Reservoirs dog AIDS therapy

Pools of latent HIV, lurking in the cells of infected people, remain untouched even by powerful drug combinations. Paul Smaglik reports on how this is forcing researchers to rethink their strategies for fighting the virus.

"Depressing." That's how Robert Siliciano, a virologist at Johns Hopkins University in Baltimore, Maryland, describes the implications of his work on 'reservoirs' of HIV. Believed to consist of viral DNA held safely inside 'resting' host cells, the stubborn resilience of these reservoirs has made many researchers pessimistic about the chances of ever completely eliminating HIV from the bodies of infected people.

As it has become clear that HIV reservoirs persist in the face of the most potent drug cocktails available, many AIDS researchers are shifting their plans of attack. "The concept of a reservoir is of paramount importance to the whole philosophy of what we wish to accomplish therapeutically," says Tony Fauci, director of the US National Institute of Allergy and Infectious Diseases in Bethesda, Maryland. The problem is that reservoirs provide a means for the virus to bounce back from assault by drugs, potentially re-emerging in a drug-resistant form.

A few researchers still hope that it will prove possible to drain the reservoirs by finding new, more powerful drugs. But potent drugs tend to have toxic side effects and, given HIV's apparent ability to evolve resistance to any drug thrown at it, a fresh spotlight is being turned onto boosting the immune system's ability to fight back against the virus. If this can be done, it might be possible to control HIV infection, even if the virus can't be eliminated, allowing infected people to live long and healthy lives.

Combined forces

The current uncertainty is a far cry from the excitement of the mid-1990s, when some researchers dared to think that the right combinations of drugs might provide a cure for AIDS. In 1995, two groups — one led by David Ho, director of the Aaron Diamond AIDS Research Center in New York, the other by George Shaw of the University of Alabama at Birmingham — presented data suggesting that HIV engages in a massive, but relatively unsophisticated assault on the body[1,2]. The virus targets $CD4^+$ cells, a particular class of white blood cell involved in the immune response. From near the start of infection, the two teams argued, around a billion new viruses are produced each day. This rampant replication kills a similar number of the $CD4^+$ cells. Over time, the researchers suggested, the immune system loses its ability to replenish $CD4^+$ cells at such a phenomenal rate. As a result, the patient's $CD4^+$ cell count falls, leading to immune deficiency, opportunistic infections — and eventually death.

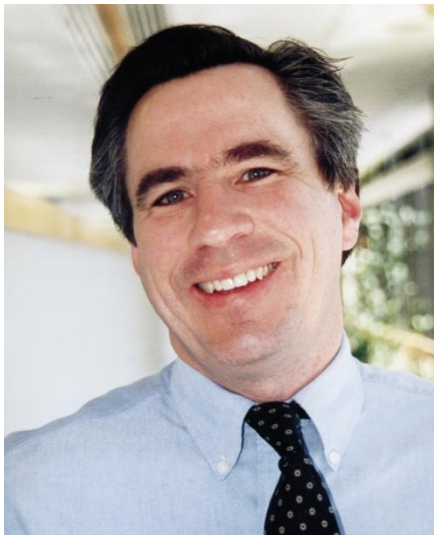
If this picture was correct, the implication was clear: hit the virus hard enough with drugs that stop its replication, wait for chronically infected $CD4^+$ cells to die, and it might just be possible to eliminate HIV from the body. The introduction of drug cocktails known as highly active antiretroviral therapy (HAART) buoyed the mood further. HAART typically combines several drugs to block the action of two enzymes — reverse transcriptase and protease — which are central to HIV's cycle of replication and infiltration of new host cells. By 1996, clinicians monitoring some patients put onto HAART were reporting anecdotally that they could find no evidence of HIV in the patients' blood.

