Solo Actions of AIDS Virus Coat

The coat protein of the AIDS virus appears to have its own set of biological effects in vitro and can be toxic to certain types of cells; researchers are exploring the implications for the natural disease process and strategies for vaccine development

One way to understand how the AIDS virus causes extensive damage in the body—to the nervous and immune systems in particular—is to determine whether individual proteins of the virus have biological effects on living cells. New evidence from four groups of researchers who are using this relatively novel approach indicates that gp120, the envelope glycoprotein that surrounds the AIDS virus, can affect cells dramatically. For instance, it is toxic to animal nerve cells in vitro and also affects human immune system cells under certain conditions. But some scientists challenge the inference that these in vitro actions of the coat protein bear any resemblance to events that occur in an infected person.

No experiments to date indicate that gp120 does have similar actions in vivo, which indicates that vaccine preparations containing the envelope protein will probably not produce toxic effects (see box). But, at the same time, none of the researchers interviewed for this article are aware of any in vivo experiments that have been designed specifically to address the newly discovered in vitro effects of gp120. Thus, if the coat protein does have similar actions in vivo, it may help to explain some of the neurological and immune system abnormalities in AIDS patients that remain largely a mystery.

The concept that the external portion of the AIDS virus coat protein has actions by itself is unusual. "The membrane-spanning portion of other viral proteins [from animal retroviruses and human leukemia viruses] may cause immune suppression or other biological effects," says Myron Essex of the Harvard School of Public Health in Boston. "But to my knowledge there is no precedence for the external glycoprotein of a retrovirus having biological activity."

No one doubts that human immunodeficiency virus (HIV), the virus that causes AIDS, infects and kills cells of the immune system that normally protect a person from many kinds of infections. Particularly vulnerable are helper T lymphocytes, which bear a high concentration of the T4 antigen, the surface protein to which HIV binds. It is also clear that the AIDS virus infects cells of the monocyte-macrophage lineage, which may harbor it for long periods of time. Thus, they can become virus factories, releasing new virus particles that can then infect other cells.

HIV-infected cells not only produce intact virus, however. They also express viral proteins, one of which is gp120. People infected with the AIDS virus make antibodies to this and other viral proteins. "The concept that viral proteins may play a role in the progression of disease is attractive and has been documented to some extent with other viruses," says Michael Oldstone of the Research Institute of Scripps Clinic in La Jolla, California. "The question is, how does it occur?"

New data—from groups led by Mark Gurney of the University of Chicago and Douglas Brenneman of the National Institute of Child Health and Human Development (NICHD)—suggest that gp120 exerts toxic effects on cultured neurons in a way that is *not* dependent on the T4 receptor. Both researchers postulate that the envelope protein may interfere with the action of a normally occurring compound required by certain neurons for their growth or maintenance.

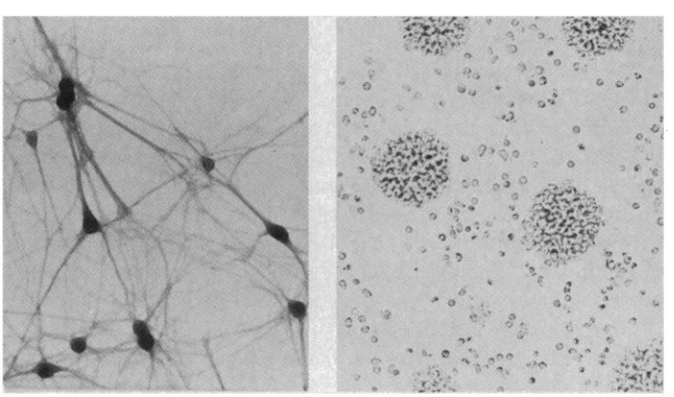
Last year Gurney, Mark Lee, and their colleagues reported that neuroleukin, which is produced by many tissues and activated T lymphocytes, acts as a nerve cell growth factor. It enhances the survival of cultured embryonic chick spinal cord neurons and dorsal root ganglion cells, sensory neurons that lie just outside the spinal cord in the intact animal. In this earlier work, the Chicago researchers also showed that part of the amino acid sequence of neuroleukin is similar (30% homology over 47 amino acids) to a conserved region of gp120. They postulated that one way in which the AIDS virus may damage the nervous system is by interfering with the actions of neuroleukin.

