New AIDS Drugs Take Careful Aim

The growing body of knowledge about the AIDS virus is leading researchers down the path of rational drug design—using the unique properties of HIV-infected cells to effect a cure

WHEN HUMAN IMMUNODEFICIENCY VIRUS (HIV) infects one of the immune system's T cells, it triggers a chain of events that ends in the destruction of the immune system itself. But HIV also puts a molecular bull's-eye on the surface of the immune cells it infects, and this is giving researchers a target to shoot at in developing some novel therapies.

Researchers at several labs around the world have been cooking up new compounds made of two separate molecules that together pack a one-two punch. The first homes in on the infected cell's bull's-eye and the second either kills the cell directly or starts a process that ends in the cell's death. One of these new compounds is undergoing preliminary tests in AIDS patients and others are likely to move into clinical trials in the coming year.

The target provided by HIV is a glycoprotein dubbed gp120. This protein sits on the virus's outer membrane, and it binds tightly to receptors on certain immune cells that it encounters. This allows the virus to get into the cell, whereupon it injects its genetic instructions into the cell's nucleus. The cell's genetic machinery then makes the proteins the virus needs to reproduce.

One of these proteins is gp120, which migrates to the infected T cell's surface, where it is anchored in place by another viral glycoprotein known as gp41. When the virus's full complement of proteins and RNA are made inside the cell, a nascent virus buds off from the infected cell, taking some of the T cell membrane—with its gp120 on the surface—along with it.

Daniel Capon, a molecular biologist at Genentech, was among the first to devise a potential therapy targeted on the gp120 glycoprotein. He developed a form of the CD4 molecule—the T cell receptor that gp120 attaches to—that could circulate in the blood. The circulating CD4, Capon hoped, would bind to both virus and infected T cells and prevent the virus from infecting new T cells.

Clinical trials of CD4 have been going on since November 1988. But CD4 alone merely acts as a passive shield, binding to gp120 wherever it occurs; it does not kill the cells or virus it encounters. And, because the soluble form of CD4 has a short half-life in the blood, it has proved hard to build up adequate concentrations. So Genentech scientists tried linking CD4 with the immunoglobulin IgG to form a class of compounds they have dubbed immunoadhesins.

This approach has two potential advantages. First, immunoglobulins have a much longer half-life than the CD4 molecule alone—on the order of days instead of hours. And second, by using a portion of the immunoglobulin molecule, the immunoadhesins can act like antibodies. When these molecules bind to free virus or infected cells, they can initiate the same set of reactions as normal antibodies to rid the body of invading pathogens.

One of these immunoadhesins began phase 1 toxicity trials in AIDS patients last August at five medical centers around the country. Genentech won't say much about how the trials are going, but sources familiar with the trials say the compound appears safe so far.

At the Basel Institute for Immunology—which is supported by the Hoffmann–La Roche pharmaceutical company—a team led by André Traunecker is experimenting with another immunoglobulin, IgM, to make a CD4 conjugate that they say is more effective than the CD4 IgG molecule.

Immunoglobulins act indirectly by priming the immune system to kill infected cells. A team at the National Institutes of Health, in a much more direct fashion, has been investigating a more direct approach by linking CD4 to a toxin produced by the common bacteria *Pseudomonas* that wipes out cells outright.

