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REVIEW

Influenza viruses and the evolution of avian influenza virus H5N1

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Summary Although small in size and simple in structure, influenza viruses are sophisticated organisms with highly mutagenic genomes and wide antigenic diversity. They are species-specific organisms. Mutation and reassortment have resulted in newer viruses such as H5N1, with new resistance against anti-viral medications, and this might lead to the emergence of a fully transmissible strain, as occurred in the 1957 and 1968 pandemics. Influenza viruses are no longer just a cause of self-limited upper respiratory tract infections; the H5N1 avian influenza virus can cause severe human infection with a mortality rate exceeding 50%. The case death rate of H5N1 avian influenza infection is 20 times higher than that of the 1918 infection (50% versus 2.5%), which killed 675 000 people in the USA and almost 40 million people worldwide. While the clock is still ticking towards what seems to be inevitable pandemic influenza, on April 17, 2007 the U.S. Food and Drug Administration (FDA) approved the first vaccine against the avian influenza virus H5N1 for humans at high risk. However, more research is needed to develop a more effective and affordable vaccine that can be given at lower doses.

Microbiology

Influenza viruses are enveloped RNA viruses from the family of *Orthomyxoviridae*, with a diameter of 80–120 nm. They have a genome that has a high mutation rate and they display a great antigenic diversity.[1] According to their core protein, they can be classified into three distinct types: A, B, and C. They have two major antigenic surface glycoproteins embedded into the membrane: the hemagglutinin (HA) and neuraminidase (NA), which induce the antibody response in humans.[1] HA enables the virus to bind to sialic acid-galactose receptors on the surface of respiratory epithelial cells and to fuse with the host membrane. The human and mammalian receptors are different but still present in different proportions in tissues of both species. NA functions as an enzyme to remove sialic acid, thus promoting dispersion during the budding of new virions from the infected cell.[1–4]

Epidemiology

An influenza virus is named by type (A, B, C), species of origin (if from human, it is not indicated), site of isolation, number of isolate, year of isolation, and HA and NA subtype in the case of influenza A viruses only (e.g., A/Chicken/Hong Kong/220/97 (H5N1)).[1,2,4]

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Antigenic drift is a relatively minor antigenic change that occurs frequently within the HA or NA of the virus, and is usually responsible for epidemic disease.[5,6] Antigenic shift brings up new viruses with different HA or NA antigens. This can be achieved by mutation or genetic reassortment (two different influenza virus strains swap their genes giving rise to a hybrid strain), which usually leads to pandemic disease.[7]

The 1918 influenza pandemic was caused by the H1N1 subtype that mutated from a purely avian virus. It was the worst pandemic by far. It killed at least 40 million people worldwide, including 675 000 people in the USA. The average life expectancy in the USA decreased by more than 10 years at that time.[8–11] The 1957 pandemic was caused by the H2N2 subtype, a product of genetic reassortment in hosts infected with both an avian and human influenza virus. It killed about 70 000 people in the USA.[12–14] The 1968 pandemic was caused by the H3N2 subtype, a product of genetic reassortment. It killed approximately 34 000 people in the USA.[14]

Avian influenza

Avian influenza is a contagious disease of animals caused by viruses that normally infect only birds, and less commonly, pigs. With the fact that both human and mammalian receptors are present in different proportions in tissues of both species, the bird influenza virus may infect humans. When mutations or reassortments occur, this could lead to the emergence of a fully transmissible strain, as in the 1957 and 1968 pandemics. Finally, they can transmit not only to humans but also to other mammals.[15]

In birds, the low pathogenic form causes only mild symptoms such as decreased egg production. The high pathogenic form can affect multiple organs and the mortality rate can reach 100%, often within 48 hours. The presence or absence of symptoms and their severity caused by low pathogenic viruses depend on the type of bird species affected. The same holds for the highly pathogenic ones: in wild and domestic ducks they can be asymptomatic, whereas they are lethal in terrestrial birds.[15]

Avian influenza H5N1

The first clinical respiratory illness of H5N1 avian influenza occurred in Hong Kong in 1997, when 18 human cases were reported during a poultry outbreak. It broke the species barrier to infect humans, cats, and tigers.[16–18] So far it has affected many countries in Asia, Africa, and Europe. According to the cases reported to The World Health Organization (WHO) appearing on the WHO website on June 29, 2007, the H5N1 virus has already infected 317 humans and has killed 191 patients worldwide, with a mortality rate exceeding 50%.[19]

Avian influenza H5N1 also affects domesticated poultry, including chickens, ducks, and turkeys. The contribution of migratory birds like wild ducks, geese, swans, and hawks (Saudi Arabia) to the spread of the H5N1 virus from Asia to Europe and Africa is still controversial, since basically all wild birds positive for H5N1 virus have been found dead or very sick, thus unlikely to have been able to fly over long distances. At the same time, more evidence supports the role of the international illegal poultry trade. It is possible that this trade contributed to the 2006 H5N1 outbreak in a large commercial farm in Kaduna State in the northern part of Nigeria. On the other hand, the country is known to lie along a flight route for birds migrating from central Asia.

