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Universal influenza virus vaccines and therapeutics: Where do we stand with influenza B virus?

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Abstract

The development of a broadly protective or universal influenza virus vaccine is currently a public health priority worldwide. The vast majority of these efforts is exclusively focused on influenza A viruses. While influenza A viruses causes the majority of all influenza cases worldwide, influenza B viruses should not be ignored. Approximately 25% of all influenza cases are caused by influenza B viruses which circulate as two distinct B/Victoria/2/87-like and B/Yamagata/16/88-like lineages. In contrast to popular belief, influenza B cases frequently cause significant morbidity and mortality, especially in children. Similar to influenza A viruses, influenza B viruses drift antigenically and the influenza B components of current vaccines have to be reformulated almost on an annual basis. A broadly protective vaccine against influenza B viruses is therefore urgently needed. Here we review both broadly protective anti-influenza B antibodies as well as the sparse attempts to create a universal influenza virus vaccine.

Influenza B viruses

Currently, vaccines are our best option in preventing influenza virus infections and disease. However, due to antigenic drift, influenza virus vaccines have to be reformulated almost every year [1]. The strain selection for these vaccines is based on surveillance and prediction, and unfortunately mismatches between selected vaccine strains and circulating viruses occur in irregular intervals. If the wrong strain is selected, vaccine effectiveness drops sharply and leaves the population vulnerable [2,3]. In addition, egg adaptation of vaccine seed viruses can cause considerable mismatches as well [4–6]. To circumvent these problems, broadly protective and universal influenza virus vaccines are under development [7,8]. However, these efforts are mainly focused on influenza A viruses with little attention paid to influenza B viruses.

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The majority of influenza cases are caused by the seasonal influenza A subtypes H1N1 and H3N2. However, approximately 25% of all human seasonal influenza cases are caused by influenza B viruses (Figure 1) [9–14]. In some cases influenza B strains can even dominate seasons (e.g. the current 2017–2018 season in Europe - Figure 1) [14,15]. Although their pathogenicity is often underestimated, it has been shown conclusively that influenza B virus infections are as severe as influenza A virus infections [16,17]. Influenza B viruses can cause significant morbidity and mortality, specifically in children who are often over-proportionately affected by these viruses [12,18]. Furthermore, influenza B virus epidemics often peak later in the season [19–21] when vaccine-induced antibody-based protection might already be waning [22]. The fact that two antigenically distinct lineages, B/Victoria/2/87-like and B/Yamagata/16/88-like, are circulating adds to the complexity of formulating influenza virus vaccines [23].

As mentioned above, broadly protective and universal influenza virus vaccines are in development, but the majority of these approaches are aimed at influenza A viruses [7,8]. However, a broadly protective influenza B virus vaccine is also urgently needed. Importantly, the smaller phylogenetic footprint and the higher sequence conservation would likely make a pan-influenza B virus vaccine a more achievable target than a universal influenza A virus vaccine (Figure 2). Below, we review broadly protective influenza B virus monoclonal antibodies (mAbs) and their epitopes as well as the sparse efforts to design a universal influenza B virus vaccine.

Broadly reactive monoclonal antibodies

In recent years, broadly neutralizing antibodies against influenza virus A virus hemagglutinin (HA) have been isolated and their protective potential has received significant attention [24,25]. Such antibodies may greatly enhance our prophylactic and therapeutic arsenal against influenza viruses while providing useful tools for informing vaccine development. MAbs with broad reactivity against influenza B viruses have also been isolated, although in small numbers.

Dreyfus *et al.* have reported three broadly cross-reactive antibodies, namely CR8033, CR8071, and CR9114. These mAbs were capable of conferring complete protection when passively transferred to mice subsequently challenged with influenza B viruses from both lineages [26]. CR8033 and CR8071 neutralized all tested influenza B viruses, with differing mechanisms across both lineages. Despite a lack of virus neutralization by CR9114, complete protection was observed *in vivo* indicating the possible contribution of Fc mediated antibody effector function [26,27]. CR8033, CR8071, and CR9114 also recognize distinct antigenic epitopes spanning the receptor-binding pocket, the vestigial esterase domain at the base of the head domain, and a highly conserved stalk epitope (across influenza A and B viruses) respectively (Figure 3). A lack of competition in binding, and strong protective efficacies suggests that these antibodies can be used in conjunction and could possibly act synergistically when administered in combination. Importantly, this study also demonstrated that conserved epitopes exist across influenza B viruses, suggesting that vaccines targeting these epitopes could induce broadly protective immunity.

MAb 5A7, another human influenza B virus HA stalk-specific antibody reported by Yasugi *et al.*, neutralized viruses spanning from the 1980s to more recent strains [28]. The binding epitope for 5A7 on the stalk is distinct from and more membrane-distal than the CR9114 epitope mapped by Dreyfus *et al.* and highly conserved with very low natural variability seen in the epitope region in sequenced isolates (Figure 3). MAb 5A7 also displayed efficacy against lethal challenge with a B/Victoria/2/87-like strain when administered therapeutically in the mouse model [28].

