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Development of Effective Vaccines against Pandemic Influenza

Commentary

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Summary

A key strategy to protect humans against an influenza pandemic is the development of an effective vaccine. However, the development of effective pandemic vaccines poses both practical and immunological challenges.

Introduction

Influenza viruses cause repeated infections in humans and are a significant cause of morbidity and mortality annually; they account for as many as 36,000 excess deaths each winter in the United States (Thompson et al., 2003; Wright and Webster, 2001). Influenza is a winter illness in temperate climates; however, it occurs in two peaks or throughout the year in tropical climates. Three types of influenza viruses, designated influenza A, B, and C, are present in nature, and of these, influenza A and B viruses cause annual epidemics. Humans are the only hosts for influenza B viruses, but influenza A viruses infect a variety of species, including birds, pigs, horses, dogs, and humans (Wright and Webster, 2001; Crawford et al., 2005). Influenza A viruses, but not influenza B or C viruses, are divided into subtypes based on the antigenicity of the two major surface glycoproteins, the hemagglutinin (HA) and neuraminidase (NA). These two proteins are the main targets of the protective immune response. The HA is a trimer with a receptor binding pocket on the globular head of each monomer, and the NA is a tetramer with an enzyme active site on the head of each monomer.

Aquatic birds represent the reservoir of influenza A viruses in nature. Viruses of all known (16 HA and 9 NA) subtypes have been isolated from waterfowl and shorebirds. However, influenza infections in waterfowl tend to be asymptomatic, and the viruses are in ecological stasis in these hosts (Webster et al., 1992). In contrast, influenza A virus infections in humans elicit an immune response that provides selective pressure and drives the virus to evolve. Influenza viruses utilize two mechanisms, referred to as antigenic drift and antigenic shift, to evade the human immune response. Antigenic drift is a continuous process of change in which mutations occur in and around the antibody (Ab)-recognition sites of the HA and NA proteins; these recognition sites allow the virus to escape neutralization by pre-existing Abs. Five Ab-combining sites have been mapped on the HA of H3 subtype human influenza A viruses (Wilson and Cox, 1990); however, less is known about HAs of avian influenza A subtypes. Antigenic shift is a rare but epidemiologically highly significant event in which a virus bearing a novel HA, with or without an accompanying novel NA, is introduced into the human population. A virus bearing a novel HA or NA has the potential to cause a pandemic if a large proportion of the population lacks immunity to the novel HA and NA and if the virus has the ability to spread efficiently from person to person. The novel HA and NA genes in pandemic influenza viruses are derived from the reservoir of avian influenza viruses in nature.

Pre-Existing Immunity against Influenza

During an influenza-virus infection, HA- or NA-specific Abs present at systemic or mucosal sites are the major mediators of resistance to the virus, whereas the cellular immune response to influenza works with the humoral immune response in viral clearance (Murphy and Chanock, 2001) (Figure 1). Abs directed at the HA and NA surface glycoproteins of the virus are effective in mediating protection that is long-lived in the absence of antigenic drift or shift. This was evident in 1977 when an H1N1 virus that had circulated in the early 1950s reappeared in the human population. Significant disease was only seen in persons born after the H1N1 virus had stopped circulating in 1957, indicating that homotypic immunity is long-lived. Because individuals born after 1957 were infected multiple times with H2N2 or H3N2 viruses that share internal protein antigens (e.g., nucleoprotein) with the H1N1 virus, it was clear that cell-mediated immunity to shared antigens, such as the nucleoprotein, played a relatively small role in resistance. Thus, homotypic Abs are highly protective and mediate significant protection in humans, whereas Abs to the HA and NA of other subtypes and cell-mediated immune responses are less effective in long-term immunity.

Heterosubtypic immunity, which is protection conferred by previous infection(s) with an influenza virus of a different subtype, is weak in humans, especially in children. Recent analysis of epidemiological data collected before and during the 1957 pandemic suggests that heterosubtypic immunity was observed in adults but not in children (Epstein, 2006). However, definitive data regarding the role that heterosubtypic immunity plays in resistance to influenza-virus infection in humans are lacking, and the mediators of such immunity in humans have not been identified.

