Brain Damage by AIDS Under Active Study

Researchers agree that the AIDS virus injures the nervous system, but whether damage results from direct viral infection of brain cells or by indirect means is subject to controversy

Since 1985, when several groups of investigators reported that the AIDS virus not only enters the cerebrospinal fluid that bathes the brain and spinal cord, but also enters the brain itself, researchers have mounted a massive effort to determine how the AIDS virus so dramatically impairs nervous system function. Within the past year they have documented the neurological symptoms that many AIDS patients develop, ranging from mild confusion and poor coordination to profound dementia and an inability to control movement. Other scientists have identified the cell types—primarily macrophages and monocytes—that contain most of the detectable virus in the brains of AIDS patients. But even with this information in hand, the mechanisms by which the AIDS virus damage the nervous system have remained an enigma.

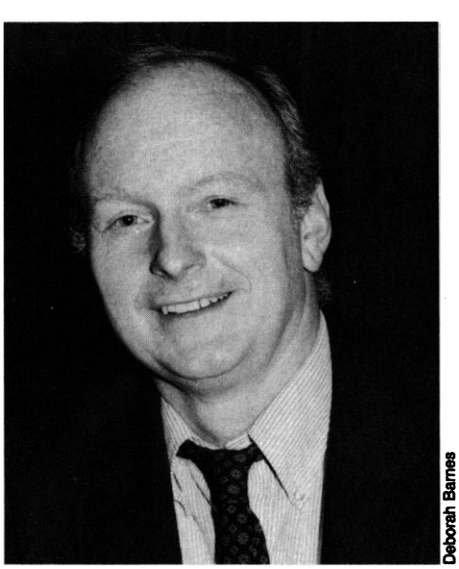
Now, new information is leading researchers to debate whether AIDS-related neurological damage is caused by direct viral infection of cells in the nervous system—glial cells in particular—or whether the virus mediates its damage indirectly, perhaps by inhibiting the actions of substances that are important for the survival or maintenance of the nervous system. Both ideas have some support, but the new evidence also calls into question the role of the T4 antigen, which on T lymphocytes binds the outer protein of the AIDS virus.

An emerging concept, new in terms of AIDS research but not new to many neuroscientists and immunologists, is that membrane proteins and responses to secreted factors that are shared by the nervous and immune systems may underlie much of the damage in AIDS. Perhaps because some of its proteins mimic the structure of naturally occurring substances, the AIDS virus may damage cells of the nervous and immune systems both directly and indirectly.

"Eventually, one-half to two-thirds of the 14,000 living AIDS patients in the United States will develop moderate to severe neurological problems." says Richard Price of the Memorial Sloan-Kettering Cancer Center in New York. Last year, Price, Bradford Navia, Carol Petito, and Enn-Sook Cho, also of Sloan-Kettering, reported that the evidence of damage to nervous system tissue, even in AIDS patients with severe symptoms, can be surprisingly subtle. Some, but not all, patients show a slight brain shrinkage, enlarged ventricles, abnormally staining white matter, or vacuolar myelopathy, a spinal cord abnormality in which the myelin sheaths surrounding nerve fibers contain abnormal spaces or vacuoles.

At about the same time, Anthony Fauci and Scott Koenig of the National Institute of Allergy and Infectious Diseases and their colleagues reported that 95% of the detectable virus in brain occurs in immune system cells, monocytes, or macrophages, some of which fuse to form multinucleate giant cells. Clayton Wiley, of the University of California at San Diego, and Jay Nelson and Michael Oldstone, of Scripps Clinic and Research Foundation in La Jolla, reported that some brain endothelial cells, which line blood capillaries, also contain virus.

To address the issue of how the AIDS virus severely impairs nervous system function, apparently without causing extensive structural damage to brain tissue, scientists are currently pursuing two major research strategies. One is to determine conclusively whether certain cells of the nervous system are susceptible to direct infection by the AIDS virus, and the other is to explore ways in which the virus may damage the nervous system indirectly.



