An Influenza A H1N1 Virus Revival – Pandemic H1N1/09 Virus

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Abstract

In April 2009, a novel H1N1 influenza A virus, the so-called pandemic H1N1/09 virus (former designations include swine influenza, novel influenza, swine-origin influenza A [H1N1] virus [S-OIV], Mexican flu, North American Flu) was identified in Mexico. The virus has since spread throughout the world and caused an influenza pandemic as defined by the criteria of the World Health Organization. This represents the first influenza A virus pandemic since the emergence of H3N2 ("Hong Kong" Flu) in 1968. Vaccine production has started, and vaccines are expected to become available during the course of 2009. Although the pandemic H1N1/09 virus originates from the triple-reassortant swine influenza (H1) virus circulating in North American pigs, it is not epidemic in pigs. Although the H1N1/09 virus pandemic is currently mild, concerns remain that it may become more aggressive during spreading. The distribution of proper information to the public on the status of the H1N1/09 virus pandemic will be important to achieve a broad awareness of the potential risks and the optimum code of behavior during the pandemic. Here, the features of pandemic H1N1/09 virus are discussed within the framework of knowledge gained from previous influenza A virus pandemics.

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Introduction

An influenza pandemic is an epidemic of an influenza virus that spreads on a worldwide scale and infects a large proportion of the human population. The World Health Organization (WHO) and the U.S. Centers for Disease Control and Prevention (CDC) have been warning that there is a substantial risk of an influenza pandemic within the next few years. In recent times, a highly pathogenic variation of the H5N1 subtype of influenza A virus as well as other avian influenza A viruses, including H7N3, H7N7, and H9N2, have been regarded as the strongest candidates for a novel influenza pandemic [1–4]. Avian influenza A virus-induced mortality, especially that of H5N1, appears to be high in humans. However, the avian viruses have not as yet fully adapted to humans. The successful adaptation of influenza viruses to humans probably results from a multistep evolutionary process, leading to a specific combination of viral factors that confer efficient replication and transmission in humans and the ability to compete with circulating human influenza viruses [5].

In the meantime, a new H1N1 strain, the so-called pandemic H1N1/09 virus (former designations include swine influenza, novel influenza, swine-origin influenza A [H1N1] virus [S-OIV], Mexican flu, North American Flu) has caused an influenza pandemic. On 11 June 2009, the WHO issued a pandemic alert level of six (of six possible levels) (current information on the status of the pandemic can be found at http://www.who.int/csr/disease/swineflu/en/index.html), indicating that all of the criteria for a pandemic had been met by the pandemic H1N1/09 virus outbreak.

The first information on the emergence of a novel H1N1 influenza A virus strain was distributed by the CDC and the WHO in April 2009 (http://www.who.int/csr/disease/swineflu/en/index.html; http://www.cdc.gov/h1n1flu). The time and location of the outbreak is still unknown, but the virus was initially identified in Mexico in mid-March (http://www.washingtonpost.com/wp-dyn/content/article/2009/05/02/AR2009050202353.html?hpid=topnews) when Mexican authorities sent samples obtained from a flu patient which they were unable to subtype to the Canadian Public Healthy Agency [6]. Following its identification, the virus was determined to have rapidly spread throughout the world and now affects people living in both the Northern and Southern Hemispheres, particularly circulating in tropical regions. Current information on the status of the H1N1/09 virus pandemic can be found at http://www.who.int. Because of the rapid spread of the

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virus, many clinics were overwhelmed by the numbers of patients requiring testing and treatment; consequently, the WHO stopped requiring countries to report all cases.

Epidemiology of H1N1 Influenza

Influenza A viruses are preferentially endemic in water birds, such as ducks, geese, and shore birds (gulls), which usually do not fall ill from this infection. Depending on the antigenicity of the two envelope spikes, which mediate first virus adsorption to target cells *in vivo* or erythrocytes *in vitro* (hemagglutinin, H) and then the release of viral progeny from the infected cells (neuraminidase, N), influenza A viruses are categorized into 16 H (H1–H16) and nine N (N1–N9) groups, resulting in – theoretically – 16×9 serologic subtypes. To date, 105 influenza A virus subtypes have been identified, all of which are endemic in water birds. However, some subtypes have adapted to other birds and mammalians (e.g. pigs, horses, humans) in species–specific strains [5].