An analysis of genetic and antigenic data on the HA from human influenza A H3N2 viruses led to the conclusions that the HA was under positive selection (Fitch et al., 1997) and that two or more amino acid changes in two or more Ab combining sites of the HA were sufficient for a virus to evade neutralization by Ab against the previously circulating strain (Wilson and Cox, 1990). Cellular immunity is directed at epitopes on several influenza-virus proteins, but this immunity is relatively short-lived (Murphy and Chanock, 2001).

Vaccine Development

Several important considerations for vaccine development follow from our knowledge of the interactions between influenza viruses and the host. First, influenza viruses replicate extremely rapidly in the host. Peak

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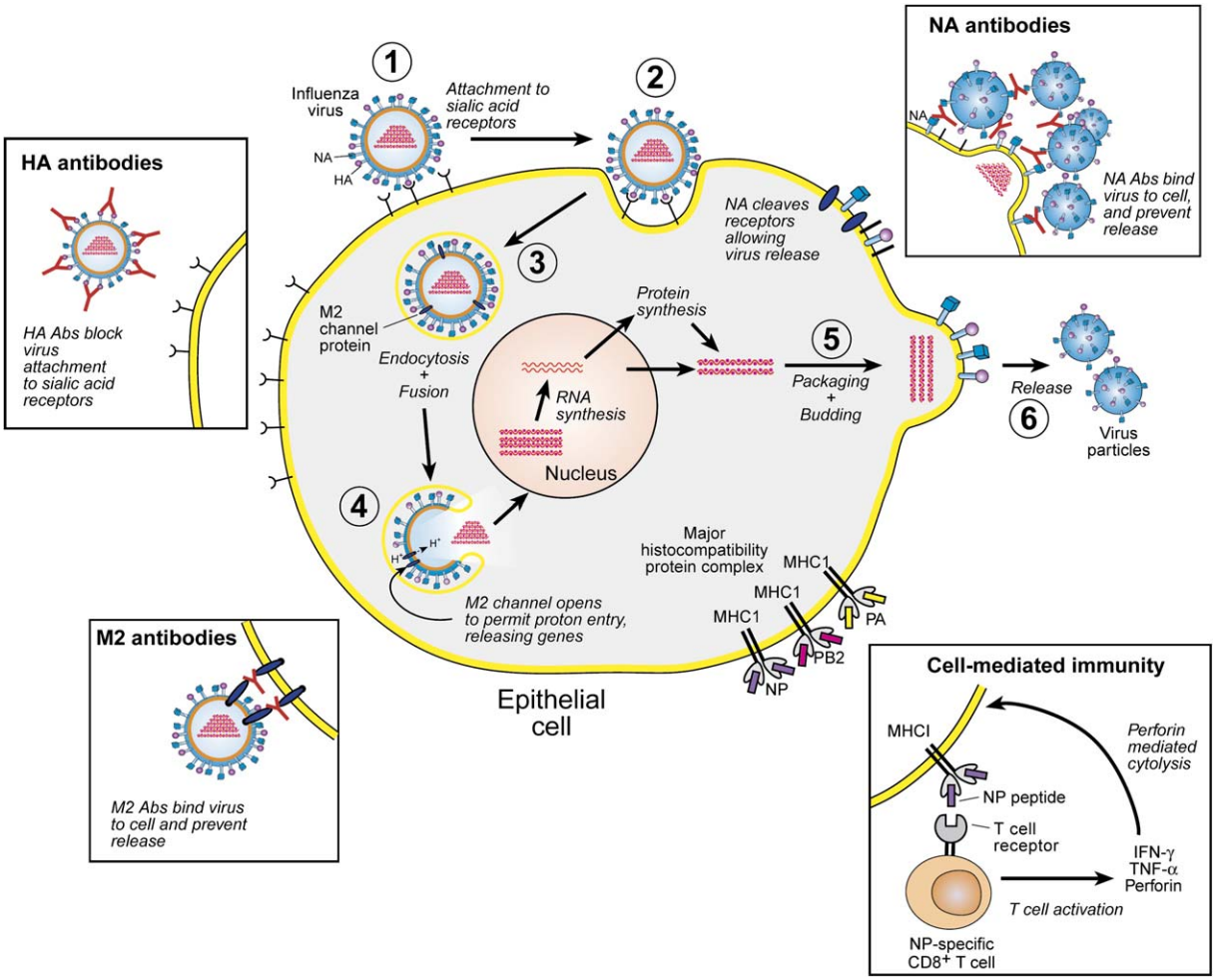

