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Influenza viremia and the potential for blood-borne transmission

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Influenza is a major cause of morbidity and mortality in the United States and worldwide. The threat of pandemic influenza recently has gained prominent attention because of widespread infection of poultry with highly pathogenic avian influenza A (H5N1) and the potential for the virus to mutate into one capable of efficient human-to-human transmission. As of March 8, 2007, 277 human cases of H5N1 infection had been reported to WHO from Asia,[1,2] Eastern Europe,[3,4] and Africa,[3] mostly as a result of close contact between humans and infected birds, although rare, unsustained human-to-human transmission has been documented. If a change in viral characteristics were to allow efficient human-to-human transmission, rapid spread and a worldwide pandemic could result. The global spread of H5N1, continuing outbreaks in birds, and sporadic infections in humans have increased concern that a pandemic virus may emerge and cause an influenza pandemic. The possibility of an influenza pandemic has focused attention on the epidemiology and pathophysiology of influenza, including its potential for transmission through the blood supply.

An infectious agent which has a blood-borne phase and can be clinically asymptomatic has the potential to be transmitted by blood transfusion. Planning to ensure an adequate and safe national blood supply during a pandemic prompted consideration of the potential for transfusion-transmitted influenza. Notably, no cases of transfusion transmission of influenza have been documented to date. The risk of transfusion transmission has been assumed to be negligible based on the premise that viremia rarely occurs and does not occur without symptoms, allowing for deferral of potentially infectious blood donors. If these assumptions are incorrect, however, and influenza infection commonly involves a viremic phase, especially before the onset of symptoms, and if influenza-infected blood resulted in clinical illness, it would have implications for blood safety. To further examine this possibility, we surveyed the literature underlying these assumptions and propose methods of redressing gaps in our knowledge.

If influenza were transmissible by transfusion, blood product recipients, who include a high proportion of immunocompromised patients, might suffer increased morbidity and mortality. In bone marrow transplant populations influenza infection appears to be associated with approximately 25 percent mortality.[5,6] If influenza were deemed to represent a transfusion transmission risk, possible screening methods would likely rely on epidemiologic query or laboratory screening. With any screening there is a risk of impacting supply. Testing of the blood supply would also become particularly important if a pandemic were to be caused by a virus similar to the highly pathogenic influenza A (H5N1). Infection in humans has thus far resulted in approximately 60 percent case fatality ratio where the virus has been isolated outside of the respiratory tract, a factor not typically seen with seasonal influenza infections. Detection of influenza in the blood of H5N1 infected humans and in animal studies has occurred predominantly during symptomatic periods and at high viral titers. A positive test might result in discarding

ABBREVIATION: CSF = cerebrospinal fluid.

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the donated unit of blood, further endangering blood product availability at a time when donors may be scarce. Such additional sources of stress on the system would need to be taken into consideration when modeling the effect of a potential pandemic.

BACKGROUND

Influenza epidemiology

Seasonal influenza represents a significant contributor to overall mortality in the United States, particularly among those 65 years and older. The rates of illness and severe complications from seasonal influenza can vary substantially from year to year. In years of high influenza activity, weekly mortality incidence due to pneumonia and influenza can peak at more than 10 percent of all cause mortality in the United States (Fig. 1).[7] During the 1990s, a mean of 36,000 influenza-related deaths occurred per year, with more than 90 percent of deaths occurring in the elderly.[8] Estimates of annual influenza illness can range from 5 to 20 percent[9] with the highest incidence generally occurring in children.[10-14] For example, during a 1976 outbreak of A/Victoria in the Houston area, an 18 percent attack rate was documented, with rates in preschool children estimated to be more than 30 percent. Influenza attack rates can be much higher in populations with close contact, with documented attack rates of 42 percent for the crew of a US Navy ship.[15] In contrast to seasonal influenza where higher illness rates occur in children compared to adults, illness rates are generally high across all age groups in a pandemic. While mean illness rates are 6 to 7 percent in healthy adults in seasonal influenza, during a pandemic illness rates are estimated to be approximately 30 percent.[16-18]

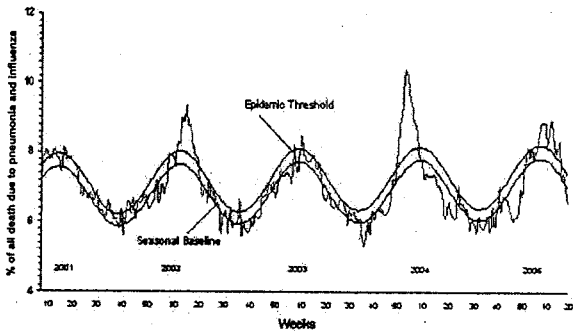

Fig. 1. Seasonal variation in influenza and pneumonia mortality for 122 cities, week ending May 21, 2005. The excess mortality seen most markedly in the winter of 2003 to 2004 is primarily attributable to influenza and secondary pneumonia associated disease.

