The AIDS Virus Can Take On Many Guises

The genetic variability of the AIDS virus may help it to escape the immune system and will complicate vaccine development

THE AIDS VIRUS, unlike the leopard, not only can change its spots but does—often and fast. Researchers have known for some time that the virus genome may differ from one infected person to the next. They are now learning that it can even show dramatic variation within a single individual. Moreover, the AIDS virus may be spinning off so many variants because it is inherently prone to make mistakes when it reproduces itself.

These genetic changes may be reflected in the behavior of the AIDS virus variants, perhaps influencing the course that the disease takes in infected individuals. In particular, they may help the virus escape from the body's immune defenses. The extreme genetic variability of the AIDS virus also appears to be still more bad news for efforts to develop a vaccine to protect against AIDS.

AIDS virus variability was the major focus of a "Conference on Genetic Variation of Immunodeficiency Viruses," which was held on 19 and 20 July at the National Institutes of Health in Bethesda, Maryland. At the conference, for example, George Shaw of the University of Alabama in Birmingham described his group's characterization of the variation in human immunodeficiency virus 1 (HIV-1), as the AIDS virus is known scientifically. That variation turned out to be extensive.

Shaw and his colleagues, including Michael Saag and Beatrice Hahn of Alabama and Wade Parks of the University of Miami School of Medicine, cloned individual virus genomes from samples isolated from two patients. Seventeen of the 27 HIV-1 clones prepared from one patient's virus turned out to be genetically different, as did 9 of the 17 clones obtained from the second patient.

The researchers estimate that the nucleotide sequences of the various cloned viruses obtained from one patient at a given time vary by 2 to 3%. "The data imply," Shaw says, "that there is no such thing as an [AIDS virus] 'isolate.' You probably have enormous numbers of slightly different viruses in an individual."

Moreover, the virus apparently continues to mutate as time goes by. The clones derived from another virus sample obtained from the second patient 16 months after the first showed even greater genetic variation. Thirteen of 18 were different and all were distinct from the earlier viruses.

The babies of women who carry the AIDS virus have about a 50% chance of being infected themselves. Steve Wolinsky of Northwestern University School of Medicine has compared the genomic sequences of HIV-1 clones from a mother and her infected infant. As expected they are very closely related, but new variants have apparently arisen in the child.

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Wolinsky's work has a wider significance, however. Diagnosing AIDS in the infants born of infected mothers is difficult. The standard diagnostic tests detect antibodies to HIV-1, and all of these children have the antibodies at birth. The half who do not carry the AIDS virus lose the antibodies around 6 months of age, but clinicians would like to know before then who is infected. Wolinsky has now shown that a new method of gene amplification, called the polymerase chain reaction (*Science*, 10 June, p. 1408), can be used for detecting the AIDS virus in the children of infected mothers.

Also at the meeting, Thomas Kunkel of the National Institute of Environmental Health Sciences (NIEHS) in Research Triangle Park, North Carolina, presented new data that may help to explain why HIV-1 mutates as readily as it does. The HIV's are retroviruses and have RNA as their genetic material. During the retrovirus life cycle, an enzyme called reverse transcriptase copies the RNA into DNA. The DNA, which may become integrated into the genomes of infected cells, then directs the synthesis of new RNA molecules for packaging into viral particles during reproduction.

Kunkel and his colleagues have been studying the reverse transcriptases of several retroviruses, now including that of HIV-1. These enzymes generally tend to be somewhat inaccurate. The enzymes that make DNA copies of DNA have a proofreading activity that allows them to recognize when they have made a mistake and then remove it. The reverse transcriptases studied so far lack this ability. "If they make a mistake, they have to live with it," Kunkel explains.

The NIEHS researchers have found that the HIV-1 enzyme errs even more often—by a factor of roughly 10—than other reverse transcriptases. "The HIV-1 reverse transcriptase is the least accurate of the enzymes we have examined," Kunkel says. The generation of numerous variants may thus be an intrinsic property of HIV-1.

