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Review

# Prospects for broadly protective influenza vaccines

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ABSTRACT

The development of vaccines that could provide broad protection against antigenically variant influenza viruses has long been the ultimate prize in influenza research. Recent developments have pushed us closer to this goal, and such vaccines may now be within reach. This brief review outlines the current approaches to broadly protective vaccines, and the probable hurdles and roadblocks to achieving this goal.



## 1. Introduction

It was only a few years after the discovery of influenza virus as the cause of the disease, that it was shown that injection of animals with a preparation of inactivated virus could protect them against subsequent exposure to influenza [1]. These observations were rapidly extended to humans, with controlled clinical trials demonstrating the protective efficacy of inactivated influenza vaccine in healthy adults as early as 1943 [2], and licensing of influenza vaccine in the United States in 1945. However, the conquest of influenza was dealt a severe blow in 1949 with the failure of the vaccine to prevent disease due to a new variant of influenza, A/Fort Monmouth/49 [3]. This new virus was antigenically very different from preceding influenza viruses, which were subsequently denoted as influenza $A_0$, while the new variant was called influenza A' (we now recognize all of these viruses as H1N1 viruses) [4]. The realization that effective vaccination against influenza might require continual reformulation of the vaccine to match antigenic changes to the virus was felt by some at the time to mean that control of influenza through vaccination was impractical.

After 70 years, nothing much has really changed. Reformulation of the vaccine is still required almost every year, putting enormous pressure on manufacturers and regulatory authorities to make decisions about formulations and have the appropriate vaccine ready in time. The complexity of the vaccine has increased, from two strains to three strains in the late 1970s, and more recently, from three strains to four strains. And, the development and stockpiling of vaccines that might provide protection against pandemic influenza A viruses with novel surface antigens, such as H5N1, H7N9, H9N2, and the like, remains a formidable challenge. Influenza vaccines that could potentially provide protection against multiple antigenic variants within a hemagluttinin subtype (heterovariant immunity), between subtypes (heterosubtypic immunity) or against both influenza type A and B viruses (heterotypic immunity) remains a very important but elusive goal, sometimes referred to as the "holy grail" of influenza vaccinology. However, recent observations on the immune response to influenza infection may be leading to a pathway toward such "universal" vaccines. This brief review will discuss the basic strategy used for current vaccines and the potential targets that have been identified as strategies for more broadly protective vaccination.

## 2. Current influenza vaccines

Current inactivated vaccines are designed to induce serum antibody directed at the globular head, or HA1 domain, of the viral hemagglutinin (HA). Antibody to the globular head region of the HA interferes with the ability of the virus to bind to its cellular receptor(s), and is reflected in assays that measure inhibition of agglutination of red blood cells by the virus (hemagglutination-inhibition, or HAI) and viral neutralization in vitro. Inactivated influenza vaccines are standardized for content based on the amount of immunologically reactive HA protein they contain, and new inactivated vaccines can receive provisional licensure based on their ability to induce specific titers of HAI antibody.

This focus on HA and HAI antibody is entirely reasonable given the repeated demonstration of a high correlation between HAI antibody and protection against influenza [5]. While it is important to recognize that inter laboratory variation in the determination of HA titers [6] can make it difficult to assign any specific titer as the

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"protective" level of antibody, it can be generally concluded that having more HAI antibody is better than having less HAI antibody [7]. Induction of HAI antibody is therefore an effective mechanism to provide protection against both influenza A and B viruses.

However, spontaneous mutations at various locations in the HA1 domain can abolish binding of HAI antibodies without compromising viral infectivity, and lead to effective escape from HA antibody based immunity [8]. This is the basis of the ongoing epidemiology of influenza, and is both a testament to the effectiveness of HA antibody in protection as well as representing the fundamental problem of influenza vaccination. Thus, the focus of development efforts for more broadly cross protective vaccines has been on inducing protective immune responses against viral targets that do not undergo such antigenic selection and evolution. In this sense, the development of a successful universal vaccine would depend on inducing immune responses that are not a major component of the response to infection, or so-called "unnatural immunity" [9].

A number of viral antigens have been identified as potential targets for such broadly protective immunity, and are being explored using various delivery platforms as candidate broadly protective vaccines. These antigens are briefly described in the table, and their potential mechanisms mediating protection are shown in Fig. 1 (Table 1).

