Research News

Debate over Potential AIDS Drug

Researchers at the National Institute of Mental Health contend that a small piece of the AIDS virus coat protein called peptide T may resemble a naturally occurring substance in brain and also have anti-AIDS virus activity, but many other scientists dispute its possible therapeutic effects

ABOUT a year ago, Candace Pert began to present her ideas on brain disease in AIDS and the prospect of treating AIDS patients with a substance she called peptide T. Pert, a researcher at the National Institute of Mental Health (NIMH), reported that in her experiments, peptide T inhibits the deadly AIDS virus from replicating. Scientific reaction to these claims ranged from great interest to great caution while AIDS patients eagerly awaited more news. Since then, many researchers' caution has turned to skepticism, while others have become convinced that the peptide does work against the AIDS virus, so far only under tightly controlled experimental conditions.

Last December, Frederick Goodwin, director of intramural research at NIMH, submitted an investigational new drug application (IND) to the Food and Drug Administration (FDA) to begin clinical testing with peptide T. "I don't think anyone can tell right now if FDA is likely to approve the IND or not," said an FDA official in an interview with *Science* on 2 July. "We are still awaiting data from the sponsor."

The major scientific basis of Pert's ideas is straightforward. It is that peptide T, which is derived from a small segment of the envelope protein (gp120) that surrounds the AIDS virus, blocks binding of the intact virus to its normal docking site on cells, the so-called T4 antigen present in high concentrations on helper T lymphocytes. Hence, she says, peptide T may interfere with the spread of the AIDS virus in an infected person, possibly making it a treatment for the disease.

Since Pert's data were published in a December 1986 issue of the *Proceedings of the National Academy of Sciences* (*PNAS*), however, the original results have been anything but straightforward to replicate. Moreover, some researchers criticize the fact that many unpublished results are being made public through the media without peer review. Others contend that ambiguous results from in vitro studies are driving peptide T toward clinical trials prematurely. But pressure to develop new drugs for AIDS is intense, and, perhaps inevitably, not all aspects of the scientific process are successfully withstanding the pressure.

During the past 6 months no less than 11 different laboratories have tested peptide T for its ability to block replication of the AIDS virus (human immunodeficiency virus or HIV) in vitro, and opinion about its efficacy varies widely. "In the systems we have tested, peptide T does nothing," says William Haseltine of the Dana-Farber Cancer Institute in Boston. By contrast, George Todaro of Oncogen in Seattle, Washington, says that "at certain concentrations of the peptide with certain concentrations of the virus on certain cell types, then it works."

Candace Pert: *"We are firmly convinced that peptide T inhibits HIV replication by blocking the binding of gp120."*

Todaro's laboratory, in collaboration with Elaine Kinney Thomas of Genetic Systems in Seattle (a sister company of Oncogen), is so far the only one outside the sphere of Pert's collaborators to report data that agree with hers. At least nine laboratories claim that peptide T is ineffective; and at least one laboratory has obtained negative preliminary results, but hestitates to conclude anything because the tests were incomplete.

Two critical aspects of the data are being challenged. One is whether peptide T blocks binding of the AIDS virus by competing with the envelope glycoprotein (gp120) for the T4 binding site. A second issue is whether the peptide effectively inhibits HIV replication in vitro.

The research began about 3 years ago, according to William Farrar of the Frederick Cancer Research Facility in Maryland. He and Pert were screening monoclonal antibodies that activate T lymphocytes for their possible reactivity with brain tissue. "One of them, OKT4, recognized brain sections," he says. Later, as other scientists reported that the AIDS virus binds to the T4 antigen recognized by the OKT4 antibody, Farrar and Pert investigated the possible connection between the receptor for the AIDS virus and a normally occurring brain protein (*Science*, 27 March, p. 1574). By using the OKT4 antibody, however, Farrar and Pert invited controversy because several research groups find that it does not label the specific binding site for the AIDS virus on T4.

Last fall, Pert asked Samuel Broder of the National Cancer Institute (NCI) and Gerald Quinnan of FDA to test whether peptide T blocks replication of the AIDS virus in vitro. Using their own assay systems, both tested samples of peptide T, obtained negative preliminary results, and reported them to Pert. Quinnan's laboratory did not pursue the research, but Broder's laboratory did. Broder plans to publish his results.

Goodwin emphasizes that researchers who obtain negative results do not use the same experimental methods that Pert reports. In a recent interview with *Science*, Pert said, "Every scientific experiment has very specific conditions under which it has to be performed." But other researchers stress that if the peptide does not work under a variety of experimental conditions, it is much less likely to work in AIDS patients.