Antigenic drift and shift and influenza reservoirs

Influenza A is a single-stranded, negative-sense RNA virus, with eight genome segments, each complexed to a nucleoprotein molecule in the mature virion. Antigenically important regions of the virus include the hemagglutinin, responsible for virus binding to sialic acid on host cells, and the neuraminidase, needed to detach newly replicated virus from host cells.[19] Antibody responses directed against the hemagglutinin protein can protect from influenza infection (reviewed by Potter and Oxford[20]), and antibodies against neuraminidase lessen disease severity.[21] There are two main types of influenza viruses, Types A and B. Type B viruses are found predominantly in humans. Type A viruses can infect multiple species including humans, birds, horses, pigs, and dogs. The primary reservoir for Type A viruses, however, is birds. Influenza A viruses are subtyped based on their hemagglutinin and neuraminidase. Both Type A and Type B viruses are included in the annual vaccine and can cause influenza epidemics.

During influenza virus replication, errors can occur and lead to genetic variation. Changes made to antigenic regions such as the hemagglutinin molecule are termed antigenic drift.[22] Antigenic drift allows individuals to have multiple infections with influenza viruses over their lifetime because antibody made against one influenza strain may not protect against distantly related drifted strains. Antigenic drift is an ongoing process and necessitates nearly yearly updates of the influenza virus strains included in influenza vaccines.

A more extreme version of antigenic variation in influenza viruses is antigenic shift, whereby genomic segments from different influenza virus strains reassort to create new combinations of gene segments.[23] The new virus can acquire a novel hemagglutinin or neuraminidase gene from the pool of 16 hemagglutinin and 9 neuraminidase genes.[24] Antigenic shift resulting in a strain capable of causing a human pandemic is a rare event. However, the two most recent influenza pandemic viruses arose through reassortment of human influenza A with avian influenza strains. Recent evidence suggests, however, that the 1918 influenza pandemic may have resulted from an avian influenza virus strain that acquired the capacity to infect humans by direct mutation rather than reassortment (Table 1).[25] A number of avian influenza strains have caused isolated infections in humans, ranging from conjunctivitis to influenza like illness and even death. Examples of contemporary strains that have caused more

TABLE 1. 20th century influenza A pandemics

Year	Name	Strain	Estimated mortality
1918	Spanish	H1N1	>20 million
1957	Asian	H2N2	70,000
1968	Hong Kong	H3N2	34,000

than one human case of infection in recent years include H5N1, first reported in 1997,[26] H9N2 reported in 1999,[27] H7N7 reported in 2003,[28] and H7N3 reported in 2004.[29] Of these, H5N1 represents the greatest current pandemic threat and will be discussed further below.

EVIDENCE FOR INFLUENZA VIREMIA IN HUMAN INFECTION

Naturally occurring human cases of influenza viremia

Relatively few studies have addressed the question of viremia caused by influenza infection, and most of these date back to the 1960s and 1970s. The first report of influenza viremia was made in 1963 by Naficy.[30] Influenza virus, Group A, Type 2 (Asian) was isolated from blood drawn on Hospital Day 2 (5 days after onset of symptoms) from a 40-year-old physician ill with fever, headache, and malaise. This report followed previous suggestions of viremia based on isolation of influenza virus from postmortem tissue samples taken from patients who had died of Asian influenza infection.[31] Subsequent to Naficy's publication, a single report in the literature describes influenza viremia in a naturally infected, asymptomatic patient.[32] Samples were prospectively obtained from 29 clinically well close contacts of 21 ill patients in a Tehran prison. Of these 21 ill subjects, 12 had influenza virus isolated from their throat washes either in the original inoculation or after the first passage in 10-day-old embryonated chicken eggs. No virus was detected in the blood specimens of these patients after two blind passages. The 29 healthy close contacts were clinically observed for 6 days and evaluated by both throat washes and blood specimens. Of 5 subjects who developed positive throat swabs, 1 had virus isolated from both blood and throat specimens collected while asymptomatic. This individual developed clinical illness 12 hours after these specimens were collected. Subsequent specimens collected at 12 and 24 hours after onset of symptoms were negative. The number of subjects studied was small, and laboratory contamination cannot be definitively ruled out, but 1 of 5 subjects observed from the time of influenza exposure who later developed documented natural influenza infection exhibited transient viremia. Seventeen symptomatic subjects with throat wash specimens position for influenza had negative blood cultures for influenza.

