Recent strategies to identify broadly neutralizing antibodies against influenza A virus

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Abstract

Recent technologies have made it possible to efficiently identify several broadly cross-neutralizing antibodies against the hemagglutinin of influenza A virus. With these advances comes a potential new age in influenza virus vaccine development and the possibility of effective, therapeutic immunotherapy.

Introduction and context

Influenza A virus is the cause of yearly seasonal epidemics and occasional pandemics with devastating effects. A current obstacle in the control of influenza is the lack of a vaccine that provides long lasting immunity from year to year, and that ideally confers protection in a pandemic situation. The challenge lies in the ability of influenza virus to antigenically change at a rapid pace, either by gradually accumulating mutations (antigenic drift) or by genetic reassortment via the exchange of viral genome segments (antigenic shift), thereby evading immune detection. Currently, influenza A virus H1 and H3 subtypes [based on the antigenicity of the viral hemagglutinin (HA)] are circulating in humans, but there are another 14 different HA subtypes circulating in birds, which theoretically could initiate a new pandemic. One logical approach to circumvent viral antigenic changes is to identify epitopes that are broadly cross-reactive among multiple influenza virus strains within a subtype or even between virus subtypes, that is, to overcome antigenic drift or antigenic shift. The feasibility of this idea, however, has long been doubted. Given the pivotal role of antibodies against the viral HA in controlling influenza infection [1–4], the prevailing belief has been that neutralizing antibodies targeting influenza viruses are directed against variable antigenic sites on the HA protein and are thus specific for a particular strain. It is noteworthy, therefore, that recent

evidence challenges this dogma. Influenza antibodies with cross-neutralizing properties can in fact be isolated using available technologies and, moreover, they may serve as a guide for vaccine design or as a source of material for passive immunization therapy.

Major recent advances

An appealing technique in the hunt for broadly neutralizing epitopes of influenza viruses is the screening of combinatorial antibody libraries, either engineered synthetically or derived from influenza virus-infected or vaccinated individuals, for reactivity with various strains. A combinatorial antibody library can represent the entire diversity of the human antibody repertoire or, more specifically, a complete history of an individual's immunological response to a pathogen. These libraries are, therefore, a rich source of fully human, monoclonal antibodies that potentially have therapeutic properties. By screening a combinatorial antibody library generated from the bone marrow of survivors of H5N1 avian influenza virus infections, Kashyap and colleagues [5] successfully obtained more than 300 unique H5N1 specific monoclonal antibodies, as well as three antibodies that neutralized both H5 and H1 subtypes of influenza viruses. A distinct advantage of these library approaches is the sheer number of antibodies produced for screening. With this vast complexity comes the opportunity to identify those rare antibodies that may

be difficult to isolate in the bulk population, but that have high affinity for a particular antigen or other unique features, for example, those that may target an epitope outside of the major antigenic sites of HA or even in another viral protein. Moreover, comparison of neutralizing versus non-neutralizing antibodies, as well as sequence analysis of these library members, could provide information on the physical interactions that govern virus neutralization.

Aside from combinatorial libraries, technologies that allow the efficient cloning of antibodies directly from humans supply an additional source of novel antibodies with cross-neutralizing or other favorable properties. Simmons *et al.* [4] cloned B-cell lines secreting neutralizing antibodies against H5N1 viruses from H5N1-infected survivors, and some of these antibodies could neutralize multiple H5N1 strains. Wrammert *et al.* [6] reported the cloning of high-affinity monoclonal antibodies from individuals following influenza vaccination. Antibodies specific for each of the three component vaccine strains could be rapidly isolated from patients within 7 days of vaccination. In a subsequent study, it was demonstrated that influenza neutralizing antibodies could be isolated from the circulating B cells of 1918 influenza pandemic survivors [7]. Among these monoclonal antibodies was at least one that cross-neutralized a highly divergent H1N1 virus from 1977. In addition, further analysis of the antibody responses in these 1918 survivors, as well as in survivors of more recent pandemics, may provide insights into the requirements for a successful immune response against pandemic influenza and a potential source of monoclonal antibody therapy.

Structural information on different HA proteins complements antibody screening for identifying novel, cross-reactive neutralization targets. Crystal structures are available for several HA subtypes, including H1, H3, and H5 HAs, and these structures can be compared to guide the rational targeting of epitopes that may be cross-reactive [8–14]. Between subtypes, this may be more feasible for the H1 and H5 HAs, which have higher homology, both at the amino acid and the structural level, compared to H3 HAs [15]. Information can also be garnered from studies examining how structure-based modification of HA affects antigenicity. Yang and colleagues [15] investigated mutations in the receptor binding domain of an avian (H5) HA that altered its specificity for particular sialic acid linkages, one of the known determinants of influenza virus host tropism. After immunization of mice with these mutants, the group isolated monoclonal antibodies capable of neutralizing lentiviral particles bearing these HA variants.

These data suggest that it may be possible to predict epitopes likely to react with emerging influenza strains based on HA structure and receptor specificity. Notably, the neutralizing antibodies that were isolated in this study targeted epitopes within the receptor-binding domain that were adjacent to, but outside of, the major antigenic sites of HA.

Future directions

Technologies that utilize combinatorial libraries and allow the rapid cloning of antibodies from humans, combined with structural analysis of HA variants, provide methods to identify novel, cross-reactive epitopes in influenza A virus. Potentially, these approaches will also yield invaluable information on the chemistry of antibody binding to epitopes, the requirements for virus neutralization, and the determinants of an effective vaccine. Characterization of the HA epitopes recognized by broadly neutralizing antibodies might provide information for the design of novel vaccines presenting only these epitopes. A vaccine that protects individuals from various strains within a single subtype would already be an improvement over the current, yearly vaccines. However, an ideal approach with the threat of an impending pandemic is one that protects or can be used as therapy across subtypes and emerging strains. Recent studies suggest that identification of anti-influenza virus monoclonal antibodies with cross-reactive properties is now within reach. However, a number of important questions remain regarding the application of these techniques in the clinical setting. For example, how can we design an immunogenic vaccine that presents influenza virus cross-reactive epitopes and provides high levels of protection against infection? Furthermore, how quickly will these sites mutate in the presence of selective pressure generated by vaccination? Also, would monoclonal antibody therapy reduce morbidity and mortality in infected individuals? These are among the many questions that are worthy of, and will surely be the subject of, future research.

Abbreviations

HA, hemagglutinin.

Competing interests

The authors declare that they have no competing interests.

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