Deborah Barnes

Richard Price *says that, "eventually, one-half to two-thirds of the 14,000 living AIDS patients in the United States will develop moderate to severe neurological problems."*

Several laboratories are now pursuing the first research strategy by determining whether neurons or glia grown in tissue culture or those in the brain of an AIDS patient can be infected with the AIDS virus. Jay Levy, Cecilia Cheng-Mayer, James Rutka, Mark Rosenblum, Thomas McHugh, and Daniel Stites of the University of California at San Francisco have preliminary evidence that the AIDS virus will infect two kinds of brain-derived cells in vitro. These are glioma cell lines, which are transformed glial cells—non-neuronal cells whose complex functions are not completely understood—and human fetal brain cells that have been subcultured several times.

"Three of the four glioma lines seem to replicate some viral isolates but not others," says Levy. "And the cultures that contain cells which stain positive for GFAP [glial fibrillary acidic protein], a marker for astrocytes, tend to be infected more often." The San Francisco group also finds that the source of a particular viral isolate does not predict the kinds of cells it will infect in vitro. Brain isolates infect some, but not all, cultures from brain and peripheral blood, and peripheral blood isolates show a similar heterogeneous pattern of infectivity.

Levy and his co-workers increase their ability to detect viral infection by adding peripheral mononuclear cells to the glioma and brain cell cultures, which Levy notes contain a variety of cell types. But even under enhanced conditions, the AIDS virus reproduces in these brain-derived cells at only 1/1,000 to 1/100,000 its level of replication in T4 lymphocytes.

Despite this low level of infection, however, Levy says that their data "clearly show that the AIDS virus will infect brain astrocytes in addition to infecting brain macrophages and endothelial cells. And cells do not need to have CD4 antigen [also known as the T4 receptor on T lymphocytes to which the AIDS virus binds] to be infectible with the virus." Levy, Nelson, and Wiley also have evidence that spinal cord oligodendrocytes, which make the fatty myelin sheaths that surround nerve cell axons, may also be infectible, possibly accounting for vacuolar myelopathy in some patients.

But David Ho of the UCLA School of Medicine is skeptical about in vitro evidence that the AIDS virus infects brain-specific cells directly. "The bulk of the evidence suggests that there is very little direct infection of neural cells," he says. "You can take

virus and add it to glial cell cultures, and if you also add T cells you can rescue the virus. But you really don't know that the virus entered the glial cells. It may have only stuck to the surface."

The notion that the AIDS virus actually infects glial cells in vivo as well as in vitro is strongly supported by new data from Joseph Melnick, Ferenc Gyorkey, and Phyllis Gyorkey of Baylor College of Medicine in Houston. They find that the AIDS virus infects two kinds of glial cells, both oligodendrocytes and astrocytes, in fresh brain biopsy tissue from AIDS patients. The researchers examined tissue from the neocortex of seven patients and found mature virus particles and staining for core protein of the AIDS virus in five of them. They indicate that mature and replicating virions, budding from infected cells, are generally rare, but occur more often in oligodendrocytes than in astrocytes.

"There is now no question that the AIDS virus is harbored in the brain," says Melnick. "What we see in our tiny samples of tissue probably represents what takes place in thousands of places in the brain of an AIDS patient." Melnick describes the Baylor group's recent efforts to find areas of brain cells that contained virus particles as "extremely laborious." He thinks that their ability to examine fresh, rather than postmortem, tissue was a key factor in making electron micrographs that show both intact virus and viral budding from brain glial cells.

Other laboratories are also trying to obtain convincing evidence that the AIDS virus infects brain cells directly in vitro, but with ambiguous results. For example, Suzanne Gartner of the National Cancer Institute (NCI) says, "We have not been able to get what I consider a productive infection in primary cultures of brain cells."

