Is It Possible to Develop a “Universal” Influenza Virus Vaccine?

Immunogenetic Considerations Underlying B-Cell Biology in the Development of a Pan-Subtype Influenza A Vaccine Targeting the Hemagglutinin Stem

Sarah F. Andrews, Barney S. Graham, John R. Mascola, and Adrian B. McDermott

Vaccine Research Center, National Institute of Allergy and Infectious Disease, National Institutes of Health, Bethesda, Maryland 20892

Correspondence: adrian.mcdermott@nih.gov

Current influenza vaccines preferentially generate B-cell responses to the variable hemagglutinin (HA) head. Focusing vaccine-induced antibody responses on epitopes in the conserved HA stem may provide better protection against future drifted and pandemic strains. Understanding the basis for the dominant HA head and subdominant HA stem-specific responses at the level of B-cell activation and differentiation will be critical for designing vaccines that induce sustained stem-specific responses. Identifying antibody lineages with broad neutralizing activity against influenza A viruses and defining the structural mode of recognition for germline precursors of those antibodies will also guide future immunogen design.

GREAT DEBATES

What are the most interesting topics likely to come up over dinner or drinks with your colleagues? Or, more importantly, what are the topics that *don't* come up because they are a little too controversial? In ***Immune Memory and Vaccines: Great Debates***, Editors Rafi Ahmed and Shane Crotty have put together a collection of articles on such questions, written by thought leaders in these fields, with the freedom to talk about the issues as they see fit. This short, innovative format aims to bring a fresh perspective by encouraging authors to be opinionated, focus on what is most interesting and current, and avoid restating introductory material covered in many other reviews.

The Editors posed 13 interesting questions critical for our understanding of vaccines and immune memory to a broad group of experts in the field. In each case, several different perspectives are provided. Note that while each author knew that there were additional scientists addressing the same question, they did not know who these authors were, which ensured the independence of the opinions and perspectives expressed in each article. Our hope is that readers enjoy these articles and that they trigger many more conversations on these important topics.

Editors: Shane Crotty and Rafi Ahmed

Additional Perspectives on Immune Memory and Vaccines: Great Debates available at www.cshperspectives.org

Copyright © 2018 Cold Spring Harbor Laboratory Press; all rights reserved; doi: 10.1101/cshperspect.a029413

Cite this article as *Cold Spring Harb Perspect Biol* 2018;10:a029413

S.F. Andrews et al.

Influenza vaccines require annual vaccinations, which have limited efficacy against strain variants, including those with pandemic potential. At present, seasonal influenza vaccination includes circulating variants from influenza B and influenza A group 1 (H1N1) and group 2 (H3N2) virus subtypes, which concurrently circulate in the population. These influenza strains constantly evolve by subtle genetic variation (antigenic drift), particularly in the apical head domain of hemagglutinin (HA) near the receptor-binding site, thereby escaping existing immune responses (Kucharski et al. 2015), and requiring frequent modification of the strains included in the seasonal influenza vaccine. Pandemic influenza strains, such as the recent 2009 H1N1, arise by reassortment events (antigenic shift) typically with completely new avian or porcine HA molecules (Yen and Webster 2009). Such drifted and shifted strains can circumvent preexisting immunity in the population as a whole. Because influenza A has been responsible for all recognized human pandemics, our research focus is to address the basic immunological questions required to advance a “universal” influenza vaccine that protects against all circulating subtypes. Our goal is development of immunogens that induce persistent antibody-mediated immunity that protects children and adults from severe group 1 or group 2 influenza A disease. We believe that achieving this goal will require advances in both immunogen design and basic B-cell immunobiology to elicit high-magnitude, long-lasting, and broadly potent serum antibody responses directed toward regions of vulnerability.

The conserved membrane-proximal stem region of the HA glycoprotein represents such a region of vulnerability that can either be a component or the primary focus of a pan-subtypic vaccine (Nabel and Fauci 2010). This HA region elicits cross-reactive and protective antibodies, and is vital in the process of membrane fusion and delivery of viral RNA into the target cell. Seasonal influenza vaccines are generally poor at eliciting high titers of HA stem-directed antibodies (Corti et al. 2010; Sui et al. 2011), because exposure to seasonal H1 and H3 subtypes predominantly generates immunodomi-