Before the report by Naficy in 1963, Minuse and colleagues[33] examined 7 college students hospitalized for Asian pandemic influenza in 1957. In spite of isolating virus from throat washes of 6 of these students and antibody evidence of infection in all 7, none of the blood specimens yielded positive results, although pooling of serially collected specimens may have diluted out a single positive specimen beyond the infectious dose for the chick embryo. Naficy, Minuse and Khakpour all used clotted blood specimens. In contrast, Poliakova and coworkers[34] isolated influenza virus from hemolyzed blood of 11 of 63 patients, all of whom were described as having moderately severe illness.[32] Ages of these 11 patients ranged from 9 months to 67 years, and virus was isolated most frequently on Day 3 after onset of symptoms (2 on Day 2, 5 on Day 3, 2 on Day 4, 1 on Day 5, 1 on Day 6, 1 on Day 7, and 1 on Day 13; 2 patients had virus isolated on 2 different days). Isolated case reports have demonstrated viremia in hospitalized patients either pre- or postmortem.[35,36] More recently, Tsuruoka and associates[37] used reverse transcription–polymerase chain reaction (RT-PCR) to identify genomic influenza RNA in peripheral blood mononuclear cells (PBMNCs) taken from 18 children aged 1 to 14 years with positive throat swabs for influenza during the 1992 to 1993 season in Japan. Three of the 18 samples were RT-PCR positive with NP and/or HA primers (subtype H3, the contemporary circulating subtype).[38] The NP gene sequence observed in 1 patient's PBMNCs was identical to that obtained from his throat swab, although the HA sequence of the other 2 isolates differed from the amplified throat nucleic acid by 3 to 9 nucleotides. This large number of nucleotide changes could signal either selection of a quasi-species of influenza virus that was not detected by culture or may represent contamination of samples. In addition, viral culture was not attempted in this study and thus did not assess presence of viable virus. During an influenza B outbreak in the 1994 to 1995 season, 5 of 17 red blood cell (RBC) and white blood cell (WBC) samples from 4 of 11 ill children yielded influenza B when cocultured with Madin–Darby canine kidney cells.[39] In this study as well, the nucleotide and amino acid sequences varied substantially between viruses isolated from throat and blood specimens. A number of studies searching for influenza viremia after the onset of illness have failed to detect virus, supporting the notion that influenza viremia is at most a rare event in the postsymptomatic period and if it exists is not generally sustained for long periods.[40-42]

Indirect evidence of influenza viremia has been suggested by reports of viral detection (isolation or PCR) from extrapulmonary sites including autopsy specimens of heart, kidney, brain, spinal cord, spleen, and liver of a pregnant 19-year-old woman who died as a result of A2/HongKong/8/68 infection.[43] Fetal heart tissue and amniotic fluid were also positive, as was the amniotic fluid of a 24-year-old woman who recovered from influenza A/Bangkok (H3N2) infection.[44] A series of autopsies

performed during the 1957 Asian pandemic also yielded positive results from spleen, lymph node, liver, kidney, heart, and tonsil.[31,45] Systemic dissemination of influenza virus is suggested in influenza-associated encephalopathy, although virus isolation from either blood or cerebrospinal fluid (CSF) from patients with encephalopathy and influenza is rare.[41,42,46,47] This may indicate that the presence of virus in the CSF is not required for the development of neurologic symptoms. Alternatively, viremia and possible infection of the CSF may precede the onset of encephalopathy. Influenza viremia has also been detected in a case of virus-associated myocarditis.[48]

Viremia after experimental human influenza infection

Experimental infection has resulted in isolation of virus from the blood of individuals infected by nasal inoculation.[40] With a cell line coculture system, virus was detected in nasal secretions of 1 of 15 subjects and was not detected in any blood samples. Samples were available for retesting from 4 subjects with existing antibodies against Asian influenza, with each of the subjects showing a fourfold or greater increase in titer following challenge. With a more sensitive egg inoculation culture system viremia was seen in all four subjects tested. Viremia preceded nasopharyngeal shedding of virus by approximately 1 day. The authors reported that symptoms began on the second or third day after challenge, which was 2 days after the initial viremia and 1 day after demonstration of virus in the nasopharynx. One of these 4 patients remained asymptomatic throughout the observation period (22 days) and was viremic through Day 3. These results imply that viremia can precede nasopharyngeal shedding of virus and can occur in asymptomatic individuals. Caution in interpreting these data is urged, however, because others have noted that a much larger dose of influenza virus is required to effect infection when intranasal inoculation is used compared to an aerosol route, and other investigators using a similar virus strain and methods were not able to detect viremia in 27 experimentally infected patients.[40,49]

Potential interaction of influenza with blood elements

Influenza typically does not cause major hematologic abnormalities aside from occasional lymphopenia, usually in the setting of a normal WBC count.[50-52] Thrombocytopenia in the setting of influenza infection has been noted in a number of case reports, often in the setting of concomitant antibiotic therapy, which can also affect the platelet (PLT) count.[50,52,53] Although influenza does not appear to cause major hematologic perturbations in patients, it has been shown to interact with blood elements.[54] Influenza has been shown to adsorb to PLTs and RBC with equal kinetics and to elute from PLTs more slowly and less completely than from RBCs.[55] Binding of live or dead influenza virus to PLTs in vitro caused morphologic signs of damage to the PLTs including swelling, ballooning, and fragmentation, with a pronounced decrease in PLT counts.[56] Intravenous infusion of influenza virus in rabbits caused transient thrombocytopenia to levels 50 percent of normal for 1 to 2 days' duration. In vitro experiments have shown that anti-hemagglutinin antibody causes lysis of influenza-treated PLTs via the classical complement pathway in the presence of autologous human serum.[57] Although hematologic abnormalities aside from lymphopenia are not typically observed during the course of influenza infection, the available data suggest that influenza can interact with WBCs, RBCs and PLTs. If viremia occurred primarily in presymptomatic or asymptomatic infection, transient disturbances of hematocrit or PLT counts would likely escape detection. Binding to cellular components within the blood in theory could facilitate influenza viremia, although evidence for the phenomenon is lacking in humans.

