

The Highly Pathogenic Avian Influenza H5N1 – Initial Molecular Signals for the Next Influenza Pandemic

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A new pandemic influenza in the human world may originate from avian reservoirs. Influenza is one of the most widely spread zoonotic infectious diseases. All avian influenza viruses are type A, and they have often caused pandemics throughout human history. The highly pathogenic H5N1 influenza A viruses have now been spreading to many countries in Asia, Europe and Africa. They have infected an increasing number of humans in at least 15 countries in the world. This paper describes recent advances in the mechanism of transmission of highly pathogenic avian influenza to humans and measures for control of a new pandemic. (*Chang Gung Med J* 2009;32:258-63)

Key words: influenza virus, hemagglutinin, H5N1, pandemic, receptor

Highly pathogenic avian influenza viruses

In May 1997, subtype H5N1 of the influenza A virus was detected in a 3-year-old infant boy who died of multiple organ failure, including acute respiratory distress syndrome (ARDS), in Hong Kong. This virus infected eighteen people, causing the death of six. Analysis of the viral gene indicated that all viruses isolated from patients were the avian influenza virus (H5N1).⁽¹⁻⁴⁾ Subsequently, the spread of the virus was temporarily halted but was again transmitted to humans in 2003, spreading to Europe through Asia and Russia, and to the Middle East and Africa.^(5,6) Since 2003, this virus has infected 424 people in 15 countries, killing 261 (WHO, as of May 15, 2009) with a fatality rate of 61.61% (Fig. 1). H5N1 also infects cats, tigers, dogs and other animals, and has been expanding its host range. However, no officially confirmed cases of infection in swine have been reported by the WHO so far.

Human H5N1 infection and the molecular mechanism of mutation causing human-to-human transmission

The type A virus caused at least four pandemics in the twentieth century – Spanish influenza (H1N1) in 1918; Asian flu (H2N2) in 1957; Hong Kong flu (H3N2) in 1968; and Russian flu (H1N1) in 1979 (Fig. 2). Spanish influenza caused the death of 40 million people around the world. The current H5N1 virus appears to be resident in Asia and may not be easily eradicable. There are a variety of differences in the nature of the avian (H5N1) and human influenza viruses which are now circulating, including host range and host recognition systems. The most important point is the difference in host receptor binding specificity.^(3,7) Viruses isolated from aquatic birds such as ducks bind to the terminal sialic acid (Sia) α 2-3galactose (Gal) linkage (2-3) in the sialyllactosamine structure of sugar chains. On the other hand, prevalent human isolates bind to the

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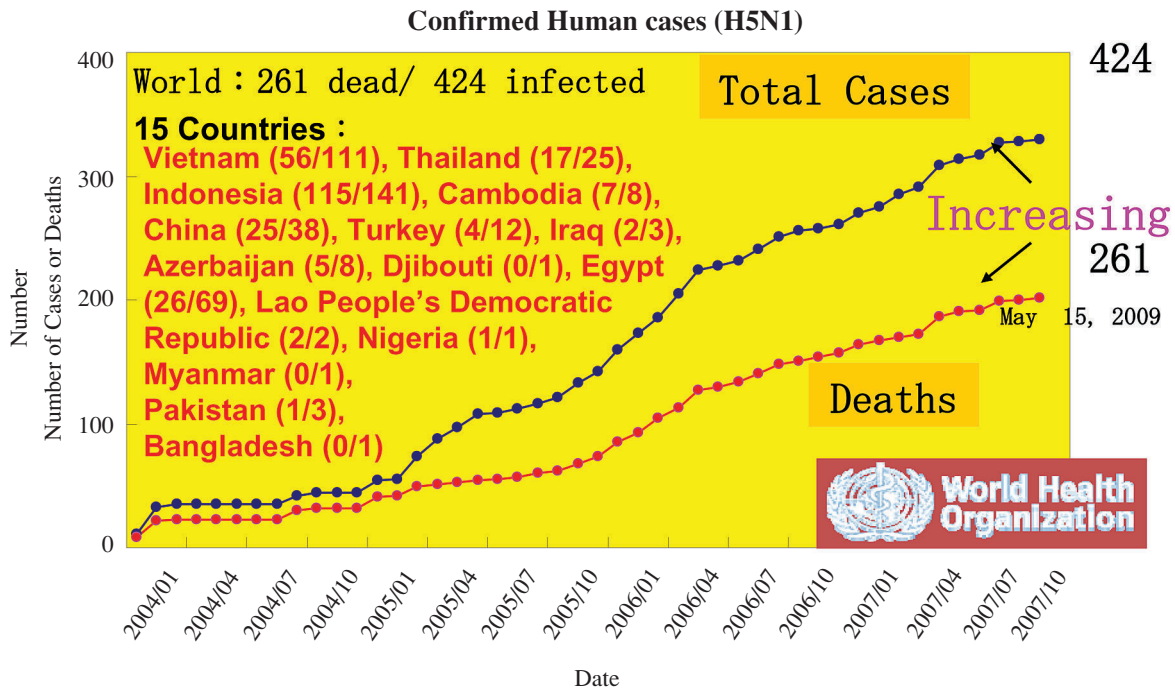


