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The Influenza Virus Enigma

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Abstract

Both seasonal and pandemic influenza continue to challenge both scientists and clinicians. Drug-resistant H1N1 influenza viruses have dominated the 2009 flu season, and the H5N1 avian influenza virus continues to kill both people and poultry in Eurasia. Here, we discuss the pathogenesis and transmissibility of influenza viruses and we emphasize the need to find better predictors of both seasonal and potentially pandemic influenza.

Introduction

Influenza is historically an ancient disease that causes annual epidemics and, at irregular intervals, pandemics. Seasonal influenza kills 36,000 persons annually in the United States. The impact of seasonal influenza caused by a virus showing antigenic variation in the major viral glycoproteins hemagglutinin (H) and neuraminidase (N) can be moderated by antigenically matched vaccines and anti-influenza drugs. The consequences of continuing genetic variation in seasonal influenza viruses are apparent in the current and prior influenza seasons. Despite intensive global surveillance, the H3N2 vaccine in the 2007–2008 season imperfectly matched the virus that emerged between vaccine selection and its use (6 months). In the current influenza season, the H1N1 virus that has become dominant is resistant to the anti-influenza drug oseltamivir (Tamiflu).

Pandemics that occur at irregular intervals can vary in severity from mild to catastrophic. The pandemics of the past century include the catastrophic H1N1 Spanish influenza of 1918 (more than 50 million deaths globally), the H2N2 Asian flu of 1957 (more than 1 million deaths globally), and the H3N2 Hong Kong flu of 1968 (~0.5 million deaths globally). The natural reservoirs of these influenza A viruses are aquatic birds, and the spread of influenza to humans occurs either by direct transmission (Spanish influenza) or by reassortment between the segmented RNA genomes of avian and human influenza viruses (the Asian and Hong Kong pandemics). Although we know the general mechanisms by which new influenza viruses emerge, our basic knowledge of how these viruses acquire human pandemic potential is minimal, and our molecular understanding of the virus and the host factors involved in successful transmission and spread is rudimentary.

A highly pathogenic H5N1 avian influenza virus has been circulating for more than a decade in Eurasia and has spread to more than 60 countries. It has infected 394 humans killing 248,

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with recent deaths reported in China, Indonesia, Vietnam, and Egypt. The occasional direct transmission of the virus to humans and its lethality suggest the possibility of a pandemic akin to the 1918 Spanish flu if consistent human-to-human transmission is achieved. We argue that it is premature to become complacent, and we identify research directions in influenza virus ecology and the molecular biology of pathogenesis and transmission that should enable the development of better predictors of seasonal and pandemic influenza and increased preparedness (Figure 1).

Reservoirs and Surveillance

The 16 hemagglutinin and 9 neuraminidase subtypes of influenza A virus are perpetuated in aquatic birds, in which they cause no apparent disease (Peiris et al., 2007). Only viruses of the H5 and H7 hemagglutinin subtypes can become highly pathogenic after transmission to alternative hosts. Each of the H5 and H7 lineages that are lethal to domestic poultry originated from nonpathogenic precursor viruses of Eurasian and American lineages (Alexander, 2007). However, until 1996, highly pathogenic H5 and H7 viruses either were eradicated or failed to persist in nature. Today, it is unknown whether the ecology of these viruses has changed and whether highly pathogenic H5N1 viruses continue to be propagated in domestic or wild bird reservoirs. The continued circulation of Asian H5N1 viruses of multiple clades (at least 10 different clades) and subclades is unprecedented.

The available evidence suggests that all of the pandemic influenza virus strains, including the Spanish 1918 (H1N1), Asian 1957 (H2N2), and Hong Kong 1968 (H3N2) viruses, originated from the avian influenza reservoir either by reassortment (swapping of viral genetic information in hosts coinfected with more than one influenza virus) or direct transfer (Kobasa et al., 2004). influenza outbreaks in domestic animals, including poultry, also originate from the avian reservoir. Our knowledge of the precursors of pandemic and panzootic influenza viruses is extremely limited. The available information indicates that viruses in their natural reservoirs undergo limited evolution, replicate primarily in the intestinal and respiratory tracts, and change their predominant subtypes every 2 years (Fouchier et al., 2003). Knowledge of the genomics of influenza viruses in this natural reservoir is fragmentary, and evidence suggests that there is continuing reassortment in nature (Dugan et al., 2008; Obenauer et al., 2006).

