JOURNAL OF VIROLOGY, Sept. 2004, p. 8951–8959 Vol. 78, No. 17
0022-538X/04/$08.00+0 DOI: 10.1128/JVI.78.17.8951–8959.2004

MINIREVIEW

Influenza: Emergence and Control

Aleksandr S. Lipatov,[1] Elena A. Govorkova,[1] Richard J. Webby,[1] Hiroichi Ozaki,[1] Malik Peiris,[2] Yi Guan,[2] Leo Poon,[2] and Robert G. Webster[1,2]*

Division of Virology, Department of Infectious Diseases, St. Jude Children's Research Hospital, Memphis, Tennessee 38105,[1] and Department of Microbiology, University of Hong Kong, Hong Kong, Special Administrative Region, People's Republic of China[2]

The natural reservoirs of influenza A viruses are the aquatic birds of the world (91), in which the viruses appear to be in evolutionary stasis—they are in equilibrium with their natural host and cause no disease. Periodically, the influenza viruses are transmitted to other hosts, including mammals, and cause transitory infections and occasionally deaths. Less frequently, influenza viruses are transmitted to other species and establish continual infections in those hosts. Thus, permanent lineages of influenza A viruses occur in humans, swine, horses, and domestic poultry. What is the difference between transitory infection occasionally causing death and the establishment of permanent lineages in the host? The molecular bases of a virus's ability to spread among a range of hosts and of the pathogenicity of influenza viruses are still unresolved. Since 1997, when H5N1 influenza virus was transmitted to humans and killed 6 of 18 infected persons, there have been multiple transmissions of avian influenza viruses to mammals. Either the whole virus is transmitted directly (12, 81) or gene segments from the avian influenza virus are acquired by mammalian strains (e.g., H3N2 triple reassortants in pigs in the United States) (37, 38, 97, 98). Widespread infections of poultry with H5N1 viruses in Asia have caused increasing concern that this subtype may achieve human-to-human spread and establish interspecies transmission. In this minireview, we consider recent interspecies transmissions of influenza A viruses and examine our limited knowledge of the contributors to the success of these viruses. Finally, we briefly consider control measures.

INFLUENZA VIRUSES TRANSMITTED AMONG HUMANS IN THE 20TH CENTURY

The most successful influenza virus of the 20th century from the perspective of transmissibility among and pathogenicity to humans was the H1N1 virus that caused the Spanish flu pandemic of 1918. This virus is thought to have killed up to 100 million persons (84). The next most successful viruses were those that caused the Asian flu pandemic in 1957 (H2N2), which killed 70,000 persons in the United States, and the Hong Kong flu pandemic in 1968 (H3N2), which killed 34,000 persons in the United States. The basis of the high pathogenicity of the 1918 Spanish flu virus remains an enigma (84); the available data point to an avian virus origin, but the precursors are still unknown. It is possible that all gene segments were from mammalian-adapted avian influenza viruses. More is known about the 1957 and 1968 human pandemic strains. Each of these newly emerged H2N2 and H3N2 viruses possessed gene segments from avian and human influenza viruses (40). Acquisition of novel surface glycoproteins (hemagglutinin [HA] and neuraminidase [NA]) allowed the viruses to circumvent the host's humoral immunity, and their possession of a novel PB1 gene implicates this gene in interspecies transmission. One recipe for success for a virus is therefore reassortment that results in the acquisition of novel surface antigens and of a novel PB1 gene and in the retention of the gene segments that enable transmissibility among humans.

TRANSITORY TRANSMISSIONS OF AVIAN INFLUENZA VIRUSES TO HUMANS SINCE 1997

Since 1997, there have been many incidents of transmission of avian influenza virus to humans. Increased surveillance may have increased the detection rate, but there is support for the notion that H9N2 influenza virus was not found in Asia in domestic chickens or in humans before the mid-1980s (62, 72). The spread of H5N1 influenza virus throughout Asia in 2004 is undoubtedly a novel event.

H5N1

The H5N1 bird flu virus that infected humans in 1997 acquired all eight gene segments from Eurasian avian sources and retained a preference for binding to α(2,3) sialic acid receptors, a feature typical of avian influenza viruses (53). The 1997 H5N1 bird flu was successfully eradicated by the slaughter of all poultry in Hong Kong. However, the donor of the HA gene in the 1997 H5N1 strain (A/goose/Guangdong/1/96 [H5N1]) continued to circulate in geese in southeastern China (8, 92), and the 1997 H5N1 virus was soon replaced by different genotypes (22) that were highly pathogenic in chickens but not in ducks. These H5N1 viruses were again eradicated by the slaughter of poultry, only to be replaced by additional genotypes in 2002 (Fig. 1). From 1997 through 2001, the HA on the various genotypes remained antigenically homogeneous, but in 2002 it underwent marked antigenic drift (23, 79). The most remarkable property of the H5N1 genotype from late 2002 was

* Corresponding author. Mailing address: Division of Virology, Department of Infectious Diseases, St. Jude Children's Research Hospital, 332 North Lauderdale St., Memphis, TN 38105-2794. Phone: (901) 495-3400. Fax: (901) 523-2622. E-mail: robert.webster@stjude.org.

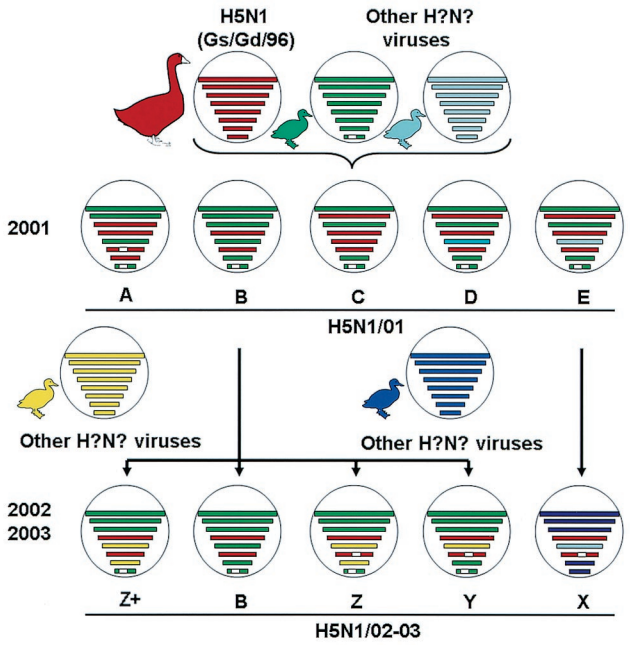

FIG. 1. The derivation of H5N1 reassortants in the years 2000 through 2003. Reassortment between influenza viruses is proposed to generate viruses with different gene constellations. Gs/Gd/96-like viruses reassorted with viruses from wild aquatic birds and multiple H5N1 genotypes appeared in Hong Kong poultry markets in 2001. Some 2001 H5N1 genotypes may have been transmitted back from domestic poultry to the wild aquatic avian reservoir, where the next reassortant events may have occurred. As a result, multiple H5N1 genotypes, mostly different from those of 2001, were isolated from domestic poultry and wild birds in Hong Kong in 2002-2003. The eight gene segments schematically shown in each virus particle encode (top to bottom) polymerase complex (PB2, PB1, and PA), HA, nucleoprotein (NP), NA, matrix (M), and nonstructural (NS) proteins. Color coding indicates virus lineages. A gap in the NA or NS gene segment denotes a deletion. Deletions within the NA gene appear to result from adaptation to poultry, although the exact role of this phenomenon is unclear. NS gene deletions in H5N1 viruses have recently been observed, but the biological significance of these deletions is unknown.

its high pathogenicity for ducks and other aquatic birds, a property rarely found in nature; a previous event of significance to aquatic birds occurred in 1961, when A/tern/South Africa/61 (H5N3) killed terns.

In early February 2003, H5N1 virus reemerged in a family in Hong Kong (2, 59). The daughter died of a respiratory infection of undiagnosed cause while visiting Fujian; the father and son developed severe respiratory illnesses after their return to Hong Kong. The father died and the son recovered (59). Infection with H5N1 influenza virus in father and son was confirmed; the strain was antigenically and molecularly similar to the antigenically drifted strain that was highly pathogenic for ducks and chickens (23).

