Published in final edited form as:

Curr Opin Virol. 2013 June ; 3(3): 316–321. doi:10.1016/j.coviro.2013.05.008.

To Dream the Impossible Dream: Universal Influenza Vaccination

Jonathan W. Yewdell

Laboratory of Viral Diseases, NIAID, Bethesda MD, 20892

Abstract

Year in and year out, influenza viruses exact a deadly and expensive toll on humanity. Current vaccines simply do not keep pace with viral immune evasion, providing partial protection, at best, among various age groups. A quantum leap in understanding the basic principles of the adaptive and innate immune responses to influenza viruses offers the opportunity to develop vaccines that forestall, and potentially ultimately defeat, influenza virus antigenic variation.

Ending Complacency

In the past decade, viral immune evasion became major topic of virology and adaptive and innate immunology research. This field began long ago, however, with the first failure of influenza A virus vaccination [1], which was clearly attributed to antigenic “drift” in what we now know are the IAV virion surface glycoproteins, the neuraminidase (NA) and particularly the (HA). Humanity received nasty surprises in 1957 and 1968 with the introduction of IAVs with highly divergent glycoproteins derived from what turned out to be an enormous reservoir of novel IAV clades in pigs, and particularly birds.

Despite these clear reminders of the devastating 1918 pandemic, complacency ruled influenza public health planning, no doubt abetted by misconceptions swirling about vaccination campaign for the 1976 swine IAV pandemic that never was (see iom.edu/Global/News%20Announcements/~media/Files/About%20the%20IOM/SwineFluAffair.pdf for a thoughtful, thorough and gripping account of this event that had an enormous negative impact on public policy regarding vaccination of epidemic preparedness).

Startled by the introduction of highly pathogenic avian influenza A virus (IAV) into humans in 1997, the world began to shake its long acceptance of the remarkable toll of influenza (influenza B virus (IBV) is also a major, though typically less severe cause of seasonal influenza) on human health and economic activity. Garden-variety seasonal influenza kills hundreds of thousands worldwide and costs hundreds of billion dollars in health care costs and lost worker days. On top of this, is the misery inflicted by this “self-limited” pathogen, one of the few viruses capable of landing substantial numbers of otherwise healthy people in bed for a week or two.

The swine origin influenza virus (SOIV) 2009 pandemic was the last straw. Although far milder than previous pandemics (in part, likely due to the extremely rapid identification of

© 2013 Elsevier B.V. All rights reserved.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

this new human pathogen and swift production of vaccine: both resulting from increased government investment in epidemiological surveillance and basic and applied influenza research), the world finally awakened to the dire need for broadly neutralizing vaccines (BNVs), *i.e.* vaccines that provide durable protection against a broad range of influenza viruses. The ultimate BNV is a “universal” vaccine providing protection against all IAVs that might become transmissible in humans and cause significant diseases.

This new energy to improve influenza vaccines could not have come at a better time. Recent years have witnessed enormous progress in basic immunological research that supports the possibility of BNVs. It is also now abundantly apparent that current influenza vaccines perform far below vaccines for other pathogens [2–4].

Here I focus on the key points of BNV research with particular emphasis on opportunities to deepen basic understanding of the principles required for rational vaccine development, and direct readers to a number of recent outstanding, detailed reviews on influenza virus basic and applied immunology [5–11].

Escaping the Box: New Vaccine Targets

The goal of current vaccines is to induce Abs against the globular head domain of HA (head reactive Abs, (HRAbs)) that neutralize viral infectivity. Vaccines are benchmarked by their induction of Abs that inhibit hemagglutination, a proxy for blocking viral attachment to cells, but a limited measure of viral neutralization (VN). Even monoclonal Abs (mAbs) specific for the defined antigenic sites on the globular domain demonstrate wide (unexplained) variation in their ratio of HI/VN activities, and only a moderate association with VN activity and binding sites [12,13]. More radically, it is now clear that a mAb described in 1993 [14] that binds the HA stem and broadly neutralizes clade I IAVs due to the much higher conservation of the stem relative to the hypervariable globular domain, is not an odd, exceptional Ab as widely assumed at the time.

Work from many laboratories demonstrates that such stem reactive Abs (StRAbs) can be readily induced and are frequently present in human and animal sera [15–18]. X-ray crystallography has been used to generate spectacular images of numerous mAbs binding to various regions of the stem, including one remarkable Ab that binds to broad range of both IAVs and IBVs [19], previously considered completely serologically distinct.