NIMH called a meeting on 30 June to enable scientists who have tested peptide T to air their data and differences of opinion.* Two major issues emerged. First, in various experimental systems peptide T has potent biological effects, which may or may not be

*The meeting sponsored by NIMH on "Strategies for the Evaluation of Anti-HIV Effects of Peptide Drugs in the Immune and Central Nervous Systems," was held 30 June at NIH.

D–Ala–Ser–Thr–Thr–Thr–Asn–Tyr–Thr–NH_2

Peptide T. *The original sequence under review at the FDA is modified chemically at both ends and has eight amino acids, but Pert now says that only the final five are required for biological activity.*

important in AIDS. Second, the reported biological effects of peptide T, including its ability to inhibit replication of the AIDS virus under certain conditions, may not depend on its ability to bind to the receptor site for HIV.

In vitro test systems for peptide T fall into three general categories: measurements of HIV replication in T lymphocytes, including assays for the enzyme reverse transcriptase or the production of viral protein; determinations of T cell fusion events, made by counting the number of multinucleated cell clumps termed syncytia; and assays of peptide activity that may or may not be related to the AIDS virus, evaluated in mouse macrophages or neuronal tissue.

"In the experiments reported in *PNAS*, Pert and her colleagues used an undefined strain of the virus and fresh peripheral blood lymphocytes," notes Joseph Sodrowski of Dana-Farber. These conditions for measuring viral infectivity can never be duplicated, he says.

According to Sodrowski, he and Haseltine and their colleagues initially used peptide T to probe whether it could inhibit gp120 binding to the T4 receptor. They tested the same batch of peptide T that Pert used, in three kinds of assays—gp120 binding, fusion of T cells, and HIV replication in vitro (*Lancet*, 20 June, p. 1428). It was ineffective at all concentrations they tested, which were higher than the optimal dose range reported by Pert and her colleagues. "In my mind I don't see any effect of peptide T," says Sodrowski.

Haseltine asserts that Pert's original reason for testing the peptide—namely her claim that it represents a conserved segment of gp120 within a region of the protein that is otherwise highly variable—is ill founded. "The so-called conserved region is not conserved at all," he says. "There is not a single amino acid that is conserved when you compare different isolates of the AIDS virus." Sodrowski and Haseltine describe gaps in the peptide T sequence at sites where Pert finds homology because the Boston researchers align the amino acid sequences somewhat differently than Pert.

In response to recent comments from Haseltine about her data (*Science*, 19 June, p. 1523) Pert says, "Absence of proof is not proof of absence. We are firmly convinced that peptide T inhibits HIV replication by blocking the binding of gp120."

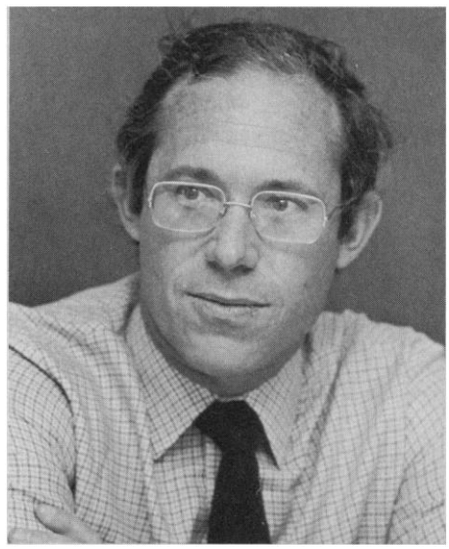
Several European investigators, including Hans Wigzell of the Karolinska Institute in Stockholm and Jaap Goudsmit of the University of Amsterdam in The Netherlands, have also been unable to reproduce Pert's results. "We made a very thorough study," says Wigzell. "The peptide didn't do anything. We have not been able to confirm a rational basis for using it."

Five other research groups, represented by Lawrence Lasky of Genentech in South San Francisco, Steven McDougall of the Centers for Disease Control in Atlanta, Angus Dagleish of Northwick Park Hospital near London, Thomas Matthews of Duke University in Durham, and Christine Ljungren of Roslagstull Hospital in Stockholm, reported at the recent NIMH meeting that peptide T is ineffective in their experimental systems, all of which differ somewhat from Pert's. In particular they note that it fails to block the HIV binding site on the T4 receptor and therefore fails to block the biological processes—fusion of T cells, for example—that apparently depend on this interaction. Sodrowski and McDougall also say that small peptides in general, including peptide T, are not likely to block gp120 binding, because they cannot mimic the complex interaction of this much larger protein molecule with the T4 receptor that depends on the folded three-dimensional structure of gp120.