Another antibody C12G6, a chimerized human IgG1 F_c fragment containing mouse monoclonal was recently isolated and described by Shen *et al.* [29]. MAb C12G6 was also shown to broadly neutralize influenza B viruses spanning both lineages and strains from the ancestral lineage as far back as 1940 [30]. The neutralization activity was mediated by hemagglutination inhibition and robust virus targeting remained consistent *in vivo* in mice and ferrets in therapeutic and prophylactic administrations in the context of lethal viral challenge. Compared to generic versions of the aforementioned mAbs, C12G6 portrayed a significantly higher therapeutic efficacy indicated by complete protection, negligible weight loss, and substantially lower lung titers upon 25 MLD₅₀ virus challenge with viruses from both lineages [29]. Similar to CR8033, mAb C12G6 recognized a highly conserved epitope in close proximity to the receptor binding site domain across influenza B viruses. Both mAbs were found to compete for binding, which is implicative of an overlapping binding footprint [29]. Remarkably, C12G6 prevented viral entry, viral egress, and membrane fusion, and it also displayed lineage-specific antibody-dependent cell mediated cytotoxicity (ADCC), a rare and unexpected effect for an antibody with HA inhibition activity [27,31], and robust complement-dependent cytotoxicity *in vitro*, leading to the authors referring to it as ‘multimechanistic’.

In a similar manner, Chai *et al.* have previously described an anti-influenza B human antibody targeting the HA that is capable of interfering with receptor binding, blocking membrane fusion, and preventing viral egress [32]. This antibody, 46B8, bound to the vestigial esterase domain, neutralized a panel of influenza B viruses, induced ADCC *in vitro*, and conferred complete protection even when administered as late as 72 hours post infection in mice.

The influenza B virus neuraminidase (NA) has also been found to display conserved epitopes with critical residues within or adjacent to the active site and the monomer-monomer interface of the tetrameric NA. Recent work has demonstrated that such broadly reactive anti-NA antibodies can provide protection against morbidity and mortality in a mouse challenge model against influenza B viruses spanning more than 70 years of antigenic drift from both the B/Yamagata/16/88-like and B/Victoria2/87-like lineages as well as the ancestral lineage [33]. Interestingly, in a mouse model, a single therapeutic administration of an anti-NA mAb 72 hours post lethal challenge performed superiorly in terms of survival when compared to oseltamivir therapy started at the same time (the current standard of care). In addition, mAb HCA-, was reported to also bind a conserved epitope on the influenza B virus NA, but no *in vivo* data for influenza B viruses is available so far [34].

The broadly protective mAbs described above prove the existence of conserved epitopes on the head and stalk domain of influenza B virus HA and on influenza B virus NA. These mAbs could be developed into valuable therapeutics. More importantly, they underscore that it should be feasible to develop broadly protective or universal influenza B virus vaccines.

Broadly protective and universal influenza B virus vaccine approaches

Several broadly protective or universal influenza A virus vaccine strategies are in late pre-clinical or clinical development. Given the clear unmet public health need, the proven existence of conserved epitopes on the head and stalk of HA as well as the NA, and the relatively small phylogenetic footprint of influenza B virus surface glycoproteins, it would appear that a broadly protective influenza B virus vaccine is ‘low-hanging fruit’. It is therefore surprising that very few studies investigating this possibility have been published.

Bianchi *et al.* designed a universal influenza B virus vaccine by exploiting the highly conserved and exposed nature of the cleavage site and fusion peptide that connects the immature HA1 and HA2 subunits [35]. The authors conjugated this 19 amino acid-long peptide with a carrier protein, which when used as a vaccine, protected mice against lethal challenge from viruses of both influenza B virus lineages and reduced viral lung titers.

Sequential vaccination with chimeric HAs has been shown to be a promising strategy for the development of universal influenza A virus vaccines [25]. This approach has also been tested successfully for influenza B viruses [36]. Chimeric HA constructs expressing different influenza A virus HA head domains (e.g. from H5, H7, or H8) combined with an influenza B HA stalk domain were recently designed. When used in a sequential vaccination regimen that presented the same stalk domain in combination with changing head domains, this vaccination regimen refocused the antibody response towards the stalk domain and protected mice from lethal influenza B virus challenge. Cross-protection was observed against B/Victoria/2/87-like, B/Yamagata/16/88-like, and ancestral B/Lee/1940 challenge strains with negligible weight loss observed. Interestingly, the antibody response induced as a result of vaccination in this study was predominantly non-neutralizing and conferred protection by inducing ADCC in an Fc-dependent manner, which resembles the mode of action of stalk-reactive mAb CR9114 described above.

