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News & view

Are the H5N1 Viruses Prepared for Inter-human Transmission?*

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Since 1997, highly pathogenic avian influenza (HPAI) H5N1 viruses have caused serious outbreaks in poultry and markets. In human, overall mortality in HPAI H5N1 infection exceeds 60%, but human to human transmission is limited and has been only reported within family members[7,8]. There is much concern as to whether H5N1 can enhance its transmission among humans through genetic variation. Further, there is an urgent need to discover the potential mutations in viral proteins that are responsible for inter-human transmission.

ENLIGHTENMENT FROM THE TRUTH

A paper recently published in Nature[4] seems to provide an important clue for us to answer this question. In this paper, a research team led by Kawaoka found that the N158D/N224K/Q226L/T318I multisite mutations appearing in the H5 hemagglutinin (HA) protein of the recombinant virus could enhance viral transmission among ferrets. The research team obtained the N224K/Q226L double-point mutant through an *in vitro* library screening. Mutations of these two sites led to a bias binding of H5 HA from a avian (Siaα2, 3Gal)-type receptor to a human (Siaα2, 6Gal)-type receptor. They further constructed a recombinant virus containing HA from human H5N1 virus (Vietnam strain) and other segments donated by the 2009 pandemic H1N1, and found these two mutations could infect ferrets, but could not be effectively transmitted in the population. Furthermore, two other mutations, the N158D and T318I were also found in the virus during its infection in ferrets. The appearance of N158D enhanced the transmission of the recombinant viruses among ferrets, but with limited efficiency, while the appearance of mutant T318I led to effective transmission.

Other findings in this study are of great significance to revealing viral characteristics. For example, the H5 HA protein carrying the N224K/Q226L mutations could efficiently bind to the human(Siaα2, 6Gal)-type receptor *in vitro*, yet the virus could not be effectively transmitted, which suggests that viral transmission is not solely dependent on HA binding to its receptor. In addition, the N224K/Q226L mutation enhanced binding to the human receptor, but led to instability of the recombinant virus, while the emergence of N158D/T318I caused the virus to be more stable. These findings suggested that the virus can restore the influence of the existing mutations through new mutations.

SHOULD WE BE TOO WORRIED?

The scientific significance of these results is self-evident and has aroused concern about bio-terrorism. In addition, once the recombinant mutant viruses are leaked, they may lead to a pandemic. Perhaps it is not so pessimistic that we seem to be overly apprehensive.

Although the multisite mutation of the recombinant H5 HA protein enhanced viral transmission between ferrets, the transmission of influenza in humans is caused by a combined effect of more than one protein. In addition to HA, we should also consider the influences from other proteins. For example, the neuraminidase (NA) protein promotes viral release and the functional balance between HA and NA is critical for viral transmission[6]. Furthermore, viral replication proteins such as polymerase basic 1 and 2 proteins (PB1 and PB2) and the polymerase (PA) protein are also critical for viral transmission in the host[3], and their amino acid compositions may determine the proliferative ability of viruses in the host. In this study, all gene segments were donated by the 2009 pandemic H1N1 virus, except for HA. It is not hard to speculate that the H1N1 virus responsible for the 2009 pandemic may enhance transmission of the recombinant viruses in ferrets. Unfortunately, the authors did not analyze the transmissibility of the H5N1 virus, but there still exists a possibility that the H5N1 virus carrying the

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N158D/N224K/Q226L/T318I multisite mutations can enhance viral transmission.

The ferret is the best animal model for studying influenza viral infections, because its symptoms mirror those in humans. Although the viral N158D/N224K/Q226L/T318I mutant can be effectively transmitted in ferrets, compared with the 2009 pandemic H1N1 virus, the transmission ability of the recombinant virus is weak. Although leakage of recombinant viruses may occur, human transmission may not lead to an influenza pandemic such as what occurred in 2009. In addition, compared to the 2009 pandemic H1N1 virus, the pathogenicity of recombinant viruses does not increase significantly, which explains why recombinant viruses only cause mild infections in animals. These findings suggest that if viral leakage occurs, human mortality would be close to that of the 2009 pandemic H1N1 virus.

