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A Structural and Mathematical Modeling Analysis of the Likelihood of Antibody Dependent Enhancement in Influenza

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Abstract

Broadly neutralizing monoclonal antibodies (bNAbs) for viral infections, such as HIV, respiratory syncytial virus (RSV), and influenza, are increasingly entering clinical development. For influenza, most neutralizing antibodies target influenza virus hemagglutinin. These bNAbs represent an emerging, promising modality for treatment and prophylaxis of influenza due to their multiple mechanisms of antiviral action and generally safe profile. Pre-clinical work in other viral diseases, such as dengue, has demonstrated the potential for antibody-based therapies to enhance viral uptake, leading to enhanced viremia and worsening of disease. This phenomenon is referred to as antibody-dependent enhancement (ADE). In the context of influenza, ADE has been used to explain several pre-clinical and clinical phenomena. Using structural and viral kinetics modeling, we assess the role of ADE in treatment of influenza with a bNAb.

Antibodies as Antivirals and Development of Antibody-Dependent Enhancement

Recent developments in antibody discovery and protein engineering have led to the identification of highly potent, broadly neutralizing antibodies to a number of infectious agents. Several of these, including monoclonal antibodies against influenza and HIV have entered clinical development and are in phase I or phase II (*e.g.* NCT02603952, NCT02293863, NCT02468115, NCT02568215 and NCT02588586; <https://clinicaltrials.gov/>). Recent antiviral data reported for these antibodies has generated much excitement, for example, data on 3BNC117, targeting the CD4-binding site of gp120, suggests that the antibody has higher efficacy in humans than was necessarily predicted from *in vitro* or *in vivo* studies because of effector functions, including the ability to clear infected cells [1, 2]. Given the intriguing, albeit early, data on the antiviral activity of broadly neutralizing monoclonal antibodies, there is the potential for these agents to become important complements to existing, small molecule antivirals.

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For influenza, virus specific antibodies play a pivotal role in preventing and controlling viral infection. Several studies have examined the dynamics of the humoral immune response upon infection or vaccination using genetic and/or structural tools [3–9], largely focusing on antibodies to influenza hemagglutinin (HA), a surface protein, which is generally associated with a protective response [10]. Notably, although antibodies to the other predominant surface protein, neuraminidase, are also produced, these antibodies, in isolation, do not prevent viral infection but may participate in effector-mediated neutralization. These studies have identified three general properties of the virus-neutralizing humoral response to hemagglutinin: (i) the antibody response within an individual is dictated by the history of previous exposures and/or vaccinations (*i.e.* original antigenic sin); (ii) the majority of the antibody response is focused to the head of the influenza HA protein; and (iii) these antibodies tend to be strain specific due to the variability in amino acid sequence of the HA head, leading to the potential for seasonal re-infection due to alterations in virus structure through mutation and genetic reassortment.

HA is synthesized as a single polypeptide (HA0) which folds into a pre-fusion conformation and exists as a homotrimer. Maturation of the HA trimer occurs upon its cleavage into HA1 and HA2 subunits by host proteases. HA1 and HA2 subunits do not separate but instead remain as a stable complex with a disulfide bond linking the two subunits. The virus receptor binding site and the host membrane fusion peptide are located in the HA1 and HA2 subunits, respectively. As such, HA1 plays an important role in virus-host attachment, while HA2 plays an important role during virus-host membrane fusion in the endosome. In the pre-fusion conformation, most of the HA2 subunit is buried by the HA1 subunit. The head domain of the HA molecule, which is comprised of a large portion of HA1, is often the target of neutralizing antibodies. These head-directed antibodies neutralize virus by blocking attachment to the host cell. On the other hand, antibodies to the stem region of the HA molecule, primarily located within the HA2 subunit, are less frequently produced and generally act to prevent viral fusion through preventing the necessary conformational changes required for the virus and host membrane to fuse in the endosome [11, 12]. In addition to direct neutralization, stem-binding antibodies play an important role in clearing the infected host cells by recruiting effector molecules, such as complement or innate immune cells [13, 14]. Despite regions of the stem being highly conserved among various subtypes of influenza A, neutralizing antibodies targeting this region represent a minor fraction of the total humoral immune response. Furthermore, administration of novel HA stem immunogens clearly demonstrates that a humoral response to this region is protective [15, 16]. In fact, a recent study confirmed that even non-neutralizing antibodies, induced by immunization, can be protective in animal models [17].

Recently, several broadly neutralizing antibodies against the stem of influenza HA have been reported, including against group 1 of influenza A [18], group 2 of influenza A [19], and against both group 1 and group 2 [20–22]. These broadly neutralizing antibodies, either individually or in combination, have been able to treat influenza infection and/or provide prophylactic protection in various animal models. To date, the family of stem-directed broadly neutralizing antibodies has been found to employ divergent germ lines, including the VH_{1–69} germline as well as others, and targeting different, but overlapping epitopes (Figure 1). Early discovery of C179 [23] was followed over a decade later by the identification of

other stem-binding antibodies, including F10 [24], CR6261/CR8020 [18, 19], CR9114 [21], FI6 [20], 39.29 [25], 12D1[26], 6F12[27] and VIS410 [22]. Notably, these antibodies have differentiated properties including breadth of coverage and the ability to elicit effector functions through binding Fc γ receptors [17, 28].

