More Progress on the HTLV Family

As researchers learn more about the HTLV's and their relatives they find that it may be necessary to define a new viral category for them

Human T-cell leukemia viruses (HTLV's) have been linked to two serious and as yet incurable diseases—adult T-cell leukemia and acquired immune deficiency syndrome (AIDS)—and are therefore the targets of an intense research effort. On 6 and 7 December of last year, investigators discussed the status of this research at an "HTLV symposium" that was sponsored by the National Cancer Institute (NCI) and held at the National Institutes of Health in Bethesda, Maryland. There clearly has been rapid progress in understanding the molecular biology of these viruses. The structures of the viral genomes are being elucidated and researchers are beginning to understand how the viruses may alter infected cells to produce the uncontrolled proliferation seen in the leukemia or the immune cell destruction characteristic of AIDS. The new work also suggests that it may be necessary to define a new virus category to accommodate the HTLV's and their relatives.

A major question in AIDS research over the past several months concerns whether lymphadenopathy-associated virus (LAV), which was isolated and causally linked to the disease by Luc Montagnier of the Pasteur Institute and his colleagues, is the same as HTLV-III, which was isolated and causally linked to AIDS by Robert Gallo's group at the NCI. There now appears to be little room for doubt on this issue. The results obtained by the two groups are so similar that it is difficult to imagine how they could be dealing with different viruses.

The structures of the LAV and HTLV-III particles look identical. They both have the same cylindrical cores, for example. Both viruses are transmitted only by close contact. Both show the same strong preference for infecting T4 cells, a subclass of T lymphocytes that includes the helper cells needed for many immune responses. Both kill the T4 cells, which is the apparent cause of the profound immune suppression of AIDS patients. In collaborative studies, the NCI and Pasteur workers have found that the core proteins of HTLV-III and LAV are antigenically indistinguishable and the genomes are highly related as shown by molecular hybridization studies.

The NCI workers now have isolated HTLV-III from more than 100 patients with AIDS or AIDS-related complex, a condition that includes lymphadenopathy as a prominent symptom and may be a mild or early form of AIDS. The Pasteur group has isolated LAV from more than 30 such patients. Researchers on both sides of the Atlantic have generally found that 90 percent or more of patients with AIDS or lymphadenopathy carry antibodies to HTLV-III or LAV, an indication that they were infected with the virus. Less than 1 percent of healthy, nonhomosexual controls have the antibodies.

The final proof that HTLV-III and LAV are the same depends on the results of a comparison of the nucleotide sequences of the viral genomes. This will soon be possible. At least three groups have determined the genome sequence of one or the other or both viruses, although the investigators in question are reluctant to discuss the data before they are published.

> "These viruses may have put their fingers on the pulse of the replicative cycle of the T4 cell."

Despite the likelihood that HTLV-III and LAV are essentially the same virus, the genomes of the French and U.S. isolates may not prove to be absolutely identical. Work by Gallo, with Flossie Wong-Staal and George Shaw, also of NCI, and their colleagues, has already shown that the genomes of different HTLV-III isolates can show sequence variations, a finding of some concern to those who are trying to develop a vaccine to protect against the virus. If the genome variations are reflected in antigenic differences, a single vaccine may not protect against all virus variants.

Need for a vaccine to protect against AIDS is great. So far, more than 7200 cases from the United States have been reported to the Centers for Disease Control in Atlanta, and the reported number of cases lags behind the actual number by 15 percent, according to James Curran, who heads the CDC's task force on AIDS. The CDC predicts that another 8500 individuals will be stricken in 1985, Curran told the meeting participants.

One of the many puzzling aspects of AIDS has been the high frequency of degenerative brain changes, which cause dementia and other neurological problems and do not appear to be the result of the patients' opportunistic infections. New data* presented at the symposium suggest that HTLV-III itself may be the cause of the brain degeneration, which afflicts about as many as 75 percent of the patients in the later stages of the disease.