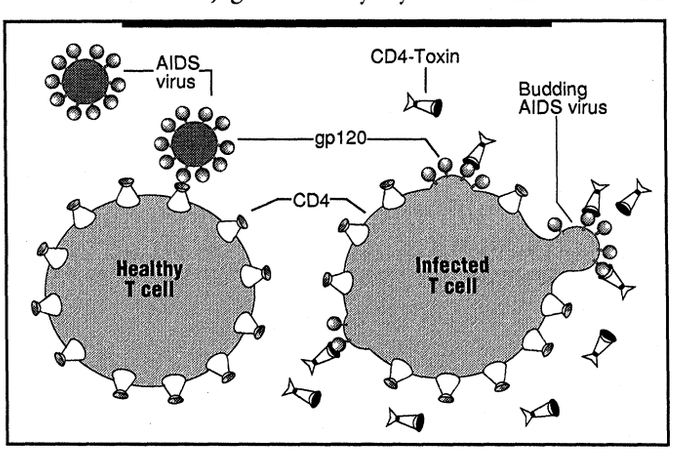
The *Pseudomonas* exotoxin is a protein with a mass of some 66,000 daltons. One region of the molecule binds to cells, another facilitates the entry of the toxin into the cell, and a third blocks a critical step in a cell's metabolic pathways. The upshot of this assault is that the cell is unable to make protein and dies.

Ira Pastan and his colleagues at the National Cancer Institute have been tinkering with the *Pseudomonas* exotoxin, turning it into a guided missile capable of seeking out and destroying specific types of cell. First, they deleted the part of the molecule that binds to cells, creating a 40,000-dalton protein called PE40 that could not by itself bind to cell receptors. Then they molecularly linked the PE40 to a molecule that would only attach to the cell they were interested in destroying. For example, by linking PE40 to interleukin-2 (IL-2), they were able to block an experimental form of arthritis in rats that was caused by an excess of cells expressing the IL-2 receptors.

Working with Edward Berger and Bernard Moss and their colleagues at the National Institute of Allergy and Infectious Diseases, Pastan created a hybrid CD4-PE40 molecule. The CD4 portion will bind to T cells that are expressing gp120 on their surface, and the PE40 then enters the cell and kills it.

That, at least, has been demonstrated in the laboratory. When the NIH researchers added CD4-PE40 molecules to a mixture of infected and uninfected cells in cultures, only the infected cells were killed, and the virus was prevented from infecting the uninfected cells.

The pharmaceutical giant Up-



Zeroing in. *Linking a toxin to a molecule that recognizes glycoproteins on an infected cell's surface should only destroy sick cells.*

john has just begun producing the genetically engineered CD4-PE40 molecule in bulk under an exclusive license from NIH. The company expects to begin animal toxicity tests early in the new year and is hoping to start clinical trials next summer.

Another toxin that has been conjugated with CD4 is ricin. Like *Pseudomonas* exotoxin, ricin is extremely lethal to cells, acting by blocking cellular protein synthesis. Like PE40, ricin A chain is a subunit of the toxin that retains its lethal qualities, while lacking the ability to bind directly to cells.

Jonathan W. Uhr and his colleagues at the University of Texas Southwestern Medical Center have shown that the CD4–ricin A chain combination can selectively target HIV-infected cells. More recent work used a combination of ricin A chain linked to antibodies that specifically recognize the gp41 molecule that anchors the gp120 to the cell membrane.

While Upjohn, Hoffmann–La Roche, and Genentech are clearly enthused enough about these new compounds to bankroll their development, Daniel Hoth, director of the division of AIDS at NIAID, is cautious about predicting success.

"There's a solid laboratory foundation," says Hoth. "All you can say is this has a lot of promise, but I don't think it's a 'drop everything' situation."

There are several hurdles researchers already know are looming. One is the immune system itself. As a foreign protein, the PE40 or ricin A chain portion of the immunotoxin molecule should, under normal circumstances, prompt an antibody response that will make the therapy ineffective. But there may be a way around this. "The strategy would be to combine immunotoxin treatment with a known immunosuppressant agent, such as cyclosporin," says Pastan. "If you give [CD4-PE40] with these agents, the antibody response would be suppressed, and you'd be able to give it longer."

Another problem is that CD4-PE40 is only expected to be effective against infected cells because it won't be administered in doses adequate to bind all free virus. Berger says this could be overcome by giving the compound along with a drug such as AZT that blocks new viral infections.

Soluble CD4 and the CD4-toxin compounds are noteworthy because they are the first drugs designed specifically to combat HIV infection and AIDS. But with new information constantly surfacing about HIV, they surely won't be the last.