Now Gurney, Lee, David Ho of Cedars-Sinai Medical Center and the University of California School of Medicine in Los Angeles, and Brian Apatoff of Columbia University College of Physicians and Surgeons in New York find that gp120 inhibits the growth-promoting effects of neuroleukin on neurons from either peripheral or central nervous system tissue. "In the culture systems we tested—dorsal root ganglion cells, septum, hippocampus, or spinal cord—gp120 is toxic to neuroleukin-dependent neurons," says Gurney. They report on page 1047 of this issue of *Science* that either purified gp120, a disrupted preparation of HIV that contains the coat protein, or a recombinant fragment of gp120 inhibits the effects of neuroleukin in dorsal root ganglion cultures. As a result, 60 to 75% fewer neurons survive than those surviving in the presence of neuroleukin alone.

Brenneman and his colleagues have preliminary data that gp120 kills embryonic

Gp120 affects cells in vitro

The AIDS virus coat protein kills mouse brain neurons (left) and induces clumping and prostaglandin release from monocytes (right). [Photos courtesy of D. Brenneman and W. Farrar]



mouse hippocampal or spinal cord neurons in 1-week-old cultures. "There are two striking things about this effect," Brenneman says. "First, the concentrations at which it occurs are very low. And second, we don't know why, but when you use higher concentrations, the effect goes away. It is a very curious dose-response curve." At lower doses, cell death occurs after 5 days.

But the new work leaves many questions unanswered. For instance, the NICHD neuroscientists do not know which cells in the neuronal cultures are affected by gp120. The envelope protein may be directly toxic to neurons or glial cells (non-neuronal cells found in the nervous system), or it may act indirectly, as Gurney's work suggests. "That would be the most reasonable hypothesis," says Brenneman. "Gp120 may be interfering with a survival or maintenance factor required by neurons."

Oldstone offers an alternative, but not necessarily mutually exclusive, hypothesis to explain why gp120 may damage nerve cells. Certain viral proteins, he says, may mimic the molecular structure of a natural host protein but at the same time be different enough to trigger antibody production. The result could be an autoimmune response, which has not yet been shown to occur in AIDS.

Another question concerns the receptors on chick and mouse cells that bind the HIV coat protein. No one has reported data indicating that cells from either mice or chickens can be infected by the AIDS virus. Instead, mouse neuronal tissue has L3T4 receptors, which do not seem to bind HIV. This means that in the neuronal cultures gp120 must be exerting its effects by some mechanism other than its interaction with the T4 receptor.

Gurney proposes that the key to this as yet unknown mechanism may lie in the part of the gp120 molecule that resembles neuroleukin. "There is something about the second conserved domain of gp120 that is necessary for infection but not for binding," he says. Gurney and Ho speculate that this conserved portion of the gp120 molecule may somehow be involved in the internalization of virus by a cell.

The notions that gp120 alone may cause biological changes in cells is reinforced by preliminary data from a third group of researchers. William Farrar and Larry Arthur of the National Cancer Institute (NCI) in Frederick, Maryland, and Larry Wahl, of the National Institute for Dental Research, show that gp120 stimulates freshly isolated human monocytes to release prostaglandins (PG) and leukotrienes, both of which are normally produced during an inflammatory response.

The NIH researchers find that gp120 binds to the T4 receptors that are present on about 26% of the monocytes in their preparation. Farrar proposes that the HIV coat protein triggers the intracellular cascade of biochemical events that normally results in PG release. By binding to T4 receptors, gp120 may activate the enzyme phospholipase A2. If Farrar is right, then he and his colleagues have also demonstrated that a viral signal, namely, the binding of the envelope protein to a surface membrane receptor, becomes transduced into an intracellular response.

"PGE2 is notorious in its ability to shut off the immune response," says Farrar. If gp120 stimulates prostaglandin release in a person infected with the AIDS virus, it could further diminish the immune response already reduced by HIV infection of T lymphocytes. Farrar speculates that various immune mediators—interleukin-1 or prostaglandins—may contribute to the spectrum of pathological effects in AIDS.