Piling surprise upon surprise, monoclonal HRAbs have been generated that are highly drift resistant. Some of these mAbs possess “atypical” projections (quotes, since the number of solved Ab structures is relatively low, and possibly biased towards what we consider “normal” Abs) that extend into the highly conserved HA sialic binding site, [20–22] the principal receptor site for IAV attachment (though attachment can also be mediated via NA [23,24] and possibly by other HA domains as well). Other Abs, however, bind to regions of the globular domain overlapping the defined 5 classical antigenic sites [8]. This should come as no surprise, since in the original studies, fully 39 of the 89 mouse mAbs tested mapped to multiple sites [25,26].

Indeed, it is time to jettison the 5-site orthodoxy of HA antigenicity. While useful as landmarks (and emotively rich to viral immunologists of a certain age), “5 discrete sites” oversimplifies HA antigenicity. A more sophisticated understanding of Ab interaction with HA (and protein antigens (Ags) in general) is clearly needed. Can general rules be gleaned for which regions of proteins are most immunogenic? For which amino acid substitutions have the greatest effects on Ab binding? For the contributions of direct physiochemical effects of amino acid substitutions at the Ab-Ag interface *vs.* conformational effects that change the interface or limit induced fit in either the HA [27] or Ab [28].

We need to complement X-ray crystallography studies with methods that measure Ab-HA interactions in more physiological conditions. Advanced electron microscopy now can visualize contact regions between antibodies and HA [29–31], and provide critical information regarding steric hindrance of StRAbs binding to epitopes in the context of virions, particularly filamentous forms that are believed to predominate naturally transmitted viruses [32]. Like all proteins, HA [27] and Abs are dynamic structures, and understanding Ab-mediated neutralization requires methods capable of discerning the contribution of molecular movements to Ab binding and HA functional impairment. These include standard biochemical analysis as well as advanced biophysical approaches:

- Small angle x-ray crystallography (SAX), which offers the possibility of visualizing Ab-AG interactions in solution at ~ 20 Å resolution.
- Hydrogen/deuterium exchange mass spectrometry, which gauges conformational changes by measuring solvent accessibility at the resolution of individual residues [33].
- Calorimetry, which measures classical thermodynamic values of enthalpy and entropy.
- Surface plasmon resonance (SPR), which measures Ab on- and off-rates, whose divisor provides the Ab association constant (K_a).

With the exception of SPR (which has been underutilized), these techniques have yet to be applied to Ab-HA interactions. Two additional factors open enormous opportunities to better understand Ab binding and ultimately, virus neutralization. First is the availability of thousands of human HA-specific mAbs from newly developed technologies to generate human B cell lines [34] or express Ab genes from sorted B cells [18,35–37]. Second, is the facility to generate recombinant viruses or isolated HA that possess whatever amino acid substitutions are desired, as chosen rationally or naturally, as revealed by sequencing tens of thousands of IAV strains circulating in humans and animal reservoirs.

Complementing this approach, we need to better define the Ab repertoire of humans to evaluate its role in immunodominance, the propensity of the immune system to respond to epitopes in a hierarchical manner. Immunodominance has been extensively studied for T cell responses to viruses (especially IAV)[38]. By contrast, B cell immunodominance has been barely studied since pioneering work with mouse anti-IAV mAbs, largely completed 20 years ago [39,40].

This state of affairs is radically changing. In a single recent study, high throughput sequencing provided an approximate 10,000-fold increase in the known sequence diversity of anti-IAV Abs [41]. Sequencing technology is poised to revolutionize understanding of Ab responses and begin to answer the central puzzle of antigenic drift, *viz* why don't other human RNA membrane viruses (e.g. parainfluenza viruses) with high mutation rates and high frequencies of escape mutants exhibit antigenic drift? Does HA antigenic drift result from Ab responses focused on an individual antigenic site, perhaps only present in the odd individual, leading to sequential selection of antigenic variants, as originally proposed, based on the low frequency of escape mutants with multiple mutations [42]?

