

## Influenza Vaccine — Outmaneuvering Antigenic Shift and Drift

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The winter of 2003–2004 will be remembered as a year in which stories about influenza dominated the news and patients young and old clamored for influenza vaccination. This intense interest is the result of the confluence of multiple circumstances. The onset of the annual influenza epidemic came earlier than expected and was accompanied by reports of severe disease in previously healthy children, associated with the isolation of antigenically variant influenzaviruses represented by the prototypic strain A/Fujian/411/2002 (H3N2). There was concern that the available influenza vaccine would not be optimally effective against this strain of virus, but at the same time, the unexpectedly high demand for vaccination led to shortages in many areas. Perhaps it should not come as a surprise that this infinitely adaptable pathogen has again found a way to thrust itself into our consciousness. How are these events linked, and how do they fit into our overall understanding of influenza?

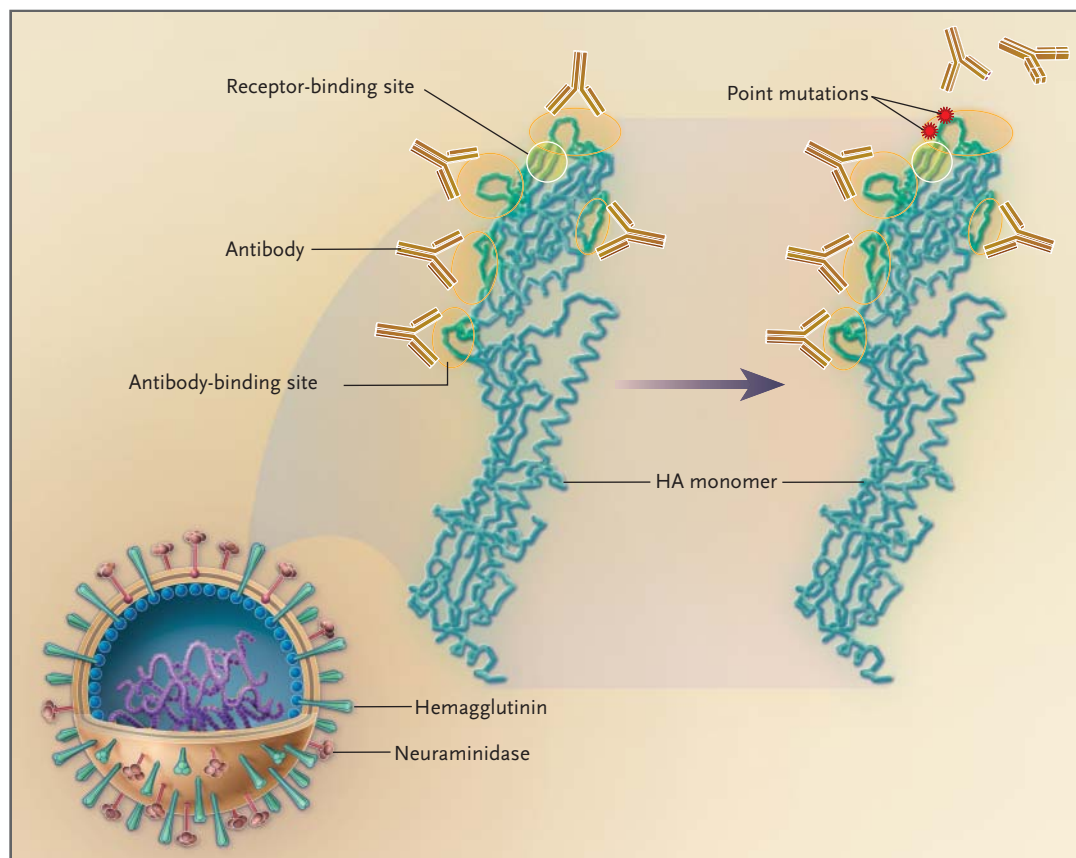
Influenza is a serious illness that causes excess morbidity and mortality in most years. It is estimated that influenza is responsible for an average of 51,000 excess deaths annually in the United States, with a range from about 7000 to about 72,000 deaths in any given year.<sup>1</sup> Influenza vaccine is protective against influenza-related death and hospitalization in all age groups. What is perhaps more noteworthy than this year's epidemic is the extent to which we routinely accept these vaccine-preventable deaths without much comment. The effects of influenza are felt most keenly at both ends of the age spectrum. Most people know that older adults have disproportionate morbidity due to influenza. But young children also have increased risks of hospitalization and complications. Because of these increased risks, the Advisory Committee on Immunization Practices recently began to encourage influenza vaccination for all children 6 to 23 months of age.