## 3. Approaches based on hemagglutinin (HA)

While the most well-characterized antibody response of humans to infection with influenza is the development of antibody directed against the HA1 region, recent studies have identified circumstances in which a significant response can be directed against the HA2, or stalk region of the HA. These responses have been primarily seen in humans who were infected with novel influenza A subtypes, such as H5N1 viruses [10]. Similar stalk directed responses were identified in humans with the emergence of the pandemic H1N1 virus (pH1N1) in 2009 [11]. In both cases, it was possible to isolate B cells or immunoglobulin genes from the peripheral blood of infected patients that made antibody that recognized the stalk of the HA. Because the stalk of the HA is largely conserved among HA proteins within a specific HA genetic group, such antibodies are highly cross reactive, for example, between H1 and H5 [12], or between H3 and H7 [13]. Cross-reactive, stalk specific antibodies have also been detected after influenza vaccination [14,15], although in lesser amounts.

It has been postulated that such stalk specific antibody responses predominantly occur when individuals are exposed to novel HA structures, while the responses to repeated infections with the same HA subtype becomes increasingly oriented toward the HA1 domain. Stalk specific antibody responses have been suggested as a mechanism responsible for the elimination of previous subtypes when new subtypes emerge [16].

Stalk specific antibodies are capable of mediating virus neutralization in vitro, potentially by inhibiting HA mediated fusion [17], and of providing passive protection against severe disease in mice. In addition, these antibodies can mediate recognition and killing of infected cells by natural killer cells, a process referred to as antibody dependent cellular cytotoxicity. Stalk-specific antibodies are detectable in the sera of most adults in low levels [18,19]. There is therefore considerable interest in vaccines that might induce such broadly cross protective antibodies as a strategy for a universal influenza vaccine [20].

Several approaches toward this goal are being pursued. Because stalk directed antibody responses are primarily detected when the host encounters a novel HA subtype, one approach has been the use of chimeric molecules in which the HA1 domain is derived from a novel subtype, and the stalk remains the same [21,22]. Priming and boosting of animals with a succession of these chimeric HAs can generate strongly cross protective neutralizing antibody, and provides protection against lethal challenge with heterologous subtypes of influenza A virus. Alternatively, stalk-only constructs could be used as immunogens [23,24]. This is more challenging, since it requires the stalk to be stabilized in the neutral pH, pre-fusion configuration, which is not stable in the absence of the head domain. However, mutations at key structural sites can stabilize stalk constructs [25], and have been used to create vaccines that generate cross protective responses in animals.

While it is becoming increasingly possible to generate strong immune responses to the HA stalk, the potential role of such responses in protection has not been proven. An alternative approach to more cross protective vaccine would be strategies that attempt to improve the cross reactivity of HA head-directed antibody. While such strategies might not be as broadly cross protective as stalk strategies, they have the advantage that head-directed antibody is known to be protective in humans.

One approach to generating broadly cross-protective responses to the head is by synthezing immunogens with consensus sequences, i.e., that represent the most common amino acid at each position from among available sequences within a subtype [26]. A variation on this approach is to use computational techniques to correct for biases introduced by oversampling of certain variants in the database, creating a so-called "computationally optimized, broadly reactive antigen" or COBRA. Immunization of ferrets with such a computationally designed synthetic H5 antigen generated a more broadly cross reactive antibody response within the H5 subtype than seen with native HA, and was protective against challenge [27,28], including in non-human primates [29]. Such an approach could be especially important in situations where multiple antigenic variants within a subtype with pandemic potential exist, such as with H5, or potentially, in providing protection against antigenic drift.

## 4. Approaches based on neuraminidase (NA)

In contrast to anti-HA antibody, anti-NA antibody does not neutralize virus infectivity but instead reduces efficient release of virus from infected cells, resulting in decreased plaque size in in vitro assays [30]. Antibody directed at the NA also has a protective role in influenza. This was perhaps most clearly demonstrated in the 1968 H3N2 pandemic. The pandemic virus was a reassortant between the previously circulating H2N2 virus and an unknown avian progenitor. In this case, the H3 gene was derived from the avian virus, but the N2 NA gene was derived from the previous human H2N2 virus [31]. Individuals with immunity to the N2, but lacking immunity to H3, were partially protected from pandemic H3N2 disease [32,33]. Subsequent studies in the human challenge model have also supported the role of NA-specific antibody in protection [34]. Such NA antibody can be protective against disease and results in decreased virus shedding and severity of illness, but is infection permissive [35].

The rate at which mutations accumulate in the NA appears to be less than that in the HA [36], suggesting that vaccines that induced substantial NA specific immunity would continue to provide protection against drifted viruses, and would need updating less often than HA-centric vaccines. Current influenza vaccines are formulated based on the content of HA, and the amount of NA protein, which is generally not very stable, is not standardized. Assessment of functional antibody against the NA, using classic neuraminidase-inhibition (NAI) assays is technically difficult and not amenable to high throughput. However, recent development of more simplified assays for NAI have facilitated assessments of the response