This points to the importance of understanding differences in Ab repertoires between individuals. How do individuals vary in their responses to the head and stem? Can HRAb responses suppress (immunodominate) StRAb responses? Moreover, how do anti-HA Ab repertoires diverge based on differences in:

- Germ line Ab genes (particularly in the VH1-69 gene family, which is overrepresented in BNAb against both the stem and receptor pocket [8]), and which is allelic in human populations [43]).
- Prior antigenic exposure to related, and even unrelated antigens (the degree of degenerate recognition among Abs is essentially unknown).
- Random somatic diversification (SD) of Ab genes, since B cells are endowed with a unique system of mutating Ab genes after the initial stages of activation, if T cell help is available.

In theory, selection of somatically diversified Abs is based strictly on antigen binding affinity as B cells compete for limiting amount of antigen. While affinity maturation is clearly a central feature of Ab responses, it is possible that other factors contribute to B cell selection. Indeed, as we approach the 50th anniversary of the discovery of affinity maturation [44,45], much remains to be learned regarding its workings in the context of primary vs. memory responses. How can affinity maturing B cells sense essentially minute differences in Ab affinity if antigen is presented in multivalent form on viruses, creating enormously avid interactions between virions and B cells [46]?

At the effector end, little is known about the contribution of Ab affinity (or more accurately, avidity, the net affinity between multivalent interacting partners) to virus neutralization and more generally, to protection (with potential contributions from Ab-dependent cellular cytotoxicity (ADCC) and Ab-based viral destruction via complement and phagocytic cells). Are avidity effects strictly linear, *i.e.* does a ten-fold increase in Ab avidity increase its biological effect 10-fold, or are there also qualitative effects that enhance or diminish the biological effects of avidity changes? With SPR it is now possible to robustly measure mAb avidities and the off-rates of serum Abs [47], and extend original studies on the anti-viral effects of affinity maturation [48].

It is critical to pay more attention to the complexities of the biological activities of serum antibodies, which comprise thousands of IAV-specific Ab species [41] that compete for binding with each other, but also can mutually enhance binding. Such Ab synergy, though not widely appreciated, is likely common and typically based on allosteric alterations in Ags that favor binding of Abs that do not sterically compete for binding [49]. Allosterity can also have the opposite effect, course, and it is predictable that some HRAbs will allosterically diminish the binding of some StRAbs (and *vice versa*). Synergistic Ab binding also results from the interaction of Fc domains on neighboring antigens [50], which is nearly certain to occur on a virion surface with hundreds of HA molecules in close, but variable proximity.

The latter mechanism points to the poorly defined role for Ab heavy chain classes in protection against IAV. In addition to the enormous differences between IgM, IgA, and IgG effector functions, what are the effects of various IgG subclasses on anti-viral activities? Adding to the complexity, subtle differences in the glycosylation of Fc domains governs ADCC activity [51]. Although StRAbs can directly neutralize virus, their activity *in vivo* may be greatly augmented by ADCC, since StRAb access to their epitopes may be limiting on virions, but not the cell surface, where HA is sterically unencumbered and also present in non-native conformations.

Understanding the role of ADCC in human influenza is of great importance in rational vaccine design. ADCC is a potential mechanism for the protection afforded by Abs directed against the M2 protein, highly expressed on infected cells (though Ab-dependent phagocytosis and complement activation may also participate [52]), and also nucleoprotein (NP) [53], an internal gene product, present on infected cells at low but still relevant levels [54]. NP and other highly conserved internal IAV gene products are major cross-reactive

targets for T cells, which can directly kill IAV infected cells, and also are essential for Ab affinity maturation and Ig class switching. The importance of cytotoxic T cells (T_{CD8+} , but also T_{CD4+} [55]) in human IAV is unclear, but a recent study extends a substantial body of work demonstrating T_{CD8+} mediated protection in mice and chickens to primates [56].

Although the ability of T_{CD4+} specific for internal gene products to help anti-HA Ab responses was shown more than 30 years ago [57], little is known how T_{CD4+} specificity might influence the help provided to HA-specific B cells, in terms of class switching, epitope specificity, and generation of various B cell subsets, including long-lived plasma cells that are needed to provide durable immunity. These questions are intimately related to the participation of professional antigen presenting cells, which are needed to activate the T_{CD4+} that activate primary B cells (due to Ab-based acquisition of antigen, secondary B cells are extremely adept at presenting antigens to the T cells that provide their help). And all these events are governed by activation of innate immune sensors by the natural or artificial adjuvants present in the immunogen. Although I have focused on antigens/immunogens, adjuvants will likely play an equally important role in developing BNABs. Adjuvants influence every aspect of the B cell response, and can enhance Ab affinity [47] and even modulate original antigenic sin (OAS) [58].