nant strain-specific head-directed antibodies. In contrast, exposure to antigenically divergent HA subtypes (e.g., H1 CA2009 or H5) elicits increased frequencies of subdominant HA stem-directed B cells. This is presumably because the stem region is conserved while there is little pre-existing immunity to the head region of a divergent HA (Sui et al. 2011; Wrammert et al. 2011; Li et al. 2012; Thomson et al. 2012; Miller et al. 2013; He et al. 2015). In addition, HA stem-specific neutralizing antibody recognition likely increases in frequency late in life and with repeated exposure (Krammer et al. 2013; Nachbagauer et al. 2016a). This demonstrates that broadly neutralizing stem-directed B cells can be elicited, but are induced at a low frequency upon vaccination, with current inactivated vaccines containing full-length HA from seasonal influenza strains. Thus, a key challenge in developing an influenza vaccine based on HA stem-specific antibodies is the difficulty in inducing and maintaining these responses. Our focus is on designing immunogens that will specifically engage HA stem-specific B cells and overcome the immunological factors leading to the subdominance of this response. We believe that an HA stem-directed antibody response will be a key component of a broadly protective influenza vaccine strategy, and that understanding the biology of immunodominance in the context of influenza will be critical for achieving universal influenza immunity.

WHAT ARE THE GENETIC CHARACTERISTICS OF THE DESIRED HETEROSUBTYPIC INFLUENZA HA STEM-SPECIFIC ANTIBODY RESPONSE?

We and others have evaluated immunoglobulin regions of group 1 HA stem-specific memory B-cell populations and found frequent use of heavy chain IGHV1-69 (Sui et al. 2009; Pappas et al. 2014; Wheatley et al. 2015; Avnir et al. 2016). Particularly, group 1 HA stem-specific IGHV1-69 sequences recovered from memory B cells following H5N1 immunization were highly conserved within the hydrophobic CDRH2 region responsible for binding. Typically, the CDRH2 region contained canonical

Is It Possible to Develop a Universal Influenza Virus Vaccine?

hydrophobic residues at position 53 and a critical phenylalanine at position 54 in the CDRH2 with little affinity maturation needed to produce high-affinity antibodies recognizing the group 1 HA stem (Lingwood et al. 2012; Pappas et al. 2014). Interestingly, IGHV1-69 exhibits copy number variability and polymorphism with 14 known alleles in addition to subtle variations among different ethnicities (Watson et al. 2013; Avnir et al. 2014). On a population basis, the allelic polymorphism of germline leucine or phenylalanine at position 54 within the IGHV1-69 CDRH2 strongly affects binding and serum titers of group 1 influenza HA stem-specific antibodies and concomitantly the overall frequency of HA stem-specific memory B cells (Pappas et al. 2014; Wheatley et al. 2015; Avnir et al. 2016). Notably, IGHV1-69 derived HA stem antibodies neutralize group 1 strains (with the exception of antibody CR9114 described below).

Recently, investigators have isolated several human HA stem-specific monoclonal antibodies

that are able to neutralize both group 1 and group 2 influenza A viruses in vitro and that are protective in experimental animal models (Clementi et al. 2011; Corti et al. 2011; Dreyfus et al. 2012; Hu et al. 2013; Nakamura et al. 2013; Wu et al. 2015; Nachbagauer et al. 2016b). The broadest of these neutralizing antibodies include CR9114 (Dreyfus et al. 2012) and FI6v3 (Corti et al. 2011), and the structural basis of this broad recognition is partially understood. Differential glycosylation patterns between group 1 and group 2 influenza HA stem regions restrict the recognition of group-specific neutralizing antibodies such as group 1-specific F10 and CR6261 or group 2-specific CR8020 (Fig. 1) (Corti et al. 2011). The contact footprints of groups 1 and 2 neutralizing antibodies differ in part as a result of the presence of glycans at position N21 and N38, respectively (Fig. 1). Developing a broadly neutralizing cross-group-specific humoral response would rely upon eliciting antibodies such as FI6v3, CR9114, 39.29 (Nakamura et al. 2013), and CT149 (Wu et al.

Figure 1. Diagram of the contact footprint of three hemagglutinin (HA) stem-binding antibodies with different influenza subtype specificity. Shown is the HA stem (highlighted by the black box on the diagram of whole HA on the far left) with two potential glycans shown in green. Group 1 HA has a glycan at N21, while group 2 HA is glycosylated at N38. Antibodies that bind the group 1 HA stem (left) have a contact footprint (shown in orange) that avoids the N21 glycan. Group 2 HA stem-binding antibodies (center, CR8020 contact footprint shown in purple) avoid the N38 glycan. Cross-group HA stem-binding antibodies (right, contact footprint of FI6 shown in light green) are able to bind in the presence of either glycan. Diagrams are based on published structural data (Ekiert et al. 2009; Sui et al. 2009; Corti et al. 2011; Dreyfus et al. 2012).

S.F. Andrews et al.

