Available online at www.sciencedirect.com

SciVerse ScienceDirect

Current Opinion in Virology

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

Masaki Imai[1] and Yoshihiro Kawaoka[2,3,4,5,6]

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, partly, owing 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.

Addresses
[1] Influenza Virus Research Center, National Institute of Infectious Diseases, Musashi-Murayama, Tokyo 208-0011, Japan
[2] Influenza Research Institute, Department of Pathobiological Sciences, University of Wisconsin-Madison, Madison, WI 53711, USA
[3] Division of Virology, Department of Microbiology and Immunology, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan
[4] Department of Special Pathogens, International Research Center for Infectious Diseases, Institute of Medical Science, University of Tokyo, Tokyo 108-8639, Japan
[5] ERATO Infection-Induced Host Responses Project, Saitama 332-0012, Japan
[6] Department of Microbiology and Infectious Diseases, Kobe University, Hyogo 650-0017, Japan

Corresponding author: Kawaoka, Yoshihiro (kawaokay@svm.vetmed.wisc.edu)

Current Opinion in Virology 2012, **2**:160–167

This review comes from a themed issue on
Emerging viruses
Edited by Erica Ollmann Saphire and Heinz Feldmann

1879-6257/$ – see front matter
Published by Elsevier B.V.

DOI 10.1016/j.coviro.2012.03.003

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.

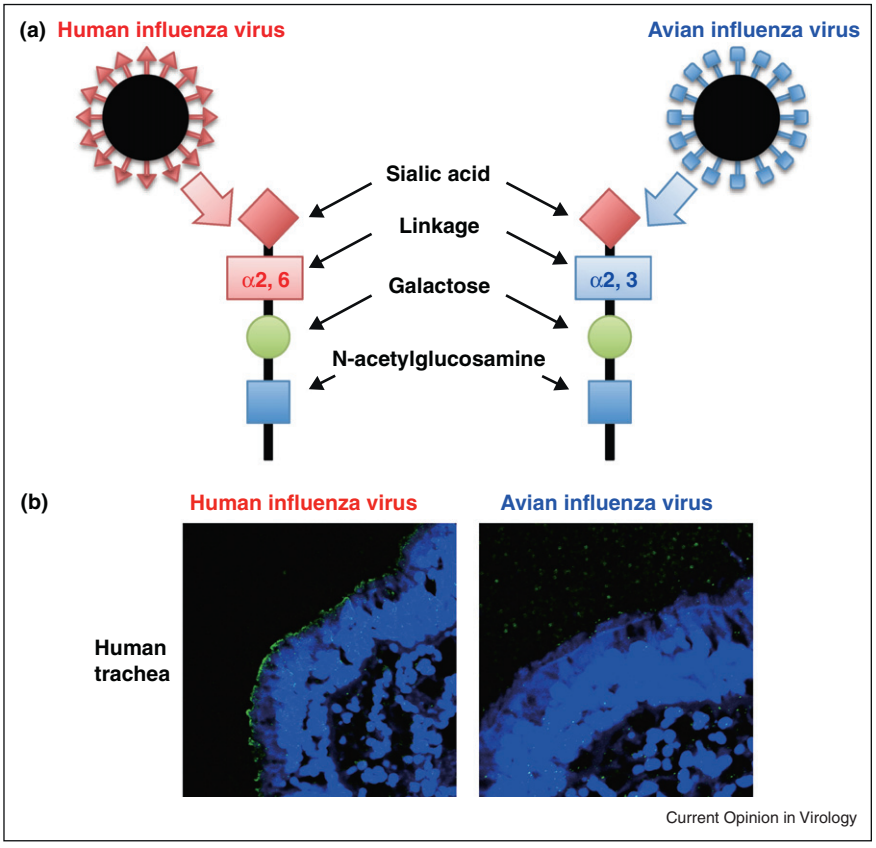
Influenza A viruses are classified into subtypes based on the antigenicity of their HA and NA. To date, sixteen known HA (H1–H16) and nine NA (N1–N9) subtypes of influenza A viruses have been isolated from aquatic birds, which are the reservoir of influenza A virus in nature [4,5]. Viruses with the HA 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 probably 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

Figure 1



The receptor-binding properties of influenza A viruses. **(a)** The HA of human isolates preferentially recognizes sialic acid linked to galactose by α2,6-linkages (Siaα2,6Gal), whereas the HA of avian isolates preferentially recognizes sialic acid linked to galactose by α2,3-linkages (Siaα2,3Gal). **(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.

