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SUPPLEMENT ARTICLE

Immunodominance and Antigenic Variation of Influenza Virus Hemagglutinin: Implications for Design of Universal Vaccine Immunogens

Seth J. Zost,[1,a] Nicholas C. Wu,[2,a] Scott E. Hensley,[1] and Ian A. Wilson[2,3]

[1]Department of Microbiology, Perelman School of Medicine, University of Pennsylvania, Philadelphia; [2]Department of Integrative Structural and Computational Biology and [3]The Skaggs Institute for Chemical Biology, The Scripps Research Institute, La Jolla, California

Influenza viruses routinely acquire mutations in their hemagglutinin (HA) and neuraminidase (NA) glycoproteins that abrogate binding of pre-existing antibodies in a process known as antigenic drift. Most human antibodies against HA and NA are directed against epitopes that are hypervariable and not against epitopes that are conserved among different influenza virus strains. Universal influenza vaccines are currently being developed to elicit protective responses against functionally conserved sites on influenza proteins where viral escape mutations can result in large fitness costs [1]. Universal vaccine targets include the highly conserved HA stem domain [2–12], the less conserved HA receptor-binding site (RBS) [13–16], as well as conserved sites on NA [17–19]. One central challenge of universal vaccine efforts is to steer human antibody responses away from immunodominant, variable epitopes and towards subdominant, functionally conserved sites. Overcoming this challenge will require further understanding of the structural basis of broadly neutralizing HA and NA antibody binding epitopes and factors that influence immunodominance hierarchies of human antibody responses.

Keywords: influenza virus, antibody response, immune imprinting, virus evolution, vaccine design.

IMMUNODOMINANCE OF THE HEMAGGLUTININ HEAD

Antibodies targeting epitopes in the HA globular head domain can protect animals and humans from influenza virus infections [20]. The majority of these antibodies neutralize by blocking viral attachment to host cells, although other neutralization mechanisms might be in play for some of these antibodies [1, 21]. Infection and vaccination typically elicit strain-specific HA-head antibodies that are often long-lived [22], but these antibodies can become ineffective when viruses acquire antigenic changes in the HA head. Such an example occurred during the 2014–2015 season when a new antigenically drifted H3N2 strain possessing a novel glycosylation site on the HA head caused dramatically reduced vaccine effectiveness [23, 24]. Although most antibodies against the HA head are directed against epitopes adjacent to the conserved HA RBS [25, 26], some antibodies are able to partially mimic the sialic acid receptor and bind to conserved residues within the HA RBS [13, 15, 16, 27, 28]. Hemagglutinin stem antibody responses constitute a small fraction of total anti-influenza virus antibodies in most humans [29]. In contrast to most epitopes on the HA head, the HA stem is less tolerant of change [30–33] and is much more highly conserved across subtypes. Although some anti-HA stem monoclonal antibodies can directly neutralize viruses through inhibiting HA proteolytic processing, pH-induced conformational changes, and viral egress [1, 21], many HA stem antibodies require Fc-mediated effector functions for in vivo protection [34].

HEMAGGLUTININ IMMUNODOMINANCE OF PRIMARY ANTIBODY RESPONSES

Antibodies against highly exposed epitopes on the HA head usually dominate the primary responses against influenza viruses (Figure 1A). Primary H3N2 infections in ferrets elicit high levels of antibodies that are directed towards HA antigenic sites A and B [35, 36], which are located in close proximity to the HA RBS [25]. Likewise, H1N1-infected young children tend to mount antibody responses to epitopes in antigenic sites near the HA RBS [37]. Although steric hindrance or inaccessibility has been suggested to contribute to the immunosubdominance of HA stem antibodies [38], recombinant HA vaccines also fail to elicit high-titer HA stem responses [39]. In fact, cryoelectron tomography has shown that the majority of the HA on influenza virions are indeed available to bind to stem antibodies [40].

[a]S. J. Z. and N. C. W. contributed equally to this work.

Correspondence: S.E. Hensley, PhD, University of Pennsylvania, 402 Johnson Pavilion, 3610 Hamilton Walk, Philadelphia, PA 19104 (hensley@pennmedicine.upenn.edu) and I.A. Wilson, PhD, The Scripps Research Institute, La Jolla, CA 92037 (wilson@scripps.edu).

