Influenza: Biology, Infection, and Control

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I had a little bird
Its name was Enza
I opened the window
And in-flew-Enza

Jump rope song (USA, 1918)

Overview

The growth of the human population has profoundly affected the global ecosystem, influencing the animal population balance, the availability of fresh water, arable land, biotic production, and atmospheric gases. The human ecological impact has significantly accelerated the evolutionary change of numerous organisms. For example, the production of human medicine and food has resulted in the rapid evolution of drug-resistant pathogenic organisms as well as plants and insects resistant to pesticides (Palumbi, 2001). Recently, the nutritional support of the human population has relied on the vast monoculture of domestic mammals and birds, which has facilitated the emergence of pathogenic enzootic organisms that infect both animals and humans. This chapter will focus on the global threat to human health represented by the highly contagious enzootic virus influenza. It will also discuss current efforts and future improvements to protect humans from global influenza epidemics and pandemics.

Human Population Growth and the Evolution of Infectious Disease

The evolution and global dispersal of human populations over many millennia have profoundly influenced the pattern and development of infectious human pathogens. McMichael and Weiss have outlined five major transitions in human ecology that have profoundly influenced the profile and properties of organisms that infect humans (McMichael, 2004; Weiss & McMichael, 2004).

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1. The primary ecological transition of the human population occurred several million years ago with the emergence of our early ancestors in Africa from arboreal habitats into the open savannah. Contact with animals in the savannah resulted in infection with new enzootic agents. This trend continued during the emergence of *Homo sapiens* and the subsequent migration of Neolithic hunter-gatherers out of Africa approximately 100,000 years ago.
2. The second transition originated about 10,000 years ago with the development of agriculture, following the domestication of animals and plants. This practice resulted in the establishment of stable settlements, in which the number and density of humans and animals increased, facilitating the transmission of pathogens between species. Ultimately, this enzootic exchange of pathogens led to the development of infectious organisms that were exclusively propagated and maintained within the expanding human population. This mechanism of evolution over the last 10,000 years has been suggested for measles, mumps, rubella, and smallpox. Recently, the emergence of HIV and Hepatitis C from animal sources, and their establishment and global transmission within human populations, have been observed.
3. The third transition was the continental expansion of human populations, which resulted in the geographic isolation of populations that evolved different types of enzootic and human pathogens. When these distinct human populations throughout the Eurasian continent made contact through trade or warfare, catastrophic epidemics ensued. These occurred during the period 1000 BCE and 1500 CE, culminating in the Black Death in 1347 CE, which killed approximately one-third of the European population.
4. The fourth transition was initiated in 1500 CE when Europeans undertook global exploration followed by widespread colonization of countries in Africa, Asia, and the New World. This resulted in the global distribution of infectious diseases and the eradication of naive native populations by the introduction of new diseases such as measles and smallpox.
5. The fifth transition started during the eighteenth century and is characterized by the uncontrolled upward spiral of the human population despite wars, famine, and disease. The growth rate of the human population rose significantly in the eighteenth century, coincident with the European Industrial Revolution, when agricultural practice was rendered more efficient, and manufacturing was facilitated by the application of power-driven machinery.

This trend intensified into the twentieth century when the global population grew from 1.6 billion in 1900 to 6.1 billion by 2000. The bulk of the global population growth occurred after the Second World War, predominantly in the developing world, namely, Asia, Africa, and South America (United Nations, 2004). In 2005, the global population was about 6.5 billion, with 5.3 billion living in the developing world. The economic and health disparities between the developed and the developing are constantly expanding, with the latter sustaining a tenfold higher infant mortality rate, more than two-thirds of the 13 million deaths due to infectious diseases, and 50% of the population living on less than $2 per day (Roberts &

Lu, 2004). During this period, urban settlements have become the predominant human habitat with estimates of 7% of the world's population living in megacities (United Nations, 2004). The UN 2000 forecasts that the world's population will increase 30% by 2030 and of these additional 2 billion people, 1.9 billion will live in the cities and towns of South America, Asia, and Africa. Toward the end of this century the majority of the world's population will be living in cities and large towns (United Nations, 2004). This trend toward urban living and the redistribution of the human population will profoundly impact the profile and spread of infectious diseases. Moreover, during the second half of the twentieth century, the development of global communication and economic interactions has led to an enormous increase in global travel both by air and sea. The redistribution and urbanization of large portions of the human population, together with the vast increase in global air travel, have already dramatically altered the pattern and kinetics of disease dissemination. During the twentieth century, this was clearly demonstrated by the global spread of the chronic diseases AIDS and Hepatitis C, caused by HIV1 and HCV, respectively. In 1999, the coronavirus that originally caused severe acute respiratory syndrome in southern China was globally distributed within 3 months (Vijayanand et al., 2004), and the flavivirus West Nile Virus introduced into the eastern USA from the Middle East was efficiently disseminated across the continent within 3 years (Petersen & Roehrig, 2001).

Biology of Influenza Virus

Current databases indicate that 1,415 pathogens cause diseases in humans, of which 61.6% are enzootic; that is, they infect humans and other animal species (Cleaveland et al., 2001). Pathogens that infect multiple hosts evolve independently in different populations, resulting in changes in their pathogenicity, host range, and transmission characteristics. Influenza virus is a major enzootic pathogen that is transmitted among humans, domestic animals, and wild animals and profoundly impacts human health and economics.

Influenza Virus Reservoirs

Influenza viruses are negative-strand RNA viruses belonging to the Orthomyxoviridae family. Based on antigenic differences in the nucleoprotein and matrix proteins, influenza viruses are divided into three distinct types, A, B, and C (Murphy, 1996). The viral genome is divided into eight negative-sense single-stranded linear segments in the type A and B subtypes, and seven segments in the type C virus.

Influenza C viruses have been isolated only from humans and swine, where they cause mild upper respiratory tract infections (Baigent & McCauley, 2003). The influenza B subtype has been isolated only from humans and seals and causes

severe disease in humans, including lower respiratory tract infections, pneumonia, and encephalitis (Baigent & McCauley, 2003). During the first 2 years of the twenty-first century, the influenza B strain variant B/Victoria/2/87 reemerged from Asia and spread globally, causing widespread human epidemic outbreaks (Shaw et al., 2002). The influenza A type viruses maintain an extensive subtype reservoir in wild aquatic birds and infect a range of mammals, avian species, and humans. Inducing significant morbidity and mortality worldwide, these influenza A type viruses are one of the major causes of human infection and are the focus of this chapter.

Influenza Type A Virus Biology

The influenza type A virus comprises eight genomic segments named according to the proteins they encode, namely HA, NA, M, PB1, PB2, NS, PA, and NP. These eight RNA segments encode 11 structural and nonstructural proteins that facilitate cellular uptake, protein synthesis, RNA replication, and the assembly and release of progeny virions. Viral subtypes are defined by the possession of 1 of 15 antigenically distinct hemagglutinins (HA) and 1 of 9 neuraminidase (NA) antigens. The 15 hemagglutinins (H1–H15) and 9 neuraminidases (N1–N9) are perpetuated in nature in shorebirds, waterfowl, and gulls (Alexander, 2000>; Hinshaw et al., 1980). In these aquatic wild bird populations, influenza viruses normally replicate without disease symptoms. This genetically diverse reservoir of influenza A subtypes in wild birds is well adapted to its natural hosts and exhibits an evolutionary stasis with minimal variation in its surface protein sequence, suggestive of a long-established and balanced host and pathogen interactions (Webster et al., 1992).

