

Towards a Universal Influenza Virus Vaccine Eliciting Broadly Neutralising Haemagglutinin Antibodies

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Abstract

The surface haemagglutinin (HA) glycoprotein is the immunogenic target for most of the influenza virus immune responses and consists of a globular head and a stalk domain. Recent advances have been made towards the design of a universal influenza virus vaccine to protect against different virus strains based on conserved domains of the HA molecule eliciting broadly neutralising antibodies (bnAb). Development of a universal vaccine for influenza that induces long-lived cross-protective immunity would displace the need for annual seasonal vaccination; prediction of circulating strains and vaccine reformulation. Intense research efforts have been focused on enhancing the potency and breadth of vaccine-induced bnAbs. However, knowledge of how such bnAbs are generated and their mechanisms of action are scarce. Experimental 2-step vaccination approaches using prime-boost regimes stimulate the production of bnAbs but they are usually limited in potency and breadth. Adjuvant enhanced vaccination strategies to elicit potent bnAb and improved B cell memory responses will have an immense impact in global health care and pre-pandemic preparation.

Keywords: Influenza; Vaccination; Universal vaccine; Broadly neutralising antibodies; Anti-haemagglutinin antibodies; Interferons

Review

Influenza is a perennial problem affecting millions of people annually together with the constant threat of emerging pandemic viruses. Conventional vaccination using trivalent inactivated or live attenuated virus vaccines against seasonal influenza is currently the main countermeasure. However, a major disadvantage of contemporary vaccines is the impact of antigenic drift necessitating constant updating of vaccine strain composition [1,2]. Thus if one could design a universal vaccine that provides broadly neutralising immunity to different influenza viruses, it would have immense global health benefits [3,4]. Given the possibility of an efficacious universal vaccine that inhibits virus replication and thereby reduces virus shedding, vulnerable populations would be protected from the spread of seasonal influenza virus and in the event of an impending influenza pandemic [5].

Classification of influenza A viruses is based on distinct phylogenetic groups: group 1 HA (H1, H2, H5, H6, H8, H9, H11-13, H16, H17) and group 2 HA (H3, H4, H7, H10, H14 and H15). Currently, there is great interest in developing vaccination strategies that induce bnAbs for cross protection from different influenza virus strains and types, since influenza A (H1N1, H3N2) viruses cause ongoing problems of infection in human populations due to their constant evolution of viral surface antigens which escape immunity. Therefore, antigenic drift challenges immune memory requiring annual development of vaccines to contain specific HA- and neuraminidase-like proteins of predicted circulating influenza virus strains. Despite the availability of reformulated vaccines, approximately 500,000 annual deaths occur from seasonal influenza in mostly the young and elderly in both hemispheres [6]. Also, given the

ever-present threat of a pandemic from emerging or re-emerging influenza viruses, vast numbers of people around the world remain vulnerable, lacking immunity [7]. Indeed, although H1N1 viruses have been circulating in the human population for over three decades, in 2009 a new H1N1 strain emerged as an atypical mild pandemic pathogen with fatalities. In addition, avian influenza viruses with high pathogenicity (H5N1 and H7N9) causing human deaths have recently been identified to possess pandemic potential. Besides lack of immunity in the human population, virus transmissibility and virulence are also important factors in pandemics. Indeed a reassortant virus created in the laboratory, containing the viral PB2 gene from H1N1 within a constellation of genes from the high pathogenicity avian influenza (HPAI) H5N1 virus, was shown to have greater virulence than the wildtype HPAI H5N1 virus with increased transmission and hence higher classification of pandemic potential [8].

The viral surface HA glycoprotein is the immunogenic target for most of the antiviral immune responses to influenza. The globular head is highly variable whereas the stalk domain remains mostly conserved between virus strains [9]. Universal influenza vaccines that target invariant regions of the viral antigens and induce effective broadly neutralising immune responses could potentially provide wide cross-protective antiviral immunity. However, most neutralising antibodies are induced by the HA head domain and are restricted in neutralising only strain-matched influenza viruses. These antibodies may block binding of the virus to sialic acid receptors on host cells thus preventing virus entry, prohibit virus escape from infected cells, or inhibit fusion of the virus once inside the target cell. On the contrary, bnAbs that target conserved regions of the HA stalk are cross-protective and can neutralise different viruses within or spanning virus subtypes [10,11]. Unfortunately, activated B cells that produce antibody responses to shared regions of the HA protein are not high in frequency and their antibody levels are generally low in titre following either natural virus infection or contemporary vaccination [12,13],

warranting further investigations into optimisation [14]. This review focuses on a novel adjuvant approach to vaccination against influenza, to reach the objective of optimising bnAbs to HA with enhanced potency and breadth.