Anthony Fauci of the National Institute of Allergy and Infectious Diseases (NIAID) is skeptical that purified gp120 has specific effects on monocytes. "Anything that tickles a monocyte might make it secrete prostaglandins," he says. Before he accepts the notion that the cells are responding specifically to gp120, Fauci would like to see a control experiment in which other viral proteins fail to activate monocytes.

"The concept that viral proteins may play a role in the progression of disease is attractive . . . [but] how does it occur?"

A fourth research group, Dani Bolognesi and his co-workers at Duke University Medical Center in Durham, North Carolina, has just reported that gp120 may convert uninfected lymphocytes into targets for immune attack. "We find that you can use bound gp120 as a target for immune attack, for example, by antibody-dependent cytotoxicity or natural killer cells," says Bolognesi. "You can kill lymphocytes that way. It raises the question that if gp120 was present, as might occur in an infected person, could it bind to uninfected cells and mediate their death? There is no evidence that it happens in vivo. But you can certainly demonstrate that it happens in vitro."

"Gp120 is unusually loosely bound and is shed into the culture medium of infected cells," says Bolognesi. "But does that mean that there is free gp120 in an AIDS patient?" These measurements have not been reported, he says, possibly because any soluble gp120 in the circulation might immediately attach to available T4 receptors on blood cells, making it difficult if not impossible to detect. Still, he contends, the bound protein might cause cell damage.

If these events do occur in vivo, the new data may help to explain why cells with the T4 receptor, particularly T4 lymphocytes, become severely depleted in AIDS patients. The effects of gp120 on T cells and monocytes apparently depend on binding of the coat protein to the T4 receptor. But neither Farrar nor Bolognesi rules out the possibility that some of the effects of gp120 on cells of the immune system—even those that have the T4 receptor—may be mediated by receptors other than T4.

Another issue is whether researchers can find a definitive link between the in vitro effects of gp120 alone and events in an HIV-infected person. Until they do, any potential role for the envelope protein in the disease process will be uncertain. For example, Gurney underscores the point that although gp120 is toxic to certain chick and mouse neurons in vitro, the same effects may not occur in vivo. "There is no documented evidence that neurons in an AIDS patient are killed by the virus," he says.

In fact, recent reports from Samuel Broder and Robert Yarchoan of NCI and their colleagues seem to indicate just the opposite. Some patients who have clear neurological deficits improve with AZT (3′-azido-3′-deoxythymidine) treatment. Gurney interprets this improvement, even if it is transient, to mean that at least some neuronal damage can be reversed. A recovery of this kind would be much less likely if the AIDS virus killed large numbers of neurons in the central nervous system, he reasons.

This leaves at least three possible explanations as to why the AIDS virus, or gp120 alone, appears to kill nerve cells in vitro but does not seem to have the same effect in vivo. One is that neurons in AIDS patients *are* killed, which may account for some of the tissue shrinkage seen on their brain scans, but no one has yet demonstrated an actual reduction in nerve cell number.

A second possibility is that the neurons in vitro may be more susceptible to gp120-induced toxicity because they are still differentiating. If developing neurons, as opposed to mature ones, really are more vulnerable, it may help to account for some of the neurological damage in children who are born with HIV infections.

And a third possibility is that the virus or some of its proteins alter nerve cell function in vivo, perhaps indirectly, rather than actu-

ally killing neurons. Both Gurney and Brenneman have measured the ability of gp120 to cause nerve cell death, however; neither has looked for any milder changes in function that the envelope protein might induce.

All four groups that have studied the physiological actions of gp120 obtained the viral coat protein from Larry Arthur and Peter Nara, also of NCI in Frederick. In their preparation, the protein appears to retain its native three-dimensional configuration and carbohydrate residues. But some researchers question whether gp120, even in its native state, has significant biological effects on cells in vivo.

"I don't think the in vitro results with gp120 can be extrapolated to the in vivo situation at all," says Robert Gallo of NCI. "There is no evidence that they can." In their attempts to develop a vaccine for AIDS, numerous researchers, including some in Gallo's laboratory, have injected gp120 into animals and have not observed toxic effects.

Still, the new in vitro data on gp120 actions raise the issue of whether a vaccine preparation that contains the coat protein could affect nerve cells, T lymphocytes, or monocytes in an immunized person. Fauci thinks it is unlikely, with the possible exception that monocyte activation could be induced. The coat protein administered in a vaccine preparation is unlikely to enter the central nervous system and affect nerve cell function or survival, he says. And T cells are not likely to be targeted for immune attack because the vaccine—at least in the trial that has just been approved—will be given to uninfected persons who lack antibodies to the AIDS virus at the time of immunization.