Peter Nara of the Frederick (Maryland) Cancer Research Facility of the National Cancer Institute described results that further buttress this idea—and also bode ill for efforts to develop an AIDS vaccine. As part of the NCI vaccine project, Nara and his colleagues injected chimpanzees with HIV-1. The researchers then began isolating HIV-1 from the animals at 2-week intervals. The very first HIV-1 samples recovered were already resistant to neutralizing antibodies raised specifically against the parent HIV-1 strain even though the animals had not yet mounted detectable antibody responses to the virus.

One theory proposed to explain the emergence of new forms of HIV-1 in AIDS patients holds that immune system activity provides a selective pressure that essentially drives the production of resistant variants. But the Frederick group's results indicate that antibody-resistant HIV-1 variants can arise in chimpanzees in the absence of immune selection. "Neutralizing antibodies don't seem to be the driving force for variant production in this species," Nara says. The parent HIV-1 strain used for these experiments was not a pure cloned virus, but Nara says that it was extensively analyzed for resistant variants and none were detected.

Even though HIV-1 variants may not be arising as a result of immune selection, their generation may nonetheless help the virus escape control by the immune system. David Looney of the Walter Reed Army Institute in Washington, D.C., in collaboration with Flossie Wong-Staal and Robert Gallo of NCI, has recently shown that a change of a single amino acid in the HIV-1 envelope protein is sufficient to make the virus resistant to antibody neutralization.

Equine infectious anemia virus (EIAV), like the HIV's, belongs to the lentivirus family, the members of which cause diseases with a slow course of development. The equine virus also shows a high degree of

genetic variation, according to Ronald Montelara of Louisiana State University in Baton Rouge, and this helps it avoid destruction by the immune system of infected animals for a time.

There is a major difference between EIAV and HIV-1 infections, however. Most infected horses eventually win their battle with EIAV. "Despite the variation, the animals routinely bring the virus under control," Montelara points out.

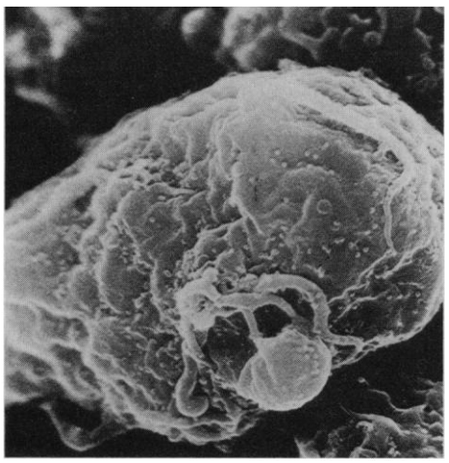
This does not happen in AIDS patients, nor apparently in the chimpanzees infected with HIV-1, according to Nara. When ordinary viruses invade their hosts, they first elicit the production of counteracting antibodies, but once the infection is brought under control, the antibody concentrations decline. The chimpanzees, however, have made neutralizing antibodies to the original HIV-1 parent strain continuously for as long as 4 years. "This suggests that the parent virus is around and replicating," Nara says. How horses manage to control EIAV, whereas humans and chimpanzees fail to do so for HIV-1 is an interesting question that remains to be answered.

In addition to showing altered resistance to antibody neutralization, the genetic variants of HIV-1 may also differ in their biological activities. These differences may influence the course that AIDS takes in infected individuals. In some patients, for example, suppression of the immune system is the principal manifestation of the disease, whereas for others neurological degeneration may be dominant. What happens may be determined by the type of cells infected by AIDS virus variants, which researchers are now finding to differ in their cell preferences.

In one such demonstration, Wong-Staal, Gallo, and Amanda Fisher, who is also a member of the NCI group, took envelope gene sequences from six of the variant HIV-1 clones produced by Shaw and his colleagues and used them to construct hybrid HIV-1's that were identical except for their envelope proteins. The researchers found that the hybrids differed widely in their ability to grow in cells, including T cells and monocytes, which are the major cell types that HIV-1 infects in AIDS patients.