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 α2,6-linkages (Siaα2,6Gal), whereas the HAs of avian influenza virus strains prefer oligosaccharides that terminate with a sialic acid linked to galactose by α2,3-linkages (Siaα2,3Gal) [19–21] (Figure 1a). Correspondingly, sialic acid-specific lectin staining of tissue sections has revealed that epithelial cells in the human upper respiratory tract express predominantly Siaα2,6-Gal, whereas those in duck intestine, where avian viruses replicate, express predominantly Siaα2,3Gal [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 (Figure 1b). By contrast, avian influenza viruses bind poorly to these cells. Thus, the relative lack of Siaα2,3Gal in the human upper respiratory tract is thought to restrict the efficient replication of avian influenza viruses. A shift from Siaα2,3Gal-binding to Siaα2,6Gal-binding specificity is probably 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 probably varies among avian species. In aquatic birds, including ducks and geese, avian-type receptors (Siaα2,3Gal) dominate in tracheal epithelial cells [28–30]. On the contrary, in terrestrial birds, including chickens, turkeys and quails, both avian-type (Siaα2,3Gal) and human-type (Siaα2,6Gal) 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.

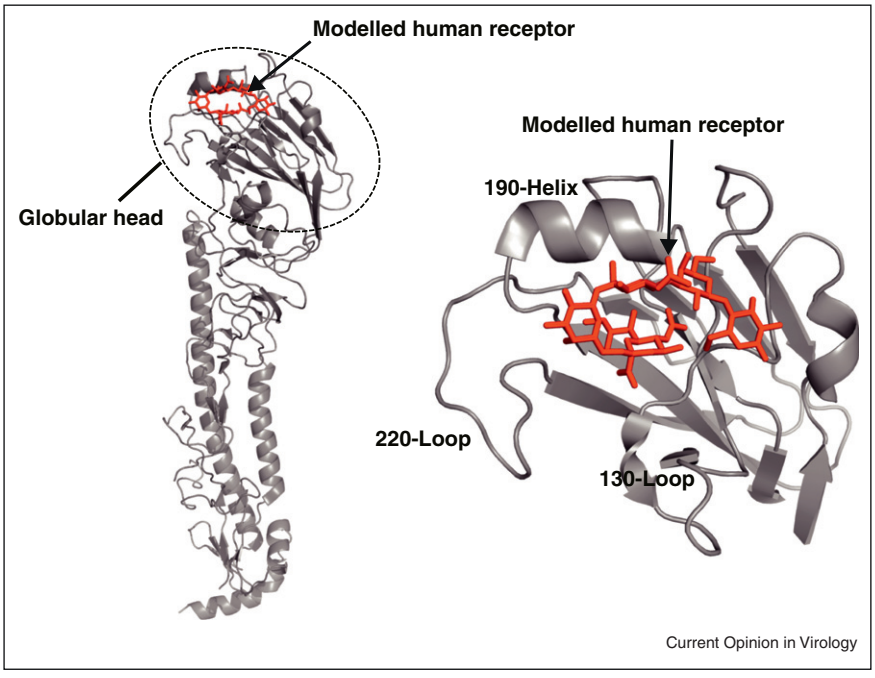
Traditionally, pigs have been considered as intermediate hosts or mixing vessels for the reassortment of avian and human influenza viruses owing to their susceptibility to infection with both avian and human isolates [34]. Initially, tracheal epithelial cells from pigs were reported to express substantial 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 α2,6-sialylated glycans are expressed predominantly on primary swine respiratory epithelial cells [37]. Thus, the receptor distribution in the respiratory tract of swine is probably 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 α2,6-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 (Figure 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 α2,3-glycan to α2,6-glycan

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/].

binding [20,40–42]. In the case of H1 HAs, the E190D and D225G mutations are critical for the shift from α2,3-glycan to α2,6-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 [43,45–48,49••]. However, viruses with these mutations retain their receptor binding preference for α2,3-glycans. A recent reverse genetic study demonstrated that Q196R/Q226L/G228S mutations in an H5 HA resulted in a shift from α2,3-glycan to α2,6-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 α2,3-glycans and α2,6-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 α2,6-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 α2,6-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 α2,6-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 α2,6-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-type 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 α2,6-glycan binding and decreased binding to α2,3-glycan [51]. In addition, H9N2 viruses that exhibit an α2,6-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