The unprecedented magnitude of the bird flu epidemic in Asian countries in 2004, with H5N1 in China, Japan, South Korea, Thailand, Vietnam, Indonesia, Cambodia, and Laos; H7N3 in Pakistan; and H5N2 in Taiwan, has, at the time of writing, resulted in the destruction of hundreds of millions of poultry, mainly chickens (Fig. 2). In most countries, outbreaks of highly lethal H5N1 avian influenza were confined to poultry, but in at least two countries the virus was transmitted to humans and most of the persons infected have died (15 deaths in Vietnam and 8 deaths in Thailand). This H5N1 virus was first detected in wild migrating aquatic birds in Hong Kong in November 2002, when dead egrets (*Egretta garzetta*), gray herons (*Ardea cinerea*), and Canada geese (*Branta canadensis*) were shown to be infected with it. The viruses isolated from these birds were shown to be antigenic drift variants of earlier H5N1 viruses from Hong Kong and had acquired the ability to cause lethal infection in ducks (see below) (79). One of the unusual features of this episode was that highly lethal H5N1 influenza viruses were detected in migrating wild birds overwintering in Hong Kong in the winter of 2002-2003. We predicted that widespread transmission of this H5N1 virus might occur when the migrating waterfowl returned to their summer habitats (79). The first cases of H5N1 were detected in poultry in Vietnam, Indonesia, and Thailand in July 2003, but these cases were not reported officially until the disease in poultry got out of control in January 2004 (Fig. 2).

The H5N1 virus currently circulating in Asia is genetically similar to the Z genotype (shown in Fig. 1) that became dominant in Hong Kong in 2003, but it has drifted antigenically. This drift has necessitated the creation of a new human vaccine strain. The dominant H5N1 virus in Asia appears to be antigenically and genetically similar to A/Vietnam/1203/04 (H5N1) and contains eight gene segments of Eurasian avian origin. This is the virus genotype that has spread to humans in Vietnam and Thailand (Fig. 2).

Multiple opportunities for the successful mammalian transmission of H5N1 influenza viruses are provided by their continuing evolution in Asia, their propensity for reassortment, the generation of multiple genotypes of H5N1 viruses (Fig. 1), antigenic drift in the HA of H5N1 viruses, and the acquisition of high pathogenicity for aquatic birds. If an opportunity for reassortment with human influenza strains occurs, then the likelihood of successful transmission between humans is high.

H9N2

In 1999, H9N2 influenza virus was transmitted to two children in Hong Kong and caused mild influenza; the children recovered (48, 60). At about this time, reports of infection with H9N2, again with mild illness, came from mainland China (28). These incidents were caused by antigenically different H9N2 viruses. The human infections in Hong Kong were caused by a virus that was related to A/quail/HK/G1/97 (H9N2) and possessed a genotype related to the H5N1/97 virus. The infections on the mainland (28, 29) were caused by a virus that was antigenically related to A/duck/HK/Y280/97 (H9N2) and to A/chicken/HK/G9/97 (H9N2) and had a distinct avian-like genotype. The H9N2 influenza viruses have acquired a preference for binding to $\alpha(2,6)$ sialic acid receptors (like human strains) (54) and have been detected in pigs in Hong Kong (58). The H9N2 influenza viruses are now panzootic in domestic poultry in Eurasia (1); their presence in such a host represents the widening of their host range since the 1980s.

Analysis of H9N2 strains identified since 1999 reveals that most of them are antigenically related to chicken or duck (A/duck/HK/Y280/97 [H9N2-like]) strains but have acquired a

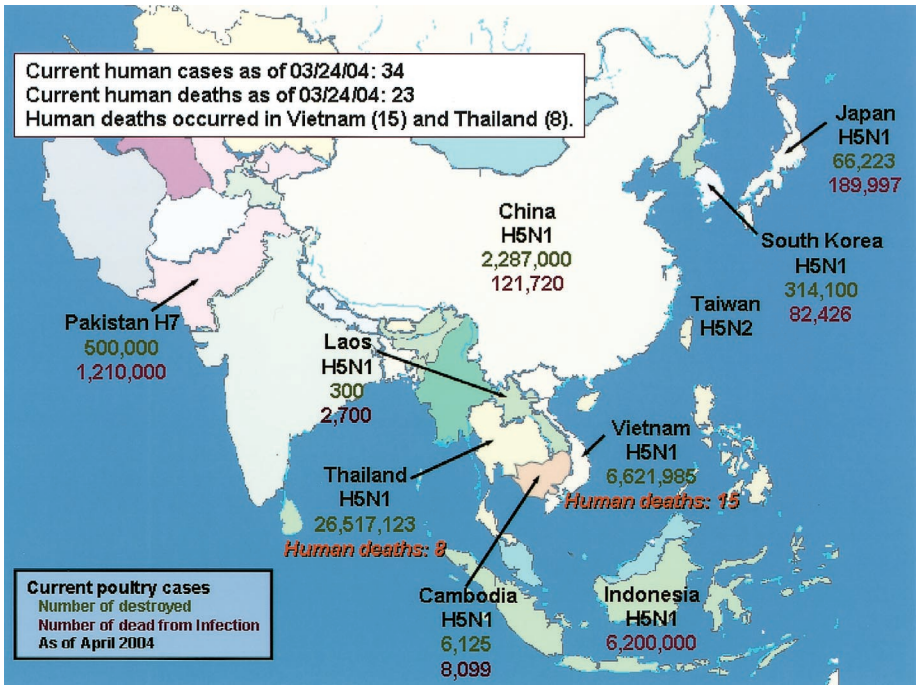

FIG. 2. Locations and dates of avian influenza outbreaks in Asia in 2004. The information presented was obtained from http://www.who.int (for human cases) and http://www.oie.int (for avian data).

multiplicity of different genotypes (unpublished data); some have internal genes of H5N1/97-like origin. In December 2003, a child in Hong Kong was infected with one such genotype, which caused mild respiratory disease (unpublished finding).

The widespread distribution in Eurasia of the H9N2 viruses that have acquired human receptor specificity [i.e., $\alpha(2,6)$ sialic acid binding] and the ability of these viruses to be transmitted among pigs and cause mild infection in humans indicate that their transmissibility in humans may not require many additional changes. H9N2 could become successful at transmission between humans without causing significant disease.

H7N7 AND H7N2

The transmission of H7N7 influenza viruses to poultry in The Netherlands in March 2003 caused severe losses of domestic poultry (45). The virus also was transmitted to and caused conjunctivitis in at least 82 persons. There was evidence of limited human-to-human transmission, and one veterinarian died (16, 45). Additionally, there was serologic evidence of infection of swine.

Since 1994, H7N2 influenza viruses have circulated in live bird markets in New York (80). The 1994 H7N2 isolate had two basic amino acids at the HA cleavage site, and over the subsequent 2 years the virus progressively acquired two additional basic amino acids and spread to commercial poultry farms (80). Although these viruses were characterized as being of low pathogenicity, the U.S. poultry industry wisely eliminated the virus from commercial poultry farms. These H7N2 influenza viruses continue to circulate in poultry in the northeast United States and are again causing problems in 2004.

The H7N2 and H7N7 influenza viruses were controlled by the culling and quarantine of domestic poultry. During the H7N7 outbreak in The Netherlands, humans were vaccinated with the human vaccine available at that time to prevent reassortment, and the prophylactic use of antineuraminidase drugs minimized the possibility of transmission between persons. The H7N7 influenza outbreak in poultry and humans in The Netherlands provides a model for the transmission and control of an emerging influenza virus. Such control minimizes the possibility that the virus will achieve human-to-human transmission.

At the time of the submission of this article, a highly pathogenic influenza virus has emerged in domestic poultry in British Columbia, Canada. This virus is a H7N3 virus which is lethal in chickens and turkeys. Over 350,000 birds had been culled at the time of writing, and one human case had been confirmed, with clinical signs in 10 other poultry cullers being reported. The clinical signs included conjunctivitis and mild respiratory symptoms; vaccination with the current human vaccine and the use of the influenza antiviral agent oseltamivir has been recommended to minimize reassortment and human transmission.