Figure 1. Life Cycle of Influenza Virus and Role of the Adaptive Immune Response during Infection

Influenza virus attaches to the epithelial cell surface through binding of the viral hemagglutinin (HA) protein to cell surface sialic acid receptors (1, 2). The virion is internalized through endocytosis and fusion (3). Opening of the M2 channel allows proton flow across the viral membrane (4), triggering fusion of viral and endosomal membranes and release of viral genes into the cytoplasm, from where they travel to the nucleus. Viral proteins produced in cytoplasm assemble with viral genes and bud from the cell membrane as progeny virions (5). Release of new virus particles (6) requires the viral neuraminidase (NA) protein, which cleaves sialic acid receptors from the cell membrane. Antibodies (Abs) to the HA protein block virus attachment (inset, upper left), thereby decreasing the number of cells infected. They can also function to prevent fusion (4). Abs to the NA protein (inset, upper right) bind virus to the cell, preventing release of new virions. Abs to the M2 protein bind virus to the cell and prevent release of viral particles into the extracellular fluid (inset, lower left). Cell-mediated immunity contributes to resistance when $CD8^+$ T cells specific for viral proteins such as nucleoprotein (NP) or polymerase proteins (PB2 and PA) recognize viral peptides presented by MHC class I proteins, resulting in the release of cytokines with antiviral activity (IFN-γ and TNF-α) and perforins that mediate cytolysis of the infected cell (inset, lower right). Lysis of the infected cell decreases the amount of virus released by the cell. The latter three mechanisms, NA Abs, M2 Abs, and $CD8^+$ T cells, operate after a cell becomes infected. Only HA Abs prevent infection; this is likely to be why they are the most effective in vivo.

titers (which correlate with disease) are achieved before a cell-mediated immune response can be generated de novo or from memory to restrict replication (Figure 2). Therefore, the major goal of the currently licensed influenza vaccines is to induce, prior to infection, Abs that function to dampen virus replication. Second, influenza is a respiratory-tract infection, and Abs induced by vaccine that restrict replication throughout the upper and lower respiratory tract are desired. Intranasally administered live, attenuated vaccines efficiently induce a mucosal as well as a systemic Ab response. Mucosal Abs are more effective than systemic Abs in restricting replication of influenza virus in the upper respiratory tract. In contrast, parenterally administered inactivated vaccines primarily induce systemic (serum) Abs that restrict replication of virus in the lower respiratory tract. Therefore, inactivated vaccines are effective in prevention of severe disease and complications of influenza but are less effective than previous natural infection and live, attenuated virus vaccine in protection of the upper respiratory tract. Third, the ability of the virus to drift and evade immune detection and the paucity of HA conserved epitopes that induce cross-reactive neutralizing or protective Abs pose a challenge for vaccine development. Currently licensed human influenza vaccines are updated annually to keep up with antigenic drift that is identified through virologic surveillance. Fourth, clinical studies have established that two doses of currently formulated inactivated vaccine are required to elicit protective Ab titers in immunologically naïve individuals. The

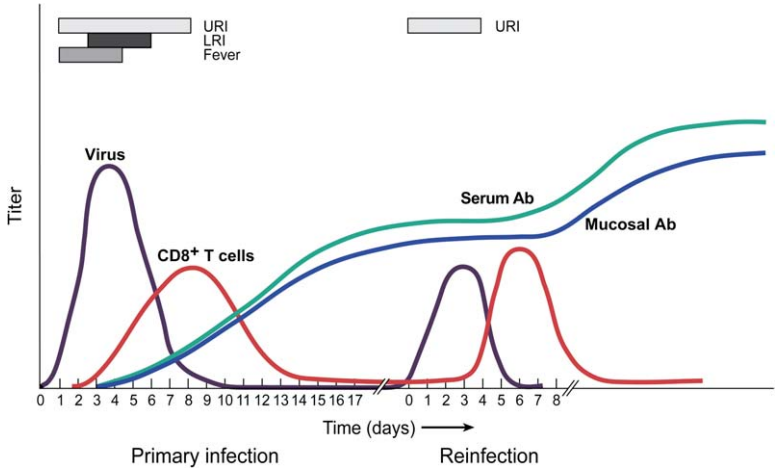

Figure 2. Course of Immune Response during Influenza Infection

Influenza virus titers peak at approximately 3 days after infection, at which time antibodies (Abs) and T cell responses begin to appear. Activated T cell responses peak on days 6–9 during the primary infection and then subside into a memory or resting state, whereas serum and mucosal Ab concentrations are sustained. Abs present at the time of reinfection result in lower viral titers and a reduction in symptoms. Upper respiratory infection, URI, lower respiratory infection, LRI.

