Lessons from the 1918 influenza

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Recent studies of the 1918 influenza virus shed light on the risks of avian influenza virus.

The clock is counting down to the next influenza pandemic. Since 1997, the avian influenza H5N1 virus has infected over 250 people worldwide with a fatality rate of ~60%¹, although so far without sustained human-to-human transmission. Two recent studies of another influenza virus, the H1N1 virus responsible for the 1918 pandemic, provide important clues about the potential of H5N1 to spark a modern-day equivalent. In January, Kobasa *et al.*² reported in *Nature* that the 1918 virus triggers an aberrant innate immune response in primate hosts that probably contributes to its lethality and that may be similar to the response induced by the H5N1 virus. The following month, a report in *Science* by Tumpey *et al.*³ showed that a two-amino-acid substitution in the 1918 viral hemagglutinin protein, which mediates binding to target cells, abolishes transmission among ferrets. These papers, together with other recent work on the H5N1 virus, highlight important similarities and differences between the two viruses.

Tumpey *et al.* studied the relationship between the 1918 H1N1's receptor binding preference and its transmissibility (Fig. 1a,b). Mutation of two amino acid residues (190 and 225) in the hemagglutinin protein that switched the virus' receptor binding preference from human α -2,6 sialic acid to avian α -2,3 sialic acid prevented transmission among ferrets without affecting viral replication and lethality. This very significant observation supports the argument that the viral receptor is necessary and sufficient to determine mammalian transmissibility, at least for the 1918 H1N1. How applicable are these findings to H5N1?

Although H5N1 can be detected in the upper airways during human infections⁴, lack of transmission among individuals has been attributed to a paucity of α -2,3-sialic acid glycoconjugate receptors in the human upper respiratory tract, implying that viral replication is confined to the lower tract, preventing the spread of viral progeny. Several studies have confirmed increased density of α -2,3 receptors in the lower airways of humans⁵ and other mammalian model systems for H5N1 (ferrets and macaques)⁶.

However, assessment of receptor distribution by the lectin binding assays used in these studies may be imprecise given the wide spectrum of glycoconjugates found in human tissues and overlying mucus. In addition, H5N1 viruses have diverse receptor binding properties⁷, the complexity of which has been demonstrated by glycan array analysis⁸. Indeed, recent work by Nicholls *et al.*⁹ shows that a human H5N1 isolate can replicate in upper airway cells that have little evidence of α -2,3 receptors on their surface, although the cells' morphology is not well characterized. Thus, receptor preference may not be the only determinant of H5N1

virus entry and replication. What constitutes an infectious dose of H5N1 in humans, which cells may be targets in the upper airways and the effects of respiratory tract mucus rich in α -2,3-linked glycoconjugates on virus tropism remain unknown.

Strains of H5N1 in which the hemagglutinin protein is altered toward the human α -2,6 sialic acid binding preference have already been found in both bird and human viral isolates. However, the mechanism of binding-preference shift observed in H1 and H3 hemagglutinin may not be structurally feasible for the H5 hemagglutinin¹⁰. The amino acid substitutions

Figure 1 Adaptation of avian flu to humans. (a) Avian flu predominantly binds α -2,3 sialic acid on glycan arrays, but may interact with α -2,6 and other sialic acids (left panel). On the other hand, human influenza primarily binds α -2,6 sialic acid (right panel). (b) Depiction of α -2,3- and α -2,6-sialic acid-galactose-protein conjugate. As α -2,6 sialic acid is bulkier than α -2,3 sialic acid, the hemagglutinin receptor expressed by avian flu needs to change sufficiently to accommodate the predominantly human α -2,6-sialic acid glycoconjugates. (c) In human patients infected by nonadapted viruses, such as avian flu and 1918 human influenza, the innate immune response is uncoupled from viral replication (left panel). The immune response in humans infected by adapted viruses, such as human H1N1 influenza, correlates with viral replication and effectively eradicates the virus.

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required to alter H5 binding from α -2,3 sialic acid to α -2,6 sialic acid have not been fully elucidated. The lack of mammalian transmissibility of H5N1 viruses with an avian receptor preference was confirmed by a study that investigated whether mixing viral gene segments of human and avian viruses is necessary or sufficient for acquiring transmission capability¹¹.

Together, the studies on H5N1 (refs. 10,11) and on the 1918 H1N1 (ref. 2) underscore the importance of receptor binding preference, a mutable biological property, in determining transmissibility. The thin dividing line between an avian influenza infection in a dead-end human host and a strain with full pandemic potential is all too apparent, although other viral components may have a role. It is extremely worrying that a small coding change in an influenza virus genome could be the key to the genesis of a pandemic strain. If this is correct, an extremely important control measure to prevent the emergence of an H5N1 pandemic must be limitation of virus replication in diverse avian hosts to avoid sporadic transmission of a virus with a human receptor preference to a human host.

Transmission capability is only one aspect of a pandemic influenza strain. A study of previous pandemic viruses indicated that soon after emergence into humans, further adaptation takes place¹². This may result in altered cell tropism¹³, perhaps relevant in determining the virulence of infection. Not all pandemic influenza strains are highly pathogenic: the 1957 and 1968 pandemics, for example, caused far fewer deaths than the 1918 pandemic, which led to over 20 million deaths.

Although the entire sequence of the influenza genome has been known for ~30 years, why individuals die from infection remains obscure. Virulence is associated with several different

viral gene segments. Array-based studies of cytokine responses in various cells have expanded knowledge of human immune responses to infections. Applying this approach to influenza virus infections in primates, Kobasa *et al.* found that infection with human seasonal H1N1 virus leads to synchronous responses between host gene expression and virus replication, whereas infection with the 1918 H1N1 virus does not (Fig. 1c), suggesting that replication of the latter virus is uncoupled from or resistant to antiviral host immune responses. Similar findings for H5N1 in both experimental mammalian model systems¹⁴ and in actual human infections⁴ point to dysregulation of interferon or other immune response in mammals infected with highly virulent influenza viruses.

Recent interest has also focused on the viral replication complex, which contains viral polymerase proteins and nucleoproteins. Adaptation of viral replication from an avian to a mammalian environment probably requires changes in viral replication proteins. Key residues in the polymerase PB2 are associated with mammalian cell adaptation. Novel technical approaches to structure-function analysis¹⁵ using array technology coupled with protein expression libraries suggest roles for several residues in the C terminus of this protein already associated with host range specificity. These residues may be involved directly in interactions with cell factors. Such technical developments may help elucidate hitherto cryptic host-viral interactions that influence replication efficiency or immune response.

Finding a final common cellular pathway that explains the virulence of different influenza A subtypes in humans will be important for understanding the severity of human or zoonotic infections. Although a unifying

hypothesis is some way off, identification of specific host genes switched on early in infection is essential for pinpointing prognostic indicators of disease severity. Immune intervention strategies are badly needed for treatment of zoonotic H5N1 and may ultimately be simpler to apply than antiviral regimes, which are currently the only countermeasure available for severe influenza infections, whether human or animal in origin.

In the last two years, the circulation of H5N1-infected birds has extended from Southeast Asia into Africa, Europe and the Middle East, bringing an increasing danger of sporadic transmission of avian flu to humans. In the face of this threat, and given the unpredictability of viral evolution and the relative lack of global preparedness, the effort to understand what defines a pandemic influenza strain has become a matter of extreme urgency.

COMPETING INTERESTS STATEMENT

The author declares competing financial interests: details accompany the full-text HTML version of the paper at www.nature.com/naturebiotechnology/.

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