PIGS AND THEIR ROLE IN TRANSMISSION

The susceptibility of pigs to experimental infection with avian viruses (42) and the demonstration that serial passage of avian viruses in pigs can lead to human virus-like traits (35) have strongly implicated pigs in the emergence of human influenza viruses. Supporting this implication are the numerous reports of swine influenza viruses infecting, and in some cases causing disease in, humans (6). Although passage in pigs may well adapt an avian virus to the infection of humans, it is becoming clear that the features required to establish a virus in

swine populations are not necessarily the same as those for human populations. The increasing number of reports describing self-limiting human infection with swine viruses strongly argues that swine viruses are not well adapted for transmission in humans. It is also likely that the opposite is true, that human viruses are not well adapted to pigs. Because of these differences in host range, the genetic compositions of viruses in swine and human reservoirs are becoming increasingly divergent.

Although the same virus subtypes (H1N1, H3N2, and their reassortants) have established lineages in pigs and humans (we have not considered H9N2 an established lineage in swine), pigs host a more genetically diverse group of viruses. Two particularly successful interspecies transfer events in the past 30 years have contributed significantly to this divergence in swine and human virus gene pools.

During the late 1970s, swine populations in Europe became hosts to a new avian H1N1 virus (61, 65, 67). This virus was antigenically and genetically distinct from the classic H1N1 virus, which was a descendant of the 1918 Spanish influenza virus. Over the subsequent few years, the new avian virus replaced the classic H1N1 virus and continued to evolve through reassortment with other swine and human viruses (5–7). A common feature of the resulting reassortants was the abundance of genes (other than the HA and NA genes) derived from the avian-like H1N1 virus. This feature suggested that the reassorted viruses had gained some advantage in possessing the genes derived from the avian H1N1 virus.

Another new lineage of virus has recently appeared in pigs in the United States. During the latter part of the 1990s, genes from human and avian viruses were introduced into the U.S. swine virus gene pool (37, 38, 97, 98). These genes were first identified in viruses containing the HA, NA, and PB1 genes of human viral origin; the NP, NS, and M genes of classic swine viral origin; and the PA and PB2 genes of avian viral origin. These new viruses were quickly established in the pigs of North America (88); further reassortment with classic swine H1N1 viruses has been documented (11, 37). As observed in Europe, these reassortant viruses all maintained the genetic components of the avian virus.

Although the lack of establishment of the new European or North American swine viruses in humans argues against common requirements for their establishment in pigs and humans, pigs may still play a central role in the ecology of influenza viruses by acting as mixing vessels in which viruses of different origins can reassort (66). Our current knowledge does not allow us to predict whether such reassortant viruses will affect humans, but it seems unlikely that they will, for the pig H3N2 strains are related to those in humans and humans are likely to be immune.

PATHOGENICITY OF AVIAN INFLUENZA VIRUSES FOR MAMMALS

The factors determining the pathogenicity of influenza viruses are unresolved. Most of the available knowledge comes from studies of highly pathogenic avian influenza viruses; these studies have shown that broad tissue tropism and an ability to replicate systemically are important factors determining high pathogenicity in domestic chickens. One of the molecular determinants of high pathogenicity in avian influenza viruses is the presence of a multibasic cleavage site in the HA; the correlation between the structure of the HA cleavage site and viral pathogenicity has been extensively studied (9, 43, 78, 90). The highly pathogenic avian H5N1 viruses that have infected humans and the highly pathogenic avian H7N7 virus transmitted to humans in The Netherlands in 2003 all possessed HAs with multiple basic amino acids at the cleavage site.

The pathogenicity of H5N1/97 viruses has been studied in a number of mammalian models, including mice (17, 51, 86), outbred ferrets (99), pigs (73), and cynomolgus macaques (63), but the results obtained with these models are conflicting. Unlike other human and avian influenza A viruses, the human and avian H5N1/97 isolates do not require adaptation to be pathogenic in mice and are categorized as viruses that either are of high pathogenicity and replicate systemically (including in the brain) or are of low pathogenicity and replicate only in the lungs and upper respiratory tract of mice (17, 51). In general, the pathogenicity of H5N1/97 isolates in mice has corresponded to the severity of disease in humans (39). Furthermore, Lys627 in PB2 is crucial for high virulence and systemic replication of A/Hong Kong/483/97 (H5N1) virus in mice (30). The amino acid at position 627 of PB2 determines the efficiency of viral replication in mouse (not avian) cells, but this amino acid does not determine viral tropism toward different organs in the mouse (71). Although structural aspects of PB2 and HA have been associated with the pathogenicity of H5N1/97 in mice, other genotypes of H5N1 that emerged in 2001 and are neurotropic in mice do not posses a Lys at residue 627 of PB2. These highly pathogenic H5N1 variants had mutations in all gene segments except those encoding the PB1, NP, and NS1 proteins, but no common set of mutations was found (49). Therefore, multiple gene constellations and residues are associated with the pathogenicity of influenza viruses in mice. Despite the differential pathogenicity of H5N1/97 influenza viruses in mice, all of these viruses cause systemic infection in ferrets (99). In studies with cynomolgus macaques, these viruses caused severe respiratory disease but did not spread systemically (63), and in studies with pigs the H5N1/97 viruses replicated (to modest titers) only in the respiratory tract and caused no disease signs (73). All of these findings indicate that multiple gene constellations are involved in influenza virus pathogenicity and that the outcome of infection is host dependent.

Recent findings showed that the NS gene of Hong Kong H5N1/97 viruses has a role in the determination of high pathogenicity in mammals. Seo et al. (69) demonstrated that the NS gene of H5N1/97 virus dramatically increases the pathogenicity of A/PR/8/34 (H1N1) virus in pigs. These authors hypothesized that the NS gene of H5N1/97 viruses confers resistance to the antiviral effects of interferons (IFNs) and tumor necrosis factor alpha (TNF-α) (68, 69). The NS gene segment of influenza A viruses encodes two proteins: NS1 and nuclear export protein. NS1 contributes to viral pathogenesis by allowing the virus to disarm the host's IFN defense system in multiple ways (reviewed by Garcia-Sastre [18, 19] and Krug et al. [46]). In mice, A/WSN/33 (H1N1) reassortants with the complete NS gene or with only the NS1 segment of the gene of the 1918 pandemic influenza virus were less pathogenic than the original A/WSN/33 virus (3). On the other hand, a virus containing the

NS gene of the 1918 pandemic strain blocked the expression of IFN-regulated genes in human lung cells more efficiently than its parental A/WSN/33 virus did (20).

In contrast, a study in which primary human monocyte-derived macrophages served as an in vitro model showed that transcription of TNF-α and IFN-β genes was induced by viruses containing the NS gene of H5N1/97 viruses as well as particular constellations of their internal genes rather than the genes encoding the surface proteins (10). The authors of that study concluded that the induction of cytokine transcription contributed to H5N1 pathogenesis. We recently found that a virus having the NS gene of H5N1/97 on a PR/8/34 backbone is highly pathogenic in mice and causes an overall cytokine imbalance (unpublished data); a reassortant virus carrying the NS gene of H5N1/97 induced an increase in the concentration of inflammatory cytokines but a decrease in the concentration of the anti-inflammatory cytokines in infected mouse lungs.

Studies of reassortant viruses containing the NS gene of the highly pathogenic H5N1/97 virus in two mammalian models support the theory that the NS gene of H5N1/97 viruses can confer and support high pathogenicity when it is inserted into a virus (e.g., A/PR/8/34) that is pathogenic in one model (in this case, mice) and nonpathogenic in another (i.e., pigs) (69). The idea that high pathogenicity of viruses containing the NS gene of the H5N1/97 virus may result from the induction of a cytokine imbalance is supported by findings from a detailed pathological examination of two persons who died of H5N1 pneumonia in Hong Kong in 1997 (85, 95). A cytokine imbalance could explain, at least partially, the unusual severity of illness caused by infection with H5N1/97 influenza virus.

It is unlikely that the products of the NS gene of the H5N1/97 virus are unique in causing a cytokine imbalance: other gene products certainly play roles, especially in different hosts. Studies of human H5N1/03 isolates, which possess an NS gene different from that of H5N1/97 viruses, show that these human influenza viruses also induce high levels of TNF-α and IFN-induced protein 10 in infected patients and in a cell model in vitro (23, 59). Therefore, the ability to induce a cytokine imbalance is probably an important factor in influenza virus pathogenicity and a polygenic property that is significantly influenced by host factors.

