Published in final edited form as:
*Future Virol*. 2006 ; 1(4): 415–418. doi:10.2217/17460794.1.4.415.

# Influenza hemagglutinin attachment to target cells: 'birds do it, we do it…'

**Jeffery K Taubenberger**
*Department of Molecular Pathology, Armed Forces Institute of Pathology, Rockville, MD 20306 USA*

## Past & future influenza

Influenza A viruses have been much in the news lately, and rightly so. This divergent group of viruses has caused occasional and unpredictable pandemics, some with horrific consequences, and also annual epidemics in humans with over 36,000 fatalities per year in the USA alone [1]. Other strains of influenza cause significant outbreaks in domestic animals, including horses, pigs, dogs, chickens, turkeys and other poultry. In the last 2 years, an evolving strain of highly pathogenic avian influenza viruses, of the H5N1 subtype, has spread from South-East Asia to much of the world, and has been associated with devastating outbreaks in domestic poultry and some species of wild birds. More alarmingly, this virus has caused over 200 known human infections with a reported case fatality rate of over 50%. It is the risk that a virus from this lineage may become adapted to humans and cause a new pandemic that is driving the concern of the international public health community.

Influenza A viruses are negative-strand RNA viruses. Their segmented genome consists of eight RNA segments encoding 11 open reading frames. Two glycosylated proteins, hemagglutinin (HA) and neuraminidase (NA), are involved in viral attachment to cells. These two proteins are quite divergent, both antigenically and genetically, in avian influenza isolates. There are currently 16 known subtypes, or serotypes, of HA (H1–H16), and nine subtypes of NA (N1–N9). Influenza viruses are identified by the combination of HA and NA subtypes they express, such as H3N2 or H5N1. All 16 HAs and all nine NAs have been observed in avian influenza viruses (but not all possible combinations have been found). However, only a much more limited set of influenza subtypes have been observed in humans and other mammals. Whether only particular subtypes can adapt to mammals is currently unknown.

HA can change in order to evade previously acquired immunity either by antigenic drift, whereby mutations of the currently circulating HA gene disrupt antibody binding, or by antigenic shift, in which the virus acquires a HA of a new subtype.

Much has been learnt about the basic biology of influenza A viruses since their initial isolation in the early 1930s, but much more needs to be elucidated. It is known that wild birds, including waterfowl like ducks and geese, and shorebirds, including gulls and terns, are probably the natural reservoir of these viruses. Ongoing surveillance and the recent application of genomic analysis will help define the range and extent of these viruses in their host species. Numerous questions remain, however, before we can understand the ecobiology of these viruses in their wild bird hosts: how is such a divergent pool of viruses maintained? Are particular strains adapted to particular hosts? What kinds of immune response are mounted? Is there an

taubenbe@afip.osd.mil.
Current address: Laboratory of Infectious Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, MD 20892, USA.

environmental reservoir for these viruses? What role do bird migrations play in the distribution of influenza viruses? As interesting as these questions are, more significant questions remain unanswered.

## Host switching

The most important of these questions is: how do influenza viruses switch hosts? What changes are needed in the genome of a virus adapted to the gastrointestinal tract of wild waterfowl to become respiratory viruses of domestic poultry, horses, swine and, most importantly, humans? A question everyone wants answered is: can the current H5N1 viruses adapt to become transmissible in humans to form a new pandemic? Unfortunately, these questions cannot be answered with available data. We can conclude with certainty that avian influenza viruses have adapted to new hosts, and novel host-adapted poultry, equine, swine and human viral lineages derived from avian influenzas have emerged in the past. Two things are clear from this:

- It is possible for avian influenza viruses to adapt to new hosts
- The relative paucity of these events suggests that it is a complex, multistep process

The complete genomes of the last three pandemic influenza viruses in humans, those of the 1968 'Hong Kong' H3N2 pandemic virus, the 1957 'Asian' H2N2 virus and the 1918 'Spanish' H1N1 virus are known. The two most recent pandemics were reassortant viruses, in which the core of the human-adapted viral lineage derived from the 1918 virus acquired two or three gene segments from an unidentified avian influenza virus. The 1918 virus was likely an entirely avian-like virus adapted to humans. These genomes, however, are the 'finished products' of this process and we do not know how any of these pandemic viruses formed; whether they used an intermediate host animal during the process of adaptation to humans, or for that matter, how long this process of host adaptation took. In no case in the past was surveillance adequate to identify precursor strains in order to construct a workable hypothesis as to how such pandemic strains emerge.

Employing sequence analysis of the 1918 virus with subsequent human influenza viruses, and the increasing number of avian influenza genomes publicly available, a number of mutations have been hypothesized to play important roles in human adaptation for each of the genes of the virus. Experiments to test the significance of these changes are underway, but our understanding of the processes of host switching is still very primitive. Two changes have been shown to be important for mammalian adaptation experimentally: a single amino acid change in the polymerase PB2, although the underlying mechanism favoring the accumulation of this mutation in mammalian influenza viruses has not been adequately explained, and the receptor-binding site of HA, which has also be studied intensively.