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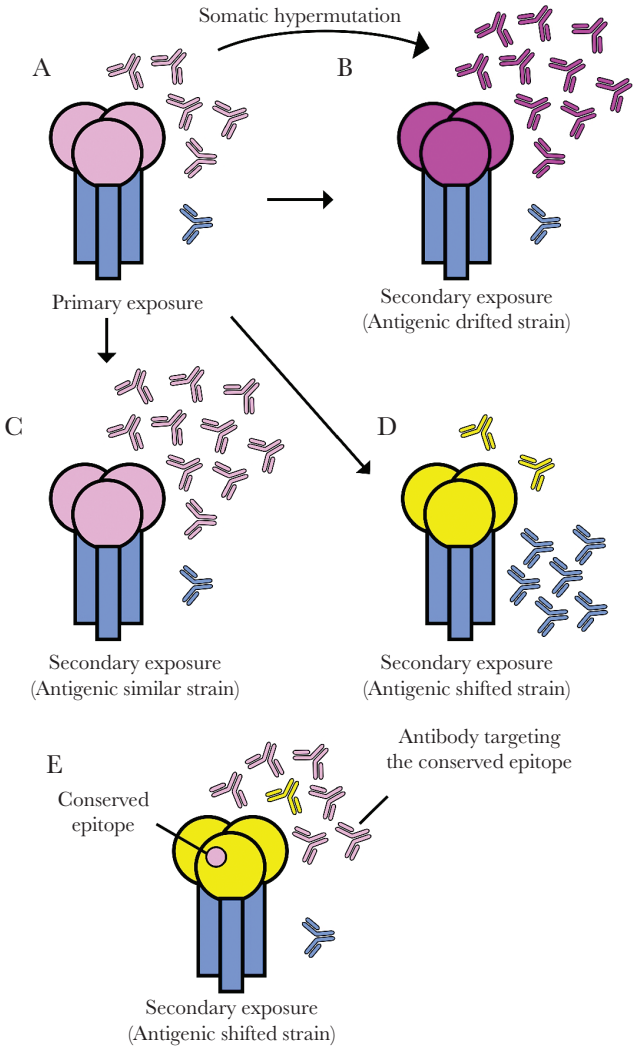

Figure 1. Immunodominance of primary responses and recall responses against influenza hemagglutinin (HA). (A) The HA head domain (pink) is immunodominant in primary responses, whereas antibodies against the stem domain (blue) are rare. (B–C) Antibodies against the HA head remain dominant after exposure to antigenically similar (B) and antigenically drifted (C) seasonal viral strains. Antibodies elicited by antigenically drifted seasonal influenza virus strains often have high levels of somatic hypermutations that allow recognition of altered epitopes. (D–E) Antibodies against new pandemic viral strains tend to be more dominant initially against the (D) conserved HA stem, and (E) rare conserved epitopes, if any, in the HA head. Memory B cells producing antibodies against these conserved epitopes are preferentially boosted upon exposure to new pandemic viral strains. The color similarity of the HA head domain represents the similarity of the antigenicity in all figure panels.

Some HA stem antibodies can be polyreactive [38], and it is possible that selection against B-cells specific for HA stem epitopes contributes to HA stem antibody immunosubdominance. Recent data suggest that the fine specificity of influenza virus antibody responses in mice changes over time [41]. Angeletti et al [41] found that (1) antibodies against epitopes near the top of the HA head dominate the early response and (2) antibodies against other epitopes increase later in the response. Given that most studies have only examined a limited range of timepoints, it is likely that shifts in antibody immunodominance dynamics have yet to be fully explored.

Almost all immunological studies of influenza virus have been carried out in organisms that make immunoglobulin (Ig)-based humoral responses. To test whether some features of immunodominance are antigen-intrinsic, Altman et al [42] studied immune responses in lampreys that were immunized with influenza virus. Lampreys, a jawless fish, lack Ig genes but encode variable lymphocyte receptors (VLRs), which are an entirely different system of humoral adaptive immunity based on Leu-rich repeats rather than Ig domains. Remarkably, lamprey VLR responses were found to be focused on the same HA epitopes as those that have been observed in mice [42]. The similarity of antibody and VLR responses against HA in mice and lamprey suggest that properties of the HA protein itself contribute to antibody immunodominance hierarchies.

NEW HEMAGGLUTININ STEM-BASED UNIVERSAL VACCINE APPROACHES

Several universal vaccines are being developed to elicit antibodies against the immunosubdominant HA stem. One approach is to generate stable "headless" HA constructs that lack the head domain [10] and, as a result, induce antibody responses exclusively directed against HA stem epitopes [2, 3]. Another approach is sequential immunization with chimeric HAs that express divergent head domains with the goal of refocusing antibody responses towards the HA stem domain [12] (also see Krammer and Palese in this issue). This approach is promising because chimeric HAs selectively recall subdominant HA stem-reactive B-cells in the absence of HA head-reactive immunity. Both of these approaches have shown protection in animal models, but their success in humans will likely depend on their ability to induce protective responses in the context of differing pre-existing immunity in different individuals and age groups.