Analysis of the multiple lineages of highly pathogenic H5N1 viruses supports the contention that all of them arose in Southeast Asia (Kilpatrick et al., 2006; Smith et al., 2006). For example, the H5N1 virus that emerged at Qinghai Lake, China spread (probably in wild birds) to Europe, Africa, and India (Li et al., 2004). Similarly, the lineage that spread to Indonesia can be traced to China's Hunan Province (Wang et al., 2008). The domestic duck may be the "Trojan horse" of the H5N1 viruses, for many ducks show no signs of disease yet shed virus for up to 17 days after infection and propagate influenza virus anti-genic variants with low pathogenicity (Hulse et al., 2005). This hypothesis will be resolved only by detailed molecular epidemiological studies.

To date, there is no influenza surveillance system in lower animals and birds that is comparable to the well-organized, interactive Global influenza Surveillance Network (GISN) for human influenza. The pandemic threat of H5N1 influenza has resulted in closer collaborations between international agricultural and human health organizations. However, the lack of a counterpart of GISN at the human-animal interface is a serious shortfall in pandemic preparedness. A genomic library of all subtypes of influenza viruses in wild and domestic birds, continuously updated by high-throughput sequencing and analysis, is badly needed to identify predictors of pandemics.

Host Range and Transmission

Influenza viruses probably undergo genetic changes to spread from the wild bird reservoir to other hosts. Such changes are facilitated when multiple species of birds and mammals are housed in close proximity in live animal markets (Webster, 2004). It is unclear whether influenza viruses are transmitted directly from natural reservoirs to mammals, including humans. Notably, chickens are not susceptible to most of the low-pathogenicity subtypes, including nonpathogenic H5 and H7 strains, without adaptation (Swayne, 2007). The involvement of intermediate hosts, including the quail and the pig, has been suggested (Matrosovich et al., 1999), but there is no smoking gun. A suggested transmission scenario might follow this sequence: wild waterfowl → domestic waterfowl → quail/pig → chicken → human. All of these birds and some mammals are found in various live markets. Information about the molecular profiles that permit transmission between these species is emerging (Perez et al., 2003), but there is much still to learn. Expansion of the host range of the Asian H5N1 avian influenza virus to felines, viverrids, stone martens, and dogs has been associated with high pathogenicity and systemic spread (Rimmelzwaan et al., 2006; Songserm et al., 2006). Extension of the host range to fielid species remains to be elucidated at the molecular level; if domestic cats can serve as intermediate hosts, their infection would promote the selection of variants transmissible to humans.

The pig may be an intermediate host for interspecies spread; the replication of all avian viruses in pigs supports this notion, as does the presence of avian-type and mammalian-type virus receptors in pigs (Ludwig et al., 1995). The periodic transmission of avian influenza viruses to pigs in the absence of disease and the spread of human H1N1 and H3N2 viruses to pigs (Ma et al., 2007) are also consistent with the “mixing-vessel” hypothesis, but to date the pig has not been directly implicated in the generation of pandemic influenza viruses.

Viral Factors in Pathogenesis and Transmission

Receptor Specificity

A major enigma of influenza virus is whether alteration of viral specificity for host cell receptors (sialic acids) can generate a pandemic strain of virus. The viral hemagglutinin surface glycoprotein preferentially binds to certain sialic acid residues on host cells, making hemagglutinin a determinant of host range. Specific amino acid changes in hemagglutinin have been identified as important in sialic acid receptor specificity and pathogenicity (Matrosovich et al., 1999; Stevens et al., 2006b; Yamada et al., 2006). The hemagglutinin of human influenza virus isolates typically binds preferentially to α2,6-linked sialic acids, whereas that of avian influenza virus isolates has a higher affinity for α2,3-linked sialic acids (Ito, 2000). Interestingly, sialic acid receptors are distributed differently in the respiratory tracts of humans and other host species (Matrosovich et al., 2004; Shinya et al., 2006; van Riel et al., 2007). The human and ferret upper respiratory tract, believed to be the primary site of influenza infection, carries primarily α2,6-linked sialic acids, which gives human viral isolates a binding advantage. Receptor specificity must be studied at the level of the cell type to discern the relative susceptibility of cells to infection on the basis of sialic acid expression. This information will be of particular interest, as some H5N1 viruses cause systemic infection, including infection of brain cells.

