The AIDS Virus—Well Known But a Mystery

The natural history of the AIDS virus makes it extremely well suited for evading the immune system—and a hard target for vaccine development

If knowledge is power, then the research community should be gaining a firm ascendancy over the virus that causes AIDS. In the 4 years since that virus, now called human immunodeficiency virus 1 (HIV-1), was discovered, its genome has been cloned and sequenced, and the various genes have been identified, including at least five "accessory" genes that are not found in more ordinary viruses. A great deal has also been learned about the transmission of HIV-1 and the body's responses to it. "Never has so much knowledge been generated in such a short time as in the case of the human retroviruses and AIDS," as Erling Norrby of the Karolinska Institutet in Stockholm, Sweden, put it in his introduction to the session on AIDS research at the seventh annual DNA/Hybridoma Congresses held recently in San Francisco.

Much of that knowledge leads to the inexorable conclusion, however, that the AIDS virus has an insidious nature that makes it well equipped to resist immune attack. The virus destroys the immune system instead. The San Francisco meeting, for example, reflected a growing recognition that the virus may spread, both from person to person and also to various types of cells within an individual, by direct cell-to-cell transmission. Moreover, it is apparently able to lurk within some of these cells in latent form. All this means that developing a vaccine to protect against AIDS may be even more difficult than is already thought.

Moreover, despite the large gains in knowledge, many gaps remain. Some of these concern the functions of the HIV-1 accessory genes. Learning how these genes work is important because they provide potential targets for therapeutic drugs. Another unresolved issue concerns the two viral relatives of HIV-1 that were discovered about a year ago. The main question is whether these viruses, which are at least very closely related to one another, are a major cause of AIDS.

Until now, most efforts to develop an AIDS vaccine have focused on the production of antibodies capable of neutralizing the free virus before it infects cells. However, there are indications that the virus may be transmitted not just as the free virus but by direct cell-to-cell contacts. "The most important mode of transmission from person to person is the virus-infected cell," says Jay Levy of the University of California at San Francisco (UCSF). This would be consistent with the relative difficulty of person-to-person transmission of the AIDS virus.

There is a lot of confusion about what these genes do.

Infection occurs primarily by sexual contact or direct introduction of contaminated blood into the bloodstream or by transfer from infected mother to child in the womb or during birth, but not by casual contact.

Cell-to-cell contacts may also be instrumental in disseminating the AIDS virus once it is inside the body. Individuals can develop full-blown AIDS, for example, even though they have neutralizing antibodies that might be expected to control the free virus.

Although HIV-1 was originally thought to be specific for infecting a particular type of immune cell—the helper T cell—research over the past year or so has shown that the virus can enter many kinds of cells. A partial listing includes additional types of immune cells, such as macrophages and antibody-producing B cells, the endothelial cells that line blood vessels, and also certain nonneuronal brain cells. Robert Gallo of the National Cancer Institute and his colleagues have suggested that the macrophage may be the primary culprit in HIV-1 dissemination.

Even though HIV-1 infects many cell types, it preferentially kills helper T cells, the consequent loss of which is the primary cause of the collapse of the immune system in AIDS patients. The susceptibility of helper T cells to killing by HIV-1 has been something of a mystery, especially because virtually all the cells eventually die in AIDS patients even though only a small percentage appear to be infected by the virus. According to William Haseltine of Harvard's Dana-Farber Cancer Institute, the susceptibility of the helper cells may be related to the presence on their surfaces of large quantities of an antigen designated CD4.

Investigators have shown that this antigen is the receptor by which the AIDS virus binds to many of the cells it infects, although work by Richard Axel of the College of Physicians and Surgeons of Columbia University, Robin Weiss of the Chester Beatty Laboratories in London, and their colleagues indicates that the binding is necessary, but not sufficient, for the virus to enter cells. Another, as yet unidentified molecule may be required in addition to CD4. Moreover, according to Levy, the brain cells infected by the AIDS virus show no traces of CD4 antigen production, a finding which suggests the existence of an additional, as yet unidentified receptor.

The complete HIV-1 particle is covered by a membranous envelope containing a complex of two major viral glycoproteins called gp120 and gp41. When the virus attaches to the CD4 antigen on target cells, it apparently does so by means of gp120.

During replication of the virus in infected cells, the glycoproteins are incorporated into the outer cellular membrane and eventually become part of the viral envelope when the particles bud from the cell. Haseltine proposes that the presence of large quantities both of CD4 and envelope glycoproteins on helper cell surfaces can lead to cell death in either of two ways. By binding to CD4 molecules on the same cell, the glycoproteins may cause portions of the membrane to fuse with one another, thereby disrupting the membrane and killing the individual cell.