IMMUNE HISTORY SHAPES SECONDARY IMMUNE RESPONSES

It has been known since the 1950s that antibodies elicited by primary influenza virus exposures are highly strain-specific, whereas antibodies elicited by secondary exposures with antigenically distinct viral strains tend to be highly cross-reactive with the first strain encountered [43–45] (Figure 1B and C). This observation was originally referred to as "original antigenic sin" [45], and it has been more recently referred to as "antigenic seniority" [46] or "immune imprinting" [47]. Although the mechanisms behind original antigenic sin have yet to be fully elucidated, it is thought that cross-reactive B cells elicited by previous influenza virus exposures are preferentially recalled upon exposure with an antigenically distinct viral strain (Figure 1D).

Several studies suggest that prior seasonal H1N1 exposures influenced the fine-specificity of antibodies elicited against the antigenically distinct 2009 pandemic H1N1 virus in humans

[48–53]. In many adults, the 2009 pandemic H1N1 strain preferentially boosted HA stem antibodies [48, 49, 54, 55], likely because this strain possessed a radically different HA head but a similar HA stem compared with previously circulating seasonal H1N1 strains. However, HA stem antibodies were not the only antibody type that was preferentially recalled in humans exposed to the 2009 pandemic H1N1. In some individuals, the 2009 pandemic H1N1 virus elicited antibody responses that were highly focused on rare HA head epitopes that were conserved in seasonal H1N1 strains to which they were exposed in childhood [50–53] (Figure 1E). More importantly, different aged individuals were found to mount antibody responses of different specificities upon exposure to the 2009 pandemic H1N1 virus, due to differences in seasonal H1N1 exposure histories. Age-related differences in antibody specificity appeared to play a role during the 2013–2014 season when a drifted pandemic H1N1 strain acquired an HA mutation in an epitope that was preferentially targeted by middle-aged individuals and, as a consequence, caused a disproportionate amount of disease in this population [51, 56, 57]. Animals sequentially infected with seasonal H1N1 and the 2009 pandemic H1N1 strains produce antibodies that have similar specificities compared with those elicited in humans who were likely sequentially exposed to these viruses [50, 51].

These findings are consistent with a recent study by Gostic et al [47] who used epidemiological data to demonstrate a correlation between the probability of first exposure in early childhood to either a group 1 or group 2 HA and susceptibility to avian H7N9 and H5N1 viral strains, respectively. They found that individuals who were likely exposed to a virus with a group 1 HA in childhood appeared to be protected from H5N1 but susceptible to H7N9, whereas individuals who were likely exposed to a virus with a group 2 HA in childhood appeared to be protected from H7N9 but susceptible to H5N1. Thus, it appears that individuals exposed in childhood to group 1 HAs are more likely to respond well to group 1 HA stem antigens, whereas individuals exposed in childhood to group 2 HAs are more likely to respond well to group 2 HA stem antigens. A deeper understanding of the complexities of human prior exposure and the interplay with universal vaccine candidates will likely be required to design better vaccines and vaccine regimens.

DIFFERENCES BETWEEN INFLUENZA GROUP 1 AND GROUP 2 HEMAGGLUTININ STEMS

Although a few HA stem-reactive antibodies can target both group 1 and 2 HAs [27, 58–64], many are group specific [58, 63–70]. Several structural features are conserved within, but not across, group 1 or group 2 HAs. For example, the *N*-glycosylation site at HA1 Asn38 is highly conserved in group 2, but it is not present in group 1 HAs (Figure 2). In addition, the orientation and positioning of HA2 Trp21 differ between group 1 and group 2 HAs (Figure 2). A higher variability can be observed in other