Notably, the 1918, 1957, and 1968 pandemic strains all preferentially bind to α2,6-linked sialic acids (Stevens et al., 2006a), and so preferential affinity for these receptors may be necessary for emergence of a pandemic strain carrying an avian-derived hemagglutinin gene. However, avian isolates that bind preferentially to α2,3-linked sialic acids are lethal in humans and mammals and replicate well in the upper respiratory tract. Thus, it remains an open question whether H5N1 viruses must acquire specificity for binding to α2,6-linked sialic acids to become

pandemic. Interestingly, cultured human respiratory epithelial cells lacking α2,3-linked sialic acids could be infected ex vivo with H5N1 viruses (Nicholls et al., 2007). This finding together with advances in glycan array technology (Stevens et al., 2006a) suggest that receptor specificity may involve factors other than binding to α2,3-linked and α2,6-linked sialic acids. However, the biological relevance of receptor binding particularly for viral entry, replication, spread, tissue tropism, and transmission still needs to be determined.

Replication Efficiency

What other viral factors increase the virulence or transmission of influenza virus, and by what mechanism? Certain H5N1 viruses with a hemagglutinin that preferentially binds to α2,3-linked sialic acids replicate in humans and can be lethal, suggesting that genes other than that encoding hemagglutinin are crucial for virulence. The replication efficiency of influenza virus correlates with its virulence. Specific amino acid sequences encoded by the polymerase genes alone are sufficient to make a virus lethal in animal models (Gabriel et al., 2005; Hatta et al., 2001; Salomon et al., 2006). The best-described marker of pathogenicity is lysine at position 627 of polymerase subunit protein PB2 (Hatta et al., 2001; Subbarao et al., 1993). This residue enhances the growth efficiency of avian H5N1 viruses in the upper and lower respiratory tracts of mice. As the importance of specific polymerase residues to lethality is identified, it will be crucial to elucidate the mechanism by which these residues affect replication efficiency. Each of the eight negative-sense RNA segments of influenza virus is transcribed into mRNA by the viral ribonucleoprotein (RNP) complex comprised of the PB2, PB1, PA, and NP proteins (Figure 2). Crystal structures of portions of the RNP complex have already shed light on how these proteins work (He et al., 2008; Noda et al., 2006). However, more biochemical research into the structure of the complex is needed to reveal why certain residues affect the interaction of the polymerase proteins, viral RNA, and host proteins. Elucidating how receptor specificity and polymerase-driven replication affect the pathogenicity and transmission of H5N1 viruses will yield important clues about host adaptation, pandemic potential, and the development of antiviral drugs.

Transmissibility

What are the requirements for human-to-human transmission of a potentially pandemic highly pathogenic avian influenza virus, and what mechanisms are involved? The absence of efficient human-to-human transmission of H5N1 viruses to date may explain why the circulating avian influenza virus has not caused a pandemic. Ferrets, which are naturally susceptible to influenza, have been used as a model to investigate transmission of H5N1 viruses. In both humans and ferrets, respiratory epithelial cells express primarily α2,6-linked sialic acids, H5N1 viruses bind preferentially to epithelial cells in the lower respiratory tract, and infection causes acute respiratory illness (Matrosovich et al., 2004; Shinya et al., 2006; van Riel et al., 2007). Pathogenic H5N1 virus was not transmitted from infected to contact ferrets regardless of the α2,3- or α2,6-linked sialic acid receptor binding affinity (Yen et al., 2007b). In another study, acquisition of the surface glycoproteins of efficiently transmissible H3N2 human influenza viruses did not alter transmission of poorly transmissible H5N1 avian viruses (Maines et al., 2006), suggesting that H5N1 transmission involves multiple genetic adaptations.