Table 1

Transmission of avian influenza viruses in a ferret model

Virus	Avian or human virus gene[a]								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	NS		α2,3-glycans	α2,6-glycans		
Avian H5N1 wild-type	A	A	A	A	A	A	A	A	–	+	–		[58,59]
Avian H5N1 wild-type	A	A	A	A	A	A	A	A	–	+	+	227N	[58]
Avian H5N1 mutant	A	A	A	A	A	A	A	A	–	–	+	187G/190D/193S/226L/228S	[59]
Avian H5N1 mutant	A	A	A	A	A	A	A	A	–	–	+	196R/226L/228S	[50•]
Avian H5N1/human H3N2 wild-type	H	H	H	A	H	A	H	H	–	+	–		[58]
Avian H5N1/human H3N2 mutant	A	A	A	A	A	H	A	A	+	–	+	196R/226L/228S	[50•]
Avian H5N1/human H1N1 mutant	A	A	A	A	A	H	A	A	–	–	+	196R/226L/228S	[50•]
Avian H7N7 wild-type	A	A	A	A	A	A	A	A	–	+	–		[51]
Avian H7N2 wild-type	A	A	A	A	A	A	A	A	–	+	+		[51]
Avian H9N2 wild-type	A	A	A	A	A	A	A	A	–	+	+	226L	[60]
Avian H9N2/human H3N2 wild-type	H	H	H	A	H	A	H	H	–	+	+	226L	[60,61•]
Ferret-adapted avian H9N2/human H3N2	H	H	H	A	H	A	H	H	+++	ND[b]		226L	[61•]
Avian H9N2/human H1N1 wild-type	H	H	H	A	H	A	H	H	+++	+	+	226L	[62]

[a] PB2, PB1, and PA, polymerase proteins; HA, hemagglutinin; NP, nucleoprotein; NA, neuraminidase; M, matrix protein; NS, nonstructural protein; A, avian origin; H, human origin.
[b] ND, not determined.

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 α2,6-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.

Acknowledgements

We thank S. Watson for editing the manuscript. This work was supported by a Grant-in-Aid for Specially Promoted Research, by the Japan Initiative for Global Research Network on Infectious Diseases from the Ministries of Education, Culture, Sports, Science, and Technology of Japan, by grants-in-aid from Health, Labor, and Welfare of Japan, by grants-in-aid from the Ministry of Health, by ERATO (Japan Science and Technology Agency), and by National Institute of Allergy and Infectious Diseases Public health Service Research grants.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Laver WG, Webster RG: **Ecology of influenza viruses in lower mammals and birds**. *Br Med Bull* 1979, **35**:29-33.

2. Palese P, Young JF: **Variation of influenza A, B, and C viruses**. *Science* 1982, **215**:1468-1474.

3. Skehel JJ, Wiley DC: **Receptor binding and membrane fusion in virus entry: the influenza hemagglutinin**. *Annu Rev Biochem* 2000, **69**:531-569.

4. Fouchier RA, Munster V, Wallensten A, Bestebroer TM, Herfst S, Smith D, Rimmelzwaan GF, Olsen B, Osterhaus AD: **Characterization of a novel influenza A virus hemagglutinin subtype (H16) obtained from black-headed gulls**. *J Virol* 2005, **79**:2814-2822.

5. Webster RG, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y: **Evolution and ecology of influenza A viruses**. *Microbiol Rev* 1992, **56**:152-179.

6. Claas EC, Osterhaus AD, van Beek R, De Jong JC, Rimmelzwaan GF, Senne DA, Krauss S, Shortridge KF, Webster RG: **Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus**. *Lancet* 1998, **351**:472-477.

7. Fouchier RA, Schneeberger PM, Rozendaal FW, Broekman JM, Kemink SA, Munster V, Kuiken T, Rimmelzwaan GF, Schutten M, Van Doornum GJ *et al.*: **Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome**. *Proc Natl Acad Sci USA* 2004, **101**:1356-1361.

8. Lin YP, Shaw M, Gregory V, Cameron K, Lim W, Klimov A, Subbarao K, Guan Y, Krauss S, Shortridge K *et al.*: **Avian-to-human transmission of H9N2 subtype influenza A viruses: relationship between H9N2 and H5N1 human isolates**. *Proc Natl Acad Sci USA* 2000, **97**:9654-9658.