Molecular evidence suggests that specific viral subtypes from this avian reservoir have been transmitted to domestic birds, ocean-dwelling mammals, seals and whales, and land-dwelling mammals including horses, pigs, and humans (Webster et al., 1992). A broad spectrum of influenza A subtypes infects domestic poultry, the majority causing mild respiratory disease; yet subtypes H5N1, H5N2, H7N1, and H7N3 have mutated and become highly pathogenic and cause lethal infections (Capua & Alexander, 2004). In land-dwelling mammals, the influenza A subtypes H7N7 and H3N8 cause epidemic disease in horses, H1N1, H1N2, and H3N2 in pigs, and H3N2, H2N2, and H1N1 in humans (Webster et al., 1992).

The transmission and replication of influenza subtypes in new host animals or birds depend on the interaction of the virus and its encoded proteins with cells and tissues of the host that support virus propagation and spread within the population. The two viral surface proteins HA and NA interact cooperatively to define this host specificity, by determining the efficiency of viral attachment to, entry into, fusion with, and release from, host cells. The cell surface receptor recognized by HA is sialic acid attached to galactose by either alpha-2,3 Gal or alpha-2,6 Gal linkages (Gambaryan et al., 1995). The HA of influenza viruses isolated from the avian reservoir bind to sialic acid linked to galactose by an alpha-2,3 linkage and those that infect mammals recognize sialic acid linked to galactose by an alpha-2,6

linkage. The release of viral progeny relies on the NA cleavage of cell surface sialic acid that is linked to galactose in the same structural conformation recognized by the HA. This clearance of sialic acid residues from the cell surface obviates virus–cell interactions and facilitates the release of virions from infected cells.

The infection of cells by influenza virus relies on the cleavage of the precursor HA into proteins HA1 and HA2 by host cell trypsin-like proteases. The tissue distribution of trypsin-like proteases within the host organism restricts virus replication to distinct sites, generally the mucosal surfaces of the respiratory and gastrointestinal tracts. The efficiency of HA cleavage is influenced by the structure of its protease cleavage site, and the presence of multiple basic amino acids flanking this site facilitates cleavage by ubiquitous proteases such as furin. The cleavage of HA by ubiquitous proteases that are widely distributed in the host organism permits viral replication at sites throughout the body, resulting in a lethal disease causing major organ and tissue damage. The mutational introduction of basic amino acids flanking the protease cleavage site that enhance and broaden protease recognition has been clearly demonstrated as a major factor in the evolution of nonvirulent avian viruses into highly pathogenic avian influenza viruses in domestic poultry (Capua & Alexander, 2004).

The replication of viral genetic information within infected cells requires compatible interactions between viral proteins and cellular components to facilitate the unraveling of viral ribonucleic acid complexes, the replication of viral RNA segments, and virion assembly and release.

The specificity of the infection of animals by influenza virus subtypes illustrates the subtlety of interactions that define the host range. This is elegantly illustrated in the antibody profile of workers exposed to poultry infected by different avian influenza subtypes. The presence of antibodies in human serum samples demonstrated that they were infected productively by the avian virus subtypes, H7N7, H7N3, and H5N1, but not H7N1 or H5N2 (Hayden & Croisier, 2005; Puzelli et al., 2005).

The lethality of an influenza strain is a function of its transmission efficiency in the population and its pathogenicity, replication, and tissue tropism within the infected host. Viral pathogenesis is complex and contributed by a number of different determinants including the efficiency of HA cleavage by proteases (Steinhauer, 1999), viral polymerase activity (Almond, 1977), the viral gene products NP (Bean & Webster, 1978; Oxford et al., 1978; Scholtissek & Murphy, 1978) and NS (Treanor et al., 1989) that interact to facilitate the growth, dissemination, and transmission within the host species.

In new host organisms, the influenza viral genomes are unstable and accumulate sequence alterations that evolve into new variants. Sequence changes in influenza A virus occur by two mechanisms, the continuous accumulation of mutations within genomic segments called “genetic drift” and the exchange of entire genomic segments between different viruses referred to as “genetic shift.”

The continuous accumulation of sequence alterations by genetic drift facilitates the rapid development of resistance of influenza to small-molecule drugs. This phenomenon is illustrated by the widespread resistance to amantidine in circulating human influenza viruses and the rapid appearance of cases of resistance to

oseltamivir in humans infected with H5N1 (Bright et al., 2006; Le et al., 2005). This questions the wisdom of investing in the development of small-molecule drugs whose effective treatment of highly mutable RNA viruses, such as influenza, is short lived.

During the coinfection of a host permitting the multiplication of mammalian and avian influenza viruses, "genetic shift" occurs, when the exchange of complete genomic segments results in the formation of new reassortants. Those reassortants that exhibit a new surface HA and/or NA are new subtypes that can propagate widely in susceptible avian and mammalian populations. The emergence of a new influenza subtype that infects naive humans causes the establishment of global pandemic disease. Following pandemic disease in humans, the new subtype continues to circulate in the population and accumulates genomic mutations by genetic drift that is disseminated in the viral population by replication and recombination. This process results in the establishment of subtype variants that cause annual epidemics in humans. In a similar fashion, genetic variation occurs in influenza strains that circulate in other mammals and birds, and cause periodic epidemic outbreaks.

The extraordinary genetic malleability of this segmented RNA virus has resulted in the evolution of an extensive reservoir of subtypes in wild aquatic birds, which spread selectively and mutate in domestic poultry and mammals, causing significant disease outbreaks.

Influenza Type A Infections of Horses and Swine

In horses, epidemic disease is caused by infection with genetic variants of the subtypes H7N7 and H3N8 (Webster et al., 1992). Equine influenza was first recognized in 1956 in a widespread epidemic in Eastern Europe caused by subtype H7N7 (Daly et al., 2004). This influenza virus continued in circulation, and its last confirmed outbreak occurred in 1979. In 1963, a new subtype, H3N8, was identified in a disease outbreak in the USA, which spread globally, and its genetic variants remain the major cause of equine influenza (Daly et al., 2004).

Influenza was first recorded in pigs during the pandemic outbreak of 1918 (Koen, 1919), and the virus was isolated in 1930 (Shope, 1931). During most of the twentieth century, swine influenza was caused by the genetic drift of the predominant circulating subtype H1N1. However, in 1998, a severe influenza outbreak in swine in the USA was caused by a new pathogenic subtype H3N2. This subtype was shown to be a triple reassortant H3N2, containing the HA, NA, and PB1 segments from humans, the M, NS, and NP segments from swine, and the PA and PB2 segments from birds (Webby et al., 2000). This subtype became endemic in swine and, by coinfection with the H1N1 subtype, created a further reassortant H1N2 (Karasin et al., 2000). Currently, the three subtypes H1N1, H1N2, and H3N2 circulate worldwide in swine and their variants cause epidemic outbreaks. The evolution of subtypes and their variants in swine illustrates the extensive genetic change that can occur rapidly in the segmented RNA genome of the influenza virus.