Factors beyond the viral genome may also contribute to transmissibility. For example, virus is thought to be transmitted in droplets generated by coughing or sneezing. In a guinea pig model of human infection, H3N2 influenza virus was indeed transmitted via an aerosol, and aerosol transmission was enhanced by lower humidity and temperature (Lowen et al., 2007). These findings shed light on the seasonality of human influenza outbreaks. Importantly, however, H3N2 influenza virus is transmitted among guinea pigs without coughing and sneezing, which is not true in ferrets. If H5N1 viruses do acquire efficient human-to-human transmissibility, it will be important to understand the full range of factors that can modulate transmission.

Coinfection

The coinfection of a human by a seasonal H3N2 influenza virus with efficient transmissibility and an avian H5N1 virus with poor transmissibility has the potential to generate a reassortant H5N1 virus with efficient transmission or pandemic potential. In a hallmark study, the reassortant viruses generated from swapping the genes of H5N1 and H3N2 influenza viruses did not yield influenza viruses with efficient transmissibility in ferrets (Maines et al., 2006). Nevertheless, there is still concern about coinfection of humans and the emergence of H5N1 viruses with efficient human-to-human transmission. Multiple different genotypes of avian H5N1 viruses continue to emerge and the possibility of coinfection of humans with both avian H5N1 and seasonal H1N1 or H3N2 influenza virus is a continuing possibility. The possibility of genetic reassortment between these influenza viruses resulting in an H5N1 virus with increased ability to transmit between humans indicates that increased surveillance is needed to capture these coinfections of H5N1 and other influenza viruses and to elucidate which genetic reassortments will result in an influenza virus with pandemic potential.

The contribution to pathogenesis of coinfections with influenza virus and bacteria is another intriguing research area. Evidence suggests that a majority of deaths during the 1918 Spanish flu pandemic were due to secondary bacterial pneumonia (McCullers, 2006; Morens et al., 2008). Major knowledge gaps exist in our understanding of the complex interactions of multiple pathogens with each other and with the coinfected host. Thus, research and pandemic preparedness will require a focus on secondary bacterial infection and treatment.

Host Factors in Pathogenicity

The Immune Response

The pathology induced by some strains of influenza A virus has been correlated with an excessive immune response (de Jong et al., 2006). Studies of innate immune cells (dendritic cells, monocytes, natural killer cells, and neutrophils) and of the CD4, CD8, and B lymphocytes during infection with H5N1 avian influenza virus are necessary to understand the protective and pathologic effects of the adaptive immune response and to inform the design of vaccines. The fundamental questions are the tissues in which these immune cells act, the effector functions they perform, and the requirements and mechanisms that regulate these functions.

The rapid accumulation of proinflammatory cytokines (“cytokine storm”) after infection, with either the currently circulating highly pathogenic avian influenza virus or the 1918 Spanish influenza virus, is thought to play a prominent role in morbidity and mortality (Cheung et al., 2002; de Jong et al., 2006; Kash et al., 2006b). Levels of mRNAs encoding TNFα, RANTES (regulated on activation, normal T cell expressed, and secreted), MIP1α and 1β (macrophage inflammatory protein), and CCL2 (monocyte chemotactic protein-1 MCP-1) were markedly higher in primary human macrophages infected with H5N1 virus than in those infected with human-adapted H3N2 or H1N1 viruses (Cheung et al., 2002). Similarly, primary human bronchial and alveolar epithelial cells secreted significantly more IP-10 (interferon-γ-inducible protein-10), IL6 (interleukin-6), and RANTES when infected with H5N1 compared with H1N1 influenza virus (Chan et al., 2005). Mice and macaques infected with the 1918 pandemic strain of influenza virus showed increased expression of proinflammatory cytokine mRNAs and proteins (Kash et al., 2006b).