Another strategy towards designing a universal influenza B virus vaccine involves focusing the immune response on targeting the viral NA rather than the immunodominant HA. In contrast to influenza A virus NAs, which have diverged into nine distinct subtypes, the influenza B virus NAs are similar enough that vaccination with recombinantly expressed protein provides protection against homologous and heterologous virus challenge. It was shown that vaccinating mice with NA from a virus in the B/Yamagata/16/88-like lineage protected them against morbidity and mortality after lethal challenge with viruses in the from both lineages and an ancestral strain [37]. Passive transfer of sera from vaccinated animals conferred protection against lethal challenge in naïve animals, suggesting that the humoral immune response in this context is sufficient to safeguard against weight loss and mortality. The broadly protective antibodies induced by recombinant NA-based vaccination likely target similar epitopes as the anti-NA mAbs described above. Novel vaccines that, in

contrast to current inactivated vaccines, induce robust anti-NA responses could therefore enhance the breadth of protection against influenza B viruses.

Conclusions

Influenza B virus infections are a significant public health concern. Historically, despite the existence of two different influenza B virus lineages, only one influenza B virus component was included in influenza virus vaccines. Due to the co-circulation of influenza B virus lineages during influenza seasons, this led to frequent mismatches [10]. Ultimately, quadrivalent vaccines which include a B/Victoria/2/87-like component and a B/Yamagata/16/88-like component were developed and are now in use along with trivalent vaccines. Nevertheless, influenza B viruses from both lineages drift, which could lead to vaccine mismatches. In addition, the vaccine strains have to be frequently updated. As described above, broadly protective antibodies targeting the HA and NA of influenza B virus have been isolated and have implications for designing a universal vaccine. In contrast to influenza A viruses, where the majority of broadly protective mAbs target the stalk domain of the HA with only a few exceptional mAbs binding to conserved epitopes on the head domain, broadly-protective anti-head antibodies are more common for influenza B viruses. The reason for this might be the higher conservation of the HA. Taking all information regarding the phylogenetic diversity of the antigenic glycoproteins and the presence of conserved epitopes of influenza B viruses together, it seems that designing a broadly protective vaccine should be achievable. However, very little work towards that direction has been performed. Given the impact that influenza B viruses makes on human health, efforts to design universal influenza B virus vaccines should be intensified. In contrast to influenza A viruses, influenza B viruses have no animal reservoir. Given the right vaccine strategy that induces broad immunity and high vaccination rates, it is - at least hypothetically - possible to eradicate this virus.

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Highlights

- Conserved epitopes on influenza B virus HA head, HA stalk and NA exists.
- Broadly protective antibodies that target these epitopes have been isolated and might be developed into novel therapeutics.
- Very little work has focused on the development of broadly protective influenza virus vaccines so far, despite the presence of conserved epitopes and a small phylogenetic footprint.

Figure 1. Influenza-positive clinical specimens by influenza virus type

Panel **A** shows seasonal surveillance data from public health and clinical laboratories reported to the United States Centers for Disease Control and Prevention (CDC) from the 1997–1998 season to the current 2017–2018 season with preliminary data up to and including week 6, ending February 10, 2018 (adapted from <https://www.cdc.gov/flu/weekly/pastreports.htm>). Panel **B** represents seasonal surveillance data from the World Health Organization (WHO) European Region from sentinel and non-sentinel specimen sources reported to the European Centre for Disease Prevention and Control (ECDC) from the 2014–2015 season to the current 2017–2018 season with preliminary data up to and including week 6, ending February 11, 2018 (adapted from <http://flunewseurope.org/Archives>). Panel **C** includes annual surveillance data submitted to the Global Influenza Surveillance and Response System (GISRS) and FluNet from 1999 (data from three countries) to 2018 (data from 109 countries) with preliminary data up to and including data submitted by February 19, 2018 (adapted from <http://apps.who.int/flumart/Default?ReportNo=12>).

Figure 2. Phylogenetic trees representing HA diversity of all influenza A subtypes and human H3 HAs compared to influenza B HAs

Panel A represents the diversity of the HA and phylogenetic footprint of influenza A virus HAs. Panels B and C show the same for H3 HAs and influenza B HAs sequences for human isolates over time, respectively. The ancestral B/Lee/1940 strain, and antigenically distinct B/Victoria/2/870-like and B/Yamagata/16/88-like lineages are indicated in panel C. Scale bars have been normalized across all three trees and represent 1% change in amino acid sequence. Sequences were obtained on FluDB or GISAID, aligned in Clustal Omega following sequence cleanup, aligned in FigTree, and compiled in Adobe Illustrator.

Figure 3. Reported antibody binding epitopes or footprints on influenza B HA

Antibody binding epitopes for **CR8033** (yellow), **CR8071** (orange), **CR9114** (green), **5A7** (purple), **C12G6** (red), and **46B8** (blue) are highlighted on a surface representation of the influenza B/Brisbane/60/2008 virus HA (PDB: 4FQM - [26]) using PyMOL. The globular head domain of the HA is highlighted in dark grey relative to the stalk domain which is shown in light grey. CR8033 and C12G6 show a similar binding footprint in proximity to the receptor binding site, while 46B8 and CR8071 show a similar footprint on the vestigial esterase domain.