Guest Editorial

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Human Retroviruses and Adult T-Cell Leukemia–Lymphoma

The last several years have seen exciting developments concerning the presence of retroviruses in humans and its association with certain types of T-cell leukemia–lymphoma. Retroviruses have long been identified in many species and have been shown to induce leukemias and lymphomas or have been closely implicated in the etiology of these diseases in a number of species. Transmission of leukemia with cell-free filtrates was reported for chickens in 1908 by Ellerman and Bang (1). Gross (2) later showed the induction of leukemia in inbred strains of mice with murine leukemia virus. Induction of leukemia was demonstrated in cats with FeLV by Jarrett et al. (3), and seroepidemiologic studies among outbred cat populations by Cotter et al. (4) showed that transmission of FeLV is responsible for most, if not all, naturally occurring leukemias in cats. Retroviruses are primary causes of naturally occurring leukemias in at least several other species, including cattle, turkeys, and gibbon apes [for reviews, see (5, 6)].

There are two general categories of retroviruses based on their life cycles. Endogenous viruses are contained in the germ line of a species, transmitted genetically, not usually expressed into RNA, protein, or extracellular virus, and do not seem to be pathogenic except in some inbred animals. The retroviruses associated with leukemias and lymphomas, in contrast, are transmitted by infection with exogenous virus. These retroviruses can also be subdivided into 2 general groups based on their biologic activity, a reflection, of course, of differences in their genome. One group is competent for virus replication, causes disease after a long latent period, and does not appear to directly transform cells. BLV and FeLV belong to this group. The other group is generally replication-defective and is comprised of viruses that have undergone recombination with host cell DNA sequences. These host cell sequences, called "onc" genes, allow these viruses to directly transform cells in vitro, and disease onset is rapid. Sarcoma viruses and Abelson murine leukemia virus belong to this group. Replication is possible only in the presence of competent "helper" viruses.

Identification and Characterization of a Human Retrovirus

Until recently, retroviruses had never been unequivocally demonstrated in humans, although data suggestive of their presence were frequently reported. For example, definitive evidence was obtained in human leukemias for virus-like reverse transcriptase (7), and extra, presumably acquired, DNA sequences were also reported in this disease (8). No convincing evidence or demonstration of a human retrovirus, however, was reported. It is now evident that the inability to show the existence of retroviruses was in part due to the uncertainty of where to look (tissue type, cell type, and type of diseases) and in a large part due to the inability to successfully culture the appropriate types of cells in vitro.

Our laboratory had long been interested in learning how to grow different types of hematopoietic cells. In particular, the growth of T-cells seemed of interest since many virally induced leukemias of animals are of T-cell origin (9). In 1976, we reported the discovery of a factor from short-term cultures of mitogen-stimulated peripheral blood human lymphocytes. This factor (TCGF) supported the long-term growth of human mature T-cells (10, 11). TCGF has been purified to near homogeneity and is a protein whose monomeric molecular weight is 12,000–13,000 (12).

With the use of purified or partially purified TCGF, mature T-cells from normal sources could be grown only after mitogen activation, indicating that TCGF is a second signal in T-cell growth (12) due to lack of TCGF receptors on unactivated T-cells. It was soon realized, however, that purified TCGF alone was sufficient to grow mature T-cells from the blood of patients with cutaneous T-cell lymphoma–leukemia and certain other T-cell cancers (13). Some of the biochemical markers of these cells, as well as the requirement for prior activation of normal T-cells for TCGF-supported growth, suggest that the T-cells grown with purified TCGF consist, at least in part, of the malignant T-cells of these patients. The lack of a requirement for prior activation of these cells appears to be due to their constitutive expression of receptors for TCGF (14).