Because a dysregulated cytokine response has been linked to the severity of disease caused by some strains of influenza A virus, therapy that blocks the cytokine cascade could prove beneficial. Administration of an immunomodulatory statin, gemfibrozil, 4–10 days after inoculation with an H2N2 virus increased survival of mice by 50% (Budd et al., 2007). In another study, disruption of the TNFα signaling cascade in mice reduced morbidity after inoculation with the H5N1 avian influenza virus (Szretter et al., 2007). However, although

survival was prolonged, mortality was not significantly reduced. Further, mice that lacked CCL2, IL6, or TNFα succumbed as often as wild-type mice to infection with a lethal H5N1 virus (Salomon et al., 2007). Interestingly, recent investigations into the role of cytokines in ferrets infected with the H5N1 virus indicated that treatment with a chemokine receptor inhibitor, AMG487, reduced morbidity and modestly delayed mortality (Cameron et al., 2008). Together, these results suggest that inhibition of a single immune signaling molecule is unlikely to improve morbidity or survival following infection with highly pathogenic avian influenza virus. However, therapies targeting an overexuberant immune response may yet prove beneficial. A more complete understanding of the cause and effect of the cytokine storm may aid in the development of new therapeutics (Table 1).

Other Host Factors

Influenza virus encodes only ten viral proteins but replicates successfully in a broad range of avian and mammalian species by exploiting host cell functions (Figure 2). The interactions of the virus and host cell proteins are crucial to viral replication, assembly, and trafficking (Ludwig et al., 2006). Further understanding of the complex signal transduction pathways induced by viral and host protein interactions may provide new targets for antiviral therapy. The identity of the specific host factors involved in resistance and susceptibility to avian influenza virus can be solved only by the combined efforts of virologists, immunologists, geneticists, and biochemists.

We know that type I interferons (IFNα/β) are a crucial innate defense against viruses because of their potent antiviral and immunoregulatory effects. *Mx1* is an antiviral host gene induced by IFNα/β, and inbred mouse models of influenza usually lack this gene. In two recent studies, these mice were protected from infection with lethal human H5N1 virus and from the reconstructed 1918 pandemic virus by a mechanism that reduces polymerase activity and is enhanced by IFNα/β (Tumpey et al., 2007). Further research into this protective mechanism may reveal how it can be exploited therapeutically.

A key component of innate immunity is pattern recognition receptors, some of which specifically detect viral components. One such receptor, retinoic acid-inducible protein I (RIG-I), was recently implicated in the recognition of influenza RNA and the activation of antiviral pathways. This protein is crucial for production of IFNα/β in response to influenza, and mice genetically deficient in RIG-I show increased susceptibility to influenza. RIG-I specifically recognizes 5′-phosphorylated viral genomic single-stranded RNA. However, the nonstructural NS1 protein of influenza virus has multiple functions including inhibiting the host immune response by forming a complex with RIG-I and blocking the induction of type I interferons (Pichlmair et al., 2006). Further investigation of these innate sensors and the host cofactors involved in inducing the anti-influenza virus response is needed. A point of interest is whether the highly lethal H5N1 viruses and other influenza viruses with pandemic potential have mechanisms to reduce the effectiveness of innate immune sensors.

Another host factor inhibited by the NS1 protein of influenza A is CPSF30 (cleavage and polyadenylation specificity factor), which is required for processing of cellular pre-mRNAs including IFNβ mRNA. Recent structural data suggest that drugs targeting the interaction of CPSF30 and NS1 may be useful in the treatment of influenza (Das et al., 2008). Other interferon-induced host factors involved in protection against influenza viruses include 2′-5′ oligo (A) synthetase and protein kinase R. The cellular signal transduction pathways activated during infection with highly pathogenic avian influenza virus need further elucidation to reveal the key biochemical mechanisms that are important during infection (Kash et al., 2006a; Ludwig et al., 2006). Although Mx1, RIG-I, CPSF30, 2′-5′ oligo (A) synthetase, and protein kinase R have clear roles in the host response to influenza viruses, many other host factors that affect resistance or susceptibility to these viruses probably remain to be discovered. Recent

innovative work reveals host factors that are involved specifically in influenza virus replication (Hao et al., 2008). Priority should be given to identifying these factors, some of which may explain the elevated pathogenicity or the absence of transmission observed in certain populations.

