COMMENTARY

Antibody immunoprophylaxis and immunotherapy for influenza virus infection: Utilization of monoclonal or polyclonal antibodies?

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ABSTRACT

Control programs for emerging influenza are in urgent need of novel therapeutic strategies to mitigate potentially devastating threats from pathogenic strains with pandemic potential. Current vaccines and antivirals have inherent limitations in efficacy, especially with rapid evolutionary changes of influenza viruses. Antibody-based antiviral protection harnesses the natural power of the immune system. Antibodies present prophylactic and therapeutic intervention options for prevention and control of influenza, especially for at-risk populations. Specific monoclonal antibodies are well defined in purity and initial efficacy but polyclonal antibodies are easier to scale-up and cost-effective with long-term efficacy, using batches with broadly neutralizing properties against influenza variants. This review presents the pros and cons of monoclonal versus polyclonal antibody therapy for influenza.

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Introduction

Influenza is a serious global public health burden with disease complications requiring hospitalization of patients with 250,000 deaths worldwide per annum.¹ Currently, influenza A H1N1, H3N2 and influenza B viruses are circulating in both hemispheres in human populations. However, recent zoonosis of avian H5 and H7 influenza A viruses have become emerging diseases. Vaccination requires yearly updates of the vaccine virus strains as circulating influenza viruses mutate and undergo antigenic drift and/or reassort to create new virus subtypes, for which people have inadequate immunity. Development of universal vaccines that provide cross protective immunity against all antigenic variants is a critical unmet need. A universal vaccine that provides protection from Group 1 and Group 2 influenza viruses would circumvent the current annual requirement for new vaccines.² However, experimental animal studies show varying efficacies of vaccine strategies that induce broadly neutralizing antibodies (bnAb).^{3–5} For post-exposure treatment, antiviral drugs are available but limited in efficacy and use as influenza virus variants become resistant, rapidly changing active target-binding sites. Furthermore, they are often less active when administered more than 72 hours after virus infection. Stockpiles of now ineffective antivirals (TamifluTM and RelenzaTM) by governments have been rendered obsolete in the case of an emergency during a pandemic.⁶ Thus there is a clear demand for a better option in control of influenza virus epidemics in the face of virus escape from clinical use of licensed vaccines and antivirals.⁷

Vaccines elicit antibody-mediated immunity, both neutralizing and binding antibodies, as the major mechanism of protection from morbidity and mortality. Passive vaccination with

neutralizing antibodies against the major surface glycoproteins of influenza viruses, haemagglutinin (HA) and neuraminidase (NA), has been shown to be effective in protection of the respiratory tract from disease. The degree of protection from lethal infection is dependent on the target binding sites on the virus. Antibodies to the HA1 head subunit block virus attachment to host cells via sialic acid-containing receptors whereas antibodies to the HA2 stem subunit inhibit virus fusion after endocytosis and hence interrupt the intracellular virus replication cycle. Most neutralizing antibodies produced to the immunodominant HA globular head are specific for highly variable loops surrounding the receptor-binding site. However recently, neutralizing antibodies to the highly conserved HA stem regions, have been shown to provide full protection against homologous and intrasubtypic influenza virus infection in mice.⁸ Such bnAbs are induced by prime-boost vaccination strategies to drive activation of B cells to produce antibodies with heterosubtypic cross reactivity. Delivery of antibody provides immediate antiviral effects, reducing virus replication in epithelial cells and preventing virus shedding and spread to susceptible hosts. Importantly, immunotherapy with antibodies for severe influenza was not shown to interfere with the development of antiviral adaptive immunity,⁹ allowing the formation of memory responses from exposure to influenza viruses in the future. Monoclonal antibodies (mAbs) raised against active binding sites of the HA have been studied in animals and clinical trials. Some specific activities were shown to be superior to others at inhibiting viral load and reducing signs of disease in animal models of influenza. Polyclonal antibodies (pAbs) with a wide spectrum of activities in blocking virus attachment and penetration in host cells have also demonstrated efficacy in

protection from influenza in animal models. Although anti-influenza specific antibodies from convalescent sera or intravenous immunoglobulin preparations from pooled human plasma have also shown efficacy they are not currently recommended as therapy for influenza for safety reasons.¹⁰ Here we discuss the comparative benefits of mAbs and pAbs in passive vaccination and treatment for influenza.