9. Peiris M, Yuen KY, Leung CW, Chan KH, Ip PL, Lai RW, Orr WK, Shortridge KF: **Human infection with influenza H9N2**. *Lancet* 1999, **354**:916-917.

10. Subbarao K, Klimov A, Katz J, Regnery H, Lim W, Hall H, Perdue M, Swayne D, Bender C, Huang J *et al.*: **Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness**. *Science* 1998, **279**:393-396.

11. Kandun IN, Wibisono H, Sedyaningsih ER, Yusharmen, Hadisoedarsuno W, Purba W, Santoso H, Septiawati C, Tresnaningsih E, Heriyanto B *et al.*: **Three Indonesian clusters of H5N1 virus infection in 2005**. *N Engl J Med* 2006, **355**:2186-2194.

12. Koopmans M, Wilbrink B, Conyn M, Natrop G, van der Nat H, Vennema H, Meijer A, van Steenbergen J, Fouchier R, Osterhaus A *et al.*: **Transmission of H7N7 avian influenza A virus to human beings during a large outbreak in commercial poultry farms in the Netherlands**. *Lancet* 2004, **363**:587-593.

13. Olsen SJ, Ungchusak K, Sovann L, Uyeki TM, Dowell SF, Cox NJ, Aldis W, Chunsuttiwat S: **Family clustering of avian influenza A (H5N1)**. *Emerg Infect Dis* 2005, **11**:1799-1801.

14. Tran TH, Nguyen TL, Nguyen TD, Luong TS, Pham PM, Nguyen VC, Pham TS, Vo CD, Le TQ, Ngo TT *et al.*: **Avian influenza A (H5N1) in 10 patients in Vietnam**. *N Engl J Med* 2004, **350**:1179-1188.

15. Ungchusak K, Auewarakul P, Dowell SF, Kitphati R, Auwanit W, Puthavathana P, Uiprasertkul M, Boonnak K, Pittayawonganon C, Cox NJ *et al.*: **Probable person-to-person transmission of avian influenza A (H5N1)**. *N Engl J Med* 2005, **352**:333-340.

16. Glaser L, Stevens J, Zamarin D, Wilson IA, Garcia-Sastre A, Tumpey TM, Basler CF, Taubenberger JK, Palese P: **A single amino acid substitution in 1918 influenza virus hemagglutinin changes receptor binding specificity**. *J Virol* 2005, **79**:11533-11536.

17. Matrosovich M, Tuzikov A, Bovin N, Gambaryan A, Klimov A, Castrucci MR, Donatelli I, Kawaoka Y: **Early alterations of the receptor-binding properties of H1, H2, and H3 avian influenza virus hemagglutinins after their introduction into mammals**. *J Virol* 2000, **74**:8502-8512.

18. Matrosovich MN, Klenk HD, Kawaoka Y: **Receptor specificity, host-range, and pathogenicity of influenza viruses**. In *Influenza Virology: Current Topics.* Edited by Kawaoka Y. Caister Academic Press; 2006:95-138.

19. Rogers GN, Paulson JC: **Receptor determinants of human and animal influenza virus isolates: differences in receptor specificity of the H3 hemagglutinin based on species of origin**. *Virology* 1983, **127**:361-373.

20. Connor RJ, Kawaoka Y, Webster RG, Paulson JC: **Receptor specificity in human, avian, and equine H2 and H3 influenza virus isolates**. *Virology* 1994, **205**:17-23.

21. Stevens J, Blixt O, Glaser L, Taubenberger JK, Palese P, Paulson JC, Wilson IA: **Glycan microarray analysis of the hemagglutinins from modern and pandemic influenza viruses reveals different receptor specificities**. *J Mol Biol* 2006, **355**:1143-1155.

22. Ito T, Couceiro JN, Kelm S, Baum LG, Krauss S, Castrucci MR, Donatelli I, Kida H, Paulson JC, Webster RG *et al.*: **Molecular basis for the generation in pigs of influenza A viruses with pandemic potential**. *J Virol* 1998, **72**:7367-7373.

23. Shinya K, Ebina M, Yamada S, Ono M, Kasai N, Kawaoka Y: **Avian flu: influenza virus receptors in the human airway**. *Nature* 2006, **440**:435-436.

24. Chutinimitkul S, van Riel D, Munster VJ, van den Brand JM, Rimmelzwaan GF, Kuiken T, Osterhaus AD, Fouchier RA, de Wit E: **In vitro assessment of attachment pattern and replication efficiency of H5N1 influenza A viruses with altered receptor specificity**. *J Virol* 2010, **84**:6825-6833.

