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Challenges of selecting seasonal influenza vaccine strains for humans with diverse pre-exposure histories

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Abstract

Seasonal influenza vaccine strains are routinely updated when influenza viruses acquire mutations in exposed regions of the hemagglutinin and neuraminidase glycoproteins. Ironically, although thousands of viral isolates are sequenced each year, today's influenza surveillance community places less emphasis on viral genetic information and more emphasis on classical serological assays when choosing vaccine strains. Here, I argue that these classical serological assays are oversimplified and that they fail to detect influenza mutations that facilitate escape of particular types of human antibodies. I propose that influenza vaccine strains should be updated more frequently even when classical serological assays fail to detect significant antigenic alterations.

Introduction

Influenza infections and conventional influenza vaccines elicit neutralizing antibodies (Abs) predominantly directed against the hemagglutinin (HA) and neuraminidase (NA) glycoproteins. Although human influenza Ab responses can be extremely long lived [1], influenza vaccines must be frequently reformulated [2,3] since viruses continuously accumulate mutations in Ab binding sites of HA and NA through a process termed 'antigenic drift' [4]. Current seasonal influenza vaccines usually provide some level of protection, but vaccine efficacy varies greatly between different influenza seasons and among different individuals [5]. Great progress has been made towards the development of 'universal' influenza vaccines that elicit immunity against antigenically stable viral epitopes [6-8]. However, until a universal influenza vaccine is brought to fruition, vaccine manufacturers must continuously update influenza virus strains. Here, I focus on the challenging process of identifying vaccine strains that are antigenically matched to most circulating strains.

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Selection of seasonal influenza strains

Seasonal vaccines include only 3 or 4 viral strains (one H1N1 influenza A, one H3N2 influenza A, and one or two influenza B viruses). Twice a year, the World Health Organization (WHO) recommends which strains to include in seasonal vaccines and this recommendation is made 7-8 months in advance of the northern and southern hemisphere influenza seasons [2,3]. The Global Influenza Surveillance Network determines the antigenic properties of thousands of viral isolates each year [9]. Antigenic characterizations of viral isolates are largely based upon hemagglutination-inhibition (HAI) assays, which measure reference sera's ability to prevent binding (agglutination) of influenza virus isolates to sialic acid receptors on red blood cells [10]. Virus neutralization (VN) assays are also completed with select virus isolates, however these assays are more time-consuming compared to relatively simple HAI assays [11]. Enormous amounts of viral sequence data are analyzed and geographical distributions of specific variants are taken into consideration when selecting vaccine strains.

Antigenic characterizations of viral isolates require reference anti-sera. Reference sera for HAI assays are routinely produced in ferrets recovering from primary influenza infections [12]. Although antigenic characterizations are mostly determined using ferret anti-sera, additional HAI and VN assays are also completed using sera isolated from humans vaccinated with current vaccine formulations [11]. For studies using human sera samples, surveillance laboratories consider a reduction of 50% or more in geometric mean titers as significant [11]. Using this approach, it is difficult to identify viral variants capable of escaping Abs that are only elicited in a subset of the human population. For studies using ferret sera samples, there is no gold standard of what constitutes a significant antigenic change. Although 2-fold HAI titer differences can be reproducibly measured using ferret anti-sera, most laboratories do not recognize 2-fold changes as significant.

HA antigenic sites

The first monoclonal Abs specific for the A/Puerto Rico/8/34 (PR8) H1N1 strain were isolated in the 1970s, and shortly thereafter, Gerhard and Webster were able to isolate variant viruses in the presence of a monoclonal Ab after a single passage in eggs [13]. Monoclonal Ab mapping studies revealed that there are 5 independent antigenic sites on HA [14-16] (FIG 1). Alterations in a single antigenic site abrogates binding of most monoclonal Abs specific to that site, but does not affect binding of monoclonal Abs to the other antigenic sites. Crystallography studies confirmed that HA point mutations locally distort single antigenic sites without affecting neighboring antigenic sites [17]. Based on variability in nature, Gerhard named the most variable H1 sites Sa and Sb (S refers to 'strain-specific') and the more conserved sites Ca1, Ca2 and Cb (C refers to 'cross-reactive') [15].

