A Possible Mutation that Enables H1N1 Influenza A Virus to Escape Antibody Recognition

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***Abstract*—The H1N1 influenza A 2009 pandemic caused a global concern as it has killed more than 18,000 people worldwide so far. Studies that have found cross-neutralizing antibodies between the 1918 and 2009 pandemic flu elicit a basis of pre-existing immunity against the 2009 H1N1 virus in old population. The cross-reactivity occurs due to conserved antigenic epitopes shared between the two pandemic viruses. However, evolutionary mutation can enable the virus to elude human immunity system, making these antibodies probably no longer effective. In our study, we found that a possible mutation in B-cell epitope (the sequence PNHDSNKG) could be the chance for the virus to escape the 1918 antibody recognition. Hence, this finding can be helpful for further vaccine designs against the H1N1 2009 influenza A virus.**

Keywords: H1N1 influenza A virus; mutation; protein docking; antibody recognition

I. INTRODUCTION

The outbreak of H1N1 influenza A 2009 was caused by a re-assorted strain from swine, avian, and seasonal human flu viruses [1-2]. Various studies have been conducted, especially on Hemagglutinin (HA) protein which is essential for virus to attach and infect cells [2], making it a key target for developing neutralizing antibodies and vaccines to prevent the infection. The studies also revealed that the 2009 HA contains conserved epitopes that existed in the 1918 Spanish flu HA protein [3-5]. The conservation has explained cross-reactivity of antibodies between the two pandemic viruses and prior immune memory against the current pandemic strain of the older population [5-6]. However, no research could make sure that evolutionary mutation would not render the antibodies ineffective and enable the virus to escape human immunity system. In this paper we examined and analyzed variability in HA sequences of current and past strains of pandemic influenza virus. A potential mutation in B-cell epitope was found to show possible escape mechanism of the H1N1 influenza A virus from the current antibody recognition.

II. METHODS

A. Phylogenetic Analysis of Pandemic (H1N1) 2009 Strains

The HA protein sequences of the pandemic (H1N1) 2009 strains were retrieved from NCBI Influenza sequence database [7]. MAFFT version 6 [8] was used to do multiple sequence alignment and construct the Neighbour Joining tree (bootstrap value = 1,000). The tree was visualized using Archaeopteryx tree viewer [9]. The potential epitopes were predicted using the IEDB Analysis Resource Tools, and the B-cell candidate was found using linear epitope prediction tools such as Chou & Fasman Beta-Turn Prediction [10], Emini Surface Accessibility Prediction [11], Karplus & Schulz Flexibility Prediction [12], Kolaskar & Tongaonkar Antigenicity [13], Parker Hydrophilicity Prediction [14], and Bepipred Linear Epitope Prediction [15]. The candidate with score higher than the default threshold and predicted by most of the methods was chosen for further investigation. Homology modeling was then done with I-TASSER [16-17] on the selected B-cell epitope.

B. Molecular docking for the predicted epitopes

The binding ability of the mutated epitope and antibodies was demonstrated using ClusPro [18-19], a docking server of Boston University. ClusPro v.2 is a multistage protocol docking program. It performs rigid docking using DOT or ZDOCK programs, filters and clusters docked

conformations, and refines the results using CHARMM minimization [20]. The dockings were performed using all default parameters and antibody docking mode was applied. The lowest docked energy model was selected for detection with an assumption that the minimum binding energy-obtained model is assumed to be the best docked conformation and nearest to the native binding state.

III. Results and Discussion

A. *Phylogenetic relationship of current pandemic influenza*

Typically, HA subtypes of the pandemic influenza strains differ from that of circulating seasonal strains. To demonstrate that, multiple sequence alignment and phylogenetic analysis of HA H1 clade subtype 1 to strains of subtype 2 and 3 were done and it confirmed that the clade of the current pandemic virus strains is distinct from strains of seasonal influenza strains. The current pandemic strains also differ from WHO recommended strains that had been used for vaccine development and group 2 HA subtype 14 (Fig. 1). Group 2 but not Group 1 HA subtypes are recognized by tert-butyl hydroquinone (TBHQ), a small molecule that inhibits virus entry [21]. Considering that the binding sites of monoclonal antibody CR6261 is close to the TBHQ binding site, it is unlikely that passive immunization would protect against the current pandemic strains. Molecular docking further confirmed that the CR6261 did not recognize the presence of the epitope in the current pandemic HA proteins.

