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Combing Through HIV's Family Tree for Ways to Block "Immune Escape"

In the battles that rage between the human immunodeficiency virus (HIV) and an infected patient's T cells, the rules of engagement are always changing. The T cells adapt continuously to recognize HIV proteins and alert the immune system to launch an attack. But the virus perseveres because it has an exceptional capacity to mutate - to take on mutant forms that can escape immune surveillance.

Immune escape renders HIV more dangerous because the virus cannot be reigned in by the immune system. As researchers work toward developing a vaccine that will assist the immune system in its battle against HIV, they want to know exactly which mutations enable the virus to evade detection.

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— **Bruce D. Walker**

Researchers had initially believed that by cross-sectionally analyzing mutations in HIV one could define those that had arisen as a result of immune escape, and pinpoint regions of the viral genome that are important for recognition by T cells.

New research by a team of scientists that includes Howard Hughes Medical Institute investigator Bruce Walker has now established that the accuracy of this type of analysis can falter due to the many subtypes of HIV that circulate globally. This is because some of the mutations represent historical subtype or lineage differences rather than mutations that have arisen as a result of immune selection pressure.

Walker and his colleagues have found that identifying the presence of these multiple lineages of HIV can greatly improve the accuracy of the genetic analyses. Furthermore, statistical methods for elucidating such phylogenetic relationships among viral genome sequences will give virologists new

insights into the evolution of viruses and how viruses alter themselves as they adapt to the immune system.

The researchers published their new approach in the March 16, 2007, issue of the journal *Science*. Bette Korber of Los Alamos National Laboratory and the Santa Fe Institute was the senior author of the article. Walker is at the Partners AIDS Research Center of Massachusetts General Hospital, Harvard Medical School. Other co-authors were from Microsoft Research, the University of Washington and Royal Perth Hospital in Australia.

In a previous study, co-author Simon Mallal and colleagues at Royal Perth Hospital revealed that viral genetic sequences isolated from a group of patients in Perth showed clear evidence for host-driven immune selection during infection.

That was a seminal study, because it showed that the biology of the human immune system was impacting how HIV was evolving because of these immune genes, said Korber. But the methods used didn't take into account the possibility that different lineages of virus might be present, and might affect the analysis, she said.

The challenge for HIV vaccine design is to determine the precise mutational pathways the virus uses to escape detection, Walker said. Existing methods did not differentiate between actual immune escape and historical differences in the HIV subtypes.

In the new study, Korber, Walker and their colleagues reanalyzed the Perth data using a new statistical technique that could trace how multiple genetic subtypes of the virus had evolved in individual patients. Taking these lineage effects into account, said Korber, led to a very different portrait of the specific nature of the immune selection than they had reported in the original paper.

The researchers focused on the viral genes for two HIV proteins. The genetic portrait that emerged from those experiments revealed that the sample — formerly believed to be homogenous — actually contained more than one genetic subtype of the virus. The presence of multiple subtypes would compromise the accuracy of the genetic analysis, said Korber and Walker. Even within these subtypes, the researchers found genetically distinct subclusters that would further compromise the accuracy of genetic analyses of immune escape. The new methodology should improve the accuracy of future analyses by taking into account phylogenetic relationships among viral genetic sequences, they said.

These new bioinformatics techniques, together with functional immunology data being generated in real time as the epidemic expands, will be critical for HIV vaccine design to deal with the tremendous variability of the virus, said Walker.

This methodology is generally applicable to other viruses and to other HIV studies, said Korber. For example, my colleagues and I are now using it to

ask whether at the moment of transmission, there are genetic differences in the transmitted virus, compared to the virus that exists in a chronic infection. All of this kind of information can help vaccine developers make informed decisions about what components need to be included in a vaccine to give maximum population coverage.

Korber said the new analysis also indicated that the same genetic characteristics of HIV that may enable the virus to escape immune detection in one person may make the virus susceptible to detection in another person. Such insights will also be important in formulating vaccines with sufficient variation to be maximally effective in many people, she said. If you have those variants in a vaccine, you might not only be able to get better population coverage in the first place but you might be able to block common immune escape routes, she said.

This project is a great example of people from multiple disciplines working together to gain new insight into a disease that is devastating large areas of the globe, said Walker.

Korber and her colleagues are now applying their method to analyze the genes for all of the proteins produced by the HIV genome, besides the two reported in the *Science* study, to gain further insights into the machinery of immune escape.