

VIRAL INFECTION

Universal Flu Vaccines: *Primum non nocere*

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Envisioning universal influenza vaccines that induce antibodies to conserved viral epitopes is exciting, but first we need to better understand the balance of effects caused by neutralizing and nonneutralizing antibodies (Khurana *et al.*, this issue).

“First, do no harm” is one of the core principles of medical practice and dates back to about the fourth century B.C.E. New work by Khurana *et al.* (1) illustrates why we would do well to consider this maxim in our 21st-century scramble to thwart influenza virus epidemics and threatened pandemics.

PUBLIC ENEMY

Vaccination against influenza is a vexing problem. Reinfection by influenza viruses is common despite previous infection or seasonal immunizations. Part of the obstacle to achieving durable immunity to influenza is that the viral antigenic determinants that give rise to neutralizing antibodies vary from year to year through antigenic drift [caused by point mutations in the genes that encode surface proteins hemagglutinin (HA) or viral neuraminidase (NA)] or major antigenic shift (caused by genetic reassortment that dramatically changes these same genes and creates entirely new viral subtypes). The public has become increasingly aware of the likely threat of major human pandemics caused by avian influenza viruses (such as influenza type A virus subtypes H5N1 and H7N9) that transmit directly from birds to humans (2), the adaptation of avian strains to allow study of the basis for human-to-human transmission (3, 4), and exposure to other animals, such as pigs, that harbor influenza viruses. Thus, there is a high level of interest in the development of vaccines that induce potent, durable immunity to many, if not all, influenza viruses.

In years past, influenza viruses were thought to be so variable that it would not be possible to identify antigens that induce broad immunity against the diverse strains circulating in nature. However, recent groundbreaking studies in the influenza immunity field have pinpointed highly conserved regions of the HA protein

(5–7). This molecule has a globular head that faces outward and a stem domain that inserts into the viral lipid membrane and is the most conserved region of the HA protein. The stem domain is the target for many naturally occurring human monoclonal antibodies (mAbs) that cross-react with multiple influenza strains from diverse subtypes and are often encoded by a single antibody variable-gene segment designated V_H1-69. Single mAbs that mediate neutralization of influenza type A viruses of more than one antigenic subtype have been isolated, and indeed, some of these antibodies bind to representative HA molecules from all known influenza A subtypes.

These unexpected results have led innovative scientists to pursue the development of recombinant mimetics of the stem region of HA that could serve as the antigenic basis of a universal influenza vaccine. The rationale behind these efforts is that the highly variable HA head domain is immunodominant and diverts the human immune response away from the more conserved epitopes in the stem region, which could be more immunogenic if presented in a more immune system-accessible form. Of course, achieving a safe, immunogenic influenza vaccine that induces durable immunity to diverse virus subtypes would be one of the major accomplishments in the history of biomedicine, and the possibility that we may see this outcome during our professional lives is tantalizing.

FLU FEVER

In our zeal to plunge forward in the development and deployment of next-generation influenza vaccines, we should remain true to the core principles of vaccine development and testing, which include a strong focus on safety and minimization of risk. In this issue of *Science Translational Medicine*, Khurana *et al.* (1) used a pig model of pandemic influenza H1N1 infection to investigate the effect of high-level induction of HA stem region-specific antibodies in pigs that

were vaccinated with a whole-inactivated H1N2 virus vaccine and subsequently challenged with the pandemic H1N1 virus, which is antigenically mismatched. In this experimental setting, the H1N2 vaccine induced antibodies that bound to an immunodominant epitope in the HA stem region that mediated enhanced H1N1 viral fusion to host cells and were associated with more frequent and more severe pneumonia in vaccinated animals. This observation suggests that we need to better understand the molecular basis for neutralization of influenza viruses in polyclonal responses *in vivo* before assuming that immunogenic HA stem-region antigens will always be safe immunization targets simply because such vaccines are immunogens that do not grow in cells like live viruses do. I distinctly recall the sobriety of a moment early in my training in the 1990s, when asking several of the investigators in the Laboratory of Infectious Diseases at the U.S. National Institutes of Health what it was like to have directed the phase I trial of the ill-fated formalin-inactivated respiratory syncytial virus vaccine in the 1960s that was associated with the subsequent death of several children (8). The quiet wisdom that I received was to pay attention when experimental vaccinologists state, “this vaccine might not work, but we are sure it will be safe” before conducting clinical studies of new nonreplicating vaccines.

The need to understand the molecular forces that drive antibody-enhanced virus replication or altered immune response is not limited to vaccine settings. In fact, it follows from the evidence presented by Khurana *et al.* that antibody-enhanced disease may occur in nature with an incidence that is hard to determine because of the high baseline morbidity caused by influenza infection itself. Studies during the 2009 H1N1 pandemic suggest that cross-reactive human antibodies may have played a role in enhanced severe disease observed in otherwise healthy young adults, with similar findings also present in preserved lower-airway tissues from fatal cases during the 1957 H2 influenza pandemic (9). Therefore, we should not interpret the new findings to indicate that influenza vaccines, in general, are of particular risk compared with natural infection. Current influenza vaccines do prevent a substantial amount of disease when the antigens are well matched. Nevertheless, inducing antibodies focused to the influenza HA stem region in the ab-

