mouse brain at that stage is only a few millimeters thick—sufficiently well to perform the transplants. "A lot of people who have come to our institute and seen us doing these procedures have been getting excited," says Turnbull. "Everybody sees the future applications where we can start introducing labeled cells into mutant mice, to look at how the differentiation process is altered."

Perhaps the greatest excitement at the NICHD workshop, however, was sparked by another effort to image individual cells. Researchers at the University of Iowa's W. M. Keck Dynamic Image Analysis Facility in Iowa City have melded a confocal microscope with a 3D movie camera and a computer to create the world's only instrument for monitoring the full range of movements and shape changes that cells undergo during development. The microscope changes its focal plane 30 times per second, and the computer records the resulting "optical sections" for reconstruction into a 3D computer model that highlights cell membranes and internal surfaces such as those of the nucleus, mitochondria, and vesicles. The process is repeated every 2 seconds, and a QuickTime movie is the result.

So far, the technique has been used only on cells that can crawl in a lab dish. David Soll, director of the Keck facility, handed out red-and-blue glasses at the workshop and treated viewers to a 3D movie of the sluglike colonies that the normally unicellular slime mold *Dictyostelium* forms when it needs to reproduce. Like all amoeboid creatures, the *Dictyostelium* colony moves by continuously assembling and disassembling its internal skeleton, made of the protein actin. Soll's movie showed how the colonies pulsate as their actin-filled pseudopods appear and disappear, dragging along the entire mass.

Using the 3D motion analysis system, Soll and his collaborators have demonstrated that *Dictyostelium* strains engineered to lack certain of the proteins known to regulate actin display specific flaws in the way they create or absorb pseudopods. These flaws are a sign that the numerous cytoskeletal regulators aren't redundant, but specialized. According to George Washington's Moody, that result would probably elude a researcher viewing mutant *Dictyostelium* colonies under a conventional 2D microscope.

And that, in the end, may be the strongest rationale behind the new surge in developmental imaging. Researchers using 2D still images of their subjects have to spend years acquiring an ethereal, intuitive "feeling for the organism" before they can understand its behavior in three dimensions over time, Moody argues. "But having these new technologies out there means that people will quickly be able to form a visual understanding they can rely on. ... It's going to be terrific."

–Wade Roush

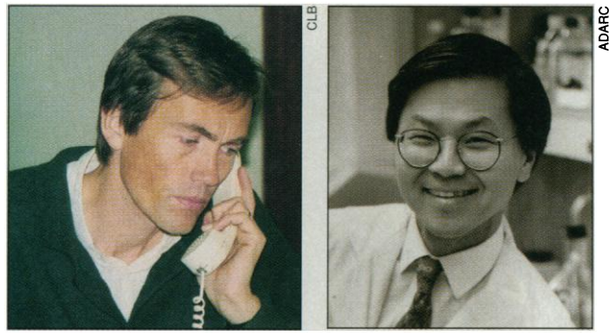
MEETING BRIEFS

How Does HIV Overcome the Body's T Cell Bodyguards?

MARNES-LA-COQUETTE, FRANCE—In 1854, Emperor Napoleon III created an elite squadron called the "Cent Gardes" for his own protection. Thirty years later, Louis Pasteur turned one of the squadron's barracks in this small town just outside Paris into laboratories. Pasteur died here in 1895, but his disease-fighting tradition lives on: Today, the Cent Gardes building hosts one of the world's most prestigious AIDS meetings. At this year's gathering,* an elite squadron of researchers grappled with still-unsolved questions about how HIV destroys the immune system and how they can fend off its attacks.

The Life and Times of T Cells

It might be said that AIDS researchers have come to know the virus that causes the disease, HIV, inside and out. They have isolated its proteins, sequenced its genome, and identified the receptors it uses to dock onto the CD4 T lymphocytes that are the virus's primary target. Yet the central mystery of AIDS remains unresolved: How does the virus cause the severe loss of CD4 cells, which wrecks the immune system, that is the hallmark of the disease? This question has stimulated heated discussion in recent years, and new findings presented at the meeting by David Ho, director of the Aaron Diamond AIDS Research Center in New York City, and immunologist Paul Johnson of Harvard Medical School in Boston are fanning the flames of the debate.



Different calls. Frank Miedema (*left*) and David Ho disagree about how T cells are lost.