There has been significant conversation within scientific and regulatory bodies on the potential risks associated with the use of antibodies in the treatment of influenza; discussions at the Pan American Health Organization 2014 meeting on monoclonal antibodies highlighted the controversy, with a special session on this topic being convened at the annual meeting of the International Society for Influenza and Other Respiratory Virus Diseases (2015, Austin, Texas). While antibodies against influenza have demonstrated the ability to mitigate lethal influenza infection in animals, the clinical use of monoclonal antibodies for the prevention and/or treatment of acute influenza has raised the question of whether binding by a therapeutic antibody could potentially exacerbate disease either by enhancing virus uptake and propagation or by promoting fusion in the endosome [29]. In the former scenario, engagement of Fc γ receptors at the cell surface by antibody-bound virus would lead to uptake of the virus; however, in the event that the virus is not neutralized (either because of low affinity binding or through targeting a non-neutralizing epitope), antibody binding would result in virus escape and hence, enhanced infection. Alternatively, enhancement could occur after virus binding to the cognate cellular receptor (sialic acid in the case of influenza) and internalization by stem binding antibodies that enhance fusion of the virus with the endosome, and result in enhanced infection.

This so-called antibody-dependent enhancement (ADE) of viral infection has been invoked for many viruses including dengue [30, 31], respiratory syncytial virus (RSV) [32, 33], influenza [34, 35] and others [36–38], to describe multiple clinical phenomenon. For example, in the case of dengue, ADE is invoked to explain the fact that previously exposed individuals who are infected with a heterologous serotype are at increased risk for severe disease. At a mechanistic level, enhanced disease in the presence of non-neutralizing antibodies has been explained by antibody binding promoting enhanced virus output, resulting from the attachment of immune complexes to Fc γ I and IIa receptors, uptake of such complexes, and resulting infection [39, 40]. In the case of dengue, animal models are able to replicate this phenomenon [41, 42]. Conversely, in the case of RSV, immune enhancement was observed upon vaccination with formalin-inactivated virus which resulted in enhanced disease during breakthrough infections. In contrast to dengue, the target cells for RSV are pulmonary epithelial cells and not Fc γ -bearing cells. In this case, it is thought that non-neutralizing antibodies to the F (and potentially G) protein, elicited through vaccination, resulted in stimulation of RSV-primed Th2 polarized cells, immune complex formation, and increased cell/tissue damage [43]. This article discusses the link between ADE and influenza and offers perspectives on likelihood of ADE with different anti-influenza antibodies using a mathematical modeling approach.

ADE and Influenza

ADE associated with the influenza A virus was first described by Ochiai and colleagues[44], where they characterized the antibody-mediated growth of influenza A virus in macrophages

using rabbit serum isolated from animals that had been exposed to influenza virus. Later studies with serum from animals infected with various influenza A viruses showed that the subtype cross-reactive, non-neutralizing antibodies to both HA and neuraminidase molecules contributed to ADE *in vitro*[45]. This analysis was further elaborated in pigs, where enhanced disease was found to be caused by various influenza vaccination protocols (*i.e.*, vaccine-associated enhanced respiratory disease, VAERD)[34]. More specifically, this study demonstrated that when pigs were vaccinated with an inactivated H1N2 virus and then challenged with 2009 H1N1, the pathological findings of the resulting infection exhibited enhanced pulmonary lesions. Additional analysis indicated that the increased pathology was the result of VAERD. VAERD in humans has been suspected[46], where the seasonal 2008–09 trivalent inactivated influenza vaccination was found to potentially be associated with increased risk of illness associated with the pandemic 2009 H1N1 virus. Notably, the observations of disease enhancement were in response to immunization or polyclonal sera.

Given these observations, both within influenza and with other viruses, could a monoclonal antibody-based solution for the treatment of influenza elicit a VAERD-like response through promotion of enhanced disease? To address this question, one has to consider the different potential mechanisms that have been described to cause enhancement. One potential mechanism, as in dengue, would be that antibodies targeting influenza HA could potentially mediate enhanced uptake of virus into Fc γ -expressing cells and, if non-neutralizing, could lead to escape of the virus from the endosome. Endosomal escape would result in either productive infection in cells that do not normally sustain influenza infection and/or promote additional infection in susceptible cell populations. Alternatively, antibodies targeting certain epitopes on the HA stem could be directly pro-fusogenic, by stabilizing the presentation of fusion peptides and facilitating the low pH conformational change in HA that results in fusion of the viral and host cell membrane. Finally, a third possibility, as has been invoked for RSV, the presence of non-neutralizing antibodies could form immune complexes that lead to polarization of the immune response to a pro-inflammatory, pathogenic response, resulting in enhanced disease.

HA2-Binding Antibodies, Depending on Epitope, Can Enhance Fusion

The broadly neutralizing anti-influenza HA antibodies in clinical development target the stem region and inhibit virus-host endosomal membrane fusion. This class of antibodies does not inhibit virus-host receptor attachment. Since such antibodies target the fusogenic potential of the virus, it is important to assess the ability of stem binding antibodies to directly enhance infection.

A previous study molecularly characterized the antibodies associated with VAERD in pigs in an effort to address the underlying mechanisms of VAERD [35]. While the sera from H1N2-vaccinated pigs could enhance pH1N1 infection of MDCK cells, the sera did not inhibit agglutination of red blood cells (RBCs) by H1N1 virus. This result indicates the sera had little or no cross-reactive binding antibodies that blocked HA-glycan receptor interactions. Through additional studies they found this serum to be capable of enhancing H1N1 infection in MDCK cells. Fractionation of the serum antibodies identified that a polyclonal pool of antibodies (pAb) bound epitopes mapping to amino acids 32 to 77 of HA2 as responsible for

enhancing virus infection. Given the lack of Fc γ receptors on MDCK cells and the mapping of activity to HA2-directed pAb, the ability of the pAb to enhance RBC hemolysis and the enhancement of infection was attributed to an Fc γ receptor-independent pathway, where the polyclonal response directly promotes virus membrane fusion activity that in turn enhances replication and ultimately increased lung pathology.

