

REVIEW ARTICLE

Influenza: Forecast for a Pandemic

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Recently, great speculation about a possible influenza pandemic has been made. However, the facts supporting the possibility of this threat are less discussed. During the last decade highly pathogenic strains of avian influenza virus, including the H5N1 subtype, crossed the species barriers from birds to humans and caused fatal disease. The Z strain of H5N1 subtype is characterized by pathogenicity to a larger number of animal species and by resistance to the older class of antiviral drugs. At present, two out of three general conditions for the onset of a pandemic have been met; namely, the emergence of a new virus and its ability to replicate in humans causing serious illness. Should the virus achieve efficient human-to-human transmission, the next influenza pandemic might occur. This review addresses these biological and epidemiological aspects of influenza in the context of history and characteristics of previous epidemics, as well as concrete actions that can be undertaken considering current understanding of influenza pathogenesis, treatment, and control possibilities. © 2005 IMSS. Published by Elsevier Inc.

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Emergent Infections

Infectious diseases are a continuing threat to all persons, regardless of age, sex, lifestyle, ethnic background, or socioeconomic status (1). They cause suffering and death and impose a financial burden on society. Although the incidence of some diseases has decreased due to the use of antibiotics and vaccines, new diseases are constantly emerging, whereas others reemerge in drug-resistant forms. Because no one knows what new diseases will emerge, the public health systems must be prepared for the unexpected (2). In addition, emergent infections frequently occur in poor, resource-constrained places where it is more difficult to achieve an effective response.

Emergent diseases may be placed into three categories, each of which requires a different type of research response: (i) new emergent infections, (ii) rare infections, which may re-emerge occasionally or be considered a biodefense threat,

and (iii) common infections, which may increase in significance owing to issues such as social instability or resistance development (3). For new emergent diseases we need efficient surveillance and response systems with the capacity to rapidly identify the nature of any new disease. The development of networks of research institutions and centers for disease control is imperative, as seen during the SARS outbreak (4).

Among new emergent diseases, influenza deserves particular attention. An influenza pandemic is a global outbreak of disease that occurs when a new type A influenza strain emerges in the human population, causes serious illness, and then spreads easily from person to person worldwide. Pandemics are different from seasonal outbreaks of influenza, as the latter are caused by subtypes of influenza viruses that are already present among people, whereas pandemic outbreaks are caused by new subtypes or by subtypes that have not circulated among people for a long time. Pandemics are caused by a highly contagious virus to which populations have little or no immunity, so they benefit from almost universal susceptibility to infection.

Avian influenza viruses pose significant threats to animal and human health. Influenza is caused by viruses that undergo continuous antigenic change and that have an animal

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reservoir. The genome of influenza A viruses consists of eight single-stranded RNA segments, and the viral particle has two major glycoproteins on its surface: hemagglutinin (HA) and neuraminidase (NA). With at least 15 different hemagglutinins, each one able to combine with any of the nine different neuraminidase subtypes, there is considerable antigenic variation among influenza viruses. Previous pandemics in humans have occurred as a result of changes in the surface glycoproteins of the virus.

During the last decade, highly pathogenic strains of avian influenza virus, including the H5N1 subtype, crossed the species barriers from birds to humans and caused fatal disease. Major epizootics have been reported in poultry and mammals. Within this context, influenza experts agree that another influenza pandemic is inevitable and may be imminent.

Previous Influenza Virus Pandemics

Past influenza pandemics have led to high levels of illness, death, social disruption, and economic loss. Three occurred in the last century. In 1918, the ‘Spanish’ influenza, a highly contagious and deadly disease, had a major global impact. In fact, this pandemic, caused by an H1N1 influenza virus, is now known to have been the most deadly in history, with an estimated death toll of 40 million people in less than a year (5).

Spanish flu caused most deaths in young and healthy persons in the age range of 15–35 years. In a complete reversal of previous patterns, 99% of deaths occurred in people younger than 65 years. As expected, many of the deaths in 1918 were from pneumonia caused by secondary bacterial infections. But Spanish flu also caused a form of primary viral pneumonia. Antibiotics, which could have prevented many deaths from bacterial pneumonia, had not yet been discovered, and an effective vaccine was not available. During the course of the pandemic, an estimated 25–30% of the world population fell ill. Life expectancy dropped by 10 years or more (6).