TRANSMISSIBILITY OF INFLUENZA VIRUSES

The capacity of influenza virus to infect and efficiently replicate in a susceptible host and cause disease (i.e., its pathogenicity) is important but not the primary factor determining the virus's emergence as a new influenza subtype in humans. A key feature of a potentially pandemic influenza virus is its ability to spread efficiently from infected to noninfected hosts (i.e., its transmissibility). The molecular basis of influenza virus transmissibility remains unresolved. Studies of human H3N2 viruses showed that amino acid changes accompanying transmission among humans accumulated in HA (24). These changes may be related to antibody pressure. Experimental transmission studies of H3N2 viruses in ferrets that were seronegative for influenza virus also showed the accumulation of amino acid changes in HA (33). Experiments with reverse-genetically derived H9N2 reassortant viruses have shown that amino acids in the HA control the efficiency of transmission of H9N2 influenza viruses in quail and chickens (52, 62). The available evidence therefore supports the role of the HA of influenza virus as one determinant of virus transmission. However, the data are currently meager and it is probable that other molecular determinants in the virus and the host determine the efficiency of transmissibility.

VACCINES

The ideal way to combat the emergence of new influenza viruses in humans is to inhibit or at least reduce the likelihood of interspecies transfer. The culling of infected poultry is the time-honored method of achieving this goal. This strategy was successful in Hong Kong in 1997 and in The Netherlands in 2003, and time will tell whether it will be successful in Asia in 2004. The culling of infected poultry reduces the viral load and the likelihood of transmission to humans. Unfortunately, attempts to control emerging influenza viruses by quarantine and physical containment are unlikely to be successful. Since 1997, much has been learned about the role of domestic poultry in human disease through research and increased surveillance. Although the information obtained has led to some practical changes, particularly in Hong Kong's live bird markets, human infection with H5N1, H7N7, and H9N2 viruses has continued. Despite the widespread emergence of H5N1 influenza viruses in poultry in many countries in Asia in early 2004, there were no outbreaks of H5N1 influenza in poultry or humans in Hong Kong during this time. This lack of outbreaks probably reflects changes in live poultry marketing practices since 1997 in Hong Kong (75), improvement in biosecurity, and the use of inactivated H5N1 vaccine on poultry farms in the region (50, 93). These farms also utilize unvaccinated sentinel birds in each flock to ensure that vaccinated birds are not shedding transmissible levels of H5N1 virus. While the optimal method of eradication of H5N1 influenza is the culling of poultry when the outbreak is widespread, this course of action may not be possible and the alternative strategy is culling plus vaccination. In the absence of an ability to stop interspecies transfer, emphasis must be placed on alternative vaccine strategies and rapid vaccine production capabilities.

Egg-grown inactivated influenza vaccine makes up the bulk of the human vaccines currently used. Although in most interpandemic influenza seasons this method of production works well, it has inherent limitations (13, 89, 94). Of particular concern is that these limitations are unavoidable and in some instances prohibitive to the production of vaccines against some potentially pandemic strains. Indeed, many of the limitations are not specific to egg-grown inactivated vaccine but apply to other methods of vaccine production as well.

The viruses that pose the greatest problems in vaccine production are the highly pathogenic H5 and H7 subtypes (i.e., those that in our opinion have the greatest pandemic potential). The main problems are the requirement for high-level biocontainment facilities to handle these viruses and, in some cases, an inability to obtain high yields of virus in embryonated chickens' eggs (64, 94, 96). Recent advances in plasmid-based reverse-genetics technologies (15, 34, 57) do, however, provide ways in which these obstacles may be overcome. Plasmid-based systems allow the alteration of a prime determinant of virulence in H5 and H7 viruses: the connecting peptide in HA (9,

43, 78, 90). Experimental H5N1 vaccines have been produced by using these technologies and have been shown to be safe and efficacious in animal models (50, 82).

The best preparation for an influenza outbreak arising from a newly emerging virus requires the availability of all possible avenues of vaccine production and manufacture. Official approval of reverse-genetics methodology, cell-based production systems, and the use of alternative adjuvants to create vaccine will greatly expand current capabilities for vaccine production with live attenuated and inactivated egg-grown vaccine (see Kemble and Greenberg [41] for a review of alternative vaccine strategies). The time to introduce and approve these techniques is now. Although this review concentrates on the pandemic potential of emerging influenza viruses, many of the new technologies could be useful during interpandemic periods.

One of the greatest needs in 2004 is for standardized vaccines that can be used for poultry in Asia. Commercially available vaccines to A/chicken/Mexico/232/94 (H5N2) are efficacious against H5N1 viruses from 2002 (50). So far in 2004, a lack of H5N1 on farms in Hong Kong suggests that these vaccines are also efficacious against the 2004 H5N1 strain. Therefore, despite only 94% homology between the HA of the A/chicken/Mexico/232/94 (H5N2) vaccine virus and that of the 2003 H5N1 viruses, the vaccine is efficacious. Chicken vaccines apparently do not have to be as well matched antigenically as human vaccines, but the mechanism underlying this heterotypic immunity is unresolved.

It should be noted that the eradication of an emerging virus is the optimal strategy of control. In agriculture, this aim is achieved by the culling of infected flocks. However, vaccination is an option when widespread infection has occurred.

Of great concern is that agricultural vaccines are not standardized for antigen content and that substandard vaccines may be used. There is some speculation that the use of substandard vaccines may have led to the selection of the H5N1 variants currently circulating in Asia. Experience in Hong Kong in 1997 showed that cross-protective immunity can prevent disease signs but not the shedding of virus (70). Indeed, the 1997 outbreak of H5N1 influenza in humans was epidemiologically and virologically traced to live poultry markets that contained apparently healthy birds that had been infected with H9N2 virus that provided cell-mediated protection from death (70), but the birds continued to shed virus in their feces.

ANTIVIRAL DRUGS

Although vaccination is the ideal way to reduce the interspecies spread of influenza viruses, the preparation of a new vaccine takes 6 months or more. In the interim, antiviral drugs are the only option.

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 (87). 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 (56, 74).

The main drawbacks to the use of M2 blockers are that drug-resistant variants develop rapidly and that these agents are ineffective against influenza B virus (31, 32). By days 5 to 7 of therapy, 16 to 35% of isolates from treated patients may be resistant, and these drug-resistant variants are fully pathogenic and transmissible to close contacts (14). Amantadine- and rimantadine-resistant mutants are characterized by mutations in the transmembrane domain of the M2 protein; single-amino-acid mutations have been identified at residues 26, 27, 30, 31, and 34, and mutations in codon 31 are the most common (36, 44). NA inhibitors are more costly, but they are active against influenza A and B viruses and elicit fewer side effects; in addition, the emergence of drug-resistant variants has been reported in fewer than 1% of treated adults (55). Influenza virus mutants with reduced susceptibility to zanamivir or oseltamivir carboxylate have been selected in the presence of increased concentrations of the drug in vitro. Two mechanisms of resistance were identified: NA-independent and NA-dependent resistance. The former mechanism is accompanied by mutations in or near the HA receptor-binding site and reduces the efficiency of virus binding to cellular receptors (27); the latter mechanism leads to amino acid substitutions at the conserved residues in the active site of NA, most frequently at positions 292 (Arg→Lys) and 119 (Glu→Gly) (4, 55, 77, 83).

In the face of an emerging new influenza virus, antiviral drugs would clearly be the most important short-term resource, especially if effective vaccines were not available. A number of factors can make an antiviral drug useful in response to novel viruses, including a broad antiviral spectrum and potency, prophylactic and therapeutic effectiveness, favorable pharmacokinetics, availability to the population at risk, and tolerability and safety. 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 (unpublished data). The NA inhibitors have been tested against a few avian influenza viruses: zanamivir was shown to protect mice against lethal challenge with A/HK/156/97 (H5N1) influenza virus and to protect chickens from a highly pathogenic A/chicken/Victoria/1/85 (H7N7) virus (25, 26). The use of the orally administered NA inhibitor oseltamivir is an effective treatment for H5N1 and H9N2 influenza virus infections in mice (21, 47). The efficacy of therapy for infection with highly pathogenic influenza virus could be improved by the use of combination therapy with two different classes of anti-influenza drugs to target different viral proteins, providing that the virus is not resistant to either drug. The possible role of antiviral drugs in response to pandemic influenza will depend on a number of issues, of which one of the most important is whether enough drugs will be available for the target population. In the face of a natural outbreak or a bioterrorism event involving highly pathogenic influenza viruses, information on the optimal use of limited supplies and long-term stockpiling of antiviral drug supplies are essential.

