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How HIV "Exhausts" Killer T Cells

American and South African scientists working at the epicenter of the AIDS epidemic in South Africa have discovered how the human immunodeficiency virus (HIV) "exhausts" killer T cells that would otherwise attack the virus. The researchers found that HIV can simply turn off fully functional T cells by flipping a molecular switch on the cells. In test tube studies, however, the scientists showed that they could reinvigorate the killer T cells by blocking that inhibitory switch, which is called programmed death-1 (PD-1).

The study's senior author, Bruce Walker, a Howard Hughes Medical Institute researcher at Massachusetts General Hospital, said that clinical testing of drugs that block the PD-1 switch could begin very soon, since such drugs exist already. However, he cautioned that these kinds of drugs could cause serious side effects, including autoimmune reactions that trigger the immune system to attack the body. Walker added that the researchers' findings will also likely have application in understanding other chronic viral diseases.

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— **Bruce D. Walker**

The findings by Walker and his colleagues were published in an advance online publication on August 20, 2006, by the journal *Nature*. Walker is also at the Partners AIDS Research Center and Harvard Medical School. Other co-authors were from the University of KwaZulu Natal in South Africa, Oxford University, Dana-Farber Cancer Institute of Harvard Medical School, Emory University School of Medicine, and The Wistar Institute.

It's long been known that people with HIV infection have a lot of HIV-specific immune cells that one would think would be actively combating the virus, said Walker. But a major puzzle has been that even in late-stage illness, when one can still measure great numbers of these immune cells, they don't seem to be controlling the virus at all.

An important clue to why killer T cells stop functioning after infection came from earlier studies in mice, which were published by co-author Rafi Ahmed of Emory. Ahmed found that chronic viral infection actively inhibits T cells by switching on the cells' inhibitory PD-1 pathway. He also found that blocking the PD-1 pathway in the mice restored T cell function and reduced the amount of virus in the animals' blood, which is known as viral load. Ahmed's studies showed that blocking PD-1 rejuvenated killer T cells, which directly attack viruses, but did not investigate helper T cells, which mobilize the immune system against infection.

Ahmed's findings prompted Walker and his colleagues to explore the role of the PD-1 pathway in a large population of HIV-infected patients in the KwaZulu-Natal province of South Africa. The province is at the epicenter of the HIV epidemic in sub-Saharan Africa; a third of pregnant women there are infected with HIV. The researchers conducted much of their work at the Doris Duke Medical Research Institute in Durban. Durban is the largest city in KwaZulu-Natal, and HIV prevalence among pregnant women in the city exceeds 50 percent.

The studies at the lab in South Africa found that PD-1 was significantly activated in 71 HIV-infected patients who had not yet begun antiviral treatment. In a separate study, the researchers demonstrated that PD-1 is also activated in the T cells of people with Epstein-Barr virus, which is a persistent infection, but much lower on T cells of persons immunized with vaccinia virus, a live virus vaccine against smallpox that is effectively cleared by the immune system. According to Walker, those findings indicate that activation of the PD-1 pathway occurs during the immune system's general response to viral infection.

In test tube studies, the researchers showed that higher PD-1 expression was associated with more severe functional exhaustion of HIV-specific killer T cells. The studies clearly demonstrated that the greater the PD-1 expression, the higher the patient's viral load and the lower the count of helper T cells, said Walker.

When they compared PD-1 activation in blood samples from four patients before and after antiviral therapy, they found that PD-1 expression dropped when treatment began. It became very clear from this analysis that the virus was actually driving these high levels of PD-1 expression, and that you could actually change the PD-1 level by getting rid of the viral antigen with drugs, said Walker. An antigen is a protein on a virus or other infectious agent that triggers an immune reaction.

Walker said one of the team's key findings emerged from their test tube studies showing that blocking the PD-1 pathway could restore the function of exhausted killer T cells. We wanted to determine whether these T cells had been irreparably damaged or misprogrammed, he said. And we found that they are capable of functioning; they've just been turned off.

Similarly, the researchers also found that blocking PD-1 restored helper T cell function. This was the most striking finding, because the majority of

patients we have studied had no detectable levels of these HIV-specific cells; but as soon as we blocked the PD-1 pathway, they had a ton of them, said Walker.

The researchers' discoveries could lead to immediate clinical application, although Walker cautioned against over-optimism. Obviously, the big question is whether you could manipulate this pathway in HIV-infected people to turn these T cells back on and better control the virus, he said. And drugs to block this pathway have already been developed for cancer, so that question should be able to be addressed in the very near future.

However, one has to proceed with real caution, because if you turn back on an immune regulatory switch that the body has decided to turn off, you could trigger serious immunological problems such as autoimmunity, Walker noted. And while an ideal clinical strategy would be to seek to switch off PD-1 only in HIV-specific T cells, techniques for such a targeted approach do not exist, he said.

The researchers are also exploring PD-1 measurement as a diagnostic tool, said Walker. Currently, we just count the number of helper T cells to decide when to treat someone, but we are excited about the possibility that adding PD-1 measurement might tell us more about the likelihood of progression of the disease and need for treatment in infected people, he said.

Walker said that scientists know little about the evolutionary purpose of a pathway that would deactivate such an important component of the immune system in the face of a viral attack. It may be that in the heat of a battle that it sees it's not winning, the body makes an adjustment to try to co-exist, he said. But we really do not know at this point.

Walker emphasized that the study could not have been done without his group's ongoing collaboration with the University of KwaZulu Natal. These are studies that simply could not have been done in the United States, he said. Despite the fact that these patients in Africa are living in poverty and in rural areas, it is easier for us to obtain their cooperation in such studies than with patients in the United States.

It was with the key support of the Doris Duke Charitable Foundation and the Nelson Mandela School of Medicine that we have been able to build a biomedical research institute and develop this rich international collaboration that enables us to study the HIV epidemic at its epicenter, to gain knowledge that we could not obtain anywhere else, he said.