A Controversial Shot in the Arm

Possible AIDS vaccine taps an unlikely protein called Tat

By JOHN TRAVIS

In 1890, in work that later won him a Nobel prize, the German scientist Emil von Behring successfully immunized rabbits and mice against tetanus and diphtheria, two serious illnesses that commonly afflicted people. Instead of vaccinating the animals with weakened copies of the bacteria responsible for the diseases, Behring made use of the fresh discovery that the microbes cause sickness by secreting toxins into the bloodstream.

When he and a colleague injected harmless amounts of the toxins into his animals, their immune systems made antibodies that bound and neutralized the toxins. More importantly, the antibodies prevented illness when the researchers later injected the rabbits and mice with the bacteria.

Physicians quickly began to treat infected people with antitoxin antibodies generated in animals. Several decades later, scientists adapted the antitoxin strategy for mass immunization by inoculating people with toxoids, inactive versions of the toxins made by the diphtheria and tetanus bacteria.

AIDS investigators embracing this century-old strategy argue that the HIV protein Tat is also a secreted toxin, one that dangerously subdues the immune system. They therefore predict that vaccinations with Tat, a molecule previously recognized as helping HIV turn on its genes, may prevent HIV infection or keep AIDS symptoms from developing in a person already infected with the virus.

"We don't think Tat is just another player in the immunosuppression [caused by HIV], we think it's the key player," says David I. Cohen of Queen's College in Flushing, N.Y. "Unless we get immunity to Tat, and that isn't going to be easy, we're not going to get a good protective vaccine."

Controversy swirls around Tat, however. There's an ongoing debate, even among those who advocate using the protein in a vaccine, about what the secreted form of Tat actually does in a person infected with the AIDS virus. Consequently, many scientists think that Tat vaccinations are unlikely to succeed.

"I'm not impressed by the cumulative body of evidence," says John P. Moore of Rockefeller University in New York. "Yes, there are effects in [test-tube] systems for extracellular Tat. Now, is that physiologically relevant to HIV infection? There's no good evidence to say so."

Disregarding such strong opinions, however, a few research teams have taken a dramatic step. They've begun to inject people with inactivated forms of Tat.

At first glance, Tat makes an unlikely candidate for a component of an AIDS vaccine. Unlike components of other viral vaccines, Tat isn't present on the surface of the viral particles that roam the bloodstream of an infected individual. HIV makes Tat only after the virus gets inside a host cell. The protein then activates viral genes crucial to HIV replication. It's difficult for most scientists to imagine how an immune response would reach a viral protein inside a cell and control an HIV infection.

In the late 1980s, however, some scientists began to observe higher concentrations of Tat outside infected cells than could be accounted for by the bursting of cells dying from the virus. They eventually concluded that cells infected with HIV actively secrete Tat.

The burning question then became, What is Tat doing out there? For the past decade, biologists have tried to address that issue by exposing various human cells to Tat and documenting their responses. It's an understatement to say that there's a lack of consensus about the results of those efforts.

"How Tat functions inside the cell is now fairly well understood. But what it is doing outside the cell is controversial," says Andrew P. Rice of the Baylor College of Medicine in Houston. "There are enough fairly credible publications that one has to take seriously the concept that Tat has effects outside the cell. What those may be is a morass in the scientific literature."

Many factors contribute to the confusion over Tat. For one, the protein isn't easy to keep in its biologically active state. "A lot of people said that Tat didn't do anything, but you can trace that to the fact that they didn't know how to handle the molecule properly," argues C. David Pauza of the University of Wisconsin Medical School in Madison. "It rapidly reverts to an inactive form."

"It's a nightmare to work with," agrees Rice.

Another complication may result from there being two forms of Tat, one with 72 amino acids and one with 101. The two proteins can trigger profoundly different responses, says Eric M. Verdin of the University of California, San Francisco. In fact, some of his experiments suggest opposing roles for the two Tats. The long version seems to act as an immune suppressor, whereas the short one stimulates immune cells.

Then, there's the thorny issue of the appropriate laboratory dose of Tat. In many of their experiments, scientists have used far larger amounts of Tat than cells would appear to encounter in the body. Yet researchers caution that it's impossible to gauge how much Tat exists in the microenvironments between cells.