In 1957, a new influenza virus with two unknown surface proteins appeared: H2N2. The hemagglutinin glycoprotein (H2 subtype) of the 1957 ‘Asian’ influenza virus showed only a 66% amino-acid sequence identity with the hemagglutinin H1 subtype. The N2 subtype neuraminidase of the 1957 virus shared an overall sequence identity of only 37% with the N1 subtype neuraminidase. Thus, in 1957, after 39 years of H1N1 viruses, there was little or no pre-existing immune protection in the human population against this new influenza virus (5). The pandemic that began in 1957 was caused by a milder virus than the one responsible for the 1918 pandemic. In addition, the world was much better prepared to cope. Vaccines for seasonal epidemics had been developed and had already proven their value as the most effective method for prevention. Antibiotics were available to treat

complications, including bacterial pneumonia. Nevertheless, estimates suggest that 70,000 people died in the U.S. during the Asian influenza pandemic caused by this new H2N2 virus (7). The total excess mortality globally has been estimated at more than 2 million deaths (6).

Eleven years later, in 1968, another change in the surface glycoproteins again caused the virus to become pandemic, resulting in high morbidity and mortality rates worldwide. In this 1968 virus, only the gene that encodes hemagglutinin, HA, and the *PBI* gene re-assorted to yield an H3N2 subtype (8). The H3 and H2 hemagglutinins differed in more than 60% of their amino acids. The conservation of the neuraminidase in the 1968 H3N2 virus conferred partial protection to the population who had been previously exposed to H2N2. The pandemic that began in 1968 was even milder than that in 1957. The virus spread from a focal point within mainland China. The epidemic in the U.S. began in September in California, carried there by troops returning from Vietnam. Although accurate mortality estimates are not available, global excess mortality was probably around 1 million (6).

There was still a fourth influenza pandemic in the last century, caused by an H1N1 strain that appeared in 1977. It caused disease mostly in people born after 1950, because the older population had protective immunity resulting from prior contact with H1N1 strains. This H1N1 strain and its descendants have been circulating ever since, and at present both H3N2 and H1N1 influenza viruses continue to be present in the human population (5).

Have the Conditions for the Next Influenza Pandemic Been Met?

Prior to 1997, the H5N1 strain of avian influenza virus began circulating in poultry populations of certain regions of Asia. H5N1 initially caused only mild disease with symptoms, but after months of circulation in chickens, the virus mutated to a highly pathogenic form that could kill chickens within 48 h, with a mortality approaching 100% (6).

Since the first avian influenza outbreak in 1997, the main concern has been that the highly pathogenic influenza A (H5N1) virus might either mutate and adapt to allow efficient transmission during the infection of mammals or reassort its gene segments with human influenza viruses during the coinfection of a single host, resulting in a new virus that would be both highly lethal and transmissible from person to person. Such events are believed to have preceded the influenza pandemics of 1918, 1957, and 1968 (9).

Outbreaks of highly pathogenic avian influenza A (H5N1) occurred among poultry in eight countries in Asia (Cambodia, China, Indonesia, Japan, Lao, South Korea, Thailand and Vietnam) during late 2003 and early 2004. At that time, more than 100 million birds either died from the disease or were culled. From December 30, 2003 to March 17, 2004, 12 confirmed human cases of avian influenza A (H5N1)

were reported in Thailand and 23 in Vietnam, resulting in a total of 23 deaths (10). As reported by the World Health Organization (WHO) in May 2005, the total number of deaths in Asia was 52 (Table 1) (11). The mortality associated with human H5N1 infection is remarkably high (72%) as compared with an estimated 2.5% for Spanish influenza (12). The most vulnerable population has turned out to be rural subsistence farmers and their families, and these people constitute the true risk group. H5 viruses are becoming more capable of causing disease (pathogenic) for mammals than earlier H5 viruses and are becoming more widespread in birds in the region. Ducks infected with H5N1 are now shedding more virus for longer periods of time without showing any symptoms of illness. This has implications for the role of ducks in transmitting disease to other birds and, possibly, to humans as well. Additionally, other findings have documented H5 infection among pigs in China and H5 infection in felines (experimental infection in house cats in The Netherlands and isolation of H5N1 viruses from infected tigers and leopards in Thailand), suggesting that cats could host or transmit the infection. These findings raise particular concern in the context that reassortment of avian influenza genomes is most likely to occur when these viruses demonstrate a capacity to infect multiple species, as is now the case in Asia (10). Thus far, two conditions for the next influenza pandemic have been met, namely, the generation of an unknown virus and its ability to replicate in humans.