## Hemagglutinin receptor binding

Many of the glycan chains making up the mammalian glycocalyx terminate in sialic acids. Consequently, these sialic acids are used as attachment sites for many pathogens. They are attached by different sialyltransferases to glycans via $\alpha$2–3, $\alpha$2–6, $\alpha$2–8 or other linkages. It is thought that the binding specificity of avian influenza virus HA, regardless of subtype, is preferentially for sialic acids in an $\alpha$2–3 linkage to the underlying galactose (SA $\alpha$2–3Gal). Human-adapted influenza viruses, of H1, H2 and H3 subtypes, derived from the last three pandemics, have a preference for sialic acids bound via an $\alpha$2–6 linkage (SA $\alpha$2–6Gal). Thus, a switch in specificity from SA $\alpha$2–3Gal to SA $\alpha$2–6Gal has been proposed as essential for host adaptation [2].

Recent studies employing a number of novel techniques have helped examine this issue in more detail. While these studies generally support this underlying avian-human receptor-

binding dichotomy, they have shown that the interactions between virus and host are very complex.

A number of studies published over the past decade have examined the distribution of SA α2–3Gal and SA α2–6Gal in tissue sections of different animal species by lectin histochemistry. Several studies have examined the distribution of sialic acids on human respiratory epithelium, and have shown that ciliated cells of the upper airway express predominantly SA α2–6Gal. Staining for SA α2–3Gal was limited mainly to mucin-producing goblet cells, compatible with the finding that secreted respiratory mucins express α2–3-linked sialic acids, and thus may serve as a barrier to zoonotic influenza viral infections [2–5]. As expected, avid binding of human influenza viruses was shown to upper respiratory epithelium while viruses with avian-like receptor specificity bound mainly to mucin-producing cells. In the lower respiratory tract, type II alveolar cells were shown to express SA α2–3Gal. The staining pattern was shown to be reversed in the upper airways of gorillas and mice, but the pattern in ferrets was analogous to humans, possibly providing one explanation for the suitability of ferrets as an animal model for human influenza. Swine and quail were both shown to express SA α2–3Gal and SA α2–6Gal in their respiratory epithelia, thus supporting a possible role as an intermediate host in adaptation [6,7].

By contrast, recent studies employing primary human tracheobronchial airway cultures found that human influenza viruses bound predominantly to non-ciliated, mucin-producing cells, whereas avian influenza viruses bound predominantly to ciliated cells [8]. Resolving the apparent conflict between these results and those employing fixed tissue sections is still needed. Two recent studies showed that H5N1, and other avian influenza viruses, bound poorly to the upper respiratory tree, but did bind to some alveolar epithelial cells [4,5]. Together, these studies demonstrate that the epithelium of the respiratory tracts of different animals show different expression patterns of SA α2–3Gal and SA α2–6Gal and provide some explanation for the evident barriers to host switching. On the other hand, the presence of both SA α2–3Gal and SA α2–6Gal in the human respiratory tree suggests the possibility that humans may serve as their own ‘mixing vessel’ or intermediate host in the coinfection of human and avian influenza viruses that could yield a reassortant virus with pandemic potential.

Adding another level of detail are recent studies using glycoarrays that have begun to assess in great detail the binding specificity of particular influenza HA proteins [9–11]. The use of lectins specific for SA α2–3Gal and SA α2–6Gal in the above studies does not distinguish the expression of the particular glycans terminating in these linkages. The arrays have shown that the binding pattern of two viral HAs can be quite different, even if they both have a preferential binding to SA α2–6Gal. In other words, different viruses can each bind different subsets of glycans terminating in SA α2–6Gal.

It has been shown that the sequenced isolates of the 1918 HA differ at a single amino acid in the receptor-binding site, with three of the five sequences showing a change from the conserved avian sequence at residue 225 from a glycine to an aspartic acid. The remaining two sequences retain the avian glycine at that site, but all five sequences share another change from the avian configuration, a glutamic acid to aspartic acid change at residue 190. Interestingly, the 1918 variant with both 190 and 225 changes was found to bind only SA α2–6Gal, like a contemporary human H3 HA. The 1918 variants with only the 190 change bound both SA α2–3Gal and SA α2–6Gal. Curiously, no difference in pathology, clinical course or chronology of infection was noted for these 1918 cases. The significance of a pandemic human virus with only limited specificity for the ‘human’ SA α2–6Gal receptor is currently unknown. Mutating this site back to the conserved avian residue revealed a binding pattern typical of avian HAs with broad specificity to glycans terminating in SA α2–3Gal, with or without fucose or sulfate side-chains on the sugars internal to the penultimate galactose [10,11].