B. *Investigation of interactions between known neutralizing antibodies and epitope-containing HA protein*

Using Epitope prediction tools in Immune Epitope Database (IEDB), B-cell epitope PNHDSNKG was found (Table 1). This epitope sequence obtained highest prediction score and predicted by multiple tools indicating that it is likely to be recognized by the immune system.

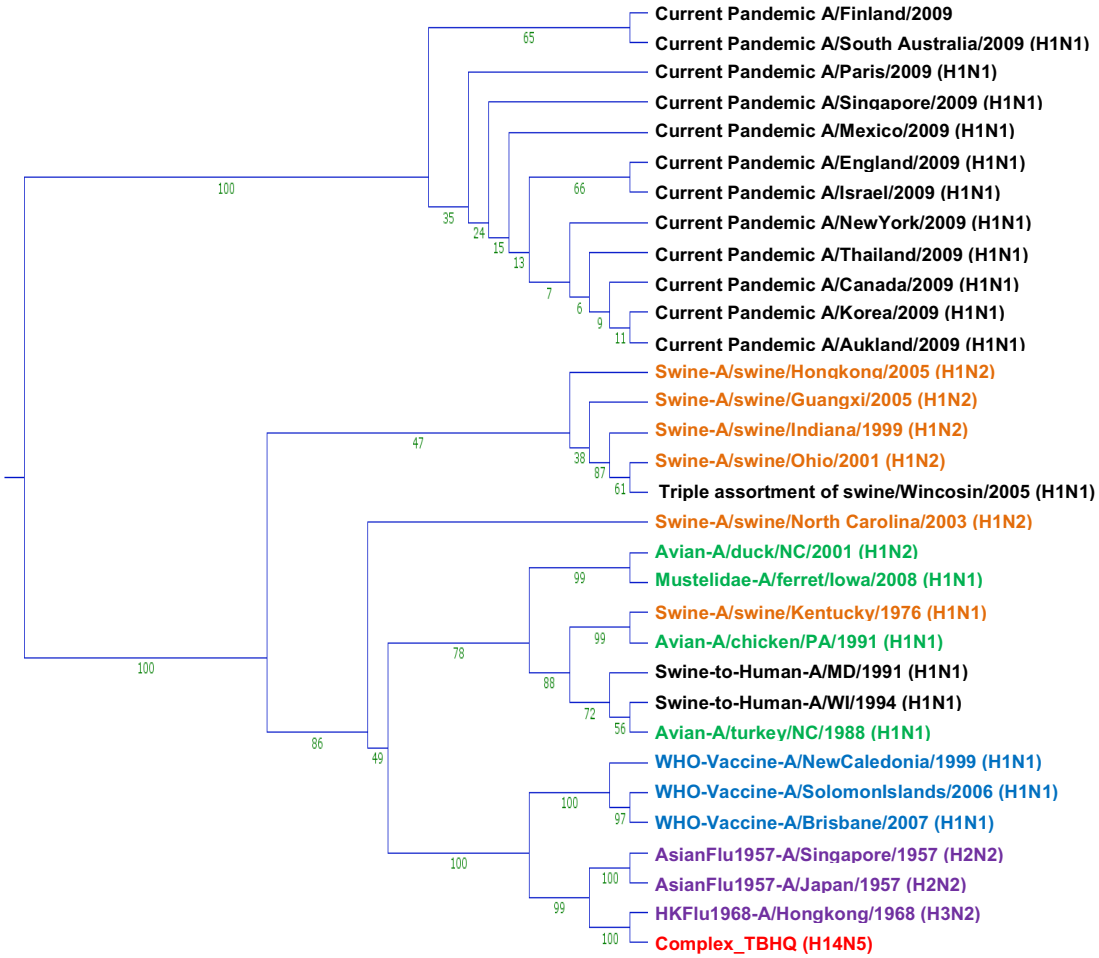

Figure 1. Neighbor-joining tree of H1, H2, H3, and H14 subtype HA sequences with 1,000 bootstrap replicates. The current strains (black) are distinct to seasonal influenza strains used in WHO vaccines (blue) and the past pandemic influenza (purple). HA of strains derived from swine (orange) and birds (green) are relatively close to current pandemic strain subtype H14 HA (red) which is known to bind to TBHQ.

