Universal epitopes of influenza virus hemagglutinins?

Taia T Wang & Peter Palese

The influenza virus has proved an elusive target in the development of broadly protective vaccines. A new study identifies an antibody with broad neutralizing activity against influenza viruses of different subtypes. The antibody recognizes a highly conserved region on the viral hemagglutinin that may be targeted to prevent infection.

Influenza viruses remain a significant cause of morbidity and mortality worldwide. Circulating virus strains demonstrate a dramatic increase in resistance to available drugs, making the development of new treatments, both prophylactic and therapeutic, a priority. The influenza vaccine is by far the most effective means of preventing infection, but current vaccines leave room for improvement as they require near-annual reformulation and accurate prediction of circulating strains for the upcoming season. A universal influenza virus vaccine would represent a tremendous medical advancement; however, owing to the remarkable ability of the virus to tolerate changes in antigenic structure, a 'one shot' vaccine remains theoretical. The search for a common surface epitope between strains that elicits a neutralizing immune response has turned up little of promise; antibodies in the literature that protect against multiple strains of influenza remain extremely rare. An elegant study by Sui and colleagues now demonstrates that a universal immune-based treatment or vaccine may not be out of reach. They show that specific antibodies that prevent viral fusion with the host cell are able to neutralize across hemagglutinin subtypes including both avian H5 viruses and the 1918 pandemic H1 virus[1].

An adaptive immune response to influenza elicits antibodies to many viral proteins, a small subset of which confer protective immunity. Most virus-neutralizing antibodies (nAbs) bind the hemagglutinin molecule, one of three proteins on the viral envelope (**Fig. 1**). Anti-hemagglutinin nAbs work either by blocking attachment of virus to the host cell or by disrupting fusion of hemagglutinin with the endosome, preventing release of viral RNA into the cytoplasm[2,3] (**Fig. 2**). Most nAbs bind specific antigenic sites surrounding the receptor binding pocket of hemagglutinin, preventing viral attachment to the host cell (**Fig. 1b**). Escape variants with mutations in the antigenic sites easily avoid neutralization by existing host antibodies, leading to seasonal influenza outbreaks. The emergence of antigenic variants also dictates the frequent production of new vaccines containing relevant virus strains.

Sui *et al.* demonstrate that a region in the stem of the hemagglutinin molecule containing the fusion peptide is structurally conserved across multiple viral subtypes. They isolated a panel of single-chain Fv (scFv) antibodies against this region that show an unprecedented degree of cross-reactivity.

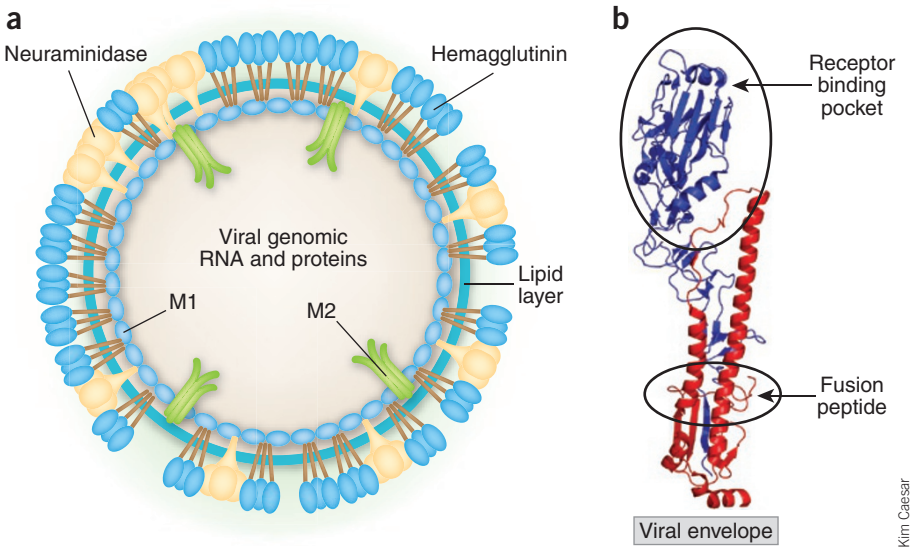

Figure 1 Influenza virus hemagglutinin. (**a**) Influenza virion. The hemagglutinin is a trimeric glycoprotein of the viral envelope. (**b**) Structure of a hemagglutinin monomer. Each monomer is made up of two segments, HA1 (blue) and HA2 (red), which includes the fusion peptide. The area containing the antigenic sites, the region eliciting the majority of neutralizing antibodies, is circled on the top of the structure, with the ligand binding site indicated by an arrow. The region of F10 binding is circled on the bottom of the structure, with the fusion peptide indicated by an arrow.

