

SEPTEMBER 16, 2005

Learning How SARS Spikes Its Quarry

Researchers have determined the first detailed molecular images of a piece of the spike-shaped protein that the SARS virus uses to grab host cells and initiate the first stages of infection. The structure, which shows how the spike protein grasps its receptor, may help scientists learn new details about how the virus infects cells. The information could also be helpful in identifying potential weak points that can be exploited by novel antiviral drugs or vaccines.

The SARS (severe acute respiratory syndrome) coronavirus was responsible for a worldwide outbreak in 2002-2003 that affected more than 8,000 people and killed 774 before being brought under control. Public health experts worry about another outbreak of the virus, which originates in animals such as civet cats.

The research team, led by Howard Hughes Medical Institute investigator Stephen C. Harrison at Children's Hospital and Harvard Medical School, and colleague Michael Farzan, also at Harvard Medical School, reported its findings in the September 16, 2005, issue of the journal *Science*. Lead author Fang Li in Harrison's laboratory and Wenhui Li in Farzan's laboratory, also collaborated on the study.

"One of the critical issues in a SARS epidemic would be to predict whether a given variant of the virus will jump species or move laterally from one human to the other."

- Stephen C. Harrison

According to Harrison, prior to these studies, researchers knew that one of the key steps in SARS infection occurs when the virus's spike protein attaches to a receptor on the surface of target cells. Attachment of the spike protein permits the virus to fuse with a host cell and inject its RNA to infect the cell.

A detailed understanding of how the spike protein complexes with its receptor, ACE2 (angiotensin-converting enzyme 2), could have important clinical implications. “The interest in understanding this complex has to do with the fact that this virus jumps from animals to humans, laterally among humans, and in some cases from animals to humans but without subsequent human-to-human transmission,” said Harrison. “And we know that those modes of transmission depend on specific mutations in the spike protein that affect spike-receptor interaction.

“One of the critical issues in a SARS epidemic would be to predict whether a given variant of the virus will jump species or move laterally from one human to the other. Understanding the structure of this complex will help us understand what these mutations in the spike protein mean in terms of infectivity,” Harrison said.

According to Harrison, Farzan and his colleagues laid the scientific groundwork for determining the structure of the spike-ACE2 complex. In 2003, Farzan's team discovered that the ACE2 protein is the receptor for the SARS virus. They also identified a specific fragment of the spike protein that is involved in viral attachment.

As a result of those studies, researchers in Harrison's and Farzan's laboratories could concentrate their efforts on creating crystals of the relevant fragments of the spike protein in complex with the ACE2 receptor. After they had crystallized the protein complex, the crystals were then subjected to structural analysis using x-ray crystallography. In this widely used technique, x-rays are directed through crystals of a protein. The resulting diffraction pattern is analyzed to deduce the atomic structure of the protein or protein complex under study.

The x-ray structure revealed that the spike protein fragment showed a slightly concave surface that fits a complementary surface on the receptor, said Harrison. There was nothing surprising about the interaction itself, he noted. However, the studies revealed important new information about two specific amino acids on the spike protein. These were the amino acids that Farzan and his colleagues had previously determined to be the most critical for determining how the SARS virus adapted from infecting only civets to infecting humans.

“Both of these critical amino acids turned out to be right in the middle of the interface between the spike protein and the receptor,” said Harrison. Thus, the structure reveals details about how even small mutations in the spike protein gene that alter the identity of amino acids at those sites can affect the virus's ability to infect humans. Such mutations enable viral transmission by altering the shape of the spike protein, which affects how well it binds to the ACE2 receptor, explained Harrison. In particular, he said, the new structure shows how mutation at one of the two sites can enable the animal SARS virus to infect humans, but by itself this mutation does not appear to allow subsequent human-to-human transmission.

“The observation is that a dramatic epidemiological difference can result from what looks like an almost trivial mutation,” said Harrison. “These findings give us the beginnings of information needed—if a new virus were isolated—to make predictive guesses about infectivity, so that we can better give advance warning.”

He also noted that laboratory studies indicate that the fragment of the spike protein they used could provide the basis of a vaccine against SARS, since it appears to be recognized by the immune system of the host.

In future studies, Harrison and his colleagues plan to explore the steps that occur after the spike protein attaches to the receptor. The researchers know that the spike protein undergoes a conformational change that enables the virus to fuse with the host cell.

“When there's a conformational change, it gives you an opportunity to explore the possibility of antiviral therapeutics,” said Harrison. “When you have two conformational structures, you can think about how to prevent infection by inhibiting the transition from one state to another.”