In addition, the envelope glycoproteins displayed on one cell may bind with CD4 molecules on the surfaces of other, uninfected T cells to form the fused cell complexes called syncytia. "In this way, one infected cell can kill up to 500 uninfected cells," Haseltine says. "It helps to explain how you can get total T-cell ablation when only a few are infected."

In a development consistent with much else in the history of AIDS research, Haseltine's explanation of how HIV-1 kills T cells is not accepted by all researchers. For example, Gallo and his colleagues do not think that syncytia formation contributes to cell death. Flossie Wong-Staal of the Gallo group points out that they have identified an HIV-1 mutant that causes syncytia formation but does not kill cells. The NCI workers suggest that an interaction between the CD4 and gp120 molecules of the same cell leads to the death of that cell. They have evidence suggesting that a portion of the gp41 molecule is also necessary for the lethal interaction.

Even if fusions between infected and uninfected cells do not result in cell death, they may at least contribute to the spread of HIV-1. Macrophages and B cells, for example, carry much smaller numbers of CD4 molecules than helper T cells. Macrophages and B cells can therefore be fusion partners, but are more likely to be infected without being killed as a result. These and the other cells that are infected by HIV-1, but not killed, may serve as reservoirs for maintaining the virus in the body and transmitting it to other cells, including any newly made helper cells that might otherwise serve to restore an AIDS patient's immunity.

The importance of cell-to-cell transmission of HIV-1 has implications for vaccine development. "Any strategy for a vaccine against AIDS will have to deal not only with the free virus, but with virus-infected cells," says Dani Bolognese of Duke University Medical Center in Durham, North Carolina. To be effective, a vaccine will have to elicit immune cells capable of recognizing and killing infected cells, in addition to eliciting neutralizing antibodies.

The results regarding the ability of HIV-1 to stimulate cell-mediated immunity are both encouraging and discouraging with regard to potential vaccine development. On the encouraging side, infection with the virus apparently leads to the generation of killer cells. At the meeting, Bernard Moss of the National Institute of Allergy and Infectious Diseases described work in which Bruce Walker and Martin Hirsch of Harvard Medical School found that individuals who have been infected with the AIDS virus produce cells that can attack cells bearing HIV-1 proteins. They do not see the killer cells in persons who have not been infected by HIV-1.

More discouraging, however, is the finding that the killer cells do not necessarily prevent AIDS development. They are found in AIDS patients, as well as in infected individuals who remain healthy.

There are indications that another type of immune cell, which is called the suppressor cell because it acts as negative regulator of immune responses, may help suppress the spread of HIV-1 and perhaps the development of full-blown AIDS. Levy and his colleagues found that they could isolate HIV-1 from blood cells from some healthy, infected individuals and from a patient with a stable case of the AIDS-related cancer Kaposi's sarcoma only when they first removed the suppressor T cells from the samples. "When you have infected people who have gone relatively long times in clinically good health you may have a difficult time in getting the virus out of blood," Levy explains. "The suppressor T cells are keeping the virus in check." They may be doing this by producing an antiviral agent such as an interferon.

The suppressor T cells do not wipe out the virus, however. Once they are removed the virus can be recovered.

Another potential problem with regard to developing a vaccine that elicits cell-mediated immunity is the large number of cell types that can apparently harbor HIV-1 in latent form. Cells that are not making and displaying appreciable amounts of viral proteins might well escape the immune system's killer cells.

This is one of the reasons why researchers are very interested in finding an explanation of how HIV-1 manages to remain latent for long periods of time without either killing the host cells directly or eliciting an immune attack that destroys them. The genes that control the synthesis of the viral proteins provide an obvious place to look for the answer, and the HIV-1 genome comes equipped with a wealth of possibilities in the five accessory genes that are not found in ordinary retroviruses.

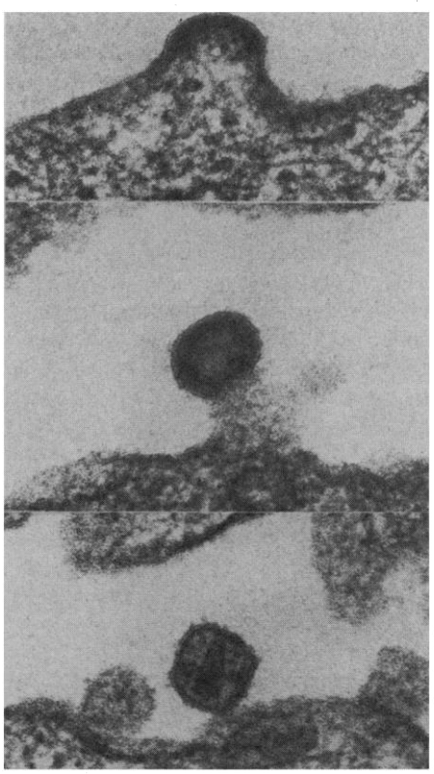
Understanding how the genes work may also be important from a therapeutic point of view. The genes are potential targets for therapeutic drugs for AIDS. "While every viral gene is a target for intervention the accessory genes are unique to the AIDS viruses and work late," Wong-Staal notes. The only drug now approved for treating AIDS patients is AZT (3′-azido-3′-deoxythymidine), which acts early in the viral life cycle by blocking the reverse transcription of the RNA genome of HIV-1 into DNA. Combining an early-acting drug such as AZT with one that acts to inhibit a late component of the the viral life cycle may produce a more effective therapy.