Influenza Type A Infections of Domestic Poultry

A highly pathogenic disease of domestic birds called fowl plague was identified in Italy in 1878 (Perroncito, 1878), which led to the isolation of the first influenza virus type A in 1902 (Horimoto & Kawaoka, 2001). Domestic fowl are infected by a wide range of influenza A subtypes, the majority of which exhibit infections of low virulence characterized by mild respiratory disease and lowered egg production. Highly pathogenic avian influenzas are generally confined to subtypes bearing the surface HA H5 or H7 that cause highly lethal disease with flock mortalities up to 100% (Capua & Alexander, 2004). Highly pathogenic subtypes display HA H5 or H7 on their surface, which contains multiple basic amino acids proximal to the protease cleavage site. The efficient cleavage of the HA facilitates widespread infection of the avian tissues and the rapid onset of lethal disease. Recent scientific results support the conclusion that wild bird populations propagate influenza strains that exhibit no disease symptoms or those that cause only mild infections (Rohm et al., 1995; Banks et al., 2000, 2001). However, highly pathogenic H5 and H7 influenza strains arise in domestic poultry as a result of mutations in nonlethal viruses introduced from wild birds (Garcia et al., 1996; Perdue et al., 1998).

Records kept since 1959 identified 19 highly pathogenic primary influenza isolates in domestic fowl, which were either self-limiting or controlled by culling flocks (Fouchier et al., 2003). Diagnostic assays recently identified the mutation of low pathogenic subtypes into highly pathogenic variants, which caused widespread outbreaks in domestic fowl and severely impacted animal health and regional economies. These disease outbreaks occurred in Pennsylvania, USA, in 1983 (Webster & Kawaoka, 1988), Mexico in 1993 (Villarreal & Flores, 1997), Pakistan in 1994 (Naeem, 1998), Italy in 1999 (Capua & Marangon, 2000), and Chile in 2002 (Rojas et al., 2002), and demonstrate the global distribution of highly pathogenic influenza infections in domestic poultry stocks. In the period from 1997 to 2004, the incidence of worldwide infections with highly pathogenic strains of H5 and H7 has exceeded 28 lethal outbreaks in domestic poultry (Capua & Alexander, 2004).

In 1996, a highly pathogenic H5N1 virus was isolated from an infected goose in southern China, which reassorted with the other seven genes from the avian influenza H6N1. This virus caused disease in chickens in Hong Kong and was controlled by the culling of millions of chickens in southern China. Despite these containment efforts, this H5N1 strain spread widely in Southeast Asian countries including Japan, Vietnam, Laos, Cambodia, Thailand, and Indonesia, causing widespread lethal infections of domestic poultry (Writing Committee of the World Health Organization Consultation on Human Influenza A/H5, 2005). Wild ducks were shown to propagate and transmit the highly pathogenic H5N1 virus without manifesting disease symptoms, implying their potential role in the dissemination of this lethal subtype throughout Southeast Asia (Sturm-Ramirez et al., 2005). During mid-2005, it was reported that the H5N1 pathogenic virus caused a lethal epidemic in migratory waterfowl that led to the death of thousands of waterfowl in northwestern China, over 3,000 miles away from its epicenter of origin in southern China (Chen et al., 2005). In this case, the avian vector of the pathogenic H5N1 viruses was not

clearly established. However, the ongoing global dissemination of pathogenic H5N1 virus is due to its transmission from domestic poultry to wild migratory aquatic birds. Currently, the spread of H5N1 from northwestern China has resulted in the lethal infection of wild birds and domestic poultry in Siberia, the Urals, Turkey, Romania, Iraq, Greece, Italy, and Africa and is threatening the vast domestic poultry stocks in Europe and the Americas.

The reason for the recent escalation of highly pathogenic avian influenza virus infections in domestic poultry is not known. It could be due to their population size, genetic composition, conditions of containment, or other factors associated with the monoculture of poultry. However, this increase in highly pathogenic strains of influenza in poultry in the second half of the twentieth century parallels the 244% increase in poultry meat production during this period. Currently, 65% of this meat is generated by four poultry producers, the USA, China, EU, and Brazil, with production in the developing world exceeding that in the developed world in both enclosed and free-range facilities (Tilman, 1999). This domestication of poultry has established ideal conditions for the growth and rapid evolution of enzootic pathogens such as influenza virus. This has resulted in two unprecedented occurrences: highly pathogenic H5N1 influenza virus evolved in domestic poultry in 1996 and established global transmission in wild migratory aquatic birds; this pathogenic H5N1 virus grows, directly infects, , and produces lethal disease in humans.

Characteristics of Influenza Infections in Humans

The history of influenza infections in humans during the twentieth century is well recorded, and this information is reviewed in order to understand the issues and obstacles that confront efforts to control outbreaks in the future. The majority of the information is derived from documentation in the developed countries, as information from the developing regions is more difficult to assemble and consequently less complete. Even the information from countries with well-established public health systems is not completely reliable, especially during pandemic outbreaks. The compiling of clinical and lethality records was difficult during pandemic outbreaks due to the social disruption caused by the rapid onset of acute disease and its impact on the general population. In addition, it appears that in some instances clinical and lethality records were distorted because of the implementation of inappropriate social and political pressures (Barry, 2004). Despite the shortcomings of this historical database, it is an important record of the timing and impact of influenza disease within society and highlights the tasks implicit in the control of this disease. However, it would be imprudent to rely on this information as being predictive of the nature and characteristics of future influenza outbreaks, especially because there are dramatic changes in the size, distribution, and disease susceptibility of the human population and its ongoing activities that are driving major evolutionary changes in the biology and pathology of influenza viruses.

Influenza Infections in Humans

Influenza is a highly contagious acute respiratory infection that is efficiently transmitted between humans by the inhalation of contaminated droplets or by direct contact. Records show it to be one of the most significant infectious diseases of humans. The causative agent of influenza disease was finally established in 1933 by the isolation of a filterable agent, characterized as influenza type A virus, from human patients (Smith et al., 1933). In the 1940s, influenza virus grown in embryonated eggs was purified and formulated into an inactivated vaccine that was shown to produce protective immunity in human clinical studies. Influenza immunity in humans is principally related to protective antibodies that inactivate the surface protein HA.

However, a variation of influenza HA was shown in 1947 to undermine this variant-specific immunity, requiring the preparation of new vaccines to protect against these variants (Francis et al., 1947). This observation led to an initiative from participants at the International Congress of Microbiology in 1947 to recommend that WHO organize an international effort to survey and collate the global spread of influenza viruses. As a consequence, a program was established by WHO to monitor the global epidemiology of influenza and to isolate new strains and make them available to vaccine manufacturers (Payne, 1953). Currently, the program is coordinated by five Collaborating Centers in Atlanta, Memphis, London, Melbourne, and Tokyo, collating information from over 110 national laboratories in 82 countries. Sensitive and rapid methods of nucleic acid and protein sequencing and immune-based assays for antibody identification have been harnessed to identify influenza subtypes and variants that infect humans. The WHO Global Influenza Surveillance System coordinates this information on the antigenic variation and the epidemiology of influenza viruses, and this database is used to select new strains and variants for influenza vaccines.