live, attenuated virus vaccine is significantly more immunogenic than inactivated virus vaccine in naïve individuals. In practical terms, each winter, previously unimmunized children should receive two doses of vaccine one month apart, whereas a single vaccine dose can protect previously primed children and adults.

Pandemic Influenza Preparedness

Recent events in Asia have highlighted the pandemic potential of avian influenza viruses and the need to prepare for an antigenic shift in influenza A viruses. Although antiviral drugs can be effective in prophylaxis, vaccines are the preferred strategy for the prevention of a pandemic because pandemic viruses might be resistant to available antiviral drugs or, even if initially sensitive, can rapidly develop drug resistance (Le et al., 2005; de Jong et al., 2005). A realistic goal of a pandemic influenza vaccine is to prevent mortality and severe morbidity with acceptance of the fact that infections associated with mild illness will not be prevented. This requires the development of vaccines that, at the least, elicit systemic Abs of sufficient titer to restrict virus replication in the lower respiratory tract and thereby prevent pneumonia and its associated complications.

Although principles that have been established from basic and applied research in human influenza can be applied to pandemic influenza vaccine development, several critical gaps in knowledge remain. For example, the antigenic sites on avian HAs and the immune correlates of protection from avian influenza-virus infections are not known. Additionally, the HA proteins of avian subtypes of influenza A viruses are not as immunogenic as human influenza A HA subtypes for unknown reasons; therefore, approaches to enhance the immunogenicity of the avian HA in a pandemic virus may be needed to achieve a protective level of immunity. Such new approaches will be needed in addition to the two doses of vaccine now required to successfully immunize a naïve population.

Currently, two classes of vaccines are licensed for interpandemic influenza in the US: parenterally delivered inactivated virus vaccines (whole virus or subunit) and a live, attenuated vaccine delivered as a nasal spray. Both types of vaccines are trivalent and contain an influenza A H1N1 subtype virus, an influenza A H3N2 subtype virus, and an influenza B virus to protect against each of the co-circulating strains of influenza. Vaccines against potential pandemic strains of influenza are now being developed based on both of these strategies. Seed viruses for inactivated vaccines have been generated against influenza viruses of H5, H7, and H9 subtypes. Preclinical data have been generated for all three subtypes, and H5 and H9 subtype vaccines have been evaluated in phase I clinical trials (Hehme et al., 2002; Nicholson et al., 2001; Stephenson et al., 2003). The investigational H5 and H9 inactivated vaccines are less immunogenic than interpandemic influenza vaccines (H1 and H3 subtypes). The amount of HA required in pandemic vaccines to elicit a serum Ab response of a magnitude similar to that of the licensed interpandemic influenza vaccine is likely to exceed the 15 μg present in the current inactivated virus vaccines. This increase in dose will determine the number of doses of vaccine available in the event of a pandemic and could strain manufacturing capacity.

In addition to seed viruses being made beforehand and their safety and immunogenicity being evaluated, several important applied vaccine research issues should be explored to ensure the availability of enough doses of appropriately immunogenic influenza vaccines to protect the population against potential pandemic strains of influenza. These include an exploration of ways to reduce the amount of HA antigen required to elicit protective Ab titers by investigation of alternative routes of vaccine administration; use of known and novel adjuvants to enhance immunogenicity; and consideration of a strategy of pre-emptive vaccination to prime the population for an Ab response to a novel HA. Preliminary results from a phase I clinical trial indicate that inactivated H9N2 vaccine administered with adjuvant is significantly more immunogenic than vaccine without adjuvant.

