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Toward universal influenza virus vaccines: from natural infection to vaccination strategy

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Conceptually, a universal influenza vaccine should elicit broadly protective antibody responses targeting highly conserved epitope(s) shared by various virus strains. Strategically directing antibody immunity to the conserved hemagglutinin stalk has recently emerged as a promising approach that is substantiated by the identification of naturally occurring, stalk-reactive human antibodies capable of conferring broad protection against influenza virus challenge in animal models. Despite all the advancements, future realization of this strategy still faces many challenges, particularly whether it is able to induce enough of cross-reactive antibody response to protect against pandemic viruses. In this respect, recent in-depth dissections of human immune responses to H7N9 virus and vaccination provide much-needed new insights.

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Introduction

Vaccination provides the most cost-effective measure for preventing influenza and represents the only feasible means to control outbreaks of influenza infection at herd level. The protection provided by current influenza vaccines is largely dependent on the induction of neutralizing antibody against the globular head domain of the viral surface protein hemagglutinin (HA) to block viral entry. Given that the HA head domain is highly variable among different virus strains, current seasonal vaccines are only effective against well-matched circulating virus strains and provide little or no protection against pandemic influenza. There are a total of 18 HA subtypes in influenza A virus which are categorized into two groups, group 1 (H1, H2, H5, H6, H8, H9, H11, H12, H13, H16, H17 and H18) and group 2 (H3, H4, H7, H10, H14, and H15). Influenza B HAs, distinct from influenza A HAs, are classified into two lineages, namely the Yamagata and Victoria lineages. The HA diversity is further expanded along with the virus diversity introduced by antigenic shift as the result of reassortment of different influenza viruses and antigenic drift in which amino acid alterations are constantly introduced by the error-prone viral polymerase.

The exploration for a broadly protective influenza vaccine has been focused on highly conserved influenza viral protein sequences that can elicit either broad cellular or humoral responses. Here, we are focusing on the emerging strategies which are designed to induce humoral immunity against the stalk domain of HA, which unlike its head domain is highly conserved among different influenza A subtypes. Recently, largely owing to the increased capability to dissect the human immune repertoire with the advent of new technologies, researchers have discovered many HA stalk-targeting human antibodies with broadly neutralizing activities [1]. For the majority of these antibodies, the breadth is generally restricted to one group. Such group restriction is well explained by structural studies [2–5]: group 1-specific and group 2-specific neutralizing antibodies display different stalk recognition modes, which partially stems from an epitope shielding effect posed by the presence of group-specific N-glycan modifications. Rare cross-group neutralizing antibodies were also identified and they have been shown to evolve special binding mode(s) to accommodate variations in the stalk including not only different N-glycosylation sites but also amino acid changes [6–8,9••].

Recognizing the natural existence of HA stalk antibodies has subsequently fueled the development of immunization strategies aimed at selectively inducing those antibodies. Currently, two approaches are extensively explored. The first approach is simply using stalk-only, also called headless, HA constructs as immunogens [10]. The second approach explores chimeric HAs (cHAs) composed of a conserved stalk and mismatched heads for consecutive immunization [11]. Until now the stalk-only approach has been only applied to group 1 HA [12,13], we will therefore only focus on the sequential cHAs approach which has been generally applied to both group 1 and 2 HA as well as influenza B HA with success in animal models [14–17].

Compared to seasonal influenza vaccines, universal influenza vaccines are expected to not only mitigate

suboptimal humoral immunity against drifted influenza viruses but also afford protection against novel, pandemic influenza, which is unattainable with seasonal influenza vaccines. Thus, an improved design of HA stalk-based vaccines would certainly benefit from a better understanding of the human immunity against pre-pandemic or pandemic influenza. In this review, we will discuss recent studies, including our own, on dissecting human immune responses to infection and/or vaccination of pre-pandemic H7N9 and their potential impacts on the development of HA stalk-based universal influenza vaccines.

