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Distinct evolution patterns of influenza viruses and implications for vaccine development

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Influenza A (H5N1), particularly the clade 2.3.4.4b, caused a panzootic outbreak starting in 2022, resulting in 40 human infections from January 2022 to September 2024. Among these cases, 15 have been confirmed to be of the clade 2.3.4.4b. Despite the availability of three FDA-approved A(H5N1) vaccines, these vaccines, based on earlier strains of other clades, have shown reduced hemagglutination inhibition (HAI) titers against clade 2.3.4.4b due to antigenic drift.[1] In 2023, the EMA approved a vaccine containing an A(H5N8) strain of clade 2.3.4.4b. However, a recent study reported a decrease in HAI titers of serum induced by this strain against recent 2.3.4.4b strains.[1] These findings highlight the urgent need for developing an effective vaccine.

DISTINCT EVOLUTION PATTERNS OF INFLUENZA VIRUSES

Monitoring antigenic evolution is crucial to mitigate the impact of antigenic drift on future vaccine development (Figures 1A and 1B). The antigenic maps of A(H1N1) and A(H3N2) (Figures 1C and 1D) show a unidirectional evolution with multiple clusters of strains over time, indicating a punctuated antigenic evolution driven by significant alterations in the hemagglutinin (HA) protein. This evolution trajectory is reflected in their "ladder-like" phylogenetic trees, resulting from strong immune selection of seasonal influenza viruses.[2] Dominant strains in one season can generate immune selection against circulating strains in humans, leading to the emergence of immune escape-driven "winner" strains in the next season, creating a ladder-like evolutionary tree and a punctuated antigenic trajectory. Thus, it is theoretically possible to select well-matched vaccine strains when a new ladder of strains appears in the phylogenetic tree or a new punctuated strain emerges on the antigenic map.

In contrast, identifying a clear, unidirectional evolution path for A(H5Nx) is challenging, indicating fewer antigenic drifts (Figure 1E). Unlike A(H3N2) and A(H1N1), the evolution trajectory of A(H5Nx) is multidirectional, consistent with the shape of their relatively balanced non-ladder-like trees, which exhibit high standing genetic variation.[2] This trajectory is a characteristic of a panzootic affecting multiple hosts worldwide. The diverse hosts and patched geographic distribution of A(H5Nx) weaken immune selection. Additionally, A(H5Nx) does not exhibit seasonal spread, making it challenging to generate consistent unidirectional selection across hosts and geographic areas. Therefore, the selection of vaccine strains should consider more details rather than directly selecting the recently emerging strains.

Further analysis of A(H5Nx) reveals that different clades are antigenically distinct, indicating relatively weak cross-neutralization between these clades. Fortunately, strains isolated from the recent outbreak in bovine and the bovine-to-human case are antigenically close to the WHO-recommended vaccine strains. Given the distinct evolution pattern of A(H5Nx), it is less suitable to directly apply past experience from A(H3N2) and A(H1N1) to develop an effective A(H5N1) vaccine.

DISTINCT PATTERNS REQUIRE DIFFERENT VACCINE DEVELOPMENT STRATEGIES

The distinct evolution patterns of viruses necessitate tailored vaccine development strategies. For human influenza viruses (Figure 1A), a major evolution lineage can be identified, allowing for the selection of a well-matched candidate vaccine virus at specific intervals to keep up with antigenic evolution. This approach has proven effective for A(H3N2) and A(H1N1), where updating vaccines to match the current circulating strains has been successful. However, this strategy has not been as successful for SARS-CoV-2, despite the use of more instant vaccine platforms like mRNA vaccines. In contrast, A(H5Nx) exhibits a model similar to SARS-CoV-2 (Figure 1F), requiring a different approach to vaccine development.[3] The evolution pattern of A(H5Nx) indicates that more than one circulating clade has the potential to spill over from animals to humans. This complexity makes selecting a vaccine seed strain more challenging, as one or two seed strains may not provide adequate protection against all variants from different hosts (Figure 1B).

The impact of pre-existing immunity on influenza vaccine development

Pre-existing immunity, resulting from prior exposure to viruses, can significantly influence the effectiveness of subsequent vaccines. This phenomenon, known as immune imprinting, can either weaken or enhance immune responses to later exposures. Prior exposure may weaken the immune response to new antigenically related strains, but it can also provide a "back-boost" effect, where a secondary exposure enhances the immune response generated by a previous exposure, leading to higher HAI titers against both old and new strains.[4]

To leverage the pros and cons of pre-existing immunity, strategies such as displaying multiple antigens (e.g., recombinant nanoparticles or mRNA multivalent vaccines)[5] and selecting an antigen with proper antigenic distance from previously exposed strains[3] are proposed. These approaches aim to weaken immune imprinting and optimize vaccine design. Additionally, the immune imprinting induced by different vaccine platforms varies. For instance, repeated Omicron exposures can overcome the immune imprinting of ancestral SARS-CoV-2 induced by inactivated vaccine, while imprinting by an mRNA vaccine cannot be altered. A well-designed vaccination process alternating between different vaccines can effectively modify immune responses as needed.