Structure-based analysis of this phenomenon can enable us to further elucidate the mechanism by which enhancement may be occurring. As a first step, mapping the recognized epitope on HA2 is essential to describe its function. This is particularly important since HA2 exists in two different conformational forms - the pre-fusion conformation on the extracellular virus and the post-fusion conformation inside the host cell endosome. Structure-based analysis indicates that residues 58–76, within the 32–77 epitope, are completely buried by the HA1 subunit in the pre-fusion conformation and exposed only in the post-fusion conformation, suggesting interaction of the polyclonal response to the post-fusion conformation of HA2 (Figure 2). Indeed, the recombinant HA2 subunit, such as the one used in this study to fractionate the enhancing polyclonal fraction[35], is known to exist only in the post-fusion conformation when it is expressed as either membrane-bound or in a soluble form[47].

In the endosome, when HA undergoes conformational change, the first step of this process is the separation of the HA1–HA2 interface. The disulfide bond anchor keeps the two subunits attached, but the interface separation allows for the dramatic conformational change within the HA2 subunit. Because it is membrane bound, the distal fusion peptide is initially prevented from reaching and interacting with the host membrane to initiate membrane fusion (Figure 3). Cryo-electron tomography of influenza at neutral and acidic pH confirms this analysis[48]. At neutral pH, HA is found in an ordered array at the surface of the virus. Conversely, at acidic pH, the density associated with HA is fused and individual trimers cannot be resolved. To initiate a successful membrane fusion, then, HA molecules at this point must act cooperatively with their fusion peptides to re-orient themselves towards the host membrane, enabling membrane fusion (Figure 3). At the same time, the low pH form of HA is more susceptible to proteolysis, resulting in cleavage and release of HA1, thereby enhancing the ability of HA2 to cooperatively undergo membrane fusion. In this model, pro-fusogenic antibodies, if present, would bind and crosslink HA2 molecules, enabling them to order and orient towards the host membrane and enhance viral fusion efficiency.

In direct contrast to the enhancing antibodies described above, neutralizing, stem-binding anti-influenza A antibodies bind to a distinct set of epitopes on the pre-fusion conformation of HA with their binding centered on the hydrophobic cleft found adjacent to the exposed B-helix of HA2[49]. Antibody binding prevents viral infection of host cells by inhibiting the conformational change of HA in the endosome required for fusion of viral and host membranes. Although these antibodies do not inhibit virus entry; their binding prevents HA from undergoing the conformational change in the endosome. Taken together, the fact that neutralizing antibodies bind to a region of HA2 exposed in the pre-fusion conformation that is distinct from the epitope known to be pro-fusogenic indicates that these antibodies are highly unlikely to elicit ADE by promoting fusogenic activity.

Fc γ -Mediated Uptake of Influenza Virus

As an alternative to direct stabilization of the pro-fusogenic state of HA, a monoclonal antibody could elicit enhanced viremia through immune complex formation and uptake through Fc γ receptors, such as is observed for dengue virus. Of note, influenza virus primarily targets sialic acid receptors present on epithelial cells of the respiratory tract, rather than Fc γ -bearing cells of the adaptive and innate immune system[50]. Several cell culture-based studies have shown that innate immune cells, such as macrophages and dendritic cells, are generally not supportive of productive influenza infection for most seasonal and low-pathogenic strains but may support infection of some highly pathogenic strains[50]. Furthermore, quantitative estimates indicate that such events are unlikely during the natural course of influenza infection. Quantification of receptor presentation indicates that there are approximately 4×10^8 target cells for influenza virus[51], each containing 10^4 – 10^5 sialic acid receptors[52]. The engagement of virus HA with cell surface sialic acid is polyvalent with an apparent K_d of 0.4–0.5 nM[53]. This is in comparison to monovalent binding of antibody (IgG_{1,2, and 4}) to Fc γ RI, which binds with a maximal dissociation constant of 15 nM[54], approximately 30-fold weaker, and where lung monocytes (total number of 2.3×10^7 cells[55]) have approximately 1.3×10^4 Fc γ RI receptors on their surface[56]. Therefore, based on absolute number of receptors (~100-fold lower for Fc γ receptors compared to sialic acid receptors) and their relative affinity/avidity, influenza binding and endosomal uptake is expected to largely occur as a function of HA binding to its cognate receptor form of sialic acid (if present) as opposed to engagement through Fc γ receptors.

A further distinction can be made based on the particular epitope bound by the antibody. Most antibodies generated from a humoral response to vaccination or infection are directed to the HA head, which serve to neutralize virus by preventing attachment through the predominant sialic acid pathway. Binding of such antibodies could facilitate virus entry through the Fc γ R pathway; in this case, such antibodies do not have any mechanism to stop viral infection in the endosome[13]. Such a phenomenon would be largely unapparent with a neutralizing, head-directed antibody but would become more apparent in the event that the antibody is non-neutralizing. For example, HA1-directed antibodies that do not target the major antigenic sites surrounding the receptor binding site but rather the “side” of the head are broadly binding but non-neutralizing[57].

In contrast, stem-directed antibodies that bind to the pre-fusion form and prevent HA conformational changes would not influence cell attachment through the ‘normal’ sialic acid pathway; hence internalization through the Fc γ R binding would be predicted to be minimal; in essence this pathway would be outcompeted by the more prevalent, higher avidity interaction between the receptor binding site on HA1 with human sialic acid receptors. Then, in the event that such a stem-directed antibody did not neutralize the virus (*i.e.* did not prevent virus-cell fusion), active viral replication would occur to the same extent as if there was no antibody present.