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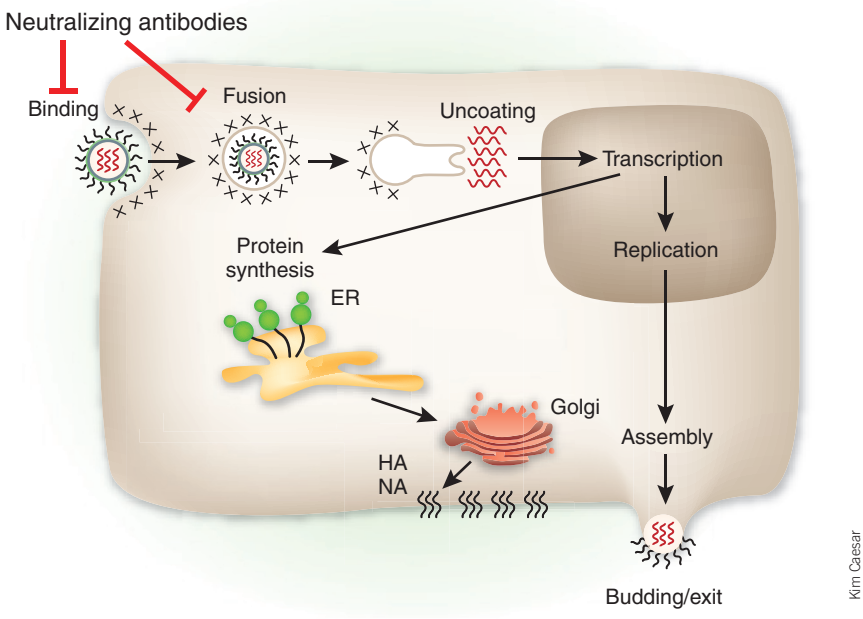

Figure 2 Antibody-mediated inhibition of influenza virus replication. Neutralizing antibodies inhibit either binding to the host cell or fusion to the endosome. ER, endoplasmic reticulum; HA, hemagglutinin; NA, neuraminidase.

One monoclonal antibody (mAb), F10, binds representative viruses from 8 of the 16 hemagglutinin subtypes. F10 is also shown to protect mice against multiple H5 and H1 viruses. In addition, the authors were unable to select escape variants to these mAbs, suggesting that the region targeted is resistant to genetic variation. This may be due to electrostatic and structural properties that are required to enable pH-dependent fusion to occur, while also making the region not readily permissive to amino acid changes. This would make the fusion peptide an ideal target: a viral Achilles' heel. An antibody such as F10 is intriguing for its potential to be used in the treatment of influenza disease, including pandemic influenza.

Why are broadly neutralizing mAbs rarely described in the literature? The success by Sui *et al.* may lie in the method they used for selection of antibodies. Although mAbs to influenza have been studied for decades, most are isolated after immunization with virus, a procedure that biases antibody production toward those that react with the most immunogenic regions of the viral surface. The antigenic sites of hemagglutinin, located in the membrane-distal region of the molecule, are readily accessible for B cell activation. In contrast, the fusion peptide is located proximal to the viral membrane and is therefore less likely to be available to B cell receptors (**Fig. 1b**). The antigenic properties of hemagglutinin on the viral surface probably culminate in minimal humoral immunity directed toward the fusion peptide region of the molecule. Sui *et al.* selected from a human scFv library and they panned with purified hemagglutinin protein, thus eliminating steric restrictions inherent in the membrane-bound hemagglutinin configuration.

By resolving the crystal structure of an H5 trimer bound to F10, Sui *et al.* show that residues from the HA1 and HA2 segments (**Fig. 1b**), including the fusion peptide, are involved in binding. The characterization of the binding epitope may guide production of a vaccine with the potential to provide significant heterosubtypic immunity. Technically, generating such a vaccine may not be a straightforward task, as the noncontiguous epitope would have to be built into a construct in such a way as to render it immunogenic.

It also remains to be seen whether a vaccine that would elicit a fusion-inhibiting immune response would be safe. In their study, Sui *et al.* found biased usage of *VH1-69*, encoding an immunoglobulin heavy chain that is found in less than 2% of normal human B cells[4] but that was used by nine out of ten antibodies selected. Use of the *VH1-69* gene has also been observed in previous studies of anti-hemagglutinin antibodies that neutralize across influenza subtypes[5,6]. Protective immune responses mediated by antibodies using a restricted heavy-chain repertoire have been observed: a particularly well-characterized example is the response to *Haemophilus influenzae* type b polysaccharide, which shows preferential use of the *VH3-23* gene[7]. Interestingly, the *VH1-69* gene has also been shown to be preferentially used in the response to the E2 glycoprotein of the hepatitis C virus and in neutralizing antibodies to the HIV gp120 glycoprotein[8,9]. Of possible significance, the *VH1-69* gene is used in anti-DNA autoantibodies from patients with systemic lupus erythematosus[10,11]. *VH1-69* is also overrepresented among natural IgM antibodies that cause mixed cryoglobulinemia occurring secondarily to hepatitis C virus infection[12] and in antibodies against ADAMTS13, found in autoimmune thrombocytopenic purpura[13]. Because of the involvement of *VH1-69* in specific autoimmune conditions, the fine specificity that may be elicited by an influenza fusion peptide–based (or HIV gp120–based) vaccine is a possible concern.

A limitation in the findings by Sui *et al.* is the lack of reactivity of their mAbs against many influenza subtypes that could cause pandemic disease, in particular H3 viruses, which are at present responsible for a significant fraction of seasonal influenza[14]. The work by Sui *et al.*, however, does represent an important advance in the characterization of conserved epitopes of the influenza virus hemagglutinin and brings us closer to the development of a universal influenza virus vaccine.

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