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Fully human broadly neutralizing monoclonal antibodies against influenza A viruses generated from the memory B cells of a 2009 pandemic H1N1 influenza vaccine recipient

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ABSTRACT

Whether the 2009 pandemic H1N1 influenza vaccine can induce heterosubtypic cross-protective anti-hemagglutinin (HA) neutralizing antibodies is an important issue. We obtained a panel of fully human monoclonal antibodies from the memory B cells of a 2009 pandemic H1N1 influenza vaccine recipient. Most of the monoclonal antibodies targeted the HA protein but not the HA1 fragment. Among the analyzed antibodies, seven mAbs exhibited neutralizing activity against several influenza A viruses of different subtypes. The conserved linear epitope targeted by the neutralizing mAbs (FIEGGWTGMVDG-WYGYHH) is part of the fusion peptide on HA2. Our work suggests that a heterosubtypic neutralizing antibody response primarily targeting the HA stem region exists in recipients of the 2009 pandemic H1N1 influenza vaccine. The HA stem region contains various conserved neutralizing epitopes with the fusion peptide as an important one. This work may aid in the design of a universal influenza A virus vaccine.

Introduction

Because of their highly flexible genomes, influenza A viruses cause annual epidemics and sometimes pandemics around the world. For nearly 100 years, influenza A viruses have been a global threat to humans (Palese, 2004). Based on the antigenicity of the hemagglutinin (HA) protein, influenza A viruses are classified into two groups and at least 16 different subtypes (H1-H16). The HA protein is the functional protein that mediates the entry of influenza viruses into susceptible host cells and thus contains various epitopes that are recognized by neutralizing antibodies (Skehel and Wiley, 2000). However, heterosubtypic neutralizing or protective antibody responses are rarely observed in the general population, largely because of the high mutation rate of the HA protein, especially in the globular head (HA1) region, which is the primary target of the humoral immune response. Consequently, when a new reassortant influenza virus emerges that the human immune system has not previously encountered, a pandemic may occur. The 2009 swine-origin H1N1 influenza is an example of such a pandemic.

The 2009 pandemic H1N1 influenza virus contains gene segments that are in both the American and the Eurasia swine genetic linkages (Garten et al., 2009). Nucleotide sequence alignment has shown that the HA sequence of the 2009 pandemic H1N1 influenza virus is divergent from the sequences of the seasonal H1 influenza viruses that have previously been

Abbreviations: mAb, monoclonal antibody; $TCID_{50}$, 50% tissue culture infective dose

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circulating in humans. The antigenicity of the HA in this strain is also highly distinct from that of the previously circulating H1 influenza viruses (Garten et al., 2009; Hancock et al., 2009). People, especially young people, generally lacked protection against this new virus (Hancock et al., 2009), and the 2009 pandemic H1N1 influenza vaccines have been proven effective in inducing neutralizing antibody responses against the pandemic influenza virus (Liang et al., 2010; Zhu et al., 2009).

It is important to determine whether cross-reactive neutralizing antibodies against both seasonal and pandemic influenza viruses are present in individuals who were infected with or vaccinated against 2009 pandemic H1N1 influenza. Recently, Wrammert et al. discovered that plasmablasts from 2009 pandemic H1N1 influenza patients produced cross-subtype neutralizing antibodies that targeted both the HA stalk and the head domain (Wrammert et al., 2011). We examined whether such antibodies existed in individuals vaccinated against pandemic influenza.

In this study, we used the full-length HA protein from the 2009 pandemic H1N1 influenza virus to raise fully human neutralizing mAbs. We obtained 19 monoclonal antibodies from the memory B cells of a 2009 pandemic H1N1 influenza vaccine recipient and confirmed that all 19 of the monoclonal antibodies recognized the lysates of both the pandemic virus and the recently circulating seasonal H1N1 influenza virus. Seven of the human monoclonal antibodies were further found to have apparent neutralizing effects against different subtypes of influenza A viruses, including viruses belonging to both group 1 and group 2 and the pandemic influenza virus. Interestingly, we found that most of the monoclonal antibodies, including the seven neutralizing mAbs, bound to the HA stem region (HA2), which is relatively conserved among different influenza A virus strains. These findings indicate that a broad cross-subtype neutralizing antibody response targeting the HA stem region exists in individuals vaccinated against 2009 pandemic H1N1 influenza and that these broadly reactive memory B cells may be important for protecting humans from infection with different influenza A viruses. A functional analysis revealed that the HA2 region contained several (at least four) conserved neutralizing epitopes that could be recognized by the raised mAbs. Further experiments showed that one of them was a linear epitope (FIEGGWTGMVDGWYGYHH), which was in the region of the fusion peptide on HA2. These results may be helpful in the design of universal influenza vaccines.