Antibody-based prophylaxis

In a natural infection with influenza virus, the adaptive immune system responds with activation of naïve and memory B cells reliant on the extent of re-exposure or recognition of similar antigens of the invading virus strain or clade. Immuno-gen-based vaccination also relies on the ability of the humoral immune response to recognize viral antigenic determinants introduced by parenteral routes for protection against subsequent influenza virus challenge. This process of activation requires CD4⁺ T cell help and costimulatory signals from the innate immune system in a reaction to a hierarchical order of viral antigen immunodominance.¹¹ The influenza A viruses with H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17 or H18 are classified into Group 1 and viruses with H3, H4, H7, H10, H14 or H15 into Group 2.⁸ The head/globular region is immunodominant over the stem regions of the HA molecule, located on the surface of the enveloped virion. Antibodies binding five major globular-domain sites on the HA head are capable of blocking attachment of the HA to sialic acid-containing residues on host cell receptors. Importantly, CD4⁺ T cells influence the duration and level of the antibody response but not the immunodominance from intranasal infection with influenza virus.¹¹ However, the routes of virus immunization and host genetic background modulate the immunodominance of antibody epitopes as demonstrated in a mouse model of influenza. Interestingly, co-stimulatory signals from type I interferon subtypes, as potent innate cytokines, drive antibody class switching in activated B cells.¹² This provides an opportunity to exploit select interferon subtypes as immunotherapeutic adjuvants for vaccines to generate desirable antibody responses, including bnAbs to HA. Antibodies directed toward the different antigenic sites in the HA head show distinct functionalities in HI and neutralization assays. On a cautionary note, pre-existing antibodies to HA sites, either by vaccination or natural infection, influence the immunodominance in recall responses to reinfection with influenza viruses. Therefore, assessment of efficacy of antibodies should utilize a variety of assays (both *in vitro* and *in vivo*) in addition to standard HA inhibition and microneutralization tests, as bnAbs can display various mechanisms of action to interfere with the virus lifecycle.¹³

However, there are other antibody-neutralizing targets on the influenza virus. The NA molecule is an important viral target for neutralizing antibodies in humans¹⁴ but is less immunodominant than the HA protein. Antibodies strongly bound to sites on the NA glycoprotein inhibit virus release from infected host cells and so abort successive virus replication cycles.¹⁵ Similar to the HA, NA contains both conserved and immunodominant strain-specific B cell epitopes. Another target for neutralization of influenza viruses is the ectodomain of the matrix protein 2 (M2e). Antibodies against M2e have prevented

intracellular fusion of the viral envelope with the endosome membrane, blocking further steps in viral replication within the host cell. Of particular interest are conserved regions of the M2e molecule, which have a high degree of sequence identity amongst influenza viruses.¹⁶

Advantages of monoclonal antibodies

Protection against influenza with administration of mAbs engineered to react to immunodominant and conserved regions of the HA, NA and M2e molecules has been demonstrated using *in vitro* and *in vivo* animal studies, utilizing mostly IgG in mice. The choice of mAbs for prophylaxis and therapeutic treatment is guided by varied specificities and functional attributes and has included mouse, human, chicken and camel-derived antibody sources derived from splenocytes, bone marrow and PBMCs.¹⁷ Single monotherapy and cocktails of mAbs have been used to successfully treat infections from highly mutable viruses (influenza and Ebola) bringing renewed hope in antibody-based therapeutics.