ANIMAL MODELS OF INFLUENZA INFECTION AND VIREMIA

Animal models may provide insight into the frequency of viremia after influenza infection, and the murine model of influenza infection has been used as a prototypic model of an antiviral immune response, defining which subsets of immune cells are important in protection from lethal lytic virus infection. Although the model has been widely used for study of the immune response to viruses[58] relatively few studies measure influenza viremia as a study outcome. In mice infected with influenza intravenously, viremia persists for approximately 1 week in immunocompetent mice and shows rising titers at 1 week in gamma-irradiated mice.[59] In mice intranasally infected with influenza, virus could be detected in RBC fractions on Days 1 through 5 but not in PBMNCs or plasma aliquots.[60] Virus disseminated widely to a number of organs, and viremia was blocked by treatment of mice with hyperimmune serum before infection. Local lung damage may play a role in the development of viremia. After intranasal influenza infection, no mice with histologically normal lungs developed detectable viremia, whereas 9 of 20 mice with lung congestion had influenza RNA detected in blood by RT-PCR.[61] Although the murine model has been widely used to study influenza infection, pathogenicity of influenza isolates in mice and men does not always show a correlation.[62] Simian models of influenza infection generally reflect lower pathogenicity than in humans,[63,64] and cynomolgus macaques show less systemic dissemination of highly pathogenic avian influenza virus than human hosts.[65] A model animal that may more closely parallel human influenza infection is the ferret. Human influenza

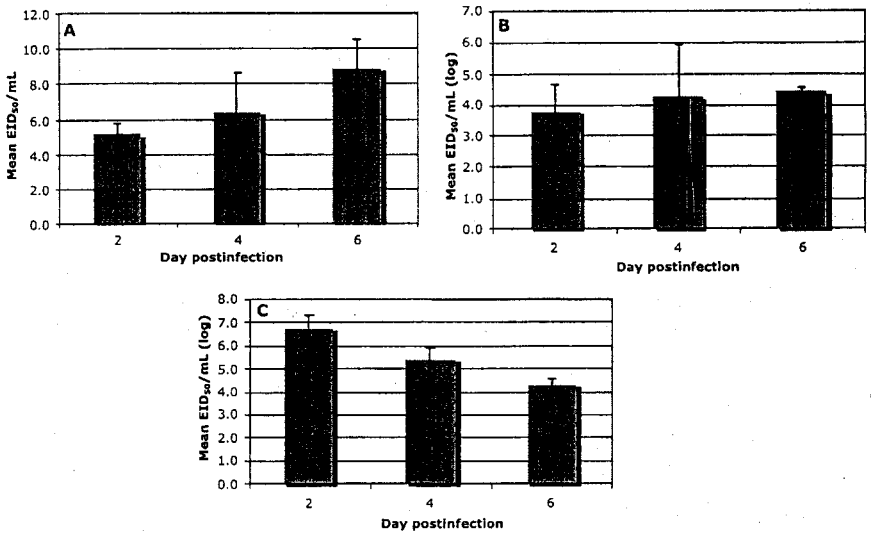

Fig. 2. Dissemination of H5N1 influenza A in ferrets. Viral loads in nasal turbinate (A), lung (B), and spleen (C) tissue from nine intranasally H5N1 (A/Vietnam/1203/2004) influenza A-infected ferrets. Ferrets were euthanized on Days 2, 4, and 6 postinfection, and tissues were immediately snap-frozen, homogenized, and inoculated onto embryonated hen's eggs for 50 percent egg infectious dose (EID_{50}) determination to assess viral load in the specified tissues.[63] Data shown represent the mean viral load in tissues from three ferrets per time point.

A strains with differential pathogenicity showed similar differential pathogenicity in ferrets, yet caused nonpathogenic infection in mice,[66] although ferrets may be more susceptible to influenza than humans.[67] Ferrets appear to be susceptible to highly pathogenic avian influenza H5N1 virus, developing severe symptoms with isolates from both fatal and mild human cases.[68] Multiple organ involvement is also observed in the ferret model of highly pathogenic avian influenza H5N1 infection. Representative original data from ferret challenge experiments with H5N1 influenza virus are included, revealing widespread productive viral replication in the brain, spleen, and upper and lower respiratory tract (Fig. 2). Ferret experiments were performed at the Southern Research Institute (Birmingham, AL) and were supported by the Canadian Institutes of Health Research.