Results

Generation of fully human mAbs and their gene usage study

A 27-year-old healthy female adult volunteer who had been vaccinated with a 2009 pandemic H1N1 influenza split-virion vaccine for one month was enrolled in this study. We used flow cytometry to separate pandemic H1N1 HA-specific memory B cells with three surface markers: CD19, IgG, and HA-specific BCR. A baculovirus-expressed HA protein was used for cell sorting. As shown in Fig. 1, the memory B cells accounted for approximately 0.6% of the total peripheral blood small lymphocytes, and less than 1% of the selected memory B cells were HA-specific .

The antibody variable genes of these memory B cells were identified with single-cell RT-PCR and nested PCR (Smith et al., 2009; Wrammert et al., 2008). Nineteen human monoclonal

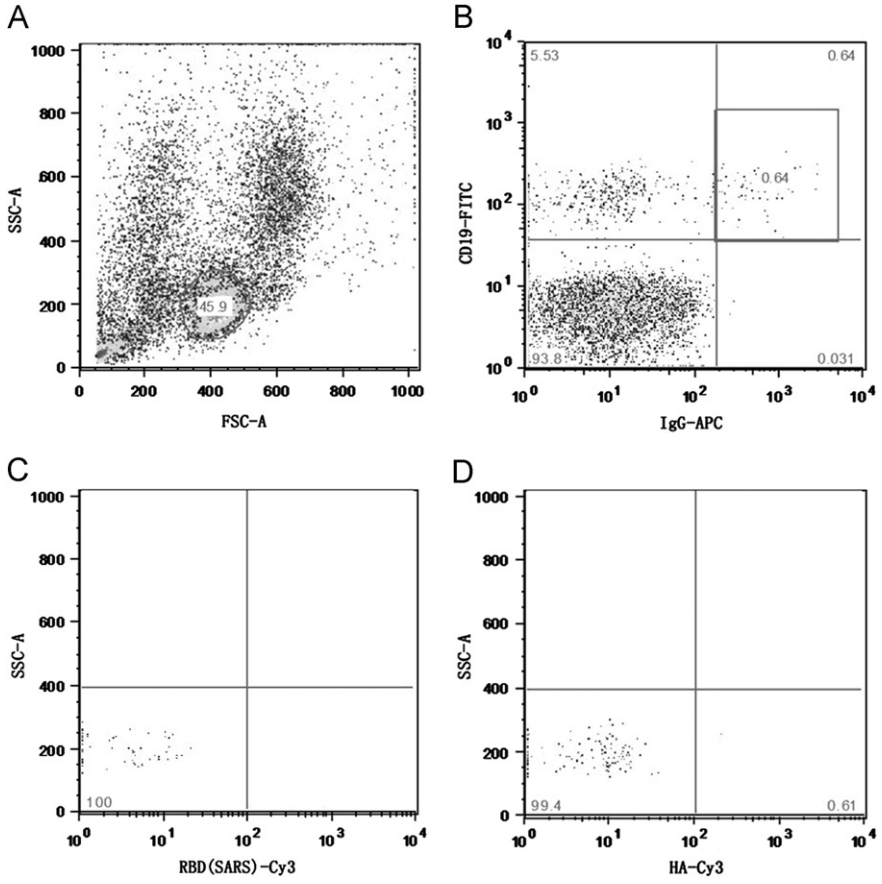

Fig. 1. The isolation of pandemic H1N1 HA-specific memory B cells. (A) Small lymphocytes were sorted from the human peripheral blood of a vaccinated individual. (B) Memory B cells were sorted using CD19 and IgG as markers. (C) Negative control: cells stained with an unrelated protein (RBD, receptor-binding domain of SARS-CoV). (D) Cells were sorted using the pandemic H1N1 HA protein. HA-specific memory B cells accounted for approximately 0.61% of the total memory B cells.