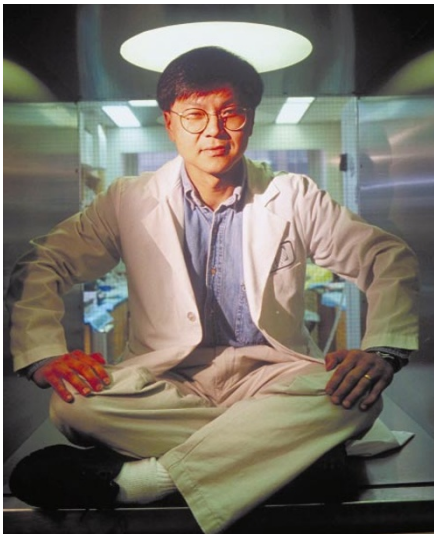
However, Siliciano's research was already throwing a spanner into the works. In 1995, before HAART came into widespread use, his team showed that a small number of infected $CD4^+$ cells enter a 'resting' state in which HIV DNA integrates into the cell's chromosomes and simply sits there[3]. In these cells, the virus does not engage in the orgy of replication and destruction described by Shaw and Ho. In 1997, working in collaboration with Ho's team, Siliciano produced one of a flurry of papers showing that these reservoirs were still present in patients on HAART[4–6]. Previous researchers hadn't detected the reservoirs because they didn't flush the virus out of resting cells. And late last year, researchers in Fauci's lab confirmed that, when HAART stops, HIV can bounce back from the reservoirs with a vengeance[7,8].

Phantom menace

The precise nature of the reservoirs remains a matter of debate. As well as resting $CD4^+$ cells, there may be other cellular reservoirs that are not fully understood. But knowledge of how the reservoirs persist is advancing. Last year, for instance, Siliciano presented evidence suggesting that latently infected $CD4^+$ cells divide slowly, copying their cargo of HIV DNA as they do so[9]. So although $CD4^+$ cells eventually die of old age, their viral legacy lives on. In addition, other $CD4^+$ cells might become latently infected because of a low level of ongoing viral replication.

Ho still hasn't given up on the idea of eradicating HIV using drugs alone. He compares a viral reservoir to a sink with a leaky plug and a dripping tap. In a best-case scenario, he suggests, patients who adhere strictly to their HAART regime might empty their reservoirs in about six years. Ho bases this conclusion on studies of eight individuals who began HAART soon after they were infected with HIV[10], and other patients who were responding particularly well to