Those caveats aside, scientists have recorded a diverse, and sometimes contradictory, array of functions for extracellular Tat. One study suggests that Tat attracts immune cells, perhaps to lure future victims of HIV. Several research teams have reported that Tat induces immune cells to make extra copies of the surface proteins through which HIV infects those cells. Another recent publication describes how Tat wakes immune cells from their resting state and makes them more vulnerable to virus replication (SN: 7/26/97, p. 53).

In terms of immune suppression, investigators have assigned several roles to Tat. A few test-tube studies suggest that it can induce uninfected immune cells to commit suicide. An Italian research team reported last year that Tat impairs the cell-destroying ability of immune sentinels called natural killer cells.

Also last year, a study concluded that the viral protein spurs immune cells called macrophages to release large amounts of alpha-interferon. This chemical seems to suppress other immune cells from making several other compounds that stymie HIV's ability to infect cells. The work came out of a collaboration led by two scientists who have long proclaimed the importance of extracellular Tat—Robert C. Gallo of the University of Maryland's Institute of Human Virology in Baltimore and Daniel Zagury of the University of Pierre and Marie Curie in Paris.

Cohen's work also draws attention to the influence of Tat on macrophages. In the Sept. 14 PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES, he and his colleagues offer evidence that Tat hinders the immune system of live animals. For exam-

ple, they report that if mice are injected with active Tat, they have a much more sluggish antibody response to the foreign protein than mice injected with an inert form of Tat do. Rodents receiving the active Tat also do a poor job of making antibodies against another HIV protein, leading Cohen's group to conclude that Tat suppresses the animals' overall immune response.

The investigators believe that macrophages lie at the heart of this blunted immune response. They found that small quantities of Tat trigger the cells to make a surface molecule called FasL. This protein, in turn, commands the suicide of some immune cells that help in the attack on HIV.

"The macrophage swallows Tat and turns into an immunosuppressive beast," contends Cohen.

Many investigators, however, remain unconvinced that any of the proposed roles for extracellular Tat take place in an infected person. "You always have to be careful to what extent these experiments mimic the reality of life. You can do a lot of things in the test tube that have little bearing on what goes on in vivo," notes Beatrice Hahn of the University of Alabama at Birmingham.

Scientists who favor using Tat to vaccinate people argue that several observations support their novel strategy, even if questions remain about how Tat acts. "There are a number of studies in humans and monkeys that indicate that the immune response to Tat correlates with nonprogression to AIDS," says Barbara Ensoli of Istituto Superiore di Sanità in Rome.

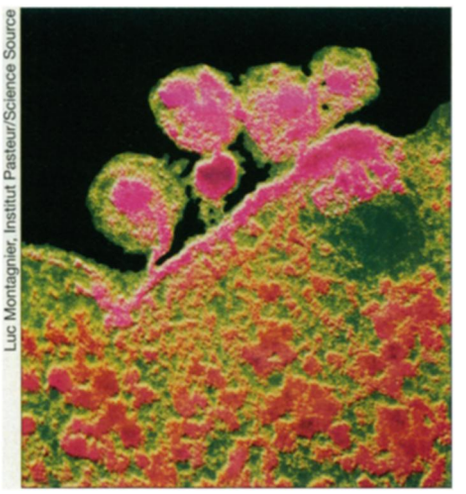
Last year, for example, Zagury, Gallo, and their colleagues reported that HIV-infected people who naturally had higher blood concentrations of Tat-neutralizing antibodies were less likely to develop AIDS rapidly.

A research group led by Ab Osterhaus of Erasmus Medical Centre Rotterdam in the Netherlands, made a similar discovery 2 years ago. Studying a dozen people with HIV, the scientists found that those who progressed quickest to AIDS had relatively few virus-killing immune cells, known as cytotoxic T lymphocytes, that recognize Tat.

Osterhaus also recently reported that a Tat-based vaccine seems to protect monkeys from SIV, a simian version of HIV. In the June VACCINE, he and his colleagues described vaccinating two monkeys with a harmless virus engineered to make the SIV forms of Tat and another protein, Rev. The scientists then injected the two monkeys, as well two unvaccinated monkeys, with SIV. In the untreated monkeys, SIV quickly established itself and replicated extensively. In the immunized pair of monkeys, however, Osterhaus' team found evidence that the virus was present only for the first few weeks. Over the next 36 weeks, the scientists didn't detect the virus in the animals' blood.