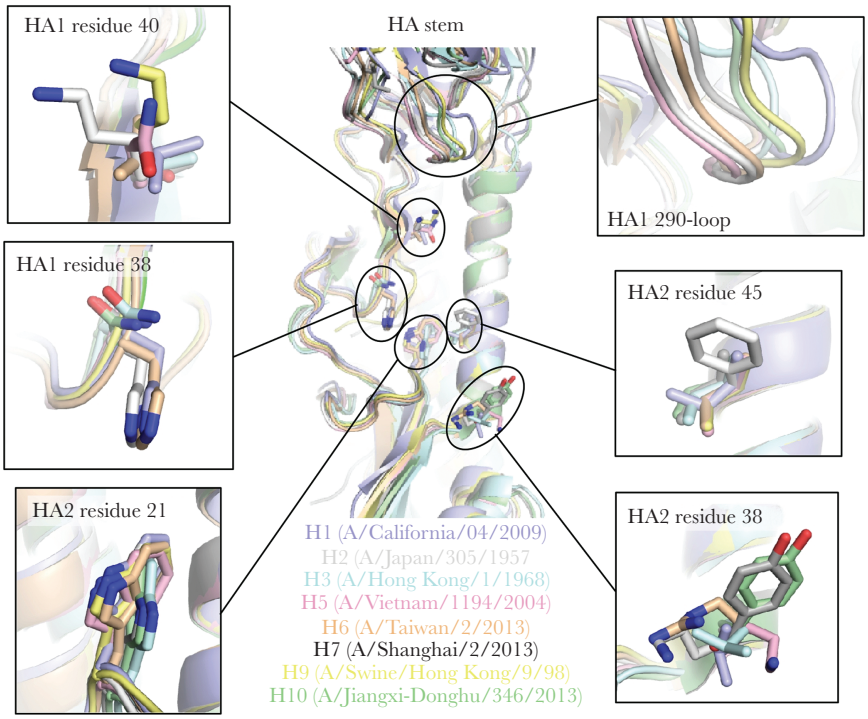

Figure 2. Structural variation in the hemagglutinin (HA) stem and neighboring regions. Structures of HA protein from subtypes that have caused human infection are aligned by the helix A (HA2 residues 38–55) in the stem region: H1 (PDB 3LZG) [53], H2 (PDB 3KU5) [93], H3 (PDB 4FNK) [16], H5 (PDB 4BGW) [94], H6 (PDB 4XKD) [95], H7 (PDB 4LN6) [96], H9 (PDB 1JSD) [97], H10 (PDB 4XQ5) [98]. Zoomed-in views for several structural features of interest are shown. PDB, Protein Data Bank.

structural features in the HA stem and proximal regions, such as HA2 residue 38 and HA1 290-loop that are often contacted by stem-reactive antibodies [58–60, 65, 69, 71]. Subtype-specific variation can also be observed. For example, whereas HA1 Ile45 is highly conserved across group 1 and 2 HAs, HAs of the human H2 subtype have HA1 Phe45 instead (Figure 2). Some of these features have been associated with limiting the breadth of HA stem-reactive antibodies. The most well known example is perhaps the group 2-specific *N*-glycosylation site at HA1 Asn38 [72], which restricts the approach angle for antibodies to the highly conserved epitope in the stem of group 2 HAs [60]. Due to the structural difference in group 1 and group 2 HAs, it is not surprising that the germline usage of group 1-specific HA stem antibodies also seems to have a different preference than that of group 2-specific HA stem antibodies [73]. Most group 1-specific HA stem-binding antibodies utilize V_H1-69 germline [64–66, 69, 70, 74], whereas group 2-specific HA stem-binding antibodies utilize a more diverse set of germlines, such as V_H1-2 [64], V_H3-53 [64], V_H1-3 [67], and V_H1-18 [75]. Nonetheless, studies have shown that the breadth of broadly neutralizing antibodies (bnAbs) can be increased during memory B cell evolution [59, 62, 70]. For example, although FI6 and MEDI8852 are both cross-group anti-HA stem antibodies, their germline versions (V_H3-30 and V_H6-1, respectively) only react with group 1 HAs [59, 61]. In fact, V_H6-1 has been proposed to be a germline that encodes a multidonor class of bnAbs [63]. Therefore, it is possible for an HA group-specific anti-HA stem antibody to evolve a cross-group breadth through affinity maturation.

Recent studies from the Vaccine Research Center at the National Institutes of Health have elucidated several classes of cross-group anti-HA stem antibodies that were commonly observed in individuals after H5N1 (group 1) or H7N9 (group 2) vaccinations [63, 64]. An additional observation is that the anti-HA stem antibodies from H5N1-vaccinated individuals are primarily group 1-specific, whereas most antistem antibodies from H7N9-vaccinated individuals can react with both group 1 and group 2 HAs [64]. Such results suggest that there is a higher chance to induce cross-group anti-HA stem antibodies from a group 2 HA stem-based immunogen than from a group 1 HA stem-based immunogen. Nonetheless, most HA stem-based immunogen designs to date [76] are based on group 1 HAs (H1 [2, 3, 5, 6, 9] and H5 [4]), although some success has come from group 2 based HAs (H3 [7, 8]). Head-to-head comparison should be performed in the future between group 2 HA stem-based and group 1 HA stem-based immunogens to see whether one is superior to the other in inducing cross-group antistem antibodies. It will also be important to establish whether sequential vaccinations with group 1 HA stem vaccines followed by group 2 HA stem vaccines elicit different types of antibodies compared with sequential vaccinations with group 2 HA stem vaccines followed by group 1 HA stem vaccines. Throughout all of these studies, it will be crucial to take into account vaccinees' year of birth and the potential effects of HA imprinting from early childhood influenza virus exposures.

