AIDS THERAPY

Failure Isn't What It Used to Be ... But Neither Is Success

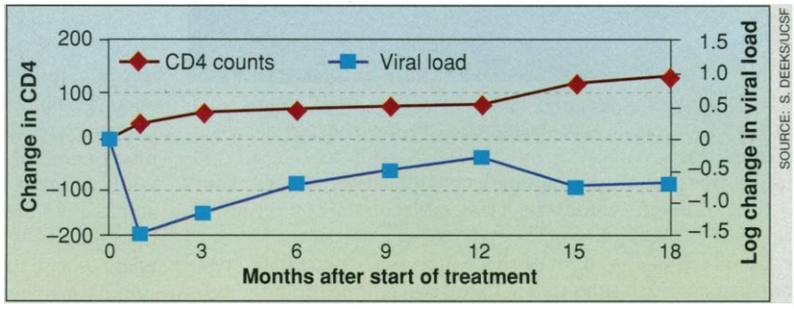
CHICAGO—In his kickoff speech to the main annual U.S. AIDS meeting, held here 1 to 5 February, retrovirologist Ashley Haase of the University of Minnesota, Minneapolis, set the tone for the gathering with a quote from novelist William Faulkner: "If it aint complicated it dont matter whether it works or not because if it aint complicated up enough it aint right." If Faulkner's logic is correct, AIDS research is on the right track: As the 3500 researchers who attended the Fifth Conference on Retroviruses and Opportunistic Infections heard over and over again, the more we know about the workings of HIV, the more complicated the picture gets.

Take the new AIDS treatments that are helping HIV-infected people live longer, healthier lives. Until now, many researchers have assumed that a treatment failed when a patient's virus levels increased, but new data suggest that it's not quite that simple. Their immune cells can remain high even when the virus is thriving—perhaps because other factors besides viral levels can affect immune-cell production, powerful new tests suggest. But there's some bad news as well: Even when drugs succeed in keeping HIV at bay, an odd, newly discovered side effect could complicate treatments, according to some physicians.

The meaning of failure. Clinicians routinely judge the success of anti-HIV treatments by their ability to drive down the amount of the virus in a person's blood (the "viral load"). A good treatment quickly knocks down viral load to the point at which the most sensitive assays can't detect it, allowing white blood cells called CD4s, which the virus selectively targets and destroys, to rebound. If the virus returns, the treatment is usually deemed to have failed. But now, several investigators have noticed something unexpected: In some patients, the resurging virus doesn't wipe out gains in CD4 counts.

Steven Deeks and his co-workers at the University of California, San Francisco (UCSF), reported a study of 45 patients taking a combination of anti-HIV drugs, including ones that inhibit the viral protease—an enzyme that HIV uses to assemble itself. These patients' viral loads had dropped at first, but after about 6 months bounced back to near-original levels. Yet, after 18 months, their CD4 counts remained higher (by roughly 125 cells per cubic millimeter of blood) than they were before treatment (see graph). In one case, the treatment failed to decrease viral load at all, yet the patient's CD4 count jumped by about 500. Because these patients had only 77 CD4s on average before treatment (the normal range is 600 to 1200), such gains may be enough to help ward off opportunistic infections that are the hallmark of AIDS. "Treatment failure is not synonymous with clinical failure," says the University of Pittsburgh's John Mellors, a leading AIDS virologist. Deeks says he does not expect the benefits to last indefinitely, however. "At some point, [these patients are] going to progress," he predicts.

Researchers floated several explanations for this disconnect between CD4 counts and viral load. David Ho, head of the Aaron Diamond AIDS Research Center in New York City, is investigating whether the similar patients he's following have developed mutant viruses that are resistant to the drugs but are less able to destroy the immune system. "The virus is trying to escape from the drugs, but by making those mutations, the virus is not fit enough," suggests Ho. Others speculated that when treatment knocks back the virus even temporarily, the immune system gets a chance to recharge itself.



Disconnect. Although the viral load in 45 patients is heading back to pretreatment levels, their gains in CD4 cell counts have held up.

The immune system at work. Endocrinologist Marc Hellerstein of the University of California, Berkeley, offered intriguing data of his own that may provide a different explanation. Hellerstein's group, working with Joseph McCune's laboratory at UCSF, has developed a powerful new tool—which it published in the 20 January *Proceedings of the National Academy of Sciences*—for determining how many CD4 cells a person makes each day. Researchers have long sought such a technique to help reveal the intricacies of the battle between the immune system and HIV (*Science*, 21 November 1997, p. 1399). "I think they've made a genuine contribution to the field," says Ho.