2015), which have progressively evolved a contact footprint to avoid both glycosylation sites by different modes. The broadly neutralizing antibodies CR9114 and FI6v3, for example, are both able to dislodge the glycan at position N38, though are rotated 90°C from each other and interact with the HA through different heavy/light chain contact points (Corti et al. 2011; Dreyfus et al. 2012). These studies highlight the importance of understanding at the atomic level structural biology that influence modes of antibody recognition of the known group 1 and 2 broadly neutralizing antibodies. This will enable rational design of immunogens that elicit potentially recurring classes of antibodies. Indeed, these kinds of epitope-focused vaccine design strategies are being employed and developed for elicitation of desirable neutralizing antibodies against EBV, RSV, and HIV (McLellan et al. 2013; Kanekiyo et al. 2015; Gorman et al. 2016).

DO INFLUENZA VACCINATED INDIVIDUALS SHARE COMMON CLASSES OF ANTIBODIES THAT WILL INFORM EPITOPE VACCINE DESIGN FOR THE GENERATION OF HETEROTYPIC NEUTRALIZING ANTIBODIES?

The use of common restricted or recurrent immunoglobulin classes, such as IGHV1-69, have been observed, not only for influenza, but also for other viral pathogens such as dengue, rotavirus, and HIV and often develop against structurally constrained epitopes (Weitkamp et al. 2005; Wu et al. 2011; Parameswaran et al. 2013; Jackson et al. 2014; Martins and Tsang 2014; Henry Dunand and Wilson 2015). To identify other possible recurring immunoglobulin classes capable of binding conserved HA stem epitopes, we investigated the circulating memory B-cell responses following influenza group 1 subtype H5N1 vaccination. After immunization, we performed antigen-specific B-cell sorting using H5 (group 1) and H3 (group 2) protein probes to identify group 1 and 2 cross-reactive circulating memory B cells, and in this way identified hundreds of novel anti-influenza stem-specific immunoglobulins

from multiple donors (Fig. 2) (Joyce et al. 2016). Notably, we found three recurrent patterns for cross-reactive group 1 and 2 HA-stem neutralizing antibodies with differing characteristics, which were present at varying frequencies in multiple vaccinated individuals (Fig. 2). Because of similarities in heavy- and light-chain gene usage and structural mode of recognition, we classified these antibodies into one of several classes. One class utilized IGHV6-1+HD3-3 genes, a second class utilized IGHV1-18+HD3-9 genes and a 15-amino-acid CDRH3, and a third class utilized IGHV1-18 and a convergent CDRH3 Gln98_{HC}-x-x-Val100a_{HC} motif (Joyce et al. 2016). Cococrystallization of representative antibodies revealed that each of the three classes evolved and employed slightly different modes or solutions for cross-neutralization of group 1 and group 2 influenza subtypes (Joyce et al. 2016). The previously described CT149 (Wu et al. 2015) and SFV005-2G02 (Li et al. 2012) HA stem-specific broadly neutralizing antibodies, which both utilize IGHV1-18, would be categorized within the Gln98_{HC}-x-x-Val100a_{HC} and IGHV1-18+HD3-9 classes, respectively. The recently described MEDI8852 HA stem-specific antibody is a member of the HV6-1+HD3-3 class (Kallewaard et al. 2016). The identification of the same classes of stem-directed neutralizing antibodies in multiple donors suggests that such antibodies could be elicited with the appropriately designed immunogen. However, we need a deeper understanding of such cross-reactive responses as analysis of large published clinical influenza trial immunoglobulin databases has shown presence of these multidonor class signatures across the human population to varying degrees (Joyce et al. 2016). Inherent B-cell repertoire differences and variation in influenza exposure history likely explain this variation.

IS IT POSSIBLE TO ACHIEVE BROAD UNIVERSAL INFLUENZA IMMUNITY BY ELICITING MULTIDONOR CLASS ANTIBODIES TARGETING THE HA STEM?

Recent data strongly suggest that influenza HA stem-specific memory B cells expressing immu-

Is It Possible to Develop a Universal Influenza Virus Vaccine?

Figure 2. Schematic of strategy used to identify recurring immunoglobulin classes that recognize conserved hemagglutinin (HA) stem epitopes. Two weeks after an H5N1 vaccine boost, peripheral blood IgG⁺ memory B cells capable of binding HA from different influenza subtypes were detected using an HA probe mutated to prevent nonspecific binding to sialic acid. B cells able to bind multiple group 1 subtypes (H5 and H1) or both group 1 and group 2 subtypes (H5 and H3) were single-cell sorted, and immunoglobulin heavy and light chains for each cell were polymerase chain reaction (PCR) amplified and sequenced. Representative heavy and light chain pairs were cloned, expressed as monoclonal antibodies and tested for binding and neutralization. The pie charts show the prominent IGHV genes expressed by B cells within each category with the number of sequences analyzed indicated in the center of each chart. The predominant IGHV gene used by group 1 HA-binding B cells was IGHV1-69. Few group 1/2 HA-binding B cells expressed IGHV1-69. Instead, three classes of cross-group HA stem-binding and neutralizing B cells encoded by IGHV1-18 and IGHV6-1 were identified in multiple donors after H5N1 vaccination as indicated. IGHV genes, including IGHV3-30 and others not referenced here, are colored in grey. More details can be found in Joyce et al. (2016).

