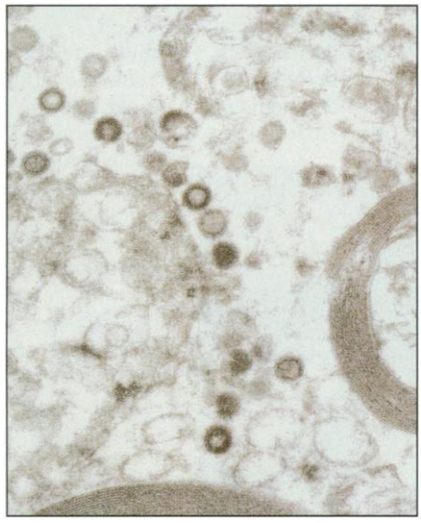
AIDS THERAPIES

Exploring How to Get at—and Eradicate—Hidden HIV

DEDHAM, MASSACHUSETTS—Arthur Ammann, head of the American Foundation for AIDS Research (AmFAR), placed a small placard next to a slide projector sitting in the back of a lecture hall at a small scientific meeting he co-organized 2 weeks ago. "Don't even think about it," the placard warned the three dozen scientists who attended the weekend gathering in this quaint Massachusetts town.

Ammann's sign was simply a warning to participants that the meeting was supposed to be a slide-free, discussion-heavy think tank. But 3 years ago, that same sentiment—don't even think about it—would have applied equally well to the topic of the conference* itself: how to eradicate HIV from an infected person. Now, the remarkable success achieved by various combinations of anti-HIV drugs—called highly active antiretroviral therapy, or HAART—has allowed researchers not only to think about whether the last bits of virus can be eliminated from the body, but also to speculate about how it might be done. "Up until now, we've been talking about an ocean of HIV in patients," said Richard Kornbluth, a researcher at the University of California, San Diego, who specializes in HIV infection of scavenger white blood cells called macrophages. "Now it's like the tide has gone out with HAART."

Despite this sea change, however, discussions at the meeting made it clear that eliminating HIV from the body may be every bit as difficult as attempting to roll back the ocean itself. The main obstacle: Even in people whose viral load is "undetectable," the virus continues to lurk in cells throughout the body. It hides out, under the immune system's radar and out of reach of antiviral drugs, in "latently" infected blood cells and in sites such as the brain, eye, and testes.



JAN MARC ORENSTEIN/GEORGE WASHINGTON UNIVERSITY

Gray matter. There are no black-and-white solutions to ridding the brain of cells like these HIV-producing glial cells.

"The issue is answering the question: Is eradication possible?" said David Ho, head of the Aaron Diamond AIDS Research Center in New York City. He and others proposed several strategies, including injecting people with immune system messengers that could flush out the virus so that the patient's own immune system could pick it off and looking for ways to sneak drugs past borders such as the one that protects the brain. "Whether you succeed or not," said Ho, "you're going to learn a lot of science."

The hiding place that should be the most vulnerable to current treatments, Ho thinks, is latently infected blood cells, which have the viral genome woven into their own but do not actively produce new copies of the virus. Keeping patients on HAART to hold the virus at bay and waiting for the latently infected cells—which include macrophages and T lymphocytes—to simply die off is one approach. Using mathematical models recently developed by his team, Ho calculates that the macrophages would die off first, leaving the more stubborn T cells around for—at a minimum—5 years. That's a long time to be on HAART, which requires taking dozens of pills a day and also can cause serious side effects.

Other results suggest that Ho's estimates about macrophages may be too optimistic. Donald Mosier, a mouse researcher at The Scripps Research Institute in La Jolla, California, has found that HIV infection somehow lengthens the life-span of macrophages in mice. And at the meeting, Suzanne Crowe from Australia's Macfarlane Burnet Center for Medical Research revealed that she still finds latently infected macrophages in "undetectable" patients who have been on HAART for up to a year. "It may well be that the macrophage is one of the toughest nuts we have to crack when it comes to eradication," said Crowe (*Science*, 29 November 1996, p. 1464).