WHAT DO WE REALLY NEED TO FOCUS ON?

Govorkova *et al.*[1] have compared the pathogenicity of H5N1 virus in ferrets and found that all human H5N1 viruses led to death of the test animals, while only few H5N1 viruses from avian origins were fatal to ferrets, most of them caused mild infections in ferrets. Accordingly, we can speculate that if H5N1 virulent strains of fowl-origin cross the species barrier to infect humans and then gain the N158D/N224K/Q226L/T318I multisite mutation during infection in human, this may lead to enhanced viral transmissibility and pathogenicity. Although the data of Kawaoka *et al*. have confirmed that currently available vaccines and medicines for H5N1 are effective in preventing and treating recombinant mutant viruses, once the mutants acquire an effective interpersonal transmission and pathogenicity, we contemplate a more serious scenario.

What we need to focus on is the likelihood of the N158D/N224K/Q226L/T318I mutations occuring in the H5N1 virus in poultry, and whether the H5N1 virus will gain other mutations following human infection that can lead to effective viral transmission. H5N1 lineage was first identified in 1996 and after that, the viruses have become established geographically and ecologically, and new clades of viruses continuously evolve. Since the outbreak of H5N1 in mainland China in 2003, the dominant epidemic strain has shifted many times. Three clades of H5 subtype, i.e. clades 2.3.4, 2.3.2.1 and 7.2, co-circulated in China in recent years, and clade 2.3.2.1 has now emerged as become the most dominant lineage by now[2,5]. Interestingly, clade 2.3.2.1 carries the N158D mutation; therefore, if the current endemic strain of H5N1 infects humans and acquires the other three mutations, it could result in person to person transmission.

Therefore, it is necessary to maintain long-term surveillance of the endemic H5N1 strain in poultry, as well as analyze the correlation of amino acid changes in the human H5N1 mutant to viral transmissibility and pathogenicity. Such work will provide important information for the prevention and control of H5N1.

References

1. **Govorkova E A, Rehg J E, Krauss S, *et al.*** 2005**.** Lethality to ferrets of h5n1 influenza viruses isolated from humans and poultry in 2004. **J Virol**, 79(4): 2191-2198.
2. **Han Y, Hou G, Jiang W, *et al.*** 2012**.** A survey of avian influenza in tree sparrows in china in 2011. **PLoS One**, 7(4): e33092.
3. **Hatta M, Gao P, Halfmann P, *et al.*** 2001**.** Molecular basis for high virulence of hong kong h5n1 influenza a viruses. **Science**, 293(5536): 1840-1842.
4. **Imai M, Watanabe T, Hatta M, *et al.*** 2012**.** Experimental adaptation of an influenza h5 ha confers respiratory droplet transmission to a reassortant h5 ha/h1n1 virus in ferrets. **Nature**, 486(7403): 420-428.
5. **Jiang W M, Liu S, Chen J, *et al.*** 2010**.** Molecular epidemiological surveys of h5 subtype highly pathogenic avian influenza viruses in poultry in china during 2007-2009. **J Gen Virol**, 91(Pt 10): 2491-2496.
6. **Wagner R, Matrosovich M, Klenk H D.** 2002**.** Functional balance between haemagglutinin and neuraminidase in influenza virus infections. **Rev Med Virol**, 12(3): 159-166.
7. **Wang H, Feng Z, Shu Y, *et al.*** 2008**.** Probable limited person-to-person transmission of highly pathogenic avian influenza a (h5n1) virus in china. **Lancet**, 371(9622): 1427-1434.
8. **Yang Y, Halloran M E, Sugimoto J D, *et al.*** 2007**.** Detecting human-to-human transmission of avian influenza a (h5n1). **Emerg Infect Dis**, 13(9): 1348-1353.