noglobulin genes, which recognize and neutralize influenza A group 1 and group 2 viruses are not as rare as previously envisioned. They commonly circulate as a subdominant memory B-cell population with the ability to rapidly expand and differentiate, especially upon immunization with novel HA subtypes. There is evidence that HA stem-specific responses induced by an HA stem-only immunogen can contribute to short-term protection following lethal influenza challenge in experimental animal models (Impagliazzo et al. 2015; Yassine et al. 2015; Valkenburg et al. 2016). However, even with immunogens designed to engage and activate HA stem-specific B cells, there are other immunological factors that might prevent robust and long-lasting stem-directed immunity. Following H5N1 vaccination, we observed a rapid expansion but also a rapid contraction in the HA stem-specific memory B cells, whereas

HA head-specific B cells were maintained for a longer period of time (Wheatley et al. 2015). We speculate that serum antibody levels against the HA stem are likely also transient. Potential explanations for the apparent weak and short-lived HA stem response have been considered. HA stem-directed antibodies have been shown to be polyreactive (Andrews et al. 2015), suggesting that HA stem-specific B cells may be restricted because of secondary tolerance mechanisms. In addition, it may be that insufficient CD4-specific T cell helps “program” the HA stem-specific response toward short-term plasma cells rather than long-term bone marrow resident plasma cells. It is also possible that the density of HA on the virion could limit access of stem-specific antibody to membrane proximal epitopes. However, direct observation suggests that the majority of HA molecules are accessible to HA stem-directed antibodies

S.F. Andrews et al.

(McCraw et al. 2016) though probably not equally to the more dominant HA head-specific antibodies. To address these fundamental questions, we are employing whole transcriptome analyses of plasma and memory B cells generated after influenza vaccination along with proteomic analyses to detect multidonor classes of antibodies in serum derived from individuals immunized with diverse HA constructs. This will allow us not only to follow the repertoire of the HA response but also the functional development of HA-specific B cells directed against the HA head and stem.

WHAT IS THE NEXT-GENERATION UNIVERSAL VACCINE DESIGN?

Design of a universal Influenza vaccine based upon the influenza HA stem would need to overcome immunodominance of the HA head region. Strategies that involve the sequential use of various chimeric HA head and stem constructs are in development, which aim to increase functional serum antibody directed toward the HA stem by mimicking successive “diverse HA” exposure (Hai et al. 2012; Krammer et al. 2013; Margine et al. 2013). Although some HA stem-only constructs have had mixed success (Lu et al. 2014; Wohlbold et al. 2015), we continue to favor this approach. Group 1 influenza HA stem trimer constructs that are stable and faithfully folded demonstrate efficacy against lethal heterosubtypic challenge from distant group 1 viruses in animal models (Impagliazzo et al. 2015; Yassine et al. 2015). As mentioned above, the group 1 HA stem responses are dominated by IGHV1-69 responses, while the other alleles, including IGHV1-18, IGHV6-1, and IGHV3-23 or IGHV3-30, include variants that more easily recognize group 1 and 2 influenza A viruses. Therefore, establishing higher precursor frequencies of long-lived plasma cells that produce these non-IGHV1-69 types of antibodies would allow the host to evolve even broader HA stem-specific responses. We have recently designed headless stabilized HA stem antigens displayed on self-assembling ferritin nanoparticles for both group 1 and group 2 subtypes (Yassine et al. 2015). Future studies will

include sequential and combined immunization schemes to investigate the best strategy to elicit cross-group HA stem-specific B cells and maintenance of associated serum antibody responses. Because there is so much preexisting influenza immunity, it may not be possible to significantly shift precursor frequency or induce new specificities to achieve the desired responses in adults or even older children. In fact, repeated immunization with conventional vaccines has been suggested to be counterproductive in some studies (Skowronski et al. 2016), potentially by expanding serotype-specific B-cell populations that compete with cross-reactive or new responses to drifted strains. Therefore, these questions may need to be addressed in studies of young children who are influenza-naïve, and who could potentially be primed in a way that would provide them more effective and broad “universal” influenza immunity throughout their lifetimes.

In summary, HA head-specific B-cell responses generated by the seasonal influenza vaccine are very effective at protecting against specific influenza strains, but cannot provide universal protection. Developments in the past few years characterizing broadly reactive and protective B-cell responses directed toward the HA stem have given new hope that we can indeed develop a “universal” influenza vaccine capable of protecting against a wide range of Influenza A strains. However, the HA stem-specific B-cell response to the current influenza vaccine is subdominant, low, and transient. The challenge for the future is designing immunogens and vaccine regimes that will generate a persistent and efficacious serum HA stem-specific antibody titer response across the human population.