Rapid Viral Evolution

The Moving Vaccine Target

The rapid evolution of the influenza A viruses continually complicates the effective use of vaccines and therapies. Because these genomically unstable negative-strand RNA viruses change so rapidly, vaccine strains can quickly become outdated and the lengthy, labor-intensive, large-scale production of vaccines in eggs is problematic. The time lag between vaccine production and seasonal flu outbreaks (often 6 months or more) can result in a mismatch between the vaccine and the circulating virus. During the 2007–2008 flu season, a mismatch in the seasonal influenza vaccine caused an increase in childhood deaths from influenza in the Northern Hemisphere. The outdated technology used to prepare vaccine strains and to mass-produce vaccines urgently requires modernizing (Table 1). Much research has been focused on alternative vaccine production systems. The plasmid-based reverse genetics system has been used to generate reference viruses for H5N1 vaccines. In addition, there have been promising advances in the development of vector, DNA, recombinant subunit, peptide-based, and virus-like particle vaccines (Subbarao and Joseph, 2007).

Lack of Immune Correlates of Vaccine Protection

How do vaccines mediate immune protection? Protection does not correlate with neutralizing antibodies after vaccination against H5N1 viruses, as it usually does after receipt of the seasonal influenza vaccine. Further, vaccines of different H5N1 clades and subtypes appear to offer crossprotection (Govorkova et al., 2006). Importantly, in the case of a pandemic, crossprotection may allow a minimum amount of vaccine to be used per person or may expand the pool of vaccine candidates. It is therefore essential to determine the mechanisms and factors required for crossprotection, including the contributions of T cell-mediated immunity and serum and mucosal antibodies. Questions also remain about which vaccination methods (e.g., intact virus versus subunit vaccines; use of adjuvants; number, dose, and route of inoculations) will reduce the amount of vaccine needed (Leroux-Roels et al., 2007). Research should also focus on resolving how age (particularly from the point of view of children and the elderly) affects vaccine efficacy and immune correlates of protection.

New strategies to induce immune protection against highly pathogenic avian influenza virus must be explored. Vaccines based on proteins other than the surface glycoproteins, such as the matrix segment, have demonstrated some protective potential (Watanabe et al., 2007). Basic research into the mechanisms of the innate and adaptive immune responses to avian influenza virus will provide the cornerstone for the development of optimally protective vaccines. Protection is currently gauged primarily by the production of neutralizing antibodies, although there is great variation in assay standards and, for certain viruses, poor correlation between the assay results and the level of protection. It is generally agreed that better immune correlates for testing vaccine efficacy are needed.

A long-standing question is whether a single vaccine could protect against influenza viruses of different subtypes. Rather than focusing on the production of neutralizing antibodies, immunologists hope to generate a vaccine that activates virus-specific cytotoxic T lymphocytes (CTLs) directed toward epitopes conserved among influenza virus subtypes. A subset of epitopes recognized by human CTLs is highly conserved among human and avian H5N1 influenza A viruses; these epitopes are on internal influenza proteins, which are less susceptible

to antigenic variation (Wang et al., 2007). However, it remains to be determined how an activated T cell population could be maintained in the lungs without inducing autoimmunity. Crossreactive vaccines that activate CTLs would be a valuable tool for controlling avian influenza viruses with pandemic potential (Rimmelzwaan et al., 2007).

Vaccine Use in Domestic Poultry

Vaccination to control the H5N1 virus in domestic poultry is controversial because of concerns that it may drive antigenic drift or mask the continued circulation of virus. However, the benefit of vaccinating poultry has been dramatically illustrated in Vietnam. By 2005, 90 humans in Vietnam had been infected with the avian influenza virus H5N1 and 39 had died. After widespread vaccination of domestic poultry, infection of humans and domestic chickens ceased. The H5N1 virus re-emerged in humans and poultry in Vietnam in 2007 due to the difficulty of maintaining poultry vaccinations, effectively immunizing domestic ducks, and controlling poultry smuggling. A program requiring vaccination of all poultry entering Hong Kong was successful for 7 years but is now less effective because the vaccine needs to be updated.

Although poultry vaccination is an important tool for control of the H5N1 virus, the ultimate goal is eradication of this virus and cessation of vaccine use. Continued vaccination promotes endemic persistence of the H5N1 virus in domestic poultry and may mask the presence of highly pathogenic strains. The absence of global standards for the antigenic content of poultry vaccines is an unresolved problem, although the antigen dose required to induce protection and prevent virus spread in different breeds of domestic fowl is easily determined. More significantly, it remains unresolved whether standardized vaccination or vaccination of immunocompromised animals promote selection of more pathogenic variants of avian influenza virus. More information is needed about the immunobiology of avian species to determine the best use of poultry vaccines.