Several such cell lines were studied from an adult black man (CR) with a particularly aggressive cutaneous T-cell lymphoma (mycosis fungoides) (15). Typical retrovirus particles were visible by electron microscopy (fig. 1), and particular reverse transcriptase activity was detected in the media. This enzyme activity had all of the biochemical parameters of retrovirus reverse transcriptase, and both the

Abbreviations used: ATL=adult T-cell leukemia–lymphoma; BLV=bovine leukemia virus; FeLV=feline leukemia virus; HTLV=human T-cell leukemia–lymphoma virus; TCGF=T-cell growth factor.

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Editor's note: Periodically, the Journal publishes solicited guest editorials as a means of transmitting to investigators in cancer research the essence of current work in a special field of study. The Board of Editors welcomes suggestions for future editorials that succinctly summarize current work toward a clearly defined hypothesis regarding the causes or cure of cancer.
particles and the reverse transcriptase activity banded in equilibrium sucrose density gradients at the appropriate density (1.16 g/cm³). The reverse transcriptase activity was not inhibited by antisera against the reverse transcriptases of several other retroviruses, including primate retroviruses. This virus was provisionally designated HTLV. The genomic RNA of HTLV was shown to be a polyadenylated 70S RNA, in common with other retroviruses. With the use of cDNA transcribed from the viral genomic RNA, it was shown that HTLV was not significantly related to any previously described animal retroviruses and that it was not endogenous to humans [i.e., not a ubiquitously germ line-transmitted human virus (16)]. Furthermore, HTLV was not transmitted via the germ line in patient CR but was acquired by postzygotic infection. This conclusion was based on the presence of HTLV provirus in the DNA of some of the T-cells from three independently derived T-cell lines from CR, including one from the laboratories of Dr. S. Broder and Dr. T. Waldmann (National Cancer Institute), and its absence from the DNA of a B-cell line from the same patient, as well as from DNA of other T-cells (17). All these lines were positively identified as having originated from patient CR by their HLA haplotype (17).

The major HTLV core protein was identified as p24, which was shown to be serologically distinct from the core proteins of all previously described viruses (18). A second core protein, p19, was also shown to be novel (19). The reverse transcriptase (also serologically distinct) is somewhat larger than that of type C retroviruses (95,000 daltons) and slightly prefers Mg²⁺ over Mn²⁺ as the divalent cation, a property shared by the reverse transcriptase of BLV (20). Consistent with this observation, the amino acid sequence of the p24 core protein has recently been determined by Oroszlan et al. (21) to be most closely homologous to that of the major core protein of BLV among all the analogous retroviral proteins tested. On the basis of this finding, HTLV appears to be distantly related specifically to BLV. However, the relationship is not sufficiently close to suggest current interspecies transmission. In fact, by conventional immunologic assays and nucleic acid hybridization assays, the proteins and nucleic acids of BLV and HTLV are not detectably related (16, 18, 19). In addition, natural antibodies to BLV in cows do not react with proteins of HTLV, and, conversely, human antibodies to HTLV do not react with BLV (22, 23).

A second isolate of HTLV was subsequently obtained from the cultured T-cells of another adult patient (MB) with a highly malignant variant of Sézary T-cell leukemia (24). This virus was shown by nucleic acid hybridization and protein serology to be very closely related or identical to HTLV. Moreover, viral proteins and proviral DNA were detected in uncultured tissue from the same patient, which virtually ruled out a laboratory contamination.

