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Implications of broadly neutralizing antibodies in the development of a universal influenza vaccine

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Abstract

Serum antibodies are the major correlate of influenza vaccine efficacy, providing short-term protection against infection. Recent efforts have been focused on studying antibody responses at a monoclonal level to understand their role in protection against influenza, and to ultimately improve vaccine strategies to provide broader, long-term immunity against influenza virus. These studies have shown that broadly neutralizing antibodies specific for the conserved stem domain of the hemagglutinin protein can target multiple strains of influenza. These antibodies show great promise both from a therapeutic perspective as well as for guiding vaccine design efforts. In this review, we will summarize past and recent findings about broadly neutralizing antibodies against influenza, and discuss how these findings may guide development of universal vaccine strategies.

Introduction

Influenza is a major cause of morbidity and mortality worldwide. In the United States alone, influenza can cause more than 200,000 hospitalizations and 36,000 deaths per year [1,2]. Occasionally, a novel influenza strain can be introduced into the population. If little or no pre-existing immunity exists towards these new strains, a pandemic can occur, increasing both the healthcare and economic burden induced by influenza, as was recently observed during the 2009 H1N1 pandemic [3]. These strains are typically a consequence of antigenic shift, in which two different strains of influenza virus exchange components of their segmented RNA genome to create a novel viral pathogen against which humans may have little to no pre-existing immunity [4,5]. While generally ineffective against these pandemic strains, the seasonal influenza vaccine has proven to be an effective preventative measure against commonly circulating influenza viruses. However, making the seasonal influenza vaccine is a complex and challenging process [6]. A new vaccine is administered every season because protection is short-lived [7,8], and the influenza virus can undergo antigenic drift, in which the virus mutates very rapidly, allowing it to produce escape mutants that can evade immune recognition by the host. Antigenic drift can occasionally prevent the vaccine from targeting the circulating virus strain, which lowers the efficacy of the seasonal

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influenza vaccine. This scenario occurred most recently during the 2014/2015 flu season with a drifted H3 virus strain [9].

The vaccine works primarily by eliciting antibodies that target the hemagglutinin protein, which consists of two domains: HA1 and HA2. HA1, the head domain, allows the virus to attach to sialic acid receptors on host cells, allowing for endocytosis and entry of the virus into the target cell. HA2, the stem domain, controls the membrane fusion process. Of the two, HA1 is the immunodominant epitope, with a large majority of antibodies targeting this domain. Unfortunately, HA1 is highly variable between influenza strains, and is also the major site for mutations leading to antigenic drift [10]. In contrast, the HA2 domain is much more conserved between virus strains and is relatively infrequently mutated [10] (Figure 1).

Based on a large body of evidence from the last several years [11–14], it is thought that preferentially targeting the antibody response against the HA2 domain will result in broadly neutralizing antibodies capable of protection against a wide spectrum of influenza viruses, including both pandemic and drifted strains of influenza. Intense efforts directed towards developing this type of “universal” vaccine are ongoing, as well as efforts to develop broadly neutralizing antibodies for use as therapeutic agents, particularly in vulnerable populations that normally do not respond well to vaccination.

Broadly neutralizing human monoclonal antibodies

One of the first broadly neutralizing influenza specific monoclonal antibodies, C179, was isolated in 1993 from a mouse immunized with an H2N2 strain of influenza virus. It was found to neutralize multiple H1 and H2 strains of influenza virus, but exhibited no hemagglutination inhibition activity. Mapping of the C179 antibody suggested that the antibody bound the HA2 stem domain [15]. Recent technological advances has allowed for high throughput generation of human monoclonal antibodies. These novel approaches include improved memory B cell immortalization [16–18] and single cell expression-cloning from either plasmablasts [19–21] or antigen-labeled memory B cells [22] (Table 1). Using these approaches, broadly cross-neutralizing antibodies have been isolated from humans infected with influenza [21,23,24], as well as from influenza vaccinees [16,17,20,24–27]. Importantly, studies in humans have not only illustrated that these antibodies exist, but also that under certain conditions they can make up a major part of the immune response [21]. One study comparing experimental infection to seasonal vaccine responses in a human challenge study also reported that infection appeared to elicit a more diverse and cross-reactive response [24]. The majority of the broadly neutralizing antibodies described to date appear to be limited to reactivity within the influenza Group 1 viruses [16,20,21,23,24,27]. Smaller numbers of additional antibodies have also been identified that neutralize Group 2 [17] or B strains of viruses [26]. Even more rare are some antibodies that can neutralize both Group 1 and Group 2 viruses, which suggest that a heterosubtypic antibody response cross-reactive with all influenza strains could potentially be induced [20,25]. Epitope mapping of these antibodies, primarily through competition ELISAs and X-ray crystallography, revealed that many are directed towards the stem domain, and neutralize by inhibiting key conformational changes necessary for the fusion process [17,23,25,28]. The antibody 5J8, isolated by EBV transformed human memory B cells, broadly neutralized H1 strains of