Gartner, Mikulas Popovic Elizabeth Read-Connole, and Robert Gallo, also of NCI, and Werner Mellert of the Institute for Biology Environmental Research Center in Munich, West Germany, are now trying "to see if we can mimic what happens in vivo," says Gartner. "In an AIDS patient, the cell type responsible for persistent brain infection is probably the macrophage. Infected macrophages are not like infected T cells; they don't die quickly, so they can continue to produce virus for a long time." To test how infected cells from the immune system might alter normal brain cells, Gartner and her co-workers add already infected T cells or macrophages to cultures of uninfected glioma and human fetal brain cells. "We have some preliminary evidence of infection, but only in a small number of cells," she says.

Their results are very preliminary, but the

Candidate AIDS Vaccine

"I do not have a vaccine for AIDS; I have a candidate vaccine," says Daniel Zagury of the Pierre and Marie Curie University in Paris, who has inoculated himself and ten Zairian volunteers with recombinant vaccinia virus containing a gene from the AIDS virus. News of the procedure elicited mixed reactions from U.S. scientists, some of whom expressed concerns about using the vaccinia virus. Zagury and his colleagues reported their findings in the 19 March issue of *Nature*.

"No complications up to now have occurred," said Zagury in a telephone interview with *Science*. Each of the people who received the vaccinia-based vaccine was initially free of antibodies to the AIDS virus, an indication that none was already infected. Zagury emphasized that his goal in the experiment was to "trigger signals that promote cell-mediated immunity" that would protect a person from different subtypes of the AIDS virus.

Vaccinia virus has been widely used to immunize people against smallpox and Zagury is testing a genetically engineered form of it that contains a gene from the HTLV-III_B isolate of the AIDS virus that codes for gp160, the envelope glycoprotein composed of gp120 and gp41. Zagury's primary immune response was to make antibodies that killed the same viral subtype in vitro, but failed to neutralize HTLV-III_{RF}, a Haitian isolate that differs genetically. The vaccine also stimulated two cell-mediated responses—lymphocyte mitosis and expression of T-cell receptors for interleukin-2—when Zagury's lymphocytes were exposed to either viral isolate in vitro.

Zagury received the biological materials necessary to produce the candidate vaccine as a result of his prior collaboration with Robert Gallo and Marjorie Robert-Guroff of the National Cancer Institute (NCI), and Bernard Moss of the National Institute of Allergy and Infectious Diseases (NIAID).

Moss sent Zagury two kinds of materials that were intended for experiments in animals, not in humans. One was a recombinant vaccinia virus that expresses the AIDS gp160 protein and the second was a plasmid preparation (which contained small circular chains of viral DNA that included the AIDS virus gene) from which Zagury could make other recombinant viruses. Moss was surprised to learn in December that Zagury had injected himself with the engineered vaccinia virus.

"This was not a collaboration on my part; Zagury simply used material intended for African Green monkeys," says Moss. The prototype vaccine has not been reviewed or approved by the Food and Drug Administration (FDA) for use in humans. It is based on the WR laboratory strain of vaccinia not normally used in humans because it is not produced under stringent laboratory conditions. Although Zagury initially used the WR vaccinia strain in his candidate vaccine, he indicates that new preparations of the vaccine are based on the Lister strain of vaccinia, which has been used in humans.

