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

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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.

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

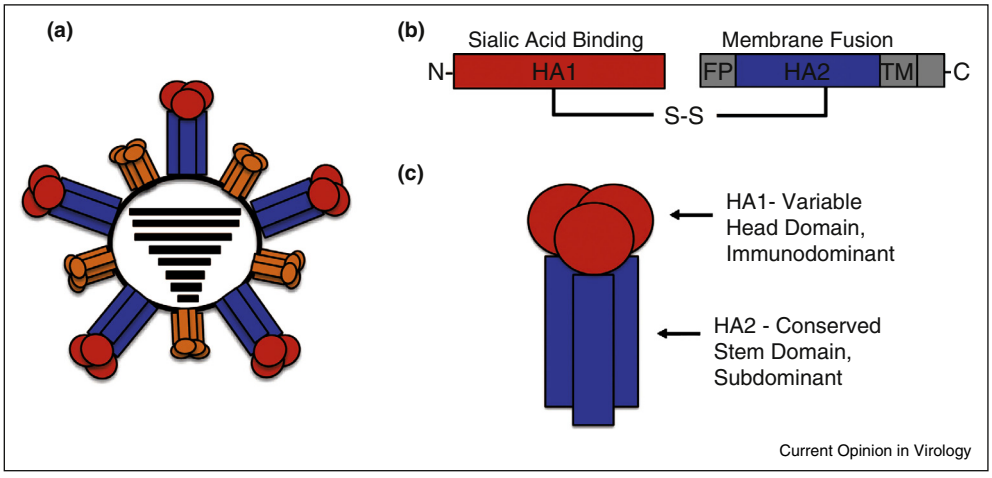
The vaccine works primarily by eliciting antibodies that target the hemagglutinin (HA) protein, which consists of two domains: HA1 and HA2. HA1, the head domain, allows the virus to attach to sialic acid receptors on host cells, allowing for endocytosis and entry of the virus into the target cell. HA2, 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).

On the basis of 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 toward 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

Figure 1



Influenza virus and the HA protein. **(a)** Influenza virus is a negative-sense, single stranded RNA virus with a genome consisting of eight RNA segments, encoding for a total of 11 proteins. Three 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.

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 vaccineess [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 influenza Group 1 viruses [16,20,21,23,24,27]. Smaller numbers of additional antibodies have also been identified that neutralize Group 2 [17]

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 virus

Isolation method	Pros	Cons	Example	Ref
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•]