According to Matthew Gonda of the NCI–Frederick Cancer Research Facility, who obtained the results with the Gallo group, the HTLV-III particle is very similar structurally to that of visna virus, which causes a slowly progressing, degenerative brain disease in sheep and goats. "In all stages of development it is difficult to distinguish HTLV-III from visna virus," Gonda says. Moreover, the genomes of the two viruses are partially homologous.

Not only does HTLV-III resemble visna virus structurally but it also infects the brains of AIDS patients, according to data presented at the symposium by Wong-Staal. The viral DNA could be detected in brain cells from 5 of 15 individuals who had died of AIDS and had showed neurological symptoms.

Whereas HTLV-III causes the death of the T4 cells it infects, HTLV-I and -II cause them to undergo malignant transformation and uncontrolled growth. About 6 months ago, researchers began getting their first clues to how HTLV-I and -II might produce these effects. William Haseltine at Harvard's Dana-Farber Cancer Institute and his colleagues obtained evidence indicating that the two viruses each produce a protein with the ability to *trans*-activate, that is, to increase the expression of genes attached to appropriate viral control sequences, whether or not those genes are integrated in the cellular genome. A protein with the ability to stimulate viral gene expression in this way might do the same for cellular genes, including genes participating in the control of cell division.

The investigators suggested that the proteins were likely to be encoded near the 3′ ends of the viral genomes in a region that had been designated "pX" because its coding function was un-

*See also M. A. Gonda *et al.* on p. 173 of this issue and G. M. Shaw *et al.* on p. 177.

known. The 3′ half of that region, which they now call the "long open reading frame" (LOR), is highly conserved between the two viruses and capable of coding for a protein. Shortly thereafter, Haseltine and his collaborators, and, independently, Irvin Chen and his colleagues at the University of California School of Medicine in Los Angeles, demonstrated that the LOR regions of the two viruses are active, directing the synthesis of proteins having a molecular weight of approximately 40,000.

Now Haseltine and his colleagues, in collaboration with Wong-Staal and Gallo, have evidence that HTLV-III also produces a factor with *trans*-activating abilities in infected cells.† The virus is about ten times more active in this regard than HTLV-I and -II. The "exuberant" *trans*-activation by HTLV-III may help to explain why this virus kills infected cells, Haseltine suggests, whereas infection by HTLV-I and -II causes transformation. HTLV-I and -II may have been infecting humans long enough to become attenuated, with the result that they reproduce poorly in cells and are less likely to kill them. Instead the viral genome integrates into the cellular genome and eventually causes transformation of some cells. Meanwhile, HTLV-III, especially if it has arisen more recently, may be less attenuated and still have cell-killing as its dominant effect.

It remains to be seen which cellular genes, if any, are regulated by the *trans*-activating factors of the various HTLV's, and whether these differ from virus to virus. The factors might be specific for different cellular genes, the identities of which would be of great interest to anyone studying the control of cell division. "These viruses may have put their fingers on the pulse of the replicative cycle of the T4 cell," as Haseltine puts it.

The HTLV's are retroviruses, meaning that they have RNA as their genetic material and that the life cycles include a step in which the RNA genomes are copied into DNA. Until recently, they have generally, although somewhat uneasily, been considered to be members of the type C oncornavirus (cancer-causing RNA virus) subgroup of the retroviruses. However, the structures of the HTLV particles are somewhat different from those of other type C retroviruses and they appear to transform by a different mechanism, namely by *trans*-activation. Of course, HTLV-III apparently does not transform at all. For these reasons, many of the researchers in the field are now proposing that the HTLV's ought to be included in a newly defined category of retroviruses. The name HTLV is also being changed to human T-cell *lymphotropic* virus in recognition of the fact that HTLV-III is not a leukemia virus, although it, like HTLV-I and -II, prefers to infect T cells.