One of the four accessory genes, which is located near the right-hand (3′) end of the viral genome and called the 3′ open reading frame (3′ORF), apparently has a negative effect on HIV-1 replication. When Levy, Cecilia Cheng-Mayer, also of UCSF, and Paul Luciw of the University of California at Davis made deletions in the 3′ORF, they found that the replication of the virus increased five- to tenfold. Wong-Staal and her colleagues have made a similar observation. The action of this gene may help to keep HIV-1 replication in check and possibly allow the virus to remain latent.

The other three accessory genes are apparently needed for the production of infectious HIV-1 particles, although just how these genes work seems less clear now than it was a year or two ago. Wong-Staal does not overstate the situation when she says, "There is a lot of confusion about what these genes do." Expression of the gene designated ***tat***, for example, increases the synthesis of the viral structural proteins. The normal immune stimulation of HIV-1–infected helper T cells apparently contributes to the death of the cells by setting in motion a train of events that result in ***tat*** gene activation and increased synthesis of the viral proteins (see also p. 393).

Haseltine and Wong-Staal originally proposed that the ***tat*** gene product works by increasing transcription of the viral genes into messenger RNA, which is the first step in protein synthesis. Then, about a year ago Haseltine and his colleagues concluded that the ***tat*** product primarily acts after transcription to increase the efficiency with which the viral messenger RNAs are used to direct protein synthesis.

At the meeting, Wong-Staal described her group's latest findings regarding ***tat*** gene action and concluded that it can increase both transcription and translation, with the amount of ***tat*** product made determining the balance between the two effects. If just a little of the ***tat*** protein is produced, the transcriptional effect predominates, whereas higher concentrations stimulate transcription and translation more equally. Haseltine, meanwhile, says that there is currently not enough information to determine whether

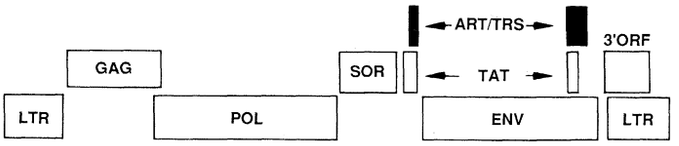


Matthew Gonda, NCI

Budding AIDS virus. *In the early stages of budding, viral proteins accumulate under the cell membrane, which begins to bulge outward (top). The bud pinches off (middle) until the free viral particle is released (bottom).*

AIDS virus genome

The genes for the structural proteins of the virus are designated gag, pol, *and* env. *The accessory genes, which are involved in controlling virus replication, are* sor, tat, art/trs, *and the 3'ORF. A fifth gene, called "R," is located before* tat *overlaps* sor. *The LTRs are the duplicated sequences known as long terminal repeats.*



the *tat* product acts directly on transcription or on post-transcriptional activities.

Haseltine and Wong-Staal also fail to see eye-to-eye on the effects of the third of the HIV-1 accessory genes, which Haseltine calls *art* and Wong-Staal calls *trs*, designations that reflect the researchers' differing views on the gene's mode of action. According to Haseltine, the unspliced messenger RNA for the HIV-1 structural proteins contains a regulatory sequence that would inhibit the synthesis of the proteins if its effects were not counteracted by the *art* gene product. He therefore named the gene *art* for "*a*nti-*r*epression *t*rans-activator" because it relieves the repressive effects of the inhibitory sequence.

According to Wong-Staal, *trs* gene activity results in decreased transcription of the viral genome, but is also necessary for correct splicing of the messenger RNAs that are formed. An active *trs* (for *t*rans-*r*egulator of *s*plicing) gene is therefore needed for synthesis of the viral proteins, even though it serves at the same time to damp down that synthesis. These ongoing controversies about the mode of action of the HIV-1 accessory genes *tat* and *art-trs* cannot now be resolved.

