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

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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.

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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 toward 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 manufactures 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.

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 twofold HAI titer differences can be reproducibly measured using ferret anti-sera, most laboratories do not recognize twofold 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 five independent antigenic sites on HA [14–16] (Figure 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]. On the basis of 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

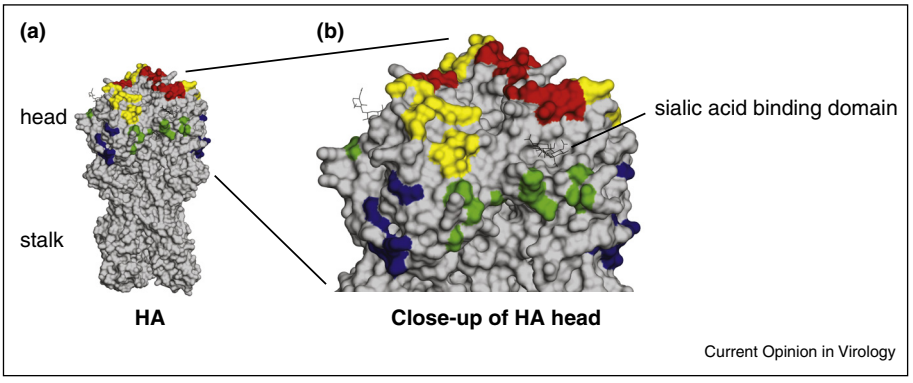
The goal of HAI assays is to detect HA antigenic mutations. Large HAI datasets involving different types of anti-sera can be difficult to analyze. A major breakthrough addressing this problem came in 2004 when Smith *et al.* used antigenic cartography to map the antigenic evolution of human H3N2 viruses [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, six of the seven 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 seventh 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 one 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 five 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 [29,30,31••,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

Figure 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.