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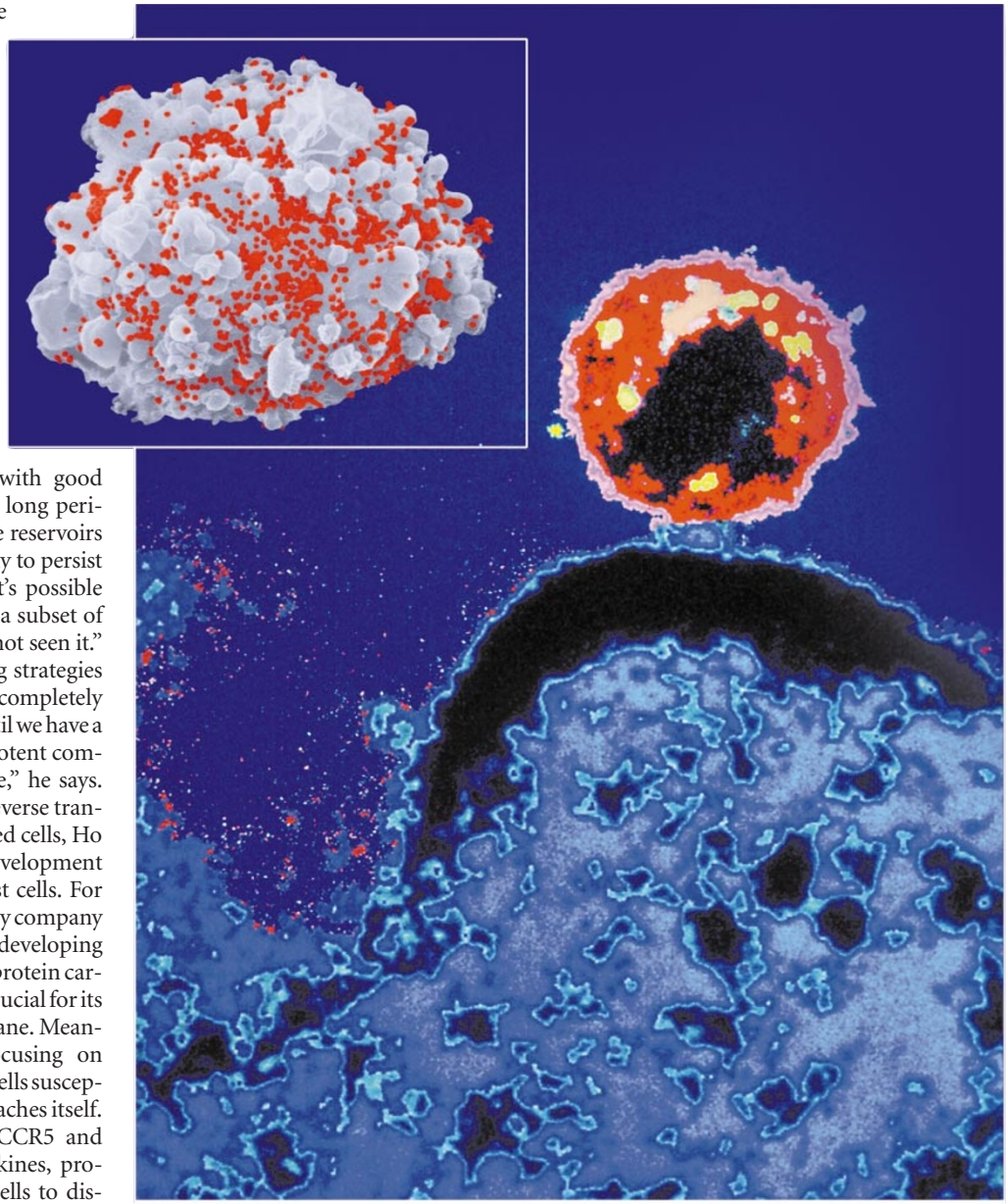
The eliminator: Ho (right) believes that HIV can be eradicated from the body, but Siliciano is sceptical.

HAART[11]. In at least some patients, Ho concludes, it might be possible to turn off the tap completely. "It's still going to be difficult," he says. "I'm not trying to underestimate the task."

Mission impossible?

But many AIDS researchers don't share Ho's optimism about eradicating the virus. Frank Miedema, an immunologist at the University of Amsterdam, is particularly outspoken. "It's completely impossible to eradicate the virus with the current drugs," he asserts. Siliciano, who has tracked about 60 patients with good records of drug compliance over long periods, has found that the size of the reservoirs decays so slowly that they are likely to persist for life[9] (see figure, overleaf). "It's possible that there is more rapid decay in a subset of people," Siliciano says. "We have not seen it."

Ho points out that many drug strategies remain untried. "We should not completely give up on the eradication idea until we have a good go at attacking with more potent combinations at all the sites available," he says. Drugs that inhibit protease and reverse transcriptase target HIV inside infected cells, Ho notes, while other drugs still in development aim to prevent HIV entering host cells. For instance, Trimeris, a biotechnology company in Durham, North Carolina, is developing two peptides that bind to gp41, a protein carried on the surface of HIV that is crucial for its fusion with the host cell's membrane. Meanwhile, other researchers are focusing on receptors found on the surface of cells susceptible to HIV, to which the virus attaches itself. Two of these receptors, called CCR5 and CXR5, normally bind to chemokines, proteins that lure immune-system cells to diseased or damaged tissues. Drugs companies are trying to find chemicals that block these receptors.



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Veiled threat: HIV (red) buds from the cells in which it is replicating (enlarged in main picture). When it does so it is vulnerable to drugs, but when it lies dormant it is untouched by therapy.

The HAART of the matter

Many researchers doubt that any new drug strategy will prove much more effective than HAART at eliminating viral reservoirs. But even if drugs alone aren't the answer, they could be used in conjunction with strategies to boost the immune system. In theory, HAART could get the virus down to manageable levels, and therapies that boost the immune response might then be able to contain it.