The mutations that occur in the surface proteins result in antigenic drift, which helps the virus escape the immunity of its host. Since the influenza A virus genome is segmented into eight parts, two or more different virus variants infecting the same cell can produce progeny virus with a mixed genome, which supports the variability of viral structures. An antigenic shift may result if two different subtypes of influenza A virus reassort their genomic segments. Pigs are highly susceptible to both human and avian viruses and are thought by some experts to be a ''mixing vessel'' for avian and human viruses. A reassortment of these may give rise to a pandemic strain [1, 5].

In humans, three subtypes of influenza A viruses resulting from genomic reassortment have been detected: H1N1, H2N2, and H3N2. These have caused pandemics of high morbidity and mortality, such as the so-called Spanish influenza (H1N1, 1918–1919, in which at least approximately 50 million people died), the Asian influenza (H2N2, 1957–1963, in which about 2–4 million people died), and the Hong Kong influenza (H3N2, 1968–1969, in which about 1–2 million people died). Newly circulating influenza viruses ultimately replace previously circulating strains; for example, H1N1 disappeared when it was replaced by the novel pandemic H2N2 avian-human reassortant virus, which vanished in turn when it was replaced with the H3N2 human-avian reassortant virus.

The disappearance of H1N1 in 1957 most likely represents competition by the emerging pandemic H2N2 strain in the face of population immunity to H1N1. In November 1977, the H1N1 strain reemerged in the former Soviet Union, Hong Kong, and northeastern China to cause the so-called Russian influenza epidemic. The H1N1 strain of Russian influenza was closely related to a 1950 strain but dissimilar to the influenza A (H1N1) strains from both 1947 and 1957. This finding suggests that the 1977 outbreak strain had been preserved since 1950 [7]. It affected mostly children and young adults because a substantial number of older individuals had sufficient immunity due to exposure to earlier H1N1 strains [8]. Although the 1977 outbreak was limited, this H1N1 virus and its descendants have been circulating ever since. Both H1N1 and H3N2 influenza viruses continue to be present in the human population; the variants currently circulating are less pathogenic, but they nevertheless have caused an increased mortality rate among elderly people in each winter epidemic wave [1, 5].

Analysis of the full genome sequences of representative influenza A (H1N1) viruses from 17 countries and five continents that were sampled between 1918 and 2006 revealed that human H1N1 virus has not acquired new gene segments from avian or other sources [9]. However, several distinct intrasubtype reassortant events were found to have occurred among viruses from various sublineages. For example, the virus responsible for the post-World War II epidemic was found to vary significantly from the 1943 H1N1 strain, especially in its H segment, where five antigenic sites were involved in amino acid changes [10]. The H segment of the 1947 virus, which was determined to have emerged through intrasubtype reassortment, was more representative of later H genotypes. In contrast, the N segment of the 1947 virus was conserved, which may have provided partial protection and thus could explain why disease severity in the 1947 influenza epidemic was attenuated despite significant changes in the H protein [9, 10].

Clinical signs of influenza in pigs were first observed in 1918, coinciding with the Spanish influenza. Similarities in the clinical presentation and pathologic features of influenza in humans and swine led to the suggestion that the human influenza pandemic in 1918 was actually adapted to the pig. The etiological agent of swine influenza, isolated by Shope in 1930, was also an H1N1 antigenic subtype [11]. Shope provided evidence that the human pandemic strain H1N1 and the infectious agent of swine influenza were closely related by showing that human adult serum could neutralize the swine influenza virus [12]. Descriptions of the 1918 swine outbreak, taken together with the recent phylogenetic analyses of the reconstituted 1918 human influenza virus sequences [13], support the hypothesis that the virus most probably spread from humans to pigs. Notably, data indicate that genetic components of the 1918 H1N1 pandemic virus were circulating freely in mammalian hosts, i.e., swine and humans, as early as 1911 and that the 1918 H1N1 pandemic virus was not likely an avian virus that appeared later [14]. Phylogenetic relationship studies suggest that the A/Brevig Mission/1/1918 virus was generated by reassortment between mammalian viruses and a previously circulating human strain, either in swine or, possibly, in humans. In addition, seasonal and classic swine H1N1 viruses were not derived directly from A/Brevig Mission/1/1918, but their precursors co-circulated during the pandemic [14]. Mean estimates of the time of the most recent