Pert strongly contests the evidence that peptide T does not block gp120 binding and she challenges the methods that lead other researchers to this conclusion. Speaking at the recent NIMH-sponsored meeting about peptide T, Pert said, "inhibition by antibody binding is really a poor technique for demonstrating a peptide binding site on a specific receptor." She also notes that peptide T itself tagged with a radioactive label can be displaced from its binding sites in brain tissue with gp120, an indication, she says, that both bind to the same T4 receptor. But Sodrowski argues that Pert has failed to demonstrate that the target for peptide T and gp120 binding in brain really is the T4 receptor.

In contrast to the negative results, however, one laboratory outside Pert's original group of collaborators confirms that peptide T blocks HIV replication in vivo. "My results are very similar to Pert's," says Thomas, whose data Todaro cites in his endorsement of peptide T. Todaro says that Oncogen is thinking about developing peptide T as an anti-AIDS drug but has not yet made a final decision on the matter.

Thomas initially wanted to compare the reported positive effects of peptide T with other peptides that Genetic Systems is testing as potential drugs for AIDS. In three experiments she used a different form of peptide T than that reported by Pert and in two more recent experiments she used the same form. The results of all her experiments indicate that by limiting the infectious dose of the virus and by preincubating cells with a low concentration of peptide T, the peptide is about 90% effective in blocking viral antigen production. It is less effective without the preincubation, which may help to explain why other laboratories that did not do the preincubation step failed to see positive effects.



William Haseltine: *"In the systems we have tested, peptide T does nothing."*

Two of Pert's original collaborators, Michael Ruff of the National Institute of Dental Research and Frank Ruscetti of NCI, have recently reproduced the results from their early work but have used different experimental systems. Ruff spent several months in Anthony Fauci's laboratory at the National Institute of Allergy and Infectious Diseases learning how to do an assay that measures HIV infection of a T cell line. At the recent NIMH-sponsored meeting he reported that at certain concentrations, peptide T inhibits by 80% replication of a French LAV isolate of the AIDS virus.

Ruscetti finds that peptide T blocks HIV infection of both freshly isolated and activated T cells as well as a T cell line. Like Thomas, he uses a limited concentration of the virus but, unlike Thomas or Ruff, he has tested the peptide against three different viral strains and finds it effective against all of them.

At least two different issues remain with respect to the ability of peptide T to block replication of the AIDS virus in vitro. One is why Pert, Thomas, Ruff, and Ruscetti see a

positive effect of the peptide within a narrow concentration range and lose the effect at higher doses. Such biphasic dose-response curves are not unprecedented, but the reason why peptide T behaves this way is yet to be explained.

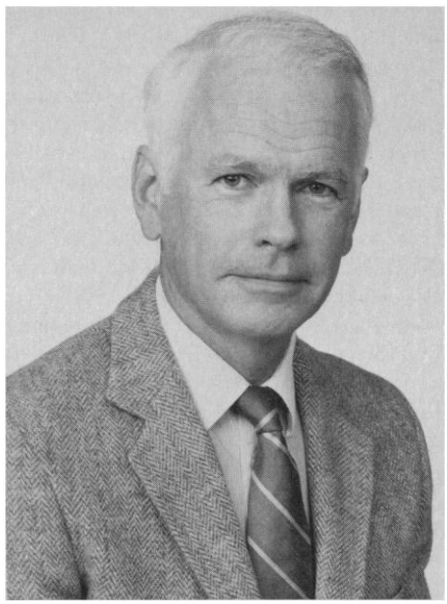
A second issue is whether the peptide's ability to inhibit viral replication under carefully controlled conditions really occurs because it blocks gp120 binding to the T4 receptor. Data from Haseltine, Goudsmit, Wigzell, Lasky, McDougall, Dagleish, Matthews, Ljungren, and Farrar suggest that the peptide does not act at the HIV binding site. "I have the feeling that peptide T is doing something different," says Goudsmit. "It could be interesting, but it needs to be straightened out before giving the compound to patients."

Two other research groups now find that peptide T has potent biological effects perhaps mediated by receptors other than T4. Douglas Brenneman of the National Institute of Neurological and Communicative Disorders and Stroke reports that very low concentrations of peptide T protect cultured mouse neurons from the toxic effects of gp120. Vasoactive intestinal peptide (VIP), which also has effects on non-neuronal cells in the central nervous system, similarly protects spinal cord and hippocampal nerve cells from the toxic effects of gp120. Whether peptide T and VIP are working through the same receptor, whether that receptor is T4, and whether any of these effects are important in AIDS patients with brain disease have yet to be determined.