Another indication that HIV-1 variants differ in their ability to infect cells comes from Yoshio Koyanagi, who works with Irvin Chen at the University of California School of Medicine in Los Angeles. Koyanagi isolated two genetically distinct HIV-1's from an AIDS patient who had had severe neurological deterioration before he died.

One variant, which had been obtained from cerebrospinal fluid, infected glial cells (a sort of accessory cell for neurons), but reproduced very poorly in monocytes. The other variant, which had been isolated from the brain, displayed the opposite cell specificity. Since monocytes probably carry HIV-1 from the bloodstream into the brain, it is not surprising to find a brain isolate with a preference for infecting monocytes.



Budding AIDS virus particles. *When the AIDS virus reproduces itself it may make numerous mistakes.*

The ability of HIV-1 to generate variants may also bear on another issue in AIDS research, namely, why such a long time is required for symptoms to develop after infection with the AIDS virus. This lag period varies, but is usually years.

Cecilia Cheng-Mayer, from Jay Levy's group at the University of California School of Medicine at San Francisco, reported at the genetic variation meeting that the HIV-1's isolated from AIDS patients become more effective at killing cultured cells as the patients' symptoms worsen. "For sequential isolates from the same patient, the later isolates replicate better and are more cytopathic than earlier isolates," Cheng-Mayer says.

The time needed for cytopathic HIV-1 variants to emerge may contribute to the long lag period between infection and the development of AIDS symptoms, although other factors, such as the ability of the infected person's immune system to fight off the AIDS virus might well be involved, too.

What researchers have not yet done is identify the particular changes in HIV-1 that influence the virus's cell specificity or ability to kill cells. Results with a feline AIDS model that were described by James Mullins of the Harvard School of Public Health may provide some clues to the features that influence the virulence of immunodeficiency viruses, however.

Mullins and his colleagues have been studying a feline leukemia virus that causes an immunodeficiency syndrome in cats that is very similar to human AIDS. The animals do not develop symptoms until a year or more after they have been infected with the virus. Variant forms of the virus always appear just before the cats get sick.

The Mullins group has cloned more than two dozen virus variants directly from the tissues of diseased cats and found that nearly all have undergone changes that have made them incapable of replicating by themselves. Somewhat surprisingly, these replication-defective variants all cause a rapid immunodeficiency disease in cats and are highly cytopathic to cultured T cells, whereas the variants that can replicate on their own do not have these effects.

Mullins suggests that the pathogenic, replication-defective variants may be minor components of the virus initially used to inoculate the cats and that their numbers increase slowly, eventually making the animals sick. Although the replication-defective viruses cannot reproduce on their own, they can do so if another virus, such as a replication-competent variant, helps out by supplying the viral functions lacking in the nonreplicating variants.

The Harvard workers have localized the genetic changes that make the replication-defective variants pathogenic to a small segment of the gene encoding the viral envelope protein. "Very subtle changes lead to very remarkable changes in the biology of the virus," Mullins says.

The feline leukemia virus work also implies that current techniques for isolating HIV-1 may not detect an important subset of pathogenic variants. The replication-defective feline viruses were cloned directly from cat tissues, whereas the HIV-1's are generally obtained by culturing cells from AIDS patients with other cells in which HIV-1 will grow. This coculture step might result in a loss of cytopathic variants, which will kill the cells they infect. Meanwhile, recipient cells that have acquired variants that are not very effective cell-killers will survive and multiply.

Researchers have begun to identify replication-defective variants of HIV-1 in AIDS patients. In work done while at the Pasteur Institute in Paris, Maureen Goodenow used the polymerase chain reaction to amplify HIV-1 genomes directly from patients' cells and then determined partial nucleotide sequences for the cloned genomes. Some of them had alterations that would make them incapable of replicating themselves. Wolinsky detected similar HIV-1 variants.

Whether these HIV-1 variants have anything to do with the development of human AIDS remains to be seen. Nevertheless, the ability of HIV-1 to generate so many variants may help to explain the unusual pathogenic features of AIDS, at the same time that it helps to frustrate researchers' efforts to produce a vaccine for the disease.

■ JEAN L. MARX