Fig. 1 Cumulative number of confirmed human cases of avian influenza A (H5N1) reported by WHO.

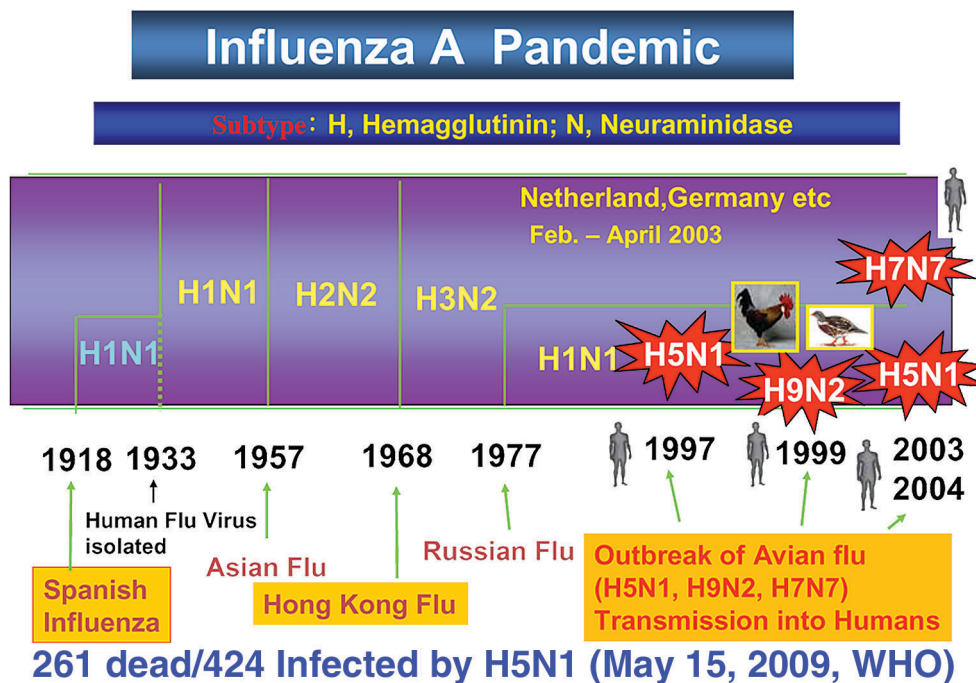


Fig. 2 Influenza pandemics in the human world, and outbreaks of avian influenza and its transmission to humans.

