Genetically distinct flu viruses grow better together in vitro

May 16, 2016

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Two influenza variants, differing only by one mutation, can cooperate to improve viral growth in cells in culture.

Image provided by Katherine Xue.

To replicate effectively, viruses must be able to enter and exit host cells. Neuraminidase (NA) is an enzyme on the influenza virus capsid that mediates exit from host cells by cleaving the virion from receptors on the host cell surface. A specific amino acid residue in the NA enzyme catalytic site, aspartic acid 151 (D151), has been shown to be required for cleavage activity. Interestingly however, different amino acids have been observed at this position in H3N2 influenza viruses sequenced since 2007. The point mutations D151G and D151N, as well as an "ambiguous" nucleotide at that position, have been documented in the GISAID EpiFlu database since 2007. Isolated strains of influenza from clinical samples are usually passaged through cells cultured *in vitro* to grow enough virus for sequencing. Because having a glycine (G) or asparagine (N) rather than aspartic acid (D) at residue 151 of NA should prevent cleavage activity and prevent the virus from exiting cells, scientists have suggested that the increased frequency of the G151 and N151 flu variants is likely an artifact of passaging the virus *in vitro*. However, researchers in Dr. Jesse Bloom's laboratory (Basic Sciences) hypothesized that genetically distinct flu viruses might work together to improve viral growth. Their research, recently published in *eLife*, provides evidence that cooperation occurs when two particular viral variants are grown in cell culture. It also suggests that cooperation between viruses could be an unappreciated facet of influenza evolution and infection in natural populations.

The scientists genetically engineered strains of human H3N2 virus with either aspartic acid or glycine (D or G) at position 151 of the NA enzyme. They inoculated human cells in culture with pure populations of either variant alone or a mixture of the two viral variants and they measured viral titer (growth). Interestingly, they found that inoculating cells with a mixture of D151/G151 viruses led to higher viral titers than those seen with either variant on its own. If the two H3N2 variants cooperate, one would expect a stable proportion of the two to appear and then be maintained over time. Thus the scientists performed serial passaging experiments with pure D151, G151, and mixed D151/G151 viruses and sequenced viruses after each passage. They found that cells initially infected with D151 virus alone accumulate G151 viruses over time and vice versa for cells starting with G151 virus. When beginning with a 50/50 mixture of D151/G151, the researchers observed that the 50/50 proportion of D151 and G151 was maintained over time, through 5 serial passages. It is of note that the G151 variant did not continue to increase in frequency through serial passage, suggesting it is not simply an adaptation to the artificial lab conditions.

Each influenza virus can only have one copy of the neuraminidase (NA) gene. Therefore, the scientists knew that if cooperation among viruses was actually happening, it should depend on the concentration of viruses, called multiplicity of infection (MOI). In agreement with this, the scientists found that during an infection with low MOI (fewer viruses), the mixed D151/G151 viruses grew indistinguishably from pure D151 H3N2 viruses.

Given the appearance of the G151 NA variant in H3N2 viruses since 2007, the scientists hypothesized that compensatory mutations in other viral genes led to the rise of G151 NA. They postulated that the G151 NA variant, which should bind but not cleave host cell surface receptors, might be adaptive for viruses with mutations that weaken receptor binding. Therefore, they cloned the sequence of the influenza receptor binding protein hemagglutinin (HA) from H3N2 viruses isolated in 2005 and 2007. As before, a mixture of D151/G151 NA viruses had enhanced viral growth when the viruses had the 2007 HA sequence. Intriguingly however, they found that the mixture of D151/G151 NA viruses did not grow better than the D151 variant in combination with the HA sequence from 2005. Finally, to test whether a 50/50 mixture of D151/G151 virus could perform both the receptor binding and cleaving functions of H3N2, they measured whether this population of viruses could grow when the HA protein could not bind receptors. Strikingly, they found that the D151/G151 NA virus population could grow well even in the absence of functional HA, while pure populations of either D151 or G151 NA virus could not grow when HA was inactivated.

So why don't we see more mutations at position 151 of NA in unpassaged flu viruses? The scientists hypothesize that the sensitivity of sequencing is not always sufficient to detect low-copy

variation in the population. To this end, they are "working now on sequencing flu from patient samples to try to understand whether cooperation is something that occurs in clinical infections," said author Katherine Xue.

This research was funded by the National Institute of General Medical Sciences (NIGMS), the National Science Foundation (NSF), and the Hertz Foundation.

Xue KS, Hooper KA, Ollodart AR, Dingens AS, Bloom JD. 2016. "Cooperation between distinct viral variants promotes growth of H3N2 influenza in cell culture." *eLife*. 2016; 5:e13974.