The next-generation universal influenza vaccine development

Developing a universal influenza vaccine aims to broaden the protective spectrum to cover more strains. However, there are two major obstacles: immune imprinting due to previous vaccinations or infections and the highly variable nature of HA proteins (Figure 1A). To address these challenges, chimeric or mosaic antigens, as well as stalk-only antigens, are employed to redirect the antibody response to the less variable stalk of the HA protein.[5] Additionally, targeting T cell epitopes can improve broad-spectrum protection, though human leukocyte antigen (HLA) polymorphism restricts the wide usage of this vaccine. These rational design strategies represent the peaks of empiricism of vaccinology (Figure 1B).

In the future, vaccine design will increasingly rely on AI. Past computational methods including computationally optimized broadly reactive antigen (COBRA)[5] cannot consider the cross-neutralization of or interference from pre-existing immunity. Utilizing large-scale influenza data, an AI model can be trained to precisely predict the cross-neutralization based on HA sequences. Thus, an

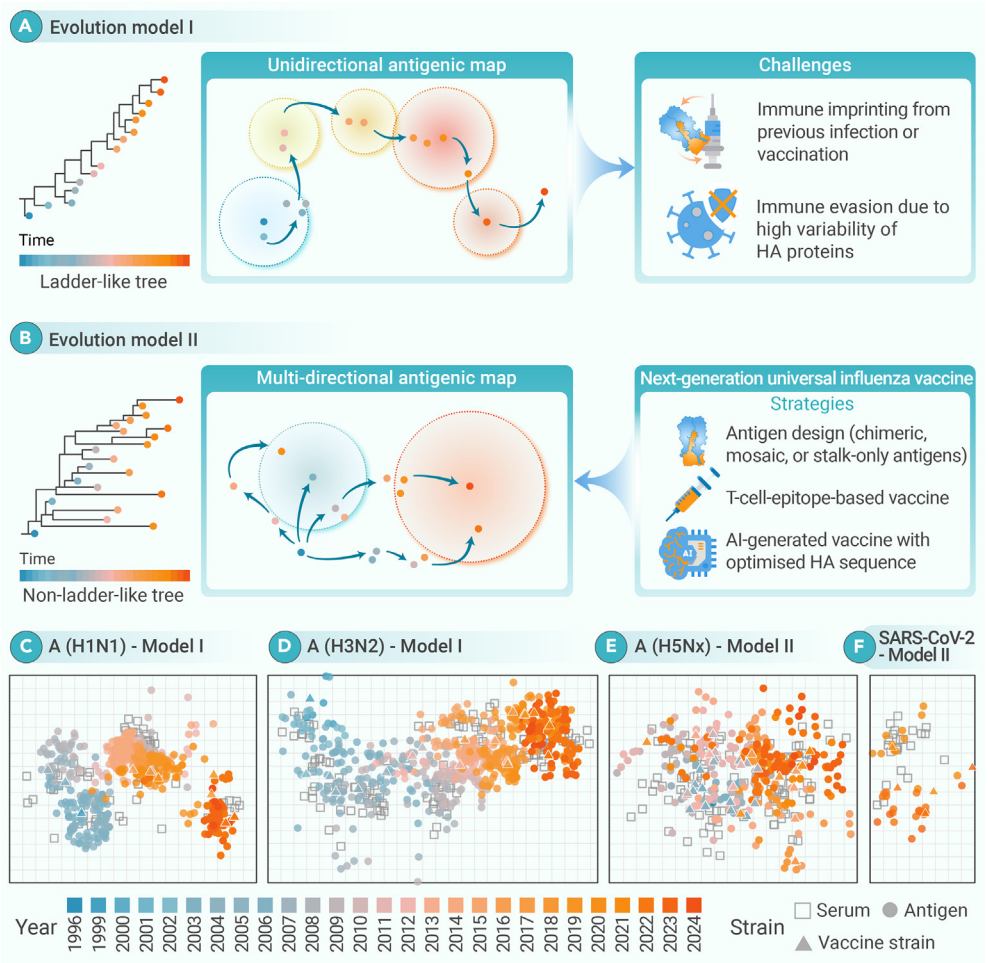

Figure 1. Evolution patterns of distinct viruses and pairwise vaccine development strategies (A and B) Two models are illustrated based on antigenic and genetic evolution patterns. (C and D) The evolution of (C) A(H1N1) and (D) A(H3N2) follows model I. (E and F) In contrast, the evolution of (E) A(H5Nx) and (F) SARS-CoV-2 follows model II. In (C)–(F), both vertical and horizontal axes indicate relative antigenic distance. Each scale is one arbitrary unit of antigenic distance, which means a 2-fold change in terms of HAI or neutralizing titer.

optimized mosaic or conserved epitope-focused antigen can be *de novo* generated by screening all possible natural or non-natural HA sequences utilizing a generative AI model. In this way, the ideal universal vaccine can be automatically designed.

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DECLARATION OF INTERESTS

Y.-F.H. is currently the CEO of BayVax Biotech Limited.