Curiously, one approach involves stopping HAART for short periods of time. HAART is a mixed blessing, explains Bruce Walker, an immunologist at the Massachusetts General Hospital in Boston. "When you're put on HAART, your immune response drops down to lower levels," he says. Although viral replication surges as soon as patients are taken off HAART, it is bouncing back from a very low level, and the concurrent boost in the patients' immune response might just allow the virus to be recognized and controlled.

Walker has tried interrupting HAART in nine volunteers. "We are very encouraged by what we are seeing," he says. The results have not yet been published, but the patients showed variable increases in the numbers of circulating cytotoxic T lymphocytes (CTLs), the 'killer' cells that can destroy HIV-infected cells. And in some patients, there were signs that these cells were having the desired effect. In one individual, the levels of circulating virus remained low for several months after the second interruption of HAART.

One appealing aspect of the interruption strategy is that occasionally stopping HAART reduces the treatment's toxicity. But at the same time, interrupting therapy can encourage the emergence of drug resistance. Stop HAART for too long, and the danger is that it won't be possible to control the virus once the treatment resumes.

Walker believes that, by itself, the interruption strategy is only likely to work in recently infected patients who were put on HAART as soon as their HIV status was known. Such patients are the 'best case', as the virus will have had little time to damage the immune system before the therapy begins.

Several teams are attempting the same strategy in chronically infected patients, who didn't begin HAART straight after infection. Published findings[12] and results presented at meetings are less encouraging than those obtained by Walker. "If there is an effect, it is only partial and in a subset of patients," says Giuseppe Pantaleo, an immunologist at the University of Lausanne in Switzerland.

Other immune-boosting treatments include infusing patients with interleukin-2 (IL-2), one of a family of proteins known as cytokines, which help coordinate our immune responses. Some AIDS researchers had hoped that intermittent treatment with IL-2, perhaps in association with other cytokines, might flush out the viral reservoirs by activating the resting $CD4^+$ cells — rendering the hidden HIV susceptible to HAART. While the latest research from Fauci's lab suggests that it won't completely eliminate the reservoirs[13], IL-2 does boost the $CD4^+$ cell response. This could make it an important component of strategies to help the immune system regain control over HIV infection.

A shot in the arm

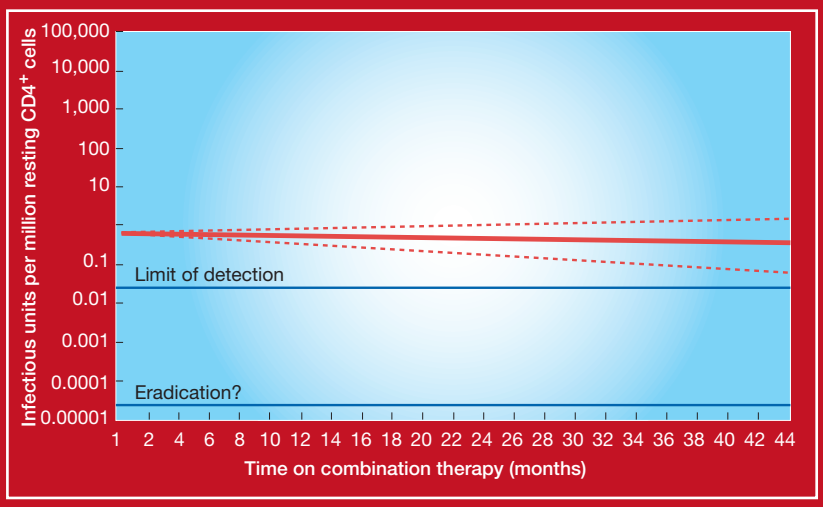
For now, however, the main growth area is in 'therapeutic' vaccine research. Rather than using vaccines in an attempt to prevent initial infection, the idea is to vaccinate HIV-positive people being treated with HAART, then halt the drug therapy. Hopefully, the vaccine will stimulate their immune systems sufficiently to bring the infection under long-term control.