The birds shed the virus in their saliva, nasal secretions, and feces. Infection can spread among birds by contact with infected birds or with their excretions. People can get infected by direct or close contact with infected poultry or surfaces contaminated with secretions and excretions from the infected birds. Human exposure occurs most often during slaughtering, de-feathering, butchering, or preparation for cooking. Raw poultry or eggs can also transmit the disease. There are no reported cases through properly cooked poultry.[15,20]

Human-to-human transmission was suggested in a family in Thailand, when an 11-year-old girl who had been in contact with an infected bird, and her mother who had provided her with nursing care, both died of the avian influenza. The girl's aunt, who had also had close contact with the girl, survived the infection after treatment with oseltamivir. H5N1 was confirmed in the mother and the aunt.[21,22] Recently there have been fears of other human-to-human transmission cases after seven family members died of the avian influenza in Indonesia. WHO confirmed that partial human-to-human transmission occurred.[20]

Other potential modes of transmission of H5N1 virus include contamination of hands from infected fomites and exposure to untreated poultry feces used as fertilizers. Further possible but not proven modes of transmission are oral ingestion of contaminated water during swimming and direct intranasal or conjunctival inoculation during exposure to water.[23]

Pathogenesis of avian influenza

The virulence of the avian influenza viruses in mammals is not well understood. As opposed to the earlier H5 viruses, the more recently circulating H5 viruses appear to be more pathogenic in mammals and birds. This is a feature that may precede the emergence of reassorted H5 strains with pandemic potential.[17,24] Although virulence determinants are polygenic traits, the possible major contributing factor is the hemagglutinin molecule. The acquisition of a multibasic amino acid sequence at the cleavage site of a hemagglutinin belonging to the H5 or H7 subtypes enables its widespread cleavage by ubiquitous tissue proteases, resulting in multi-organ infection and high pathogenicity.[25,26]

H5N1 viruses are relatively resistant to host antiviral cytokines, which leads to the production of high levels of cytokines and an excessive pro-inflammatory response causing tissue injury.[27,28] This suggests that the severity of human H5N1 infection may be related to the excessive pro-inflammatory responses that exacerbate tissue injury.[23,29] Autopsies of current human cases of H5N1 influenza have revealed necrotizing hemorrhagic pneumonia, similar to that found in the 1918 influenza cases. Like the 1918 virus, H5N1 is associated with unusually high death rates in humans; in fact the case death rate is 20 times higher than that of the 1918 virus (50% vs. 2.5%).[30] Continuous evolution of the H5N1 virus has been suggested by changes in the internal gene constellation, expanded host range, increased pathogenicity, and greater environmental stability.[31,32]

Symptoms and signs

According to one study, symptoms of H5N1 infection include typical influenza-like symptoms: fever (100%), cough and sore throat (67%), myalgias (30%), pneumonia (58%), diarrhea and vomiting (50%), and congestion of the conjunctiva (0%). Laboratory findings include elevated serum aminotransferases and pancytopenias.[23,33] Complications include acute respiratory distress syndrome (ARDS), pulmonary hemorrhage, myocarditis, pericarditis, encephalitis, multi-organ failure with renal dysfunction, and sepsis. Most deaths have been related to respiratory failure.[23]

The spectrum of disease is wide. Two children from the same family in Vietnam presented with diarrhea and encephalopathy, without any signs of respiratory compromise.[34,35] The incubation period for H5N1 may be longer than other known human influenza viruses.[23] In 1997, most cases occurred within two to four days after exposure, while reports of cases from 2004 suggest that longer intervals of up to eight days may be possible.[16,36]

Diagnosis

Diagnosis can be made with clinical findings, plus recent history of exposure to dead or ill poultry, and can be confirmed with serologic tests. Travel history is also very important. Rapid antigenic testing kits are not able to subtype influenza A, and the standard serologic test (HA inhibition test) is insensitive.[23–36] Diagnosis can be confirmed by HA-specific PCR assay or by viral culture of a nasopharyngeal aspirate obtained within three days of the onset of symptoms.[23] ELISA and western blotting are useful for epidemiologic surveillance studies and retrospective diagnosis.[37] Radiologic findings include diffuse, multi-focal or patchy infiltrates and segmental or lobar consolidation with air bronchograms.[36]

The differential diagnoses of avian influenza include atypical pneumonia, human influenza, respiratory syncytial virus, severe acute respiratory syndrome (SARS), and upper respiratory tract infections associated with conjunctivitis (e.g., adenovirus).