common ancestor also suggest that the H2N2 and H3N2 pandemic strains may have been generated through reassortment events in unknown mammalian hosts in the period preceding pandemic recognition and involved multiple avian viruses. Remarkably, numerous (less virulent) H1N1 strains have been detected in animals, including pigs and birds.

Pandemic H1N1/09 Virus Generation

Based on genomic analysis results, it appears that the pandemic H1N1/09 virus first evolved around September 2008 and circulated in the human population for several months before the first cases were detected. It emerged from a triple-reassortant swine influenza A (H1N1) virus that developed and became enzootic in pigs in North America at the end of the 1990s [15–17]. This triple-reassortant swine influenza A virus contains genes from avian, human, and swine influenza viruses. Its spread to humans was detected sporadically, but all patients recovered. Severe illnesses of the lower respiratory tract and unusual influenza symptoms, such as diarrhea, were reported [16]. The triple-reassortant swine influenza A (H1N1) virus contains classic swine RNA segments of North American lineage (hemagglutinin [H], nucleoprotein [NP], non-structural proteins [NS], neuraminidase [N], matrix proteins [M]), avian influenza RNA segments of North American lineage (polymerase basic protein 2 [PB2], polymerase acidic protein [PA]), and PB1 of human seasonal H3N2 viruses [15, 16]. In the pandemic H1N1/09 virus, the N and M segments of triple-reassortant swine influenza A (H1N1) were replaced by N and M from the Eurasian influenza A (H1N1) swine lineage [15, 16]. Therefore, in contrast to the 1918 pandemic for which the zoonotic sources of the introduced viral gene segments remain to this day ambiguous [14], it is known that pandemic H1N1/09 virus probably emerged from swine into humans. However, there were no confirmed influenza virus outbreaks in Central American pigs before the reported H1N1/09 infections in humans. The asymptomatic infection of H1N1/09 in specific pathogen-free miniature pigs (swine influenza model), despite efficient virus replication, may explain the lack of reports on H1N1/09 outbreaks in pigs prior to its transmission to humans [18].

This lack of similarity between the pandemic H1N1/09 virus and its nearest relatives indicates that its gene segments have already circulated for years [17, 19]. The genetic diversity of pandemic H1N1/09 virus is low, suggesting that its transmission to humans results from a single event or that very similar viruses were transmitted. The pandemic H1N1/09 virus lacks molecular markers thought to be associated with the adaptation of influenza A viruses to humans [19]. Consequently, pandemic H1N1/09 virus spread in humans appears to result from unidentified molecular events that warrant further studying. Moreover, the results of evolutionary analysis aimed at estimating the timescale of the origins and the early development of pandemic H1N1/09 virus epidemic revealed that the initial transmission to humans occurred several months before the recognition of the outbreak [17]. The multiple genetic ancestry of pandemic H1N1/09 virus suggests that the pandemic H1N1/09 virus has developed naturally, and no indications of artificial origin were found [17]. The pandemic H1N1/09 virus pandemic clearly proves that the mixing of genetic elements from different influenza A viruses in pigs can result in pandemic viruses and that the surveillance of influenza viruses in pigs is warranted.

