The Family of Human T-Lymphotropic Leukemia Viruses: HTLV-I as the Cause of Adult T Cell Leukemia and HTLV-III as the Cause of Acquired Immunodeficiency Syndrome

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When the first human retrovirus was isolated in 1978, and was shown subsequently to be etiologically linked to a human cancer (reviewed), there was some expectation that discovery of other human retroviruses would follow, if not through use of these first prototypes as probes to detect related viruses, then merely through provision of hope to investigators committed to this quest. Now, six years later, a family of human retroviruses has been identified, consisting of at least three diverse subgroups. All viruses of these subgroups share some common properties: first, they are extremely tropic for T lymphocytes, especially a subset of OKT4+ (Ortho, Raritan, NJ) helper cells. We named them human T cell leukemia/lymphoma virus, HTLV. Second, they all cause profound morphological and functional changes in the infected T lymphocytes, inducing the formation of multinucleated giant cells, impairing T cell function, and in some cases causing cell death. Third, their genomic organization is quite similar, although the actual nucleotide sequences are only distantly homologous to each other. The homology of the nucleotide sequences, however, is sufficient to code for a similar-size reverse transcriptase with Mg++ preference, a similar-size major core protein (p24), and immunologically cross-reactive core and envelope antigens. Despite these similarities, viruses of this family are naturally associated with two diseases that are opposite in nature. HTLV-I is the etiologic agent of adult T cell leukemia (ATL), a malignant disease characterized by abnormal T cell proliferation, and HTLV-III is the etiologic agent of acquired immunodeficiency syndrome (AIDS), a disease characterized by T cell depletion and immunosuppression. In vitro, HTLV-I causes immortalization and transformation of normal T lymphocytes, while HTLV-III is cytopathic, and kills its target cell rather than immortalizes it. HTLV-II, a rare isolate that was also obtained initially from a T cell malignancy, namely T cell hairy cell leukemia, is similar to HTLV-I in its biological functions and is of the transforming variety of HTLV. Therefore, the HTLV members are, on one hand, tangible culprits of at least two deadly diseases, but on the other hand, invaluable tools for unraveling the regulatory mechanisms of T cell proliferation and immune function.

HTLV-I AND ADULT T CELL LEUKEMIA

Two factors were paramount to the discovery and isolation of the first human leukemia viruses. These were the development of sensitive assays for virus detection, chiefly by reverse transcriptase assays (reviewed), and the capability of growing the right cell for long periods. In this respect, the discovery of a growth factor for T cells (T cell growth factor [TCGF] or interleukin-2 [IL-2]) played a direct role in the isolation of viruses of the HTLV family. When culturing leukemic T cells from patients with T cell malignancies with TCGF, it was noted that some neoplastic T cells were found to constitutively express receptors for TCGF. This later turned out to be a property of HTLV-infected T cells.

The first two isolates of HTLV belonged to a well-conserved subgroup, which we designated HTLV-I. They were obtained from two black patients from...
the United States with mature T cell malignancies originally diagnosed as mycosis fungoides and Sézary syndrome. The leukemic cell that harbors HTLV-I is most frequently an OKT4+ T lymphocyte, often with a distinct morphology (e.g., lobulated nuclei). When a cluster of T cell leukemia, called adult T cell leukemia/lymphoma (ATL or ATLL), was discovered in parts of Japan,9,10 and the leukemic cells had the same phenotype and morphology as the US cases, the analogy beckoned a search for HTLV in the Japanese ATL. In a seroepidemiologic survey using a purified HTLV-I antigen (p24) in an immunoprecipitation assay, close to 100% of the Japanese ATL patients had sera that reacted with the HTLV protein.11,12 Furthermore, HTLV was found to be endemic in the population in parts of southwestern Japan, correlating well with areas where ATL clustered. Not long after this, Japanese investigators independently obtained virus isolates from ATL patients.13 In retrospect, the clinical course of the first US patients is very similar to Japanese ATL and clearly represents the same syndrome. Other regions of the world were also found to be endemic for HTLV-I: the Caribbean Islands,14,15 Central and South America,16 and Africa17,18 (Fig 1). The disease that is associated with this virus is usually ATL, but atypical, more benign HTLV-positive T cell malignancies are sometimes found. The leukemic cells contained clonally integrated HTLV-I provirus,19,20 indicating that the virus is present prior to expansion of the leukemic clone and is not a passenger virus in the course of the disease. To date, there are over 80 isolates from ATL cases all over the world, and a few from more typical cases of Sézary syndrome. All of these isolates, except two, are extremely closely related as measured by restriction enzyme maps, nucleotide sequence analyses, and needless to say, protein homologies. A single isolate from an African ATL that was analyzed in detail showed significant changes from the prototype.21 It is not yet known whether this variant, designated as HTLV-Ib, is the common variant in Africa. Another isolate from a Caribbean ATL patient (HTLV-Ic) also had a number of restriction enzyme site changes throughout the genome, although, like HTLV-Ib, it was closely homologous to the prototype HTLV-I by molecular hybridization and protein immunology analyses (B. Hahn and F. Wong-Staal, unpublished observations, 1984).