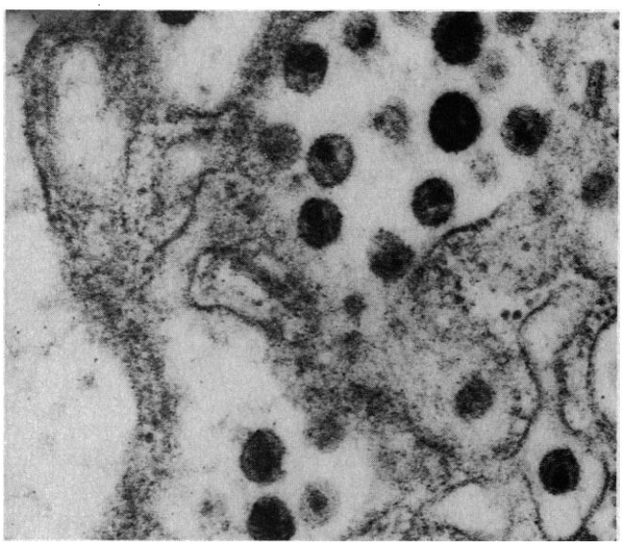
Zagury says that animal testing done in collaboration with the NCI and NIAID researchers demonstrated that the vaccine procedure was innocuous. The new work "has the full support of the Zairian ethics committee," he reports.

Anthony Fauci of NIAID describes the candidate vaccine trial as "potentially a very exciting study." He says that this limited study begins to address the concern that such a vaccine might induce death of cells carrying the T4 antigen. Researchers have worried that gp120 might cause T4 lymphocytes, which bind the protein, to fuse together, thus reducing their number and decreasing a person's ability to mount cell-mediated responses. This did not seem to occur according to Zagury's preliminary results, however. Fauci also notes that Zagury's findings do not say if cell-mediated cytotoxicity was induced. This form of immunity may play a very important role in eliminating cells in the body that are infected with the AIDS virus.

Zagury says that he also treated ten AIDS patients to try to induce cell-mediated immunity, but used an entirely different experimental protocol. Two patients in July and eight more in December "received AIDS virus-infected cells that had been fixed with formaldehyde," Zagury says. The formaldehyde rendered the cells noninfectious but preserved their ability to stimulate an immune response, according to Zagury. He did not comment further on the outcome of these trials. ■ **D. M. B.**

AIDS in vitro

The AIDS virus infects some transformed glial cells in culture, which stain darkly because they are producing viral protein. "Uninfected cells" (or cells not expressing viral protein) can be seen in the background (arrowheads). [Photo courtesy of Werner Mellert]



AIDS in vivo

An AIDS virus–infected glial cell in the brain of a patient actively produces virus particles, some of which have bar-shaped cores. The infected cell is identified as an oligodendrocyte because it is producing myelin (not seen at this high magnification; ×85,000). [Photo courtesy of Joseph Melnick]

NCI group is finding that infected macrophages do alter noninfected glioma cells, and they propose two mechanisms by which this might occur. One is that the virus infects brain cells directly. The other is that macrophages, which secrete regulatory molecules under normal circumstances, secrete abnormal factors when they are infected by the AIDS virus. "I think the viral infection is changing the macrophage and then its function is changed," Gartner says.

Not all researchers are convinced that brain macrophages play a primary role in nervous system damage, however. "Everyone is euphoric, thinking that macrophages bring virus into the brain," Price says. "But macrophages could just be an indicator of infection. Suppose a very low level of virus infects the brain. Then, the macrophages could come in and pick up virus and amplify the infection. There is no evidence that this happens, but there is also no evidence that macrophages bring virus into the brain either."

Price also questions why it is often difficult to find evidence of viral infection in patients with neurological symptoms. "We find virus in the brain of only about one-third of the demented patients," says Price. "An obvious explanation is that the detection techniques are not sensitive enough. But one of the questions that people have raised is, 'Are there some indirect methods by which the virus damages the nervous system?' "

Scientists who pursue this second major research direction envision several possibilities by which damage may occur. First, as Gartner suggests, infected cells may secrete altered factors that can no longer perform some necessary function. Second, infected cells may secrete substances that are actually toxic to some cells. Or third, the AIDS virus may compete for receptor binding sites on cells of the nervous system that would normally be occupied by necessary maintenance or survival factors.

At least two different groups of investigators, Mark Gurney and his colleagues at the University of Chicago and Candace Pert and her co-workers at the National Institute of Mental Health (NIMH), have recently described data that point to the third possibility—specifically, that gp120 (the envelope glycoprotein that surrounds the AIDS virus) may mediate damage indirectly.

