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Review

Broadly neutralizing antibodies against influenza viruses

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ABSTRACT

Despite available antivirals and vaccines, influenza continues to be a major cause of mortality worldwide. Vaccination generally induces an effective, but strain-specific antibody response. As the virus continually evolves, new vaccines have to be administered almost annually when a novel strain becomes dominant. Furthermore, the sporadic emerging resistance to neuraminidase inhibitors among circulating strains suggests an urgent need for new therapeutic agents. Recently, several cross-reactive antibodies have been described, which neutralize an unprecedented spectrum of influenza viruses. These broadly neutralizing antibodies generally target conserved functional regions on the major influenza surface glycoprotein hemagglutinin (HA). The characterization of their neutralization breadth and epitopes on HA could stimulate the development of new antibody-based antivirals and broader influenza vaccines. This article forms part of a symposium in *Antiviral Research* on "Treatment of influenza: targeting the virus or the host."

1. Introduction

Infections with influenza virus have a major impact on human health and the economy. Annual epidemics result in a substantial number of hospitalizations, with an estimated 3–5 million cases of severe disease and 300–500,000 deaths globally. During the 20th century, three major influenza pandemics occurred with a total mortality of 50–100 million people (Lambert and Fauci, 2010). Influenza types A and B are enveloped RNA viruses that belong to the *Orthomyxoviridae* family and can lead to respiratory or gastro-intestinal tract infections in mammalian or avian species. Both types are responsible for recurrent annual influenza epidemics, but only influenza A has so far led to pandemics. Influenza A viruses circulates in a variety of animals, including birds, humans, horses, pigs and sea mammals, while influenza B is restricted to humans and seals (Osterhaus et al., 2000; Webster et al., 1992).

Influenza A and B viruses contain two surface glycoproteins, hemagglutinin (HA) and neuraminidase (NA), that are embedded in the viral membrane envelope. HA mediates binding to sialic acid receptors on host cells and subsequent fusion between the virus and host membranes, while NA is responsible for virus progeny release. There are 17 different subtypes of influenza A HA (H1-H17), which are divided into two markedly distinct antigenically phylogenetic groups, group 1 (H1, H2, H5, H6, H8, H9, H11-H13, H16 and H17) and group 2 (H3, H4, H7, H10, H14 and H15). Most subtypes are present in the avian host, but only H1, H2 and H3 are or have been resident in the human population. Influenza B is classified in two distinct phylogenetic lineages, Yamagata and Victoria (Yamashita et al., 1988).

HA is synthesized as a single polypeptide and folds into a trimeric spike (HA0) that is cleaved by host proteases into HA1 and HA2 subunits. Each trimer comprises a membrane distal globular head composed of HA1, which contains the receptor-binding site, and a stem region, which houses the fusion machinery (Wilson et al., 1981) (Fig. 1). The receptor-binding site is located in a small depression on the head of the HA and mediates virus binding to host cell sialic-acid receptors. The stem region is primarily composed of HA2 and some HA1 residues and is mostly helical. Like the surface spikes of many other viruses, HA is highly glycosylated (Wiley et al., 1981; Wilson et al., 1981). Although some glycans may be required for correct protein folding (Roberts et al., 1993), most are used as a mean for the virus to circumvent the immune response. The glycans are synthesized by host enzymes and are observed by the immune system as "self-structures" and do not normally induce an adaptive immune response. Moreover, glycans can directly shield vulnerable epitopes on HA and thereby prevent immune recognition.

Vaccination provides the best method for prevention and control of influenza and normally elicits a potent neutralizing antibody response. Most vaccines are trivalent and contain representative HAs from two influenza A strains and one influenza B strain. However, FDA recently approved quadrivalent influenza vaccines containing two influenza A strains and two influenza B strains. Current licensed vaccines include trivalent inactivated vaccines,

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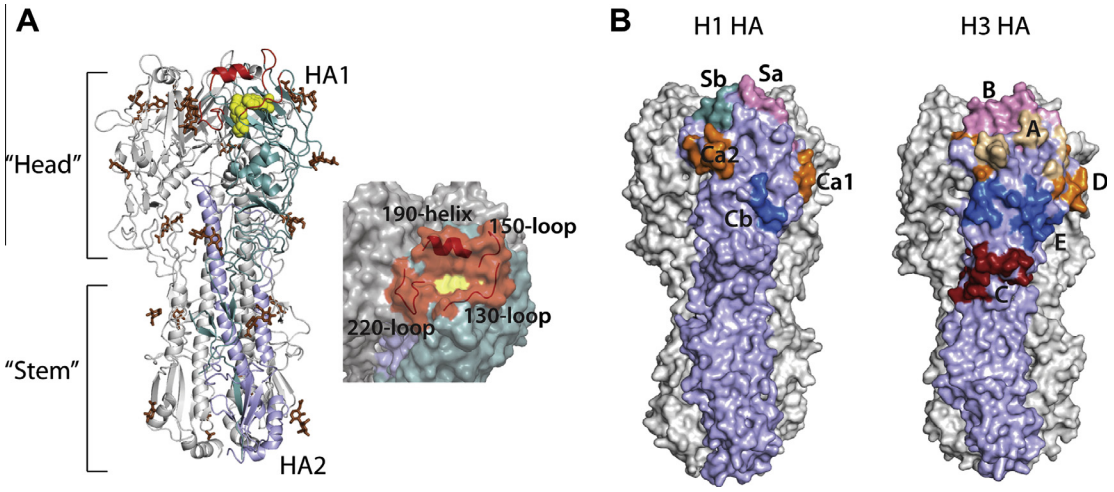

