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.

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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 A0, 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 hemagglutinin subtype (hemovariant 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 titters 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...
“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 HA1 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 HA1s 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 synthesizing 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 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.
to influenza vaccines [37]. These assays have shown that current inactivated vaccines can induce functional antibody to the NA in a dose dependent fashion [38–40]. However, the consistency of this effect from vaccine to vaccine is unclear.

It has been observed that when HA and NA are present together on virions, the strong immune response to HA may depress the response to NA [41]. Supplementation of vaccines with additional neuraminidase, or other efforts to enhance the neuraminidase response to vaccination have been suggested as a strategy to improve and enhance the breadth of vaccine protection [42,43]. Because the N1 of human seasonal H1N1 viruses shares epitopes with the H5N1 virus, strong responses to N1 can be elicited after a single dose of H5N1 vaccine [44]. Cross-reactive responses to N1 can provide protection against H5N1 in mice [45]. Preliminary results of an early study in the human challenge model have suggested that NA supplementation might improve the protective efficacy of inactivated influenza vaccines [46].

Table 1

<table>
<thead>
<tr>
<th>Target</th>
<th>Level of conservation</th>
<th>Potential mechanisms of protection</th>
<th>Evidence for protection in humans</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA1</td>
<td>Low</td>
<td>Neutralizing antibody</td>
<td>Multiple studies showing correlations between HAI and MN assays and protection in humans [5]</td>
</tr>
<tr>
<td>HA2</td>
<td>High</td>
<td>Fusion inhibition</td>
<td>None</td>
</tr>
<tr>
<td>NA</td>
<td>Modest</td>
<td>Interference with viral release</td>
<td>Protection by N2 antibody in 1968 pandemic [32,33], antibody correlates with protection in some vaccine studies</td>
</tr>
<tr>
<td>M2e</td>
<td>High</td>
<td>ADCC, interference with viral release</td>
<td>Protection by passive transfer of monoclonal antibody in human challenge model [60]</td>
</tr>
<tr>
<td>Any surface</td>
<td>High</td>
<td>ADCC</td>
<td>None</td>
</tr>
<tr>
<td>Peptides</td>
<td>High</td>
<td>CD4 and/or CD8 mediated</td>
<td>Associated with protection in human challenge model [69], pH1N1 epidemic in absence of antibody [70]</td>
</tr>
</tbody>
</table>

5. Approaches based on eM2

In the late 1980s, studies using monoclonal antibodies identified a third envelope protein on the surface of influenza A virions, referred to as the M2 protein. The M2 protein is the product of a spliced message from the M gene segment of influenza A virus, and is present on the surface of infected cells and in lesser quantities incorporated into the viral envelope. M2 is a tetrameric ion channel that acidifies the interior of the virion after entry into the endosome, playing a critical role in the uncoating process.

A small portion of the N terminus of the M2 protein protrudes from the surface of the virus, referred to as the extracellular domain, or M2e. Because this is a third surface protein, it seemed reasonable that antibody against this domain could have a protective role. In fact, monoclonal antibodies to M2e were shown to reduce plaque size in vitro and provide passive protection in the mouse model if present in large enough amounts [47].
M2e is an attractive approach to a universal vaccine because it is fairly well conserved among human influenza A viruses, although there are significant differences in the sequences of avian and human influenza A viruses. Protection mediated by M2e would probably be antibody based, which is well recognized as an effective protective mechanism in influenza. The fact that M2e does not appear to evolve over time might indicate that M2e antibody does not play an important role in natural protection, but antibody responses to M2e are not common in humans after infection [48], possibly because so little of the antigen is exposed to the immune system. Recently, more vigorous M2e specific responses have been detected following pandemic H1N1 infection [49].

The mechanism of protection provided by M2e antibody may be multifactorial. In cell culture the antibody appears to impair cell to cell spread of virus, while studies in animals have also emphasized the role of antibody-dependent cellular cytotoxicity in M2e antibody mediated protection [50].

Multiple platforms have been used to induce antibody specific for M2e, including fusion proteins with TLR ligands [51–53], multimeric proteins [54], proteins attached to virus-like particles [55–57], and other forms of nanoparticles [58]. Generally, studies in animal models have shown broad protection induced by M2 immunization. These approaches have induced variable levels of M2-specific antibodies in humans [59], although achievement of levels equivalent to those found to be protective in animal models has been difficult. Monoclonal antibodies to M2e have also been evaluated for passive protection, and a recent study in the human challenge model has suggested a potentially protective role [60]. The goal of active vaccination might then be to induce a durable antibody response at a level similar to that achieved by this passive antibody approach.

6. Mucosal approaches

Intuitively, since humans are initially exposed to influenza virus via mucosal surfaces, vaccines that induce immunity at mucosal surfaces are an attractive option for influenza immunization. Both IgA and IgG antibody specific for HA are found in nasal secretions following infection. Nasal HA-specific IgG is predominantly IgG1, and its levels correlate well with serum levels of HA-specific IgG1, suggesting that nasal IgG originates by passive diffusion from the systemic compartment [61]. Nasal HA-specific IgA is predominantly polymeric and IgA1, suggesting local synthesis [62]. Studies in mice and ferrets have emphasized the importance of local IgA antibody in protection of the upper respiratory tract [63]. Studies in humans have also suggested that the resistance to reinfection induced by virus infection is mediated predominantly by local HA-specific IgA [34].