**Presence of HTLV in Japanese ATL**

Because of the initial association of HTLV with cutaneous T-cell lymphoma (patient CR) and Sézary T-cell leukemia (patient MB), a number of serum samples were screened from each type of patient. With the use of solid-phase immunocompetition and competition radioimmunoprecipitation assays, antibodies to p24 and p19 were detected in the sera of a few of these patients and shown to be absolutely specific for HTLV (22, 23). Antibody to HTLV p24 was also found in the normal spouse of one of the HTLV-infected T-cell lymphoma patients, although all other normal sera were negative. It has become apparent that most cases readily identifiable as positive for HTLV have strikingly common clinical features: adult onset, usually a rapid disease course, often hepatosplenomegaly, lymphadenopathy, and large and usually pleomorphic lymphocytes with lobulated nuclei and mature T-cell surface phenotype markers (usually OKT4⁺), often hypercalcemia, and skin manifestations in over half. In spite of the OKT4⁺ phenotype, the T-cells from this type of disease often have a suppressor function [see CTC-16 in (25)]. A typical cell is shown in figure 2. Despite the similarity in most cases of HTLV-positive disease, however, HTLV has also been identified in a few instances in the typical, more benign Sézary syndrome (Popovic M, Sarin P, Robert-Guroff M, et al.: Manuscript in preparation). Moreover, since in most instances we have tested only for serum antibodies to HTLV and since we have identified HTLV antigen in cultured T-cells of a few antibody-negative persons, HTLV may be more prevalent in classical Sézary syndrome and mycosis fungoides than is now evident.

A T-cell cancer, representing a type of leukemia observed in adults and endemic to regions of southern Japan, was first recognized and described by Takatsuki and his colleagues (26). This disease, called ATL, has clinical symptoms very similar to those observed with HTLV-positive cases of cutaneous T-cell lymphoma and frequently has cutaneous manifestations (27, 28). Like the T-cells from cases in the United States, the T-cells from these patients are generally OKT4⁺ and function as suppressor cells. These similarities prompted us to make a seroepidemiologic survey of patients with this disease from the endemic region of Japan. It was quickly evident that almost all of these ATL patients had specific antibodies to HTLV p19 and p24, while a few normal persons from this region were also antibody-positive (29-31). Consistent with these findings were the subsequent results obtained by investigators in several laboratories in Japan. These investigators have shown the presence by electron microscopy of retrovirus particles in cultured T-lymphocytes from a few ATL patients and have demonstrated serum antibodies directed against determinants on cells producing these particles in almost all Japanese ATL patients (as well as 26% of normal persons from the endemic area of Japan) (32, 33). The similarity in the distribution of this antibody and the antibody against HTLV suggested that the two agents are very similar or identical. This relationship has now been confirmed in our laboratory in collaboration with colleagues in Japan by characterizing the virus produced by two of the cell lines, MT-1 and MT-2, used in the studies by Hinuma et al. (32) and Miyoshi et al. (33). This virus has antigenic determinants identical to those of HTLV p24 and p19, and its mRNA and provirus hybridize virtually completely to cDNA from HTLV (34).

Cocultivation of the other cell line in (33), i.e., MT-2, with cord blood T-lymphocytes results in the induction of TCGF-independent T-cell lines (35). This result suggests
that in the MT-2 cell line the virus may have acquired an oncogene, enabling it to directly transform human T-cells. We found that transmission of several of our strains of HTLV to cord blood T-cells usually results in several reproducible biologic effects, including a reduction in their requirement for TCGF, an apparent loss of the so-called "crisis period" which generally prevents these cells from growing indefinitely in vitro, the capacity to form large colonies in agar, and the ability to grow to high cell density (Popovic M, Sarin P, Robert-Guroff M, et al.: Manuscript in preparation). In at least some cases, TCGF independence is induced. Moreover, the virus apparently infects only mature T-cells and, despite repeated efforts, we have been unable to infect human B-cells, fibroblasts of human and animal origins, or human bone marrow cells.

