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Diversity of the murine antibody response targeting influenza A(H1N1pdm09) hemagglutinin

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ABSTRACT

We infected mice with the 2009 influenza A pandemic virus (H1N1pdm09), boosted with an inactivated vaccine, and cloned immunoglobulins (Igs) from HA-specific B cells. Based on the redundancy in germline gene utilization, we inferred that between 72–130 unique IgH VDJ and 35 different IgL VJ combinations comprised the anti-HA recall response. The IgH VH1 and IgL VK14 variable gene families were employed most frequently. A representative panel of antibodies were cloned and expressed to confirm reactivity with H1N1pdm09 HA. The majority of the recombinant antibodies were of high avidity and capable of inhibiting H1N1pdm09 hemagglutination. Three of these antibodies were subtype-specific cross-reactive, binding to the HA of A/South Carolina/1/1918(H1N1), and one further reacted with A/swine/Iowa/15/1930(H1N1). These results help to define the genetic diversity of the influenza anti-HA antibody repertoire profile induced following infection and vaccination, which may facilitate the development of influenza vaccines that are more protective and broadly neutralizing.

Importance: Protection against influenza viruses is mediated mainly by antibodies, and in most cases this antibody response is narrow, only providing protection against closely related viruses. In spite of this limited range of protection, recent findings indicate that individuals immune to one influenza virus may contain antibodies (generally a minority of the overall response) that are more broadly reactive. These findings have raised the possibility that influenza vaccines could induce a more broadly protective response, reducing the need for frequent vaccine strain changes. However, interpretation of these observations is hampered by the lack of quantitative characterization of the antibody repertoire. In this study, we used single-cell cloning of influenza HA-specific B cells to assess the diversity and nature of the antibody response to influenza hemagglutinin in mice. Our findings help to put bounds on the diversity of the anti-hemagglutinin antibody response, as well as characterizing the cross-reactivity, affinity, and molecular nature of the antibody response.

Introduction

Influenza viruses are common pathogens of humans and animals. In humans, influenza virus infections cause substantial morbidity and mortality through seasonal epidemics and occasional pandemics (Thompson et al., 2010; Molinari et al., 2007). Vaccination remains the key component of public health protection against influenza virus infection. However, durable protection is limited by the ability of influenza virus to undergo rapid genetic and antigenic change, allowing it to escape from pre-existing immunity. As a result, influenza vaccines require updating on a regular basis (Grohskopf et al., 2012).

Antibodies that target the principal viral surface protein, hemagglutinin (HA), play a major role in protection against influenza virus infection and provide the basis for current vaccine design and the tests by which vaccine efficacy is assessed (Ellebedy and Webby, 2009). HA is a trimeric glycoprotein consisting of the membrane-distal globular head and the stalk region. The head is responsible for receptor binding, while the HA2 subunit is required for viral fusion with cellular membranes. Antibodies that bind to the globular head of HA and block receptor binding can be detected by the hemagglutination inhibition (HI) assay, and HI antibody threshold titers ≥ 32 are associated with a reduction in the risk of influenza infection (Katz et al., 2011). There are four or five antigenic sites in the globular head region of the HA (Caton et al., 1982; Wiley et al., 1981) and antibodies binding to these antigenic sites generally have virus-neutralizing

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activity, but non-neutralizing globular-head binding antibodies also have been described (Khurana et al., 2013; Daniels et al., 1987; Laeeq et al., 1997; Temoltzin-Palacios and Thomas, 1994; Yewdell et al., 1986). Antibodies binding outside these antigenic sites, including to the stalk region of HA, have also been identified. Some of these antibodies confer protection against infection, either by directly blocking virus infectivity or by playing a role in other functions of the immune system, such as antibody-dependent cellular cytotoxicity (ADCC)-mediated activation of NK cells (Jegaskanda et al., 2013a,b; Srivastava et al., 2013) or complement (Co et al., 2012; Terajima et al., 2011).

Influenza virus can tolerate significant sequence variation in antigenic sites, and sequence changes in these regions ("antigenic drift") often reduce binding of the existing antibody repertoire. Some anti-HA antibodies, however, are broadly cross-reactive, and can confer protection to a range of viruses within a particular HA subtype or even across subtypes. These antibodies have been reported to bind to conserved regions within the globular head of HA (Corti et al., 2010; Wrammert et al., 2011; Yoshida et al., 2009; Whittle et al., 2011; Ekiert et al., 2012) or the stalk region (Corti et al., 2010, 2011; Wrammert et al., 2011; Okuno et al., 1993; Throsby et al., 2008; Sui et al., 2009; Ekiert et al., 2011; Dreyfus et al., 2012).

Clearly the goal of influenza vaccination is to increase the proportion of antibodies that are protective, and ideally to increase the proportion that are cross-protective against multiple strains. Generating antibodies after vaccination that are cross-protective against antigenically drifted strains of the same subtype may reduce the need for frequent updates of vaccine strains. Furthermore, a vaccine that could elicit broadly cross-reactive antibodies that protect against multiple subtypes of HA would be an important public health tool in the event of a newly emerged virus with pandemic potential. However, without understanding the relative frequency of strain-specific versus cross-reactive, it is difficult to preferentially stimulate an optimal antibody response.