Pandemic H1N1/09 Virus Transmission

The pandemic H1N1/09 virus is able to sustain relatively high human-to-human transmission and requires no contact with swine. Epidemiological estimates place the basic reproduction number R_0; the average number of secondary cases infected by each primary case at a defined time) at 1.4–1.6. At this level, the pandemic H1N1/09 virus does not seem to be as transmissible as the 1918 pandemic H1N1 strain, but it does appear to be substantially more transmissible than seasonal influenza. However, transmission data on the pandemic H1N1/09 virus are still poor. The generation time (the time until an infected person begins infecting others) has been estimated to be 1.3–2.7 days, which is somewhat shorter than estimates for previous pandemics. An assessment of the secondary attack rate (proportion of a defined cohort [e.g. household or school] that falls ill after contact with an infected person) in families in the USA suggests that pandemic H1N1/09 virus patients infected about 10% of family members [20] compared with 5–20% of family members afflicted with seasonal influenza.

The incubation time of the pandemic H1N1/09 virus appears to range between 2 and 7 days [15]. Although this can only be regarded as a rough and preliminary estimate, the incubation time may be longer than that of seasonal influenza A viruses, which has been calculated to be 1.4 days [21]. Based on seasonal influenza data, viral shedding might be expected from 1 day prior to disease onset until 5–7 days after first symptoms or until symptoms resolve. In certain patient groups, including immunocompromised individuals, severely ill patients, and young children, virus shedding time may be prolonged [22].

The pandemic H1N1/09 virus, such as other influenza A viruses, is believed to be transmitted from infected individuals through air by coughs or sneezes, creating aerosols containing the virus [23, 24]. Influenza can also be transmitted by saliva, nasal secretions, feces, and blood. Infections occur through contact with these body fluids or with contaminated surfaces. Initially, contradictory findings on pandemic H1N1/09 virus transmission were reported from two independent animal experiments using the ferret model. In the first experiment, the pandemic H1N1/09 virus exhibited less efficient respiratory droplet

transmission in ferrets in comparison to the high transmissible phenotype of a seasonal H1N1 virus [25], while in the second experiment the new virus spread as well as the seasonal strain [26]. A recent study reported the transmission pattern of H1N1/09 virus in the ferret model to be similar to that of two human control influenza viruses that are known to transmit among ferrets [18]. Based on data showing that 25% of pandemic H1N1/09 virus patients had diarrhea [15], the potential for fecal viral shedding and fecal–oral transmission should be considered and investigated.

Pandemic H1N1/09 Virus Pathogenicity

It is not yet possible to exactly assess the pathogenicity and mortality rate of the pandemic H1N1/09 virus since reliable data are still lacking. Data available from early analyses of the outbreak in Mexico suggest that 23,000 (range 6,000–32,000) individuals had been infected in the country by late April, giving an estimated case fatality ratio (CFR) of 0.4% (range 0.3–1.5%) based on confirmed and suspected deaths reported at that time [27]. In a community outbreak in the small district of La Gloria, Veracruz no deaths were attributed to infection, giving 0.6% as the upper 95% bound of CFR. Thus, while uncertainty remains, the clinical severity appears to be less than that seen in 1918/1919 when Spanish influenza killed at least 50 million people but comparable with that seen in the 1957 (H2N2) Asian influenza pandemic which killed 2–4 million people [27]. If 30% of the current world population ended up being infected with H1N1, which is a fairly typical proportion for an influenza pandemic, then a CFR of 0.3%–0.6% would result in around 6–12 million deaths.

The cases reported to date in the USA have been generally mild, with an age distribution that is typical for seasonal influenza; school children are the age group with the highest rates of influenza, and they spread the virus to household contacts [15]. The clinical manifestations of the pandemic H1N1/09 virus to date have also been typical of seasonal influenza, with fever in 94% of patients and sore throat in 66%. An additional common symptom is vomiting (25% of patients) or diarrhea (25% of patients) [15]. The majority of patients infected with the pandemic virus worldwide continue to experience mild symptoms, recovering fully within 1 week, even in the absence of any medical treatment. However, pregnant women seem to be at an increased risk for complications from pandemic H1N1 virus infection, with a higher estimated rate of hospital admission among this group than in the general population [28]. Other groups at an increased risk of severe or fatal illness include people with underlying medical conditions, most notably chronic lung disease (including asthma), cardiovascular disease, diabetes, and immunosuppression. Results from a number of preliminary studies suggest that obesity, and especially extreme obesity, may be a risk factor for more severe disease (http://www.who.int/csr/disease/swineflu/notes/h1n1_pregnancy_20090731/en/index.html).