Mathematical Model to Assess Impact of ADE on Viral Replication Kinetics

The above observations suggest that from a structural and biochemical binding perspective, it is unlikely that ADE occurs for antibodies that target the pre-fusion conformation of HA stem. However, to quantitatively assess ADE and its effect on viral replication with these antibodies, we adapted the recently developed mathematical models for viral kinetics in influenza infection [58–61]. The basic kinetic parameters of influenza infection have been measured or approximated previously (Table 1). We extended the target cell-limited differential equation model by Baccam *et al.* [58] by explicitly modelling the three different, mutually exclusive, modes of infection: infection by free virus (I_1), infection by antibody-bound virus via SA receptor (I_2), and infection by antibody-bound virus via Fc γ receptor (I_3) (Box 1). The infections by the three mechanisms are expressed by the second-order terms, $\beta_1 TV$, $\beta_2 TU$, and $\beta_3 TU$, where β_i is the rate of infection, T is the number of target cells, V is the number of viral particles not affected by antibodies, and U is the number of virus particles inhibited by the antibody. The infected cells then become either virus-producing cells (P) at an intrinsic rate k_i (independent of antibody binding) or are cleared at rate c_i , $i = 1, 2, 3$. The virus-producing infected cells produce new viral particles at rate p and are cleared at rate δ . The free virus is bound by antibodies at rate α or is cleared at rate c . The antibody-bound virus is cleared at rate c_0 . The depletion of free and antibody-bound virus due to infection of target cells is explicitly modelled by terms $\beta'_1 TV$, $\beta'_2 TU$, and $\beta'_3 TU$ [61]. When modelling head-binding antibody, we assume 50% efficiency of infection ($\beta_2 = 0.5 \times \beta_1$) and no clearance of infected cells ($c_1 = c_2 = c_3 = 0$). When modelling stem-binding antibody, we assume 100% efficiency of infection ($\beta_2 = \beta_1$) and 50% clearance of infected cells ($c_2 = k_2$, $c_3 = k_3$). When modeling ADE, we assume the same infection rate ($\beta_3 = \beta_1$). In contrast to Li and Handel [62], we do not explicitly model the immune response and the amount of antibodies; such effects are indirectly captured by the relative values of α and β_1 , β_2 , β_3 . Further, we do not include the contribution of antibody dependent cellular cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC) in mediating clearance of infected cells. Figure 4 shows the results from the model when antibody is administered 1 day post infection (1dpi). The results indicated that in a target cell-limited model, stem binding neutralizing antibodies pose a lower risk of increased viral load due to ADE as compared to head-binding neutralizing antibodies (1.6 fold vs. 2.2 fold increase in area under the curve (AUC)). Taken together, the mathematical model quantitatively supports the structural and biochemical rationale that stem-binding antibodies, which are able to bind the prefusion form of HA and prevent its conformational change, are unlikely to induce significant ADE through Fc γ -mediated uptake.

Box 1

Influenza Virus Model in the Presence of Antibody

T is the number of uninfected target cells, I_1 , I_2 , and I_3 are the number of infected (but not virus-producing) cells, P is the number of cells actively producing new viruses, V is the free-virus load, and U is the antibody-bound virus load. We distinguish three modes of infection, I_1 – target cells infected by free virus via sialic-acid receptor (not bound by

antibody), I_2 – target cells infected by antibody-bound virus via sialic-acid receptor, and I_3 – target cells infected by antibody-bound virus via $\text{Fc}\gamma$ receptor (ADE).

$$\begin{aligned}\frac{dT}{dt} &= -\beta_1 TV - \beta_2 TU - \beta_3 TU \\ \frac{dI_1}{dt} &= \beta_1 TV - k_1 I_1 - c_1 I_1 \\ \frac{dI_2}{dt} &= \beta_2 TU - k_2 I_2 - c_2 I_2 \\ \frac{dI_3}{dt} &= \beta_3 TU - k_3 I_3 - c_3 I_3 \\ \frac{dP}{dt} &= k_1 I_1 + k_2 I_2 + k_3 I_3 - \delta P \\ \frac{dV}{dt} &= pP - \beta'_1 TV - \alpha V - cV \\ \frac{dU}{dt} &= \alpha V - \beta'_2 TU - \beta'_3 TU - c_0 U\end{aligned}$$

Antibody Binding Leading to Enhanced Pathology

Finally, antibody binding and immune complex formation is known in certain limited cases to enhance pathology. For example, this has been hypothesized to be the case in the context of formalin-inactivated RSV, where vaccination led to enhanced pulmonary disease upon subsequent infection, likely through production of non-neutralizing antibodies that promoted a non-productive, damaging immune response[63, 64]. In the case of RSV, a Th1-driven response tends to promote cell-mediated immunity important for protection against the virus. Conversely, a Th2 response can be associated with eosinophilia, goblet cell hyperplasia, mucus overproduction, and airway hypersensitivity. In contrast to natural infection that is resolved, the formalin-inactivated RSV vaccine induced a Th2-biased response, likely a consequence of inefficient induction of interferon- γ (IFN- γ)-secreting NK cells and CD8+ T lymphocytes[65, 66]. Indeed, Th2 polarization of the immune response in RSV infection is a hallmark of more severe disease[43] and several viral gene products, including soluble G protein, promote such a response[67]. This is in contrast to influenza infection, where influenza immunity is largely driven by a Th1 response[68], with the virus suppressing efficient recruitment of Th2 cells[69]. Thus, while such an immune-modulating mechanism appears to be the case for RSV, and potentially other viruses, no information suggests that this is true for influenza. In addition, examination of the natural humoral response in animals administered a stem-directed antibody indicates that there is no detectable difference between infected animals which clear the virus naturally compared to those which are infected and then treated with a stem-directed antibody[70]. Taken together, these data argue that immune complex formation is likely not a substantial mechanism for disease enhancement for influenza.