Antiviral Therapies

The anti-influenza drugs approved for clinical use are the neuraminidase inhibitors (orally administered oseltamivir trade name Tamiflu and inhaled zanamivir trade name Relenza) and inhibitors of the viral M2 matrix protein ion channels (the adamantanes, amantadine, and rimantadine). Several other neuraminidase inhibitors (peramivir; pyrrolidine derivative A315675; and long-acting R-118958 and FLUNET compounds) are under development. Oseltamivir is effective against many avian influenza virus strains in animal models, although an optimal treatment schedule may be required for highly virulent viruses (Govorkova et al., 2007). Information about drug efficacy in humans is limited; treatment often starts late in the course of infection, and the dosage and duration of treatment are often suboptimal (Beigel et al., 2005).

The emergence of drug-resistant virus variants is one of the disadvantages of antiviral therapy. Most clade 1 H5N1 influenza viruses are now resistant to adamantanes (Hayden, 2006). Resistance to the neuraminidase inhibitors appears to be less of a problem, although oseltamivir-resistant viruses with neuraminidase mutations (H274Y and N294S) have been isolated from patients during and before drug treatment (Le et al., 2005). Further, resistant variants carrying either of these neuraminidase mutations may retain their high pathogenicity in mammalian species (Yen et al., 2007a). Emerging resistance to antivirals is of increasing concern as H1N1 seasonal influenza viruses resistant to oseltamivir appeared in the 2007–2008 flu season (Lackenby et al., 2008) and have become prevalent in the 2008–2009 influenza season. Given that neuraminidase inhibitors are the most commonly prescribed for seasonal influenza and are being stockpiled in case of an influenza pandemic, it is imperative to understand how and why these drug-resistant influenza viruses are maintaining transmissibility

and virulence. Improved approaches to antiviral drug use may include development of new antivirals, using combinations of antivirals, or optimizing existing antiviral drug regimens (dosage, duration, route of administration).

Given increasing evidence that resistance to the conventional antivirals emerges rapidly, there is an urgent need to identify new therapeutic targets. Viral polymerase activity may offer such a target, in view of the correlation between the lethality and rapid replication of certain avian influenza virus strains. Small-interfering RNAs against the genes encoding nucleoprotein or polymerase protein PA of the viral replication complex reduced virus replication and increased the survival of lethally challenged hosts (Tompkins et al., 2004). The screening of small inhibitory molecules using high-throughput viral replication assays will advance the field of anti-influenza therapy. Ribavirin and its analog viramidine, which inhibit virus-encoded RNA polymerases, may also reduce the replication efficiency of H5N1 viruses. The sialidase fusion construct DAS181 (Fludase) was recently shown to cleave sialic acid receptors for both human and avian influenza viruses and to provide a potent anti-H5N1 therapeutic effect in infected mice (Belser et al., 2007).

In the search for protective agents, some researchers have focused on familiar anti-inflammatory drugs, including statins (Fedson, 2006). Neutralizing antibodies are also being pursued as a treatment strategy. Neutralizing anti-H5N1 human monoclonal antibodies provide effective prophylaxis and therapy in mice (Hanson et al., 2006; Simmons et al., 2007). Such neutralizing crossreactive antibodies or other immunotherapies are promising avenues for treating humans infected with the H5N1 influenza virus.

Pandemic Preparedness

Influenza does not recognize man-made borders, and it is debatable whether any country should lay claim to strains of influenza virus isolated there. However, a country that shares its influenza virus isolates for global research is entitled to the benefits derived, especially if an emerging pathogen is killing its citizens. How such competing claims are to be balanced must be resolved by the World Health Organization. The core issues are ultimate ownership of the isolated viruses and associated intellectual property, and the fair distribution of vaccines derived from those viruses. Such proprietary claims conflict with the global sharing of influenza viruses and their genomic information, vaccines, and antiviral drug sensitivity data required for optimal pandemic preparedness. These issues are under intensive review by international organizations seeking to ensure that developing countries will have access to vaccines and anti-influenza drugs derived from viruses isolated within their borders and will be informed of the distribution of those viruses and their derivatives.