OAS, the tendency of the immune response to direct Abs to previously encountered immunogens [59], is typically viewed as a hindrance to IAV vaccination, since the induced Abs only weakly cross-react with the drifted re-immunizing IAV strain. Given the potential benefits of StRAbs, however, the ultimate goal should be to manipulate OAS to enable strong memory StRab responses along with primary HRAb responses to the novel strain.

Prospects for Universal Vaccination: Are We There Yet?

Yes and no. To this point, I have aimed to identify the salient basic science known and unknowns for rational IAV vaccine design. Obviously, we have a lot to learn (watch out for those unknown unknowns!). This should not, however, overshadow the enormous progress that has been made. To go forward I propose three mutually supporting strategies.

It's a feature, not a bug: I

The yearly occurrence of IAV combined with the broad use and limited effectiveness of vaccines provides an enormous opportunity for improving vaccination. What distinguishes protected from non-protected individuals among the various age groups? The central question is the relationship between the level of serum BNABs and protection, but measuring as many potentially relevant immune parameters as feasible is likely to more than pay for the increased costs of analysis (Table I). This analysis would be greatly aided by better characterization of vaccines with regard to the state of HA (fraction of fully folded *vs.* various unfolded forms), amounts of other viral proteins (NA is particularly important, since anti-NA Abs also confer protection *in vivo*, but also NP and M, will activate T_{CD4+} and T_{CD8+} cells), and viral RNA and other innate immune activators.

It's a feature not a bug: II

The limited effectiveness of vaccines also offers the possibility of supplementing existing vaccines with experimental vaccines, since additive effects should be evident. Taking advantage of the compartmentalized nature of peripherally administered antigens, administering the vaccines at sites with distinct lymph drainage will prevent adverse immunodomination effects. Indeed, just measuring the effect of splitting the trivalent/tetravalent current seasonal vaccine into its components and vaccinating at separate sites is of interest.

In general, novel approaches applied to small trials should be supported. While animal models are justly notorious for limited correlation with human outcomes, this extends to both positive and negative outcomes. Serendipity still plays an important role in medicine and science.

The Long Term

Given the enormous animal reservoir, it is unlikely that IAV will be eradicated from nature. Inasmuch as humanity plans on existing *ad infinitum*, we need to take a long-range view of IAV vaccination, with the ultimate goal of a single childhood vaccination providing life long protection against all IAV strains. This will require a long-term commitment to supporting research into basic IAV virology and immunology.

Ultimately, a universal vaccine might elicit optimal antibody and cellular immune responses based on advanced rational design of both immunogen and adjuvant. At the same time, there are enormous opportunities for developing a broad armamentarium of drugs targeted to IAV protein functional domains, based on advances in structural biology of influenza gene products.

What seems difficult and improbable today will seem obvious once discovered/invented and implemented. Indeed, future medical historians will have a field day with our dallying with such a deadly and costly pathogen.

References

1. Francis T Jr. Influenza: the new acquaintances. *Ann Intern Med.* 1953; 39:203–221. [PubMed: 13080880]
- 2. Osterholm MT, Kelley NS, Sommer A, Belongia EA. Efficacy and effectiveness of influenza vaccines: a systematic review and meta-analysis. *Lancet Infect Dis.* 2012; 12:36–44. [PubMed: 22032844]
- 3. Ohmit SE, Petrie JG, Malosh RE, Cowling BJ, Thompson MG, Shay DK, Monto AS. Influenza Vaccine Effectiveness in the Community and the Household. *Clin Infect Dis.* 2013
- 4. Skowronski DM, De Serres G, Crowcroft NS, Janjua NZ, Boulianne N, Hottes TS, Rosella LC, Dickinson JA, Gilca R, Sethi P, et al. Association between the 2008–09 seasonal influenza vaccine and pandemic H1N1 illness during Spring-Summer 2009: four observational studies from Canada. *PLoS Med.* 2010; 7:e1000258. References 2–4 raise important questions about the effectiveness of influenza vaccination demonstrating the dire need for drift-resistant vaccines. [PubMed: 20386731]
5. Burton DR, Poignard P, Stanfield RL, Wilson IA. Broadly neutralizing antibodies present new prospects to counter highly antigenically diverse viruses. *Science.* 2012; 337:183–186. [PubMed: 22798606]
6. Han T, Marasco WA. Structural basis of influenza virus neutralization. *Annals of the New York Academy of Sciences.* 2011
7. Kaur K, Sullivan M, Wilson PC. Targeting B cell responses in universal influenza vaccine design. *Trends Immunol.* 2011; 32:524–531. [PubMed: 21940217]
8. Corti D, Lanzavecchia A. Broadly Neutralizing Antiviral Antibodies. *Annu Rev Immunol.* 2013
9. Julien JP, Lee PS, Wilson IA. Structural insights into key sites of vulnerability on HIV-1 Env and influenza HA. *Immunol Rev.* 2012; 250:180–198. [PubMed: 23046130]
10. Schotsaert M, Saelens X, Leroux-Roels G. Influenza vaccines: T-cell responses deserve more attention. *Expert Rev Vaccines.* 2012; 11:949–962. [PubMed: 23002976]
11. Chiu C, Wrammert J, Li GM, McCausland M, Wilson PC, Ahmed R. Cross-reactive humoral responses to influenza and their implications for a universal vaccine. *Ann N Y Acad Sci.* 2013