Who should be tested?

High-risk individuals, such as patients with a history of travel within 10 days of symptom onset to a country with documented H5N1 avian influenza in poultry and/or humans, and patients who have radiographically confirmed pneumonia, ARDS, or any other severe respiratory illness for which an alternate etiology has not been established, should be tested.

For low-risk individuals, testing should be considered in patients with a history of contact with domestic poultry or with a known or suspected human case in an H5N1-affected country within 10 days of symptom onset, documented fever of 38 °C, and one or more of the following: cough, sore throat, shortness of breath.[23,36]

Treatment

Whenever feasible, while the number of affected persons is small, patients with suspected or proven influenza A (H5N1) infection should be hospitalized in isolation for clinical monitoring, appropriate diagnostic testing, and antiviral therapy.[38]

The majority of H5N1 isolates are still sensitive to amantadine. However, more recent viruses isolated in Thailand and Vietnam have amino acid substitutions within the M2 protein, which confer resistance to amantadine and rimantadine.[39,40] These viruses are susceptible to the neuraminidase inhibitors in animal models.[41] Oseltamivir should be started as soon as possible. In an animal study where mice were inoculated with H5N1 virus isolated from a patient who did not survive the infection, the mice that were treated for eight days with oseltamivir had significantly better survival rates in all dose ranges when compared to animals that were treated for only five days.[39] Prior animal studies demonstrated that five days of oseltamivir was effective against another H5N1 variant.[42] Thus, treatment dose and duration may vary according to the pathogenicity and virulence of the virus.[39]

An oseltamivir-resistant H5N1 strain (His274Tyr) has been reported in two cases in Vietnam. Both patients died of the infection despite early initiation of treatment in one case and proper dosage (75 mg twice daily) in both cases. Therefore, the use of higher doses, longer durations of treatment, or combination therapy may deserve further evaluation.[43] Improper use of personal stockpiles of oseltamivir may promote resistance and should be strongly discouraged.[44] Zanamivir is another neuraminidase inhibitor, and was the first neuraminidase inhibitor to be approved for influenza treatment. It is also recommended for the treatment of H5N1 infection, together with oseltamivir.

Corticosteroids have been used frequently in treating patients with H5N1 infection with uncertain effects. Other agents like peramivir, long-acting topical neuraminidase inhibitors, ribavirin,[45,46] and possibly interferon alpha, which has both antiviral and immunomodulatory activities, are under investigation.[47]

According to a recent report, patients with Spanish influenza pneumonia who received influenza-convalescent human blood products may have experienced a clinically important reduction in the risk of death. Convalescent human H5N1 plasma could have similar benefits and should be studied in clinical trials.[48]

Prevention

Although immunization with human influenza vaccine will not protect against avian influenza strains, it should be considered in poultry workers, and also be given to those traveling to affected areas, two weeks ahead of departure, to prevent co-infection and reassortment.[23]

According to Centers for Disease Control and Prevention (CDC) and WHO recommendations, people who live in affected areas should avoid all direct contact with poultry and surfaces contaminated with poultry feces and secretions, should avoid eating undercooked eggs and poultry, should wash hands carefully and frequently, and should seek medical attention if they become ill within 10 days of suspected contact.[15,20]

Post-exposure prophylaxis with oseltamivir 75 mg daily for 7 to 10 days should be recommended to household contacts of patients.[23,49,50] Although the risk of transmission from person

to person appears low, quarantining of close contacts to patients for a week after last exposure and monitoring for symptoms may help to reduce transmission rates.[23] If available, negative pressure rooms should be used for patient isolation.

