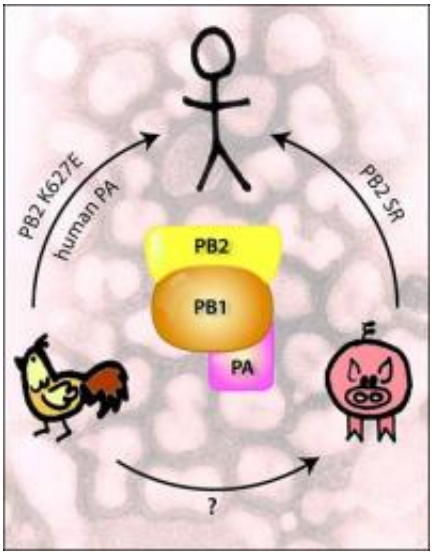
H1N1 influenza adopted novel strategy to move from birds to humans

December 8 2009, By Robert Sanders



The sequence of the three subunits of the influenza virus polymerase (center) determines whether or not the enzyme works efficiently in birds, pigs or humans. A mutation in the PB2 subunit allows the bird virus to function in humans, as does switching out the bird PA subunit for a human PA subunit. Two mutations in the PB2 subunit of 2009 H1N1 allow the pig virus to work in humans. The background is a false-color electron micrograph image of influenza virions. (Andrew Mehle/UC Berkeley)

(PhysOrg.com) -- The 2009 H1N1 influenza virus used a new strategy to cross from birds into humans, a warning that it has more than one trick up its sleeve to jump the species barrier and become virulent.

In a report in this week's early online edition of the journal Proceedings of the National Academy of Sciences, University of California, Berkeley, researchers show that the H1N1, or swine flu, virus adopted a new mutation in one of its genes distinct from the mutations found in previous flu viruses, including those responsible for the Spanish influenza pandemic of 1918, the "Asian" flu pandemic in 1957 and the "Hong Kong" pandemic of 1968.

Previous influenza strains that crossed from birds into people had a specific point mutation in the bird virus's polymerase gene that allowed the protein to operate efficiently inside humans as well. The polymerase transcribes the virus's RNA, allowing the host to express viral genes, and also copies the viral genome, needed to make new viruses.

The 2009 H1N1 virus retains the bird version of the polymerase, but has a second mutation that seems to suppress the ability of human cells to prevent the bird polymerase from working.

"We were quite shocked when we looked at the swine flu virus, which was clearly replicating in people and other mammalian systems, yet had a polymerase that looked like it was derived from a bird virus, which should not function too well in a human cell type," said UC Berkeley post-doctoral fellow Andrew Mehle of the Department of Molecular and Cell Biology. "The other mutation within the polymerase seems to compensate and allow the enzyme to function."

The researchers also discovered another strategy - one not yet adopted by any known flu virus - by which influenza virus can increase its virulence even more. When a particular human subunit is substituted for one of the three protein subunits that make up the bird polymerase, the new combination makes the polymerase more efficient in human cells.

"This is an extremely rare mutation and a rare combination, which

suggests that there may be other ways that haven't emerged yet that these viruses are going to continue to evolve," said Jennifer Doudna, UC Berkeley professor of molecular and cell biology and an investigator in the Howard Hughes Medical Institute.

"As mechanistic biologists, we are hoping that by understanding how the virus works at the molecular level, we will be able to predict with more accuracy how it will evolve."

She suggested that those monitoring influenza outbreaks around the world in search of new variants be on the lookout for this recombination of polymerase subunits, which could herald an uptick in swine flu virulence. The findings also could help scientists develop better antiviral treatments, Mehle and Doudna said.

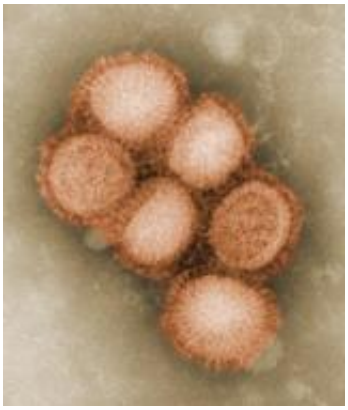
"The more we can understand the biochemistry and the particular structure of these polymerase complexes, the better we can make rational decisions about drug development," Mehle said.

H1N1, which appeared on the scene earlier this year, was dubbed swine flu because it emerged from pigs, in which human, bird and pig influenza viruses mixed, swapped genes and gave rise to a variant that could infect human cells and reproduce.

While mutations in the surface protein hemagglutinin - indicated by the H in H1N1 - are key to allowing the virus to enter human cells, mutations in the polymerase enzyme are key to the virus's ability to replicate inside human cells. All previous flu strains that entered and were transmitted in humans had a single mutation in the second subunit of the bird polymerase gene, which apparently allowed the enzyme to operate in human cells.

Last year, Mehle and Doudna showed that human cells apparently

prevent the three subunits of bird virus polymerases from assembling into a functioning enzyme. A single amino acid switch at position 627 on the second subunit of the polymerase overcomes that inhibition and allows the virus to replicate. Apparently, Mehle said, when the amino acid glutamic acid - typical of most bird virus polymerases - is changed to a lysine, typical of human polymerases, the surface charge of the subunit changes from acidic (negatively charged) to basic (positively charged) and allows assembly of the subunits. Previous studies in mammals have shown that a lysine in that position enhances polymerase activity, increases viral replication and transmission, and in some cases, is associated with increased pathogenicity and death.



A mutation in the H1N1 influenza A virus -- a pair of amino acid variants termed the 'SR polymorphism' -- was found to enhance replication of the virus in humans. Credit: Image courtesy of NIGMS

In their new study, Mehle and Doudna found that H1N1 has two rare mutations in the second subunit: a serine at position 590 and an arginine at position 591. This combination, which is most common in pigs, apparently has the same effect on surface charge as the mutation at position 627, allowing the polymerase complex to form and function in

human cells.

Mehle noted that, in addition to such point mutations, flu viruses also mix and match the three subunits. Both the 1957 and 1968 viruses had polymerases composed of a first subunit from a bird and the other two subunits from humans. H1N1 has a human-like first subunit, while the second and third are bird-like - hence the need for a mutation in the second subunit to make it more human-like.

To see which other combinations might make H1N1 more virulent, they mixed human, avian and pig subunits in culture, replicating the pig "mixing vessel," Mehle said. Several combinations with a human third subunit increased the activity of the polymerase enzyme when other mutations were not present in the second subunit. Viruses with this alteration are now being tested in human cell culture to see if they are more virulent.

"In addition to having individual amino acid changes affecting the ability of the virus to transmit across species and be more pathogenic, we need to think about these entire gene segments being exchanged back and forth," said Doudna, who also is a faculty affiliate of the California Institute for Quantitative Biosciences (QB3). "Those will affect the outcome of disease."

"We are very hopeful that the kind of basic science that we are doing here will have an impact on human health, either at the level of diagnostics or thinking forward to development of antiviral therapeutics," she added.

Mehle and Doudna continue to explore the polymerase to discover what in human cells prevents the assembly of the bird polymerase, and to determine the three-dimensional structure of the enzyme and its three subunits.

Source: University of California - Berkeley (news : web)