The technique relies on tracking the amount of DNA the body synthesizes as it makes new cells. Hellerstein and colleagues first labeled deoxyribose, a sugar that forms the backbone of DNA, with deuterium, a heavy form of hydrogen. They infused people with the labeled sugar and drew their blood for several days. When they analyzed these blood samples in a mass spectrometer, the researchers could determine how many cells had taken up deuterium in their DNA and thus calculate the rate at which new cells were being made.

Hellerstein's group applied the technique to CD4 cells separated from the blood of eight patients taking various combinations of anti-HIV drugs, including protease inhibitors; five HIV-infected people not taking protease inhibitors; and five uninfected people. A few intriguing trends emerged. The researchers found an astonishing variation from person to person in CD4 production rates: The lowest was 0.7 billion CD4s a day, and the highest was 12 billion. Those on the most potent drug regimens produced new CD4s at the highest rates, while uninfected people made them at the lowest rates. Among infected people, those with the lowest CD4 counts tended to produce the cells more slowly. Hellerstein also reported that one patient whose viral load increased while his CD4 count remained high was "grinding out the cells" at a furious rate.

Hellerstein cautions against drawing hard and fast conclusions from these early data. "I think it's more interesting that we *can* answer these questions than [that] we *have* answered them," says Hellerstein, who has high hopes that the assay—which can be used to measure production rates of any type of cell—will lead to insights well beyond AIDS. Nevertheless, he suggests that the results indicate that the treatments may stimulate CD4 production directly, and that the extent of damage to the immune system may determine how well someone responds to treatment. If so, this would challenge the popular notion that AIDS drugs work simply by preventing HIV from killing CD4 cells. "I think that's quite wrong," asserts Hellerstein. In other words, it "ain't complicated up enough."

The price of success. Treatment failure may no longer mean what it did, but neither may success. Twelve groups reported at the meeting that long-term treatment with protease inhibitors causes a puzzling redistribu-

tion of fat around the body that could be an omen of other, more serious side effects. The most detailed of these studies, reported by clinician Andrew Carr of St. Vincent Hospital in Sydney, Australia, involved 116 patients taking protease inhibitors for an average of 10 months. Of these, 72 (64%) had fat wasting of the limbs and face with fat accumulation in the gut, a syndrome called lipodystrophy. "I don't think anyone has a good concept of what the mechanism is, or how reversible it is," says Henry Masur, who heads the critical-care unit at the Clinical Center of the National Institutes of Health, where he and his colleagues have studied 18 patients with the disorder.

"The data from [the Australian group] have struck all of us," says clinician Scott Hammer of Harvard Medical School in Boston. "The excitement of success we've experienced has to be tempered by the toxicity." Carr notes that although lipodystrophy by itself does not appear to be dangerous, it suggests that the powerful protease inhibitors are producing a systemic effect that could lead to more severe problems later on. "Are these people going to get heart attacks and strokes in 10 years' time?" Carr asks. He also worries that the drugs could cause metabolic imbalances that lead to insulin resistance.

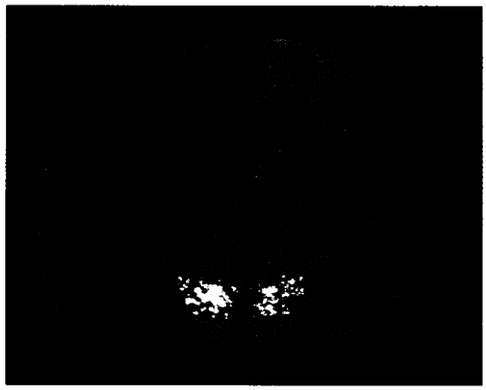
Such concerns led Carr and others to question the common wisdom that treatment should begin as soon as patients learn they are infected with HIV. "These people are going to be on drugs for years," says Carr. Once again, Faulkner's observation could apply: The common wisdom may not be complicated enough.