Concluding Remarks

In summary, the available evidence, and the known or hypothesized mechanisms that have been advanced by which antibodies (primarily non-neutralizing) can enhance viral replication and/or disease do not *a priori* support the presence of a substantial risk of ADE for neutralizing, stem-directed antibodies. To verify these findings requires, of course, clinical testing (see Outstanding Questions). However, by bringing together multiple observations that have been made for stem-directed antibodies, largely discovered within the

last eight years, with what we know about influenza infection, propagation, and pathogenesis, it is unlikely that such antibodies would enhance infection by mechanisms that have been previously posited.

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References

1. Lu CL, et al. Enhanced clearance of HIV-1-infected cells by broadly neutralizing antibodies against HIV-1 in vivo. *Science*. 2016; 352:1001–1004. [PubMed: 27199430]
2. Schoofs T, et al. HIV-1 therapy with monoclonal antibody 3BNC117 elicits host immune responses against HIV-1. *Science*. 2016; 352:997–1001. [PubMed: 27199429]
3. Laserson U, et al. High-resolution antibody dynamics of vaccine-induced immune responses. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111:4928–4933. [PubMed: 24639495]
4. Lingwood D, et al. Structural and genetic basis for development of broadly neutralizing influenza antibodies. *Nature*. 2012; 489:566–570. [PubMed: 22932267]
5. Pappas L, et al. Rapid development of broadly influenza neutralizing antibodies through redundant mutations. *Nature*. 2014; 516:418–422. [PubMed: 25296253]
6. Sobolev O, et al. Adjuvanted influenza-H1N1 vaccination reveals lymphoid signatures of age-dependent early responses and of clinical adverse events. *Nature immunology*. 2016; 17:204–213. [PubMed: 26726811]
7. Kreijtz JH, et al. Immune responses to influenza virus infection. *Virus research*. 2011; 162:19–30. [PubMed: 21963677]
8. Andrews SF, et al. Immune history profoundly affects broadly protective B cell responses to influenza. *Science translational medicine*. 2015; 7:316ra192.
9. Schmidt AG, et al. Immunogenic Stimulus for Germline Precursors of Antibodies that Engage the Influenza Hemagglutinin Receptor-Binding Site. *Cell reports*. 2015; 13:2842–2850. [PubMed: 26711348]
10. Wang TT, et al. Vaccination with a synthetic peptide from the influenza virus hemagglutinin provides protection against distinct viral subtypes. *Proceedings of the National Academy of Sciences of the United States of America*. 2010; 107:18979–18984. [PubMed: 20956293]
11. Brandenburg B, et al. Mechanisms of hemagglutinin targeted influenza virus neutralization. *PloS one*. 2013; 8:e80034. [PubMed: 24348996]
12. Otterstrom JJ, et al. Relating influenza virus membrane fusion kinetics to stoichiometry of neutralizing antibodies at the single-particle level. *Proceedings of the National Academy of Sciences of the United States of America*. 2014; 111:E5143–5148. [PubMed: 25404330]
13. DiLillo DJ, et al. Broadly neutralizing anti-influenza antibodies require Fc receptor engagement for in vivo protection. *The Journal of clinical investigation*. 2016; 126:605–610. [PubMed: 26731473]
14. DiLillo DJ, et al. Broadly neutralizing hemagglutinin stalk-specific antibodies require FcγR interactions for protection against influenza virus in vivo. *Nature medicine*. 2014; 20:143–151.
15. Impagliazzo A, et al. A stable trimeric influenza hemagglutinin stem as a broadly protective immunogen. *Science*. 2015; 349:1301–1306. [PubMed: 26303961]
16. Yassine HM, et al. Hemagglutinin-stem nanoparticles generate heterosubtypic influenza protection. *Nature medicine*. 2015; 21:1065–1070.
17. Henry Dunand CJ, et al. Both Neutralizing and Non-Neutralizing Human H7N9 Influenza Vaccine-Induced Monoclonal Antibodies Confer Protection. *Cell host & microbe*. 2016; 19:800–813. [PubMed: 27281570]
18. Ekiert DC, et al. Antibody recognition of a highly conserved influenza virus epitope. *Science*. 2009; 324:246–251. [PubMed: 19251591]