25. van Riel D, Munster VJ, de Wit E, Rimmelzwaan GF, Fouchier RA, Osterhaus AD, Kuiken T: **H5N1 virus attachment to lower respiratory tract**. *Science* 2006, **312**:399.

26. van Riel D, Munster VJ, de Wit E, Rimmelzwaan GF, Fouchier RA, Osterhaus AD, Kuiken T: **Human and avian influenza viruses target different cells in the lower respiratory tract of humans and other mammals**. *Am J Pathol* 2007, **171**:1215-1223.

27. Childs RA, Palma AS, Wharton S, Matrosovich T, Liu Y, Chai W, Campanero-Rhodes MA, Zhang Y, Eickmann M, Kiso M *et al.*: **Receptor-binding specificity of pandemic influenza A (H1N1) 2009 virus determined by carbohydrate microarray**. *Nat Biotechnol* 2009, **27**:797-799.

28. Kimble B, Nieto GR, Perez DR: **Characterization of influenza virus sialic acid receptors in minor poultry species**. *Virol J* 2010, **7**:365.

29. Kuchipudi SV, Nelli R, White GA, Bain M, Chang KC, Dunham S: **Differences in influenza virus receptors in chickens and**

ducks: implications for interspecies transmission. *J Mol Genet Med* 2009, **3**:143-151.

30. Yu JE, Yoon H, Lee HJ, Lee JH, Chang BJ, Song CS, Nahm SS: **Expression patterns of influenza virus receptors in the respiratory tracts of four species of poultry**. *J Vet Sci* 2011, **12**:7-13.

31. Wan H, Perez DR: **Quail carry sialic acid receptors compatible with binding of avian and human influenza viruses**. *Virology* 2006, **346**:278-286.

32. Yamada S, Shinya K, Takada A, Ito T, Suzuki T, Suzuki Y, Le QM, Ebina M, Kasai N, Kida H *et al.*: **Adaptation of a duck influenza A virus in quail**. *J Virol* 2012, **86**:1411-1420.

33. Matrosovich MN, Krauss S, Webster RG: **H9N2 influenza A viruses from poultry in Asia have human virus-like receptor specificity**. *Virology* 2001, **281**:156-162.

34. Kida H, Ito T, Yasuda J, Shimizu Y, Itakura C, Shortridge KF, Kawaoka Y, Webster RG: **Potential for transmission of avian influenza viruses to pigs**. *J Gen Virol* 1994, **75(Pt 9)**:2183-2188.

35. Nelli RK, Kuchipudi SV, White GA, Perez BB, Dunham SP, Chang KC: **Comparative distribution of human and avian type sialic acid influenza receptors in the pig**. *BMC Vet Res* 2010, **6**:4.

36. Van Poucke SG, Nicholls JM, Nauwynck HJ, Van Reeth K: **Replication of avian, human and swine influenza viruses in porcine respiratory explants and association with sialic acid distribution**. *Virol J* 2010, **7**:38.

37. Bateman AC, Karamanska R, Busch MG, Dell A, Olsen CW, Haslam SM: **Glycan analysis and influenza A virus infection of primary swine respiratory epithelial cells: the importance of NeuAc{alpha}2-6 glycans**. *J Biol Chem* 2010, **285**:34016-34026.

38. •• Chandrasekaran A, Srinivasan A, Raman R, Viswanathan K, Raguram S, Tumpey TM, Sasisekharan V, Sasisekharan R: **Glycan topology determines human adaptation of avian H5N1 virus hemagglutinin**. *Nat Biotechnol* 2008, **26**:107-113.
This study demonstrated that the three-dimensional structure of sialylated glycans plays a critical role in the differential recognition of sialic acid-containing receptors by avian and human influenza virus HAs.

39. Varki A: **Glycan-based interactions involving vertebrate sialic-acid-recognizing proteins**. *Nature* 2007, **446**:1023-1029.

40. Liu J, Stevens DJ, Haire LF, Walker PA, Coombs PJ, Russell RJ, Gamblin SJ, Skehel JJ: **Structures of receptor complexes formed by hemagglutinins from the Asian Influenza pandemic of 1957**. *Proc Natl Acad Sci USA* 2009, **106**:17175-17180.