The numerous precedents for a retrovirus causing leukemias and lymphomas in animals, especially the recent data indicating that malignant lymphomas of monkeys are sometimes caused by a retrovirus very closely related to HTLV-I,22 the extensive seroepidemiologic data, and the clonal integration of the provirus in the leukemic cells are all arguments that HTLV-I is the primary cause of ATL. However, the most interesting argument is that HTLV-I can immortalize and transform the same cell (OKT4+) in vitro as the primary ATL cell.

**IN VITRO TRANSFORMATION AND OTHER BIOLOGICAL EFFECTS OF HTLV-I**

Normal T cells stimulated with mitogen grow transiently in culture in the presence of TCGF. They grow as single-cell suspensions and appear as a homogeneous population of lymphoblastoid cells. Normal T cells from cord blood, peripheral blood, or bone marrow samples infected with HTLV-I are immortalized, usually grow independently of TCGF, and morphologically resemble the primary ATL cells;23,24 by displaying highly convoluted nuclei and formation of multinucleated giant cells. Furthermore, the in vitro infected cells express cell surface markers in type and quantity similar to the ATL cells and different from mitogen-stimulated normal lymphocytes. These include high densities of TCGF receptors, HLA-DR antigens, and transferrin receptors.24 Finally, although the initial infected cells are polyclonal, the cells that emerge as immortalized cells in a course of four to six weeks are invariably clonal (Hahn et al25 and our unpublished data). Taken together, these results strongly suggest...
that HTLV-I has the capacity of in vitro transformation.

Other than transformation, HTLV-I also exerts other biological effects on T cells, which in turn may modulate the function of other hematopoietic cells. First, HTLV-transformed T cell lines produce many lymphokines including colony-stimulating factor, eosinophil growth maturation activity, fibroblast-activating factor, B cell growth factor, and gamma interferon. In some HTLV-transformed T cell lines, low levels of TCGF were also detected. Production of some of these factors may in part be responsible for some of the secondary symptoms that develop in patients infected with HTLV-I. An example is hypercalcemia development due to increased osteoclast-activating factor in some patients. Second, normal TCGF-dependent clones of functional cytotoxic and helper T cells sometimes lose their immune function after infection and immortalization by HTLV-I. This may explain why HTLV-I-infected individuals are immune compromised and more prone to opportunistic infections. Third, HTLV-I-infected cells may cause polyclonal B cell activation. Fourth, Mitsuya, Broder, and their colleagues observed that HTLV-I infection may selectively kill certain tolerant cytotoxic T cells. Thus, by infecting a cell that occupies a central place in regulation of hematopoietic cell growth and immune functions, HTLV may exert profound effects on a spectrum of cells beyond the immediate target cell. In this regard, recent seroepidemiologic data suggest that HTLV-I may be indirectly involved in the origin of some B cell leukemias in an endemic area of HTLV infection, such as Jamaica.31

HTLV SUBGROUP II: ANOTHER TRANSFORMING HTLV

A retrovirus was isolated from a T cell line established from a patient with a T cell variant of hairy cell leukemia. This virus, although partially homologous to HTLV-I, showed differences in its core and envelope proteins. This virus has been designated as HTLV-II. Extensive molecular (unpublished observations) and seroepidemiologic surveys using HTLV-II reagents revealed only sporadic presence of this virus and failed to link it to any particular disease. However, HTLV-II has very similar biological properties as HTLV-I in vitro, infecting the same target cells and having most of the biological effects, including cellular transformation and abolition of T cell functions.

THE GENOME STRUCTURE OF HTLV-I AND HTLV-II

Retroviruses contain an RNA genome that is transcribed into DNA (provirus) upon infectus of host cells. The provirus then integrates into host chromosomal DNA where it may complete the replication cycle by directing the synthesis of infection virions, or it may express none or only part of its genetic information in a covert infection. The retrovirus genome consists of three structural genes coding for virion core antigens (gag), viral polymerase (pol), also known as reverse transcriptase because it uses RNA as a template for transcription into DNA, and envelope antigens (env). These genes are flanked by sequences repeated on both sides known as the long terminal repeat (LTR). Regulatory elements for transcription, including the binding site for RNA polymerase (promotor) or sequences that increase the transcriptional activity of nearby genes (enhancer) are located within the LTR. The three structural genes, along with the LTR, constitute all of the essentials for virus replication. A subclass of retroviruses known as the acutely transforming retroviruses are often replication defective because they lack one or more of these genes. Instead, these viruses have acquired transformation-specific genes (onc genes) that have been acquired from host cellular DNA by recombination.