Influenza Viruses

Influenza viruses are grouped into three types, designated A, B, and C. Viruses of the C types are common but usually cause no symptoms or only very mild respiratory illness. They are not considered of public health concern. Type B viruses cause sporadic outbreaks of a more severe respiratory disease, particularly among young children in school environments. Both B and C viruses are essentially human viruses; C viruses are stable, but A and B viruses are prone to mutation. Of greatest concern are the influenza A viruses.

Table 1. Cumulative number of confirmed human cases of avian influenza A (H5N1) from 28 January 2004 to 4 May 2005 (11)

Country/Territory	Total cases	Deaths
Cambodia	4	4
Thailand	17	12
Vietnam	68	36
Total	89	52

Note: Total number of cases includes number of deaths; WHO reports only laboratory-confirmed cases.

These viruses mutate much more rapidly than type B viruses, and this gives them great antigenic flexibility. In addition to humans, they infect pigs, horses, sea mammals, and birds. Influenza A viruses have a large number of subtypes, all of which are maintained in their natural reservoir, the aquatic birds, which provides a perpetual source of viruses and a huge pool of genetic diversity (10). The principal site of influenza virus replication in aquatic birds is the gastrointestinal tract resulting in high fecal viral titers and viral transmission in migratory feeding areas.

Influenza A viruses are identified with the particular variants they present of two sets of protein spikes that protrude from the outer surface of the virus. The hemagglutinin (HA) spike is responsible for the virus binding and entry into cells, where copies of the virus are produced. There are 15 HA subtypes, designated H1–H15. The neuraminidase or NA spike regulates the release of newly formed virus from infected cells into the host. There are nine NA subtypes, designated N1–N9. All of these HA and NA subtypes have been detected in free-flying birds. They provide a huge and highly flexible pool of genetic diversity. An individual virus strain is identified by the subtypes of HA and NA protein spikes on its surface. It is named by the letters H and N, each followed by the number of the subtype.

For the onset of pandemics, the emergence of a novel HA subtype is determinant, as it defines population susceptibility. To date, only subtypes H1, H2, and H3 are known to have circulated in humans for at least a century. As a virus from the H5 subtype will be foreign to the immune system of everyone alive today, vulnerability to an H5N1-like pandemic virus would be universal.

Clinical Characteristics of Influenza Infection: A Challenge for Diagnosis

Low pathogenic subtypes of influenza cause mainly respiratory symptoms and conjunctivitis, whereas highly pathogenic subtypes cause diverse symptoms (Table 2) (13). It is important to consider that the clinical signs and symptoms of avian influenza H5N1 may be more protean than originally described. In southern Vietnam, a 4-year-old boy presented with severe diarrhea, followed by seizures, coma, and death. The diagnosis of avian influenza A (H5N1) was established by isolation of the virus from cerebrospinal fluid, fecal, throat, and serum specimens. The patient's 9-year-old sister had died from a similar syndrome 2 weeks earlier. In both siblings, the clinical diagnosis was acute encephalitis. Neither patient had respiratory symptoms at presentation (14).

Based on these cases, it has been stressed that avian influenza H5N1 should be included in the differential diagnosis of a much wider clinical spectrum of diseases than previously considered and that clinical surveillance of influenza H5N1 should focus not only on respiratory illnesses, but also on clusters of unexplained deaths or severe illnesses

Table 2. Clinical characteristics, laboratory and radiologic findings of H5N1 infected patients on admission

Clinical characteristics	Laboratory findings	Radiologic findings
Cough	Lymphopenia	Abnormal chest X-ray in all cases (100%)
Dyspnea	Thrombocytopenia	Extensive bilateral infiltrate
Shortness of breath	Prolonged TP and TTP	Lobar collapse
Fever >38°C	Hemophagocytic syndrome	Focal consolidation
Rapid respiratory rate	Elevated ALT and AST levels	Air bronchograms
Crackles	Normal renal function	No pleural effusions noted
Myalgia	Negative blood cultures	
Diarrhea (Vietnam)	Low oxygen saturation	
Less common: odinophagia and upper respiratory tract symptoms		
Low or normal blood pressure		

Based on a report of 10 cases occurring in Vietnam, infected with a highly pathogenic H5N1 subtype (13).

of any kind. The isolation of virus from a rectal specimen is a major source of concern, since it highlights a potential route of human-to-human transmission, especially in combination with crowded living conditions and diarrhea.