Various adjuvants and immunisation strategies have been explored to seek improved generation of anti-stalk HA neutralising antibodies [15]. A potent natural adjuvant of the innate immune system is type I interferon (IFN). Using a mouse model of experimental vaccination and challenge, certain IFN- α subtype (IFNA5/A6 but not IFNA1) were shown to act as adjuvants for viral proteins in an influenza DNA vaccination/challenge mouse model and enhance IgG2a production resulting in greater protection from influenza *in vivo* [16]. The mouse model is often explored experimentally as mechanistic studies of antibody responses are precluded in other models such as ferrets because immunological laboratory tools are limited [17,18]. Furthermore, the type I IFNs enhance Fc γ R expression in humans and the mouse counterpart since mouse and human IFN and FcR systems are similar and studies in mice have been clinically translated [19,20]. However, influenza viruses have developed immune evasion strategies to avoid immunity and in particular suppress the type I IFN response. This is achieved through a variety of antagonistic viral NS1-associated IFN pathways resulting in inhibition of antiviral adaptive immune responses, highlighting the importance of IFN in host defense [21]. In the case of influenza virus infection, this suggests that the type I IFNs may activate infrequent pre-existing memory B cells, including the HA stalk-specific memory B cells. Future investigations of the type I IFN subtypes, as adjuvants to enhance the generation of bnAbs to virus vaccination, hold great potential.

Current influenza control requires efficacious virus vaccination to stimulate B cell responses to HA. Updated strain-matched vaccines are developed on an annual basis requiring seed virus preparation generated by either reassortment or reverse genetics for propagation in eggs or cultured cells [22]. However, universal influenza vaccines that target invariant regions of the virus and induce effective broadly neutralising immune responses could potentially provide more effective antiviral coverage. Successful approaches to vaccination to redirect the immune response to the invariant HA stalk protein for production of bnAbs relies on a novel 2-strep (prime-boost) vaccination strategy [23,24]. One successful approach is to immunise with chimeric HA (conserved stalk/ novel head) virus vaccines [23]. This vaccination approach using the same conserved stalk regions but different head domains expands the otherwise scarce HA stalk-specific memory B cells, which are normally outcompeted by numerous immunodominant HA head-specific memory B cells [25]. Another effective strategy is to use sequential virus immunisation with matched HA proteins but in a DNA prime and protein boost regime [24]. This latter approach resulted in increased production of bnAbs that could neutralise diverse H1N1 strains dating back from 1934 to 2007 than either vaccine component used alone but offered limited protection in both mouse and ferret models [24]. It is also suggested that the number and diversity of HA-specific CD4 T cell clones is expanded with the DNA prime- protein boost strategies with matched HAs. These different 2-step (prime-boost) vaccine studies have reported increased neutralising antibody titres (30 to 50-fold more than single virus vaccine alone) with 2-log reductions in lung viral loads [23,24,26]. However, virus replication was not absolutely prevented in vaccines.

During influenza virus infection, signals from IFN and help from activated CD4 T cells are often limited by strong viral immune evasion tactics resulting in suboptimal activation of B cells and antibody

responses [27]. As the type I IFNs provide a link between innate and adaptive immunity, acting on both B and T cells, they can restore immunity. Thus novel vaccine strategies, informed by the abovementioned approaches [23,24] in combination with IFN- α / β subtypes as vaccine adjuvants to augment the potency and breadth of bnAbs to conserved regions of the HA stalk of influenza viruses are of great interest.

Polyclonal B cell stimulation by vaccination or natural infection with influenza virus can generate circulating antibody pools with multiple epitope reactivity. Neutralising antibodies blocking HA binding proteins to cell surface receptors may prevent virus entry. Once the virus infection cycle has been abrogated by such antibody-mediated responses, virus dissemination is inhibited, virus shedding is reduced, and the infection ultimately resolved by cell-mediated immune responses [28]. Correlates of protection *in vivo* are highly functional neutralising antibodies against HA epitopes of influenza viruses [29]. As previously stated, cognate help from activated CD4⁺ T cells also stimulates B cells to secrete virus-specific antibody. Extrafollicular antibody secreting cells producing early IgM are regulated by type I IFN and cytokines, whereas mature B cells class switch to produce IgG during the course of virus infection [30]. Both memory B cells and antibody-secreting cells can produce virus-specific antibody upon re-exposure to virus [31]. Live attenuated influenza virus vaccines and trivalent inactivated vaccines mostly induce neutralising antibodies directed against the HA globular head domain, which block specific virus binding and inhibit virus entry. As such antibodies rely on variable regions, they are generally not dependent on Fc regions of the antibody molecule. However, as mentioned such antibody specificities are limited in efficacy against variable influenza virus sequences due to the high mutation rate in the HA globular head.