41. Rogers GN, Paulson JC, Daniels RS, Skehel JJ, Wilson IA, Wiley DC: **Single amino acid substitutions in influenza haemagglutinin change receptor binding specificity**. *Nature* 1983, **304**:76-78.

42. Xu R, McBride R, Paulson JC, Basler CF, Wilson IA: **Structure, receptor binding, and antigenicity of influenza virus hemagglutinins from the 1957 H2N2 pandemic**. *J Virol* 2010, **84**:1715-1721.

43. Stevens J, Blixt O, Chen LM, Donis RO, Paulson JC, Wilson IA: **Recent avian H5N1 viruses exhibit increased propensity for acquiring human receptor specificity**. *J Mol Biol* 2008, **381**:1382-1394.

44. Stevens J, Blixt O, Tumpey TM, Taubenberger JK, Paulson JC, Wilson IA: **Structure and receptor specificity of the hemagglutinin from an H5N1 influenza virus**. *Science* 2006, **312**:404-410.

45. Auewarakul P, Suptawiwat O, Kongchanagul A, Sangma C, Suzuki Y, Ungchusak K, Louisirirotchanakul S, Lerdsamran H, Pooruk P, Thitithanyanont A *et al.*: **An avian influenza H5N1 virus that binds to a human-type receptor**. *J Virol* 2007, **81**:9950-9955.

46. Gambaryan A, Tuzikov A, Pazynina G, Bovin N, Balish A, Klimov A: **Evolution of the receptor binding phenotype of influenza A (H5) viruses**. *Virology* 2006, **344**:432-438.

47. Wang W, Lu B, Zhou H, Suguitan AL Jr, Cheng X, Subbarao K, Kemble G, Jin H: **Glycosylation at 158N of the hemagglutinin protein and receptor binding specificity synergistically affect the antigenicity and immunogenicity of a live attenuated H5N1 A/Vietnam/1203/2004 vaccine virus in ferrets**. *J Virol* 2010, **84**:6570-6577.

48. Watanabe Y, Ibrahim MS, Ellakany HF, Kawashita N, Mizuike R, Hiramatsu H, Sriwilaijaroen N, Takagi T, Suzuki Y, Ikuta K: **Acquisition of human-type receptor binding specificity by new H5N1 influenza virus sublineages during their emergence in birds in Egypt**. *PLoS Pathog* 2011, **7**:e1002068.

49. •• Yamada S, Suzuki Y, Suzuki T, Le MQ, Nidom CA, Sakai-Tagawa Y, Muramoto Y, Ito M, Kiso M, Horimoto T *et al.*: **Haemagglutinin mutations responsible for the binding of H5N1 influenza A viruses to human-type receptors**. *Nature* 2006, **444**:378-382.
This study reported that some human H5N1 isolates show limited binding to human-type receptors and identified mutations in their H5 HAs associated with increased binding to human-type receptors.

50. • Chen LM, Blixt O, Stevens J, Lipatov AS, Davis CT, Collins BE, Cox NJ, Paulson JC, Donis RO: **In vitro evolution of H5N1 avian influenza virus toward human-type receptor specificity**. *Virology* 2012, **422**:105-113.
This is the first report to show that that an H5 HA can convert to an HA that supports viral transmission via respiratory droplet in mammals.

51. Belser JA, Blixt O, Chen LM, Pappas C, Maines TR, Van Hoeven N, Donis R, Busch J, McBride R, Paulson JC *et al.*: **Contemporary North American influenza H7 viruses possess human receptor specificity: implications for virus transmissibility**. *Proc Natl Acad Sci USA* 2008, **105**:7558-7563.

52. Yang H, Chen LM, Carney PJ, Donis RO, Stevens J: **Structures of receptor complexes of a North American H7N2 influenza hemagglutinin with a loop deletion in the receptor binding site**. *PLoS Pathog* 2010, **6**:e1001081.

53. Wan H, Perez DR: **Amino acid 226 in the hemagglutinin of H9N2 influenza viruses determines cell tropism and replication in human airway epithelial cells**. *J Virol* 2007, **81**:5181-5191.

54. Ha Y, Stevens DJ, Skehel JJ, Wiley DC: **X-ray structures of H5 avian and H9 swine influenza virus hemagglutinins bound to avian and human receptor analogs**. *Proc Natl Acad Sci USA* 2001, **98**:11181-11186.

55. Xu Q, Wang W, Cheng X, Zengel J, Jin H: **Influenza H1N1 A/Solomon Island/3/06 virus receptor binding specificity correlates with virus pathogenicity, antigenicity, and immunogenicity in ferrets**. *J Virol* 2010, **84**:4936-4945.