Speaking at the same meeting, Esther Sternberg of NIMH said that a narrow concentration range of peptide T and closely related peptides induces mouse macrophages to express the Ia histocompatibility antigen. But researchers question whether her results and Brenneman's involve peptide T activity at T4 receptors.

A broader question about peptide T is whether any of the in vitro results predict what the peptide will do in an AIDS patient. Participants at the NIMH meeting noted that peptide T has a very short half-life in experimental animals and that it may be difficult to give injections of the peptide and maintain the relatively narrow range of concentrations over which it has any biological effects. Also, peptide T is unlikely to cross the blood-brain barrier unless it is somehow coupled to a molecule that can transport it into the brain, a chemical modification that has not been undertaken. But these concerns anticipate clinical trials with peptide T, and the road to clinical testing has also been rocky.

The first use of peptide T in humans began under something of a procedural cloud. In an interview with *Science* in March, Pert said that Lennart Wetterberg, a psychiatrist at the Karolinska Institute in Stockholm, heard her describe data about peptide T and brain disease in AIDS last year. He asked Pert to send him samples of the peptide for testing in AIDS patients, which she did. This occurred prior to FDA's review of the compound for use in humans (*Science*, 6 March, p. 1138).



Frederick Goodwin *applied to FDA last December to begin clinical tests of peptide T.*

On 17 January, Wetterberg, Pert, and their collaborators published a paper in *Lancet* that briefly described the results of the Swedish tests. Four male AIDS patients received peptide T for 4 weeks on a "compassionate use" basis and showed some improvement. In the absence of a control population of AIDS patients who did not receive the peptide, however, it is impossible to be certain if any of the observed effects, good or bad, were caused by peptide T.

According to Sven Britton of Roslagtull Hospital, all the AIDS patients were in late stages of the disease and three died 6 to 8 months after getting the peptide. The patients received peptide T on a regular basis for only 1 month, making any long-term effects of the peptide impossible to evaluate. Wetterberg, Britton, and their colleagues have now begun a formal clinical trial of peptide T in 36 AIDS patients in Sweden and expect to be able to report some results within the next 6 months.

In an interview with *Science* in March, NIMH's Goodwin said that Pert sent the peptide to Sweden without appreciating FDA regulations on providing drugs to other countries for use in humans. "I admonished her for that," he says. He also notified the NIH Office for Protection from Research Risks and asked the Swedish researchers to send copies of the consent forms signed by the AIDS patients. Normally these procedures precede the export of a compound for clinical use in other countries. Pert then stopped her supply of peptide T to Sweden, Goodwin said.

Since Goodwin submitted an IND application to the FDA for peptide T in December, the NIMH researchers have been responding to FDA requests for more information about the peptide. Although FDA officials cannot discuss individual cases, Janet Woodcock, who is handling the application for peptide T, says that FDA looks for a scientific rationale for testing a compound, evaluates the clinical trial design, and requires information about the purity, potency, and safety of the specific product.

Reports about the precise status of peptide T with regard to FDA review and clinical trials have reflected confusion. In his "talking points" about peptide T made available to the press at the Washington AIDS conference at the beginning of June, Goodwin wrote, "The National Institute of Mental Health has received FDA approval to begin testing peptide T, an analog of a naturally-occurring brain chemical, in AIDS patients." Later, on 23 June, Peter Bridge, of NIMH, said that they "have not yet filed all the paperwork" requested by FDA on their most recent batch of peptide T. Also on 23 June, Goodwin said, "In a technical sense, we may not have an IND yet. Nevertheless, we were called by FDA officials prior to the AIDS meeting and told that the clinical trial was going to be approved, but that NIMH had to supply some additional technical details."

Three things are clear, however. First, as of 2 July, the FDA does not yet have all of the information they have requested. Second, clinical trials on peptide T cannot begin until FDA officials receive and review the information. And third, Bridge and Goodwin fully expect that clinical trials at the NIH clinical center will begin soon.

Despite the uncertainties now associated with peptide T, many researchers believe that Pert's basic ideas are sound. Cells of the nervous and immune systems *do* share similar receptors. It *is* likely that naturally occurring substances normally bind to these receptors. And the AIDS virus *can* harm cells directly by infecting them or may damage them indirectly by interfering with the action of normal substances that bind to the same receptors. Whether this formula is confirmed in the case of peptide T, however, remains to be demonstrated conclusively. ■ **DEBORAH M. BARNES**