influenza, but uniquely targeted the receptor binding pocket on the HA globular head, revealing other potential targets within the HA protein for broad neutralization [18] (Table 2). Many of the aforementioned antibodies can provide both therapeutic and prophylactic protection against multiple viral strains when tested in a mouse model of infection [16,18,21,26].

Studies of these broadly neutralizing monoclonal antibodies have been important for the influenza field. A passive transfer of broadly neutralizing stem specific antibodies may provide an effective therapeutic measure for humans, especially since this structure is so highly conserved, and appears resistant to mutational drift [23,27]. This option might be especially attractive for highly exposed healthcare workers in the context of an outbreak (before availability of a vaccine), or for individuals who are unable to respond to vaccination, such as transplant patients treated with immunosuppressants, cancer patients treated with chemotherapy, or patients with immunodeficiencies. Further, studying epitopes of broadly neutralizing antibodies has presented the stem domain as a potential target for rational design of a broadly protective influenza vaccine. The large body of work describing the isolation and characterization of broadly neutralizing antibodies from humans suggest that a universal vaccine is feasible, and multiple efforts are directed to generating such broadly protective influenza vaccines [29]

Characterizing broadly neutralizing antibodies

Not only is the epitope of broadly neutralizing antibodies significant, but the inherent properties of these antibodies are also of importance. An important finding is that many of the broadly neutralizing antibodies use a V_H1-69 immunoglobulin re-arrangement [21]. Interestingly, binding of antibodies with this re-arrangement to the stem domain has been shown to be mediated primarily by the heavy chain with little contribution of the light chain [28]. Additionally, while many antibodies are still able to retain their binding when reverted to the V_H1-69 germline sequence, the presence of multiple V_H1-69 polymorphisms that do not bind suggests that there may be a genetic bias in the ability to mount broadly neutralizing antibody responses, with certain alleles of V_H1-69 being more favorable in producing these antibodies [30,31*]. However, human-derived broadly neutralizing antibodies that do not use the V_H1-69 gene have also been characterized, signifying that cross-reactivity is not exclusively linked to the V_H1-69 allele [22]. Further identification of what, if any, common features of non-V_H1-69 broadly neutralizing antibodies exists would help to address any concerns that a genetic bias may impact in the efficacy of a universal vaccine.

Current research has also moved towards characterizing the Fc domain of antibodies and understanding their function in broadly neutralizing antibodies. A recent study showed that the glycoform of stem-reactive antibodies may have important implications in the generation of a broadly neutralizing response. Immune complexes (IC) formed by an antibody with a sialylated Fc domain and an HA antigen were able to induce inhibitory receptors on B cells, which presumably raises the threshold for the affinity required for activation. In mice, vaccination with these sialylated ICs, compared to asialylated IC, not only improved vaccine responses, but was also broadly protective against multiple influenza strains. Based on these

observations, this study proposed that B cells producing stem-reactive antibodies have a much higher activation threshold than those that bind the head domain. This may explain the paucity of broadly neutralizing antibodies *in vivo* compared to those binding the head domain [32**]. FcγR are also clearly important in the function of broadly neutralizing antibodies. Using FcγR knock-out mice, it was shown that prophylactic activity of stem-reactive antibodies, but not head-reactive ones, are mediated through FcγR in an ADCC-dependent manner [33].