For many researchers, a major clue to the riddle was revealed in January 1995 with the publication of two papers in *Nature* indicating staggeringly high rates of HIV replication and CD4 cell turnover in a typical HIV-positive patient. The findings—by Ho and his collaborator Alan Perelson of the Los Alamos National Laboratory in New Mexico, and by George Shaw at The University of Alabama, Birmingham, and his co-workers—which have since been refined in more recent papers, suggest that about 100 billion new viral particles are produced every day and 1 billion to 2 billion CD4 cells are dying and being regenerated each day as well.

These extraordinarily high numbers led Ho to propose what has come to be known as the "sink model" for CD4 cell loss. In Ho's view, the high levels of HIV production keep both the sink's tap (the immune system's production of new CD4 cells) and its drain (their destruction by the virus) wide open. Because the body's ability to generate new cells can only be stretched so far, the sink slowly empties, until the CD4 cells are lost and the immune system is exhausted.

Last year, however, the sink model was challenged by a team of researchers led by immunologist Frank Miedema of the Netherlands' Red Cross Blood Transfusion Service in Amsterdam (*Science*, 29 November 1996, p. 1543). Miedema and his co-workers made their own estimate of CD4 cell turnover by measuring changes in the length of the T cells' telomeres, the extreme ends of chromosomes, which shorten slightly each time a cell divides. The telomere length can provide an estimate of how many times a cell has divided during its lifetime, and thus an indication of overall turnover rate in a cell population. The Amsterdam team found that the telomeres in CD4 cells from HIV-infected people were not appreciably shorter than those of uninfected controls, and the team concluded that turnover rates in these two groups were essentially the same—a result that directly contradicted Ho's sink model. Miedema's team proposed that the loss of CD4 cells was not due to a major increase in their rate of destruction—an open drain—but rather that HIV was interfering with production of new cells, thus turning down the tap.

At the meeting, Ho delivered a riposte to

* 11th Colloquium of the Cent Gardes, Marnes-la-Coquette, France, 27 to 29 October 1997.

Miedema, reporting new experiments in rhesus monkeys designed to measure directly the effects of virus infection on T cell turnover. Ho's group, working again with Perelson, used a compound called bromodeoxyuridine (BrdU)—which is taken up by actively dividing cells—to label the T cells of monkeys infected with SIV, the simian version of HIV, as well as those of uninfected control animals. After 3 weeks of administering BrdU to the monkeys in their drinking water, the compound was withdrawn. The rate at which the BrdU label first increased and then disappeared from the T cell population provided a measure of the production and loss of those cells. Ho reported that the death rates of CD4 cells in the SIV-infected animals was about six times higher than in uninfected monkeys, which also provides an upper limit for the increase in turnover rate. In a separate talk, Johnson presented results from similar experiments, estimating that the overall CD4 cell turnover rate in SIV-infected monkeys was about two to three times as high as in control animals.

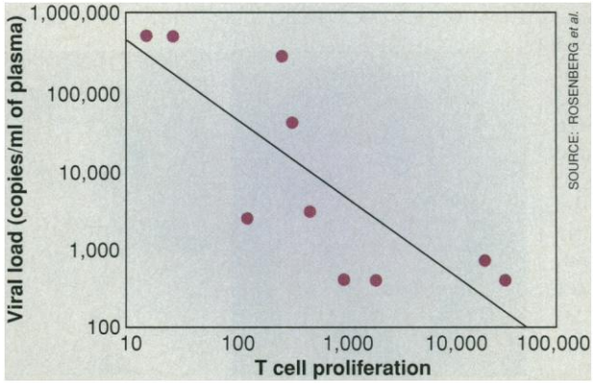
"Our results directly contradict the estimates of Frank Miedema and his colleagues," Ho told the meeting. "Our study is a direct measure of lymphocyte turnover, whereas the telomere method is very indirect." Immunologist Bruce Walker, director of the Partners AIDS Research Center in Charlestown, Massachusetts, comments that "we now have two independent studies showing that turnover rates are clearly increased" in infected animals.