A range of vaccines has been developed with the goal of preventing HIV infection — although none have yet achieved notable success. But even if vaccines can't stop people becoming infected, some might prove useful in a therapeutic context. Researchers at the Aaron Diamond AIDS Research Center have obtained intriguing results using a vaccine based on a canarypox virus, engineered to produce four HIV proteins. This is given in combination with recombinant gp160, an HIV protein involved in the virus's entry into host cells. "We have the first glimmer that there may be a role for therapeutic vaccines," says Marty Markowitz, the centre's clinical director.

Of 12 patients who received the canarypox/gp160 vaccine along with HAART, four were then taken off the drugs to see if their immune systems could control the virus. In two of these patients, levels of circulating HIV surged. But in the other two, the virus rebounded much more slowly. These two patients had a strong CTL response, while the other two did not. Markowitz acknowledges that the experiment, which has not yet been published, was small and had mixed results, but he regards it as a springboard for more research. Perhaps tinkering with the vaccine to produce more specific immune responses will do the trick, he suggests.

Another therapeutic vaccine, known as Remune, is undergoing trials in the United States, Europe and Thailand. Marketed by two companies in California, the Immune Response Corporation in Carlsbad and Agouron Pharmaceuticals of San Diego, this vaccine is based on whole HIV particles, stripped of a protein called gp120, and killed by irradiation and chemical treatment.

A life-long problem?



This plot, published last year[9], shows just how resilient latent reservoirs of HIV can be. Researchers led by Robert Siliciano of Johns Hopkins University in Baltimore monitored the size of reservoirs in 34 patients receiving potent drug combinations over extended periods. From the line of best fit for these data (shown above with 95 per cent confidence intervals), Siliciano and his colleagues calculated that, in a typical patient, it would take 60.8 years to eliminate reservoirs of HIV using current drug regimes.

Encouragingly, the vaccine causes $CD4^+$ cells to proliferate. But so far, there is no evidence that the immune response it stimulates is sufficient to control HIV infection[14].

Tit-for-tat tactics

"I think we will have to figure out a way to make these vaccines more immunogenic," says Fauci. For instance, it might be necessary to stimulate antibodies against HIV, as well as generating an effective CTL response. "I think you need both arms of the immune system," says John Moore, an AIDS researcher at the Weill Medical College of Cornell University in New York. However, Andrew McMichael, an immunologist at the University of Oxford, believes researchers should develop and test therapeutic vaccines that work on each arm of the immune system separately, so that each effect can be understood in isolation. If the two approaches are combined too early, he suggests, the results will be difficult to interpret.

One example of a candidate therapeutic vaccine that elicits antibody production as well as a CTL response is an inactivated form of an HIV protein called Tat. This protein contributes to the damage wrought by HIV in several ways: inside host cells, it directs the transcription of HIV genes; outside, it seems to hasten the death of uninfected $CD4^+$ cells and stimulates them to produce more chemokine receptors, rendering them more susceptible to infection. Based on encouraging results from primate studies[15], Robert Gallo, director of the Institute of Human Virology at the University of Maryland, believes that a vaccine based on the inactivated Tat, or Tat toxoid, should be included in a multicomponent therapeutic vaccine.

Pantaleo agrees that no single vaccine is likely to control HIV infection. He also points out that the most promising candidate vaccines being developed for prevention have not yet been tried as therapeutic vaccines. To devise an effective therapeutic strategy, he suggests, researchers may need to combine several of these newer vaccines.

Faced with these challenges, it seems that AIDS researchers are in for a long haul. But many refuse to be downcast. While the growing realization of the importance of viral reservoirs has dashed most researchers' hopes of eradicating HIV from the body, elimination may not be necessary to manage the disease. "If you could have a therapy that was easy, cheap, not toxic, not inconvenient, worked without side effects, could work lifelong, but you didn't eradicate, is that OK?" asks Gallo. "Sure it's OK." ■

Paul Smaglik is *Nature*'s Washington DC correspondent.

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