TABLE I. THE PREDICTED B-CELL EPITOPE

Epitope	Methods	Sequence 1	Sequence 2
PNHDSNKG	Bepipred	TSSWPNHDSNKGVTAACPHA GAKSF	
	Chou	TSSWPNH	PNHDSNK
	Karplus	PKTSSWP	NHDSNKG
	Parker	PNHDSNK	NHDSNKG

1) Docking with the 2D1 antibody from the complex of Fab 2D1-1918 influenza virus HA: The binding ability of the chosen epitope was predicted using a docking server ClusPro. Only clusters of docked models with minimum binding energies were considered.

The predicted epitope sequence was first modelled by I-TASSER to obtain the 3D-structures. It was then mapped back to a HA protein using homology mapping [22]. The epitope-containing HA protein was docked to a structure of a known antibody extracted from the Fab 2D1-1918 influenza virus HA complex [PDB:3LZF] [3]. The 1918 influenza virus HA was chosen because the antigenic drift of the H1N1 influenza A virus involved in the 2009 pandemic started in the year 1918 [5, 23].

The docked complex had different antibody-binding orientation from the Fab 2D1-1918 influenza virus HA complex (Fig. 2). In the docked complex, the 2D1 antibody bound to HA1 domain, but it did not interact with the predicted B-cell epitope as shown in Fig. 2A. The binding regions of the two HA protein sequences contain different residues between the positions 130-133 (Fig. 3).

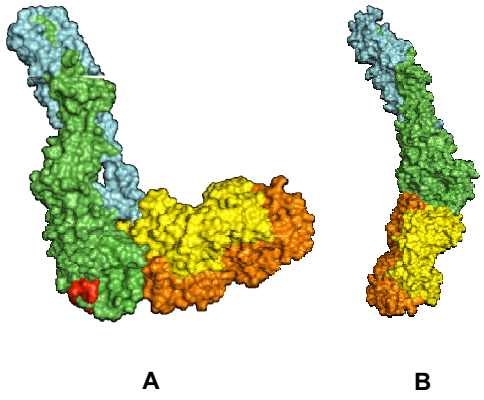

Figure 2. Different binding orientations of the 2D1antibody to the epitope-containing HA protein (A) and to the 1918 influenza HA (B). The bound complexes of antibody and HA monomer are shown as surface presentation. HA1 and HA2 domains are colored in green and cyan. The 2D1 antibody chains are shown in yellow (heavy chain) and orange (light chain). The predicted B-cell epitope is colored in red.

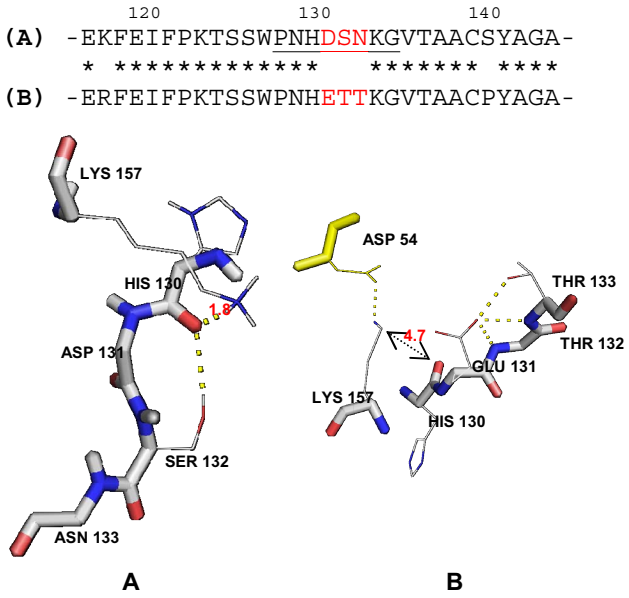

Figure 3. Influence of His^{130} side-chain on antibody binding of residue Lys^{157} in 2D1-epitope-containing HA complex (A) and 2D1-1918 HA protein complex (B). Differences in binding regions of epitope-containing HA and the 1918 HA protein is shown above. Matching residues are marked by asterisks. Mismatches in binding regions (131-133) are colored in red. The B-cell epitope is underlined. In the below panel, atom-based residue coloring: C (white), O (red), and N (blue). Backbones are presented by sticks. Side-chains are shown as lines. Interactions are presented as yellow-dotted lines. **A**. Internal contacts of Ser^{132} and His^{130} reduce the distance of His^{130} to Lys^{157} to 1.8 Å, thereby blocking binding of the antibody**. B**. Lys^{157} interacts with Asn^{54} of the antibody. The distance His^{130} is of 4.7 Å.