What is it about influenzaviruses that allows them to have this hold over our health every year? These enveloped viruses contain two important glycoproteins on the viral surface. The hemagglutinin is the viral attachment protein that also mediates the entry of the virus into the cell by fusion. The

neuraminidase is an enzyme whose main function is to facilitate the cell-to-cell spread of virus. The immunology of influenza is, in a sense, very straightforward. Antibody against the hemagglutinin is neutralizing and is very protective against infection and illness. Antibody against the neuraminidase can reduce the severity of illness.

Because solid immunity is induced by infection, influenza would quickly cease to exist except in children unless it evolved ways of defeating the immune response. The hallmark of influenzaviruses is antigenic variation, which comes in two forms: antigenic shift and antigenic drift. Antigenic shift, which is seen only with influenza A viruses, results from the replacement of the hemagglutinin and sometimes the neuraminidase with novel subtypes that have not been present in human viruses for a long time. The source of these new genes is the large reservoir of influenzaviruses in waterfowl, in which 15 antigenically distinct subtypes of hemagglutinin and 9 subtypes of neuraminidase have been identified. The consequences of the introduction of a new hemagglutinin into human viruses is usually a pandemic, or a worldwide epidemic, resulting in hundreds of thousands or millions of influenza-related deaths.

Of more relevance to this year's experience is the phenomenon of antigenic drift. Drift is a subtler process than shift and involves the accumulation of mutations within the antibody-binding sites in the hemagglutinin (see Figure), the neuraminidase, or both that abrogate the binding of some antibodies. Because of these mutations, the resulting viruses cannot be inhibited well by antibodies against previous strains, and it is easier for them to spread throughout a partially immune population. Antigenic drift occurs in both influenza A and influenza B viruses, but the pattern observed is somewhat different for the different viruses. Whereas drift variants of A (H1) viruses and B viruses often cocirculate with multiple coexisting lineages, influenza A viruses of the H3 subtype evolve more rapidly. Antigenic-drift variants of the H3 viruses occur more often, and new variants tend to replace older variants quickly, so that the evolution of the H3 hemagglutinin is more linear than that of other subtypes.<sup>2</sup>



**Figure.** Structure of a Hemagglutinin Monomer and Location of the Five Known Antibody-Binding Sites in the HA1 Subunit.

Antigenic drift involves point mutations that abolish the binding of antibody to the hemagglutinin or neuraminidase. Point mutations within the antibody-binding sites abolish binding by neutralizing antibody. The HA1 subunit of A/Fujian/411/2002 includes a total of 13 amino acid changes from the HA1 subunit of A/Panama/2007/99 virus. Mutations at positions 155 and 156 (red) seem to be of particular importance, since they are located on the top of the hemagglutinin molecule and are common to all viruses from the A/Fujian/411/2002 genetic group, though it is not clear whether these two mutations alone can account for all the antigenic differences that are seen.