CONCLUDING REMARKS

In mid-2003, an unprecedented near-simultaneous emergence of highly pathogenic H5N1 influenza viruses in poultry

occurred in Indonesia, Vietnam, and Thailand and was followed in early 2004 by outbreaks in Japan, South Korea, Cambodia, Laos, and China. These events concluded with the transmission of the virus to humans in Vietnam and Thailand (Fig. 2), with death occurring in a high percentage of confirmed cases. It is unknown whether these transmissions will result in the establishment of an H5N1 lineage in humans, but the very large number of infected poultry in many countries and the rising number of infected humans increase this likelihood. At the time of writing it appears that the culling of poultry will again be successful in reducing the likelihood of human cases of H5N1 influenza and human-to-human transmission. The role of the live animal markets in the emergence of influenza viruses is well established (reviewed by Webster [93]), and such markets may also be the source of severe acute respiratory syndrome; improvements in the sanitation and vaccination of poultry in Hong Kong show what can be done at the animal-human interface to reduce the possibility of the emergence of new strains.

Unanswered questions concerning H5N1 influenza virus in Asia at the time of writing are many and include the following.

(i) Will the H5N1 threat to humans continue to decline, or will a rare mutation or reassortant event result in human-to-human transmission?

(ii) Has the virus been transmitted to pigs, the hypothetical intermediate host, and can the virus be transmitted from pig to pig? The available information indicates that pigs can be infected, but preliminary serological surveillance in Vietnam does not support the idea of widespread infection.

(iii) Will the H5N1 virus become endemic in poultry in Asia and eventually spread worldwide?

(iv) What is the role of wild migrating aquatic birds in the spread of H5N1 virus? Crows and magpies have been implicated in the local spread of H5N1 in Japan and South Korea. The role of migrating birds in the spread of H5N1 is a key question to be resolved.

(v) Will the use of nonstandardized agricultural vaccines prevent the spread of the virus or perhaps hasten its evolution?

(vi) Can a vaccine made by using reverse-genetics technology be approved, manufactured, and tested before human-to-human spread occurs?

(vii) Can NA inhibitors provide sufficient levels of prophylactic and therapeutic effectiveness against these highly pathogenic viruses, and will antineuraminidase-resistant viruses emerge if these drugs are widely used?

The number of influenza virus H5 and H7 events in the past year including the recent outbreaks of H7N3 in poultry and humans in Canada, H5N2 in poultry in Texas, and H7N7 in 2004 in The Netherlands raises the possibility of influenza virus gene transfer to the wild aquatic bird reservoir.

We speculate that influenza virus gene combinations for transmissibility and pathogenicity have been transmitted from domestic poultry back to the influenza virus reservoir in wild migrating aquatic birds. The alternative speculation is that converging factors imposed by humans, including changing ecosystems, human demographics, ecological factors, technology and industry, and international travel and communications, as reviewed in the recent Institute of Medicine Report (76), are responsible for the upturn in the number of influenza virus infections that are a threat to humans and to their food supply.

The options available for the control of emerging influenza viruses have greatly increased with the introduction of reverse genetics to rapidly prepare vaccines and with the development of antiviral drugs. Unresolved matters are the acceptance of genetically modified vaccines for use in humans, intellectual property rights and liability issues, and the political will to continue the stockpiles of drugs. We urgently need to address these problems before a crisis situation arises.

ACKNOWLEDGMENTS

We thank Janet R. Davies for editorial assistance and Andrea Blevins for illustrations. We thank Carol Walsh and Francis Wong for manuscript preparation and administrative assistance.

Influenza virus research at St. Jude Children's Research hospital is supported by NIAID contract AI95357 and Cancer Center Support (CORE) grant CA-21765 from the National Institutes of Health and by the American Lebanese Syrian Associated Charities (ALSAC). At the University of Hong Kong, influenza virus studies were supported by contract AI95357 from the National Institutes of Health (Bethesda, Md.), by Wellcome Trust grant 067072/D/02/Z, and by the Ellison Medical Foundation.

REFERENCES

1. **Alexander, D. J.** 2003. Report on avian influenza in the Eastern Hemisphere during 1997–2002. Avian Dis. **47**(Suppl. 3)**:**792–797.
2. **Anonymous.** 2003. Avian influenza virus reappears in Hong Kong Special Administrative Region. Bull. W. H. O. **81:**232.
3. **Basler, C. F., A. H. Reid, J. K. Dybing, T. A. Janczewski, T. G. Fanning, H. Zheng, M. Salvatore, M. L. Perdue, D. E. Swayne, A. Garcia-Sastre, P. Palese, and J. K. Taubenberger.** 2001. Sequence of the 1918 pandemic influenza virus nonstructural gene (NS) segment and characterization of recombinant viruses bearing the 1918 NS genes. Proc. Natl. Acad. Sci. USA **98:**2746–2751.
4. **Blick, T. J., T. Tiong, A. Sahasrabudhe, J. N. Varghese, P. M. Colman, G. J. Hart, R. C. Bethell, and J. L. McKimm-Breschkin.** 1995. Generation and characterization of an influenza virus neuraminidase variant with decreased sensitivity to the neuraminidase-specific inhibitor 4-guanidino-Neu5Ac2en. Virology **214:**475–484.
5. **Brown, I. H., P. Chakraverty, P. A. Harris, and D. J. Alexander.** 1995. Disease outbreaks in pigs in Great Britain due to an influenza A virus of H1N2 subtype. Vet. Rec. **136:**328–329.
6. **Brown, I. H.** 2000. The epidemiology and evolution of influenza viruses in pigs. Vet. Microbiol. **74:**29–46.
7. **Campitelli, L., I. Donatelli, E. Foni, M. R. Castrucci, C. Fabiani, Y. Kawaoka, S. Krauss, and R. G. Webster.** 1997. Continued evolution of H1N1 and H3N2 influenza viruses in pigs in Italy. Virology **232:**310–318.
8. **Cauthen, A. N., D. E. Swayne, S. Schultz-Cherry, M. L. Perdue, and D. L. Suarez.** 2000. Continued circulation in China of highly pathogenic avian influenza viruses encoding the hemagglutinin gene associated with the 1997 H5N1 outbreak in poultry and humans. J. Virol. **74:**6592–6599.
9. **Chen, J., K. H. Lee, D. A. Steinhauer, D. J. Stevens, J. J. Skehel, and D. C. Wiley.** 1998. Structure of the hemagglutinin precursor cleavage site, a determinant of influenza pathogenicity and the origin of the labile conformation. Cell **95:**409–417.
10. **Cheung, C. Y., L. L. M. Poon, A. S. Lau, W. Luk, Y. L. Lau, K. F. Shortridge, S. Gordon, Y. Guan, and J. S. M. Peiris.** 2002. Induction of proinflammatory cytokines in human macrophages by influenza A (H5N1) viruses: a mechanism for the unusual severity of human disease? Lancet **360:**1831–1837.
11. **Choi, Y. K., S. M. Goyal, M. W. Farnham, and H. S. Joo.** 2002. Phylogenetic analysis of H1N2 isolates of influenza A virus from pigs in the United States. Virus Res. **87:**173–179.
12. **Claas, E. C., A. D. Osterhaus, R. van Beek, J. C. de Jong, G. F. Rimmelzwaan, D. A. Senne, S. Krauss, K. F. Shortridge, and R. G. Webster.** 1998. Human influenza A H5N1 virus related to a highly pathogenic avian influenza virus. Lancet **351:**472–477.
13. **Fedson, D. S.** 2003. Pandemic influenza and the global vaccine supply. Clin. Infect. Dis. **36:**1552–1561.
14. **Fleming, D. M.** 2001. Managing influenza: amantadine, rimantadine and beyond. Int. J. Clin. Pract. **55:**189–195.
15. **Fodor, E., L. Devenish, O. G. Engelhardt, P. Palese, G. G. Brownlee, and A. Garcia-Sastre.** 1999. Rescue of influenza A virus from recombinant DNA. J. Virol. **73:**9679–9682.
16. **Fouchier, R. A., P. M. Schneeberger, F. W. Rozendaal, J. M. Broekman, S. A.**

Kemink, V. Munster, T. Kuiken, G. F. Rimmelzwaan, M. Schutten, G. J. Van Doornum, G. Koch, A. Bosman, M. Koopmans, and A. D. M. E. Osterhaus. 2004. Avian influenza A virus (H7N7) associated with human conjunctivitis and a fatal case of acute respiratory distress syndrome. Proc. Natl. Acad. Sci. USA **101:**1356–1361.