HIGHLY PATHOGENIC AVIAN INFLUENZA A (H5N1)

Characteristics of transmission

Highly pathogenic avian influenza A (H5N1) has infected bird flocks in an almost worldwide distribution, yet transmission to humans remains rare, and human-to-human transmission has only occurred in isolated cases.[69,70] Tropism of the hemagglutinin molecule for its sialic acid receptor may influence the ease of infection and subsequent transmission of this avian influenza virus in humans. Avian influenza hemagglutinin binds to sialic acid linked to galactose by an α2,3 linkage (SAα2,3Gal), whereas human influenza hemagglutinin favors a sialic acid-galactose α2,6 linkage (SAα2,6Gal).[71,72] Upper airway epithelial cells in humans contain predominantly SAα2,6Gal, whereas SAα2,3Gal can be found in bronchiolar and alveolar cells.[73] Although clinical infection of humans with H5N1 virus rarely occurs, up to 10 percent of poultry workers in Hong Kong developed antibody responses to H5, implying that subclinical or abortive infection might occur in highly exposed subjects.[74] Exposure to live poultry was the only significant risk factor for transmission of H5N1 disease recognized in the 1997 Hong Kong H5N1 outbreak.[75] Mutations allowing use of the SAα2,6Gal receptor would likely be needed, in addition to other genetic changes, to transform the currently circulating avian H5N1 influenza virus into a human-tropic strain.

Disease manifestations in humans

Avian influenza H5N1 infection in humans causes increased morbidity and mortality compared to seasonal influenza infections in reported cases. H5N1 influenza infection in humans manifests as lower respiratory tract disease, consistent with the expression pattern of receptors for the virus.[2,70,76,77] Initial symptoms are similar to typical influenzalike illness, with fever (usually >38°C), cough, and dyspnea common.[2,77] Gastrointestinal symptoms also appear prominent in many case series, with one documented subject presenting with diarrhea but no initial respiratory symptoms.[78] An outbreak of H7N7 avian influenza was notable for conjunctivitis as a presenting symptom[28] although this has not been noted in H5N1 influenza illness. Although initial symptoms predominantly involve the respiratory system, multiple extrapulmonary tissues are affected by H5N1 avian influenza, including the gastrointestinal tract and prominent involvement of the liver and kidneys.[2,76,77] Encephalitis with virus isolated from the CSF has been reported in a child with no early pulmonary involvement.[78] Hematologic abnormalities are also prominent, with lymphopenia and thrombocytopenia frequently reported[2,76] and pancytopenia noted in one case series.[77] Marrow studies reveal hypoplastic marrow with a reactive hemophagocytic syndrome.[76] The involvement of multiple organ systems in H5N1 highly pathogenic avian influenza infection in humans suggests that subjects might show evidence of viremia. In fact, in two published studies measuring virus in the plasma or serum, virus was isolated, in the first case on Day 10 of illness (viral load not reported), and in the second a viral load of 85,000 RNA copies per mL was detected.[78,79] More recently, de Jong and coworkers[80] reported finding H5N1 viral RNA in the blood of 9 of 16 Vietnamese patients infected with avian influenza. These results suggest that viremia can occur at reasonably high levels and for prolonged periods in people with symptomatic H5N1 influenza infection. Based on limited data, it appears that symptomatic H5N1 influenza infection and infection with other new pandemic strains in humans may be more likely than currently circulating influenza A strains to result in viremia.[78,80] The risk of viremia during the incubation period of H5N1 infection or asymptomatic infection with other influenza A viruses novel to humans is unknown.

SIGNIFICANCE AND SUMMARY

With the advent of routine testing for multiple pathogens, the US blood supply has become increasingly safe. Many adverse events associated with transfusion are not related to transfusion-transmitted diseases, but rather immunologic effects (transfusion-related acute lung injury, ABO mismatch) or hemodynamic effects (transfusion-associated circulatory overload). Furthermore, the blood banks and test manufacturers have demonstrated that they can rapidly respond to interdict newly introduced infectious agents, as was seen in the response to West Nile virus introduction to the US.[81] Not all pathogens that infect blood donors, however, are successfully screened, with unknown impact on recipients. One potentially unrecognized pathogen in the blood supply is influenza virus. The incidence of viremia in blood donors is thought to be low, particularly for seasonal influenza, but has not been adequately studied. Older studies of influenza A experimental infection and one report of a naturally infected person suggest that viremia can occur before symptom onset. Even if influenza viremia occurs, the incidence is likely low during seasonal influenza outbreaks and would not pose a large risk to the safety of the blood supply.