A recent study showed that while stem-reactive monoclonal antibodies tend to have lower neutralizing activity than head-reactive antibodies, a polyclonal mixture of stem-reactive antibodies rescues the potency of neutralization. Additionally, expressing certain antibodies in an IgA backbone, as opposed to an IgG backbone, appeared to improve neutralization activity of the antibodies, which was relevant because this study detected stem-reactive IgA+ memory B cells in humans [34*]. This is important because most studies have focused on IgG stem-reactive responses, and redirecting efforts towards IgA antibodies may help us glean more information about generating broadly neutralizing antibody responses.

Boosting stem-reactive memory B cells

While stem reactive memory B cells are a part of the human B cell repertoire, they normally exist at very low frequencies, and are only boosted in the context of exposure to a very different HA protein. Presumably, robust responses would be required for sufficient protection, and thus, many studies are currently focused on understanding how to target and boost B cells that secrete these broadly neutralizing antibodies. The current theory is that stem-reactive memory B cells exist in small numbers, whereas those specific for the head domain are more abundant. When encountering a new strain of influenza which lacks the immunodominant epitopes recognized by head-reactive memory B cells, the stem-reactive memory B cells can be preferentially boosted by subdominant conserved epitopes, as these cells respond much quicker than naïve B cells that must be primed by novel epitopes. This theory has been supported through observations that broadly neutralizing antibodies seem to be boosted in individuals responding to the novel 2009 pandemic H1N1 strain [21,35]. In addition, prior vaccination and infection history seems to impact the antibody response [36]. People who have a more diverse history of influenza infection have much higher titers of antibodies specific for the HA stem domain, suggesting that prior encounters with diverse influenza virus strains greatly impact the number of protective broadly neutralizing antibodies that can be generated [37]. Another study suggests that while diverse influenza strains may boost stem-reactive antibodies, repeat exposure to common influenza strains primarily boost head-reactive responses and limit the expansion of broadly neutralizing antibodies [38*]. A recent study was able to detect stem-reactive memory B cells in healthy controls prior to a recent infection or vaccination. However, these stem-reactive memory B cells were found in much lower numbers than head-reactive memory B cells. Stem-reactive memory B cells could be boosted by an immunization with a novel H5N1 strain, whereas vaccination with the normal seasonal vaccine boosted head-reactive memory B cells with no effect on the number of stem-reactive memory B cells [39**].

Targeting specific anatomical niches to boost broadly neutralizing antibody responses may also be important. A recent mouse study suggests that memory B cells specific for broadly neutralizing epitopes may reside primarily in the lungs instead of in circulation or in secondary lymphoid organs. These lung-resident memory B cells were highly mutated, and were generated as a result of local persistent germinal centers that are responding to prolonged viral antigens found in the lung. These tissue-resident memory B cells provided robust protection against a drifted virus in a secondary challenge model, confirming the importance of these memory B cells in generating a cross-reactive, broadly neutralizing antibody response against influenza [40**]. Although these findings have not yet been confirmed in humans, it does suggest a role for tissue-resident memory B cells in providing broadly neutralizing responses. Because most studies of human broadly neutralizing monoclonal antibodies have focused primarily on B cells isolated from blood samples, a focus on lung-resident memory B cells may provide greater insight in how to generate broadly neutralizing antibodies. Current vaccination delivery strategies may want to consider how to best boost lung-resident memory B cells to elicit potent broadly protective responses.

Future prospects for universal vaccine strategies

Studies of broadly neutralizing antibodies have culminated in the generation of several vaccine strategies, reviewed in this current series by Dr. Krammer, entitled “Novel Influenza Virus Vaccine Approaches”. Early strategies focused on delivering stem-domain antigens, in the absence of the immunogenic head domain, in order to target broadly neutralizing antibodies. However, responses were suboptimal, likely because removal of the head domain destroyed important conformational epitopes, or destabilized the stem domain in such a way that it was no longer able to elicit protective antibody responses [41,42]. A recent promising strategy uses a vaccine consisting of a chimeric HA structure made of the conserved HA stem domain of an H1 or H3 strain of influenza, capped with the HA head domain from an exotic zoonotic influenza strain that has not been previously encountered by the human population. This includes those from avian virus strains such as the H5, H6, or H9 subtypes [43–45]. Because these novel head domains will be unable to activate pre-existing head-specific memory B cells, this vaccine strategy will likely direct recall responses to stimulate pre-existing stem-reactive memory B cells. This vaccination strategy has been effective in mouse models, and efforts are being made to translate these findings in ongoing human clinical trials. Another novel approach that could elicit a broadly neutralizing antibody response involves nanoparticles presenting H1 HA-stem antigens, which showed moderate protection in mice and ferret models in an H5 infection challenge model [46*]. Other laboratories have continued to try to engineer stable HA-stem antigens to deliver in a vaccine [47–49]. Recently, a group was able to create a stable trimeric stem-HA that elicited antibodies capable of broadly neutralizing both H1 and H5 influenza strains [48].