ACKNOWLEDGMENTS

This work is supported by the Intramural Research Program of the Vaccine Research Center, National Institute of Allergy and Infectious Diseases, National Institutes of Health. We thank M. Gordon Joyce for assistance in generating Figure 1 and Michelle C. Crank for thoughtful comments and review.

Is It Possible to Develop a Universal Influenza Virus Vaccine?

REFERENCES

- Andrews SE, Huang Y, Kaur K, Popova LI, Ho IY, Pauli NT, Henry Dunand CJ, Taylor WM, Lim S, Huang M, et al. 2015. Immune history profoundly affects broadly protective B cell responses to influenza. *Sci Transl Med* **7**: 316ra192.
- Avnir Y, Tallarico AS, Zhu Q, Bennett AS, Connelly G, Sheehan J, Sui J, Fahmy A, Huang CY, Cadwell G, et al. 2014. Molecular signatures of hemagglutinin stem-directed heterosubtypic human neutralizing antibodies against influenza A viruses. *PLoS Pathog* **10**: e1004103.
- Avnir Y, Watson CT, Glanville J, Peterson EC, Tallarico AS, Bennett AS, Qin K, Fu Y, Huang C-YY, Beigel JH, et al. 2016. IGHV1-69 polymorphism modulates anti-influenza antibody repertoires, correlates with IGHV utilization shifts and varies by ethnicity. *Sci Rep* **6**: 20842.
- Clementi N, De Marco D, Mancini N, Solforosi L, Moreno GJ, Gubareva IV, Mishin V, Di Pietro A, Vicenzi E, Siccardi AG, et al. 2011. A human monoclonal antibody with neutralizing activity against highly divergent influenza subtypes. *PLoS ONE* **6**: e28001.
- Corti D, Suguitan AL, Pinna D, Silacci C, Fernandez-Rodriguez BM, Vanzetta F, Santos C, Luke CJ, Torres-Velez FJ, Temperton NJ, et al. 2010. Heterosubtypic neutralizing antibodies are produced by individuals immunized with a seasonal influenza vaccine. *J Clin Invest* **120**: 1663–1673.
- Corti D, Voss J, Gamblin SJ, Codoni G, Macagno A, Jarrossay D, Vachieri SG, Pinna D, Minola A, Vanzetta F, et al. 2011. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. *Science* **333**: 850–856.
- Dreyfus C, Laursen NS, Kwaks T, Zuidgeest D, Khayat R, Ekiert DC, Lee JH, Metlagel Z, Bujny MV, Jongeneelen M, et al. 2012. Highly conserved protective epitopes on influenza B viruses. *Science* **337**: 1343–1348.
- Ekiert DC, Bhabha G, Elsliger MA, Friesen RH, Jongeneelen M, Throsby M, Goudsmit J, Wilson IA. 2009. Antibody recognition of a highly conserved influenza virus epitope. *Science* **324**: 246–251.
- Gorman J, Soto C, Yang MM, Davenport TM, Guttman M, Bailer RT, Chambers M, Chuang G-YY, DeKosky BJ, Doria-Rose NA, et al. 2016. Structures of HIV-1 Env V1V2 with broadly neutralizing antibodies reveal commonalities that enable vaccine design. *Nat Struct Mol Biol* **23**: 81–90.
- Hai R, Krammer F, Tan GS, Pica N, Eggink D, Maamary J, Margine I, Albrecht RA, Palese P. 2012. Influenza viruses expressing chimeric hemagglutinins: Globular head and stalk domains derived from different subtypes. *J Virology* **86**: 5774–5781.
- He W, Mullarkey CE, Miller MS. 2015. Measuring the neutralization potency of influenza A virus hemagglutinin stalk/stem-binding antibodies in polyclonal preparations by microneutralization assay. *Methods* **90**: 95–100.
- Henry Dunand CJ, Wilson PC. 2015. Restricted, canonical, stereotyped and convergent immunoglobulin responses. *Philos Trans R Soc Lond B Biol Sci* doi: 10.1098/rstb.2014.0238.
