What swine flu can tell us about the evolution of influenza

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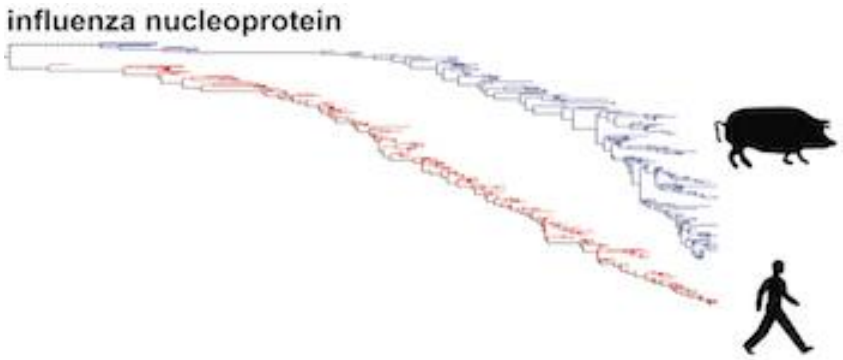

Phylogenetic tree of swine (blue) and human (red) influenza nucleoprotein.

Image provided by Heather Machkovech.

Viruses are constantly evolving to escape destruction by host immune systems. The host adaptive immune response uses antibodies as well as cytotoxic T-cells to combat viral antigens. The T-cell response is more general because T-cells recognize more conserved epitopes, or specific parts, of viral antigens. Despite an obvious pressure to evolve away from T-cell recognition, studies of human influenza virus have so far been unable to find any evidence for positive selection on epitopes that are recognized by T-cells compared to non-epitope residues. In order to further investigate this confusing observation, graduate student Heather Machkovech in the Laboratory of Dr. Jesse Bloom (Basic Sciences Division) conducted a comparative analysis of influenza viruses from human and swine lineages that was recently published in the *Journal of Virology*. The authors reasoned that swine influenza is unlikely to be under selective pressure from human T-cells and therefore provides a control for analyzing the evolution of the human virus. The idea that the evolution of epitopes on swine influenza viruses is random with respect to pressure from T-cells makes sense given the limited lifespan and thus limited number of infections pigs can experience. Furthermore, despite this difference in selective pressure, there is high homology between human and swine influenza proteins that are recognized by T-cells so the comparison is straightforward and informative.

To begin, the authors collected a list of epitopes that had been reported to elicit a response from CD8+ T-cells using Immune Epitope Database. Consistent with other reports, they found that the majority of epitopes that elicited responses were either in the nucleoprotein (NP) or the matrix protein (M1) of flu virus. They further classified each residue of the proteins with a value reflecting

how many unique epitopes that residue contributed to overall. Using these data, they confirmed that conventional methods of identifying positive selection, that use the ratio of non-synonymous (dN) to synonymous mutations (dS) as a metric for selection, do not provide evidence for positive selection in the T-cell epitope sites in NP or M1. Furthermore, they discovered that the number of non-synonymous changes at each site was not very different between epitope and non-epitope sites, suggesting that the substitution rate is not faster at residues contributing to T-cell epitopes. This can help explain the lack of evidence for change in dN/dS between epitope and non-epitope sites.

Although they found no evidence for an elevated (>1) rate of mutation at epitope vs. non-epitope sites, the authors noted an interesting trend in their data. The ratio of mutation rates at epitope vs. non-epitope sites is clearly higher in human vs. swine NP, with the human ratio being close to 1 and the swine closer to 0.5. This suggests that epitope sites are actually evolving faster in human NP compared to swine NP, even though the underlying difference in mutation rate between non-epitope and epitope sites within each viral lineage is minimal. To further test the statistical significance of this trend, they took into account the number of epitopes each residue contributed in a new statistic F, which they define as the average number of unique epitopes that are changed per substitution. They found strong statistical support ($p<0.0002$) to reject the hypothesis that epitope-changing mutations fix with equal probability between human and swine influenza virus. Instead, the probability of observing epitope-changing mutations was significantly elevated for human vs swine influenza, across the whole phylogenetic tree and especially in the "trunk" of the tree. Enrichment for epitope-altering mutations in the trunk of the phylogenetic tree of human influenza NP suggests that viruses that acquire those mutations have a wider evolutionary range.

This work identifies the CD8+ T-cell arm of the human adaptive immune response as a driver for the evolution of human influenza and informs further studies aimed at treatment and prediction of influenza infectivity. "Understanding how influenza escapes from our immunity is very important for determining which viral strains are likely to spread from year-to-year," said Dr. Jesse Bloom. "Studies in the past have focused mostly on how the virus escapes from the antibody arm of our immune system. Heather's work is the first to conclusively show that influenza also evolves to escape from the T-cell arm of the immune system." In the future, research in the Bloom Lab aims "to establish the extent to which the T-cell escape mutations that we identify enable certain viral strains to spread more effectively in the human population."

Machkovech HM, Bedford T, Suchard MA, Bloom JD. 2015. Positive selection in CD8+ T-cell epitopes of influenza nucleoprotein revealed by a comparative analysis of human and swine viral lineages. *Journal of Virology*. 89(22):11275-83. doi: 10.1128/JVI.01571-15.

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