The new work represents a different experimental approach to understanding how the AIDS virus mediates its pathological effects, particularly those that could result from the action of soluble protein products of the virus. But testing gp120 or any other viral protein for its ability to alter normal cell function is still a relatively novel idea in AIDS research. ■ **DEBORAH M. BARNES**

ADDITIONAL READING

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AIDS Vaccine Trial OKed

The National Institute of Allergy and Infectious Diseases (NIAID) and MicroGeneSys of West Haven, Connecticut, announced last week that the Food and Drug Administration (FDA) has approved the first clinical test of a potential AIDS vaccine in this country. The trial, to begin this fall, will gauge possible toxic or immune responses in people not already infected with the AIDS virus. The trial will not measure whether the candidate vaccine protects a person from the virus.

"This is a first step in what may be a very lengthy process," says Anthony Fauci of NIAID. He predicts that at best no vaccine, including this one, will be available for general use before the mid-1990s.

The vaccine preparation is based on a modified form of gp160, the AIDS virus coat protein that includes both the external portion (gp120) and most of the membrane-spanning region (gp41), according to Mark Cochran of MicroGeneSys. Other research teams working with different versions of the coat protein have also applied to the FDA to begin clinical testing of their products. Fauci suggests that MicroGeneSys gained FDA approval first because its preparation stimulates a strong immune response in animals and the company responded promptly to FDA requests for additional information.

H. Clifford Lane of NIAID will direct the study at the NIH clinical center in Bethesda. It will include "75 male homosexual volunteers who are not infected with the AIDS virus and are not practicing risk behavior," says Fauci. Volunteers will be further counseled to avoid high-risk behaviors. Sixty will receive the vaccine and 15 will receive keyhole limpet hemocyanin, an oxygen-carrying protein from shellfish, as a control. Those receiving vaccine will be divided into four groups to determine the effect of different primary and booster doses. The study will also include six heterosexual volunteers, three of whom will receive the highest dose of vaccine and three of whom will receive the control protein.

Malcom Martin of NIAID provided MicroGeneSys with an infectious clone of HIV, the human immunodeficiency virus that causes AIDS. He constructed the clone from two different HIV isolates—the French LAV isolate and NY5, a North American isolate. Cochran and Gale Smith of MicroGeneSys isolated and modified the gene for the HIV coat protein from this clone and inserted it into baculovirus, which normally infects moths and butterflies. They grew the genetically engineered virus in cultured insect cells to produce large amounts of HIV gp160 for tests in animals.

"We immunized hundreds of small laboratory animals—mice, guinea pigs, rabbits, and rhesus monkeys" says Smith. Lane and Thomas Folks, also of NIAID, found that blood samples from the animals contained antibodies, as yet unidentified, which block HIV replication in cultured human T lymphocytes. The vaccine also stimulated cell-mediated immunity (CMI), which involves lymphocyte and macrophage interaction and which may be necessary for protection against HIV infection. At FDA's request, researchers also immunized two chimpanzees that produced HIV-neutralizing antibodies and CMI in response. None of the animals had toxic reactions.

Although chimpanzees can be infected with the AIDS virus they do not develop an immunodeficiency disease. This difference leads some researchers, including Fauci, to question the necessity of showing—before initial clinical trials begin—that a vaccine intended for human use also protects chimpanzees from infection. The NIAID scientists have not yet tested the immunized chimpanzees to see if they are protected. Other groups of investigators, however, working with different preparations that have yet to be put into clinical trials, have reported that their vaccines fail to protect chimpanzees against HIV infection.

The initial stage of testing should last about 6 months, says Fauci. The next step, phase 2, will determine optimum dose and monitor immune responses more thoroughly and probably last 1½ to 2 years. But it is not until phase 3 trials, when large numbers of people receive the vaccine, that researchers will be able to evaluate whether it is effective in preventing infection with the AIDS virus. This stage of testing will be even more lengthy because it takes several years before an infected person develops disease and because volunteers will be counseled on how to avoid infection, thus reducing their risk of exposure to AIDS. ■ **D.M.B.**