Pandemic Disease in Humans

The emergence of new viral subtypes originating from the avian reservoir that transmits lethal infections in humans undermines the strain-specific immunity in the population and can instigate global pandemic disease. Potter estimated that during the last three centuries there were ten pandemic outbreaks that occurred on average once every 33 years (Potter, 2001). During the twentieth century, three pandemic strains emerged, H1N1 in 1918, H2N2 in 1957, and H3N2 in 1968; each viral subtype contained a novel HA. Retrospective molecular analyses indicate an avian origin for the HAs in the pandemic strains of 1957 and 1968 (Webster & Laver, 1972; Kawaoka et al., 1989). The origin of the 1918 HA is unclear. Its sequence is partially related to the avian HA but it may have emerged from a mammalian source and not directly from the avian reservoir (Reid et al., 2004). It is assumed that these pandemic strains derive by the reassortment of strains of avian

origin with strains that propagate in humans. These reassortants most likely deriveupon the coinfection of a mammal, possibly swine, which permit the growth of both avian and human influenza strains.

In 1918, pandemic disease appeared at about the same time in North America, Europe, and Asia, its severity increasing with time in the human population. This is the most lethal recorded pandemic with estimates of global deaths ranging from 20 to 100,000 million (Barry, 2004). During the 1918 pandemic, the number of deaths in accordance to age demonstrated a W-shaped distribution with peaks of mortality in infants, young adults, and the elderly, whereas the 1957 and 1968 pandemics data conformed to a U-shaped distribution with peaks in the deaths of infants and the elderly (Luk et al., 2001).

In 1957, the pandemic started in southern China and spread to Hong Kong by April 1957. The new subtype H2N2 responsible for the pandemic was identified after the vaccinologist Maurice Hilleman read in the *New York Times* that 250,000 people in Hong Kong had a respiratory infection (Hilleman, 1999). The first wave of disease in the USA and Europe started in August and peaked in October, followed by a second wave at the beginning of 1958. Approximately 2 million deaths were recorded worldwide with greater than 50% attack rates in children aged 5–19 years (Glezen, 1996).

The last pandemic in 1968 was initiated by the novel subtype H3N2 in Hong Kong in July and followed by infections in the USA during the winter of 1968–1969 and outbreaks in Europe during the winter of 1969–1970. This outbreak was relatively benign and it has been suggested that this was possibly due to immunity in the population supplied by the NA antigen, which was present in the new subtype H2N2 that started the previous pandemic in 1957 and remained in circulation up until 1968 (Cox & Subbarao, 2000). The global deaths exceeded 1 million, and in the USA two-thirds of all deaths occurred in persons of age 45–64 years (Simonsen et al., 1998).

Estimates from these pandemics in the twentieth century suggest that up to 30% of the total human population became infected. In susceptible populations such as schoolchildren and nursing home occupants, infection rates were as high as 40–50% (Cox & Subbarao, 2000). These records show that each pandemic exhibits a different level of lethality and that the age-related death statistics exhibited different profiles, indicating that influenza is a complex clinical syndrome in humans.

Epidemic Disease in Humans

Following pandemic disease, the new viral subtype remains in circulation in the human population for 10–40 years, and it progressively mutates to produce genetic variants that cause annual epidemics. The severity of epidemics is a reflection of the mutational changes in epitopes of the surface antigens HA and NA, and the degree of their mismatch with the protective antibodies resident in the human

population. In temperate regions, annual epidemics occur in the winter season, between November and March in the Northern Hemisphere, and April and September in the Southern Hemisphere, whereas in subtropical and tropical areas, influenza is present throughout the year (Cox & Subbarao, 2000). The CDC estimates that these epidemics are responsible for 20,000–30,000 annual deaths in the USA and the WHO estimated that 250,000–500,000 deaths occur every year around the world (World Health Organization, 2003).

Surveillance data indicate that in recent times the majority of new antigenic influenza variants originate in China from where they spread globally (Cox & Subbarao, 2000). Due to the introduction of a new viral subtype into the human population, its genetic variants form epidemic strains that progressively decline in virulence and ultimately disappear from circulation in the human population, as shown in Fig. 1 (Simonsen et al., 1998). The cause of this clearance is not known. It is assumed that strains are disadvantaged by the establishment of widespread immunity in the human population and that ultimately they reach a biological limit in the formation of viable antigenically distinct variants. The pandemic/epidemic cycle is restarted by the transmission of a new influenza subtype into the human population, which initiates the spread of pandemic disease.

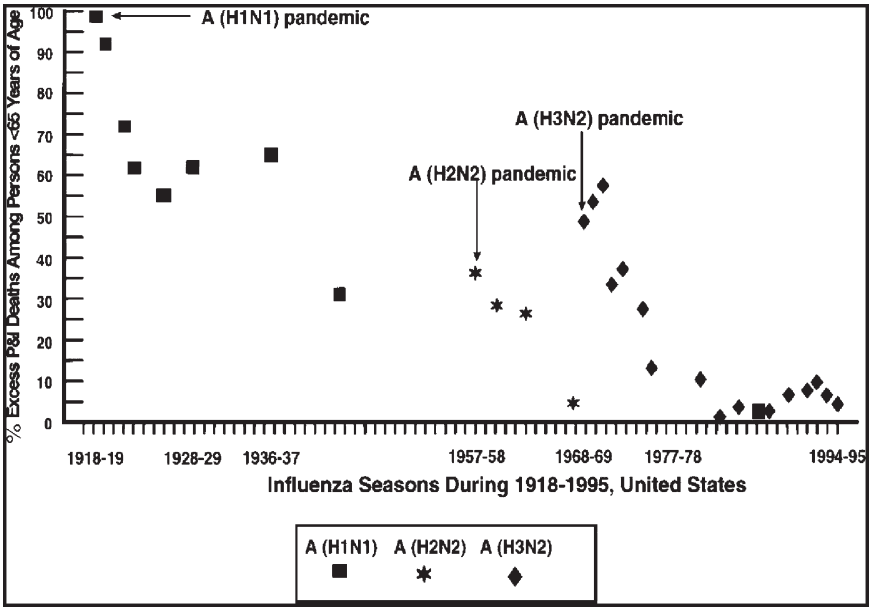

Fig. 1 The age distribution of deaths in the United States associated with the three influenza pandemics of the twentieth century and the interpandemic seasons that followed each pandemic (Reproduced with permission from Simonsen et al., 1998.)

Avian Influenza Pandemic Alert, 1997

Since the last influenza pandemic in 1968, the human population during the current interpandemic period has experienced rapid growth and redistribution to large urban areas. At the same time, global poverty and infectious diseases have increased dramatically. The support of this burgeoning human population requires enormous dedication of land resources to agriculture and energy generation that results in significant global ecological disruptions. The most dramatic impact on the ecology and evolution of influenza viruses has been caused by the vast increase in the production of domestic poultry during the last half of the twentieth century.