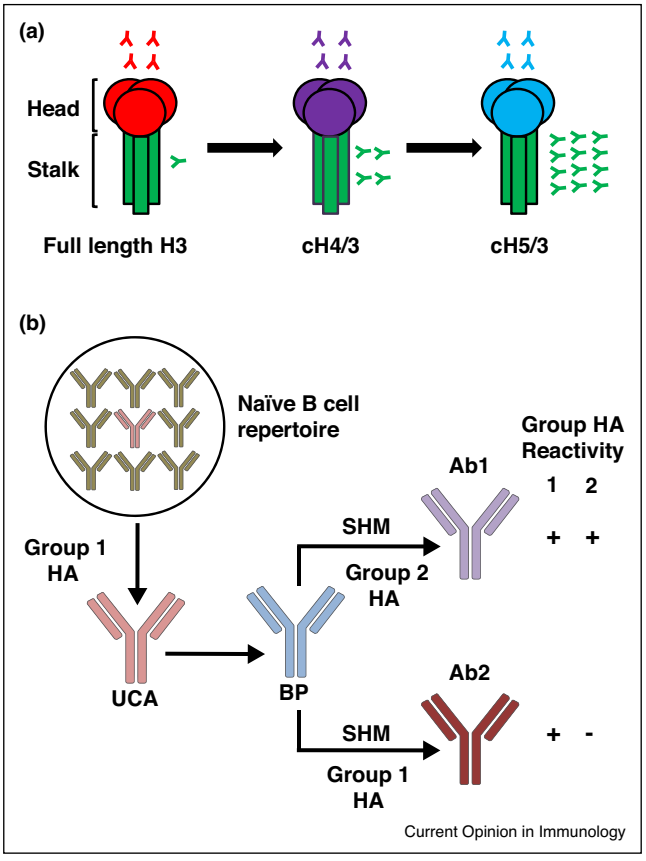
Sequential cHAs approach to direct stalk-focusing antibody responses

The primary challenge of stalk-based vaccines arises from the immundominance of the HA head over the stalk when both are available to elicit a human immune response. Consequently, the concentration of a representative stalk-specific antibody, F10, in human sera was found to only accounts for approximately 0.001% of total immunoglobin [18]. Thus, the development of a stalk-based universal vaccine requires a strategy to overcome the immundominance of the HA head.

The sequential cHAs approach for strategically focusing human antibody responses on HA stalk received an initial impetus from clinical characterization of the human humoral responses to 2009 H1N1 pandemic virus. In contrast to seasonal influenza virus infections which predominantly induce head-specific antibodies, the infection of 2009 H1N1 pandemic virus was found to be associated with a significant induction of stalk-reactive antibodies [19,20]. The stalk-focused antibody responses were later also demonstrated after 2009 H1N1 vaccination [21,22] and H5N1 vaccination [23,24]. Such remarkable difference between seasonal viruses and novel viruses led to a hypothesis: A majority of humans have a low-level HA stalk-specific memory repertoire primed by seasonal virus/vaccine exposure. An encounter with a novel HA featuring a conserved stalk and a divergent head as of 2009 H1N1 and H5N1 would render the selective expansion of the minority subpopulation of this preexisting repertoire that specifically target stalks, whereas exposure to HA with invariant or similar head would further strengthen the dominancy of head-dedicated subpopulation. This hypothesis was further validated by the observation that, on re-encountering the same novel HA, the prevalence of head-specific antibody response remerged [25].

The current immunization regimen based on sequential vaccination with cHAs consists of consecutive administrations of two to three constructs containing the conserved stalk and mismatched heads (Figure 1a). This approach was successfully demonstrated on frameworks of the H1 stalk, the H3 stalk and the Yamagata B stalk,

Figure 1



(a) Schematic illustration of sequential immunization with cHAs to achieve stalk-refocused antibody response. Shown is the modified version of vaccine regimen of H3 stalk-based cHAs with demonstrated success in protecting immunized mice against lethal H7N9 challenge [14]. The cH4/3 and cH5/3 share the H3 stalk but contain zoonotic head domains of H4 and H5 subtype respectively. Although the primary antibody response against head is much stronger than that against stalk upon the initial exposure to the full length H3 construct, only the latter was repeatedly boosted by subsequent HA exposures along with primary antibody response to the novel head domain. Consequently, the established anamnestic immune response is majorly directed against the conserved stalk. The employment of zoonotic heads in cHA constructs is critical for human vaccination because they, being novel to human, avoid the activation of pre-existing head-specific memory antibody response seeded by prior seasonal influenza infection. For humans with prior exposure to H3 influenza virus, the initial priming with H3 construct would be unnecessary, disadvantageous even. **(b)** The development of broad-spectrum HA stalk antibodies may require special prime-boost mode. Shown is the simplified version of developmental paths shared by two cross-group HA stalk antibodies, MEDI8852 and FI6 [6,9••]. An unmutated common ancestor (UCA) is initially selected from naïve B cell repertoire by exposure to group 1 HA, followed by development into a branching point (BP) via acquisition of capability to recognize a limit number of group 2 HAs. Starting from BP, the cross-reactivity breadth can be further shaped by exposure to different HAs through somatic hypermutation (SHM) process: group 2 HA exposure would direct the path toward clone (Ab1) with cross-reactivity to both group 1 and group 2 HAs whereas re-encounter with group 1 HA would promote clone (Ab2) primarily reacting to group 1 HA. The original gynecology trees described by Kallewaard *et al.* [9••] involve multiple BPs and consequently multiple independent pathways leading to ultimate acquisition of the same or similar cross-reactivity.