ESCAPE MUTATIONS TO HEMAGGLUTININ STEM ANTIBODIES

An important consideration for antiviral and vaccine development is the potential emergence of escape mutants. The HA head can tolerate a lot more mutations, additional or changing glycosylation sites, or even insertions, when compared with the HA stem [30–33]. As a result, anti-HA head antibodies are more prone to escape than anti-HA stem antibodies [77]. Nevertheless, strong escape mutations to the anti-HA stem antibodies have been isolated [66, 67, 71, 75, 78–80]. Chai et al [80] have shown that both decrease in antibody-binding affinity and enhancing membrane fusion can contribute to escape from anti-HA stem antibodies. However, not all attempts to isolate strong escape mutants to the anti-HA stem antibodies have been successful. Doud et al [77] used deep mutational scanning to systematically search for escape mutations against 2 HA stem-binding bnAbs, namely FI6v3 [59] and C179 [78], but only weak escape mutants were found. Likewise, escape mutants to HA stem-binding bnAb CR6261 were only identified after extensive passaging [66]. It remains to be resolved how strong escape can be readily identified in some studies but not in others. It is possible that certain anti-HA stem antibodies are more prone to result in viral escape. It should also be noted that different studies often use different viral strains to search for escape mutants. The escape profiles of anti-HA stem antibodies may also vary among strains and subtypes, as suggested by the differential ability of A/California/7/2009 (H1N1) and A/Perth/16/2009 (H3N2) to escape from anti-HA stem antibody 39.29 [80]. Likewise, whereas only weak escape mutants to C179 were identified in A/WSN/1933 (H1N1) [77], complete escape mutants were identified in A/Suita/1/1989 (H1N1) and A/Izumi/5/1965 (H2N2) [78]. In fact, such a phenomenon of strain- or subtype-specific mutational effects has been described in the study of the HA RBS, where the tolerability to certain mutations differs between subtypes [81] or even among strains within a given subtype [82]. Several anti-HA stem antibodies are undergoing clinical trials as therapeutics [83], and an increasing amount of resources is being invested in the development of an HA stem-based universal vaccine [84]. Therefore, a comprehensive understanding of possible escape mutations is desirable to minimize unwanted surprises. Furthermore, because combining multiple antibodies can potentially minimize the emergence of escape mutants [85–87], a universal vaccine where escape is minimized will likely require elicitation of polyclonal responses targeting different HA epitopes.

MOVING FORWARD

Although current influenza HA stem-based universal vaccine candidates are promising, the development of a universal

vaccine will likely be an iterative process, and a better understanding of the dynamics of immunodominance in humans will be essential for improving such vaccines. In the case of human immunodeficiency virus, a great deal has also been learned about the development of broadly neutralizing antibody responses by studying antibody-virus coevolution from the time of infection [88–90]. The analogous situation in influenza is more challenging, because it requires following individuals from birth in longitudinal studies and defining how immunodominance changes over the course of a response and from response to response. These longitudinal cohort studies have the potential to answer fundamental questions about (1) what antibody specificities dominate the plasmablast response versus B-cell memory and (2) which lineages are recalled in the response to an antigenically drifted strain. More importantly, these studies will also allow us to explore differences in responses elicited by infection and vaccination. Some studies suggest that B cells recalled in response to vaccination have a reduced ability to undergo somatic mutation relative to those recalled by an infection [91]. Although titers elicited by vaccination in adults exhibit modest waning [92], we know very little about the longevity of responses elicited in children.

Another major challenge will be to develop standardized assays to detect antibodies against different HA and NA epitopes. The standard assays used to select vaccine strains almost exclusively detect antibodies that bind the HA head and block viral attachment to cellular receptors. New assays to measure antibody functions such as HA stem binding and neutralization, NA inhibition, and Fc-mediated effector engagement need to be developed. Dissecting the contribution of different epitopes to protection in universal vaccine trials will allow us to precisely determine which epitopes are targeted in different individuals and whether viral escape is occurring at particular epitopes.

CONCLUSIONS

The current generation of universal vaccine candidates are the product of decades of work across multiple disciplines and represent the first attempt to control influenza immunodominance to elicit long-lived, protective responses against conserved sites. Understanding and manipulating immunodominance will be the crux of continued progress towards universal influenza immunity.

Notes

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