19. Ekiert DC, et al. A highly conserved neutralizing epitope on group 2 influenza A viruses. *Science*. 2011; 333:843–850. [PubMed: 21737702]
20. Corti D, et al. A neutralizing antibody selected from plasma cells that binds to group 1 and group 2 influenza A hemagglutinins. *Science*. 2011; 333:850–856. [PubMed: 21798894]
21. Dreyfus C, et al. Highly conserved protective epitopes on influenza B viruses. *Science*. 2012; 337:1343–1348. [PubMed: 22878502]
22. Tharakaraman K, et al. A broadly neutralizing human monoclonal antibody is effective against H7N9. *Proceedings of the National Academy of Sciences of the United States of America*. 2015; 112:10890–10895. [PubMed: 26283346]
23. Okuno Y, et al. 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]
24. Sui J, et al. Structural and functional bases for broad-spectrum neutralization of avian and human influenza A viruses. *Nature structural & molecular biology*. 2009; 16:265–273.
25. Nakamura G, et al. An in vivo human-plasmablast enrichment technique allows rapid identification of therapeutic influenza A antibodies. *Cell host & microbe*. 2013; 14:93–103. [PubMed: 23870317]
26. Wang TT, et al. Broadly protective monoclonal antibodies against H3 influenza viruses following sequential immunization with different hemagglutinins. *PLoS pathogens*. 2010; 6:e1000796. [PubMed: 20195520]
27. Tan GS, et al. A pan-H1 anti-hemagglutinin monoclonal antibody with potent broad-spectrum efficacy in vivo. *Journal of virology*. 2012; 86:6179–6188. [PubMed: 22491456]
28. Tharakaraman K, et al. Broadly neutralizing influenza hemagglutinin stem-specific antibody CR8020 targets residues that are prone to escape due to host selection pressure. *Cell host & microbe*. 2014; 15:644–651. [PubMed: 24832457]
29. Jang YH, Seong BL. Options and obstacles for designing a universal influenza vaccine. *Viruses*. 2014; 6:3159–3180. [PubMed: 25196381]
30. Whitehead SS, et al. Prospects for a dengue virus vaccine. *Nature reviews. Microbiology*. 2007; 5:518–528. [PubMed: 17558424]
31. Halstead SB. In vivo enhancement of dengue virus infection in rhesus monkeys by passively transferred antibody. *The Journal of infectious diseases*. 1979; 140:527–533. [PubMed: 117061]
32. Ponnuraj EM, et al. Antibody-dependent enhancement, a possible mechanism in augmented pulmonary disease of respiratory syncytial virus in the Bonnet monkey model. *The Journal of infectious diseases*. 2003; 187:1257–1263. [PubMed: 12696005]
33. Osowy C, et al. Antibody-dependent enhancement of respiratory syncytial virus infection by sera from young infants. *Clinical and diagnostic laboratory immunology*. 1994; 1:670–677. [PubMed: 8556519]
34. Gauger PC, et al. Enhanced pneumonia and disease in pigs vaccinated with an inactivated human-like (delta-cluster) H1N2 vaccine and challenged with pandemic 2009 H1N1 influenza virus. *Vaccine*. 2011; 29:2712–2719. [PubMed: 21310191]
35. Khurana S, et al. Vaccine-induced anti-HA2 antibodies promote virus fusion and enhance influenza virus respiratory disease. *Science translational medicine*. 2013; 5:200ra114.
36. Gould EA, Buckley A. Antibody-dependent enhancement of yellow fever and Japanese encephalitis virus neurovirulence. *The Journal of general virology*. 1989; 70(Pt 6):1605–1608. [PubMed: 2543793]
37. Gould EA, et al. Immune enhancement of yellow fever virus neurovirulence for mice: studies of mechanisms involved. *The Journal of general virology*. 1987; 68(Pt 12):3105–3112. [PubMed: 3694178]
38. Phillpotts RJ, et al. Antibody-dependent enhancement of tick-borne encephalitis virus infectivity. *The Journal of general virology*. 1985; 66(Pt 8):1831–1837. [PubMed: 2991448]
39. Ubol S, Halstead SB. How innate immune mechanisms contribute to antibody-enhanced viral infections. *Clinical and vaccine immunology : CVI*. 2010; 17:1829–1835. [PubMed: 20876821]
40. Halstead SB. Immune enhancement of viral infection. *Progress in allergy*. 1982; 31:301–364. [PubMed: 6292921]