Immunity induced by mucosal infection, such as by a live influenza vaccine, has been suggested to provide more broad protection against antigenic variants than parenteral immunization with inactivated vaccine [64]. Because of the transport mechanisms for IgA, mucosal immunization could allow for intracellular immunization [65], which might be based on conserved proteins other than HA, such as the NP or other “internal” viral proteins. Cross protection against novel subtypes has been demonstrated following mucosal immunization in animal models [66]. This potential mechanism of cross protection has not been explored in detail in humans, however.

7. Approaches based on cellular targets

Cellular (CD4 and CD8) responses to influenza infection are generated against multiple peptide epitopes, including epitopes in relatively conserved proteins such as the NP, M, and polymerase proteins, in addition to HA and NA. Because many of these epitopes are conserved, induction of cellular effectors may also be an effective strategy for cross-protective vaccines. Cellular immunity clearly does have a potential role in protection against influenza in humans. Individuals who lack cellular immunity, such as individuals with immune defects or receiving immunosuppressive therapy, are at risk for more severe influenza and can shed influenza virus for very prolonged periods after infection. In addition, studies in both the human challenge model, as well as field observations, have correlated the level of CD4 or CD8 cellular immunity with reduced illness severity in humans [67–70].

Vaccines designed primarily to elicit cellular antibodies often contain mixtures of peptides computationally identified as cellular target epitopes [71–73]. Immunization of humans with a mixture of linear peptides has been reported to induce cellular immunity and prime for subsequent vaccine responses [74]. Such epitopes could be delivered by mixtures of peptides, or by live viral vectors, such as vaccinia [75]. Virus-like particles [76], as well as gamma-irradiated whole virus [77], have also been evaluated for their ability to induce cellular immune responses.

8. Role of adjuvants

Adjuvants can be broadly defined as agents which increase the immune response to a co-administered antigen, generally without generating immune responses to themselves. Aluminum salts have been widely used as adjuvants for a variety of vaccines for many decades, although they do not appear to be particularly effective when used with influenza vaccines, for unknown reasons. Some adjuvants are designed to directly stimulate known immune mechanisms, such as toll-like receptors (TLRs), but the mechanisms of action of most commonly used adjuvants remain incompletely determined and an area of active research.

Aluminum-containing adjuvants have been shown to only modestly enhance responses to some pandemic formulations [78]. In contrast, oil-in-water based emulsions have repeatedly been shown to enhance the magnitude and the breadth of immune responses to avian influenza vaccines and provide substantial dose sparing [79,80]. These studies have shown higher titers of antibody against the vaccine virus, as well as against antigenic variants, the development of B cells that recognize a larger variety of HA epitopes, and broadened and more vigorous CD4 T cell responses [81,82].

9. Opportunities and roadblocks

The currently licensed influenza vaccines, ironically, represent both the greatest opportunity, as well as the greatest roadblock, to the development of more broadly crossprotective vaccines. It is clear that better strategies and tools are needed for the effective control of influenza. As has been reviewed extensively elsewhere, current vaccines provide less than complete protection even under the best of circumstances, and are least effective in the populations most vulnerable to the impact of influenza, such as the elderly. In addition, they require yearly reformulation, representing a considerable barrier to their effective utilization especially in settings such as developing countries, where annual immunization campaigns may not be feasible. Thus, a compelling argument can be made for the development of more effective vaccines on many levels.

However, for all of their faults, current influenza vaccines have many positive features. They are relatively simple to manufacture, and significant recent advances have improved both the speed with which new formulations can be produced as well as the robustness of the manufacturing process. Although the protection they afford is imperfect, their efficacy and effectiveness has been
Fig. 2. Potential approaches towards the clinical development of broadly cross protective vaccines. The endpoint samples for clinical evaluation would reflect the proposed mechanism of action of the vaccine, while the ultimate outcomes would reflect that goal of the vaccine approach. Development of well validated markers of protection will be an important component of the development of novel vaccines for influenza.

demonstrated repeatedly in both randomized trials and observational studies, and both inactivated and live influenza vaccines clearly do prevent influenza illness and its complications. In addition, the immunologic correlates of protection induced by current inactivated vaccines have been well established, facilitating the deployment of vaccines with similar mechanisms of action. Importantly, because these vaccines are so widely used, there is enormous documentation and confidence in their safety in all populations.

These attributes represent a relatively higher bar for new, cross protective vaccines to surpass than might be the case if effective vaccines for influenza did not already exist. Perhaps the greatest concern would be regarding safety. Clearly, large studies would be required to provide documentation of safety, as would be true of any new vaccine. The use of novel adjuvants might raise additional concerns, as the possible relationship between the use of the adjuvant AS03 and subsequent development of narcolepsy in certain populations [83].

An additional concern with vaccines that are essentially designed to induce immune responses that are different than those induced by natural infection is whether such responses might be associated with enhanced disease through some immunologic mechanisms. Observations in porcine models have suggested that enhanced disease might be seen with both M2e vaccines [84] and HA stalk vaccines [85]. The significance of these results for influenza are unclear, but suggest that surveillance for potential enhancement of influenza will also need to be a component of the clinical development of new vaccine approaches.

The role of universal vaccines in the control of influenza will raise additional questions regarding efficacy. Many of the approaches to universal influenza vaccine might be expected to protect primarily against relatively severe disease. They would have the advantage of broad protection, and a decreased need for reimmunization, but potentially might have lower levels of absolute efficacy compared to current vaccines if there is an exact match between the vaccine and circulating strains. Under such circumstances, would they be used in combination with current vaccines, or would they be viable as a stand-alone product? Some of the considerations involved in the clinical development of potential broadly protective vaccines are shown in Fig. 2. Ultimately, it will take proof of concept and a better understanding of the potential efficacy of these new products to determine the role they might play in the overall control of this continuing public health threat.

Conflict of Interest Statement

None declared.

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