Efforts are under way to develop and evaluate live, attenuated vaccines against potential pandemic strains of influenza along a track that parallels the development and evaluation of inactivated virus vaccines (Luke and Subbarao, 2006). The live, attenuated pandemic-influenza vaccine candidates contain the attenuating genes of the A/Ann Arbor/6/60 cold-adapted virus that is the backbone of the licensed live, attenuated influenza A virus vaccine. Candidate vaccines have been generated against H5 and H9 subtype viruses, and vaccines

against the other subtypes will follow. If satisfactory data are obtained from preclinical testing, these vaccines will be evaluated in phase I clinical trials for safety, infectivity, and immunogenicity. As in the case of inactivated vaccines, cell culture substrates should be evaluated as alternatives to embryonated eggs for vaccine manufacture.

Harnessing Immunological Memory

Several decades of experience with human influenza vaccines indicates that the vaccine strain must be closely related to the epidemic strain of influenza in order to be effective. Although the match between vaccine virus strains and epidemic virus strains is generally good because the evolution of human influenza viruses is continuously monitored through careful, global virologic surveillance and vaccine strains are updated based on these data, we have no basis upon which to predict which exact strain of avian influenza will cross the species barrier and cause a pandemic. This makes it unlikely that the vaccine strain will exactly match the pandemic strain. A vaccine that provides cross-reactive immunity among the H1–H16 subtypes of influenza would be preferable. Further research and development efforts are required to achieve this goal.

The development of cross-subtype, HA-based protection requires the identification of conserved H1–H16 HA sites that could induce broadly protective, highly functional neutralizing Abs. It is important to emphasize that such Abs are not regularly induced in humans by infection with influenza A viruses belonging to multiple HA subtypes, an observation that indicates the difficulty of achieving this goal. A recent advance in this area is the determination of the crystal structures of the HA from several additional subtypes of influenza A viruses. The first 15 HA subtypes fall into four clades (two groups of two), with H1, H3, H7, and H9 being the prototypes of the four clades (Russell et al., 2004.) Perhaps the commonalities within clades of HA subtypes based on HA structure can be exploited to develop immunogens and strategies that can induce cross-reactive Abs effective among HA subtypes. Another approach to inducing broadly cross-protective immunity involves identifying conserved $CD8^+$ T cell epitopes (Figure 1) that can be induced in most members of the population and maintaining the $CD8^+$ T cells in a highly functional state that can keep an infecting influenza virus from reaching high titer in vivo. This second point represents a real challenge because the genetic program of the $CD8^+$ T cell response is to transform cells from an inactive memory state into an activated state, and maintaining $CD8^+$ T cells in an activated state will have to happen in the absence of antigenic stimulus. The immunogens capable of inducing this type of response have yet to be identified but could include sequences from circulating H1 and H3 subtype influenza viruses. Immunization with such T cell vaccines could provide varying degrees of resistance to disease after infection with an H5N1 pandemic virus, as well as with circulating H1 and H3 viruses. It is essential to determine the ability to maintain this state of immunity throughout the period of circulation of the first wave of the pandemic virus. The M2 protein of influenza A viruses forms a proton channel in the virion and intracellular membrane and functions to release influenza virus genes from endosomes (Figure 1, step 4). It is highly conserved, and non-neutralizing Abs to the M2 protein protect mice from subsequent challenge (Figure 1) (Treanor et al., 1990; Neirynck et al., 1999). Clinical studies can be designed to determine whether the induction of M2 Abs prevents disease in humans. If so, efforts can be undertaken to evaluate whether a more robust and protective M2 Ab response can be achieved by immunization than by repeated infection in nature. Presentation of the M2 protein to the immune system in a more immunogenic form via vaccination than occurs in natural infection may be important in this regard.

In conclusion, two approaches to the development of vaccines for pandemic preparedness can be exploited. The first and more immediately accessible uses existing technology to generate vaccines that induce highly functional and protective Abs. Efforts in this area should focus on pre-emptive preparation of vaccine seed viruses and evaluation of their safety and immunogenicity. Strategies to augment Ab responses with adjuvants and dose-sparing immunization regimens need to be explored. The second approach will build on basic research to explore possibilities to induce cross-protective cell-mediated immunity or Abs to conserved epitopes such as those on the HA or M2 proteins, but this has a longer lag time than the first approach.

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