Thus, the broad spectrum of clinical manifestations of influenza poses a challenge for clinical diagnosis. The diagnosis of influenza A (H5N1) should be further confirmed by means of immunofluorescence (with antibodies specific for the virus), viral culture or reverse transcriptase–polymerase chain reaction with primers specific for H5 and N1, all of which are not always available at places with a confirmed risk of influenza. Therefore, training and resources for a timely and correct diagnosis must be part of a global strategy in preparation for an influenza pandemic.

Viral Subtypes for Surveillance and Control

Preliminary findings have identified the H2, H5, H6, H7, and H9 subtypes of influenza A as those most likely to be transmitted to humans. The influenza A subtypes currently circulating in humans, H1 and H3, continue to experience antigenic changes. Although these continual modifications may lead to an increase in virulence, the mildness of the past three influenza seasons suggests that the power of the H1N1 and H3N2 viruses is diminishing as their ability to cause serious disease becomes increasingly attenuated. H2 influenza viruses are included in the high-risk category because they were the causative agent of the 1957 “Asian flu” pandemic and were the only influenza A subtype circulating in humans between 1957 and 1968 (15).

Not only are the H1, H2, and H3 influenza viruses of concern. The H7 and H5 viruses have a unique ability to evolve into a form highly virulent to chickens and turkeys by acquiring additional amino acids at the HA cleavage site (HA cleavage is required for viral infectivity) (16). The highly pathogenic H7N7 influenza viruses that were lethal to poultry infected the eyes of more than 80 humans and killed one person (17).

The remaining two viral subtypes on the priority list, H6 and H9, have spread from a wild aquatic bird reservoir to domestic poultry over the past 10 years. H9N2 viruses have also been detected in humans and in pigs (18,19), and have acquired human-like receptor specificity (20). Now, for unknown reasons, H9 viruses are endemic in chickens in Eurasia, and H6 viruses are becoming endemic in both Eurasia and the Americas.

Avian influenza H10N7 seems to have crossed the species barrier from poultry to people for the first time. In Egypt, in April 2004, two infants presenting with mild febrile respiratory symptoms had H10N7 influenza viruses isolated from respiratory samples (21). Table 3 summarizes avian influenza A subtypes that crossed the species barrier, thus being transmitted to humans during 1994–2004.

The Z Genotype

The A (H5N1) influenza virus represents an increasing global concern. Changes in the virus resulted in the generation of what is termed the Z genotype, which spread to at least nine countries in East Asia and Southeast Asia. This viral strain is characterized by pathogenicity to a larger number of animal species and by resistance to the older class of antiviral drugs represented by amantadine and rimantadine (22). Bird-to-human transmission has continued, and it has been documented as the cause of 51 deaths in 88 patients with confirmed cases (Table 1).

A series of genetic reassortment events traceable to the precursor of the H5N1 viruses that caused the initial human outbreak in Hong Kong in 1997 and subsequent avian outbreaks in 2001 and 2002 were recently demonstrated. These events gave rise to the dominant H5N1 genotype Z in chickens and ducks that was responsible for the regional outbreak in 2003–2004. Domestic ducks in southern China had a central role in the generation and maintenance of this virus, and wild birds may have contributed to the increasingly wide spread of the virus in Asia (23).

Table 3. Latest influenza A subtypes of avian-to-human transmission

Strain	Symptoms	Comments	First report of transmission to humans
H5N1	Respiratory	High mortality rate, lethal	Hong Kong, 1997
H7N3	Conjunctivitis, respiratory	Two laboratory-confirmed cases among poultry workers	Canada, 2004
H7N7	Conjunctivitis, respiratory	Human-to-human transmission confirmed	UK, 1995
H9N2	Respiratory	No human-to-human transmission identified	Hong Kong, 1999
H10N7	Respiratory	Two infants (the father of one child was a poultry merchant)	Egypt, 2004

Data from Reference (27).