The seasonal influenza vaccine is efficient at eliciting antibodies that provide protection against infection. However, the response is limited in terms of longevity and breadth of protection, which is important considering the diversity and mutability of the influenza virus. The isolation and study of broadly neutralizing antibodies from humans have highlighted the stem-domain of the influenza virus as an important antigen that may provide a crucial target in generating robust protection against a wide spectrum of influenza strains.

Continued efforts to further characterize the phenotype and function of these antibodies will guide the development of a universal vaccine that could replace the seasonal influenza vaccine by providing long-lasting protection against both seasonal and pandemic influenza viruses.

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* of special interest

** of outstanding interest

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Highlights

- Novel single cell technologies have enabled rapid generation of human monoclonal antibodies
- These technologies have identified broadly neutralizing mAbs that target the HA stem domain.
- These antibodies have guided efforts to generate a universal influenza vaccine and therapeutic mAbs.

Figure 1. Influenza virus and the HA protein

A) Influenza virus is a negative-sense, single stranded RNA virus with a genome consisting of 8 RNA segments, encoding for a total of 11 proteins. 3 proteins are expressed on the virus surface, including the HA protein (red and blue), NA protein (orange), and M protein (not pictured). B) The HA protein is made of an HA1 (binds to sialic acid receptor) and HA2 (mediates membrane fusion) segment, linked by a disulfide bond. C) The HA protein is expressed as a trimer on the virus surface. The HA1 domain encodes for the immunogenic head domain, which is highly variable between strains of influenza and prone to rapid mutation. The HA2 domain is more conserved between influenza strains and is rarely mutated.

Table 1
Human broadly neutralizing antibodies

A summary of methods used to generate human monoclonal antibodies, and examples of select mAbs that were made using these methods and that exhibited in vitro broadly neutralizing activity against multiple strains of influenza.

Isolation Method	Pros	Cons	Example	Reference
EBV-transformation of memory B cells	Can identify memory B cells generated decades earlier	Must screen thousands of clones to isolate antigen-specific mAbs	FB54, FE53, FB139, FC41, FB75, FB110	[20]
			CR8020	[21]
			5J8	[22]
Phage display	Can generate a large library of mAb	Does not retain the cognate heavy and light chain pairing	D8, F10, A66	[18]
			CR8033, CR8071, CR9114	[17]
			CR6261	[19,27]
Single-cell plasmablast expression cloning	Unbiased analysis of mAb response to antigen	Must sample cells at the peak of immune response.	1003-D04, 1009-3E06, 1009-3B05, 70-1F02, 70-5B03	[26]
			EI3-2221	[25]
			FI6	[23]
			05-2G02	[24]
Antigen-specific memory B cell cloning	Identifies antigen-specific cells prior to making mAb	Limited to the quality/availability of antigen probes	310-18D5, 310-18-A3, 310-18C11, 310-18E7, 310-18C3, 310-18B10, 310-18E12	[31]

Table 2**Mechanisms of neutralization**

A brief summary on the multiple mechanisms through which an antibody is able to neutralize the influenza virus.

Target	Specificity	Mechanism
Head HA1 domain	Strain-specific	<ul style="list-style-type: none"> • Blocks virus binding to sialic acid receptors on the host cell surface, preventing entry into target cell. • Due to the variability of the head domain, these antibodies target specific strains.
Head HA1 domain	Broadly neutralizing	<ul style="list-style-type: none"> • Blocks virus binding to sialic acid receptors, preventing entry into target cell. • Targets conserved receptor binding pocket epitope. • Antibodies can neutralize multiple strains of influenza.
Stem HA2 domain	Broadly neutralizing	<ul style="list-style-type: none"> • Cannot prevent attachment to the host cell. • Impedes essential conformational changes needed for fusion. • Stem domain is highly conserved, allowing these antibodies to neutralize multiple strains of influenza.