For influenza, characterizations of over 20 different mAbs have been reported with attention focused on mAbs reactive to conserved regions of the stem and globular domain of the HA, being the most abundant surface glycoprotein of influenza virus, which in turn give rise to broadly neutralizing antibodies. However, poor immunogenicity of the stem domain limits production of natural cross-reactive antibodies in virus infection, with such antibodies binding regions outside the receptor-binding site. CR6261 and CR8020 mAbs directed against the stem inhibit membrane fusion and bind close to the cleavage site, preventing cleavage of HA0 into HA1 and HA2.¹⁸ Other bnAbs to the HA stem include F10, F16 and CR9114 mAbs. On the other hand, mAbs that bind conserved regions of the HA head (CH65, 5J8, C05 and CR8033) also show broad neutralizing activity within one influenza virus subtype. Thus activity of the key functions of the HA protein (binding and fusion) translate to genetic stability of these regions, representing potential binding sites for bnAbs. Recently, a novel mAb MEDI8852 reactive to the HA stem was shown to limit spread of aerosolised H1N1pdm09, in addition to prophylactic and therapeutic efficacies in a ferret model.¹⁹ Virus transmission is a less studied but critically important area of further work in influenza aerobiology.

MAbs 4E9 and 1H5 reactive against the NA molecule, the second most abundant surface viral glycoprotein, with reactivity to conserved epitopes on the pH1N1 and H5N1 viruses, showed only partial protection from influenza when administered prophylactically in mice (5–15 mg/kg).¹⁵ HF5 mAb specific to pH1N1, although providing greater protection, selected virus escape mutants in both prophylactic and therapeutic treatments, whereas 4E9 and 1H5 mAbs did not, highlighting its preferential role as a therapeutic. As NA-inhibiting antibodies reduce virus replication by preventing release of virions from infected cells, only mild symptoms of disease are clinically presented. Another key viral protein for interference of virus infection is the M molecule. Mouse mAbs against the M2e (IgG2a mAb 65 and IgG1 mAb 37) with similar antigen-binding affinities and specificities displayed signal transduction through activating FcRIII and FcγRIV, respectively, to contribute to full protection of mice in

receptor gene knockout studies.¹⁶ Hence, in addition to antigen binding, Fc-mediated effector functions are important protective mechanisms, often overlooked in clinical laboratory assays. Nonetheless, such antibodies may not function with high efficacy in those individuals who are immunocompromised.

However, despite great promise of clinical efficacy, human derived antibodies to influenza viral antigenic determinants have substantial logistical difficulties including supply limited by prolonged manufacturing processes involving identification, screening and validation.¹⁰ In addition, high cost and low scale-up requiring numerous batch validation have been associated with economic and safety concerns.²⁰ Generally, mAbs have high purity but five-fold higher doses of mAbs are required to reach efficacy therapeutically (5 mg/kg) than prophylactically (1 mg/kg). Novel DNA delivery for engineered antibody expression in recipient cells has displayed protection from challenge in experimental mouse models.²¹ Furthermore, mAb treatment selects escape variants, leading to prompt development of resistant influenza virus mutants. Nonetheless, mAb treatment may be useful with clinical translation in select cases (immunocompromised, newborns, elderly and pregnant women) rather than preventative measures for the general population at large.

Benefits of polyclonal antibodies

Natural advantages of passive transfer of pAbs are found in maternal immunity to protect neonates during the vulnerable phase of early life. PAbs derived from horses provide a low-cost alternative to mAbs and have been traditionally used for envenomation of snake bites and post-exposure prophylaxis for suspected infectious diseases, such as rabies, diphtheria and botulism.¹⁰ As influenza virus-specific antibodies purified from serum of immunized animals are scalable,²² they provide an alternative source to hybridoma technology-derived (humanized chimeric antibodies) or human donor selection for bone marrow-derived mAb development. Indeed, large pools of antibody mixtures can be derived from production animals, such as 10L equine antisera from a single hyperimmunised animal using plasmaphoresis, minimizing batch variability.²³ Furthermore, the half-life of equine IgG in the circulation is estimated to be about 3–4 days in humans.²⁴ Recently, equine F(ab')₂ pAbs were found to protect against avian H5N1 influenza (FabenfluTM).²⁵ An innovative approach is to utilize sheep or cattle (eg the dairy industry to supply milk-derived antibodies) from Australia/New Zealand, as a safe prion-free source of antibodies. Such xenogeneic antibodies require antibody subclass purification of IgG or IgA isotypes, which may include antibody fragments such as F(ab')₂ and validation in animal models of influenza virus infection prior to human trials for clinical efficacy and safety.