The fourth accessory gene of HIV-1 is designated *sor* and, according to Wong-Staal, has little effect on the synthesis of the viral proteins, but is necessary for the normal infectivity of HIV-1 viral particles. Finally, Wong-Staal has identified a fifth likely gene in the AIDS virus genome. The function of this "R" gene, as it is called, is currently unknown.

One of the biggest mysteries of current AIDS research concerns the pathogenecity—or lack thereof—of the HIV-1 relatives that were isolated last year by Phyllis Kanki and Myron Essex of the Harvard School of Public Health and their colleagues and by Luc Montagnier of the Pasteur Institute in Paris and his colleagues. The viruses appear to be very much alike. They both resemble simian T-cell lymphotropic virus III (STLV-III) much more closely than HIV-1. STLV-III, which is another AIDS virus relative, is found in wild African Green monkeys, although it does not appear to cause disease in the animals. It does cause an AIDS-like illness in captive macaques, however.

Moreover, the human HIV-1 relatives cannot be distinguished immunologically. They also have similar geographical distributions. They have so far been found almost exclusively in the inhabitants of western Africa, or in Europeans who have had sexual contact with western Africans, whereas the African distribution of HIV-1 is mostly confined to the countries of central Africa.

Despite these similarities, the Harvard and French groups report a critical difference between the viruses they have discovered, although the two groups used different approaches to come to their conclusions. The French workers looked for the virus they call HIV-2 in patients who have AIDS but do not show signs of HIV-1 infection. According to Montagnier, he and his colleagues have so far isolated HIV-2 from 30 such patients. Moreover, the virus kills helper T cells that are grown in laboratory dishes. In the view of French workers, the new virus is a significant cause of the immune deficiency.

Meanwhile, Essex and his colleagues have been performing an epidemiological study in which they have looked at the rate of infection with the virus they call HTLV-IV (human T-cell lymphotropic virus IV) in three groups of people in each of six western African countries. The three groups were healthy controls; hospital patients with AIDS-like symptoms; and prostitutes, who constitute a high-risk group for sexually transmitted diseases such as AIDS. In all, nearly 4300 people were included in the study.

The Essex group has not found any correlation between HTLV-IV and AIDS. In each of the six countries there was little difference between the prevalence of HTLV-IV infections in the controls and in the patients with symptoms that might be indicative of AIDS. The prostitutes generally had higher infection rates than the members of either of the other two groups, but did not show signs of disease. Moreover, HTLV-IV, unlike HIV-2, does not kill helper cells grown in culture.

How the divergent conclusions of the French and Harvard groups might be reconciled is currently unclear. One possibility, Montagnier suggests, "is that it is too early to see disease [in the populations studied by Essex]. It's just a matter of time." AIDS symptoms usually do not appear until five or more years after HIV-1 infection and HTLV-IV may have entered the populations in question too recently for a significant amount of AIDS to become apparent. Essex discounts this suggestion, however.

He and his colleagues have been doing a prospective study to look for indications of immune deficiencies in a group of prostitutes in the west African nation of Senegal who have been infected with HTLV-IV. During the 18 months the women have been studied, Essex says, they have not shown even subtle signs of immune suppression, which would be detectable in individuals infected with HIV-1 itself for that long.

Essex also notes that HTLV-IV is just as prevalent in Guinea-Bissau as HIV-1 is in the areas of central Africa where AIDS has reached epidemic proportions. Some 10 to 20 years were required for the HIV-1 infections to reach their current prevalence and if HTLV-IV behaves similarly, it should be—but is not—causing detectable AIDS. Essex concedes that HTLV-IV might rarely cause AIDS, but maintains, "It's absolutely clear that infection with this virus is not like infection with HIV-1 in Africa or in homosexuals."

Another mystery concerning the relation between HIV-2 and HTLV-IV recently emerged from work reported by James Mullins and his colleagues at Harvard School of Public Health. These researchers found that the genomes of three HTLV-IV isolates and several STLV-III isolates are nearly identical. This contrasts markedly with the situation regarding HIV-1, the genome of which has been found to vary from isolate to isolate. The same is true with regard to HIV-2. Montagnier's group has compared about ten isolates and, the French researcher says, "Our conclusion is that HIV-2 has the same type of genetic variation as HIV-1." The HIV-2 genome also shows variations compared to those of the STLV-III and HTLV-IV isolates.

The explanation for the apparently much greater stability of the HTLV-IV/STLV-III genome compared to that of the HIV-1 and HIV-2 genomes is currently unclear. There is a possibility, however, in view of the Essex group's not finding a correlation between HTLV-IV infection and AIDS, that the development of unstable viral strains is related to increased pathogenecity. More work will be needed to resolve these issues. Researchers may have come a great distance in their knowledge of AIDS, but they still have a long way to go. ■ JEAN L. MARX