12. Gerhard, W.; Yewdell, JW.; Frankel, ME., editors. An experimental approach to define the antigenic structures of the hemagglutinin molecule of A/PR/8/34. New York: Elsevier/North Holland; 1980.
13. Schofield DJ, Stephenson JR, Dimmock NJ. Variations in the neutralizing and haemagglutination-inhibiting activities of five influenza A virus-specific IgGs and their antibody fragments. *J Gen Virol.* 1997; 78 (Pt 10):2431–2439. [PubMed: 9349461]
14. Okuno Y, Isegawa Y, Sasao F, Ueda S. A common neutralizing epitope conserved between the hemagglutinins of influenza A virus H1 and H2 strains. *Journal of Virology.* 1993; 67:2552–2558. [PubMed: 7682624]
- 15. Miller MS, Tsibane T, Krammer F, Hai R, Rahmat S, Basler CF, Palese P. 1976 and 2009 H1N1 influenza virus vaccines boost anti-hemagglutinin stalk antibodies in humans. *J Infect Dis.* 2013; 207:98, 105. [PubMed: 23087428]
- 16. Wei CJ, Yassine HM, McTamney PM, Gall JG, Whittle JR, Boyington JC, Nabel GJ. Elicitation of broadly neutralizing influenza antibodies in animals with previous influenza exposure. *Sci Transl Med.* 2012; 4:147ra114.
- 17. Margine I, Hai R, Albrecht RA, Obermoser G, Harrod AC, Banchereau J, Palucka K, Garcia-Sastre A, Palese P, Treanor JJ, et al. H3N2 influenza virus infection induces broadly reactive hemagglutinin stalk antibodies in humans and mice. *J Virol.* 2013
- 18. Wrammert J, Koutsoukos D, Li G-M, Edupuganti S, Sui J, Morrissey M, McCausland M, Skountzou I, Hornig M, Lipkin WI, et al. Broadly cross-reactive antibodies dominate the human B cell response against 2009 pandemic H1N1 influenza virus infection. *J Exp Med.* 2011; 208:181–193. [PubMed: 21220454]
- 19. Dreyfus C, Laursen NS, Kwaks T, Zuijdgheest D, Khayat R, Ekiert DC, Lee JH, Metlagel Z, Bujny MV, Jongeneelen M, et al. Highly conserved protective epitopes on influenza B viruses. *Science.* 2012; 337:1343–1348. [PubMed: 22878502]
- 20. Krause JC, Tsibane T, Tumpey TM, Huffman CJ, Basler CF, Crowe JE Jr. A broadly neutralizing human monoclonal antibody that recognizes a conserved, novel epitope on the globular head of the influenza H1N1 virus hemagglutinin. *J Virol.* 2011; 85:10905–10908. [PubMed: 21849447]
- 21. Whittle JR, Zhang R, Khurana S, King LR, Manischewitz J, Golding H, Dormitzer PR, Haynes BF, Walter EB, Moody MA, et al. Broadly neutralizing human antibody that recognizes the receptor-binding pocket of influenza virus hemagglutinin. *Proc Natl Acad Sci U S A.* 2011; 108:14216–14221. [PubMed: 21825125]
- 22. Xu R, Krause JC, McBride R, Paulson JC, Crowe JE Jr, Wilson IA. A recurring motif for antibody recognition of the receptor-binding site of influenza hemagglutinin. *Nat Struct Mol Biol.* 2013 References 15–22 support the possibility of designing vaccines that elicit BNABs.
23. Laver WG, Colman PM, Webster RG, Hinshaw VS, Air GM. Influenza virus neuraminidase with hemagglutinin activity. *Virology.* 1984; 137:314–323. [PubMed: 6485252]
24. Zhu XY, McBride R, Nycholat CM, Yu WL, Paulson JC, Wilson IA. Influenza Virus Neuraminidases with Reduced Enzymatic Activity That Avidly Bind Sialic Acid Receptors. *Journal of Virology.* 2012; 86:13371–13383. [PubMed: 23015718]
25. Gerhard W, Yewdell J, Frankel ME, Webster R. Antigenic structure of influenza virus haemagglutinin defined by hybridoma antibodies. *Nature.* 1981; 290:713–717. [PubMed: 6163993]
26. Caton AJ, Brownlee GG, Yewdell JW, Gerhard W. The antigenic structure of the influenza virus A/PR/8/34 hemagglutinin (H1 subtype). *Cell.* 1982; 31:417–427. [PubMed: 6186384]
27. Yewdell JW, Taylor A, Yellen A, Caton A, Gerhard W, Bachi T. Mutations in or near the fusion peptide of the influenza virus hemagglutinin affect an antigenic site in the globular region. *J Virol.* 1993; 67:933–942. [PubMed: 7678310]
28. Rini JM, Schulze-gahman U, Wilson IA. Structural evidence for induced fit as a mechanism for antibody-antigen recognition. *Science.* 1992; 255:959. [PubMed: 1546293]
29. Du L, Jin L, Zhao G, Sun S, Li J, Yu H, Li Y, Zheng BJ, Liddington RC, Zhou Y, et al. Identification and Structural Characterization of a Broadly Neutralizing Antibody Targeting a

