Chasing HIV from Its Hiding Place

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Here's one truth about HIV: once infected, always infected. Antiretroviral therapy holds back active virus to undetectable levels but never rids the body of HIV. If people stop antiretroviral drugs, the virus returns in full force, not from new infection but from pools of HIV hidden in plain sight in memory T cells, the very immune defenders designed to fight infection. These so-called latent reservoirs stow away the virus in chemical configurations that antiretroviral therapy cannot touch.

Even though HAART (highly active antiretroviral therapy) allows people to live with AIDS, there are drawbacks. It's a chronic therapy that requires strict compliance. A person might miss one day of medicine and suffer reinfection. People on antiretroviral drugs for long periods need to switch drugs because the virus develops resistance. A recent study shows that the health consequences of HAART can include increased risk of myocardial infarction (Lang et al., 2010). Worldwide, billions of dollars are being spent on HAART. Even with the flow of money, fewer than half of the people in Africa who need antiretroviral drugs receive the therapy.

"We're facing a financial reality that we knew would happen. We cannot sustain the lifelong treatment of tens of millions of people with antiretroviral therapy throughout the world. This drives home that we need to get individual persons off of drugs, but can we cure AIDS?" asks Warner Greene, M.D., Ph.D., Director, Gladstone Institute of Virology and Immunology.

The answer to that question may not be far-fetched; for example, about 0.1% of HIV-infected people spontaneously control the virus to almost undetectable levels. Scientists are trying to figure out how this happens, but the fact that "elite controllers" exist offers good news. If scientists could find a way to reduce the size of latent reservoirs more people could come off medications. This "functional cure" wouldn't eradicate HIV but would allow the immune response to control what's left of the virus.

For such a plan to work, researchers would need to give people with HIV a compound that wakes up memory T cells harboring latent virus. Once activated, the cells turn into virus factories and die within 24 hours. Meanwhile, the antiretroviral drugs would prevent reinfection and thus avert any new reservoirs of latent virus.

Stanford's Paul Wender, Ph.D., says, "There's been a whole lot of pessimism over the last five years that this is just too big a problem and we won't make headway. But with failure of the vaccine candidates, more and more effort and attention has focused here. I think there's cause for optimism."

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Other scientists share Wender's enthusiasm. At least one compound shows potential for activating memory T cells, and researchers have good primate models for latency.

Terrorism at a Cellular Level

Early clues to latent viral reservoirs came in the 1980s on the heels of antiretroviral therapy. The drugs slashed HIV to undetectable levels, so people thought they were free of disease and stopped therapy. Within 4–6 weeks, the virus returned full force. Clearly, there had to be some source of disease that was not active virus because the therapy kills off active versions of HIV. "That's when people started to think about latency and realized this gets encoded in the genome of certain types of cells," says Wender.

Tae-Wook Chun, Ph.D., of Anthony S. Fauci's lab at the National Institutes of Health, began studying latency as part of his doctoral thesis work. In two studies published in 1997, he found the latent virus in memory T cells of HIV-infected people and provided an estimate of the number of latent cells present in any given individual (Chun et al., 1997a, 1997b).

The percentage of memory T cells that harbor HIV is relatively small, about 1%. Although the frequency is low, HIV needs only one cell that gives rise to infectious virus. That's why HIV proves so hard to eliminate. Unlike cytomegalovirus, an example of a similar virus, HIV does not have a gene that codes for latency. At some point during an HIV infection, a number of HIV-infected cells that were pumping out lots of virus survived and returned to a resting state. That's how the immune system works. Memory T cells are trained to "remember" antigens to which they were exposed so that they can respond to future encounters with the same antigen.

At this point, though, HIV is embedded into a chromatin structure that is not transcribed. Without transcription, DNA is not expressed, so no viral proteins appear on cell membranes and mark the cell for immune destruction, nor do infected resting T cells produce any virus. Viral replication, however, resumes efficiently once the same cell is activated upon meeting a specific antigen it's trained to recognize. In HIV, the latent virus slowly leaks out with a half-life estimated to be 45 months; more than 50 years would be needed to deplete reservoirs, says Wender. That's why researchers want to activate these cells with a drug that quickly depletes the virus. Unfortunately, the virus can become latent before, during, and after transcription.

Mauro Giacca, of Italy's International Centre for Genetic Engineering and Biotechnology, recently showed the mechanism for HIV pretranscription latency (Manganaro et al., 2010). Scientists

knew that HIV was inefficient at infecting nonactivated T cells, but they never knew why. Giacca's recent *Nature Medicine* paper shows that integrase, the enzyme that integrates proviral DNA into the host cell genome, doesn't work in nonactivated T cells, because such cells lack the kinase to phosphorylate integrase. Without phosphorylation, integrase can't undergo the change in shape that allows HIV to integrate into the cell's DNA.

Gone for Good?

That's why HIV researchers are so keen to find a way to activate resting T cells. One such agent under investigation is prostratin, a plant-derived small molecule that comes from the bark of a tree found in Samoa. Wender's group at Stanford found a way to synthesize prostratin in the laboratory and published the results in *Science* (Wender et al., 2008). Now, Wender is working with many groups to study the prostratin analog, including the NIH and the AIDS Research Alliance (http://www.aidsresearch.org), a not-for-profit clinical group that acquired rights to the technology developed by Wender and colleagues that allows AIDS researchers to synthesize prostratin. "We can modify prostratin in a way that gets rid of the side effects and increases beneficial effects. Now we have compounds that work much better than prostratin," says Wender.

In unpublished work, Wender's group took blood from patients with HIV/AIDS on HAART therapy. In cell cultures, prostratin converted latent virus formation to the active virus and purged the viral reservoirs. This work so far has been done in primates and in laboratory cultures and not in humans. Wender hopes to conduct phase I clinical trials in the next two years or so that will test the drug's safety.

At the NIH, Chun and colleagues are also testing prostratin. However, Chun has already tried a similar strategy with IL-2, a potent stimulator of memory T cells. In the late 1990s, Chun's group gave IL-2 to people with HIV. The treatment worked. Latent T cells in HIV patients who received IL-2 declined so much that HAART therapy was stopped. Unfortunately, the virus rebounded in every patient (Chun et al., 1999). Chun worries the same thing will happen with prostratin or with similar agents. Eradicating the virus means getting every last memory T cell activated and every last one of those cells has to die, and the spread of virus produced by those cells has to be stopped before HIV infects any new cells.

Even that may not suffice. More and more evidence shows that HIV reservoirs can be found all over the body: in tissues such as the gut and perhaps the brain. New evidence shows that hematopoietic stem cells can shelter HIV (Carter et al., 2010). "Our knowledge about the extent of these reservoirs is limited," says Chun. That's why Chun says using one agent to purge latent reservoirs may be a long shot. He suggests a strategy that tackles all three forms of latency simultaneously. Even if this strategy lowers the HIV burden as much as 1 log, HIV likely won't be eradicated. For a functional cure, the immune system will need to keep any pools of the virus in check. Proof will come later when patients stop therapy and the virus doesn't rebound for many months or years.

"You have to remember that we tried IL-2 and other groups have tried anti-CD3 antibody, an even more potent T cell activator. All cases failed to eradicate HIV," says Chun. "You have to look at all of these things very carefully, but I think it has to be done."

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