Studies using passive transfer of serum-derived ovine pAbs directed towards the HA of H1N1 PR8 virus demonstrated prophylactic potency and reduced mortality in a mouse model of infection.⁹ Such protective antibodies were shown to display a half-life of 2 weeks *in vivo*. Mice recovered from infection were also immune to reinfection by homologous virus, generating normal B and T cell responses to the virus. Thus passive transfer of pAbs did not interfere with the ability to generate host

adaptive immunity. In another study, ovine pAbs were shown to fully protect mice from H1N1 PR8 challenge by the intranasal route.²⁶ Unlike some mAbs, selection of virus escape variants is less problematic with pAbs, especially those that are broadly neutralizing. Vaccine strategies to target bnpAbs have incorporated prime-boost immunizations to enhance subdominant antibody reactivities, enabling manipulation of humoral immunity, for novel therapeutic interventions. Moreover, pAbs are cost-effective and able to be supplied in large quantities to meet demands using animals, reducing the need for validation of multiple batches as needed for mAb production. However, unlike mAb preparations with defined purity, pAbs are derived from composite mixtures of antibodies in sera targeting multiple epitopes. Despite these marked differences, mAbs and pAbs are currently being trialed in non-human primate models of infectious disease for translation to clinical use in humans as therapeutics for influenza.¹⁷

Discussion

Antibodies show various properties covering specificity of binding, affinity and ability to neutralize or prevent/reduce virus replication and transmission dependent on dose and virus strains. Influenza prevention through antibody-based prophylaxis and treatment via antibody immunotherapy has been studied using experimental passive immunization strategies in animal models and in clinical trials. Influenza can be prevented and treated by passive antibody vaccination and therapy,¹⁷ dependent on a number of variables. Manipulation of factors such as hierarchical dominance of important neutralizing epitopes on viral antigens is key to bioengineering protective immunity as prophylaxis for influenza.²⁷ Markedly, passive antibody transfer has the potential to change the immunodominance and original antigenic hierarchy of viral antigens during the progress of infection by suppressing memory B cell responses to previously immunodominant epitopes. Exploitation of specific antibody responses through orchestrated vaccination of animals to produce batches of neutralizing antibodies of desired specificities provides an immensely valuable antiviral resource. Translation of novel antibody-based immunotherapies to patients will allow effective prevention strategies against influenza virus pathogens. However, some general properties of mAbs and pAbs differ as summarized in Table 1. Regardless of mAb or pAb initial efficacies, those batches that select escape variants may become obsolete as influenza viruses undergo antigenic drift in circulating virus pools. Therefore, the antibody-based treatments must be robust in efficacy to potentially withstand rapidly evolving influenza viruses to overcome

Table 1. Generalised properties of anti-influenza viral antibodies.

Monoclonal antibodies	Polyclonal antibodies
High cost	Low cost
Hybridoma-derived	Animal-derived
Low scale-up	High scale-up
Single epitope specificities	Multiple epitope specificities
Defined purity	Undefined purity
Broadly neutralizing	Broadly neutralizing
Selection of resistant mutants	Less selection of resistant mutants
Initial efficacy	Long-term efficacy

resistance. Those antibodies that bind conserved epitopes to inhibit virus replication are likely superior and better utilized as a mixture with specificities to different epitopes to enhance efficacy and to mitigate the risk of resistance development. Thus there is great hope and promise in passive antibody-based immunity for effective control of both current and emerging influenza diseases, in particular for high-risk populations.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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