Sia α 2-6Gal structure (2-6).^(3,7) Although 2-6 receptors exist mainly in the human respiratory tract, it has recently been reported that 2-3 receptors for avian viruses also exist in the lower human respiratory tract and lungs.^(8,9) According to these results, it is believed that if humans were directly exposed to high concentrations of the highly pathogenic avian influenza virus that binds to the Sia2-3Gal receptors, the virus would accidentally bind to the Sia2-3Gal receptors in the lower human respiratory tract and subsequently develop an infection (Fig. 3). There have been at least 424 cases of H5N1 virus transmission to humans by infected birds, but human to human transmission of the virus is now poor (WHO, May 15, 2009). Intrafamilial transmission has been reported but is very limited. If the virus were to mutate into a virus transmitted among humans while maintaining high virulence, a pandemic similar to that of Spanish influenza could result. With the recent availability of the complete 1918 influenza virus coding sequence, an influenza virus bearing all

eight gene segments of the 1918 pandemic virus has been generated. The virus gene sequences are related more closely to avian viruses than any other mammalian H1N1 strains.⁽¹⁰⁾ No cases of the currently prevalent H5N1 virus have been confirmed in swine, but the virus is transmitted directly to humans. We found at least two possible mechanisms for the host range variation of influenza A viruses, selection due to the presence of antibody in the host and selection by the host cell receptor based on Sia-Gal linkage (α 2-3, α 2-6) of sialylsugar chains in host cell membranes. The host range mutation is caused by replacement of only one or two amino acids in the hemagglutinin spike molecule of the viruses.^(3,11,12) Furthermore, it has recently been reported that there are human type 2-6 receptors in the respiratory tract and intestines of domestic birds, such as quail.⁽¹³⁾ We found that H5N1 isolated from a boy who visited Fujian province in China bound to both 2-3 and 2-6,⁽¹⁴⁾ suggesting the adaptation of avian H5N1 to humans. We also detected similar mutations in avian

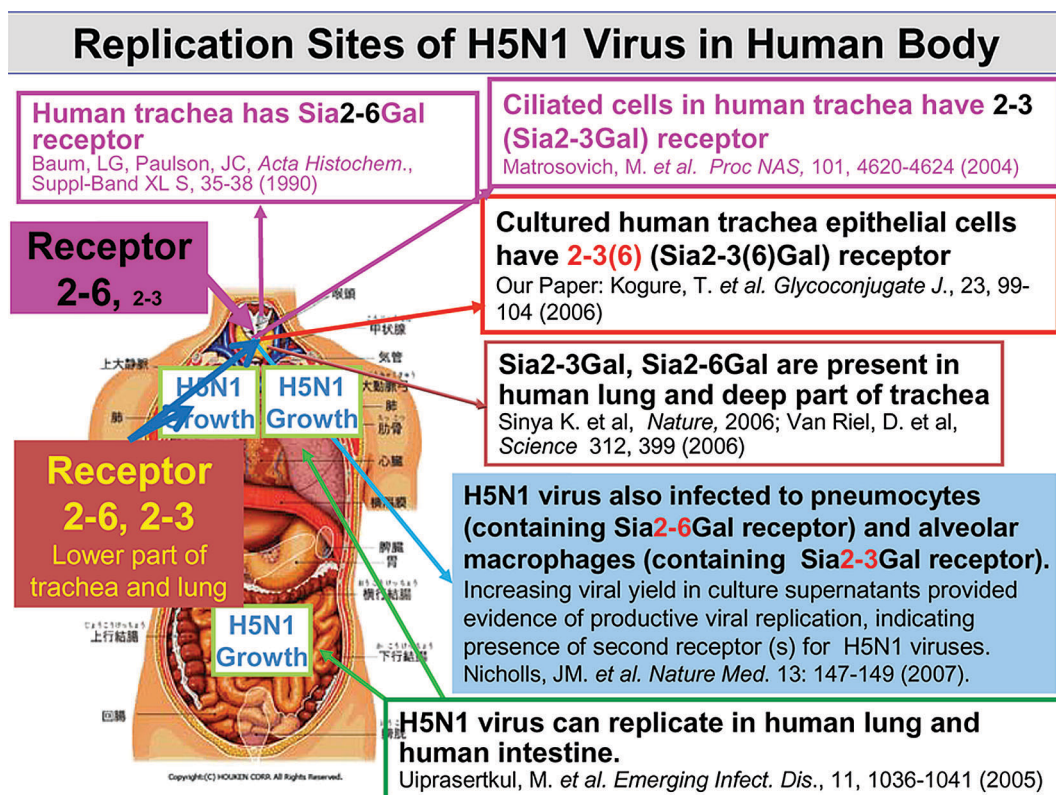


Fig. 3 Distribution of Neu5Ac α 2-3Gal and Neu5Ac α 2-6Gal receptors for influenza A viruses (H5N1) in human trachea and lung.