The genome of HTLV confirms the uniqueness of this virus family. The complete nucleotide sequence of one isolate of HTLV-I has been determined. The provirus is 9,032 nucleotides long and is bounded by a terminal repeat of 754 nucleotides. In addition to nucleotide sequences potentially coding for the three viral structural precursor proteins (gag, pol, and env), there is an unexpected region of several possible coding stretches of nucleotides (open reading frames) between the carboxyl terminus of the env gene and 3' LTR (Fig 2). This had been tentatively referred to as the pX region. At present, there is no evidence that the pX region is a recent acquisition from cellular genes of any mammalian species, including humans.

While the available sequence data on HTLV-II are still fragmentary, several conclusions can be drawn from these and other studies including heteroduplex and comparative Southern blot hybridization of HTLV-I and HTLV-II. First, HTLV-II has a genomic organization similar to that of HTLV-I. Homology throughout the genome is detected under relatively nonstringent conditions, suggesting the presence of a pX region in HTLV-II (Fig 3A). Under

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**Fig 2.** Genetic structure of HTLV-I. Diagram is based on the sequence data of Seiki et al. Sizes of viral proteins are deduced from the nucleotide sequence and from actual protein studies.
stringent conditions, the only homology between the
two genomes is a 1-kb stretch within the pX region
(Fig 3B). Nucleotide sequence comparison showed
that the pX region consists of a 5' nonconserved
sequence and a highly conserved 3' region that contains
a single long open reading (lor) frame. The predicted
protein products of HTLV-I and HTLV-II from this
region are identical in 252 of their 336 amino acids.
Since both HTLV-I and HTLV-II are capable of
lymphocyte transformation, and since this conserved
coding sequence is not part of the usual replicative
machinery of retroviruses, it has been proposed that
this 1-kb lor sequence is the transforming gene of
HTLV-I and HTLV-II. Recently, T.H. Lee et al
identified a 42-kd protein in HTLV-I-transformed
cells, and preliminary protein sequence data sug-
gested that it is encoded at least in part by the lor
sequences.

The LTR regions of HTLV-I and HTLV-II resemble
each other with respect to several structural fea-
tures, but the actual sequences of the two LTRs are
different, with homologies limited to the short
stretches at the 5' and 3' ends of the LTR; conservation
of these sequences may be essential for virus integra-
tion and transcription. In addition, there is a 21-base
pair (bp) tandemly repeated sequence conserved in the
two LTRs. Such tandemly repeated sequences often
function as transcriptional enhancer elements by
increasing the initiation of transcription of nearby
genes. Although the latter has no homology to consen-
sus transcriptional enhancer elements, its presence as a
tandemly repeated sequence upstream of the site of
RNA initiation suggests that it may function as an
enhancer in transcriptional activation. It has been
shown that retroviral enhancer sequences can
influence tissue tropism and the leukemogenicity of
leukemia viruses. A common enhancer sequence
may in part explain the T cell tropism of both HTLV-I
and HTLV-II, and perhaps, along with the lor prod-
uct, their transforming capability.

POSSIBLE MOLECULAR MECHANISMS OF
TRANSFORMATION AND LEUKEMOGENESIS BY
HTLV-I AND HTLV-II

We believe that the in vitro transformed cells pro-
vide a good model for studying the initiation of leuke-
mogenesis by HTLV-I. However, a second event may
be required for progression into full disease. This is
suggested by two observations: first, development of
ATL requires long latency periods, while HTLV-I
transforms cells in vitro with relative efficiency and
rapidity (four to six weeks). Second, while the in vitro
transformed cells usually retain a normal karyotype,
ATL cells usually exhibit clonal chromosomal abnor-
malities. However, no consistent chromosomal
change has been documented for ATL. In any event, in
considering the role of HTLV in disease causation, we
wish to dissociate the early and late stages, using the in
vitro transformed cells (early) and the frankly leu-
kemic cells (late) as models.