■ JOSEPH PALCA

ADDITIONAL READING

E. A. Berger *et al., Proc. Natl. Acad. Sci. U.S.A.* **86**, 9539 (1989).

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Is the AIDS Epidemic Slowing?

A special workshop on national projections for numbers of AIDS cases and rates of HIV infection has concluded that the rate of increase of reported cases of AIDS slowed in the middle of 1987, especially among homosexual and bisexual men who were not illegal drug users. This dip, taken together with new surveillance data, has prompted epidemiologists to cut by approximately 15% the number of AIDS cases predicted over the next 3 years.

These conclusions, contained in a draft report obtained by *Science* of the 2-day meeting sponsored by the Centers for Disease Control in Atlanta on 31 October to 1 November, have sparked a debate over what factors are causing the good news.

For Sten Vermund, chief of the epidemiology branch in the division of AIDS at the National Institute of Allergy and Infectious Diseases, the answer was clear: "We have seen what we had always hoped we would see: some kind of measurable public health benefit from these huge investments that we are making."

But that doesn't answer which of three factors have brought about the dip in the number of cases: the effect of therapy, an acute drop in reporting of AIDS cases, or a decrease in the rate of HIV transmission from earlier years.

Vermund believes the answer is therapy. He bases this in part on work by Mitchell Gail at the National Cancer Institute. Gail uses a technique called back calculation to estimate the number of HIV infections that must have occurred in the past to produce the number of AIDS cases for a given point in time. For 1987, Gail's model says there should be more AIDS cases than there actually were, even assuming that public education efforts drastically reduced transmission of the virus within certain high-risk groups as early as 1983. Vermund says this points strongly toward drug therapy as the likely cause.

"I am so excited about it that I am spending not insubstantial parts of my work week trying to track down data from our many contractors to provide data to reinforce Mitch's hypothesis," says Vermund.

But others believe that education, not treatment, is responsible for the rate of new AIDS cases. "I'm very strongly in the behavior modification camp," says Mac Hyman, an AIDS epidemic modeler at Los Alamos National Laboratory. "If you estimate when you'd expect to see the effects of behavior modification, even though the mean time from infection to AIDS may be 8 to 10 years, you should see an effect in about 4 years." That, says Hyman, coincides nicely with the number of years that have passed since the start of behavior modification campaigns in high-risk groups. If drug treatment were the cause of the declining numbers, you'd have to suppose that people who were infected with HIV, but who had no AIDS symptoms, were taking AZT or some other drug as early as 1986. That's not likely, says Hyman.

Whatever its cause, the dip in the number of AIDS cases in 1987 may substantially change forecasts for future years. According to Hyman, until 1987, the epidemic history best fit a steeply rising cubic growth equation. Since 1987, a more gradually increasing quadratic function provides a better fit. According to draft figures from the CDC workshop, the latest estimates are that there will be 40,000 to 50,000 newly diagnosed cases of AIDS in 1989, a number that rises to between 67,000 and 90,000 in 1993. These are lower than predictions made only last year, though the raw numbers are hardly comforting.

HIV infection rates are also likely to be adjusted downward. In 1986, the Public Health Service estimated that between 1 million and 1.5 million people in the United States were infected with the AIDS virus. But four different back calculation models now put the number in 1985 closer to 600,000. Similar estimates for the number presently infected with HIV range from approximately 700,000 to 1,310,000.

In addition to back calculation, CDC uses a variety of surveillance programs to determine HIV prevalence. Data from military recruits, hospital trauma patients, childbearing women, Job Corps entrants, and federal prisoners also provide data for inferring the rate of infection in the general population. Preliminary analyses of these data put the current prevalence between 800,000 and 1,300,000, very close to the estimate derived by back calculation.

The draft report is currently being reviewed by meeting participants, and insiders say the numbers may change a bit. A final report—to be delivered to the head of the Public Health Service—is expected in January 1990. ■ JOSEPH PALCA