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Host versus flu: antibodies win a round?

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Abstract

Structural and functional analyses of three neutralizing antibodies against influenza virus H2 HA may explain why this HA subtype has disappeared from circulation in the human population and point to a potential new avenue for anti-flu therapeutics.

Year in and year out, seasonal influenza A virus infection exacts a deadly and costly toll around the world. Several times per century, the public health impact of influenza infections increases dramatically, in the form of pandemics (Fig. 1a) caused by the introduction into the human population of antigenically new strains out of those circulating in the enormous animal reservoir¹.

Influenza viruses are classified on the basis of their subtype of hemagglutinin (HA), the major surface glycoprotein that initiates infection by attaching viruses to host-cell terminal sialic acid receptors and by promoting viral-host membrane fusion. Antibodies to HA are the key to virus- or vaccine-induced protective immunity. The rapid antigenic evolution of the HA (termed antigenic drift) prevents effective immunization, necessitating vaccine reformulation nearly every year. Only 3 of the 17 known HA subtypes (H1, H2 and H3) are present among human influenza A viruses. Of these, H2 is unique for circulating for only 12 years following its emergence during the 1957 pandemic (Fig. 1a). By contrast, H1 and H3 have circulated for combined periods of 75 and 43 years, respectively, since their appearances in humans in 1918 and 1968 (ref. 2). The absence of H2-specific antibodies in individuals born after 1968 (the year H3 supplanted H2 in circulating human influenza A viruses) has raised fears of a severe pandemic caused by reintroduction of H2 viruses into humans³ (H2 viruses may have circulated in the late nineteenth century as well⁴). Understanding the factors that govern the emergence and circulation of human H2 viruses is critical in influenza biology.

In this issue, Xu *et al.*⁵ use X-ray crystallography to describe the interaction between H2 HA and three human monoclonal antibodies (mAbs) that demonstrate broad neutralizing activity against drifted H2 strains, and in one case against H3 strains, despite the large antigenic and evolutionary distance separating H2 and H3 HAs. This analysis reveals a tantalizing clue that could explain why H2 was so quickly supplanted by H3 in humans. The work also provides the foundation for developing a much-needed new class of anti-influenza drugs.

HA consists of a variable globular head domain atop a much more conserved stem that attaches the virus to viral and cellular membranes. Nestled among the hypervariable loops of the globular head lies the highly conserved receptor-binding site (RBS). Nearly all broadly neutralizing antibodies that have been discovered to date target either the stem or the RBS⁶.

A key feature of H1 and H3 viruses that limits protective immunity following infection or vaccination is that most induced antibodies bind hypervariable epitopes on the globular head of the HA molecule, promoting antigenic drift⁷⁻⁹ (Fig. 1b).

In an intriguing contrast, H2 appears to strongly induce antibodies specific for epitopes in the conserved RBS (Fig. 1b) in both humans and other animals^{10,11}. Xu *et al.*⁵ show that each of the examined mAbs insert loops into the RBS to stabilize binding through interaction between hydrophobic amino acids. Remarkably, the heavy chains of two of the mAbs derive from VH1-69 germ line genes. This gene family has recently become notable for its predilection for generating broadly neutralizing antibodies to the HA stem and also to conserved regions of HIV gp160 (ref. 12). The present results amplify the importance of this gene family in antiviral immunity and raise important questions regarding the specialization of antibody heavy chains in recognizing certain structural features in viral proteins. These findings also highlight the field's near-total ignorance of immunodominance in antibody responses: why are some epitopes more immunogenic than others? Is this strictly because of antibody repertoire and epitope structures, or do other features of the antigen (or pathogen) contribute as well, either by modulating antigen presentation or changing the cytokine environment?

If the H2 RBS is a frequent target for antibodies, then it should be subject to rigorous selection pressure in humans. Xu *et al.*⁵ demonstrate that for each antibody tested, a single amino acid substitution in the epitope is sufficient for viral escape from neutralization. Because these changes also greatly affect HA receptor specificity—HA binding is highly influenced by the oligosaccharide linkage and structure of the sialic acid receptor—they are likely to have a negative impact on viral fitness, thus limiting escape in humans, and indeed are present in only a few sporadic human H2 strains. Therefore, the immunodominance of the RBS, combined with the constrained mutation space of the RBS to allow proper receptor specificity or affinity, may limit the ability of H2 HAs to outrun the host antibody response, a hypothesis eminently testable in animal models in future studies.

Another important feature of this study is that the chemistry of antibody binding to the RBS provides valuable insights into developing RBS-binding drugs. Efforts to design competitive inhibitors based on the structure of the natural Neu5Ac sialic acid ligands are hobbled by the naturally low affinity (mM range) of HA-oligosaccharide interactions. By comparing three mAb–RBS structures, Xu *et al.*⁵ derive a rational framework for designing drugs that broadly bind influenza A (and even influenza B) HA RBSs, on the basis of the π - π stacking interaction between antibody residues (or potentially drugs) with a highly conserved aromatic residue surrounded by other highly conserved amino acids.

The efficacy of anti-flu therapeutics is threatened by the ascendance of drug-resistant strains¹³. Through error-prone viral polymerase, rapid generation time and the capacity of influenza A viruses to regain fitness through epistatic mutations¹⁴, drug-resistance mutations can arise rapidly. After decades of working in the dark, scientists are finally illuminating influenza's weak points, owing to increased government investment into basic influenza virology. Promising new drugs against additional targets are in the pipeline, but they are likely to face many of the same limitations^{15,16}. It would be a grave error to repeat the complacency of the past with regard to continuously developing new strategies to minimize the impact of this serious human pathogen. Xu *et al.*⁵ herald a potential new class of therapeutics by exploiting a chink in the armor of influenza A virus that allows efficient targeting of the H2 RBS by the human immune system. This also demonstrates how basic research can serendipitously lead to useful practical applications that cannot be predicted in a grant application.

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Figure 1.

Immune responses induced by influenza A virus. **(a)** Timeline of influenza A HA subtypes in human circulation since 1918. **(b)** Primary antibody response against H1- or H3-subtype viruses is primarily targeted to the hypervariable regions in the globular head (residues marked in blue), with very little targeted to the receptor-binding site (residues in red). This antibody repertoire has minimal activity during subsequent infection with a virus in which the hypervariable regions have drifted (from blue to green). In contrast, a greater share of the antibody response to H2-subtype viruses appears to be targeted toward the receptor-binding site, thus a greater degree of cross-reactivity is seen with drifted viruses.