- Hu W, Chen A, Miao Y, Xia S, Ling Z, Xu K, Wang T, Xu Y, Cui J, Wu H, et al. 2013. Fully human broadly neutralizing monoclonal antibodies against influenza A viruses generated from the memory B cells of a 2009 pandemic H1N1 influenza vaccine recipient. *Virology* **435**: 320–328.
- Impagliazzo A, Milder F, Kuipers H, Wagner MV, Zhu X, Hoffman RM, van Meersbergen R, Huizingh J, Wannin-gen P, Verspuij J, et al. 2015. A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. *Science* **349**: 1301–1306.
- Jackson K, Liu Y, Roskin KM, Glanville J, Hoh RA, Seo K, Marshall EL, Gurley TC, Moody AM, Haynes BF, et al. 2014. Human responses to influenza vaccination show seroconversion signatures and convergent antibody rearrangements. *Cell Host Microbe* **16**: 105–114.
- Joyce MG, Wheatley AK, Thomas PV, Chuang G-YY, Soto C, Bailer RT, Druz A, Georgiev IS, Gillespie RA, Kanekiyo M, et al. 2016. Vaccine-Induced antibodies that neutralize group 1 and group 2 influenza A viruses. *Cell* **166**: 609–623.
- Kallewaard NL, Corti D, Collins PJ, Neu U, McAuliffe JM, Benjamin E, Wachter-Rosati L, Palmer-Hill FJ, Yuan AQ, Walker PA, et al. 2016. Structure and function analysis of an antibody recognizing all influenza A subtypes. *Cell* **166**: 596–608.
- Kanekiyo M, Bu W, Joyce MG, Meng G, Whittle JR, Baxa U, Yamamoto T, Narpala S, Todd JB, Rao SS, et al. 2015. Rational design of an Epstein–Barr virus vaccine targeting the receptor-binding site. *Cell* **162**: 1090–1100.
- Krammer F, Pica N, Hai R, Margine I, Palese P. 2013. Chimeric hemagglutinin influenza virus vaccine constructs elicit broadly protective stalk-specific antibodies. *J Virol* **87**: 6542–6550.
- Kucharski AJ, Lessler J, Read JM, Zhu H, Jiang CQ, Guan Y, Cummings DA, Riley S. 2015. Estimating the life course of influenza A(H3N2) antibody responses from cross-sectional data. *PLoS Biol* **13**: e1002082.
- Li G-MM, Chiu C, Wrannert J, McCausland M, Andrews SE, Zheng N-YY, Lee J-HH, Huang M, Qu X, Edupuganti S, et al. 2012. Pandemic H1N1 influenza vaccine induces a recall response in humans that favors broadly cross-reactive memory B cells. *Proc Natl Acad Sci* **109**: 9047–9052.
- Lingwood D, McTamney PM, Yassine HM, Whittle JR, Guo X, Boyington JC, Wei CJ, Nabel GJ. 2012. Structural and genetic basis for development of broadly neutralizing influenza antibodies. *Nature* **489**: 566–570.
- Lu Y, Welsh JP, Swartz JR. 2014. Production and stabilization of the trimeric influenza hemagglutinin stem domain for potentially broadly protective influenza vaccines. *Proc Natl Acad Sci* **111**: 125–130.
- Margine I, Krammer F, Hai R, Heaton NS, Tan GS, Andrews SA, Runstadler JA, Wilson PC, Albrecht RA, García-Sastre A, et al. 2013. Hemagglutinin stalk-based universal vaccine constructs protect against group 2 influenza A viruses. *J Virol* **87**: 10435–10446.
- Martins AJ, Tsang JS. 2014. Random yet deterministic: Convergent immunoglobulin responses to influenza. *Trends Microbiol* **22**: 488–489.
- McCraw DM, Gallagher JR, Harris AK. 2016. Characterization of influenza vaccine hemagglutinin complexes by cryo-electron microscopy and image analyses reveals