17. **Gao, P., S. Watanabe, T. Ito, H. Goto, K. Wells, M. McGregor, A. J. Cooley, and Y. Kawaoka.** 1999. Biological heterogeneity, including systemic replication in mice, of H5N1 influenza A virus isolates from humans in Hong Kong. J. Virol. **73:**3184–3189.
18. **Garcia-Sastre, A.** 2001. Inhibition of interferon-mediated antiviral responses by influenza A viruses and other negative-strand RNA viruses. Virology **279:**375–384.
19. **Garcia-Sastre, A.** 2002. Mechanisms of inhibition of the host interferon α/β-mediated antiviral response by viruses. Microbes Infect. **4:**647–655.
20. **Geiss, G. K., M. Salvatore, T. M. Tumpey, V. S. Carter, X. Wang, C. F. Basler, J. K. Taubenberger, R. E. Bumgarner, P. Palese, M. G. Katze, and A. Garcia-Sastre.** 2002. Cellular transcriptional profiling in influenza A virus-infected lung epithelial cells: the role of the nonstructural NS1 protein in the evasion of the host innate defense and its potential contribution for pandemic influenza. Proc. Natl. Acad. Sci. USA **99:**10736–10741.
21. **Govorkova, E. A., I. A. Leneva, O. G. Goloubeva, K. Bush, and R. G. Webster.** 2001. Comparison of the efficacies of RWJ-270201, zanamivir, and oseltamivir against H5N1, H9N2, and other avian influenza viruses. Antimicrob. Agents Chemother. **45:**2723–2732.
22. **Guan, Y., J. S. M. Peiris, A. S. Lipatov, T. M. Ellis, K. C. Dyrting, S. Krauss, L. J. Zhang, R. G. Webster, and K. F. Shortridge.** 2002. Emergence of multiple genotypes of H5N1 avian influenza viruses in Hong Kong SAR. Proc. Natl. Acad. Sci. USA **99:**8950–8955.
23. **Guan, Y., L. L. M. Poon, C. Y. Cheung, T. M. Ellis, W. Lim, A. S. Lipatov, K. H. Chan, K. M. Sturm-Ramirez, C. L. Cheung, Y. H. C. Leung, K. Y. Yuen, R. G. Webster, and J. S. M. Peiris.** 2004. H5N1 influenza: a protean pandemic threat. Proc. Natl. Acad. Sci. USA **101:**8156–8161.
24. **Gubareva, L. V., D. V. Novikov, and F. G. Hayden.** 2002. Assessment of hemagglutinin sequence heterogeneity during influenza virus transmission in families. J. Infect. Dis. **186:**1575–1581.
25. **Gubareva, L. V., C. R. Penn, and R. G. Webster.** 1995. Inhibition of replication of avian influenza viruses by the neuraminidase inhibitor 4-guanidino-2,4-dideoxy-2,3-dehydro-N-acetylneuraminic acid. Virology **212:**323–330.
26. **Gubareva, L. V., J. A. McCullers, R. C. Bethell, and R. G. Webster.** 1998. Characterization of influenza A/Hong Kong/156/97 (H5N1) virus in a mouse model and protective effect of zanamivir on H5N1 infection in mice. J. Infect. Dis. **178:**1592–1596.
27. **Gubareva, L. V., L. Kaiser, and F. G. Hayden.** 2000. Influenza virus neuraminidase inhibitors. Lancet **4:**827–835.
28. **Guo, Y., J. Li, X. Cheng, M. Wang, Y. Zhou, X. H. Li, F. Cai, H. L. Miao, H. Zhang, and F. Guo.** 1999. Discovery of humans infected by avian influenza A (H9N2) virus. Chin. J. Exp. Clin. Virol. **13:**105–108.
29. **Guo, Y. J., S. Krauss, D. A. Senne, I. P. Mo, K. S. Lo, X. P. Xiong, M. Norwood, K. F. Shortridge, R. G. Webster, and Y. Guan.** 2000. Characterization of the pathogenicity of members of the newly established H9N2 influenza virus lineages in Asia. Virology **267:**279–288.
30. **Hatta, M., P. Gao, P. Halfmann, and Y. Kawaoka.** 2001. Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. Science **293:**1840–1842.
31. **Hayden, F. G., and H. J. Hay.** 1992. Emergence and transmission of influenza A viruses resistant to amantadine and rimantadine. Curr. Top. Microbiol. Immunol. **176:**119–130.
32. **Hayden, F. G.** 1996. Amantadine and rimantadine—clinical aspects, p. 59–77. *In* D. D. Richman (ed.), Antiviral drug resistance. John Wiley and Sons, Ltd., New York, N.Y.
33. **Herlocher, M. L., S. Elias, R. Truscon, S. Harrison, D. Mindell, C. Simon, and A. S. Monto.** 2001. Ferrets as a transmission model for influenza: sequence changes in HA1 of type A (H3N2) virus. J. Infect. Dis. **184:**542–546.
34. **Hoffmann, E., G. Neumann, Y. Kawaoka, G. Hobom, and R. G. Webster.** 2000. A DNA transfection system for generation of influenza A virus from eight plasmids. Proc. Natl. Acad. Sci. USA **97:**6108–6113.
35. **Ito, T., J. N. Couceiro, S. Kelm, L. G. Baum, S. Krauss, M. R. Castrucci, I. Donatelli, H. Kida, J. C. Paulson, R. G. Webster, and Y. Kawaoka.** 1998. Molecular basis for the generation in pigs of influenza A viruses with pandemic potential. J. Virol. **72:**7367–7373.
36. **Iwahashi, J., K. Tsuji, T. Ishibashi, J. Kajiwara, Y. Imamura, R. Mori, K. Hara, T. Kashiwagi, Y. Ohtsu, N. Hamada, H. Maeda, M. Toyoda, and T. Toyoda.** 2001. Isolation of amantadine-resistant influenza A viruses (H3N2) from patients following administration of amantadine in Japan. J. Clin. Microbiol. **39:**1652–1653.
37. **Karasin, A. I., M. M. Schutten, L. A. Cooper, C. B. Smith, K. Subbarao, G. A. Anderson, S. Carman, and C. W. Olsen.** 2000. Genetic characterization of H3N2 influenza viruses isolated from pigs in North America, 1977–1999: evidence for wholly human and reassortant virus genotypes. Virus Res. **68:**71–85.
38. **Karasin, A. I., J. Landgraf, S. Swenson, G. Erickson, S. Goyal, M. Woodruff, G. Scherba, G. Anderson, and C. W. Olsen.** 2002. Genetic characterization of H1N2 influenza A viruses isolated from pigs throughout the United States. J. Clin. Microbiol. **40:**1073–1079.
