Diversifying Selection Analysis Identifies Immune Epitopes Driving Evolution of Influenza A Virus in Humans using the Influenza Research Database (IRD, www.fludb.org)

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Introduction

Influenza A virus continues to be an important human pathogen due to its ability to undergo rapid antigenic evolution. Seasonal influenza thwarts pre-existing immunity induced by natural infection or vaccination through the process of antigenic drift, which involves the accumulation of amino acid substitutions in regions of the hemagglutinin (HA) protein that mediate antibody neutralization of viral infectivity.

Hypothesis: While a large number of immune epitopes in the influenza hemagglutinin (HA) protein have been defined using various experimental systems, we propose that only a subset of these epitopes are critical for protective immunity to natural infection in humans and that these can be identified by a data mining approach to identify diversified B-cell/Ab epitopes.

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Test Diversified B-cell/Ab Epitopes

- If these diversified B-cell/Ab epitopes are critical targets of protective immunity, then we would predict that sites mutated during evolution of the pandemic H1N1 lineage would be located in these epitopes
- Early 2009 pandemic outbreak isolates were compared with recent 2012-2013 and 2013-2014 flu season isolates using the meta-CATS statistical analysis tool3 to identify sites mutated during H1N1 pandemic lineage evolution

- 10 sites (vertical lines) in the HA protein were found to be significantly different between early and recent H1N1 pandemic isolates by meta-CATS
- 3 of these 10 sites (red lines) are co-located in diversified B-cell/Ab epitopes, including the Caton Sa and Sb epitopes

Structural Co-location

- The locations of B-cell/antibody epitopes, diversified sites and pandemic mutated sites were compared on the HA 3D protein structure separately for A) Caton Sa, B) IEDB epitope ID: 159269, and C) Caton Sb. Only the diversified sites and significant pandemic (pdm) mutation sites are numbered here.

- 16 sites that experienced diversifying selection as the pre-pandemic H1N1 HA sequences evolved were identified
- 11 of these 16 sites (vertical lines) were co-located in experimentally-determined B-cell/Ab epitopes (horizontal bars) - these sites have thus been termed diversified B-cell/Ab epitopes
- Even for amino acid sites that do not precisely overlap (red, yellow, blue), their physical proximity suggests that additional diversified or mutated positions could influence B-cell receptor/Ab binding

Phylogenetic Analysis

- Highlighting strains with mutated positions acquired during H1N1 pandemic evolution (red) in phylogenetic trees illuminates their persistence over time
- 3 mutated sites (114, 180, 202) were located in diversified B-cell/Ab epitope regions
- The remaining 7 mutated sites were either linked to these 3 sites (a, d), were located in functional sequence features that may influence influenza viral fitness (e, f) or showed weak evidence of selection due to the lack of temporal persistence (c, g)

Hemagglutination Inhibition (HI)

- Performed HI assay using antisera from 10 patients vaccinated with pH1N1 vaccine (A/CA/07/09)
- Amount of antiserum required to inhibit agglutination of late pandemic isolates is 3-4 fold higher (lower dilution) than the amount required to inhibit the early pandemic isolates

Summary

- Diversifying selection analysis identified B-cell/AB epitopes that are likely to be critical targets of protective immunity
- These diversified epitopes have been targeted for mutation during the evolution of the recent influenza A H1N1 pandemic lineage
- Antisera raised by vaccination against the early A/California/07/2009 isolate in humans show reduced reactivity to more recent isolates
- These findings have important implications for improved vaccine candidate strain selection

References