It is worth pointing out that some of the Abs used to map these antigenic sites were isolated from animals recovering from primary viral exposures, while other Abs were isolated from animals sequentially exposed to different influenza strains. Similar antigenic mapping studies were completed with HA from H3N2 [18,19] and NA from H2N2 [20]. Several studies suggest that influenza viruses can also avoid Abs by acquiring HA mutations that

increase virus-cell binding avidity and that many of these mutations are located in classic antigenic sites [21-23].

Genetic variation versus antigenic variation

The goal of HAI assays is to detect HA antigenic mutations. Large HAI datasets involving different types of anti-sera can be difficult to analyze. A major breakthrough addressing this problem came in 2004 when Smith et al. used antigenic cartography to map the antigenic evolution of human H3N2 viruses [24]. The landmark study suggests that H3N2 antigenic evolution is more punctuated than genetic evolution, and that small genetic changes can disproportionately affect antigenicity. The same group published a study last year indicating that antigenic drift of H3N2 viruses from 1968 to 2003 was caused mainly by single amino acid substitutions at only 7 HA residues [25]. Remarkably, 6 of the 7 residues attributed to H3 antigenic drift are located in a single antigenic site near the HA receptor binding domain (antigenic site B; analogous to the H1 Sb antigenic site) [25]. The 7th HA residue in this study (residue 145 in antigenic site A) influences HAI assays by modulating receptor binding avidity [26]. Bedford et al. extended these antigenic cartography studies by developing methods that simultaneously analyze genetic and antigenic influenza data [27].

H3N2 viruses routinely acquire mutations in all 5 HA antigenic sites. Why is this the case if only 1 antigenic site is antigenically important? One possible explanation is that mutations in non-immunodominant antigenic sites offset viral fitness costs associated with mutations in immunodominant antigenic sites [25,28]. An alternative explanation is that all 5 HA antigenic sites are actually antigenically relevant, but that the ferret reference sera used to create antigenic maps in these studies are not fully representative of human immunity.

Human immunity is complicated

Reference sera for HAI assays are routinely produced in ferrets that are pre-screened to verify that they have not been previously infected with influenza viruses [12]. However, unlike captive ferrets, most humans are repetitively infected with different influenza viruses, and prior influenza exposures influence the development of new Ab responses against drifted influenza virus strains [29-32]. In the 1950s Thomas Francis Jr. discovered that the human immune system preferentially generates Abs that cross-react to previously circulating strains at the apparent expense of generating new Ab responses that specifically recognize newer viral strains [33]. This process, termed 'original antigenic sin' has been experimentally recapitulated in a variety of animal models [34-36]. More recent studies of humans infected with the 2009 pandemic H1N1 (pH1N1) virus demonstrated that prior infections with seasonal H1N1 (sH1N1) viruses influence the development of anti-pH1N1 Ab responses [32,37-40]. For example, there are many somatic mutations in the immunoglobulin genes of most monoclonal Abs derived from adult humans infected with the pH1N1 virus [37-39], which is indicative of recall B cell responses. Furthermore, most of these pH1N1-induced monoclonal Abs bind with a high affinity to older circulating sH1N1 viruses [37-39].

Given that humans are routinely pre-exposed to different influenza strains, do most human influenza Abs have the same specificities compared to anti-sera obtained from ferrets

recovering from primary influenza virus infections? We addressed this by directly comparing Ab specificities in sera isolated from humans and ferrets infected with the 2009 pH1N1 strain [32]. Consistent with previous studies [41], we found that sera isolated from ferrets recovering from a primary 2009 pH1N1 infection were narrowly dominated against the top of the Sa antigenic site. The majority of human sera tested did not have this specificity. Instead, we identified an age-specific human Ab signature that likely resulted from sequential infections with an earlier sH1N1 strain and the 2009 pH1N1 strain. Importantly, we were able to recapitulate the specificity of human Abs in ferret anti-sera by sequentially infecting animals with an older sH1N1 strain and the 2009 pH1N1 strain. These studies raise the possibility that primary ferret anti-sera currently used to guide the choice of seasonal vaccine strains may not be representative of human immunity.