Evaluating the anti-influenza repertoire in humans is complicated by the fact that, with increasing age, individuals are repeatedly exposed to antigenically diverse influenza viruses, including both different subtypes and drifted strains within a subtype. Exposure to any particular HA is likely to preferentially stimulate and expand previously primed B cells, eclipsing the stimulation of naive B cells and thereby potentially impairing the generation of strain-specific, high-affinity antibodies that provide a more protective response.

At the molecular level, mouse antibody responses are generally similar to that of humans, but the exposure of laboratory mice to influenza viruses can be controlled. To understand the antibody repertoire arising from infection with influenza virus, we infected naïve C57BL/6 mice with the 2009 pandemic influenza A virus (H1N1pdm09) and boosted them with an inactivated influenza vaccine. B cells specific to HA were purified, and the immunoglobulin (Ig) heavy and light chain variable regions were cloned from single B cells to characterize the diversity and specificity of the anti-HA antibody repertoire.

Results

Anti-HA B cell identification and purification

To induce an anti-HA response, C57BL/6 mice were administered a sub-lethal infection with OH/07(H1pdm), and 3 weeks later, the mice were boosted with 2011/2012 TIV. Three days post-boost, spleens from four mice were harvested and pooled. Antigen-positive B cells (1.5–8% of the CD19/IgM-population: Fig. 1D vs. Fig. 1B) were single cell-sorted onto glass slides based on their ability to bind CA/04(H1pdm) recHA. As a control, lymphocytes were stained with the distantly related recHA of DE/68(H13) (Fig. 1A and C).

Anti-HA IgH and IgL gene segment usage

The amplification of the IgH gene from the single cells yielded 54 heavy chains for which respective VDJ_H germline genes were identified according to the international ImMunoGeneTics database (IMGT) (Lefranc et al., 2009). In total, genes representing 4 of the 15 murine V_H families were identified (Fig. 2A). The V_H chains displayed a strong bias toward the use of the VH1 family (74%), which represents approximately 50% of the V_H gene repertoire available and is located most distal from the diversity genes (Schroeder, 2006; de Bono et al., 2004; Johnston et al., 2006). Within the four V_H families, the V_H genes included 14 of the possible 92 functional C57BL/6 V_H genes (de Bono et al., 2004), of which IGHV1-82*01 was the most common (26%), followed by IGHV5-4*01 (13%) (Fig. 2B). The D_H segment of IgH most frequently employed was the DH1-1*01 gene (37%) (Fig. 2C). For the J_H gene segment, genes 1–4 were used with similar frequencies, ranging from 20 to 30% each (Fig. 2D).

Because C57BL/6 mice predominately use the kappa light chain (Schroeder, 2006), and preliminary experiments confirmed that the anti-HA response in our experiments was composed of 85–90% kappa light chain usage (data not shown), we only amplified these V_L chains. Of the 18 known V_K families, 10 (55.5%) were identified in our screen with a bias toward the use of the VK14 family (36%) (Fig. 2E). VK3 and 4 were also well represented, with 24% and 16% of germline sequences used, respectively. Of the possible 93 murine-expressed V_K genes, 19 were recovered from the HA-specific B cells (20%) (Fig. 2F), and of these, segment IGKV14-130*01 was obtained most frequently (26%), followed by IGKV3-4*01 and 14-11*01 (10% each). All four possible IgL J genes were used, with JK5 represented most often (44%) (Fig. 2G).

Germline gene combinations and CDR3 sequence analysis of the IgH and IgL chain anti-HA repertoire

The 54 individual anti-HA IgH chains included 32 distinct VDJ_H combinations, with 11 being employed multiple times (from 2 to 6 times) (Fig. 3A). The length of the CDR3 region (produced through joining the 3′ end of V_H, a D segment, and the 5′ end of J_H) ranged from 8 to 17 amino acids (Fig. 3B). As well as a number of identical CDR3 sequences, clusters of similar CDR3 sequences were observed, mainly but not solely driven by similar VDJ usage (Fig. 3A). For example, the sequence C[T/A][T/S]GGSSGYGGAYW was seen five times, and was generated by the use of the same V, D, and J gene segments (14-4*01, 3-2*02, and 3*01 respectively), while the sequence CVRRGDYYFDYW, identified twice, was generated from either 1-50*01/1-1*02/3*01 or 1-50*01/1-3*01/2*01 V/D/J regions.

The 50 IgL chains encode 25 unique VJ_L combinations, 12 of which were represented multiple times (from 2 to 9 instances) (Fig. 3C). For IgL, the CDR3 region is formed by the 3′ end of V_L and the 5′ end of J_L. The length of the IgL CDR3 ranged from 8 to 11 amino acids, with 90% encoding nine residues (Fig. 3D). The V_L CDR3 sequences were less diverse than those of V_H, with three major clusters identified, including nine that had identical sequences (Fig. 3C). As with IgH, some evidence for convergence on sets of CDR3 sequences was observed. Similar CDR3 sequences were usually generated by identical V/J germline usage (e.g., nine instances of CLQFYEFPLTF generated by 14-130-01/5*01), but in some cases, similar CDR3 sequences were generated by different V and J germlines (CQQS[K/N]E[V/D]P[L/W]TF generated by 3-2*01/5*01 or 3-4*01/1*01).

In 18 cases we amplified both the IgH and IgL chain from the same B cell, allowing analysis of the complete antibody gene