39. **Katz, J. M., X. Lu, T. M. Tumpey, C. B. Smith, M. W. Shaw, and K. Subbarao.** 2000. Molecular correlates of influenza A H5N1 virus pathogenesis in mice. J. Virol. **74:**10807–10810.
40. **Kawaoka, Y., S. Krauss, and R. G. Webster.** 1989. Avian-to-human transmission of the PB1 gene of influenza A viruses in the 1957 and 1968 pandemics. J. Virol. **63:**4603–4608.
41. **Kemble, G., and H. Greenberg.** 2003. Novel generations of influenza vaccines. Vaccine **21:**1789–1795.
42. **Kida, H., T. Ito, J. Yasuda, Y. Shimizu, C. Itakura, K. F. Shortridge, Y. Kawaoka, and R. G. Webster.** 1994. Potential for transmission of avian influenza viruses to pigs. J. Gen. Virol. **75:**2183–2188.
43. **Klenk, H.-D., and W. Garten.** 1994. Host cell proteases controlling virus pathogenicity. Trends Microbiol. **2:**39–43.
44. **Klimov, A. I., E. Rocha, F. G. Hayden, P. A. Shult, L. F. Roumillat, and N. J. Cox.** 1995. Prolonged shedding of amantadine-resistant influenza A viruses by immunodeficient patients: detection by polymerase chain reaction-restriction analysis. J. Infect. Dis. **172:**1352–1355.
45. **Koopmans, M., R. Fouchier, B. Wilbrink, A. Meijer, G. Natrop, A. D. M. E. Osterhaus, J. E. van Steenbergen, M. du Ry van Beest Holle, M. A. E. Conyn van Spaendonck, and A. Bosman.** 2003. Update on human infections with highly pathogenic avian influenza virus A/H7N7 during an outbreak in poultry in the Netherlands. Eurosurveill. Wkly. **7:**1–4.
46. **Krug, R. M., W. Yuan, D. L. Noah, and A. G. Latham.** 2003. Intracellular warfare between human influenza viruses and human cells: the roles of the viral NS1 protein. Virology **309:**181–189.
47. **Leneva, I. A., N. Roberts, E. A. Govorkova, O. G. Goloubeva, and R. G. Webster.** 2000. The neuraminidase inhibitor GS4104 (oseltamivir phosphate) is efficacious against A/Hong Kong/156/97 (H5N1) and A/Hong Kong/1074/99 (H9N2) influenza viruses. Antivir. Res. **48:**101–115.
48. **Lin, Y. P., M. Shaw, V. Gregory, K. Cameron, W. Lim, A. Klimov, K. Subbarao, Y. Guan, S. Krauss, K. Shortridge, R. Webster, N. Cox, and A. Hay.** 2000. Avian-to-human transmission of H9N2 subtype influenza A viruses: relationship between H9N2 and H5N1 human isolates. Proc. Natl. Acad. Sci. USA **97:**9654–9658.
49. **Lipatov, A. S., S. Krauss, Y. Guan, M. Peiris, J. E. Rehg, D. R. Perez, and R. G. Webster.** 2003. Neurovirulence in mice of H5N1 influenza virus genotypes isolated from Hong Kong poultry in 2001. J. Virol. **77:**3816–3823.
50. **Liu, M., J. M. Wood, T. Ellis, S. Krauss, P. Seiler, C. Johnson, E. Hoffmann, J. Humberd, D. Hulse, Y. Zhang, R. G. Webster, and D. R. Perez.** 2003. Preparation of a standardized, efficacious agricultural H5N3 vaccine by reverse genetics. Virology **314:**580–590.
51. **Lu, X., T. M. Tumpey, T. Morken, S. R. Zaki, N. J. Cox, and J. M. Katz.** 1999. A mouse model for the evaluation of pathogenesis and immunity to influenza A (H5N1) viruses isolated from humans. J. Virol. **73:**5903–5911.
52. **Makarova, N. V., H. Ozaki, H. Kida, R. G. Webster, and D. R. Perez.** 2003. Replication and transmission of influenza viruses in Japanese quail. Virology **310:**8–15.
53. **Matrosovich, M., N. Zhou, Y. Kawaoka, and R. Webster.** 1999. The surface glycoproteins of H5 influenza viruses isolated from humans, chickens, and wild aquatic birds have distinguishable properties. J. Virol. **73:**1146–1155.
54. **Matrosovich, M. N., S. Krauss, and R. G. Webster.** 2001. H9N2 influenza A viruses from poultry in Asia have human virus-like receptor specificity. Virology **281:**156–162.
55. **McKimm-Breschkin, J. L.** 2000. Resistance of influenza viruses to neuraminidase inhibitors—a review. Antivir. Res. **47:**1–17.
56. **Mendel, D. B., C. Y. Tai, P. A. Escarpe, W. X. Li, R. W. Sidwell, J. H. Huffman, C. Sweet, K. J. Jakeman, J. Merson, S. A. Lacy, W. Lew, M. A. Williams, L. Zhang, M. S. Chen, N. Bishofberger, and C. U. Kim.** 1998. Oral administration of a prodrug of the influenza virus neuraminidase inhibitor GS 4071 protects mice and ferrets against influenza infection. Antimicrob. Agents Chemother. **42:**640–646.
57. **Neumann, G., T. Watanabe, H. Ito, S. Watanabe, H. Goto, P. Gao, M. Hughes, D. R. Perez, R. Donis, E. Hoffmann, G. Hobom, and Y. Kawaoka.** 1999. Generation of influenza A viruses entirely from cloned cDNAs. Proc. Natl. Acad. Sci. USA **96:**9345–9350.
58. **Peiris, J. S., Y. Guan, D. Markwell, P. Ghose, R. G. Webster, and K. F. Shortridge.** 2001. Cocirculation of avian H9N2 and contemporary "human" H3N2 influenza A viruses in pigs in southeastern China: potential for genetic reassortment? J. Virol. **75:**9679–9686.
59. **Peiris, J. S. M., W. C. Yu, C. W. Leung, C. Y. Cheung, W. F. Ng, J. M. Nicholls, T. K. Ng, K. H. Chan, S. T. Lai, W. L. Lim, K. Y. Yuen, and Y. Guan.** 2004. Re-emergence of fatal human influenza A subtype H5N1 disease. Lancet **363:**617–619.
60. **Peiris, M., K. Y. Yuen, C. W. Leung, K. H. Chan, P. L. Ip, R. W. Lai, W. K. Orr, and K. F. Shortridge.** 1999. Human infection with influenza H9N2. Lancet **354:**916–917.
61. **Pensaert, M., K. Ottis, J. Vandeputte, M. M. Kaplan, and P. A. Bachmann.** 1981. Evidence for the natural transmission of influenza A virus from wild ducks to swine and its potential importance for man. Bull. W. H. O. **59:**75–78.