Ensoli and her colleagues recently took a different tack. They injected monkeys with an active form of Tat and later inoculated the monkeys with an SIV-HIV hybrid virus, or SHIV. Only two of seven vaccinated monkeys developed a persistent SHIV infection, while a pair of unvaccinated animals both did, Ensoli's group reported in the June NATURE MEDICINE.

The apparent success of the vaccine drew front-page headlines in Europe long before the researchers published their data. AIDS scientists, however, have proven to be a much tougher audience. Both publicly and privately, they've criticized the report for several shortcomings. Even researchers pursuing Tat-based vaccines have complained, for example, that the SHIV strain used by Ensoli does not offer a stiff challenge to a monkey's immune system. "It's barely growing in the animals to begin with, so it's not that difficult to protect against," says Pauza.


Luc Montagnier, Institut Pasteur/Science Source

Copies of HIV bud from a white blood cell, as seen in a false-color micrograph.

Ensoli rejects that argument, stressing that unvaccinated monkeys given the SHIV virus develop a vigorous infection and suffer a dramatic loss of immune cells. Half of her control monkeys have already died, she told SCIENCE NEWS.

Another criticism focuses on Ensoli's use of an apparently active form of Tat. The scientists convinced that the viral protein is immunosuppressive argue that inactivated forms are safer and still generate an impressive immune reaction.

"Why would you ever give a poison if you can get just as good an immune response with a toxoid?" asks Gallo. At a meeting last month, for example, Pauza presented unpublished data showing that immunization with an inactivated Tat protects rhesus monkeys from the effects of an infection with a strain of SHIV.

Ensoli, who believes that HIV uses extracellular Tat to spread to other cells and replicate more efficiently, argues that the protein itself isn't harmful. "We never saw any kind of toxic effect in all the monkeys we vaccinated with Tat," she says.

Researchers who split on whether Tat is a toxin also disagree about how a Tat-based vaccine may protect a person. Ensoli and Osterhaus contend that Tat-recognizing immune cells are most crucial, while Pauza, Gallo, and Cohen stress the importance of antibodies that neutralize the protein.

Despite the disagreements, investigators have already begun to test a Tat vaccine in people in Europe. In the past 2 years, several dozen HIV-infected and uninfected people have received injections of inactive Tat. They suffered no ill effects and developed a strong antibody response to the viral protein, says Gallo, who is collaborating with Zagury and other researchers on the study.

These initial trials were intended solely to test the safety of the vaccine and its ability to trigger an immune response to Tat. Gallo, Cohen, and other investigators plan to start U.S. trials next year that would begin to evaluate whether the immunization strategy helps HIV-infected people avoid AIDS. Study subjects will be patients who either aren't responding to conventional drug therapy or can't tolerate its side effects.

Investigators express the hope that periodic immunizations with Tat could offer a practical alternative for HIV-infected people in developing countries that cannot afford the expensive daily drug regimens available in the United States and Europe. "Our mind is on trying to help the Third World," says Gallo.

He and others even envision that a Tat-based vaccine, in conjunction with other vaccines being developed, can prevent infection with HIV. If Tat-neutralizing antibodies defuse the protein's ability to suppress the immune system, the body's response to components of other AIDS vaccines now being developed might defeat the virus.

"Tat is going to be a very important addition to conventional vaccines against HIV and has the potential to push them over the top. I think it will enhance vaccines using other viral proteins," says Pauza. "I was very skeptical when we started. Now, the data have beaten me into submission."

For many AIDS scientists, however, those same data aren't nearly convincing. Still, given that the other vaccines currently in clinical trials aren't expected to succeed, most investigators are unwilling to simply dismiss the idea that Tat-based vaccines can make an impact in the fight against HIV.

"I want to keep an open mind," says Gary Nabel, director of the newly created Vaccine Research Center at the National Institutes of Health in Bethesda, Md. "We're not so far along in vaccine development that we can afford to ignore any option." □