Last fall, Gurney, Mark Lee, and Brian Apatoff reported that neuroleukin, a novel factor secreted by stimulated T lymphocytes, promotes the survival of a population of embryonic chick sensory neurons that is insensitive to nerve growth factor. The researchers suggested that, because a region of the neuroleukin protein is partly homologous to gp120 and because neuroleukin can activate B lymphocytes to secrete immunoglobin, there may be a common mechanism by which a product of the AIDS virus both alters immune system function and interferes with neuronal function.

Now, Gurney, Lee, Apatoff, Gregory Spear, and Indre Rackauskas, also of the University of Chicago, in collaboration with Ho have preliminary evidence that fragments of the AIDS virus envelope glycoprotein inhibit the ability of neuroleukin, but not nerve growth factor, to enhance the survival and maintenance of certain nerve cells in vitro. Their new work also shows that brain neurons, specifically septal and hippocampal neurons, respond to neuroleukin, indicating that the trophic factor may work in the central, as well as the peripheral, nervous system.

As yet, however, Gurney and his coworkers have not been able to show that neurons have specific membrane receptors for neuroleukin or to determine whether the AIDS virus envelope glycoprotein competes with neuroleukin for specific binding sites on nerve cells. Additionally, the researchers do not know if adult neurons in the human brain require neuroleukin as a survival factor. But, says Gurney, "with neuroleukin, you have a growth factor that is highly homologous in different species—it affects human B lymphocytes, embryonic mouse brain neurons, and embryonic chick sensory neurons. It may be that a gene product of the AIDS virus, the envelope glycoprotein, is released by infected macrophages in the brain and that it inhibits the function of neuroleukin."

Pert and Joanna Hill, also of NIMH, and William Farrar of the Frederick Cancer Research Facility in Maryland, along with their colleagues also have evidence that the AIDS virus may interfere with the action of a normally occurring substance in the brain. They suggest that a peptide—which Pert and Michael Ruff, of the National Institute of Dental Research, now think is vasoactive intestinal peptide—binds to the T4-like antigen the group has recently identified in brain tissue from rats, squirrel monkeys, and humans. "It could be that parts of the brain—the hippocampus, dentate gyrus, amygdala, and outer layers of the cerebral

cortex in particular—are areas of attachment for the AIDS virus," says Hill. "This could mean that they are sites for infection."

But the accumulating evidence about where and how the AIDS virus affects the brain sometimes points in different directions. For instance, Price's group finds viral antigen staining in AIDS patients' brains, not at the cortical sites that contain T4 receptor, but in the gray matter underlying the cerebral cortex and in the white matter.

Two possible resolutions of this apparent contradiction are that the brain T4 antigen is not a binding site for the AIDS virus or that it is a site that allows binding but not infection. Pert and her colleagues favor the latter explanation and speculate that the AIDS virus may indirectly damage brain neurons important for intellectual function and emotions. "There is a possibility that the T4 antigen is acting as a receptor for an endogenous peptide," Hill says. "But when it is covered up with the AIDS virus, then the endogenous substance may not be able to bind." By this indirect mechanism, the AIDS virus could cause brain damage because it prevents the interaction of brain cells with a substance, perhaps vasoactive intestinal peptide or a related peptide, that is necessary for their survival or function.

Adding to the body of evidence that a T4 receptor is a component of brain tissue, Charles Gerfen and Paul St. John of NIMH have preliminary data suggesting that the T4 receptor "is located on brain neurons and not on glia." They find that staining for the T4 antigen occurs on cells that appear to be neurons in cultures of rat hippocampus. The cells with T4 antigen look like those that "have neuronal morphology and stain positive for neurofilament and tetanus toxin [two markers for nerve cells]," Gerfen says, although he and St. John have not yet demonstrated T4 staining and neuronal markers in the same cells.