The HA molecules of most genotype Z viruses isolated since late 2002 in Asia acquired a potential N-linked glycosylation site at positions 154–156. Glycosylation at this site, adjacent to the receptor binding, is capable of altering the receptor-binding profile and may help the virus to evade the host antibody response (23). It is notable that in the short time since its emergence, genotype Z has replaced genotypes A–E, X and Y to become dominant in both aquatic and terrestrial poultry in this region. The genetic stability of this new gene constellation indicates that genotype Z viruses have not yet fully adapted to poultry, and this raises the possibility that they may continue to evolve through mutation or reassortment to achieve greater viral fitness (24). Another relevant mutation, also found in the Z strain, is Lys 627 in the PB2 protein. This mutation has been associated with increased virulence of H5N1 viruses in mice (25) and with H7N7 viruses in humans (17). Thus, the molecular modifications yielding the Z strain make it potentially very harmful due to its particular ability to respond to environmental changes. It could be easy to speculate that in Asian countries where humans live in close proximity to ducks and pigs, the Z strain has been induced, by selective pressure, to infect humans.

Will H5N1 Be Able to Acquire Potent Human-to-Human Transmissibility?

Whether H5N1 will be able to acquire potent human-to-human transmissibility is the pressing issue. So far, H5N1 has shown an extraordinary evolutionary ability, but it still lacks an efficient human-to-human transmissibility mechanism. No evidence of efficient person-to-person transmission has yet been reported, though possible person-to-person transmission has been suspected (26). Should the virus improve its transmissibility, everyone in the world would be vulnerable to infection by a pathogen (transmitted by a cough or a sneeze) entirely novel to the human immune system (6).

The hemagglutinin of human influenza viruses preferentially binds to sialic acid receptors containing α 2,6-galactose linkages, whereas avian influenza viruses preferentially bind to those containing α 2,3-galactose linkages. Although the molecular mechanisms responsible for receptor-binding specificity are poorly defined, it is believed that hemagglutinin of avian origin must acquire human receptor-binding

specificity to generate influenza strains capable of sustained human-to-human transmission (27). Site-directed mutagenesis studies have shown that only one or two aminoacid mutations are required for this change (28). In human beings, the limited passage of a virus possessing an avian hemagglutinin, such as occurring in Asia, may be sufficient to generate such a change (29).

Influenza A viruses may acquire efficient human-to-human transmissibility as a result of their unique features that confer a great genetic variability. The first consists of mutations introduced during replication. As they lack a proofreading mechanism, the small errors that occur when the virus copies itself are left uncorrected. This way, influenza A viruses undergo constant changes. This survival strategy, known as antigenic drift, is used by the virus to keep populations susceptible to infection. These small changes are sufficient to evade the immune system.

The second and third mechanisms leading to diversity are recombination (use of another nucleic acid segment as template from same or coinfecting virus during replication) and reassortment (packaging of different genetic segments during coinfection of two viruses) respectively. Recombination leads to the same result as reassortment, namely, the acquisition of large sections of foreign genetic material, albeit through a different route. The genetic material of these viruses is segmented into eight genes, which facilitates the exchange of gene segments during coinfection with human and avian influenza viruses. The resulting new combinations generate changes on the HA and/or NA proteins on the surface of the virus, resulting in a new influenza A virus subtype that will be unknown for the human immune system. Due to the immunological consequences of this mechanism, known as antigenic shift, the virus gets large populations of susceptible hosts. Influenza A viruses regularly cause seasonal epidemics in humans, and at recurring unpredictable intervals they cause pandemics.

Yet, the acquisition of an efficient human-to-human transmissibility might not be so easy, and probably the virus should pay a cost for it. Viruses may need to adapt in numerous ways to cross the species barrier successfully. Once H5N1 reassorts with human influenza viruses and thus gets efficient human-to-human transmissibility, it may lose virulence that would in turn allow their persistence. Avirulence could

Table 4. Changes in virulence upon cross-species transmission of viruses

Virus	Old host	New host	Virulence
Influenza H5N1	Bird	Human	Lethal→ lethal
Nipah	Bat	Human, pig	Mild→ lethal
Lassa fever	Rodent	Human	Mild→ lethal

Data from Reference (30).

be a particular feature of persistent infections that have co-evolved for million years with their “natural hosts”. Cross-species transfer can then be catastrophic (Table 4) (30).

We are beginning to understand at least some of the obstacles that pathogens must overcome in order to emerge in the human population. In general terms, for a virus to emerge successfully in a human population it must achieve two feats. The first feat is replication in human cells. This replication requires a virus to accomplish at least five steps: first, contact with a human host; second, entry into the appropriate cell type; third, production of more copies of itself; fourth, overcoming any immediate host response; and fifth, exiting from the cell and transmitting to another. The second feat is human-to-human transmission (31).