56. Pappas C, Viswanathan K, Chandrasekaran A, Raman R, Katz JM, Sasisekharan R, Tumpey TM: **Receptor specificity and transmission of H2N2 subtype viruses isolated from the pandemic of 1957**. *PLoS ONE* 2010, **5**:e11158.

57. •• Tumpey TM, Maines TR, Van Hoeven N, Glaser L, Solorzano A, Pappas C, Cox NJ, Swayne DE, Palese P, Katz JM *et al.*: **A two-amino acid change in the hemagglutinin of the 1918 influenza virus abolishes transmission**. *Science* 2007, **315**:655-659.
This study demonstrated that the respiratory droplet transmission of influenza A virus in mammals can be controlled by the sialic acid binding specificity of HA.

58. Maines TR, Chen LM, Matsuoka Y, Chen H, Rowe T, Ortin J, Falcon A, Nguyen TH, Mai le Q, Sedyaningsih ER *et al.*: **Lack of transmission of H5N1 avian-human reassortant influenza viruses in a ferret model**. *Proc Natl Acad Sci USA* 2006, **103**:12121-12126.

59. Maines TR, Chen LM, Van Hoeven N, Tumpey TM, Blixt O, Belser JA, Gustin KM, Pearce MB, Pappas C, Stevens J *et al.*: **Effect of receptor binding domain mutations on receptor binding and transmissibility of avian influenza H5N1 viruses**. *Virology* 2011, **413**:139-147.

60. Wan H, Sorrell EM, Song H, Hossain MJ, Ramirez-Nieto G, Monne I, Stevens J, Cattoli G, Capua I, Chen LM *et al.*: **Replication and transmission of H9N2 influenza viruses in ferrets: evaluation of pandemic potential**. *PLoS ONE* 2008, **3**:e2923.

61. • Sorrell EM, Wan H, Araya Y, Song H, Perez DR: **Minimal molecular constraints for respiratory droplet transmission of an avian-human H9N2 influenza A virus**. *Proc Natl Acad Sci USA* 2009, **106**:7565-7570.

This study demonstrated that a reassortant virus possessing the HA and NA gene segments of an avian virus in a human virus backbone can transmit via respiratory droplet in mammals.

62. Kimble JB, Sorrell E, Shao H, Martin PL, Perez DR: **Compatibility of H9N2 avian influenza surface genes and 2009 pandemic H1N1 internal genes for transmission in the ferret model**. *Proc Natl Acad Sci USA* 2011, **108**:12084-12088.

63. Bussey KA, Bousse TL, Desmet EA, Kim B, Takimoto T: **PB2 residue 271 plays a key role in enhanced polymerase activity of influenza A viruses in mammalian host cells**. *J Virol* 2010, **84**:4395-4406.

64. Hatta M, Gao P, Halfmann P, Kawaoka Y: **Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses**. *Science* 2001, **293**:1840-1842.

65. Li Z, Chen H, Jiao P, Deng G, Tian G, Li Y, Hoffmann E, Webster RG, Matsuoka Y, Yu K: **Molecular basis of replication of duck H5N1 influenza viruses in a mammalian mouse model**. *J Virol* 2005, **79**:12058-12064.

66. Mehle A, Doudna JA: **Adaptive strategies of the influenza virus polymerase for replication in humans**. *Proc Natl Acad Sci USA* 2009, **106**:21312-21316.

67. Yamada S, Hatta M, Staker BL, Watanabe S, Imai M, Shinya K, Sakai-Tagawa Y, Ito M, Ozawa M, Watanabe T *et al.*: **Biological and structural characterization of a host-adapting amino acid in influenza virus**. *PLoS Pathog* 2010, **6**:e1001034.

68. Wagner R, Matrosovich M, Klenk HD: **Functional balance between haemagglutinin and neuraminidase in influenza virus infections**. *Rev Med Virol* 2002, **12**:159-166.

69. Yen HL, Liang CH, Wu CY, Forrest HL, Ferguson A, Choy KT, Jones J, Wong DD, Cheung PP, Hsu CH *et al.*: **Hemagglutinin-neuraminidase balance confers respiratory-droplet transmissibility of the pandemic H1N1 influenza virus in ferrets**. *Proc Natl Acad Sci USA* 2011, **108**:14264-14269.