Healthcare workers should wear N-95 masks (non-oil-proof respirators with at least 95% efficiency in filtering particles more than 3 μm in diameter), gloves, long sleeved cuffed gowns, and eye protection when within 3 feet of patients.[51]

Quarantine and depopulation or culling of affected poultry is the preferred way of eradication. The use of inactivated H5N1 vaccines in chickens is an additional step but should be done with caution.[52]

Vaccine

Currently, there is no commercially available vaccine against H5N1 virus. Due to safety and technical reasons, and since H5N1 is highly virulent and lethal to eggs, traditional methods of production are not feasible. Furthermore, it is impossible to predict whether the currently circulating H5N1 strain will cause the next pandemic. If successfully produced, vaccines would likely be the most important health tools to decrease morbidity, mortality, and the economic effects of pandemic influenza. Resistance to oseltamivir makes vaccines even more important.[23,53]

The H5N1 viruses can be divided into clade 1 and clade 2; the latter can be further subdivided into three subclades. These clades and subclades probably differ sufficiently in their antigenic structure to warrant the preparation of different vaccines. Studies in ferrets suggest that vaccination against one clade will not protect against infection with another clade, though it will protect against influenza-associated death.[54]

A new vaccine prepared from an egg-grown recombinant influenza A virus, composed of the hemagglutinin and neuraminidase genes from a human H5N1 isolate inserted into a laboratory-adapted human influenza A strain, achieved only 54% presumably protective micro-neutralization titers of 1:40 following use of the maximum tested dose (90 μg). This dose is 12 times that of the seasonal influenza vaccine, making mass production untenable with current manufacturing capacity. Current seasonal vaccines contain 15 μg HA/strain/dose. Whether this vaccine could induce cross-protection against other H5N1 strains is unclear. Furthermore, the death of a Chinese woman infected with an H5N1 strain markedly different from those now being used to develop vaccines has recently been reported.[55–57]

Studies of different dose levels of vaccines administered with adjuvants like aluminum hydroxide are urgently needed to improve immunogenicity and increase the number of doses available (if lower doses are effective).[57–59] Recently, promising progress has been made in industry-supported research in France and the UK, where the safety and immunogenicity of a monovalent, inactivated split-virion vaccine, derived from a highly pathogenic strain of H5N1 influenza, has been investigated; this vaccine was administered with and without a fixed dose of aluminum hydroxide as an adjuvant. On day 42, 67% of subjects who received the highest dose HA with adjuvant exhibited detectable antibody titers, and 61% had positive HA-inhibition responses.[60]

Other promising approaches to vaccine development involve DNA, adenovirus vectors, and cell manufacturing techniques to increase the speed and capacity of vaccine production.[61,62]

On April 17, 2007, the U.S. Food and Drug Administration (FDA) approved a human vaccine against the H5N1 influenza virus, marking the first such approval in the USA. Should H5N1 develop the ability to spread readily from person to person, this vaccine may provide early limited protection in the months before a vaccine tailored to the pandemic strain of the virus can be developed and produced. The vaccine will be kept in a federal stockpile and will be made available only through public health officials; it is approved for those aged 18 to 64 years who are at increased risk for H5N1 exposure.[63]

Pandemic risk

For a pandemic to occur, three conditions must be met: a new influenza virus subtype must emerge, this must infect humans and cause serious disease, and finally spread easily among humans.[20] The first two conditions have already been met, and there are new suggestions of human-to-human transmission in Thailand and Indonesia. However, there is no evidence to-date of the easy human-to-human transmission that is key for a pandemic.[20–23] With the emergence of influenza virus H5N1, the threat of an influenza pandemic seems to be real and inevitable, but no one can predict when it might happen. According to a study by the Congressional Budget Office, the consequences of a severe pandemic could, in the USA, include 200 million people infected, 90 million clinically ill, and 2 million dead. The study estimated that 30% of all workers would become ill and 2.5% would die, resulting in a decrease in the gross domestic product of 5%. Furthermore, 18 to 45 million people would require outpatient care, and the economic cost would total approximately $675 billion.[64]

Conclusions

The world is facing the real threat of another influenza pandemic with a virus that has a great pathogenicity and a mortality rate in humans exceeding 50%. Despite the high level of technology and ongoing research, at the present time there is no highly effective vaccine against avian influenza H5N1 virus that can be manufactured commercially on a large scale for use at low doses. As is always said, ''a dime of prevention is better than a pound of treatment''. Responsibility should be taken at all levels and preventative steps should be implemented immediately. Healthcare providers should be up-to-date with the pandemic risk and educate their patients. Such information can be found at www.cdc.gov/flu. Other sources of information include www.defra.gov.uk, www.fda.gov, www.who.int, and www.eurosurveillance.org.

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