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Diverse antigenic site targeting of influenza hemagglutinin in the murine antibody recall response to A(H1N1)pdm09 virus

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ABSTRACT

Here we define the epitopes on HA that are targeted by a group of 9 recombinant monoclonal antibodies (rmAbs) isolated from memory B cells of mice, immunized by infection with A(H1N1)pdm09 virus followed by a seasonal TIV boost. These rmAbs were all reactive against the HA1 region of HA, but display 7 distinct binding footprints, targeting each of the 4 known antigenic sites. Although the rmAbs were not broadly cross-reactive, a group showed subtype-specific cross-reactivity with the HA of A/South Carolina/1/18. Screening these rmAbs with a panel of human A(H1N1)pdm09 virus isolates indicated that naturally-occurring changes in HA could reduce rmAb binding, HI activity, and/or virus neutralization activity by rmAb, without showing changes in recognition by polyclonal antiserum. In some instances, virus neutralization was lost while both ELISA binding and HI activity were retained, demonstrating a discordance between the two serological assays traditionally used to detect antigenic drift.

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Introduction

Influenza viruses are common pathogens of many species, including humans, in whom seasonal influenza epidemics are a significant cause of global disease with a high annual public health and economic burden (Molinari et al., 2007). Vaccination is the most effective public health counter-measure against influenza. Antibodies play an important protective role against influenza infections, and the goal of immunization against influenza viruses is to induce a protective antibody response against the immunodominant surface protein, hemagglutinin (HA). As a small number of mutations in HA allow the virus to avoid neutralization by antibodies, influenza viruses rapidly evolve resistance to population immunity, so that influenza vaccines need to be reformulated on a regular basis. In addition to the antigenic match between vaccine and circulating virus, the protective efficacy of influenza vaccination can vary depending on the age, history of exposure and health status of the vaccinee.

HA binds to the viral receptors, sialic acids (SA), on host cells facilitating virus entry, and also brings about membrane fusion between the virus and cellular membranes that is triggered by the low pH of the endosomal compartment. Structurally, HA is composed of a globular head that includes the receptor-binding site (RBS) and previously defined antigenic sites, and a stem region that includes the membrane fusion peptide, as well as transmembrane and cytosolic regions. A number of lines of evidence, including the generation of antibody escape mutants and measurement of binding to specific mutants, have shown that protective antibodies tend to bind to specific antigenic sites on the globular head (Wan et al., 2014; Rudneva et al., 2010). For the H1 subtype of HA, the sites are termed Sa, Sb, Ca1, Ca2, and Cb (Caton et al., 1982; Yewdell and Gerhard 1981). The residues comprising these antigenic sites tend to be highly variable over time and, since mutations in these sites are well tolerated by viruses, this allows mutant viruses to arise that are capable of infecting individuals immune to the parent influenza strain. Protective antibodies can also bind to regions outside of the defined antigenic sites on the HA head (Zhu et al., 2013; Matsuzaki et al., 2014). In many cases, these regions of HA are more conserved than the antigenic sites, and antibodies targeting these regions may provide cross-reactive protection against multiple strains of influenza (Lee et al., 2014; Whittle et al., 2011; Hong et al., 2013; Krause et al., 2011).

Immunity to influenza is assessed using serological assays, such as hemagglutination inhibition (HI) or virus neutralization (VN) assays, that are correlated with protection against disease(Tsang et al., 2014; Hobson et al., 1972; Coudeville et al., 2010; Cox, 2013). These assays measure the "average" specificities and affinity of a complex mixture of antibodies. In principle, sequence analysis of the variable regions of antibodies in combination with their

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functional characteristics may provide more detailed information on the predicted immune response, including identification of antibodies that are present below the limit of detection of conventional assays but that have the potential for cross-protective or broadly reactive immunity.

Antibodies are comprised of a heavy and light chain. The heavy chain is the result of genomic recombination of a variable (IGHV), diversity (IGHD), and a joining (IGHJ) gene, while the light chain is composed of a variable (IGKV or IGLV) and joining (IGKJ) gene recombination. In a previous study, we analyzed the diversity and nature of the mouse antibody response against influenza HA by cloning IgH and IgL chains from individual B cells (Wilson et al., 2014). This work demonstrated that the antibody response targeting the HA of A(H1N1)pdm09 (following this particular immunization regimen) is relatively narrow, being dominated by approximately 100 heavy chain VDJ germline sequences and approximately 35 light chain VJ germline sequences. Here, we define the epitopes on HA that are targeted by a group of nine representative recombinant monoclonal antibodies (rmAbs) from this response and begin to define antigen and genomic sequence information associated with antibody recognition and neutralizing activity.

Results

rmAb recognize multiple epitopes on HA

In previous work we immunized mice by sub-lethal infection with A(H1N1)pdm09 virus and boosted with TIV, leading to a robust antibody response that predominately targeted the HA of pdm09 viruses (Wilson et al., 2014). We used single-cell cloning from memory B cells to identify a number of rmAbs, including nine that bound H1N1pdm recHA with sub-nanomolar steady-state affinity (Wilson et al., 2014). These rmAb contain the most common IgH VDJ and IgK VJ rearrangements identified in that screen, and each displayed HI activity toward H1N1pdm virus (Wilson et al., 2014). To determine the binding sites of these rmAbs, we generated a panel of 20 recHAs, each containing a single point mutation within or near the defined antigenic regions of CA/07 (Fig. 1A and B), and measured binding affinity of the rmAbs to each by BLI. Control monoclonal antibodies 26-D11 and Y2_50132_1C04 ("C04") bound with high affinity to the wild-type HA, and as expected their binding was reduced by mutations in antigenic site Sa (S121K) and in the stalk region (I372K) respectively (Fig. 2B and data not shown). For 6 of the 9 rmAbs, binding was compromised by mutation(s) in a single antigenic site, and 2 other rmAbs showed reduced binding to recHA with mutations in two (145-C09) or three (146-B09) antigenic sites (Fig. 2A and B).