Three possible scenarios have been described in this section. The first one is that in a short period H5N1 will acquire potent efficient human-to-human transmissibility by reassortment with human influenza viruses. The second is that by means of adaptive mutation during infection of humans or other mammals, the virus will slowly and gradually improve its transmissibility among humans. The third, less likely, is that H5N1 gradually becomes avirulent, thus reaching equilibrium with the human host. However, many other scenarios should be considered as the outcome of the evolving interaction of H5N1 with the different species. It must be considered that the molecular basis of interspecies transmission constitutes a complex interaction between virus and host and between the genomes of both.

Vaccines: The First Line of Defense

Vaccines are universally regarded as the most important medical intervention for preventing influenza and reducing its health consequences during a pandemic. In the past, however, vaccines were never available early enough and in sufficient quantities to have an impact on morbidity and mortality during a pandemic. The manufacturing capacity of vaccines is still inadequate and problems related to the nature of pandemic vaccines remain (6).

Annual Influenza Vaccines

There are two approaches for the prevention of influenza: the live, attenuated, cold-adapted, intranasally administered

influenza vaccine (CAIV-T [FluMist; Med-Immune Vaccines]) and traditional trivalent inactivated vaccine (TIV). The second appears to mediate protection almost exclusively through induction of serum antibody. The degree of immunity induced by CAIV-T is predicted to be lower in adults than in children (32).

TIV vaccines are produced from virus grown in embryonated hen eggs and are of three types: whole-virus, “split-product”, or subunit “surface-antigen” formulations. Trivalent vaccines contain 15 mg each of two A subtypes (H1N1 and H3N2) and one B strain (33). TIV elicit a relatively strain-specific humoral response, have reduced efficacy against antigenically drifted viruses, and are ineffective against unrelated strains. The influenza A virus components of annual influenza vaccines are typically derived from egg-grown reassortment viruses that have the relevant hemagglutinin and neuraminidase genes of the antigenically relevant strain, and the six remaining gene segments from A/Puerto Rico/8/34 (H1N1). This process requires large numbers of eggs and many companies lack the flexibility to respond rapidly to a pandemic event (27). In addition, highly pathogenic H5 and H7 viruses cannot be grown in large quantities because they are lethal to chicken embryos (34). Several approaches have been attempted for avian influenza vaccine development. In this review, only the reverse genetics will be described as it is likely to produce the most rapid response in an emerging pandemic.

Vaccines for Highly Pathogenic Subtypes (H5 and H7)

The most promising method to accelerate the response to pandemic influenza is by means of plasmid-based reverse genetic systems to construct influenza virions and vaccines. As viable viruses can be generated from individually cloned cDNA copies of each of the eight viral RNA segments, reassortment can be prospectively defined and directed, and the extra amino acids at the HA cleavage site (which are associated with high virulence) can be removed to allow rapid generation of a vaccine seed strain in eggs. Plasmids encoding the internal genes of the base vaccine are already available. A strain-specific vaccine can thus be created by cloning the appropriate hemagglutinin and neuraminidase genes from the target virus, altering its HA connecting peptide if necessary, and transfecting an appropriate cell line (15). The next step is to take these plasmid-derived influenza vaccines through clinical trials to address crucial questions such as number and quantity of doses and the role of adjuvants.

Reverse genetic systems introduce new challenges. One of the most limiting of these is the need to use cell lines. Perhaps the only cell line that meets all criteria for international use at this time is the African green monkey kidney cell line Vero, and these cells must be of certified quality for human vaccine production. A second new challenge is the use of a genetically modified virus seed strain. Because

the traditional vaccine strains are made by natural reassortment, they have escaped being labeled “genetically modified.” This difference, although largely semantic, may affect the acceptance of the new vaccines (15) because of laws and regulations limiting the human use of genetically modified organisms.

Safety and legal problems should be solved before reverse genetic systems can be used for vaccine development. A risk to be considered by vaccine manufacturers is the occurrence of adverse reactions in a percentage of recipients. These reactions may be due to the vaccine, to the host, or to a unique combination of the vaccine and the host. Legislative measures can be taken to reduce the impact of liability exposure. In addition, intellectual property rights on reverse genetics technology are held, and licenses may need to be granted for commercial use of vaccines.