Battle plans. Instead of simply waiting for the latently infected blood cells to die off, some researchers proposed a more active approach. The idea is to compel latently infected cells to produce HIVs, drawing the attention of the immune system so that it can destroy the infected cells. HAART, meanwhile, would theoretically prevent any new HIV that was "flushed out" from infecting virgin cells.

Latently infected T cells might be flushed, proposed Aaron Diamond's Martin Markowitz, by injecting patients with the mouse antibody OKT3, which specifically tells T cells to copy themselves. Latently infected macrophages might be unmasked, others suggested, with immune system messengers like granulocyte-macrophage colony-stimulating factor (GM-CSF), tumor necrosis factor, and interleukin-12, all of which stimulate macrophage reproduction.

Harvard immunologist Abul Abbas suggested yet another strategy, aimed at the whole class of quiescent T cells that can harbor latent virus. "Identify stimuli that are particularly good at keeping these memory cells alive and then inhibit them," said Abbas. He gave the example of interleukin-15 (IL-15), which in mice blocks the "death by neglect" that memory cells usually undergo if they don't receive growth stimulation. Theoretically, then, blocking an IL-15–like substance could speed the death of memory cells.

On the road to eradication, the last hairpin turn requires stopping HAART. It's possible that if there are still tiny amounts of virus left, the person's immune system can then mop them up, ultimately clearing the infection. But Markowitz and Ho worry that the immune system may not do its part. In several of their patients, HAART has so successfully controlled the infection that they no longer mount strong immune responses against HIV. To try to thwart this resurgence, Ho and Markowitz conclude that it

ERADICATING STUBBORN RESERVOIRS OF HIV

Virus Location	Treatment Ideas
Brain	Develop drugs that permeate blood-brain barrier
Latently infected T cells	Flush with OKT3
Latently infected macrophages	Flush with GM-CSF, IL-12, TNF
Systemic	Boost immunity with HIV-specific vaccine

* Cellular and Systemic Reservoirs for HIV Replication Under Highly Active Antiretroviral Therapy, Massachusetts Institute of Technology's Endicott House, organized by AmFAR and Treatment Action Group, 27 February–1 March.

makes sense to boost these patients' immune systems with an HIV vaccine. "Before you stop the drug, wouldn't it be wise to enhance the immune response?" asked Ho.

John Coffin, a leading retrovirologist from Tufts University, didn't think so, sparking the hottest debate of the meeting. Coffin argued that while such vaccinations might hamper any remaining HIV's attempt to spread, they could not prevent it. "You're not going to eradicate the virus with an immune response," Coffin contended. "So far that hasn't been good enough to remove the infection from a single individual." Ho countered with a calculation suggesting that in patients who only have latently infected cells, a few cells—50 to 75—come out of latency each day and produce HIV. Vaccinating, he proposed, would give the immune system enough punch to clear that small number of producing cells and, over time, eradicate the infection.

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>
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But several researchers suggested that complete eradication of HIV from the blood may be the wrong target in any case. Instead, they asked, why not enlist the immune system—rather than drugs—to control the virus for extended periods? That goal, Scripps's Mosier proposed, could be achieved by cycling patients with both undetectable viral loads and waning HIV immunity off therapy, and then cycling them back on when their virus reemerges. Several studies, he noted, have shown that such "treatment holidays" do not allow the virus to develop resistance to the drugs. And this repeated exposure to the virus would keep boosting immune responses to it. "It surprises me that you're even actually thinking about vaccines [to boost immunity] when you have got the natural infection of immunized people to begin with," said Mosier.

Brain drain. Even if HIV can be eliminated from the blood, however, it might not be possible to rid the body of it altogether because of the virus's propensity for skulking around the central nervous system. "I'm very worried that there's a viral reservoir in the brain," said Stuart Lipton, who studies HIV neuropathogenesis at Harvard's Brigham and Women's Hospital. There's no accurate way to measure levels of HIV in the brain, where it can stimulate the production of toxins that produce dementia. But it seems to be protected there by the so-called "blood-brain barrier," which keeps both drugs and immune cells that might fight the virus out of the brain.