41. de Alwis R, et al. Dengue viruses are enhanced by distinct populations of serotype cross-reactive antibodies in human immune sera. *PLoS pathogens*. 2014; 10:e1004386. [PubMed: 25275316]
42. Zellweger RM, Shresta S. Mouse models to study dengue virus immunology and pathogenesis. *Frontiers in immunology*. 2014; 5:151. [PubMed: 24782859]
43. Caballero MT, et al. TLR4 genotype and environmental LPS mediate RSV bronchiolitis through Th2 polarization. *The Journal of clinical investigation*. 2015; 125:571–582. [PubMed: 25555213]
44. Ochiai H, et al. Antibody-mediated growth of influenza A NWS virus in macrophagelike cell line P388D1. *Journal of virology*. 1988; 62:20–26. [PubMed: 3334744]
45. Ochiai H, et al. Infection enhancement of influenza A NWS virus in primary murine macrophages by anti-hemagglutinin monoclonal antibody. *Journal of medical virology*. 1992; 36:217–221. [PubMed: 1564451]
46. Skowronski DM, et al. Association between the 2008–09 seasonal influenza vaccine and pandemic H1N1 illness during Spring-Summer 2009: four observational studies from Canada. *PLoS medicine*. 2010; 7:e1000258. [PubMed: 20386731]
47. Swalley SE, et al. Full-length influenza hemagglutinin HA2 refolds into the trimeric low-pH-induced conformation. *Biochemistry*. 2004; 43:5902–5911. [PubMed: 15134464]
48. Fontana J, et al. Structural changes in Influenza virus at low pH characterized by cryo-electron tomography. *Journal of virology*. 2012; 86:2919–2929. [PubMed: 22258245]
49. Wyrzucki A, et al. Alternative recognition of the conserved stem epitope in influenza A virus hemagglutinin by a VH3-30-encoded heterosubtypic antibody. *Journal of virology*. 2014; 88:7083–7092. [PubMed: 24719426]
50. Short KR, et al. The fate of influenza A virus after infection of human macrophages and dendritic cells. *The Journal of general virology*. 2012; 93:2315–2325. [PubMed: 22894921]
51. Sidorenko Y, Reichl U. Structured model of influenza virus replication in MDCK cells. *Biotechnology and bioengineering*. 2004; 88:1–14. [PubMed: 15384040]
52. Nunes-Correia I, et al. Interactions of influenza virus with cultured cells: detailed kinetic modeling of binding and endocytosis. *Biochemistry*. 1999; 38:1095–1101. [PubMed: 9894006]
53. Fei Y, et al. Characterization of Receptor Binding Profiles of Influenza A Viruses Using An Ellipsometry-Based Label-Free Glycan Microarray Assay Platform. *Biomolecules*. 2015; 5:1480–1498. [PubMed: 26193329]
54. Bruhns P. Properties of mouse and human IgG receptors and their contribution to disease models. *Blood*. 2012; 119:5640–5649. [PubMed: 22535666]
55. Crapo JD, et al. Cell number and cell characteristics of the normal human lung. *The American review of respiratory disease*. 1982; 126:332–337. [PubMed: 7103258]
56. Antal-Szalmas P, et al. Quantitation of surface CD14 on human monocytes and neutrophils. *Journal of leukocyte biology*. 1997; 61:721–728. [PubMed: 9201263]
57. Thornburg NJ, et al. H7N9 influenza virus neutralizing antibodies that possess few somatic mutations. *The Journal of clinical investigation*. 2016; 126:1482–1494. [PubMed: 26950424]
58. Baccam P, et al. Kinetics of influenza A virus infection in humans. *Journal of virology*. 2006; 80:7590–7599. [PubMed: 16840338]
59. Boianelli A, et al. Modeling Influenza Virus Infection: A Roadmap for Influenza Research. *Viruses*. 2015; 7:5274–5304. [PubMed: 26473911]
60. Dobrovolny HM, et al. Assessing mathematical models of influenza infections using features of the immune response. *PloS one*. 2013; 8:e57088. [PubMed: 23468916]
61. Cao P, et al. Innate Immunity and the Inter-exposure Interval Determine the Dynamics of Secondary Influenza Virus Infection and Explain Observed Viral Hierarchies. *PLoS computational biology*. 2015; 11:e1004334. [PubMed: 26284917]
62. Li Y, Handel A. Modeling inoculum dose dependent patterns of acute virus infections. *J Theor Biol*. 2014; 347:63–73. [PubMed: 24440713]
63. Collins PL, Graham BS. Viral and host factors in human respiratory syncytial virus pathogenesis. *Journal of virology*. 2008; 82:2040–2055. [PubMed: 17928346]

64. Connors M, et al. Enhanced pulmonary histopathology induced by respiratory syncytial virus (RSV) challenge of formalin-inactivated RSV-immunized BALB/c mice is abrogated by depletion of interleukin-4 (IL-4) and IL-10. *Journal of virology*. 1994; 68:5321–5325. [PubMed: 8035532]
65. Hsussell T, et al. CD8+ T cells control Th2-driven pathology during pulmonary respiratory syncytial virus infection. *European journal of immunology*. 1997; 27:3341–3349. [PubMed: 9464822]
66. Johnson TR, et al. Respiratory syncytial virus (RSV) G glycoprotein is not necessary for vaccine-enhanced disease induced by immunization with formalin-inactivated RSV. *Journal of virology*. 2004; 78:6024–6032. [PubMed: 15141000]
67. Boyoglu-Barnum S, et al. Prophylaxis with a respiratory syncytial virus (RSV) anti-G protein monoclonal antibody shifts the adaptive immune response to RSV rA2-line19F infection from Th2 to Th1 in BALB/c mice. *Journal of virology*. 2014; 88:10569–10583. [PubMed: 24990999]
68. Deng N, et al. Cytokine diversity in the Th1-dominated human anti-influenza response caused by variable cytokine expression by Th1 cells, and a minor population of uncommitted IL-2+IFNgamma- Thpp cells. *PloS one*. 2014; 9:e95986. [PubMed: 24788814]
69. Wohlleben G, et al. Influenza A virus infection inhibits the efficient recruitment of Th2 cells into the airways and the development of airway eosinophilia. *Journal of immunology*. 2003; 170:4601–4611.
70. Baranovich T, et al. The Hemagglutinin Stem-Binding Monoclonal Antibody VIS410 Controls Influenza Virus-Induced Acute Respiratory Distress Syndrome. *Antimicrobial agents and chemotherapy*. 2016; 60:2118–2131. [PubMed: 26787699]

Figure 1.

Surface Representation of HA Homotrimer Molecule. Each monomer shown in green, cyan, or light green illustrating the neutralizing epitopes (shown in red) of the corresponding stem-binding broadly neutralizing antibodies. The epitopes were identified using the PISA server based on antibody co-crystal structures with the HA molecule.

Figure 2.

Epitopes for Antibodies to HA2 in Pre- and Post-fusion Conformation. Epitope mapping by phage display had shown that the anti-HA2 antibodies bind to residues 32–76 of HA2 molecule [35]. These residues are shown in red on one molecule of the homotrimer in the (a) pre-fusion and (B) post-fusion conformations of the HA2 crystal structure. The fusion loop residues are shown in black on the pre-fusion form of HA. In the post-fusion state, the fusion peptide is not observed in the crystal structure and is therefore shown as a schematic (black line). In the pre-fusion conformation, most of the HA2 subunit residues are covered by the HA1 subunit, for clarity one HA2 subunit is shown in the insert. More specifically, the HA2 subunit residues 58–76 are covered by the HA1 subunit (shown in orange); therefore, these residues are not available for antibody binding. Conversely, in the post-fusion conformation, in addition to the fusion peptide, the stem residues 32 to 76 of HA2 subunit are found on the surface and are available for antibody binding.