Finally, viral evolution should be considered during the development of new vaccines. The group of Derek J. Smith quantified and modeled the antigenic evolution of influenza A (H3N2) from its introduction into humans in 1968 through 2003. They described a method that readily allows monitoring of antigenic differences among vaccine and circulating strain and thus can estimate the effects of vaccination. Further, this approach offers a route to predicting the relative success of emerging strains, which could be achieved by quantifying the combined effects of population level immune escape and viral fitness on strain evolution. The authors tested the accuracy of the method using blind prediction. Such quantitative analyses have potentially wide-ranging implications for strain surveillance, vaccine strain selection, and for applied and basic research involving antigenically variable pathogens (35).

Antiviral Therapy

An additional important resource to face influenza is the existence of antiviral agents. Two classes of drugs are currently available for prophylaxis and treatment of influenza virus infection: M2 ion channel blockers (amantadine and its derivative rimantadine) and NA inhibitors (zanamivir and oseltamivir). Amantadine and rimantadine block the ion channel activity of the M2 protein of most influenza A viruses, and viral replication is inhibited by the blockade of hydrogen ion flow, principally when virus enters the host's cells (36). The NA inhibitors interrupt an established infection in its late stages by inhibiting the release of virions from infected cells, which results in the aggregation of virions at the cell surface and the consequent inhibition of viral penetration of mucous secretions and spread to other cells (37).

The main disadvantages to the use of M2 blockers are that drug-resistant variants develop rapidly and that these agents are ineffective against influenza B virus (38). By days 5–7 of therapy, 16–35% of isolates from treated patients may be

resistant, and these drug-resistant variants are fully pathogenic and transmissible to close contacts (39). Initial studies indicate that both H5N1 influenza viruses (the 2003 and 2004 human strains) currently being isolated from humans are naturally resistant to amantadine and rimantadine (7).

NA inhibitors are more costly, but they are active against both influenza A and B viruses and elicit fewer side effects; in addition, the emergence of drug-resistant variants has been reported in less than 1% of treated adults (40). One pediatric study of oseltamivir treatment reported that 5.5% of influenza isolates had evidence of neuraminidase resistance (41).

As described, antiviral drugs may be of benefit in protecting individuals in essential services while waiting for an effective vaccine to be prepared. However, supply and cost issues would limit their effect on the course of a pandemic (27). These drawbacks should also be overcome if an effective response to a major influenza epidemic is desired.

Concluding Remarks

Biomedical research on influenza has identified three prerequisites for the start of a pandemic: 1) A novel virus subtype must emerge to which the general population will have little or no immunity. 2) The new virus must be able to replicate in humans and cause serious illness. 3) The new virus must be efficiently transmitted from one human to another. At present, all prerequisites for the start of a pandemic had been met, except one, the onset of efficient human-to-human transmission (6).

It is impossible to anticipate when the next influenza pandemic might occur or how severe its consequences might be. What if the next pandemic started tomorrow? We would not have appropriate vaccines for many months and very limited stockpiles of antiviral drugs would be available. Thus, we would be facing a 1918-like scenario. There would be no surge capacity for health care, food supplies, and many other products (i.e., mechanical ventilators). We would face inadequacy of services as well because many countries do not have biosafety laboratories for influenza diagnosis. Health care workers would become ill and would die at rates similar or even higher than those observed in the general population (42).

Clinical, epidemiologic, and laboratory evidence suggests that a pandemic caused by the current H5N1 strain would be more likely to mimic the 1918 pandemic than those that occurred more recently (43).

If we translate the rate of death associated with the 1918 influenza virus to that in the current population, there could be 180 million to 360 million deaths globally (42). Therefore, the public health system must be prepared for the unexpected.

Among the most urgent needs towards an unpredictable scenario, the following might be considered: 1) Countries should have a large supply of anti-influenza virus drugs to

reduce the severity and spread of infection. Antiviral drugs are available, but no country has yet invested in stockpiling. 2) It is necessary to overcome regulatory, safety, and legal problems in order to produce genetically modified vaccines for use in humans matching the subtype of the emerging pandemic influenza strain. 3) The vaccine produced by means of reverse genetic systems should be tested in clinical trials, and its production should be flexible to reach a massive scale.

Vaccine development is mandatory as vaccines may reduce the risk of coinfection with avian and human influenza viruses. In consequence, the possibility of genetic recombination or reassortment would be decreased together with the evolution of human-to-human transmission. The political will to modify intellectual property rights, liability, regulatory, safety, and legal issues will be determinant while facing an evolving virus: influenza.

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