62. **Perez, D. R., W. Lim, J. P. Seiler, Y. Guan, M. Peiris, K. F. Shortridge, and R. G. Webster.** 2003. Role of quail in the interspecies transmission of H9 influenza A viruses: molecular changes on HA that correspond to adaptation from ducks to chickens. J. Virol. **77:**3148–3156.
63. **Rimmelzwaan, G. F., T. Kuiken, G. van Amerongen, T. M. Bestebroer, R. A. Fouchier, and A. D. Osterhaus.** 2001. Pathogenesis of influenza A (H5N1) virus infection in a primate model. J. Virol. **75:**6687–6691.
64. **Rowe, T.** 2000. Biocontainment of highly pathogenic avian influenza viruses, p. 16–34. *In* J. Richmond (ed.), Anthology of biosafety, vol. II. Facility design considerations. American Biological Safety Association, Mundelein, Ill.
65. **Scholtissek, C., H. Burger, P. A. Bachmann, and C. Hannoun.** 1983. Genetic relatedness of hemagglutinins of the H1 subtype of influenza A viruses isolated from swine and birds. Virology **129:**521–523.
66. **Scholtissek, C.** 1990. Pigs as the "mixing vessel" for the creation of new pandemic influenza A viruses. Med. Princ. Pract. **2:**65–71.
67. **Schultz, U., W. M. Fitch, S. Ludwig, J. Mandler, and C. Scholtissek.** 1991. Evolution of pig influenza viruses. Virology **183:**61–73.
68. **Seo, S. H., and R. G. Webster.** 2002. Tumor necrosis factor alpha exerts powerful anti-influenza virus effects in lung epithelial cells. J. Virol. **76:**1071–1076.
69. **Seo, S. H., E. Hoffmann, and R. G. Webster.** 2002. Lethal H5N1 influenza viruses escape host anti-viral cytokine responses. Nat. Med. **8:**950–954.
70. **Seo, S. H., and R. G. Webster.** 2001. Cross-reactive, cell-mediated immunity and protection of chickens from lethal H5N1 influenza virus infection in Hong Kong poultry markets. J. Virol. **75:**2516–2525.
71. **Shinya, K., S. Hamm, M. Hatta, H. Ito, T. Ito, and Y. Kawaoka.** 2004. PB2 amino acid at position 627 affects replicative efficiency, but not cell tropism, of Hong Kong H5N1 influenza A viruses in mice. Virology **320:**258–266.
72. **Shortridge, K. F.** 1992. Pandemic influenza: a zoonosis? Semin. Respir. Infect. **7:**11–25.
73. **Shortridge, K. F., N. N. Zhou, Y. Guan, P. Gao, T. Ito, Y. Kawaoka, S. Kodihalli, S. Krauss, D. Markwell, K. G. Murti, M. Norwood, D. Senne, L. Sims, A. Takada, and R. G. Webster.** 1998. Characterization of avian H5N1 influenza viruses from poultry in Hong Kong. Virology **252:**331–342.
74. **Sidwell, R. W., J. H. Huffman, D. L. Barnard, K. W. Bailey, M. N. Wong, A. Morrison, T. Syndergaard, and C. U. Kim.** 1998. Inhibition of influenza virus infections in mice by GS4104, an orally effective influenza virus neuraminidase inhibitor. Antivir. Res. **37:**107–120.
75. **Sims, L. D., T. M. Ellis, K. K. Liu, K. Dyrting, H. Wong, M. Peiris, Y. Guan, and K. F. Shortridge.** 2003. Avian influenza in Hong Kong 1997–2002. Avian Dis. **47**(Suppl. 3)**:**832–838.
76. **Smolinski, M. S., M. A. Hamburg, and J. Lederberg (ed.).** 2004. Microbial threats to health. Emergence, detection, and response. National Academies Press, Washington, D.C.
77. **Staschke, K. A., J. M. Colacino, A. J. Baxter, G. M. Air, A. Bansal, W. J. Hornback, J. E. Munroe, and W. G. Laver.** 1995. Molecular basis for the resistance of influenza viruses to 4-guanidino-Neu5Ac2en. Virology **214:** 642–646.
78. **Steinhauer, D. A.** 1999. Role of hemagglutinin cleavage for the pathogenicity of influenza virus. Virology **258:**1–20.
79. **Sturm-Ramirez, K. M., T. Ellis, B. Bousfield, L. Bissett, K. Dyrting, J. E. Rehg, L. Poon, Y. Guan, M. Peiris, and R. G. Webster.** 2004. Re-emerging H5N1 influenza viruses in Hong Kong in 2002 are highly pathogenic to ducks. J. Virol. **78:**4892–4901.
80. **Suarez, D. L., E. Spackman, and D. A. Senne.** 2003. Update on molecular epidemiology of H1, H5, and H7 influenza virus infections in poultry in North America. Avian Dis. **47**(Suppl. 3)**:**888–897.
81. **Subbarao, K., A. Klimov, J. Katz, H. Regnery, W. Lim, H. Hall, M. Perdue, D. Swayne, C. Bender, J. Huang, M. Hemphill, T. Rowe, M. Shaw, X. Xu, K. Fukuda, and N. Cox.** 1998. Characterization of an avian influenza A (H5N1) virus isolated from a child with a fatal respiratory illness. Science **279:**393–396.
82. **Subbarao, K., H. Chen, D. Swayne, L. Mingay, E. Fodor, G. Brownlee, X. Xu, X. Lu, J. Katz, N. Cox, and Y. Matsuoka.** 2003. Evaluation of a genetically modified reassortant H5N1 influenza A virus vaccine candidate generated by plasmid-based reverse genetics. Virology **305:**192–200.
83. **Tai, C. Y., P. A. Escarpe, R. W. Sidwell, M. A. Williams, W. Lew, H. Wu, C. U. Kim, and D. B. Mendel.** 1998. Characterization of human influenza virus variants selected in vitro in the presence of the neuraminidase inhibitor GS 4071. Antimicrob. Agents Chemother. **42:**3234–3241.
84. **Taubenberger, J. K., A. H. Ried, T. A. Janczeqski, and T. G. Fanning.** 2001. Integrating historical, clinical and molecular genetic data in order to explain the origin and virulence of the 1918 Spanish influenza virus. Philos. Trans. R. Soc. Lond. B **356:**1829–1839.
85. **To, K. F., P. K. Chan, K. F. Chan, W. K. Lee, W. Y. Lam, K. F. Wong, N. L. Tang, D. N. Tsang, R. Y. Sung, T. A. Buckley, J. S. Tam, and A. F. Cheng.** 2001. Pathology of fatal human infection associated with avian influenza A H5N1 virus. J. Med. Virol. **63:**242–246.
86. **Tumpey, T. M., X. Lu, T. Morken, S. R. Zaki, and J. M. Katz.** 2000. Depletion of lymphocytes and diminished cytokine production in mice infected with a highly virulent influenza A (H5N1) virus isolated from humans. J. Virol. **74:**6105–6116.
87. **Wang, C., K. Takeuchi, L. H. Pinto, and R. A. Lamb.** 1993. Ion channel activity of influenza A virus M2 protein: characterization of the amantadine block. J. Virol. **67:**5585–5594.
88. **Webby, R. J., S. L. Swenson, S. L. Krauss, P. J. Gerrish, S. M. Goyal, and R. G. Webster.** 2000. Evolution of swine H3N2 influenza viruses in the United States. J. Virol. **74:**8243–8251.
89. **Webby, R. J., and R. G. Webster.** 2003. Are we ready for pandemic influenza? Science **302:**1519–1522.
90. **Webster, R. G., and R. Rott.** 1987. Influenza virus A pathogenicity: the pivotal role of hemagglutinin. Cell **50:**665–666.
91. **Webster, R. G., W. J. Bean, O. T. Gorman, T. M. Chambers, and Y. Kawaoka.** 1992. Evolution and ecology of influenza A viruses. Microbiol. Rev. **56:**152–179.
92. **Webster, R. G., Y. Guan, M. Peiris, D. Walker, S. Krauss, N. N. Zhou, E. A. Govorkova, T. M. Ellis, K. C. Dyrting, T. Sit, D. R. Perez, and K. F. Shortridge.** 2002. Characterization of H5N1 influenza viruses that continue to circulate in geese in southeastern China. J. Virol. **76:**118–126.
93. **Webster, R. G.** 2004. Wet markets—a continuing source of severe acute respiratory syndrome and influenza? Lancet **363:**234–236.
94. **Wood, J. M.** 2001. Developing vaccines against pandemic influenza. Philos. Trans. R. Soc. Lond. B **356:**1953–1960.
95. **Yuen, K. Y., P. K. S. Chan, M. Peiris, D. N. C. Tsang, T. L. Que, K. F. Shortridge, P. T. Cheung, W. K. To, E. T. F. Ho, R. Sung, A. F. B. Cheng, and members of the H5N1 Study Group.** 1998. Clinical features and rapid viral diagnosis of human disease associated with avian influenza A H5N1 virus. Lancet **351:**467–471.
96. **Zambon, M.** 1998. Laboratory containment for influenza A H5N1 viruses: level 2, level 3, or level 3+? Commun. Dis. Public Health **1:**71–72.
97. **Zhou, N. N., D. A. Senne, J. S. Landgraf, S. L. Swenson, G. Erickson, K. Rossow, L. Liu, K. Yoon, S. Krauss, and R. G. Webster.** 1999. Genetic reassortment of avian, swine, and human influenza A viruses in American pigs. J. Virol. **73:**8851–8856.
98. **Zhou, N. N., D. A. Senne, J. S. Landgraf, S. L. Swenson, G. Erickson, K. Rossow, L. Liu, K. J. Yoon, S. Krauss, and R. G. Webster.** 2000. Emergence of H3N2 reassortant influenza A viruses in North American pigs. Vet. Microbiol. **74:**47–58.
99. **Zitzow, L. A., T. Rowe, T. Morken, W.-J. Shieh, S. Zaki, and J. M. Katz.** 2002. Pathogenesis of avian influenza A (H5N1) viruses in ferrets. J. Virol. **76:**4420–4429.