The earliest Mexican fatalities were alleged to be mainly young adults, usually under the age of 50 years, without underlying medical conditions. The susceptibility of this age group is a common trait of pandemic influenza (http://www.newscientist.com/article/dn17025-deadly-new-flu-virus-in-us-and-mexico-may-go-pandemic.html). From 24 March through 24 April 2009, a total of 18 cases of pneumonia and confirmed pandemic H1N1/09 virus infection were identified among 98 patients hospitalized for acute respiratory illness at the National Institute of Respiratory Diseases in Mexico City [29]. More than half of the 18 case-patients were between 13 and 47 years of age, and only eight had pre-existing medical conditions. All patients had fever, cough, dyspnea or respiratory distress, increased lactate dehydrogenase levels, and bilateral patchy pneumonia. Other common findings were an increased creatinine level and lymphopenia. Twelve patients required mechanical ventilation, and seven died. In countries other than Mexico, a small number of otherwise healthy people also experienced very rapid progression to severe and often fatal illness. Predictors of this pattern of severe disease have not yet been identified, although studies are ongoing (http://www.who.int/csr/disease/swineflu/notes/h1n1_pregnancy_20090731/en/index.html).

There is as yet no clarification for the relatively greater severity of the Mexican H1N1/09 influenza outbreak and some of the H1N1/09 patient-cases from other countries. However, differences in virus genetics do not seem to account for the greater severity as demonstrated by a comparison of the genomic sequences of different virus isolates. A possible explanation may be that a second influenza A strain accounts for some of the unusual patterns of illness observed in Mexico. In support of this hypothesis, a variant of the seasonal H3N2 virus has been observed by Canadian researchers and tentatively linked to a case-patient returning from Mexico in mid-March. It has also been suggested that mortality during influenza pandemics can vary widely between different countries, with mortality being concentrated in the developing world [30]. Nutritional status of the host may not only influence the host response to the pathogen but also the genetic make-up of the viral genome [31]. Moreover, bacterial co-infections are considered as possible cause for differences in the outcome of influenza infection in different locations. The majority of deaths during the 1918–1919 influenza pandemic more likely resulted from secondary bacterial pneumonia caused by common upper respiratory-tract bacteria than from ''primary'' viral pneumonia (i.e., with little or no bacterial growth) [32]. In the current H1N1/09 outbreak, the first deaths were diagnosed as atypical pneumonias, i.e. pneumonias that may have been very massive due to the influenza virus and bacteria working in concert. Such severe pneumonias require early

and elaborate treatment, including antibiotic and intensive care, which may be less available in some areas of the world, resulting in increased morbidity and mortality. As consequence, the availability of antibiotics should be considered in preparedness strategies for influenza pandemics.

The severity of the pandemic H1N1/09 virus has been investigated in two independent experiments using ferrets, which show responses closely similar to those of humans. In one study, conducted at the U.S. CDC in Atlanta, viruses isolated from patients in California, Texas, and Mexico, respectively, were intranasally inoculated in ferrets (six ferrets in each group, 18 in total) [25]. In the second study, a Dutch team at the Erasmus Medical Center in Rotterdam infected six ferrets with a virus isolated from the first Dutch patient [26]. Both research groups used a seasonal H1N1 strain as a control, and both found that the pandemic virus caused more severe disease, as evidenced by its increased morbidity and more extensive replication in the respiratory tract than seasonal H1N1 virus. The replication of the pandemic H1N1/09 virus was detected in the trachea, bronchi, and bronchioles, while the replication of seasonal H1N1 virus was confined to the nasal cavity. Moreover, the pandemic H1N1/09 virus – but not the seasonal H1N1 virus – was recovered from the intestinal tract. A study conducted in Japan demonstrated that the H1N1/09 virus replicates more efficiently and causes more severe pathological lesions in the lungs of infected mice, ferrets, and non-human primates than a currently circulating human H1N1 virus [18]. These observations suggest that the H1N1/09 virus may be more pathogenic than seasonal influenza viruses but that it is not as dangerous as the 1918 pandemic virus or H5N1 avian influenza.