Coincident with the growth of the commercial monoculture of poultry, there has been an emergence of highly pathogenic avian influenza viruses that cause sporadic lethal outbreaks in poultry flocks. Intensive surveillance studies have catalogued the mutational changes in genomic and protein sequences of avian influenza viruses and their relationship to transmission, circulation, and pathogenesis among domestic poultry, wild birds, and humans. The data indicate that avian subtypes containing HAs H5 and H7 are highly pathogenic in domestic fowl. This correlates with the substitution of multiple basic amino acids proximal to the protease cleavage site, which facilitates the efficient cleavage of the HA precursor HA0 resulting in widespread infection of the avian tissues and the rapid onset of lethal disease. Avian influenza subtypes bearing HAs H5 and H7 demonstrate direct transfer and replication in humans, yet currently are limited in secondary spread within the human population. Workers exposed to infected poultry were shown to have antibodies against the avian strains H7N7, H7N3, and H5N1 but not H7N1 or H5N2 (Hayden & Croisier, 2005; Puzelli et al., 2005). These studies indicate that only specific viral subtypes are able to infect humans, implying the subtlety of interactions between viral and cellular proteins that define host range.

To date, the only subtype that demonstrates significant lethality in humans is H5N1. In 1996, a highly pathogenic H5N1 virus was isolated from an infected goose in southern China, which reassorted with the other seven genes from the avian influenza H6N1. In 1997, this strain caused disease in domestic chickens in Hong Kong and was transmitted to humans, killing 6 of 18 infected people (Writing Committee of the World Health Organization Consultation on Human Influenza A/H5, 2005). This event resulted in the declaration of a Phase 3 Pandemic Alert in accordance with the WHO Pandemic Preparedness Plan (World Health Organization, 1999). Thereafter, the virus was controlled by the culling of millions of chickens in southern China; yet in 2003 H5N1 infections occurred in two people in Fujian Province, China, resulting in the death of one person. This H5N1 strain spread widely in the southeast Asian countries including Japan, Vietnam, Laos, Cambodia, Thailand, and Indonesia, causing 112 infections and 57 deaths between December 2003 and August 2005 (Writing Committee of the World Health Organization Consultation on Human Influenza A/H5, 2005). The cumulative H5N1 infections in these countries amounted to 132 people with severe clinical symptoms of whom 64 died, indicating a lethality of 48% for this

new strain in humans. The lethality of H5N1 infections is derived from a small database, which reflects the lethality in individuals that demonstrate clinical symptoms and does not include infections with mild symptoms that were not reported. The transmission of H5N1 strains to humans was correlated in numerous countries by contact with infected domestic birds on farms and in wet markets; convincing evidence of transmission between humans has not been established.

Lectin-binding studies of cells along the human airway demonstrated that sialic acid bound by human HAs occurred mainly in nasal mucosal cells, whereas those recognized by the avian HAs were found in the lower airway on epithelial cells in the paranasal sinuses, pharynx, trachea, bronchi, and alveoli (Shinya et al., 2006; Van Riel et al., 2006). These observations are consistent with autopsy data on a patient who died from avian influenza in Thailand (Uiprasertkul et al., 2005). The attachment of H5N1 virus to the lower respiratory tract may explain the requirement for close contact with diseased birds for the infection in humans. Moreover, the inability of the virus to propagate in the upper respiratory airways may limit viral transmission in the human population in water droplets generated by coughing and sneezing.

An H5N1 virus isolated in 2004 from a Vietnamese who died from avian influenza demonstrated the standard trimeric HA structure at a resolution of 2.95Å. It displayed the characteristic receptor-binding domain embedded in a globular head and a membrane proximal domain containing the signature alpha helical stalk and HA1/HA2 cleavage site, which is a major contributor to its pathogenesis. Superimposition studies of HA domains from this Vietnamese isolate with those of human, swine, and avian viruses demonstrated that its was most closely related to the H1 HA, reconstructed from the virus responsible for the 1918 pandemic (Stevens et al., 2006).

Molecular studies demonstrate that influenza viruses switch from avian to mammalian receptor specificity, by the substitution of one or two amino acids in the receptor-binding region of the HA. This was established for the conversion of avian HAs into the H1, H2, and H3 that caused the twentieth-century human pandemics, and H1 in swine and H3 in horses and seals responsible for recent epizootic disease (Rogers et al., 1983; Connor et al., 1994; Matrosovitch et al., 2000; Nobusawa et al., 2000). Mutational analysis of the Vietnamese H5N1 isolate indicates that the replacement of individual amino acids in the receptor-binding domain modulates the avian alpha 2,3 sialic acid receptor selectivity (Stevens et al., 2006). However, the introduction of two amino acid substitutions into the receptor-binding region of the progenitor duck avian influenza virus reduced binding to 2,3 sialic acid but retained binding of 2,6 sialic acid equivalent to the human virus control (Harvey et al., 2004). These studies imply that in the future the substitution of a small number of appropriate amino acids in the receptor-binding domain of HA could alter the avian/human receptor selectivity of the H5N1 virus and facilitate its efficient human transmission.

The rapid global dissemination of H5N1 virus by aquatic wild birds has significantly increased its replication in domestic poultry, and their human contact facilitates its progressive accumulation of genetic alterations and enhances the probability of the emergence of lethal pandemic disease.

Influenza Surveillance: Implications and Applications

Surveillance studies illustrate a complex picture of the ecology, evolution, and transmission of pathogenic avian influenza viruses in domestic poultry, wild birds, and humans. To date, our understanding of the molecular determinants of host range, transmission between species, and pathogenesis is rudimentary, and consequently, sequence alterations are not predictive. There are trends in this data that have implications for the health of both domestic animals and humans.

1. The growth in the domestic poultry industry fosters the evolution of the contagious enzootic pathogen influenza, threatening the health of domestic poultry and humans. During the twentieth century, virulent influenza infections in domestic poultry have gravitated from local self-limiting outbreaks to worldwide dissemination of pathogenic strains that persist and are not controlled by large-scale culling of stocks. This trend threatens the domestic poultry industry and the nutritional welfare of humans dependant on this abundant and affordable meat source. It would appear logical for authorities regulating animal and human health to develop methods of controlling influenza infections of domestic poultry.
2. Pathogenic influenza strains that evolve in domestic poultry have recently shown large-scale transmission into wild waterfowl populations (Chen et al., 2005). The ecology of this phenomenon is complex. It appears that some species, such as ducks, transmit highly pathogenic H5N1 strains without disease symptoms. This indicates that migratory aquatic birds could be responsible for the distribution of H5N1 throughout south-east Asia and its ongoing global spread. Furthermore, the lethal infection of wild waterfowl could result in selective pressures within these populations that destabilize their reservoirs of influenza strains. The 15 subtypes of HA (H1–H15) and 9 subtypes of NA (N1–N9) identified in shorebirds, waterfowl, and gulls (Hinshaw et al., 1980) could diversify their sequences and hasten the establishment of new strains that infect domestic poultry and humans.
3. Highly pathogenic avian influenza virus strains from domestic fowl have shown direct transmission to and lethal infection of humans. However, to date, they are unable to affect secondary transmission between humans. The coinfection of humans or swine with both avian and human influenza viruses could result in a reassortant virus that transmits efficiently between humans. The pandemic reassortant virus will display the avian HA and transmit between humans, and its lethality in humans will depend on the final composition of its avian and human genomic segments. Alternatively, the direct adaptation of a pathogenic avian strain during the infection of humans could result in a variant comprising exclusively avian genomic segments, which efficiently transmits between humans. If this variant is derived by minimal sequence alterations, it may retain its high lethality in humans. Moreover, the adaptation of an avian virus comprising exclusively avian genomic segments that transmits efficiently in humans raises the possibility that this strain may cocirculate between and within the human and bird populations. Such a variant would dramatically enhance the transmission

characteristics of infection in both the bird and human populations and make the task of local containment and control of the global spread of pandemic disease more difficult. The probability of the adaptation in humans of an avian strain with these lethality and transmission characteristics is not known but should not be discounted. Our knowledge of the ecology and epidemiologic development of influenza is rudimentary. Few influenza authorities would have anticipated the unprecedented emergence and global spread of a highly pathogenic avian influenza strain from domestic poultry that induced lethal disease by the direct infection of humans and wild waterfowl in 1996.