- Novel Conserved Epitope on the Influenza Virus H5N1 Hemagglutinin. *J Virol.* 2013; 87:2215–2225. [PubMed: 23221567]
30. Ekiert DC, Kashyap AK, Steel J, Rubrum A, Bhabha G, Khayat R, Lee JH, Dillon MA, O’Neil RE, Faynboym AM, et al. Cross-neutralization of influenza A viruses mediated by a single antibody loop. *Nature.* 2012; 489:526–532. [PubMed: 22982990]
 31. Harris AK, Myerson JR, Matsuoka Y, Kuybeda O, Moran A, Bliss D, Das SR, Yewdell JW, Sapiro G, Subbarao K, et al. Structure and accessibility of HA trimers on intact 2009 H1N1 pandemic influenza virus to stem region-specific neutralizing antibodies. *Proc Natl Acad Sci U S A.* 2013 in press.
 32. Noda T. Native morphology of influenza virions. *Frontiers in Microbiology.* 2012; 2
 33. Guttman M, Kahn M, Garcia NK, Hu SL, Lee KK. Solution structure, conformational dynamics, and CD4-induced activation in full-length, glycosylated, monomeric HIV gp120. *J Virol.* 2012; 86:8750–8764. [PubMed: 22674993]
 34. Corti D, Suguitan AL, Pinna D, Silacci C, Fernandez-Rodriguez BM, Vanzetta F, Santos C, Luke CJ, Torres-Velez FJ, Temperton NJ, et al. Heterosubtypic neutralizing antibodies are produced by individuals immunized with a seasonal influenza vaccine. *J Clin Invest.* 2010; 120:1663–1673. [PubMed: 20389023]
 35. Thomson CA, Wang Y, Jackson L, Olson M, Wang W, Liavonchanka A, Keleta L, Silva V, Diederich S, Jones RB, et al. Pandemic H1N1 influenza infection and vaccination in humans induces cross-protective antibodies that target the hemagglutinin stem. *Frontiers in Immunology.* 2012; 3
 36. Throsby M, van den Brink E, Jongeneelen M, Poon LLM, Alard P, Cornelissen L, Bakker A, Cox F, van Deventer E, Guan Y, et al. Heterosubtypic neutralizing monoclonal antibodies cross-protective against H5N1 and H1N1 recovered from human IgM+ memory B cells. *PLoS ONE.* 2008; 3:e3942. [PubMed: 19079604]
 37. Sui J, Hwang WC, Perez S, Wei G, Aird D, Chen LM, Santelli E, Stec B, Cadwell G, Ali M, et al. Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. *Nat Struct Mol Biol.* 2009; 16:265–273. [PubMed: 19234466]
 38. Yewdell JW. Confronting complexity: real-world immunodominance in antiviral CD8+ T cell responses. *Immunity.* 2006; 25:533–543. [PubMed: 17046682]
 39. McKean D, Huppi K, Bell M, Staudt L, Gerhard W, Weigert M. Generation of antibody diversity in the immune response of BALB/c mice to influenza virus hemagglutinin. *Proc Natl Acad Sci U S A.* 1984; 81:3180–3184. [PubMed: 6203114]
 40. Staudt LM, Gerhard W. Generation of antibody diversity in the immune response of BALB/c mice to influenza virus hemagglutinin. I. Significant variation in repertoire expression between individual mice. *J Exp Med.* 1983; 157:687–704. [PubMed: 6600489]
 41. Jiang N, He J, Weinstein JA, Penland L, Sasaki S, He XS, Dekker CL, Zheng NY, Huang M, Sullivan M, et al. Lineage structure of the human antibody repertoire in response to influenza vaccination. *Sci Transl Med.* 2013; 5:171ra119.
 42. Yewdell JW, Webster RG, Gerhard WU. Antigenic variation in three distinct determinants of an influenza type A haemagglutinin molecule. *Nature.* 1979; 279:246–248. [PubMed: 86955]
 43. Johnson TA, Rassenti LZ, Kipps TJ. Ig VH1 genes expressed in B cell chronic lymphocytic leukemia exhibit distinctive molecular features. *J Immunol.* 1997; 158:235–246. [PubMed: 8977195]
 44. Eisen HN, Siskind GW. VARIATIONS IN AFFINITIES OF ANTIBODIES DURING THE IMMUNE RESPONSE. *Biochemistry.* 1964; 3:996–1008. [PubMed: 14214095]
 45. Eisen HN, Chakraborty AK. Immunopaleontology reveals how affinity enhancement is achieved during affinity maturation of antibodies to influenza virus. *Proc Natl Acad Sci U S A.* 2013; 110:7–8. [PubMed: 23284164]
 - 46. Lingwood D, McTamney PM, Yassine HM, Whittle JR, Guo X, Boyington JC, Wei CJ, Nabel GJ. Structural and genetic basis for development of broadly neutralizing influenza antibodies. *Nature.* 2012; 489:566–570. First study to investigate how IAV encounters naïve B cells, with added bonus of providing insight into the activation B cells secreting the Ig V1-69 family heavy chain that are over-represented among BNabs. [PubMed: 22932267]