Furthermore, the benefits of anti-viral antibodies as agents of prophylaxis for protection against influenza are widely known [reviewed in 32,33]. Recently, light has been shed on the protective mechanism of anti-stalk bnAbs, which is thought to be different to those reacting with the HA head. Traditionally, neutralising antibodies function exclusively through their variable regions but Fc γ R-mediated mechanisms may also be involved [34]. Characterisation of five IgG bnAbs targeting the conserved stalk region of the HA molecule *in vivo* found that they exhibited cell cytotoxicity dependent on Fc γ R whereas anti-head immune complexes did not. These studies point to new findings of neutralising antibody mechanisms in blocking influenza virus infection via antibody-mediated cell cytotoxicity. In light of recent discoveries of Fc dependency, reengineering of neutralising anti-stalk monoclonal antibodies to selectively interact with Fc γ R may augment protective activity.

In conclusion, the identification of influenza vaccine adjuvants to induce bnAbs is a high priority research area. By restoring the power of innate immunity, that is normally diverted during natural virus infection, utilising the versatility of type I IFN subtypes as enhancers for the production of bnAbs to invariant stalk domains of the HA protein of influenza virus could be a productive avenue of research to enhance anti-HA bnAb responses to vaccination. New rational vaccination approaches that provide universal protection against multiple influenza viruses will transform influenza control.