Of the rmAb affected by mutations in single antigenic sites, 3 (145-D11, 146-C07 and 146-D11) were affected by the same two point mutations (A139K and K142S) within antigenic site Ca2. Binding of two other rmAbs (069-A09 and 139-22a) were reduced by Sa antigenic site mutations, G155K and K160E. 139-22a showed less reduction in binding with either mutation (Fig. 2A and B), and consistent with this observation, we have previously shown that 139-22a binds to recHA with about 100 times higher affinity than does 069-A69 (Wilson et al., 2014). Reduced HA binding to rmAb 065-C05 was observed by a T72K substitution, which is within antigenic site Cb.

Of the two rmAb affected by mutations in multiple antigenic sites, 145-C09 binding is affected by residue changes in sites Ca2 and Sb, since mutations K142S and T184K independently reduce binding. Similarly, mutations K160E (Sa), T184K (Sb), and R221E (Ca2) all reduced binding by rmAb 146-B09 and indicated that the binding footprint may span the monomer–monomer interface of the HA trimer (Fig. 2B). Although antigenic sites are defined as functionally distinct (Caton et al., 1982), anti-HA antibodies that span multiple sites (Matsuzaki et al., 2014; Tsibane et al., 2012) as well as those that cross the monomer-monomer interface (Barbey-Martin et al., 2002; Iba et al., 2014) have been described. Such antibodies might not be identified when using a classical antigenic mapping approach via mAb escape mutants, as a single mutation would probably be sufficient to avoid neutralization (Caton et al., 1982; Matsuzaki et al., 2014).

Binding of the remaining rmAb, 065-D01, was not markedly affected by any of the point mutations in the recHA panel; thus the epitope for this rmAb could not be determined in this assay (Fig. 2A). However, further analysis (see below) suggests that this rmAb binds at least partly within the Sa antigenic site.

rmAb germline gene usage and CDR3 sequence indicate constraints on the light chain

We previously sequenced and reported the germline gene analysis data for these rmAb (Wilson et al., 2014). Two rmAb in this panel (146-C07 and 146-D11) may be clonally related in that they have the same IgH and IgL germline segments, but have different somatic mutations. Both of these rmAb are equally affected by the same two residue changes in antigenic site Ca2 (Table 3). A third rmAb, 145-D11, employs a closely-related IgL V/J combination and has a similar IgL CDR3 sequence as 146-C07 and 146-D11, but has a different IgH V/D/J combination (Table 3), and this rmAb is also affected by the same two residues. Similarly, 069-A09 and 139-22a share identical IgL V/J genes and CDR3 sequence, but show distinct IgH gene usage and CDR3 sequences, and are both specific for the Sa antigenic site, further suggesting that the light chain may be important in HA antigenic targeting. However, since this panel of antibodies does not include a pair with closely-related heavy chain VDJ and CDR3 and different light chain usage, further studies are needed to determine if the light chain is particularly important in determining the antibody's antigenic target.

rmAb reactivity against drifted H1N1pdm09 viruses isolated from humans

Based on the epitope profile of the panel of rmAb, we selected a group of eight H1N1pdm virus natural isolates that contained variant residues likely to affect rmAb binding activity (Table 2). Ferret post-infection antisera are typically used to antigenically characterize influenza viruses and a reduction in HI titer of 8-fold or greater relative to the homologous titer of the vaccine strain is generally considered to be a significant antigenic difference and may warrant an update of the seasonal vaccine strain (Russell et al., 2008; Garten et al., 2009). CA/07 ferret antisera, and pooled sera from the mice used to prepare rmAb, had similar HI activity against CA/07 (vaccine strain) and Ukr/2011, Nor/2009, Mass/2011, Ind/2012 and Penn/2010 (Table 4), indicating that these viruses are antigenically similar to CA/07. HI titers against Ont/2012 were more than 8-fold lower than to CA/07 using both mouse and ferret antisera; ferret antisera also had 8-fold reduced HI titer against Par/191 while mouse sera had 4-fold reduced HI titers against Mex/2009 (Table 4). Ferret antisera MN activity paralleled that of HI activity against this virus panel, with the exception of a $\geq$16-fold drop in MN titer against Mex/2009 and Ind/2012 that did not correlate with the HI titer. Due to the limited amount of mouse sera, MN could not be determined with mouse sera for this panel of viruses.

We further tested ELISA, HI and MN activity of each of the 72 possible rmAb/virus combinations, relative to the rmAb activity against CA/07 (Table 5 and Supplementary Table 1). ELISA binding analysis revealed the rmAbs mainly bound as predicted (i.e. virus containing a significant amino acid change in the identified rmAb antigenic binding site disrupted binding (Table 5)). Exceptions