There is great concern that mutations in pandemic H1N1/09 virus genes (antigenic drift) over the coming months may change its pathogenic potential. The pandemic H1N1/09 virus may be regarded as ''unstable'', meaning it could mix and swap genetic material when exposed to other influenza A viruses, thereby giving rise to more pathogenic reassortants (antigenic shift). Factors that contribute to virulence and determine the pathogenicity of avian influenza in humans and other mammals depend on all steps of virus replication. Strains of influenza A viruses that are virulent in humans have undergone alterations in any one of three viral proteins: the major surface protein of the virus HA, the viral polymerase complex (including the PB1, PB2, and PA proteins), and the non-structural protein NS1 (Table 1). However, as of early June, isolates from a wide geographic area (from the Americas, Europe, Asia, and New Zealand) did not show genetic variations, and no changes characteristic of highly pathogenic influenza A viruses have yet been found. In addition, the pandemic H1N1/09 virus does not have a version of the protein PB1-F2 encoded by the PB1 gene introduced as a potential virulence factor, which could play a role in the pathogenesis of infection with pandemic influenza viruses [33, 34].

Pandemic H1N1/09 Virus Vaccine

The novel genetics of pandemic H1N1/09 virus reduce the probability of humans developing a substantial immunity, although some individuals who have been infected naturally with H1N1 viruses may be afforded some protection [4]. Antibodies to the matrix M2 protein, which is conserved within A-type influenza viruses, are cross-protective between different subtype-virus infections, although the level of protection is limited. Moreover, peptides generated from influenza endogenous antigens, such as NP (nucleoprotein), which are targets for cytotoxic lymphocytes (CTLs), may elicit immune responses that show cross-reactivity in their recognition of the different subtypes of human influenza A viruses [4, 35]. At the present time, both H1N1 and H3N2 influenza viruses continue to be present in the human population as representatives of variants with decreased pathogenicity.

The epidemiologic features of the current pandemic H1N1/09 virus infections suggest multiple previous influenza infections may provide partial protection. In the USA, 60% of the patients with confirmed cases of pandemic H1N1/09 virus were $\leq$ 18 years of age. A first study investigating the cross-reactive antibody response to pandemic H1N1/09 virus in individuals was performed at the U.S. CDC [36]. Vaccination against recent H1N1 seasonal influenza viruses clearly did not induce antibodies that reacted with the pandemic H1N1/09 virus. However, one-third of blood samples taken from individuals > 60 years of age had antibodies that reacted with the pandemic H1N1/09 virus [36]. The results of a study conducted in Japan suggest that infection with human H1N1 viruses closely related to viruses circulating in 1918 confers a neutralizing antibody activity to the pandemic H1N1/09 virus [18]. It is therefore possible that older seasonal influenza viruses or vaccines may have generated cross-reactive antibodies in some of the older study participants. In addition, immunoinformatic comparisons demonstrated conservation of T-helper and CTL epitopes between the H and N proteins of novel H1N1/09 virus and circulating H1N1 strain (A/California/04/2009) [37]. These findings support the assumption that not only the T-cell response to cross-reactive (conserved) T-cell epitopes of virus endogenous proteins but also that to surface N and H proteins, due to vaccination or exposure to infection, may have the capacity to attenuate the course of H1N1/09-induced disease in the absence of cross-reactive antibody response.

The development of a vaccine providing protection from pandemic H1N1/09 virus infection is regarded to be crucial for the control of the pandemic. The production of an inactivated pandemic H1N1/09 virus vaccine has started, and the first vaccination programs are expected to start in 2009. The Strategic Advisory Group of Experts (SAGE) has published first recommendations for a vac-

Table 1
Virulence determinants of the pandemic H1N1/09 virus in humans.