–Jon Cohen

OBESITY RESEARCH

New Appetite-Boosting Peptides Found

As the popularity of the ill-fated diet drug combination fen/phen showed, people who are morbidly, or just unhappily, overweight are hungry for a pill to help them shed pounds. At the other end of the spectrum, people whose health is threatened by a lack of appetite, because of chemotherapy or illness, would benefit from an appetite-boosting drug. Given what's likely to be a billion-dollar demand, pharmaceutical companies are scrambling to find drugs to control appetite. Now, researchers at the University of Texas Southwestern Medical Center in Dallas and SmithKline Beecham Pharmaceuticals have found a potential new drug target: In today's issue of *Cell*, the team, led by Masashi Yanagisawa of UT Southwestern, reports the discovery of two related peptides, from the brains of rats, that trigger eating by the animals.



Hunger zone. Orexin messenger RNA (bright spots) is concentrated in the lateral hypothalamus.

S. WILLIAMS, M. DAVIS, J. RICHARDSON, M. YANAGISAWA/UT SOUTHWESTERN MEDICAL CENTER AND HHMI

The peptides—which the researchers named orexins, from the Greek word for appetite—aren't the first ones found to turn up hunger, but researchers see them as especially intriguing. For one thing, unlike most of the others, these peptides are made only in a brain area called the lateral hypothalamus (LH), previously identified as the brain's "feeding center" because its destruction caused experimental animals to stop eating and starve to death. The orexins may be key to that brain area's normal function.

What's more, because researchers already know the identity of the receptors through which these peptides work, they can begin to look for drugs that either mimic or block their appetite-enhancing effects. "I think it's terrific," says Jeffrey Friedman, of Rockefeller University in New York City, whose team discovered the appetite-suppressing protein leptin. "All the available evidence says there are many molecules" involved in the control of eating behavior, "and these two clearly need to be added to that list."

Yanagisawa's team members weren't planning to enter this hot field when they began their work. They were collaborating with researchers at SmithKline Beecham's labs in Pennsylvania and the United Kingdom in a search for peptides that activate so-called "orphan receptors." These are proteins whose amino acid sequences show the structural hallmarks of cell surface receptors but whose triggering molecules are as yet undiscovered.

Out of this search came two related peptides that activate two related orphan receptors. Further study revealed that the peptides seem to be made exclusively in the lateral hypothalamus. "That was the obvious clue that they may be involved in feeding behavior," says Yanagisawa.

To test that hypothesis, Yanagisawa's team injected the peptides directly into the brains of rats. They proved to be powerful appetite boosters, causing the animals to eat from three to six times more than control rats did for several hours. Next, the researchers looked to see what effect starvation has on brain levels of the orexins. If the peptides really govern feeding, Yanagisawa says, those levels should go up when the animals are hungry. "We did the starvation experiment," says Yanagisawa, "and lo and behold [orexin] was upregulated."

The discovery should give physiologists new clues to the puzzle of how the brain controls appetite. "Every new discovery [like the orexins] adds to an understanding of how the system works," says physiologist Larry Bellinger, who studies feeding behavior at the Baylor College of Dentistry in Dallas.

Until recently, the LH has been overshadowed by a neighboring area, the ventromedial hypothalamus (VMH). Dubbed the "satiety center" because experiments done in the 1940s showed that its destruction turns animals into chronic overeaters, the VMH has been a hot spot of research for the past few years. Not only are receptors for leptin found there, but so are neurons containing a molecule called peptide Y, which is known to enhance appetite.

But the idea of satiety or feeding centers is now recognized as too simplistic. Indeed, Bellinger says, the hypothalamus is criss-crossed with "a huge wiring diagram" of neurons receiving and passing on information about the body's nutritional state, such as how many fat cells there are or how much sugar the blood contains. And the LH is almost certainly relaying some of these signals. For example, low blood-sugar levels activate some LH neurons. When triggered, those neurons might release the orexins or another appetite-stimulating peptide discovered 2 years ago in the LH—melanin-concentrating hormone.

A better understanding of the wiring that controls feeding is essential to developing safe appetite-controlling drugs, says Bellinger. But there is already reason to hope that the orexins and their receptors will make good targets for such drugs. Because the orexins appear to be only in the LH, they may have fewer other functions in the brain than does peptide Y, whose broad distribution and multiple functions make it harder to find drugs that block only its appetite-inducing effects. "There is no guarantee that [the orexins] won't do other things either," says Friedman, but the fact that they seem to be restricted to the LH is "good news." And it's the kind of news that drug companies are sure to pounce on.

–Marcia Barinaga