Our studies identified an Ab signature that is specific for individuals born between 1983-1996 [32]. The reason why this particular Ab specificity is limited to humans born during this time period is because the Ab footprint was fully intact in sH1N1 viruses that circulated from 1983-1986, but was mutated in most sH1N1 viruses that circulated during other time periods [32]. It is expected that humans born before 1983 or after 1996 have different Ab specificities due to unique pre-exposure histories, however the specificities of Abs from other age groups remain poorly characterized.

Can we make the vaccine strain selection process better?

The WHO is faced with an extremely difficult task in choosing seasonal influenza vaccine strains, and the scientific community should be proud of the international collaborative effort that goes into this decision. Landmark studies of Smith et al [24] and others have changed the way we think about influenza virus antigenic drift and these studies have positively impacted the process of selecting vaccine strains. Here, I do not intend to be overly critical of current surveillance approaches, as there are many non-scientific practical issues that must also be considered during the vaccine strain selection process. I propose that the following simple points should be further considered:

1. A greater emphasis should be placed on sera and monoclonal Abs derived from humans when antigenically characterizing circulating viral strains. There are dramatic specificity differences between human and ferret Abs, and these differences are due in part to pre-exposure history. Current surveillance efforts do utilize human sera, but most experiments are not designed to detect antigenic mutations that facilitate escape of Abs present in a subset of the human population. Furthermore, most antigenic mapping studies continue to be dependent on anti-sera prepared in ferrets.
2. Sera obtained from sequentially infected ferrets should be included in panels used to characterize viral isolates. These types of sera panels are easy to generate and studies have already demonstrated that they can more closely resemble human immunity compared to sera obtained from animals recovering from primary influenza infections [32].

3. A greater emphasis should be placed on sequence data. It is ironic that in today's genomics age we now put more emphasis on HAI and other serological data rather than genetic data when selecting vaccine strains. Given that we don't know the identity of human Abs that are elicited by different types of sequential infections and we don't have a handle on the extent of Ab diversity that exists due to genetic differences between individuals, the community might be placing too much of an emphasis on limited sera panels. An HA mutation that prevents binding of Abs elicited in one individual (or ferret) may not prevent binding of Abs in another individual. We now have beautiful crystal structures of HA and an extraordinary amount of sequence data. I propose that we turn back the clock and pay more attention to antigenic sites defined in early experimental studies (for example [16] which defines antigenic sites using monoclonal Abs following primary and sequential exposures), and that we complete additional studies that further define footprints of different types of human Abs. Along these lines, Luksza and Lassig recently developed fitness models that predict influenza evolution from genomic data [42]. The ultimate goal is to identify rapidly evolving antigenic novel mutations that are located on exposed regions of HA even if these mutations do not prevent the binding of reference ferret Abs.

The process of selecting seasonal influenza vaccine strains can only be improved if we consider these issues related to the complexity of human influenza Ab responses.

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Highlights

- Selection of influenza vaccine strains is difficult because of ‘antigenic drift’.
- Human antibodies against influenza viruses are influenced by prior immune exposures.
- Ferret anti-sera might not be fully representative of human immunity.

Fig 1.

Antigenic sites of H1N1 HA. (A) The crystal structure of PR8 HA is shown (PDB: 1RVZ). The top portion of HA is commonly referred to as the 'head' and the portion of HA that is more proximal to the virus membrane is commonly referred to as the 'stalk'. Antigenic sites on the HA head are shown in yellow (Sa), red (Sb), green (Ca1 and Ca2), and blue (Cb). The influenza virus receptor, sialic acid, is shown in black. (B) A close-up view of the antigenic sites of the HA head is shown.