Control of Influenza Infections in Humans

Vaccines prevent many infectious diseases in humans and are regarded as the most successful medical intervention to date. Despite the value they represent to society, vaccines only comprise 2% of the total global pharmaceutical market (Rappuoli et al., 2002). The main reason for this paradox is financial in that successful vaccines provide a low return on investment because they progressively eliminate disease and ultimately erode their own market. Influenza is an exception in that vaccines against epidemic disease induce protective immunity against the surface HA of circulating variants. New genetic influenza variants are not recognized by the protective antibodies in the human population and require the annual development of new vaccines to control epidemic disease. Pandemics are initiated in humans by the introduction of an influenza subtype that displays a novel HA, which transmits infection to naive members of the population. The control of pandemics is difficult because disease outbreak in the population must occur before the causative strain can be identified and the isolate made available for manufacturers to initiate vaccine development. Thereafter, it is a race to produce sufficient vaccine to protect the population before the highly contagious acute disease spreads globally and achieves peak infection rates. In contrast, genetic variants of the pandemic strain circulate in the human population causing annual epidemics. These strains are identified by surveillance and the candidates selected early in the spring, allowing time for vaccine production for the coming winter influenza season.

Control of Influenza Epidemic Infections

Annual influenza epidemics are caused by genetic variants of the influenza type A and B viruses circulating in the human population. The predominant A type virus variants currently circulating are derived from subtype H3N2 which initiated the 1968 pandemic and the 1918 pandemic strain H1N1 which reemerged in Tianjin, China, in 1977 (Cox & Subbarao, 2000). The influenza B strain variants currently circulating are derived predominantly from B/Victoria/2/87 which reemerged in

Asia in 2000–2001 and spread globally, causing widespread human epidemic outbreaks (Shaw et al., 2002). The control of epidemics caused by these variants is achieved by the use of two vaccine types: an inactivated viral vaccine and a cold-adapted attenuated live vaccine. New vaccines are developed annually because of genetic variants that circumvent the HA-specific protective immunity in the human population.

The current inactivated vaccine contains antigen from the three viral strains, two influenza A strains and one influenza B strain, selected annually by the Vaccine and Related Biological Products Advisory Committee (VRBPAC) of the FDA using the WHO Influenza Surveillance Data. The selection of strains occurs in spring and the vaccine is available for distribution in late fall. The annual trivalent vaccine is prepared by a process developed in the 1940s in which vaccine virus grown in germ-free embryonated chicken eggs is inactivated chemically, purified, disrupted, and formulated for delivery by injection. This process requires specialized facilities for handling large quantities of eggs and is limited by the availability of germ-free eggs, the generation of egg-adapted viruses, and the retention of sterility during egg inoculation and viral harvest. Following the selection of variant viruses, the preparation of the annual vaccine and its safety and potency testing takes 7–8 months. The estimated total global production of epidemic influenza vaccine in 2003 was approximately 300 million doses, 65% of which was produced in Europe by Sanofi Aventis, Glaxo Smith Kline, and Novartis/Chiron (PAHO Meeting, 2005).

Improvements in Inactivated Influenza Vaccines

The growth of virus for vaccines in cell culture is the subject of research and development to improve the quantity and quality of the antigen (Brown et al., 1999). This is important because the supply of germ-free eggs is limited and difficult to manipulate and retain sterility. Moreover, a significant portion of the human population has acquired allergies to egg proteins, which obviate their use in current influenza vaccines. The clinical evaluation and marketing of a cell-based influenza vaccine is anticipated within the next decade. The use of adjuvants that enhance immune responses and reduce the amount of antigen in inactivated vaccines is under evaluation by US and European agencies. These advances will improve the production process and the yield of inactivated influenza vaccines.

Live Attenuated Cold-Adapted Influenza Vaccine

Recently, attenuated live vaccines have been developed using cold-adapted virus as an alternative annual vaccine for prevention of epidemic influenza (Maassab & Bryant, 1999). The HA and NA of the three variants selected for the annual vaccine are expressed in cold-adapted viruses that are delivered to recipients by nasal spray.

The manufacturer of this vaccine, Medimmune, estimated in 2006 a production capacity of 15 million doses per month. These vaccines are effective and currently represent a small fraction of the influenza vaccine sales, limited by novelty and cost. There is also a concern that the circulation of live influenza vaccines in the human population could facilitate the formation of pathogenic reassortants with a coinfecting influenza virus in recipients.

Both of these vaccines are primarily marketed in the developed world and segments of the developing world population. Despite the annual distribution of new vaccines, epidemics cause approximately 20,000–30,000 deaths annually in the USA. In a survey in the USA from 1972 to 1992, the cumulative epidemic influenza deaths were 426,000 individuals (Simonsen et al., 1997). The reason for this was problems with distribution methods and complacency among recipients. In the USA, annual direct costs due to influenza hospitalizations in 1981 were $1–3 billion and the socio-economic burden was $10–15 billion (Szucs, 1999). It is difficult to determine the annual global deaths due to epidemics, but WHO estimates are between 250,000 and 500,000 individuals worldwide each year (World Health Organization, 2003). The major cause of mortality is the lack of vaccine availability and its distribution throughout the developing world. The cumulative deaths due to epidemic influenza indicate its equivalent importance to the pandemic disease and the need for improved vaccines and their distribution.

Control of Pandemic Influenza Outbreaks

Pandemic disease is initiated when a new viral subtype spreads within the human population due to a lack of immunity against its novel HA. Once the pandemic strain is identified and made available to vaccine manufacturers it takes 6–8 months to prepare the vaccine for distribution. The key issue for the protection of the human population is that the vaccine be available before the global spread of pandemic disease and the establishment of major infections within the population. The records from previous pandemic outbreaks during the twentieth century indicate that major pandemic diseases were prevalent or at peak level before adequate stocks of vaccine were produced.

In 1957, the disease started in southern China and Hong Kong in April. By August, within 3 months of the availability of the vaccine strain for manufacture, production was at maximum capacity in the USA.. However, the first pandemic disease wave started in the USA in August and peaked in November, and only 48 million doses of vaccine were produced. So within 6 months, insufficient vaccine had been prepared to control the first disease wave (Wood, 2001).