Gene	Protein	Function	Note
H	Surface glycoprotein	Binding to receptor(N-acetylic neuraminic acid (SiA)	Similar to seasonal H1N1/H3N2 viruses, the H of pandemic H1N1/09 virus is cleaved by trypsin-like proteases in a limited number of organs (especially the lungs)
		Cleaved by cellular proteases in functional H subunits important for release of viral RNA	No multibasic cleavage sites that are recognized by multiple cellular proteases and typical of highly pathogenic viruses (e.g., H5N1)
NS1	Nonstructural protein	Inhibits host interferon response in a species-specific manner May interact with function of cellular regulatory proteins Inhibition of apoptosis by PI3 K/Akt activation	No changes associated with highly pathogenic viruses (e.g., H5N1) were found in NS1 of pandemic H1N1/09 virus
PB2, PB1, PA	Members of polymerase complex	Copies genomic RNAs into viral mRNAs and catalyzes replication of genomic RNAs	No changes associated with highly pathogenic viruses (e.g., H5N1) were found in PB2, PB1, or PA of pandemic H1N1/09 virus
PB1-F2	Encoded by an alternative reading frame in PB1	Induces apoptosis in macrophages, thereby down-regulating host immune response	Not expressed by pandemic H1N1/09 virus
M2	Ion channel	Involved in hydrogen transport during the release of viral RNA	No changes associated with highly pathogenic viruses (e.g., H5N1) were found in M2 of pandemic H1N1/09 virus

cine strategy (http://www.who.int/csr/disease/swineflu/notes/h1n1_vaccine_20090713/en/index.html). Two separate immunizations will be required to induce protective immunity in immunologically naïve persons, and it is likely that four shots will be necessary in children < 9 years.

Antiviral Therapy

In addition to vaccination, specific disease control strategies rely mostly on the prophylactic and therapeutic use of antiviral drugs. There are two approved classes of antiviral agents that are used against seasonal influenza: N inhibitors, such as oseltamivir (Tamiflu) and zanamivir (Relenza), and M2 protein inhibitors (adamantane derivatives, such as amantadine and rimantadine) [3]. N inhibitors are currently preferred for seasonal influenza virus infections since they are less toxic and more effective [3, 5].

Data on the clinical effectiveness of anti-influenza drugs against pandemic H1N1/09 virus are currently lacking. As of 5 May 2009, the CDC suggested that therapy with neuraminidase inhibitors should be given with priority to hospitalized patients and individuals at high risk of developing complications from seasonal influenza (http://www.cdc.gov/h1n1flu/antiviral.htm). The WHO states that insufficient information is available to make recommendations on the use of the antivirals for the prevention and treatment of the pandemic H1N1/09 virus infection [35]. Clinicians should make decisions based on a clinical and epidemiologic assessment of the patient and on the harm and benefits of the specific prophylaxis/treatment.

Although many nations and the WHO have stockpiled oseltamivir, shortages may arise in the pandemic setting. Pharmacokinetic investigations have revealed that co-administration of oseltamivir with probenecid (a substance registered for the treatment of gout) results in decreased elimination of oseltamivir carboxylate (the anti-influenza effective metabolite of oseltamivir) and, therefore, in increased oseltamivir carboxylate levels [38, 39]. Therefore, a combination therapy of oseltamivir with probenicid may enable the delivery of oseltamivir treatment to more influenza patients.

There is also concern that treatment of the pandemic H1N1/09 virus infection with N inhibitors may result in the development of drug-resistant virus strains. On