Presence of HTLV in ATL in the Caribbean

In collaboration with Dr. W. Blattner and his colleagues (Environmental Epidemiology Branch, National Cancer Institute), Dr. D. Catovsky and Dr. D. Galton (Medical Research Council Leukemia Unit, Hammersmith Hospital, London), and Dr. M. Greaves (Imperial Cancer Research Fund Laboratories, London), our laboratory has found that the Caribbean basin is a second region in which HTLV is unusually prevalent. A relatively high incidence of a disease clinically similar to ATL was identified among blacks of Caribbean origin in Great Britain. All cases tested to date have antibody to HTLV (36) and, when available, the leukemia cells have released infectious HTLV. T-cell leukemia--lymphoma also seems to be of relatively high incidence in the Caribbean itself, and antibody to HTLV is found in those patients with the disease, as well as in some normal persons (37). Various types of T-cell leukemia--lymphoma in conjunction with HTLV have now been found in the southeastern United States, the Boston area, the Seattle area, Japan, the Caribbean, Venezuela, Brazil, Guyana, Ecuador, Israel, and Alaska (38). It seems likely that, as other populations are tested, HTLV will also be identified in other parts of the world.

How might HTLV induce ATL? One possibility is that insertion of a viral RNA transcriptional promoter switches on cellular genes whose inappropriate expression results in the leukemia phenotype. This phenomenon has been shown to occur in avian bursal lymphomas and has been termed "downstream promotion" (39, 40). It has been suggested that infection with HTLV may result in the downstream promotion of the gene for TCGF, causing its constitutive and inappropriate expression, and some but not all of the available evidence favors this model (41). Obviously, infection with HTLV is not in itself sufficient to induce ATL, since not only do many nonleukemic persons manifest persistent serum antibodies to HTLV (23, 29–32, 37), but also, in at least some cases, they harbor HTLV in some of their peripheral blood T-cells (Robert-Guroff M, Kalyanaraman VS, Blattner WA, et al.: Submitted for publication). Host-specific or environmental factors, age at first infection, repeated infections, route of infection, or even random chance, as for example, the appropriate integration site, may play a role in pathogenesis by HTLV. Estimates of HTLV infection and association with leukemia or lymphoma have been made by Suchi (personal communication) who believes that about 1 in 2,000 infected people develop disease. This incidence is similar to BLV induction of bovine leukemia. Perhaps HTLV is involved in other diseases, including some of a nonneoplastic nature.

Are all isolates identical? The three CR isolates, the MB isolate, and the MT-1 isolate are certainly very closely related (24, 34). In addition, five other isolates (from the Caribbean, the United States, Japan, and Israel) are very closely related by serology and molecular hybridization (Popovic M, Sarin P, Robert-Guroff M, et al.: Manuscript in preparation). One isolate, however, from the Mo cell line (42) (originating from a patient in the United States described as having a T-cell variant of hairy cell leukemia) appears to differ in the serologic behavior of its p24. Although this virus thus also belongs to the HTLV group, it may represent a second subtype (HTLV-III). It is possible that others of this type, or yet different types, will be identified in the future. Our provisional nomenclature refers to each strain of HTLV in the order of establishment as a separate strain by Roman numeral followed with letters for the patient or cell line [e.g., HTLV-I(CR)]. A detailed comparison of all the HTLV-I isolates and the possible example mentioned above of HTLV-II will be greatly facilitated by the molecular cloning of a portion of the HTLV genome which has recently been achieved (Wong-Staal F, Manzari V, Gallo RC: Manuscript in preparation).

How is HTLV transmitted? In a substantial portion of the population in the endemic area is indeed infected, as judged by serum antibodies to HTLV, there is probably no need for a nonhuman reservoir to maintain the presence of HTLV. It seems that HTLV is not readily infectious and may require prolonged intimate contact for transmission. This transmission might occur within families (e.g., mother's milk and sexual contact) or could result from, for example, blood transfusions or insect vector transmission. Although a large body of data has become available on HTLV, obviously a great deal of work remains. The identification of this virus, however, raises the possibility of eventually being better able to find, treat, and prevent ATL. Moreover, it may also stimulate thoughts about certain other human neoplasias and the possible role of this type of virus.

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FIGURE 1.—HTLV particles from CR T-cell line HUT-102. × 105,000
Figure 2.—Cells from CR T-cell line CTC-16. X 7,000