Research projects are ongoing to address whether virus can be detected in blood collected during periods of transmission within the community. As part of the Retrovirus Epidemiology Donor Study II (REDS II), we are examining the incidence of influenza viremia in a retrospective cohort of blood donors, the REDS Allogeneic Donor and Recipient (RADAR) repository. The RADAR repository contains more than 120,000 whole-blood and plasma specimens that are linked to donor zip code and date of blood donation, but delinked from personal donor identifiers. Access to the repository will allow identification of samples collected during periods of widespread influenza activity in the community. Detection of influenza in the blood presents a challenge due to the lower viral loads found in blood compared to nasopharyngeal secretions. RBC or PLT fractions may contain more influenza than plasma or WBC fractions due to interaction with the hemagglutinin moiety of influenza.[57] We will first determine the blood fraction that contains the highest level of virus. Once the appropriate blood fractions are identified, samples from RADAR repository obtained during periods of known epidemic influenza A outbreak, and samples obtained during periods of very low influenza activity will be tested for the presence of influenza A RNA. This ongoing project will quantify the risk of occult influenza viremia in blood donors during periods of high-level transmission of influenza in the community. If viremia is detected then the issue of iatrogenic transmission of influenza by transfusion will need to be reassessed. In addition, further studies of people ill with seasonal and avian H5N1 virus infection are needed to evaluate the presence of viremia both by nucleic acid amplification and viral culture in comparison to viral isolates obtained from the respiratory tract to understand possible strain-specific and genetic features of viruses isolated from both sites.

Because pandemic influenza might be more likely to cause viremia and have higher pathogenicity than seasonal influenza, the risk of viremia during circulation of a new pandemic strain of influenza is more worrisome than

for possible transfusion-associated infection with currently circulating influenza, particularly given high rates in the population of antibody to current strains. More data is clearly needed to understand strain-specific characteristics that may predispose to viremia in infected persons. Based on limited available data, it appears that H5N1 virus infection and infection with newly circulating pandemic viruses in humans may be more likely than circulating seasonal human influenza viruses to cause a viremic phase. It is unclear how often asymptomatic infection occurs in avian influenza, although this may also be strain-specific as studies of human infection with earlier H5N1 strains found more mild or asymptomatic infections than studies of more recent H5N1 strains.[74,82] If and when a new pandemic appears, and the causative strain becomes evident, assuring the safety of the blood supply through study of donor viremia would be an important step. Widespread illness associated with an influenza pandemic among both donors and blood bank staff would stretch the ability of blood banks to provide a safe and adequate blood supply, and broad and indiscriminate screening based on exposure would only worsen potential product shortages. As part of pandemic planning, the resources to rapidly evaluate and implement screening measures for influenza viremia should be developed, allowing a rapid response to this potential threat to blood safety and availability.

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PATHOLOGY OF HUMAN INFLUENZA REVISITED

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Summary

The pathology of human influenza has been studied most intensively during the three pandemics of the last century, the last of which occurred in 1968. It is important to revisit this subject because of the recent emergence of avian H5N1 influenza in humans as well as the threat of a new pandemic. Uncomplicated human influenza virus infection causes transient tracheo-bronchitis, corresponding with predominant virus attachment to tracheal and bronchial epithelial cells. The main complication is extension of viral infection to the alveoli, often with secondary bacterial infection, resulting in severe pneumonia. Complications in extra-respiratory tissues such as encephalopathy, myocarditis, and myopathy occur occasionally. Sensitive molecular and immunological techniques allow us to investigate whether these complications are a direct result of virus infection or an indirect result of severe pneumonia. Human disease from avian influenza virus infections is most severe for subtype H5N1, but also has been reported for H7 and H9 subtypes. In contrast to human influenza viruses, avian H5N1 virus attaches predominantly to alveolar and bronchiolar epithelium, corresponding with diffuse alveolar damage as the primary lesion. Viremia and extra-respiratory complications appear to be more common for infections with avian H5N1 virus than with human influenza viruses. Further understanding and comparison of the pathology of human and avian influenza virus infections only can be achieved by directed and careful pathological analysis of additional influenza cases.

Keywords

influenza; human; pathology; pathogenesis

Introduction

An understanding of the pathology of influenza A virus infections in humans is important to improve diagnosis and to understand how these viruses cause disease. This knowledge also is important to evaluate animal models that adequately represent the disease in humans, and so to further unravel the pathogenesis and to test potential antiviral drugs and vaccines. We here review the pathology of human influenza A virus infections, both pandemic and seasonal, as well as that caused by infections with avian influenza A viruses such as H5N1 virus.

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Uncomplicated influenza

Human influenza A virus infections for which the pathology is described include H1N1, H2N2, and H3N2, which caused pandemics in 1918, 1957, and 1968, respectively [1]. Each time that a new subtype enters the human population it replaces the previously circulating subtype. The exception is the reintroduction in 1977 of H1N1, which has continued to co-circulate with H3N2.