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References

1. Webster RG, Govorkova EA (2014) Continuing challenges in influenza. *Ann N Y Acad Sci* 1323: 115-139.
2. Subbarao K, Murphy BR, Fauci AS (2006) Development of effective vaccines against pandemic influenza. *Immunity* 24: 5-9.
3. Kirchenbaum GA, Ross TM (2014) Eliciting broadly protective antibody responses against influenza. *Curr Opin Immunol* 28: 71-76.
4. Zhang H, Wang L, Compans RW, Wang BZ (2014) Universal influenza vaccines, a dream to be realized soon. *Viruses* 6: 1974-1991.
5. Laursen NS, Wilson IA (2013) Broadly neutralizing antibodies against influenza viruses. *Antiviral Res* 98: 476-483.
6. Salomon R, Webster RG (2009) The influenza virus enigma. *Cell* 136: 402-410.
7. Taubenberger JK, Morens DM (2009) Pandemic influenza--including a risk assessment of H5N1. *Rev Sci Tech* 28: 187-202.
8. Imai M, Watanabe T, Hatta M, Das SC, Ozawa M, et al. (2012) Experimental adaptation of an influenza H5 HA confers respiratory droplet transmission to a reassortant H5 HA/H1N1 virus in ferrets. *Nature* 486: 420-428.
9. Ekiert DC, Bhabha G, Elsliger MA, Friesen RH, Jongeneelen M, et al. (2009) Antibody recognition of a highly conserved influenza virus epitope. *Science* 324: 246-251.
10. Margine I, Krammer F, Hai R, Heaton NS, Tan GS, et al. (2013) Hemagglutinin stalk-based universal vaccine constructs protect against group 2 influenza A viruses. *J Virol* 87: 10435-10446.
11. Okuno Y, Isegawa Y, Sasao F, Ueda S (1993) A common neutralizing epitope conserved between the hemagglutinins of influenza A virus H1 and H2 strains. *J Virol* 67: 2552-2558.
12. Throsby M, van den Brink E, Jongeneelen M et al. (2008) Heterosubtypic neutralizing monoclonal antibodies cross-protective against H5N1 and H1N1 recovered from human IgM+ memory B cells. *PLoS One* 3: e3942.
13. Sangster MY, Baer J, Santiago FW, Fitzgerald T, Ilyushina NA, et al. (2013) B cell response and hemagglutinin stalk-reactive antibody production in different age cohorts following 2009 H1N1 influenza virus vaccination. *Clin Vaccine Immunol* 20: 867-876.
14. He W, Mullarkey CE, Duty A, Moran TM, Palese P, Miller MS (2015) Broadly-neutralizing anti-influenza virus antibodies: enhancement of neutralizing potency in polyclonal mixtures and IgA backbones. *J Virol* 89: 3610-3618.
15. Goff PH, Eggink D, Seibert CW, Hai R, Martinez-Gil L, et al. (2013) Adjuvants and immunization strategies to induce influenza virus hemagglutinin stalk antibodies. *PLoS One* 8: e79194.
16. James CM, Abdad MY, Mansfield JP et al. (2007) Differential activities of alpha/beta IFN subtypes against influenza virus in vivo and enhancement of specific immune responses in DNA vaccinated mice expressing haemagglutinin and nucleoprotein. *Vaccine* 25: 1856-1867.
17. Rockman S, Maher D, Middleton D (2012) The use of hyperimmune serum for severe influenza infections*. *Crit Care Med* 40: 973-975.
18. Friesen RH, Koudstaal W, Koldijk MH, Weverling GJ, Brakenhoff JP, et al. (2010) New class of monoclonal antibodies against severe influenza: prophylactic and therapeutic efficacy in ferrets. *PLoS One* 5: e9106.
19. Pfeffer LM, Dinarello CA, Herberman RB, Williams BR, Borden EC, et al. (1998) Biological properties of recombinant alpha-interferons: 40th anniversary of the discovery of interferons. *Cancer Res* 58: 2489-2499.
20. Haller O, Kochs G, Weber F (2007) Interferon, Mx, and viral countermeasures. *Cytokine Growth Factor Rev* 18: 425-433.
21. Kochs G, Garcia-Sastre A, Martinez-Sobrido L (2007) Multiple anti-interferon actions of the influenza A virus NS1 protein. *J Virol* 81: 7011-7021.
22. Milne GJ, Halder N, Kelso JK (2013) The cost effectiveness of pandemic influenza interventions: a pandemic severity based analysis. *PLoS One* 8: e61504.
23. Krammer F, Palese P (2014) Universal influenza virus vaccines: need for clinical trials. *Nat Immunol* 15: 3-5.
24. Wei CJ, Boyington JC, McTamney PM, Kong WP, Pearce MB, et al. (2010) Induction of broadly neutralizing H1N1 influenza antibodies by vaccination. *Science* 329: 1060-1064.
25. Halliley JL, Khurana S, Krammer F, Fitzgerald T, Coyle EM (2015) High-affinity H7 head and stalk domain-specific antibody responses to an inactivated influenza H7N7 vaccine after priming with live attenuated influenza vaccine. *J Infect Dis* 212: 1270-1278.
26. Margine I, Hai R, Albrecht RA et al. (2013) H3N2 influenza virus infection induces broadly reactive hemagglutinin stalk antibodies in humans and mice. *J Virol* 87: 4728-4737.
27. Alam S, Knowlden ZA, Sangster MY, Sant AJ (2014) CD4 T cell help is limiting and selective during the primary B cell response to influenza virus infection. *J Virol* 88: 314-324.
28. Iwasaki A, Medzhitov R (2010) Regulation of adaptive immunity by the innate immune system. *Science* 327: 291-295.
29. Plotkin SA (2008) Vaccines: correlates of vaccine-induced immunity. *Clin Infect Dis* 47: 401-409.
30. Sundararajan A, Huan L, Richards KA, Marcelin G, Alam S, et al. (2012) Host differences in influenza-specific CD4 T cell and B cell responses are modulated by viral strain and route of immunization. *PLoS One* 7: e34377.
31. Goodnow CC, Vinuesa CG, Randall KL, Mackay F, Brink R (2010) Control systems and decision making for antibody production. *Nat Immunol* 11: 681-688.
32. Berry CM, Penhale WJ, Sangster MY (2014) Passive broad-spectrum influenza immunoprophylaxis. *Influenza Research Treatment* 2014: e267594.
33. Rinaldi C, Penhale WJ, Stumbles PA, Tay G, Berry CM (2014) Modulation of innate immune responses by influenza-specific ovine polyclonal antibodies used for prophylaxis. *PLoS One* 9: e89674.
34. Dilillo DJ, Tan GS, Palese P, Ravetch JV (2014) Broadly neutralizing hemagglutinin stalk-specific antibodies require FcR interactions for protection against influenza virus in vivo. *Nature Med* 20: 143-151.