Pandemic preparedness is an ongoing process that continually incorporates emerging information. Most countries have pandemic plans, but their effectiveness will depend on availability of the expanding knowledge base to veterinary and public health officials, the transfer of knowledge to industry, and ongoing communication with leaders in commerce, industry, and transportation.

There is concern that if an H5N1 pandemic does not occur, scientists will lose public credibility and pandemic planning will be supplanted by more pressing public health programs. Scientists have alerted the public to observations that call for special vigilance. However, an influenza pandemic is no more predictable than the human use of biological pathogens or chemical agents. Nations can help to ensure the sustainability of their preparedness programs by establishing permanent pandemic planning staff positions in their health and security departments.

The stockpiling of antiviral agents is an important element of influenza pandemic planning, but the stability of the active ingredients, capsules, and inert carrier components must be monitored. Oseltamivir appears to be extremely stable (Monto et al., 2006). It would be unrealistic to consider replacement of stockpiled drugs, but their stable components could be recycled. Are pandemic planners making arrangements for the maintenance of these valuable national resources? Although the H5N1 avian influenza virus may never acquire full human pandemic potential, another influenza virus certainly will. Anti-influenza drugs will remain our first line of defense.

Although it is currently impossible to predict which influenza virus will cause the next epidemic or pandemic, the pathogenic potential of these viruses can be anticipated more precisely with continued research and development in surveillance, diagnostics, and genomic studies of the virus and its key hosts.

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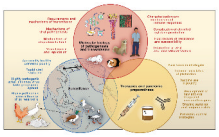

Figure 1. Spheres of Progress in influenza Research

Shown are the three major areas of influenza research: (1) the molecular basis of pathogenicity and transmission, (2) surveillance, and (3) therapies and pandemic preparedness. Points of overlap among the three circles illustrate how the findings in each area have implications for the other two areas. The major challenges within each area of research are noted around the periphery of that circle.

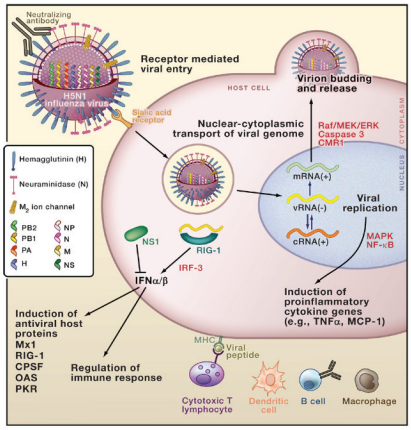

Figure 2. Molecular Basis of influenza Pathogenesis

The life cycle of the influenza virus begins with binding of the virus to sialic acid receptors on the surface of the host cell via the viral surface glycoprotein hemagglutinin (H). This step contributes to pathogenesis, transmission, and host range restriction. Replication of the eight negative-strand RNA segments that comprise the influenza genome is central to viral pathogenesis and could be a potential therapeutic target. The release of the virion from the host cell is a hallmark of successful completion of the influenza virus life cycle. Key molecular proteins and pathways that are activated during influenza virus infection of the host cell are also depicted. Potential host signal transduction factors are indicated in red.

Table 1

Strategies for Vaccines and Antiviral Therapies

Vaccines	**Antiviral Therapies**
Techniques	
Inactivated vaccine	Neuraminidase inhibitors
Subunit vaccine	M2 ion channel blockers
Live attenuated vaccine	Monoclonal antibodies
DNA-based vaccine	Immunomodulatory therapy
Vector-based vaccine	siRNAs
Virus like particles	Sialic acid receptor cleavage/ sialidases
	Inhibitors of virus-induced signaling pathways
	Inhibitors of viral polymerase
Challenges	
Non-egg-based production	Emergence of resistance
Targeting generation of virus-specific CTLs	Timeframe of efficacy
Increasing immunogenicity and adjuvants	Stockpiling
Rapid production	Accessibility and affordability
Dosage	Dosage
Administration route	Administration route
Immune correlates of protection	Duration of therapy
	Combination therapy