Transmission of human influenza virus occurs by inhalation of infectious droplets or airborne droplet nuclei and, perhaps, by indirect (fomite) contact followed by self-inoculation of the upper respiratory tract or conjunctival mucosa. The relative importance of these routes is still debated [2]. Receptors for which human influenza viruses have a preference are long glycans terminating in sialic acids linked to galactose by an alpha-2,6 linkage [3]. These receptors are expressed on epithelial cells throughout the respiratory tract—nasal mucosa, paranasal sinuses, pharynx, trachea, bronchi, bronchioles, and alveoli—but their abundance varies per site [4]. In the tracheo-bronchial tree, human influenza viruses attach predominantly to ciliated epithelial cells, and attach more abundantly to tracheal and bronchial epithelium than to bronchiolar epithelium (Fig. 1) [5]. In uncomplicated influenza in humans, the cell types in which human influenza virus replicates *in vivo* only has been determined for the nasal mucosa, where both ciliated and non-ciliated cells are infected [6]. However, *ex vivo* human tissue cultures have shown that epithelial cells of nasopharynx, adenoids, tonsil [7], bronchus and pulmonary alveolus [4] are permissive. *In vitro*, primary cell cultures of human tracheal epithelial cells have shown replication in both ciliated and non-ciliated cells [8].

One of the only descriptions of histologic lesions associated with uncomplicated influenza in humans is from a study of tracheal and bronchial biopsies obtained from six young adults between 1 and 7 days after onset of symptoms [9]. They had a diffuse, superficial, necrotizing tracheo-bronchitis, which was progressively more severe further down the tracheo-bronchial tree. Lesions were already visible at 1 day after onset of symptoms. Damage to the respiratory epithelium ranged from vacuolization, edema, and absence of cilia to extensive desquamation of epithelial cells. In the lamina propria, there was prominent edema and hyperemia, and infiltration with primarily lymphocytes and histiocytes. Inflammatory cell infiltration was limited compared to the extent of epithelial damage. From 2 days after the onset of symptoms, epithelial repair was visible in the form of epithelial metaplasia.

The changes to the bronchial epithelium from influenza virus infection are short-lasting. In a study of bronchial biopsies from patients between 1 and 6 weeks after onset of symptoms, the only significant differences between influenza patients and healthy controls were thickened surface epithelium and slight increase in lymphocytic infiltration of the lamina propria, corresponding with epithelial regeneration and bronchial inflammation, respectively [10;11].

The typical signs and symptoms of uncomplicated influenza are both local (nasal obstruction, cough, sore throat) and systemic (headache, fever, chills, anorexia, myalgia) [1]. These signs and symptoms are due both to the damage at the site of virus replication and to the local and systemic release of cytokines and other inflammatory mediators [12;13].

Primary complication: viral pneumonia

The most common complication of influenza is extension of the viral infection distally to the lung, resulting in pneumonia. In contrast to damage to the tracheo-bronchial epithelium in uncomplicated influenza, damage to the alveolar epithelium has severe consequences for the gas exchange function of the respiratory tract. This damage to alveolar epithelium—consisting of type I and type II pneumocytes—is due to a combination of the direct cytolytic effect of viral infection and the indirect effect of host response [14]. Type I pneumocytes prevent leakage

of fluid across the alveolar-capillary barrier, and type II pneumocytes both resorb fluid from the alveolar lumen and produce lung surfactant that is important for reducing alveolar surface tension. Therefore, damage to these cells allows fluid from the alveolar capillaries to flood into the alveolar lumina. This causes severe, and in some cases fatal, respiratory dysfunction [15].

Risk factors for the development of influenza viral pneumonia include lack of previous exposure to influenza virus with related surface glycoproteins, age greater than 65 years, pulmonary disease, cardiovascular disease, and pregnancy [1]. Individuals who have not been previously exposed to an antigenically related influenza virus lack the protection of the lung against viral infection conferred by specific IgG, which reaches the alveolar lining fluid by transudation from the serum [16–18]. Important chronic underlying pulmonary diseases that predispose influenza patients to hospitalization are chronic obstructive pulmonary disease, asthma, and pulmonary fibrosis [19], which involve remodeling of airways or distal lung parenchyma and thus reduce pulmonary defense against infectious pathogens [20]. There are no clear explanations for the increased risk of influenza viral pneumonia from cardiovascular disease or pregnancy. It has been speculated that pulmonary hypertension secondary to cardiovascular disease or from the increased blood volume in pregnancy may predispose the lung to pulmonary oedema when the alveolar septa are damaged by the virus [21].

Based on attachment studies [5], the primary target cells of human influenza virus in the lower respiratory tract are type I pneumocytes and ciliated bronchiolar epithelial cells, although attachment does occur less frequently to non-ciliated bronchiolar epithelial cells, type II pneumocytes, and alveolar macrophages (Fig. 1). This corresponds with *ex vivo* infection of alveolar epithelial cells by human influenza virus [4]. *In vivo* descriptions of the target cells of influenza virus in fatal pneumonia from any of the three influenza pandemics of the last century are very rare. Specific fluorescence was visible in alveolar epithelial cells and alveolar macrophages in lung tissue of two adult women who died with human influenza virus H2N2 pneumonia during or just after the 1957 pandemic [22;23]. Fluorescence-positive interstitial macrophages were detected in the interstitium and alveolar exudate of 7 of 29 lungs from people who died of influenza in Boston during the 1957 pandemic [24].