Because much research effort is directed toward showing what brain cells have T4 antigen, a critical question is whether the presence of that receptor means that the cell displaying it is susceptible to infection and damage by the AIDS virus. Many researchers think that cells must have the antigen. But, because Levy and his co-workers find that several isolates of the AIDS virus can infect cells lacking detectable levels of T4 antigen, Levy does not think that the T4 receptor "is the sole factor underlying infectivity. Instead," he says, "something about the ability of a viral isolate to replicate makes it different and therefore affects its ability to damage different cells." He proposes that the crucial differences among viral isolates that make them more infectious lie in the core or the 3′ *orf* end of the genome, rather than in the envelope glycoprotein, which is known to interact with T4 receptors on lymphocytes.

Farrar suggests that the AIDS virus may infect cells by more than one mechanism and proposes two possible modes of entry. One is the result of binding to the T4 receptor, and the other is "probably not receptor-mediated," he says. "In general, all receptor-mediated processes will occur quickly. But a process that is more amorphous—I like to call it random binding—may occur more slowly. Virus could be incorporated into a cell as a part of the pinocytotic or cell-drinking process, if there is enough of it around." Whether cell types, such as neurons, that actively recycle membrane at their synaptic terminals, take up virus by this method has yet to be determined.

Farrar also thinks that certain products of the AIDS virus genome may, in themselves, be damaging to cells, and he leans toward gp120, the envelope glycoprotein that surrounds the AIDS virus, as the culprit. "We find that purified gp120 by itself has physiological consequences," he says. Although Farrar and Douglas Ferris, also of Frederick, have yet to test the effects of gp120 on neural tissue, his hypothesis adds another possible dimension to the kind of damage the AIDS virus may do to the nervous system—specifically, that direct damage mediated by viral proteins, rather than by the intact virus, might occur.

Still other research points to the idea that much nervous system damage in an AIDS patient is due to infections by different agents, particularly cytomegalovirus (CMV). Because the AIDS virus attacks T4 lymphocytes and suppresses immune system function, many patients with neurological problems are susceptible to multiple brain infections. Wiley proposes that, by infecting the brain of an AIDS patient, CMV can elicit an immune response which then may draw AIDS virus–infected mononuclear cells into the brain.

"Sixty-seven percent of our patients have CMV infections," says Wiley. He and his colleagues are reexamining brain tissue from AIDS patients collected over the past 5 years and find that, for some unexplained reason, an increasing percentage of the brain samples have cytomegalovirus infection and damage. Although CMV brain infections are not common, they arise frequently in AIDS patients, and the DNA herpes-like virus infects both neurons and glia, he says.

Until recently, many researchers were pessimistic that AIDS-related damage to the nervous system could be reversed. But within the past year, Samuel Broder of NCI and his colleagues and Dannie King of Burroughs Wellcome in Research Triangle Park, North Carolina, have reported that 3′-azido-3′-deoxythymidine (AZT) appears to slow, or even reverse, some of the neurological symptoms in a subset of AIDS patients (see *Science*, 20 March, p. 1462).

Broder and his co-workers report that six of seven patients with neurological symptoms who received AZT improved in intellectual or peripheral nerve function. Some improved only temporarily, probably because their doses of AZT had to be lowered, and Broder is cautious about overinterpreting the preliminary results. "These data should not be taken as a final answer, but as an encouragement to do the necessary large-scale studies. Then we may be able define what kinds of neurological improvements we can expect."

Researchers still do not know precisely how the AIDS virus damages the nervous system, but within the past year they have proposed several hypotheses and are actively testing their ideas. The intact AIDS virus or its protein products may injure the nervous system directly, damage it indirectly, induce nonneural cells to secrete substances that are toxic to neural tissue, or act through a combination of mechanisms. As scientists learn more about how the virus affects cells of the immune system, they may find that many of the same mechanisms are responsible for nervous system damage. ■

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