- 47. Khurana S, Verma N, Yewdell JW, Hilbert AK, Castellino F, Lattanzi M, Del Giudice G, Rappuoli R, Golding H. MF59 adjuvant enhances diversity and affinity of antibody-mediated immune response to pandemic influenza vaccines. *Sci Transl Med*. 2011; 3:85ra48. First study to relate the nature of the vaccine to the avidity of the Ab response.
- 48. Schofield DJ, Stephenson JR, Dimmock NJ. High and low efficiency neutralization epitopes on the haemagglutinin of type A influenza virus. *J Gen Virol*. 1997; 78 (Pt 10):2441–2446. [PubMed: 9349462]
- 49. Lubeck M, Gerhard W. Conformational changes at topologically distinct antigenic sites on the influenza A/PR/8/34 virus HA molecule are induced by the binding of monoclonal antibodies. *Virology*. 1982; 118:1–7. [PubMed: 6177092]
- 50. Greenspan NS, Cooper LNJ. Complementarity, specificity and the nature of epitope and paratopes in multivalent interactions. *Immunology Today*. 1995; 16:226–230. [PubMed: 7540013]
- 51. Nimmerjahn F, Ravetch JV. Antibody-mediated modulation of immune responses. *Immunol Rev*. 2010; 236:265–275. [PubMed: 20636822]
- 52. Schmitz N, Beerli RR, Bauer M, Jegerlehner A, Dietmeier K, Maudrich M, Pumpens P, Saudan P, Bachmann MF. Universal vaccine against influenza virus: linking TLR signaling to anti-viral protection. *Eur J Immunol*. 2012; 42:863–869. [PubMed: 22531913]
- 53. Lamere MW, Lam HT, Moquin A, Haynes L, Lund FE, Randall TD, Kaminski DA. Contributions of Antinucleoprotein IgG to Heterosubtypic Immunity against Influenza Virus. *J Immunol*. 2011; 186:4331–4339. [PubMed: 21357542]
- 54. Staerz UD, Yewdell JW, Bevan MJ. Hybrid antibody-mediated lysis of virus-infected cells. *Eur J Immunol*. 1987; 17:571–574. [PubMed: 3494618]
- 55. Kaplan DR, Griffith R, Braciale VL, Braciale TJ. Influenza virus-specific human cytotoxic T cell clones: heterogeneity in antigenic specificity and restriction by class II MHC products. *Cellular Immunology*. 1984; 88:193–206. [PubMed: 6236884]
- 56. Weinfurter JT, Brunner K, Capuano SV, Li C, Broman KW, Kawaoka Y, Friedrich TC. Cross-Reactive T Cells Are Involved in Rapid Clearance of 2009 Pandemic H1N1 Influenza Virus in Nonhuman Primates. *PLoS pathogens*. 2011; 7:e1002381. [PubMed: 22102819]
- 57. Russell SM, Liew FY. T cells primed by influenza virion internal components can cooperate in the antibody response to haemagglutinin. *Nature*. 1979; 280:147–148. [PubMed: 317881]
- 58. Kim JH, Davis WG, Sambhara S, Jacob J. Strategies to alleviate original antigenic sin responses to influenza viruses. *Proc Natl Acad Sci U S A*. 2012; 109:13751–13756. Insightful study that paves the way for manipulating OAS to improve vaccination. [PubMed: 22869731]
- 59. Francis T Jr. On the doctrine of original antigenic sin. *Proceedings of the American Philosophical Society*. 1960; 104:572–578.