The pathological changes to the lung from influenza viral pneumonia have been most commonly described during pandemics and have been recently been reviewed [25]. The acute alveolar injury (diffuse alveolar damage) caused by influenza virus infection is similar to that caused by many other agents that are noxious for alveoli. In the early stage, there is necrosis of alveolar epithelium, characterized by denudation of the alveolar septum and the presence of desquamated pneumocytes in the alveolar lumen. These desquamated cells are shrunken and show pyknosis or karyorrhexis and cytoplasmic vacuolation or hypereosinophilia. The alveolar lumina are flooded by edema fluid with variable admixture of fibrin and erythrocytes (intra-alveolar hemorrhage) (Fig. 2A). In some alveolar lumina, there are many alveolar macrophages. Characteristically, alveoli and alveolar ducts are lined by hyaline membranes, consisting of fibrin-rich edema fluid mixed with the cytoplasmic and lipid remnants of necrotic epithelial cells (Fig. 2B). The alveolar septa are widened due to hyperemia of alveolar capillaries, interstitial edema, and leukocyte infiltration, mainly neutrophils as well as a few eosinophils. These leukocytes also may be present in alveolar lumina. Fibrinous thrombi may be present in the capillaries of alveolar septa and alveolar ducts, as well as in small pulmonary blood vessels (Fig. 2C). Possibly as a result of these thrombi, alveolar septa may be necrotic. The late stage of influenza viral pneumonia is characterized by re-epithelization of the alveoli by type II pneumocytes (type II pneumocyte hyperplasia), interstitial fibrosis of alveolar septa, and infiltration by mononuclear leukocytes, predominantly lymphocytes and plasma cells (Fig. 2D).

In addition to the above alveolar changes, the bronchioles show a necrotizing bronchiolitis, characterized by epithelial necrosis, the formation of hyaline membranes, and infiltration by variable numbers of neutrophils. Changes to the trachea and bronchi are similar to those of uncomplicated influenza. Chronic changes of influenza pneumonia may include squamous metaplasia and interstitial fibrosis [25].

Influenza viral pneumonia often occurs together with, or is followed by, bacterial pneumonia. Prior influenza virus infection may predispose the respiratory tract to bacterial infection by different mechanisms and, vice versa, bacterial infection may enhance influenza virus infection [26]. The bacterial infection results in a different type of inflammation than that caused by influenza virus, with a more prominent infiltration of neutrophils and production of pus: suppurative bronchopneumonia (Fig. 3). A recent review of over 8,000 published autopsy case results from the 1918 pandemic found that the majority of deaths (96%) likely resulted from secondary bacterial pneumonia (Morens D.M., Taubenberger, J.K., Fauci, A.S., unpublished data). As in 1918, most deaths in the 1957 pandemic were due to secondary bacterial pneumonia, although negative autopsy lung cultures were more common than in 1918, possibly due to the widespread administration of antibiotics [27;28]. In one study of the 1957 pandemic, 111/148 (75%) of confirmed fatal cases of influenza had bacteriological and histological evidence of a bacterial pneumonia, mainly due to *Staphylococcus aureus* or pneumococci [29]. In the same study, 30/148 (20%) of fatal cases were considered due to influenza viral pneumonia.

Complications outside the respiratory tract

Human influenza virus primarily infects and causes disease in the respiratory tract. However, human influenza virus infection also is associated with disease in other organs, albeit to a lesser extent. Given the recent reports of extra-respiratory disease from highly pathogenic avian influenza H5N1 virus infection (see below), it is important to revisit these complications of human influenza virus infection.

In general, there are two explanations for the pathogenesis of influenza-associated extra-respiratory complications. The first is that influenza virus spreads via blood to these tissues and replicates there. A likely route for influenza virus to reach blood is by crossing the alveolar-capillary barrier damaged by influenza viral pneumonia. It remains controversial whether viremia routinely occurs during pandemic or seasonal influenza infection. As recently reviewed [30], viremia has been previously reported in influenza virus infection of humans [31–35]. However, several other studies [36–38] failed to detect viremia after onset of illness, suggesting that influenza viremia is rare after onset of symptoms and, if it occurs, is not sustained for long periods [30].

Evidence for replication of influenza virus in extra-respiratory tissues usually comes from detection of virus in these tissues by virus isolation or fully-nested RT-PCR. However, these methods do not exclude the possibility that detected virus originated from blood. The only proof is *in situ* detection of virus by direct immunofluorescence, immunohistochemistry, or *in situ* hybridisation in the tissue concerned. Such reports are rare (e.g., brain: [39;40]; heart: [41]) and further confirmation of the ability of human influenza virus to replicate in extra-respiratory human tissues *in vivo* is badly needed.

The second explanation for the pathogenesis of influenza-associated extra-respiratory complications is suggested by the link between acute respiratory distress syndrome (ARDS) and multi-organ dysfunction syndrome (MODS). ARDS, which may be caused by a variety of insults to the lungs, including influenza virus infection, commonly progresses to MODS [42]. The hepatic, renal, central nervous, gastrointestinal, hematologic, and cardiac systems are most commonly affected [43]. The pathogenesis of MODS has not been elucidated, but is thought