Anti-HIV drugs that do reach the brain are a double-edged sword, warned immunologist Angela McLean of Oxford University in the United Kingdom. Only low concentrations of the drugs likely make it past the blood-brain barrier, she noted, and "suboptimal" levels of anti-HIV drugs allow the virus to develop resistance to the agents. "The last thing you want is intermediate drug levels," cautioned McLean.

To make matters worse, the brain is but one "sanctuary site" for HIV. Other areas of the body that have similar barriers include the testes, the eye, the thymus, and the spinal cord. And even less is known about HAART's impact on HIV in these sites. "The sanctuary issue is still up in the air," said Ho.

The think tank participants proposed a practical way to learn much more about the impact of anti-HIV drugs on these remote compartments: autopsies of relatively healthy, HIV-infected people. Although many autopsies have been done on people who have died from AIDS, by definition they have failed treatment and their bodies hold no clues to how HAART affects viral levels in the brain and other hard-to-access sites. Retrovirologist Ashley Haase of the University of Minnesota Medical School in Minneapolis suggested that researchers make a concerted effort to find HIV-infected people who have died from car accidents and other traumas. "One thing that might come out of this meeting is the need to develop such a tissue bank," said Haase.

In the end, researchers could not agree whether eradication is possible, but they clearly are nowhere done thinking about it.

–Jon Cohen

BIOSENSORS

Getting an Inside Look at Cells' Chemistry

New microscopic sensors can size up the chemical status of a living cell. Unveiled early this month in New Orleans at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy by a group at the University of Michigan, Ann Arbor, the sensors fit inside the cell and monitor its changing chemistry on the spot. Among other things, they could help researchers understand how an individual cell responds to a dose of drugs or toxins.

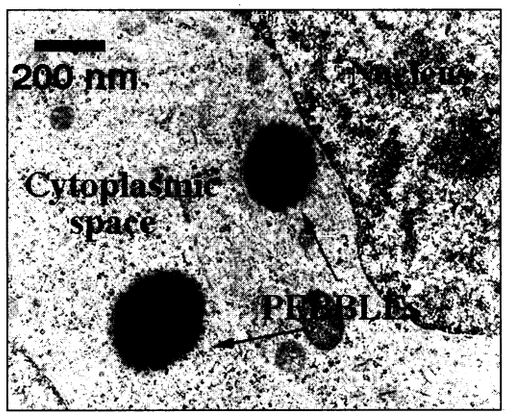
Cells constantly adjust their level of ions such as calcium or potassium in response to external stimuli. Biologists usually measure these changes by injecting dyes into a cell or poking it with electrodes. But Michigan chemist Raoul Kopelman and his colleagues wanted to find a less invasive method.

The key turned out to be a class of molecules, called ionophores, which normally latch onto positively charged ions and help pull them across the cell membrane. Kopelman and his grad student Heather Clark packaged ionophores specific for different ions into the surfaces of particles less than 100 nanometers across, made of a plasticlike polymer. As each ionophore sucks up its target positive ion, it compensates by releasing positively charged protons, which activate a fluorescent dye embedded in the particle.

To test their minisensors, dubbed PEBBLEs (Probes Encapsulated By BioListic Embedding), the researchers injected them into brain cells or mouse eggs with a "gene gun," which is normally used to shoot DNA into a cell via a puff of helium. The brightness of the PEBBLEs' glow revealed the concentrations of the ions they were tailored to detect. Cells don't seem to mind the PEBBLEs, says Kopelman, probably because they take up little space—each occupies only a millionth of an average cell's volume.

"I think it's beautiful," says Stephen Weber, a chemist at the University of Pittsburgh. He notes that because PEBBLEs are used with a microscope, researchers can determine the exact three-dimensional position of each sensor as well as its readout. Says Kopelman: "You can now follow chemistry on a single-cell level and see how any change affects it within seconds, minutes, or hours."

–Kevin Boyd



UNIV. OF MICHIGAN

Inside job. Polymer-encapsulated sensors spy on ions within the cytoplasm of a cell.

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