The HA crystal structure of a recent highly pathogenic H5N1 virus was determined and found to be very similar to the structure of the 1918 H1 HA. Despite this similarity in overall structure and structure of the receptor-binding site, mutation of the H5 HA receptor-binding site at the 190 and 225 residues (analogous to the 1918 virus) did not enhance its binding to SA $\alpha$2–6Gal in the array. Surprisingly, the mutation of two other receptor-binding residues, 226 and 228 (those changes observed in the 1957 H2 and 1968 H3 pandemics) allowed the H5 HA to bind two SA $\alpha$2–6Gal glycans on the array at the same time, thus reducing its preference for SA $\alpha$2–3Gal. Therefore, none of the mutations tested fully converted the H5 HA to SA $\alpha$2–6Gal specificity [11]. Would such a change in receptor-binding specificity in H5, in the context of a virus with all the other changes it required for human adaptation, be sufficient to allow the virus to be transmitted person-to-person? Does the blended specificity observed with one form of the 1918 HA provide a historical precedent for a similar future adaptation?

No H5 isolates have been reported with the mutations at 226 and 228 studied in this in vitro system. There have been several isolates with a change at residue 227 however. What is the significance of this mutation? Different studies have produced conflicting results. The glycoarray study found no enhanced binding to SA $\alpha$2–6Gal, but other studies have reported enhanced SA $\alpha$2–6Gal binding with this H5N1 variant [12].

## Future questions

Clearly, a number of fundamental questions remain unanswered. The glycoarray studies have shown that it is inadequate to consider only the linkage of the terminal sialic acid in regard to determining the specificity of different influenza HA proteins. But what then is the distribution of these diverse glycans on the respiratory tree? Which particular glycans are on which particular cells and at what level of expression? How much variability in expression patterns is observed between animals? Are there changes with age or other physiological states? Can all avian HA subtypes acquire mutations that would allow them to bind SA $\alpha$2–6Gal? If not, which ones? How much SA $\alpha$2–6Gal specificity is required for a virus to become an efficient human virus? What is the significance of the 1918 variants? Were these variants equally transmissible in humans? Where they equally pathogenic? Is reduced SA $\alpha$2–3Gal binding and marginally enhanced SA $\alpha$2–6Gal binding good enough for H5N1 to cause a pandemic should it acquire the ability to switch hosts in humans either by reassortment or via polygenic adaptation in toto? We have the tools to answer many of these questions. It is urgent that we solve them.

## Bibliography

1. Taubenberger JK, Morens DM. 1918 Influenza: the mother of all pandemics. Emerg Infect Dis 2006;12 (1):15–22. [PubMed: 16494711]
2. Baum LG, Paulson JC. Sialyloligosaccharides of the respiratory epithelium in the selection of human influenza virus receptor specificity. Acta Histochem Suppl 1990;40:35–38. [PubMed: 2091044]
3. Gagneux P, Cheriyan M, Hurtado-Ziola N, et al. Human-specific regulation of $\alpha$2–6-linked sialic acids. J Biol Chem 2003;278(48):48245–48250. [PubMed: 14500706]
4. Shinya K, Ebina M, Yamada S, Ono M, Kasai N, Kawaoka Y. Avian flu: influenza virus receptors in the human airway. Nature 2006;440(7083):435–436. [PubMed: 16554799]
5. van Riel D, Munster VJ, de Wit E, et al. H5N1 virus attachment to lower respiratory tract. Science 2006;312(5772):399. [PubMed: 16556800]
6. Ito T, Couceiro JN, Kelm S. Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. J Virol 1998;72(9):7367–7373. [PubMed: 9696833]
7. Wan H, Perez DR. Quail carry sialic acid receptors compatible with binding of avian and human influenza viruses. Virology 2006;346(2):278–286. [PubMed: 16325879]

8. Matrosovich MN, Matrosovich TY, Gray T, Roberts NA, Klenk HD. Human and avian influenza viruses target different cell types in cultures of human airway epithelium. Proc Natl Acad Sci USA 2004;101(13):4620–4624. [PubMed: 15070767]

9. Blixt O, Head S, Mondala T. Printed covalent glycan array for ligand profiling of diverse glycan binding proteins. Proc Natl Acad Sci USA 2004;101(49):17033–17038. [PubMed: 15563589]

10. Stevens J, Blixt O, Glaser L, et al. Glycan microarray analysis of the hemagglutinins from modern and pandemic influenza viruses reveals different receptor specificities. J Mol Biol 2006;355:1143–1155. [PubMed: 16343533]

11. 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(5772):404–410. [PubMed: 16543414]

12. Shinya K, Hatta M, Yamada S. Characterization of a human H5N1 influenza A virus isolated in 2003. J Virol 2005;79(15):9926–9932. [PubMed: 16014953]