H5N1 isolated from humans in Hanoi, Vietnam⁽¹⁵⁾ which showed an adaptation of receptor binding specificity of the H5N1 virus to 2-6 receptors (human type). Very recently, we also determined the amino acid substitutions (Asn182Lys and/or Gln192Arg) or (Leu129Val and Ala134Val) in hemagglutinin spike which are responsible for the binding of H5N1 viruses to human-type receptors (Sia2-6Gal).^(16,17) These mutations introduce molecular signs of the next influenza pandemic in the human world. Moreover, Matrosovich et al's study⁽¹⁸⁾ and our study⁽¹⁹⁾ confirmed that the avian influenza virus (subtype H9N2) strain isolated from quail in China has already mutated into a human virus with properties capable of binding with Sia2-6Gal receptors in the human respiratory tract. These results suggest that a newly mutated highly pathogenic influenza virus which is able to bind to human type receptors (2-6) will be generated in the infected body of birds with 2-6 receptors (e.g., quails) or the body of humans with multiple 2-6 receptors in the respiratory tract by selection of viruses by host cell receptor sialyl-sugar chains as described above. Thus, in addition to gene and antigenic analyses, surveillance for mutations of receptor binding specificity of highly pathogenic avian influenza viruses to human type specificity, should be started globally as early as possible. Recently, it was found that the amino acid substitution (Glu627Lys) of PB2 (viral RNA polymerase) gave avian H5N1 viruses the capability of efficient replication in mice.^(20,21) Accordingly, for the highly pathogenic avian influenza viruses to become a human pandemic, multiple mutations need to occur simultaneously.

Measures to predict, prevent, and control the development of new influenza viruses

Establishment of a mutation surveillance (monitoring) system for receptor binding specificity

As described above, in order for the highly virulent avian influenza virus to spread among humans, mutation of receptor binding specificity from bird type (Sia2-3Gal) into human type specificity (Sia2-6Gal), and mutation for effective replication of the virus in the human body must occur. Therefore, it is crucial to establish a system to monitor these mutations with high sensitivity. Currently, surveillance of H5N1 in the world is limited to antigenic and gene analysis of H5N1 viruses. However, these surveil-

lance systems are insufficient to detect a mutation of the avian H5N1 virus into a human type virus that recognizes human receptors. Therefore, we need to develop monitoring procedures (phenotype analysis) to examine the mutation of the receptor binding specificity. We recently developed a system to differentiate the receptor binding specificity (2-3, 2-6) of avian and human viruses with high sensitivity without using expensive equipment.⁽²²⁾ This method must be adopted on a global scale (Fig. 4). This will allow detection of virus mutations at an early stage before a pandemic starts, as well as prevent a pandemic by measures such regional quarantine.

Creation of a vaccine for a new virus

A vaccine is the most effective method of preventing viral infections. However, to create a vaccine for a new virus, safe antigens of a pandemic virus are needed. It may take several months to complete and distribute a new vaccine. While working to produce it, the new virus may spread on a global scale. Viruses spread very quickly in the modern era of high-speed transportation. Clinical studies are currently being conducted to create H5N1 vaccines through reverse genetic engineering,⁽²³⁾ and the creation of prototype H5N1 vaccines has been progressing.⁽²⁴⁻²⁶⁾