But Miedema—who was not at the Cent Gardes meeting—told *Science* that the Ho and Johnson findings are roughly consistent with a new mathematical analysis of his telomere data performed in collaboration with theoretical biologist Rob de Boer at the University of Utrecht in the Netherlands. According to the analysis, CD4 cell turnover rates up to three times as high as normal would not give rise to overall telomere shortening in an HIV-infected cell population, because some cells would die before their telomeres could shorten. While Miedema concedes that this reworking of his data is consistent with higher HIV-induced turnover rates than suggested in his original *Science* paper, he argues that neither these rates nor those derived from the BrdU results are high enough to support the sink model. Indeed, both rates are much lower than those estimated in Ho's and Shaw's *Nature* papers. The new data from Ho and Johnson are "not compatible with the idea of the renewal capacity of the immune system becoming exhausted" by high CD4 cell production rates, Miedema says. "The drain might be slightly more open, but the tap must be running more slowly due to HIV infection."

Although the Ho-Miedema dispute sparked a lively discussion at the meeting, many participants were reluctant to take sides. For example, Norman Letvin, an immunologist at Harvard Medical School, says that neither Miedema's telomere data, nor Ho and Johnson's BrdU results, provide definitive support for their competing models of CD4 cell loss. "If you don't have enough CD4 cells, it's either due to decreased production or increased destruction," Letvin says. "These studies are reasonable attempts to quantify T cell turnover rates, but they can't distinguish between these two possibilities." Thus, in the minds of many AIDS researchers, the riddle of CD4 cell loss remains unresolved. "We are still very confused about the mechanisms that lead to CD4 depletion," says Johnson. "But at least now we are confused at a higher level of understanding."

A Little Help From Friends

In HIV-infected people, loss of CD4 T cells is a key marker for progression to full-blown AIDS. But the immune system suffers subtle dysfunctions even in earlier stages of infection, before symptoms appear. Long before they are lost, the CD4 T cells—also known as T helper cells—begin to lose their ability to help fight off infections. The job of the T helper cells is to recognize foreign antigens, such as proteins from viruses or bacteria, and alert other immune cells—particularly CD8 T cells, also known as cytotoxic T lymphocytes (CTLs)—so they can move in and kill the invaders. Unfortunately, the immune systems of most HIV-infected people mount at best a weak attack against HIV antigens, a finding that has led researchers to conclude that the specific subset of T helpers that are primed to identify these proteins are either lost very early in infection or are never really produced in significant numbers.



Lightening the load. Patients (solid dots) with stronger T-helper responses control HIV better.

But new work presented at the meeting by immunologist Bruce Walker of the Partners AIDS Research Center in Charlestown, Massachusetts—and published on page 1447 of this issue—indicates that HIV-specific T helpers may be playing a key role in controlling the virus in HIV-infected people known as long-term nonprogressors, those who do not develop AIDS even after many years. Even better, Walker and other researchers believe the new results provide some hope that these T helpers could be boosted even in people who show little evidence of having them, possibly by combining powerful antiviral therapies with anti-HIV vaccines.

Walker says that he and his colleagues began the study when they were contacted by a hemophiliac who had been infected with HIV for 18 years, but had normal CD4 counts and undetectable amounts of the virus in his blood. When the team exposed his blood cells to HIV proteins, HIV-specific T helpers rapidly proliferated. The team went on to study other HIV-infected individuals with a wide range of virus loads in their blood and found that the strength of their T helper responses directly correlated with how well they were controlling the virus. Finally, Walker and his co-workers tested patients who had been put on powerful antiviral therapies very early in their HIV infection, even before they began to form HIV antibodies, and found that this group was able to generate strong anti-HIV T helpers once their viral loads were reduced to undetectable levels.

"From the standpoint of HIV-infected patients, Bruce's talk was the most exciting at the meeting," says virologist Andreas Meyerhans of the University of Freiburg in Germany. "You can now think about taking patients whose viral loads are already being controlled by antiviral therapy and vaccinate them [with an anti-HIV vaccine] to try to reboost these anti-HIV helper responses."

Walker says his findings provide strong evidence that if antiviral drugs are given very early, these T helper responses may be preserved. And they may also help explain why patients ultimately fail to control the virus, even though they continue to show anti-HIV CTL responses throughout much of their infection. "The quirk of HIV is that it precisely targets immunologically activated cells," he says. As a result, the very HIV-specific T helpers that march in to meet the viral challenge are among the first to become infected and die, "leaving those CTLs alone, without helpers to battle the virus over the course of infection."

But the good news, Walker says, is that AIDS researchers and clinicians can now start thinking about creative new ways, such as therapeutic vaccines, to get the lost helpers back. Says Meyerhans: "We might be able to turn HIV progressors into long-term survivors. That is a very attractive consequence of what Bruce is saying."

–Michael Balter