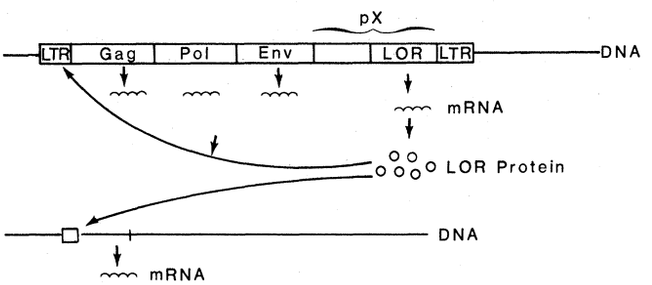
Other viruses that might also be included in the new category include visna virus, which had previously been placed in the lentivirus (slow virus) subgroup of retroviruses. In addition to its other similarities to HTLV-III, recent work from Janice Clements' laboratory at Johns Hopkins University School of Medicine indicates that visna virus produces a *trans*-activating factor.

Another candidate for inclusion is bovine leukemia virus, which resembles the HTLV's in a number of ways, according to Arséne Burny of the University of Brussels. "It induces in cows a disease that is very like adult T-cell leukemia in humans," he explains, "although in cows it is a B-cell leukemia."

The overall arrangement of the RNA genome of bovine leukemia virus is very similar to that of HTLV-I and -II. It also contains a LOR region near the 3′ end, for example. Although the exact nucleotide sequence of the bovine leukemia virus genome shows considerable variation from those of the two HTLV's, Burny and others find that the protein products of the bovine virus genes can be antigenically similar to HTLV gene products, including those of HTLV-III. "There is significant divergence at the nucleic acid level, but at the protein level there are regions that are well conserved," he concludes. Finally, Burny with the Haseltine group has evidence for *trans*-activation by bovine leukemia virus, although it is less effective in this regard than HTLV-I and -II.

David Derse and James Casey of the NCI–Frederick Cancer Center and Salvatore Caradonna of Louisiana State Medical Center in New Orleans have made observations concerning bovine leukemia virus that are similar to those of Burny and Haseltine. They, too, find that expression of genes linked to the viral control sequence only occurs in infected cells. However, they propose a different interpretation of the result, namely, that such gene expression requires specific cellular, rather than viral, *trans*-activating factors. They base this interpretation partly on the biological behavior of the virus in tumor cells from infected animals. These cells do not make viral products even though the viral genome is present and integrated in the cellular genome.

This seems hard to reconcile, Casey points out, with the hypothesis that the virus itself makes a factor that stimulates transcription of viral genes, which might be expected to produce explosive viral reproduction. In addition, there must be a way to account for the initial production of a viral *trans*-activating factor. Casey suggests that just certain types of infected cells, perhaps only rarely and at a particular stage of development, can produce the correct activating factor. Subsequently, a product of a viral gene, most likely to be a pX (LOR) region gene, might initiate cancerous transformation but would not be required for maintenance of the transformed state. In previous work, Chen had also proposed that cell-specific factors might contribute to activation of HTLV-II transcription.



Trans-*activation* model

According to the model, the protein product of the LOR gene may activate transcription of cellular, as well as viral, genes.

Haseltine takes issue with these proposals. He concedes that the question of why there is viral gene expression in tumor cells is a good one—and an active subject of investigation in his laboratory and elsewhere. But he maintains that for bovine leukemia virus and all three HTLV's, "the *trans*-activation effect is manifest whether or not the cell is a target cell."

These matters may take some time to resolve. In any event, even if cellular factors participate in the original activation of viral genes, a virus might still produce its own *trans*-activating factor. Infection by certain DNA-containing tumor viruses, including adenovirus and SV40, features both types of events. As Burny points out, "The [HTLV and BLV] family is structurally similar to the RNA viruses, but functionally they are much more reminiscent of the DNA viruses."—**JEAN L. MARX**

†J. Sodroski *et al.*, p. 171 of this issue.