenza viruses are now endemic in poultry populations in parts of Asia and the Middle East, and numerous H9N2 viruses have acquired human-type receptor binding specificity. Influenza researchers, therefore, speculate that the next pandemic might be caused by H5N1 viruses that have adapted to humans, or by H9N2 viruses. However, we do not yet know the full range of factors that can modulate the transmission of influenza A viruses. In particular, it is not clear whether the avian-human reassortant viruses that can transmit between ferrets can support sustained human-to-human transmission. Novel approaches are needed to better understand the molecular basis of host range restriction and transmission of influenza A viruses.

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Highlights

- The hemagglutinin (HA) of influenza viruses mediates receptor binding.
- Receptor binding specificity is a major determinant of host range restriction.
- Avian influenza viruses do not transmit efficiently from human to human.
- Changes in binding specificity are required for cross-species transfer.

Figure 1. The receptor-binding properties of influenza A viruses

a, The HA of human isolates preferentially recognizes sialic acid linked to galactose by $\alpha 2,6$ -linkages (Sia $\alpha 2,6$ Gal), whereas the HA of avian isolates preferentially recognizes sialic acid linked to galactose by $\alpha 2,3$ -linkages (Sia $\alpha 2,3$ Gal). **b**, Binding of influenza A viruses to human respiratory tissues. A human virus and an avian virus were incubated with human trachea tissue sections and then stained with appropriate antibodies. All sections were subsequently incubated with fluorescent-labeled secondary antibodies and Hoechst dye (blue). The green stain indicates virus binding.

Figure 2. Structural model of H5N1 virus HA in complex with human receptor analogs
Left panel, the monomer of A/Vietnam/1203/2004 HA [Protein Data Bank (PDB) accession 2FK0]. Right panel, close-up view of the globular head of A/Vietnam/1203/2004 HA. The human receptor analog [derived from its complex with H9 HA (PDB accession 1JSI)] is docked into the structure (shown in red). Images were created with MacPymol [<http://www.pymol.org/>].

Table 1

Transmission of avian influenza viruses in a ferret model

Virus	Avian or human virus gene [*]							Transmissible in ferrets via respiratory droplets	Receptor binding specificity		HA amino acid residue related to human-type receptor binding specificity	References
	PB2	PB1	PA	HA	NP	NA	M		α 2,3-glycans	α 2,6-glycans		
Avian H5N1 wild-type	A	A	A	A	A	A	A	-	+	-		[58, 59]
Avian H5N1 wild-type	A	A	A	A	A	A	A	-	+	+	227N	[58]
Avian H5N1 mutant	A	A	A	A	A	A	A	-	-	+	187G/190D/193S/226L/228S	[59]
Avian H5N1 mutant	A	A	A	A	A	A	A	-	-	+	196R/226L/228S	[50]
Avian H5N1/human H3N2 wild-type	H	H	H	A	H	A	H	-	+	-		[58]
Avian H5N1/human H3N2 mutant	A	A	A	A	A	H	A	+	-	+	196R/226L/228S	[50]
Avian H5N1/human H1N1 mutant	A	A	A	A	A	H	A	-	-	+	196R/226L/228S	[50]
Avian H7N7 wild-type	A	A	A	A	A	A	A	-	+	-		[51]
Avian H7N2 wild-type	A	A	A	A	A	A	A	-	+	+		[51]
Avian H9N2 wild-type	A	A	A	A	A	A	A	-	+	+	226L	[60]
Avian H9N2/human H3N2 wild-type	H	H	H	A	H	A	H	-	+	+	226L	[60, 61]
Ferret-adapted avian H9N2/human H3N2	H	H	H	A	H	A	H	+++		ND [†]	226L	[61]
Avian H9N2/human H1N1 wild-type	H	H	H	A	H	A	H	+++	+	+	226L	[62]

^{*} PB2, PB1, and PA, polymerase proteins; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix protein; NS, nonstructural protein; A, avian origin; H, human origin.

[†] ND, not determined.