December 12, 2008, the U.S. CDC released data showing a high level of oseltamivir resistance among nearly all seasonal influenza A/H1N1 isolates [40]. Of major concern is the fact that these oseltamivir-resistant viruses appear fit and readily human-to-human transmissible [41, 42]. In this context, zanamivir is an attractive antiviral drug because of its non-overlapping resistance patterns with oseltamivir [3] since the oseltamivir-resistant H1N1 isolates have been found to retain their susceptibility to zanamivir [40, 41]. Initially, the pandemic H1N1/09 virus isolates tested were susceptible to both oseltamivir and zanamivir as well as to peramivir and A-315675, two NA inhibitors under investigation. However, sequencing of the pandemic H1N1/09 virus M2 gene revealed a Ser-31-Asn mutation that is known to confer resistance to the M2 proton channels inhibitors [43]. Several pandemic H1N1/09 virus isolates have been obtained recently obtained from patients treated with oseltamivir [44]. In one Hong Kong case, an oseltamivir-resistant virus was isolated from a patient who had never taken the drug, suggesting that the pandemic H1N1/09 virus may be able to retain fitness despite having the mutation. Notably, this virus was also still sensitive to zanamivir. Among more than 1,000 pandemic H1N1/09 viruses tested, six have been found to be resistant to oseltamivir but sensitive to zanamivir. All resistant viruses had the characteristic mutation in the N gene at position 274/275, which is known to be associated with oseltamivir resistance of the seasonal H1N1 virus (http://www.who.int/csr/disease/swineflu/notes/h1n1_antiviral_resistance_20090708/en/index.html). Consequently, there is currently no evidence to indicate the development of widespread antiviral resistance among pandemic H1N1/09 viruses (yet).

However, the development of alternative therapies remains desirable. Antiviral drugs not normally used for the treatment of seasonal influenza, such as interferons or ribavirin, have already been shown to possess anti-influenza activity in humans. Moreover, a number of non-antiviral drugs (e.g., statins, macrolide antibiotics, gemfibrozil, and acetylsalicylic acid) or even natural products (flavonoids, flavones, and polyphenols) may help to decrease morbidity and mortality due to their ability to control virus replication and/or the severe respiratory inflammation that accompanies infection with highly pathogenic influenza A viruses [5].

Prevention and Control Measures

Prevention and control measures for pandemic H1N1/09 virus are based on our understanding of seasonal influenza and a consideration of potential modes of transmission [45]. The CDC and WHO have published a number of recommendations, including not touching the mouth, nose, or eyes, as these are primary modes of transmission, coughing into a tissue, immediately throwing away a used tissue, and hand washing. In general, individuals should wash their hands often with soap and water, especially after being in public places or in gatherings. Alcohol-based hand cleaners are also effective. Healthcare workers who provide direct care for patients with known or suspected pandemic H1N1/09 virus infection should observe contact and droplet precautions, including the use of gowns, gloves, eye protection, face masks, and fit-tested disposable respirators. Patients with confirmed or suspected pandemic H1N1/09 virus infection should be placed in a single-patient room with the door kept closed, and airborne-infection isolation rooms with negative-pressure handling should be used whenever an aerosol-generating procedure is being performed (http://www.cdc.gov/h1n1flu/guidelines_infection_control.htm. http://www.who.int/csr/disease/swineflu/en/index.html; http://www.cdc.gov/h1n1flu/).

Conclusion

The pandemic H1N1/09 influenza virus is now the dominant strain in most areas of the world. The majority of H1N1/09 patients still experience mild symptoms and fully recover within 1 week (even in the absence of any medical treatment). Nevertheless, the H1N1/09 pandemic still raises questions and concerns. The virus may persist in the coming influenza season(s) and replace the currently circulating seasonal influenza A viruses. Since it has been estimated that at least 3% of humans who develop symptomatic seasonal influenza are co-infected by two different influenza viruses, pandemic H1N1/09 virus may reassort with other influenza A viruses, such as the H3N2 virus, to produce yet another variant. In particular, the exchange of genetic material between pandemic H1N1/09 viruses and highly pathogenic avian influenza A H5N1 strains is of great concern. Hybrids of the aggressive H5N1 virus (mortality rate of between 60% and 70%) and the readily human-to-human transmissible pandemic H1N1/09 virus could emerge. In addition to experiencing antigenic shift, the pandemic H1N1/09 virus may further adapt to humans by point mutations, resulting in a virus causing more severe diseases. The current H1N1/09 pandemic strongly stresses the necessity to have effective preparedness plans and coordinated responses at a global level. The provision of adequate information to the public is a cornerstone of control programs for pandemics [46, 47].

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