In 1968, the strain was available in September and vaccine production started within 2 months, in November. However within 4 months of starting vaccine production, the disease was at peak within the USA with only 20 million doses of vaccine available (Murray, 1969).

In 1976, a pandemic alert was initiated by an outbreak of "swine flu" in Fort Dix and vaccine manufacturers prepared 150 million doses of vaccine within 3 months, sufficient to protect the entire US population. This outstanding yield of vaccine was achieved using a new reassortant virus that showed high growth in eggs and the assurance by the government of a market for all the vaccine produced (Wood, 2001). The process was extended by 2 months because of the passing of indemnification legislation for the assurance of vaccine sales and new vaccine safety and standardization procedures and independent vaccine testing by the FDA. Despite improved production capacity, the overall period for vaccine distribution was still 7–8 months (Barry et al., 1977).

In 1997, the occurrence of 18 human infections by the avian influenza virus H5N1 in Hong Kong resulted in the declaration of a pandemic alert, consistent with the new WHO Pandemic Preparedness Plan (World Health Organization, 1999). Due to the lack of human-to-human transmission of H5N1, large-scale vaccine production was postponed and only the early stages of vaccine development were undertaken. Yet, to produce the first samples of vaccine for small-scale clinical evaluation it took 7 months, much longer than in alerts in 1957, 1968, and 1976. A reason for this delay was that the pathogenicity of the H5N1 isolate in humans required the stringent use of biological containment facilities (BSL 3+) to manipulate the viral isolates. In addition, the lethality of H5N1 virus in embryonated eggs required the application of reverse genetics to produce an attenuated surrogate to produce vaccine virus in eggs (Wood, 2001).

An important issue with pandemic vaccines is that, because the protective HA antigen is new, many recipients in the population are immunologically naive. During the pandemics of 1957 and 1968, the infants and young adults were naive, whereas the older members of the population had been primed by previous infection with related strains. In contrast, in the current H5N1 pandemic alert, the entire global population is immunologically naive to the HA H5 in the new subtype. Immunity against the NA N1 may reduce disease severity and exhibit a biphasic distribution in the human population represented by two distinct age groups. These populations include individuals over 50 years of age who were infected by H1N1 before its disappearance from circulation in humans in 1957 and young adults under 29 years of age infected by H1N1 which inexplicably resumed circulation in 1977.

Clinical studies in the last quarter of the twentieth century using three subtypes H1N1, H2N2, and H5N3 in naive recipients demonstrated a need for high antigen concentration in single-dose vaccines or two inoculations of vaccine with a lower antigen dose (Wood, 2001). This indicates that much more antigen will be required to be produced to generate vaccines for unprimed recipients. Moreover, time must be allocated to evaluate clinically the antigen dose required to induce protective immunity in a naive population.

So despite the early vaccine development efforts already accomplished for a H5N1 vaccine, it will require tremendous coordination to produce and distribute vaccine to control an H5N1pandemic outbreak. First, the viral strain must be isolated in containment facilities and the surface antigens manipulated and attenuated for safe and effective growth of vaccine virus in secured stocks of germ-free eggs.

Following the coordinated production of vaccine by different manufacturers, the safety and efficacy of the final product must be evaluated in animal and human studies. Finally, when vaccine is produced, it must be distributed and administered to the population in an orderly sequence to protect individuals at high risk, infants and the elderly, and those who fulfill essential social functions, followed by general distribution. Vaccine distribution and administration rely on the public health infrastructure within each country. Even in developed countries, there has been a progressive erosion of public health services, which is evidenced by the difficulties many countries, including the USA, have in administering the annual epidemic influenza vaccine. The situation in many developing countries is dire because the deficiencies in their public health personnel and infrastructure make the distribution of vaccine to their populations extremely difficult (Brugha et al., 2002).

It is clear that during pandemic alerts it is very difficult to prepare sufficient vaccine before the disease peaks in the population. This problem is more pronounced now than in 1957 and 1968 because the population has grown significantly, requiring more vaccine and the disease will spread more rapidly because of the vast increase in global travel (Garrett, 1994).

Containment of Pandemic Outbreaks at Site of Origin

Recently, two independent groups investigated the concept of attempting to contain and eliminate emerging pandemic disease at the source from which the viral variant originated by computer modeling (Ferguson et al., 2005; Longini et al., 2005). Both groups advocate a combination of quarantine (social distancing) and antiviral therapy using oseltamivir to treat infected individuals and as a prophylactic for healthy contacts and the local population. Longini et al. also suggest using a poorly matched H5N1 vaccine if it is available. The modeling data suggest that if the exercise is started within weeks of the first transmissible infection and the disease spread is relatively slow, it would be possible within their programming parameters to contain the spread of the pandemic variant. This computer-based outcome relies on the early identification of a single outbreak epicenter in rural Thailand, the rapid and coordinated delivery of antivirals and vaccine, the staff to implement medical treatment and enforce quarantine, and the cooperation of the local indigenous population. It also assumes the efficacy of antiviral treatment of infections caused by the highly genetically variable influenza viruses and does not take into account that resistance to this antiviral has been detected in certain H5N1 infections of humans in southeast Asia (Le et al., 2005). These modeling data are encouraging and suggest that under ideal circumstances this approach may contain an emerging pandemic or at least delay its spread, and thus increase the time available for the production of an effective vaccine. Yet from a practical point of view, a disease spreading with predetermined characteristics and constraints within the confines of a computer program is far removed from the reality of its dissemination in the tropical rural communities of an impoverished southeast Asian country.

Pandemic Preparedness Plans

The procedures that define the stages of pandemic disease outbreak and international and national responses are outlined in the WHO Pandemic Preparedness Plan (World Health Organization, 1999) and reports prepared by a number of national health agencies. The WHO defines six phases in the establishment of a pandemic: two phases are interpandemic, during which old and new viral strains that pose a risk to humans are confined to animal populations; three phases of pandemic alert, during which a new virus from animals infects humans and gradually evolves the capacity to transmit between humans; and one phase of pandemic, which is declared following increased and sustained transmission of disease within the general population. The current situation (in 2006) is a pandemic alert phase 3, declared in 1997, in which the H5N1 from domestic birds infects humans without significant transmission between humans and is spreading around the globe because of the infection of wild migratory aquatic birds.

During a pandemic outbreak, the Preparedness Plan requires the coordination of rapid and disciplined responses by international and national agencies, pharmaceutical companies, healthcare workers, and essential government and service employees. This outcome may be difficult to achieve, given the history of poor interactions and territorial disputes among many agencies and the complex chain of command that would impede prompt and focused actions by essential civilian and military personnel. Responses to natural disasters on the national and international levels are replete with examples of these problems.

In an upcoming pandemic, it is not possible to predict either the number of infections in the population that will need medical treatment or the timing and extent of lethal infections. Most preparedness plans do not articulate a clear organizational response to outbreaks with either different levels of infections in the population that require medical treatment or hospitalization, or the coordination of social and essential services required to handle various levels of lethality of disease in the population. The majority of preparedness plans anticipate a disease profile of low-lethality infections in humans. This seems peculiar when confronted by the global spread of an avian strain H5N1 which exhibits unprecedented lethal infections of humans (Writing Committee of the World Health Organization Consultation on Human Influenza A/H5, 2005). Assuming the upcoming pandemic would exhibit transmission and lethality characteristics similar to the pandemic of 1918, with the present-day population six times larger, the lethality estimates would range from 120 to 600 million.