In the 2D1 antibody - 1918 HA protein complex, the antibody interacts with residue Lys^{157} of the HA protein. The residues Thr^{132} and Thr^{133} interact with the upstream Glu^{131} residue, pulling the His^{130} forward and creating a distance of 4.7 Å between the His^{130} and the Lys^{157}, therefore making the Lys^{157} free to bind to the antibody.

In case of the epitope-containing HA protein, it was noticed that (Fig. 3A) the Lys^{157} made internal polar contacts of 1.8 Å distance with the His^{130}. Epitope binding indirectly changes the side-chain orientation of His^{130} via Ser^{132}, thereby inhibits the interaction of Lys^{157} with the antibody in our docking experiment.

2) Docking with neutralizing antibody CR6261: Another docking of epitope-containing HA protein was performed with the neutralizing antibody (CR6261) of the 1918 H1N1 pandemic influenza. CR6261 differs from other antibodies because it does not interfere with the binding of virus to the host cell. The antibody inhibits the conformational rearrangement of the HA protein that is necessary for membrane fusion. The heavy chain of the antibody made contacts with the helical turns of the HA2 domains of the epitope-containing HA (Fig. 4) as it did in case of the 1918 H1N1 viral HA protein, confirmed by Ekiert et al. (2009) [4].

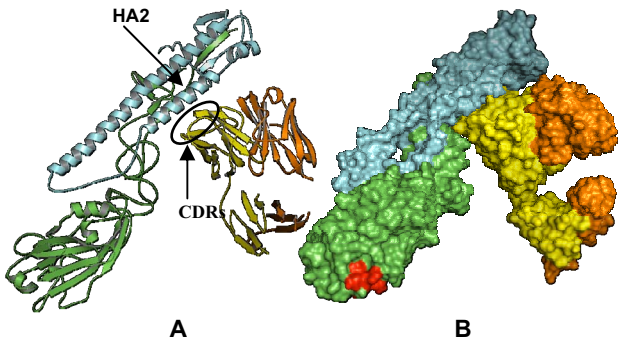

Figure 4. Binding of CR6261antibody heavy chain to helical turn of HA2 domain of the epitope-containing HA protein. The interaction is shown in ribbon (A) and surface presentation (B). HA1: green, HA2: cyan, heavy chain: yellow, light chain: orange, and B-cell epitope: red. The CDRs region was marked by the black oval.

Generally, docking results of neutralizing antibodies to the epitope-containing HA protein showed that the HA protein was inactivated by the CR6261 antibody; however, the predicted B-cell epitope was not involved in this binding. This suggests a possibility that future H1N1 strains with these mutations might escape an adaptive immune response. If the currently effective epitopes accumulate this mutation, existing vaccines are likely to become ineffective.

IV. Conclusion

Variability analysis of H1N1 2009 influenza A HA proteins highlighted their phylogenetic distinctness to other seasonal influenza and the WHO recommended vaccine strains. It is also genetically distant from HA subtype H14 which is inhibited by TBHQ. Therefore HA subtype H1 is not considered as a potential inhibitory drug target as confirmed by Russell et al [21].

The B-cell epitope was predicted using HA protein sequences of the current virus strains and it was assumed to have undergone an evolutionary mutation. In our study, it was shown that this B-cell epitope failed to be recognized by the antibodies. Intermediate neighbour contacts of the Lys157 residue make it unavailable for binding to the antibody. The docking results of the CR6261 antibody and the epitope-containing HA protein showed that the epitope was not involved in inactivating the function of the HA protein. Therefore, if the virus actually obtained this mutation, the antibodies could no longer recognize it. This finding could be useful for vaccine designs in later years.

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