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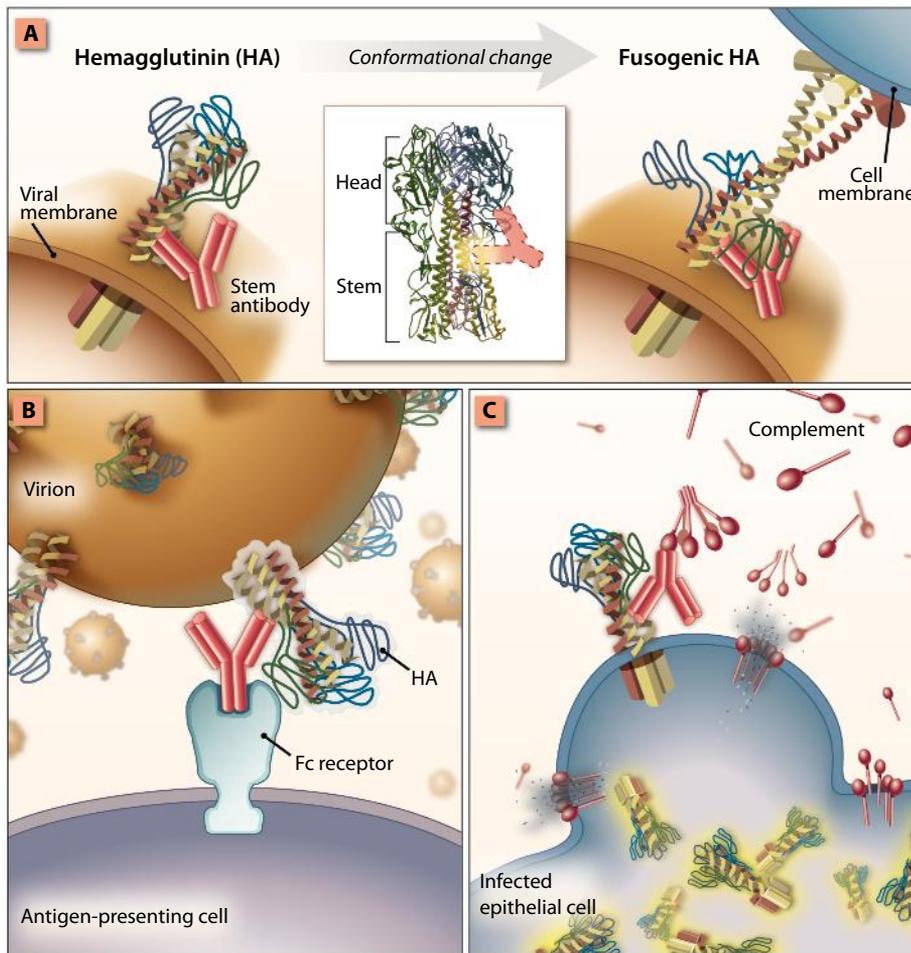


Fig. 1. Potential mechanisms for antibody-enhanced disease. (A) Enhanced fusion. Binding of an HA stem-specific antibody to the HA protein on the surface of the influenza virion induces a more efficient conformational conversion to the fusogenic form of HA. This conversion exposes a fusion peptide in HA that inserts into the cell membrane of a host cell, which is then infected by the virus. This mechanism is suggested in studies by Khurana *et al.*, in this issue (1). (B) FcR. Nonneutralizing HA stem-specific antibodies bind to HA on the surface of the influenza virus and direct the virus to Fc receptor-bearing cells that might then become infected through Fc receptor-mediated endocytosis. (C) Complement. Antibodies might cause enhanced disease without altering virus replication. For example, antibodies can bind to HA on the surface of influenza virus-infected cells and induce antibody-mediated complement activation, which leads to inflammation and cell lysis. Such a mechanism was suggested by studies in (8).

sence of antibodies to the head domain is a special strategy that deserves further study.

The next steps in this field are to conduct experiments that help us to understand in detail the molecular basis for how antibodies neutralize or enhance influenza infection *in vivo*. Several mechanisms for enhanced disease are possible (Fig. 1). The studies of Khurana *et al.* in the pig model suggest that some stem-region-specific antibodies alter the function of the HA protein so that it fuses more readily to susceptible cells; thus, the antibodies may facilitate higher levels of virus replication (Fig. 1A). Another pos-

sible mechanism is that nonneutralizing antibodies might bind the virus and direct it to Fc receptor-bearing cells that become infected through Fc receptor-mediated endocytosis (Fig. 1B). This mechanism is commonly studied in dengue virus models, but it is not clear whether influenza A virus replicates efficiently in Fc receptor-bearing cells, such as professional antigen-presenting cells. Further, antibodies might enhance disease without altering virus replication. For example, viral antibodies can bind to virus-infected cells, spur deposition of complement proteins, and cause antibody-mediated

complement activation, which leads to direct tissue damage (Fig. 1C).

The promise of universal influenza vaccines has excited biomedical scientists around the globe. Work in this field should proceed and will. The imperative now is to define the mechanisms of antibody-mediated neutralization and enhancement and to understand the complex balance of these forces that occur in polyclonal antibody responses *in vivo*. The recent explosion of reductionist studies of the structural and chemical basis of the action of influenza mAbs is helpful, but we also need methods for tweezing apart the polyclonal responses of B cell populations in the context of viral infections *in vivo*. The mAbs directed to influenza HA protein epitopes that have been studied to date merely represent members of large antibody “swarms” of related somatic variant antibodies that occur in human responses (10). Understanding the balance of forces within diverse populations of antibodies will be challenging, but newly emerging techniques will allow us to tackle these problems. High-throughput sequence analysis of the gene segments that encode antibody repertoires, proteomic studies of the protein sequences of secreted antibodies, and other evolving techniques will provide the high-information-content tools we need to develop, test, and use next-generation influenza vaccines that provide protection without harm.

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