Despite advances in medical treatments, the health system would be soon overwhelmed by the number of acute infections that would occur over a short time span. In addition, the social and essential services dealing with the large number of deaths over a short timeframe would face enormous logistical difficulties. These are clearly complex and sensitive issues, but failure to adequately address them undermines the concept of preparedness and fails to inform the general population of the constructive roles they could undertake to minimize the disruption of essential social services and unrest within the population.

Development of Cross-Protective Influenza Vaccines

Since the last influenza pandemic in 1968, there have been great advances in the technologies that identify and sequence new influenza variants, define their transmission within bird and mammalian populations, and unravel their infection and growth characteristics in cells. However, little progress has occurred in the generation of new influenza vaccines for the prevention of epidemic and pandemic outbreaks. The derivation of an influenza vaccine that induces cross-protective immunity against all viral subtypes that infect humans would yield a universal vaccine impervious to variations in the surface glycoproteins HA and NA. The ideal cross-protective vaccine would induce long-lived immunity against all subtypes of human influenza virus and avian subtypes that infect humans, and thereby eliminate epidemic and pandemic diseases. This concept was first established by researchers at CDC in 1995 (Slepushkin et al., 1995) and has been subsequently confirmed and extended by academic and pharmaceutical groups reviewed later.

The influenza virus displays three membrane proteins, the HA and NA which demonstrate continuous sequence variation and the M2 protein whose sequence is highly conserved. The 97 amino acids of the M2 protein comprise three domains: 24 extracellular residues, 19 transmembrane residues, and 54 intracellular residues (Lamb et al., 1985). The M2 protein is a homotetramer composed of two disulfide-linked dimers that assemble across the cell membrane to form a proton-selective ion channel (Sugrue & Hay, 1991). Sequencing studies show that M2 protein is highly conserved and that the extracellular domain shows very little change in all human viruses sequenced since the first human virus was isolated in 1933 (Ito et al., 1991; Fiers et al., 2004). The M2 protein is present in low concentration on the virion surface but is abundantly represented on the surface of influenza-infected cells (Lamb et al., 1985; Zebedee & Lamb, 1988). M2 mediates proton influx into endosomes which results in the dissociation of the viral ribonucleoprotein from the matrix protein facilitating the replication of viral RNA (Helenius, 1992). Consistent with this role in viral multiplication, a monoclonal antibody that attaches to the M2 extracellular domain inhibits viral replication in cell culture and in infected mice (Zebedee & Lamb, 1988; Treanor et al., 1990).

The analysis of convalescent sera from humans infected with the influenza A virus H3N2 demonstrated the presence of antibodies to the M2 protein (Black et al., 1993). Studies at CDC demonstrated that an M2/insect fusion protein induced heterosubtypic antibodies in mice that conferred protection against a lethal heterologous viral challenge (Slepushkin et al., 1995). These data were confirmed and it was demonstrated that cross-protective immunity could be induced by the 24 highly conserved amino acids of the extracellular domain of M2 when presented in soluble fusion proteins and virus-like particles (Nierynck et al., 1999; Mozdzanowska et al., 2003; Fan et al., 2004; Liu et al., 2004; Ionescu et al., 2006). These studies demonstrated that the cross-protective immune response based on antibodies was long lasting and could be significantly enhanced by the presentation of multiple copies of the M2 sequence in soluble recombinant proteins or virus-like particles and when formulated with appropriate adjuvants. It was shown that these M2

recombinant proteins induced cross-protective antibodies in rodents, ferrets and rhesus monkeys. Moreover, conserved sequences have been identified from other influenza proteins including HA, nucleoprotein, and matrix proteins that induce cross-protective immunity in rodents (Levi & Arnon, 1996; Epstein et al., 2002).

The databanks of the influenza genome sequences of human and avian subtypes and variants (Ghedin et al., 2005; Obenauer et al., 2006) can be utilized to collate the entire repertoire of conserved sequences and define their subtype distribution. Those sequences shown individually to induce immunity would be assembled into a multivalent recombinant protein. The arrangement and number of individual epitopes within the recombinant protein can be adjusted to assure the optimal induction of robust cross-protective immunity against all human viral subtypes and those avian subtypes that infect humans. These recombinant proteins can be prepared as stable soluble products or virus-like particles in bacterial or insect expression systems and formulated with appropriate adjuvants for clinical evaluation in humans. A successful clinical outcome would lead to an affordable subunit vaccine for administration to children within the pediatric schedule, which, with appropriate boosting, would confer lifelong immunity against influenza infection. Ideally, a successful universal influenza vaccine would eliminate both epidemic and pandemic diseases and the associated mortality and socioeconomic burdens.

The same approach could be used to define the equivalent conserved sequences in avian, swine, and equine influenzas, and develop universal vaccines that protect these domestic animals and limit the enzootic spread of influenza viruses.

The successful development of universal influenza vaccines would ideally confine influenza strains to the wild aquatic bird population and obviate disease in domestic animals and humans. The investment to develop such vaccines would be minimal compared to funds currently dedicated to global surveillance, annual epidemic vaccine development, and the effort of preparing for upcoming pandemics, not to mention the direct costs and the socioeconomic burden of annual epidemic and periodic pandemic disease.

The achievement of this goal will require the formation of an international organization with an unwavering dedication to the development of vaccines for the global control of infectious diseases including influenza. This new organization would need adequate funding, with a mandate to oversee the global control of infectious disease without national, regional, or a for-profit bias. This concept of a Global Infectious Disease Authority has been previously described (Roberts & Lu, 2004).

Conclusion

The control of influenza infections in humans is in serious need of modernization and improvement. The control of annual epidemics relies on antiquated vaccine production methodologies that generate insufficient quantities of vaccine for the effective protection of the human population. The control of pandemic outbreaks by vaccination is improbable because experience from the pandemics of 1957 and

1968 clearly demonstrates that the global spread and the peak of infection in the population preceded the manufacture of significant quantities of vaccine. The growth of the human population and its global mobility will assure rapid pandemic spread and will require more vaccine in a shorter time period than in previous outbreaks.

The large-scale monoculture of domestic birds has facilitated the unprecedented evolution and widespread dissemination of highly pathogenic avian viruses that cause not only lethal infections of poultry but also directly transmit disease to wild birds and humans. The infection of humans by the avian subtype H5N1 causes severe disease that is highly lethal but it is currently limited by the lack of transmission between humans. A variant of this highly pathogenic strain that transmits efficiently between humans would result in the global spread of this highly contagious infection that could rapidly overwhelm medical and societal infrastructures and lead to widespread deaths and political and social unrest. The threat posed by influenza infections to human society transcends that of most infectious diseases and dwarfs natural disasters and terrorist threats. The recent changes in influenza evolution and transmission calls for a global initiative to develop more effective vaccines that produce broad cross-protective immunity and eliminate epidemic and pandemic diseases in humans.

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