Figure 3.

Influenza Fusion with the Host Endosomal Membrane. On the virus surface HA molecules in their pre-fusion conformation are found as an ordered array of molecules, oriented to bind to the host cell surface receptor molecules. In the pre-fusion conformation (shown in state A), the HA2 subunit exists in a meta-stable conformation, anchored by the receptor-bound HA1 subunit, and is ready to undergo conformational changes (this is referred to as the *spring loaded state*). Upon virus internalization into the endosome of the host cell, HA undergoes a significant conformational change where the HA1 subunit first separates from the HA2 subunit (shown in state B) allowing the fusion peptide to be deployed towards the host endosomal membrane to initiate viral-host membrane fusion shown in state C). To achieve successful host infection, both the pre- and post-fusion conformations are essential, for example lack of a protease cleavage within HA0 inhibiting formation of HA1 and HA2 prevents viral fusion. Interestingly, when HA1 and HA2 subunit proteins are expressed separately, only the HA1 molecule can be folded into its pre-fusion conformation; the HA2 molecule, expressed either with the viral transmembrane domain or only as a soluble domain (residues 376–481 HA0 numbering or 1–331 HA2 numbering), folds only into its post-fusion conformation.

	<i>AUC (TCID₅₀/mL x days)</i>	
	Without ADE	With ADE
Head antibody	88233	189902
Stem antibody	53186	85383
No antibody	608916	

Figure 4. Comparison of Virus Infection Models with Head- and Stem-Binding Antibodies. (*Gray*) Target cell-limited model with delayed virus production (Baccam *et al.*)[58]. (*Black*) Model with no antibodies ($\alpha = \beta_2 = \beta_3 = 0$) and explicitly modelled virus consumption (Cao *et al.*) [61]; parameters V_0 , p , and c optimized to match Baccam model. (*Red*) Head-binding antibody added at 1dpi ($\beta_2 = 0.5 \times \beta_1$, $\beta_3 = \beta_1$, $c_2 = c_3 = 0$). (*Pink*) Head-binding antibody added at 1dpi without accounting for ADE ($\beta_3 = 0$). (*Blue*) Stem-binding antibody added at 1dpi ($\beta_2 = \beta_3 = \beta_1$, $c_2 = k_2$, $c_3 = k_3$). (*Cyan*) Stem-binding antibody added at 1dpi without accounting for ADE ($\beta_3 = 0$).

Table 1Numerical Values of the Model Parameters^a

Parameter	Description	No Antibody	Head-binding Antibody	Stem-binding Antibody
β_1	rate of SA-receptor infection by free virus	3.2×10^{-5}	3.2×10^{-5}	3.2×10^{-5}
β_2	rate of SA-receptor infection by antibody-bound virus		1.6×10^{-5}	3.2×10^{-5}
β_3	rate of Fc γ -receptor infection by antibody-bound virus		3.2×10^{-5}	3.2×10^{-5}
β'_1	rate of free-virus consumption by binding to target cells	8×10^{-5}	8×10^{-5}	8×10^{-5}
β'_2	rate of antibody-bound virus consumption by binding to target cells via SA receptor		4×10^{-5}	8×10^{-5}
β'_3	rate of antibody-bound virus consumption by binding to target cells via Fc γ receptor		8×10^{-5}	8×10^{-5}
k_1	rate of transition from free-virus infected to virus-producing cells	4.0	4.0	4.0
k_2	rate of transition from cells infected by antibody-bound virus via SA receptor to virus-producing cells		4.0	4.0
k_3	rate of transition from cells infected by antibody-bound virus via Fc γ receptor to virus-producing cells		4.0	4.0
c_1	clearance rate of cells infected by free virus		0	0
c_2	clearance rate of cells infected by antibody-bound virus via SA receptor		0	4.0
c_3	clearance rate of cells infected by antibody-bound virus via Fc γ receptor		0	4.0
δ	clearance of virus-producing cells	3.45	3.45	3.45
p	rate of new virus production	2.04×10^2	2.04×10^2	2.04×10^2
a	rate of antibody binding		1.28×10^5	1.28×10^5
c	clearance rate of free virus	3.72×10^4	3.72×10^4	3.72×10^4
c_0	clearance rate of antibody-bound virus		3.72×10^4	3.72×10^4

^aThe influenza virus infection model values of β_1 and k_1 were taken from the cell-limited model with delay by Baccam *et al.*[58], the virus consumption rate is set at $\beta'_1 = 2.5 \times \beta_1$, and values for p , δ , and c are optimized to match the Baccam *et al.* model. To model the role of antibody, the antibody concentration was assumed to be such that it results in 10:1 ratio of antibody-bound vs. free virus, which resulted in $a = 1.28 \times 10^5$, values for β_1 , β'_1 , k_1 , δ , p , and c the same as in the no-antibody model, $\beta'_2 = 2.5 \times \beta_2$, $\beta'_3 = 2.5 \times \beta_3$, $k_2 = k_3 = k_1$, and $c_0 = c$. For head-binding antibody, $\beta_2 = 0.5 \times \beta_1$ and $c_1 = c_2 = c_3 = 0$. For stem-binding antibody, $\beta_2 = \beta_1$ and $c_2 = k_2$, $c_3 = k_3$. When modeling ADE, $\beta_3 = \beta_1$ was assumed.