Prospecting the Influenza Hemagglutinin to Develop Universal Vaccines

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(See the article by Sui et al, on pages 1003–1009.)

Influenza pandemics are among the most serious infectious threats to public health. The 1918–1919 influenza pandemic caused up to 500,000 deaths during a single influenza season in the United States, representing the most fatalities within such a short period in US public health history. The 2009 H1N1 influenza pandemic caused much less mortality but nevertheless exposed important limitations of global pandemic preparedness. [1, 2, 3]. First, the 2009 H1N1 virus was not detected by animal health surveillance systems for 10 years, exposing the inadequacy of current surveillance for viruses in animals that have pandemic potential for humans [4]. Second, the emergence of a pandemic virus with the same HA and NA subtypes as viruses that had been circulating in humans for decades (seasonal H1N1 virus) underscored the need to better understand immunity to emerging influenza viruses in the human population [4]. Finally, the 2009 H1N1 pandemic exposed our current inability to develop, manufacture, deliver, and administer billions of doses of vaccine directed against a newly emerging virus in the 4 months or less that would have been required to mitigate the impact of the spread of the 2009 H1N1 virus. [5]. Thus, several strategic changes in pandemic vaccine preparedness plans have been recommended [6]. The proposed changes are costly and often not affordable for low- and middle-income countries. Thus, sustainable long-term solutions will require the development of novel immunization strategies. The concept of a universal influenza vaccine that would elicit protection against a broad range of antigenically diverse influenza viruses, including different subtypes, is particularly attractive for pandemic preparedness [7, 8]. Unlike currently licensed influenza vaccines, a universal influenza vaccine would remain an effective response to emerging pandemics regardless of viral subtype. In addition, such a vaccine could greatly improve seasonal influenza control by eliciting long-lived protection, reducing costs, and increasing vaccine uptake.

Solid protective immunity against influenza in humans depends on the activity of neutralizing antibodies. The vast majority of these antibodies bind to the viral envelope glycoprotein, the hemagglutinin (HA). The technological challenge to develop an HA-based universal vaccine is intimidating. Ages of influenza virus evolution have resulted in 16 known subtypes of the hemagglutinin that circulate globally in aquatic birds, any of which could theoretically initiate a pandemic. Indeed, both major phylogenetic groups of HA have previously caused pandemics. H1 and H2 subtypes (responsible for the pandemics of 1918 and 1957, respectively) belong to the Group 1 genes, which also include H5, H6, H8, H9, H11, H12, H13, and H16 subtypes. The H3 subtype (pandemic of 1968) belongs to Group 2 and includes H4, H7, H10, H14, and H15 HAs. The 16 subtypes were defined on the basis of their serologic distinctiveness, and therefore, protective immunity across all subtypes would not be expected [8]. The key question is: how could a vaccine be developed to neutralize an extremely divergent group of HA antigens?

One answer is to search for conserved protective epitopes on which to focus immune effector mechanisms and subsequently engineer an antigen eliciting such immunologic memory. Neutralizing antibodies serve as functional probes for epitopes conserved across multiple HA subtypes, making them the ideal targets for universal vaccine development. The most remarkable of these probes that have thus far been investigated are human monoclonal antibodies that inhibit the membrane fusion
process and neutralize most viruses with group 1 HA subtypes [9–11]. Structural and functional analyses have defined a highly conserved epitope in the stem of HA that encompasses the “fusion peptide” and elements of HA1 and HA2 that lock the peptide into place and inhibit viral fusion [12]. Most importantly, only 2 forms of the epitope were identified among all 16 HA subtypes of HA, raising the possibility that a bivalent antigen formulation could constitute the elusive “universal vaccine”. However, the absence of broad cross-subtype protection after influenza infection implies that this epitope may not elicit production of protective antibodies in vivo.

The article by Sui et al [13] in this issue provides tantalizing evidence that this conserved epitope is targeted by human serum antibodies that neutralize viral infectivity in vitro. In the first part of their innovative study, Sui et al [13] addressed the problem by analyzing the molecular specificity of human serum antibody responses to inactivated H5N1 vaccine. Volunteers vaccinated with a monovalent split virion H5N1 vaccine developed increased levels of serum antibodies that bound to H5 HA (group 1) and also recognized epitopes in H7 HA (group 2). They also found that pre-vaccination serum samples contained antibody to H3, H5, and H7 HA subtypes, presumably induced by past seasonal influenza infections or vaccination. Most importantly, H5N1 vaccination also elicited antibodies that competed in enzyme-linked immunosorbent assay (ELISA) with binding of human neutralizing monoclonal antibody (mAb) F10 to its target epitope on the H5 HA stem, suggesting that these volunteers had developed broadly neutralizing antibodies to group 1 viruses as a result of H5N1 vaccination. These ELISA results were in agreement with those of follow-up neutralization assays, which showed an increase in serum neutralizing antibody to H5N1 in serum samples from vaccinees, establishing the expected functionality of the antibodies. Although these studies are consistent with recent results using seasonal influenza vaccination [14], Sui et al [13] did not determine, using these assays, whether neutralization of the divergent subtype was indeed mediated by antibodies to the fusion peptide on the HA stem or by antibodies to a different epitope.

Sui et al [13] tackled this question by investigating whether antibodies in serum samples from a US serum donor pool (unlikely exposed to avian influenza) competed with human mAb F10 for binding to the H5 HA. To this end, they developed an interesting method to purify human antibodies bound to the fusion peptide epitope on the H5 HA. Based on earlier studies that indicated that pooled human immunoglobulin (Ig) G preparations (intravenous Ig [IVIG] for therapeutic use) contained antibodies to multiple influenza subtypes, they used IVIG to extract the polyclonal human Ig molecules bound to the H5 HA epitope by competitive elution with human mAb F10. This remarkable approach enabled the analysis of the polyclonal antibodies in a neutralization assay. Indeed, the IVIG antibodies eluted from H5 HA by mAb F10 competition could neutralize pseudotyped H1 and H5 viruses subtypes (group 1). However, the representation of these antibodies in the serum from the general population (IVIG donors) was extremely low (.001% of total Ig), which is consistent with the view that the majority of neutralizing antibodies elicited by infection or vaccination are HA subtype–specific [15]. Interestingly, the VH1-69 Ig gene family that encodes the heavy chain of broadly neutralizing antibody F10 and is very frequently represented in serum samples from the general population suggests that the target epitopes are inaccessible to B cell receptors, possibly due to dense packing of HA spikes on virus particles [13, 15]. The observation that neutralizing antibodies to epitopes present in multiple HA subtypes (eg, the fusion peptide) are part of the normal human immune repertoire is auspicious for universal influenza vaccine development, because it implies that broad protection may be elicited by enhancing the immunodominance of epitopes on the stem of engineered HA antigens. These promising approaches to developing a universal influenza vaccine are currently being intensively investigated in academic, government, and industrial research laboratories [8, 16, 18].

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