S.F. Andrews et al.

- structural polymorphisms. *Clin Vaccine Immunol* **23**: 483–495.
- McLellan JS, Chen M, Joyce MG, Sastry M, Stewart-Jones GB, Yang Y, Zhang B, Chen L, Srivatsan S, Zheng A, et al. 2013. Structure-based design of a fusion glycoprotein vaccine for respiratory syncytial virus. *Science* **342**: 592–598.
- Miller MS, Gardner TJ, Krammer F, Aguado LC, Tortorella D, Basler CF, Palese P. 2013. Neutralizing antibodies against previously encountered influenza virus strains increase over time: A longitudinal analysis. *Sci Transl Med* **5**: 198ra107.
- Nabel GJ, Fauci AS. 2010. Induction of unnatural immunity: Prospects for a broadly protective universal influenza vaccine. *Nat Med* **16**: 1389–1391.
- Nachbagauer R, Choi A, Izikson R, Cox MM, Palese P, Krammer F. 2016a. Age dependence and isotype specificity of influenza virus hemagglutinin stalk-reactive antibodies in humans. *MBio* **7**: e01996–15.
- Nachbagauer R, Miller MS, Hai R, Ryder AB, Rose JK, Palese P, Garcia-Sastre A, Krammer F, Albrecht RA. 2016b. Hemagglutinin stalk immunity reduces influenza virus replication and transmission in ferrets. *J Virol* **90**: 3268–3273.
- Nakamura G, Chai N, Park S, Chiang N, Lin Z, Chiu H, Fong R, Yan D, Kim J, Zhang J, et al. 2013. An in vivo human-plasmablast enrichment technique allows rapid identification of therapeutic influenza A antibodies. *Cell Host Microbe* **14**: 93–103.
- Pappas L, Foglierini M, Piccoli L, Kallewaard NL, Turrini F, Silacci C, Fernandez-Rodriguez B, Agatic G, Giacchetto-Sasselli I, Pellicciotta G, et al. 2014. Rapid development of broadly influenza neutralizing antibodies through redundant mutations. *Nature* **516**: 418–422.
- Parameswaran P, Liu Y, Roskin KM, Jackson K, Dixit VP, Lee J-Y, Artiles KL, Zompi S, Vargas M, Simen BB, et al. 2013. Convergent antibody signatures in human Dengue. *Cell Host Microbe* **13**: 691–700.
- Skowronski DM, Chambers C, Gilca R, De Serres G. 2016. Repeat influenza vaccination and high-dose efficacy. *Clin Infect Dis* doi: 10.1093/cid/ciw544.
- Sui J, Hwang WC, Perez S, Wei G, Aird D, Chen LM, Santelli E, Stec B, Cadwell G, Ali M, et al. 2009. Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. *Nat Struct Mol Biol* **16**: 265–273.
- Sui J, Sheehan J, Hwang WC, Bankston LA, Burchett SK, Huang CY, Liddington RC, Beigel JH, Marasco WA. 2011. Wide prevalence of heterosubtypic broadly neutralizing human anti-influenza A antibodies. *Clin Infect Dis* **52**: 1003–1009.
- Thomson CA, Wang Y, Jackson LM, Olson M, Wang W, Liavonchanka A, Keleta L, Silva V, Diederich S, Jones RB, et al. 2012. Pandemic H1N1 influenza infection and vaccination in humans induces cross-protective antibodies that target the hemagglutinin stem. *Front Immunol* **3**: 87.
- Valkenburg SA, Mallajosyula VV, Li OT, Chin AW, Carnell G, Temperton N, Varadarajan R, Poon LL. 2016. Stalking influenza by vaccination with pre-fusion headless HA mini-stem. *Sci Rep* **6**: 22666.
- Watson CT, Steinberg KM, Huddleston J, Warren RL, Malig M, Schein J, Wilsey AJ, Joy JB, Scott JK, Graves TA, et al. 2013. Complete haplotype sequence of the human immunoglobulin heavy-chain variable, diversity, and joining genes and characterization of allelic and copy-number variation. *Am J Hum Genet* **92**: 530–546.
- Weitkamp J-H, Kallewaard NL, Bowen AL, LaFleur BJ, Greenberg HB, Crowe JE. 2005. VH1–46 is the dominant immunoglobulin heavy chain gene segment in rotavirus-specific memory B cells expressing the intestinal homing receptor $\alpha 4\beta 7$. *J Immunol* **174**: 3454–3460.
- Wheatley AK, Whittle JR, Lingwood D, Kanekiyo M, Yassine HM, Ma SS, Narpala SR, Prabhakaran MS, Matus-Nicodemus RA, Bailer RT, et al. 2015. H5N1 vaccine-elicited memory B cells are genetically constrained by the IGHV locus in the recognition of a neutralizing epitope in the hemagglutinin stem. *J Immunol* **195**: 602–610.
- Wohlbolt TJ, Nachbagauer R, Margine I, Tan GS, Hirsh A, Krammer F. 2015. Vaccination with soluble headless hemagglutinin protects mice from challenge with divergent influenza viruses. *Vaccine* **33**: 3314–3321.
- Wrammert J, Koutsouanos D, Li GMM, Edupuganti S, Sui J, Morrissey M, McCausland M, Skountzou I, Hornig M, Lipkin WI, et al. 2011. Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection. *J Exp Med* **208**: 181–193.
- Wu X, Zhou T, Zhu J, Zhang B, Georgiev I, Wang C, Chen X, Longo NS, Louder M, McKee K, et al. 2011. Focused evolution of HIV-1 neutralizing antibodies revealed by structures and deep sequencing. *Science* **333**: 1593–1602.
- Wu Y, Cho M, Shore D, Song M, Choi J, Jiang T, Deng YQQ, Bourgeois M, Alml L, Yang H, et al. 2015. A potent broad-spectrum protective human monoclonal antibody crosslinking two haemagglutinin monomers of influenza A virus. *Nat Commun* **6**: 7708.
- Yassine HM, Boyington JC, McTamney PM, Wei CJ, Kanekiyo M, Kong WP, Gallagher JR, Wang L, Zhang Y, Joyce MG, et al. 2015. Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection. *Nat Med* **21**: 1065–1070.
- Yen HL, Webster RG. 2009. Pandemic influenza as a current threat. *Curr Topics Microbiol Immunol* **333**: 3–24.