Highlights

- Influenza A virus vaccines must become more effective
- Recent advances in basic immunology and immunology offer new strategies for improved vaccines
- The stem region of the viral hemagglutinin is the most promising target for vaccines that resist antigenic drift
- Many basic questions must be answered to rationally develop optimized vaccines

Table I**Benchmarking Universal IAV Immunity**

Immune Parameter	Comments
Serum HI activity	Flawed measure of protective Abs, measures ability of Abs to block virus attachment to erythrocyte receptors.
Serum VN activity	Better measure of protective Abs, many variations to include/exclude Abs that block attachment, fusion, release. Virus like particles must be used cautiously due to differences in glycoprotein density and arrangement. For that matter, more attention must be paid to affects of virion morphology (filamentous vs. pleomorphic) on VN.
Serum Ab ELISA vs. purified HA	Measures Abs against various HA conformations formed during Ab attachment to solid substrate. Resistance of Abs to release by chaotropic agents (e.g. urea) provides an approximate measure of Ab off rates.
Serum Ab SPR vs. purified HA	Provides actual Ab off rates, a decent proxy for Ab avidity, which is typically governed by off rates
Serum anti-HA Ab epitope specificity	Measurable via several strategies, including competition with Fab fragments specific for defined sites, ELISA/functional assays with viruses engineered to maintain a defined set of epitopes, or large scale Ig gene cloning/expression from HA specific B cells. Essential information for understanding antigenic drift in humans.
Serum anti-NA responses	Ability to block NA activity can be measured by a number of a methods; important to use purified NA or detergent disrupted virus to prevent confounding effects of anti-HA Abs
Serum ELISA vs. purified viral proteins	Provides a measure of Ab responses to potentially important viral gene products, including NA, M2, and NP.
Serum anti-IAV Ig heavy chain use	Can be measured in the various ELISAs described above, but consideration must also be given to the precise nature of the oligosaccharides present, which govern interaction with various Fc receptors
Serum ADCC	Provides a measure of potential protection afforded by cytotoxic immune cells with Fc receptors (NK cells, macrophages, neutrophils). Abs specific for any IAV antigen expressed on the cell surface (HA, M2, NA, NP) can exhibit activity
T cell responses	Can be extensively characterized by specificity and function based on MHC-peptide tetramer technology using multicolor flow cytometry to measure cytokine/chemokine secretion and cell surface expression of marker activation/memory markers
NK responses	IAV-specific NK cells can be measured by flow cytometry and functional assays.