The extent of antigenic drift is usually assessed by determining the nucleotide sequence of the hemagglutinin of new variants and evaluating the ability of antiserum against the old virus to inhibit the new virus. Variants of epidemiologic significance typically have multiple amino acid changes in the hemagglutinin, occurring at more than one antigenic site.<sup>3</sup> As compared with the previously circulating H3 viruses represented by A/Panama/2007/99, the A/Fujian/411/2002 virus has 13 amino acid changes involving multiple antigenic sites. Two changes, at positions 155 and 156 in the top-most antigenic site (see Figure), are characteristic of all the A/Fujian-like viruses that have been isolated so far (Klimov A: personal communication). Antibodies raised

against the A/Panama virus have titers one quarter as high when tested against the Fujian virus — a finding that is consistent with a moderate degree of antigenic drift.

Epidemiologically significant antigenic drift, particularly in H3 viruses, is often accompanied by the relatively early onset of a more severe influenza epidemic. A similar phenomenon was observed when the A/Sydney/97 viruses replaced A/Wuhan/95-like viruses in 1997. Other factors may also be influencing the shape of this year's influenza epidemic. In particular, there has been relatively little H3 virus activity in the United States for the past three years; there is therefore a relatively large cohort of susceptible children, which is probably playing

an important role in transmission. In fact, the most dramatic reports of severe disease this year have been those of influenza in young children.

In addition to influencing the nature of the yearly influenza epidemic, antigenic drift must be taken into account when strains are being considered for inclusion in influenza vaccines. Strain selection is an ongoing and complex process, involving the coordinated efforts of national and international organizations. Surveillance for new influenza strains is conducted year-round by the Centers for Disease Control and Prevention and the World Health Organization. The HA1 regions of the hemagglutinin gene of new viruses are sequenced, and their reaction with serum from experimentally infected ferrets is tested. The extent of epidemic activity of new viruses is assessed in an attempt to predict which viruses will emerge as predominant in the next year's influenza season. If a substantially new variant is identified, it must be prepared as an influenza vaccine in time for the next vaccination season.

Influenza vaccines are produced in eggs, but not all new antigenic variants replicate well in this system — a case in point being the A/Fujian virus. Because no suitable high-growth A/Fujian-like virus was available when this year's vaccine had to be produced, the vaccine was made with the A/Panama/2007/99 virus as the H3 component. It is important to realize that even though this strain is not an optimal match for the epidemic virus, it is still likely that it will provide some protection, particularly in adults. It is not possible to predict accurately at this time how much protection will be provided.

One way to generate updated influenza vaccines more rapidly would be to use substrates other than eggs, such as mammalian cells, for vaccine production. One might use recently described genetic techniques to generate viruses that have better growth characteristics in eggs. Considerable effort in these areas is under way. In addition, there is continued research into vaccines that might provide better protection against drift variants or that might not require annual updating at all. Recent data suggest that live influenza vaccines, such as the nasally administered cold-adapted vaccine, may induce a

broadened antibody response that could provide protection against drift variants within the same subtype.<sup>4</sup> Other strategies for inducing mucosal immunity have also been reported to offer potential protection against multiple subtypes. Finally, some investigators are pursuing vaccines based on conserved antigens, such as the M2 protein, that may be a route toward a universal influenza vaccine.<sup>5</sup>

Ultimately, the experiences of 2003–2004 may help us deal with influenza epidemics more effectively. The public awareness and media attention that accompanied reports of severe illness in children have resulted in greater recognition of both the severity of influenza in all age groups and the benefits of influenza vaccine. This recognition may spur increased use of vaccination and help us achieve the goals for vaccine coverage encompassed by the Healthy People 2010 initiative. In turn, increased demand for vaccine will encourage manufacturers to continue producing it, possibly in greater quantities. Increased production is a critically important path toward developing the surge capacity that will be needed to deal with new pandemic viruses when they occur. Finally, more manufacturers may become interested in this area, spurring the development of additional types of influenza vaccines. Influenza will probably never be completely subjugated, but with appropriate effort and resolve, there should be optimism about the control of this important pathogen in the future.

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