Fig. 1. Crystal structure of HA. (A) Structure of the trimeric HA spike (PDB code; 4FNK) (Ekiert et al., 2012). One protomer is colored in cyan (HA1) and light blue (HA2). The receptor binding site is colored in yellow and the surrounding loops and helix in red. Glycans are brown (left). Surface representation of the receptor binding site and its surroundings (right). (B) The antigenic sites on HA. Antigenic sites Sa (pink), Sb (cyan), Ca1 and Ca2 (orange), and Cb (blue) on H1 HAs (left) (PDB code; 3LZG) (Xu et al., 2010). Antigenic sites A (wheat), B (pink), D (orange), E (blue) and C (red) on H3 HAs (right) (PDB code; 4FNK) (Ekiert et al., 2012).

live-attenuated vaccines and subunit vaccines. The trivalent inactivated vaccines contain killed influenza viruses and induce a protective serum antibody response, but a poor cell-mediated response, while the live attenuated vaccine contains weakened viruses and induce both a humoral and cellular immune response. These vaccines are grown in chicken eggs, which is relatively time consuming. The subunit vaccine contains purified baculovirus-expressed HA0 protein and, thus, circumvents the lengthy process of egg adaption of influenza virus (He et al., 2006).

Most antibodies, which are generated upon vaccination or infection, are targeted towards the highly variable head of HA and are often strain-specific. As a consequence, new formulations of the vaccine are generated almost annually when a new strain starts to dominate (Fiore et al., 2010). The strains to include in the upcoming vaccine are predicted by the WHO and the efficiency of the vaccine thus depends on the match between the vaccine strains and the circulating strains. Influenza undergoes continual antigenic drift, in which mutations are accumulated in HA due to an error-prone RNA polymerase, and the selective pressure from the host immune system. Additionally, co-infection of a single host with more than one virus strain can result in an antigenic shift, in which re-assortment of genes from different viruses generates novel subtypes (Carrat and Flahault, 2007). As a result, cross-species transmission of newly adapted strains might occur, as in the case of the highly pathogenic avian H5N1 virus (Claas et al., 1998). If little or no immunity exists in the bulk of the human population, as with the 1918 Spanish flu, new pandemics can arise. The emergence of new influenza strains and lack of herd immunity in the population therefore remain a persistent threat to human health.

Given the sporadic occurrence of oseltamivir-resistant viruses, the characterization of zanamivir-resistant viruses and the lack of heterovariant vaccines, alternative treatment strategies for influenza are urgently needed (de Jong et al., 2005; Hurt et al., 2009; Monto et al., 2006). Immunotherapy with monoclonal antibodies represents a complementary strategy to current antivirals. The use of monoclonal antibodies for the treatment of medical conditions, including viral diseases such as hepatitis and respiratory syncytial virus infection, is well established (Sawyer, 2000). Monoclonal antibody therapy could be employed alone for the treatment of infection with influenza virus strains that are resistant to current antivirals, or in combination with antivirals in the case of patients with severe infections. Prophylactic administration of antibodies could be valuable in the case of a pandemic with a highly pathogenic virus such as H5N1, especially for persons who are particularly susceptible to illness, such as elderly and immunocompromised individuals, and those with a higher risk of infection, such as health care workers.

Recently, exciting new influenza monoclonal antibodies have been identified that are capable of neutralizing a wide range of influenza viruses (Corti et al., 2011; Dreyfus et al., 2012; Ekiert et al., 2009, 2011, 2012; Kashyap et al., 2010; Krause et al., 2012; Lee et al., 2012; Sui et al., 2009; Tan et al., 2012; Throsby et al., 2008; Tsibane et al., 2012; Wang et al., 2010b; Yoshida et al., 2009) (see below). These broadly neutralizing antibodies show an unprecedented breadth of cross-reactivity, enabling them to neutralize many different strains within a subtype, group or even between different groups and types of influenza virus. The therapeutic and prophylactic efficacy of these antibodies have been characterized in both mouse and ferret models, and show promising results. The molecular basis of influenza virus recognition has also been elucidated for many of these antibodies through biochemical and structural studies, as discussed below.

2. Antibody recognition of the HA head

The globular membrane distal head of HA is highly immunogenic and is the main target of antibodies generated during vaccination. However, the variability of HA leads to annual influenza epidemics. Five distinct antigenic sites have been characterized for the H1 subtype in the head and are designated Sa, Sb, Ca1, Ca2 and Cb, while those in the H3 subtype are called A through E (Caton et al., 1982; Wiley et al., 1981) (Fig. 1B). These sites are hypervariable and are the prime locations for escape from host immune detection. In contrast, the receptor binding site is relatively conserved, as it is functionally constrained. The binding site is located in a small, shallow pocket at the top of the HA, bordered by the 130-loop, the 150-loop, the 190-helix and the 220-loop. This site represents a possible target for broad-spectrum antibodies. However, the typical footprint of an antibody is rather large, and would inevitably contact the less conserved areas outside the receptor-binding site. Notwithstanding, broadly neutralizing antibodies, which target the receptor binding site and its surroundings, have recently been identified (Ekiert et al., 2012; Lee et al., 2012;