Cold Spring Harbor Perspectives in Biology

Is It Possible to Develop a "Universal" Influenza Virus Vaccine?: Immunogenetic Considerations Underlying B-Cell Biology in the Development of a Pan-Subtype Influenza A Vaccine Targeting the Hemagglutinin Stem

Sarah F. Andrews, Barney S. Graham, John R. Mascola and Adrian B. McDermott

Cold Spring Harb Perspect Biol 2018; doi: 10.1101/cshperspect.a029413 originally published online June 29, 2017

Subject Collection [Immune Memory and Vaccines: Great Debates](#)

Is There Natural Killer Cell Memory and Can It Be Harnessed by Vaccination?: Can Natural Killer and CD8 T Cells Switch Jobs?

Christine A. Biron and Marcus Altfeld

Is There Natural Killer Cell Memory and Can It Be Harnessed by Vaccination?: Vaccination Strategies Based on NK Cell and ILC Memory

Megan A. Cooper, Todd A. Fehniger and Marco Colonna

Is It Possible to Develop Cancer Vaccines to Neoantigens, What Are the Major Challenges, and How Can These Be Overcome?: Neoantigens as Vaccine Targets for Cancer

Haydn T. Kissick

Is It Possible to Develop Cancer Vaccines to Neoantigens, What Are the Major Challenges, and How Can These Be Overcome?: Neoantigens: Nothing New in Spite of the Name

Olivera J. Finn and Hans-Georg Rammensee

Is There Natural Killer Cell Memory and Can It Be Harnessed by Vaccination?: NK Cell Memory and Immunization Strategies against Infectious Diseases and Cancer

Joseph C. Sun and Lewis L. Lanier

Is There Natural Killer Cell Memory and Can It Be Harnessed by Vaccination?: Natural Killer Cells in Vaccination

Harold R. Neely, Irina B. Mazo, Carmen Gerlach, et al.

Is It Possible to Develop Cancer Vaccines to Neoantigens, What Are the Major Challenges, and How Can These Be Overcome?: Targeting the Right Antigens in the Right Patients

Stephen P. Schoenberger

Which Dengue Vaccine Approach Is the Most Promising, and Should We Be Concerned about Enhanced Disease after Vaccination?: There Is Only One True Winner

Scott B. Halstead

For additional articles in this collection, see <http://cshperspectives.cshlp.org/cgi/collection/>

**All Modifications and
Oligo Types Synthesized**
Long Oligos • Fluorescent • Chimeric • DNA • RNA • Antisense

Oligo Modifications?
Your wish is our command.

Which Dengue Vaccine Approach Is the Most Promising, and Should We Be Concerned about Enhanced Disease after Vaccination?: The Challenges of a Dengue Vaccine

Gavin Screaton and Juthathip Mongkolsapaya

Which Dengue Vaccine Approach Is the Most Promising, and Should We Be Concerned about Enhanced Disease after Vaccination?: The Path to a Dengue Vaccine: Learning from Human Natural Dengue Infection Studies and Vaccine Trials

Aravinda M. de Silva and Eva Harris

Is It Possible to Develop a "Universal" Influenza Virus Vaccine?: Potential for a Universal Influenza Vaccine

James E. Crowe, Jr.

Is It Possible to Develop a "Universal" Influenza Virus Vaccine?: Outflanking Antibody Immunodominance on the Road to Universal Influenza Vaccination

Davide Angeletti and Jonathan W. Yewdell

Which Dengue Vaccine Approach Is the Most Promising, and Should We Be Concerned about Enhanced Disease after Vaccination?: Questions Raised by the Development and Implementation of Dengue Vaccines: Example of the Sanofi Pasteur Tetravalent Dengue Vaccine

Bruno Guy

Which Dengue Vaccine Approach Is the Most Promising, and Should We Be Concerned about Enhanced Disease after Vaccination?: The Risks of Incomplete Immunity to Dengue Virus Revealed by Vaccination

Stephen S. Whitehead and Kanta Subbarao

Is It Possible to Develop a "Universal" Influenza Virus Vaccine?: Immunogenetic Considerations Underlying B-Cell Biology in the Development of a Pan-Subtype Influenza A Vaccine Targeting the Hemagglutinin Stem

Sarah F. Andrews, Barney S. Graham, John R. Mascola, et al.

Is It Possible to Develop a "Universal" Influenza Virus Vaccine?: Potential Target Antigens and Critical Aspects for a Universal Influenza Vaccine

Florian Krammer, Adolfo García-Sastre and Peter Palese

For additional articles in this collection, see <http://cshperspectives.cshlp.org/cgi/collection/>

All Modifications and Oligo Types Synthesized
Long Oligos • Fluorescent • Chimeric • DNA • RNA • Antisense

Oligo Modifications?
Your wish is our command.