Development and stock of new anti-influenza drugs

Currently, there are two types of neuraminidase-inhibitors (zanamivir and oseltamivir phosphate), and proton channel blockers, (amantadine and remantadine) which are clinically used as anti-influenza drugs. Amantadine (Symmetrel) is a functional inhibitor the viral M2 proton channel protein, which is a membrane protein of the type A virus (the pathogenic avian influenza virus is also type A) which is prone to create resistant viruses. A type A virus resistant to this drug is currently spreading throughout the world and thus it should not be used for treatment or prevention of new viruses.^(25,27,28) Generation of new viruses that are resistant to drugs and their circulation will lead to serious problems, because the first patients will be helped, but afterward, the drugs may have no efficacy. Therefore, constant monitoring of the development of resistant viruses is absolutely necessary. Oseltamivir (Tamiflu) is currently targeted for measures against a pandemic caused by new viruses in several countries

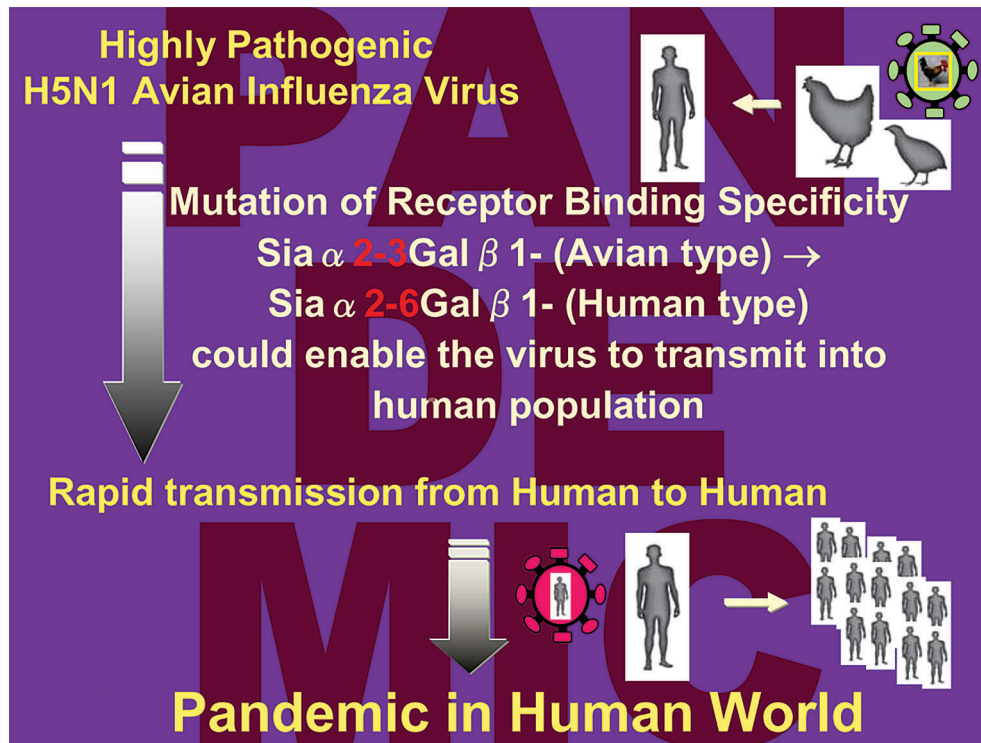


Fig. 4 Possible process to human influenza pandemic.

and is listed in WHO guidelines as the treatment of first choice for H5N1. Zanamivir (Relenza) is the second choice. Total chemical synthesis of Tamiflu recently has been successful, opening the way to mass production of the drug.^(29,30) However in one report, when Tamiflu was administered prophylactically, resistant strains of the highly pathogenic avian influenza virus (H5N1) were isolated for the first time.⁽¹⁵⁾ Zanamivir (Relenza) is effective as a substitute for Tamiflu to treat resistant strains.⁽¹⁵⁾

Conclusion

The molecular mechanism of the mutation of the highly pathogenic avian influenza virus, H5N1 into a new human type virus, which could cause the next pandemic, and its preventive measures were described. Influenza viruses have highly variable properties which lead to drug-resistant mutations. There is a possibility that the current anti-influenza drugs may not be effective in the future. Therefore, development of next-generation anti-influenza drugs that can overcome virus mutation is crucial.

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