Almost all exogenous, pathogenic animal retrovi-
ruses belong to one of two categories. The chronic
leukemia viruses are replication competent, require a
long latency period for disease induction, and lack
transforming activity in vitro. Their genomes contain
only the three structural genes (gag, pol, and env)
required for virus replication. These viruses induce
disease by a cis-acting mechanism, ie, the promoter or
encoder sequences of the viral LTR activate tran-
scription of specific cellular genes by proximal integration of the virus genome. To accomplish this end, the virus needs to integrate at a specific site, an inefficient process often leading to transformation of a rare cell, giving rise to a monoclonal tumor. The acute leukemia or sarcoma viruses are usually replication defective, induce disease rapidly in vivo, and transform appropriate target cells efficiently in vitro. They also carry a cell-derived gene (\textit{onc} gene) that codes for a product, which is necessary for the initiation and usually also maintenance of the transformed phenotype. In other words, these viruses do not require a specific integration site, and act by a \textit{trans}-mechanism. HTLV-I and possibly also HTLV-II belong to a new category of retroviruses, which have assorted properties of the acute and chronic leukemia viruses. As mentioned earlier, HTLV-I requires a long latency period for the induction of ATL, but it also has the capacity to transform cells in vitro. HTLV-I and HTLV-II are nondefective and do not carry a cell-derived \textit{onc} gene, but they do contain a \textit{lor} sequence that is not part of the virion structural genes. In spite of the monoclonality of the transformed cells, the lack of a specific integration site in the provirus in different transformed cell populations\textsuperscript{46, 51} indicates a \textit{trans}-acting mechanism.

In order to define the events required for early transformation, we have followed the course of in vitro transformed cells from the time of infection either with cell-free virus or by co-cultivation with infected cells for a time course of two to three months (our unpublished data with A. Aldovini, C. Grandori, and G. Franchini). During this time, the infected cell population expands and progresses from polyclonality to monoclonality, often becoming independent of exogenous TCGF. Interestingly, this expansion and clonal selection of infected cells frequently occur in the absence of detectable viral expression (eg, reverse transcriptase, core or envelope antigens). Therefore, the infected cells have a growth advantage over the uninfected cells, but none of this involves active virus replication. If a diffusible viral protein product is required for transformation, ie, if the virus is \textit{trans}-acting, it is not one of the three viral structural proteins (\textit{gag}, \textit{pol}, or \textit{env}). The only remaining candidate is the \textit{lor} product alluded to above. Unfortunately, expression of \textit{lor} sequences in these cells could not be examined because of limited cell material for RNA experiments and lack of reagents (eg, antisera) for specific detection of the \textit{lor} product at the time. However, this gene product is likely to be important in the transformation process in view of the high degree of conservation of this gene between the two transforming HTLV subgroups (I and II), and the fact that it is frequently expressed as a separately spliced mRNA in established in vitro transformed cells.\textsuperscript{52} In this regard, Sodroski et al have described a \textit{trans}-acting factor for transcriptional activation of HTLV-LTR-linked genes, specifically in HTLV-infected cells.\textsuperscript{53} Again, the \textit{lor} product is the likely candidate for this factor. This is analogous to the transforming proteins of some DNA tumor viruses that can exert positive or negative control of gene expression by interacting with a subset of viral and cellular promoters.\textsuperscript{54, 55} If the \textit{lor} gene product functions similarly, it can activate specific T cell genes (eg, the TCGF receptor gene, which is consistently expressed at high levels in HTLV-I- and -II-infected cells), either directly or through interaction with HTLV enhancer or promoter sequences (Fig 4).

Most (about 80\% of tested samples) primary frank leukemic cells do not express detectable levels of viral mRNA, including \textit{lor} sequences. In addition, although each of these ATL cell samples is monoclonal, containing one to three copies of HTLV, the integration sites of these proviruses vary from sample to sample. So the virus does not appear to function either in a \textit{cis}- or \textit{trans}-manner in maintaining the leukemic state. It is likely that HTLV has already set in motion a chain of events in the infected cells such that its continued expression may not be required at this stage to maintain the disease. Alternatively, expression of \textit{lor} may only be needed when cells go into cycle and actively proliferate, and in 80\% or so of the samples randomly obtained, the leukemic cells are mostly in a nonproliferative state. In this case, expression of a viral protein, eg, the \textit{lor} product, is still required for growth but is turned off when cells are not in cycle.

![Fig 4. Transcriptional activation of viral and cellular genes by the \textit{lor} protein: a model for the mechanism of transformation by HTLV.](www.bloodjournal.org)
ACTIVATION OF CELLULAR GENES IN HTLV-TRANSFORMED CELLS

Because of the precedence of activation of proto-oncogenes in tumors, sometimes specifically by chronic leukemia viruses, we had examined this possibility in the HTLV-transformed cells (our unpublished data with J. Horneff). Using cloned probes of sis, myc, myb, fes, abl, src, H-ras, and K-ras, we failed to find alteration at the DNA level, or consistent enhanced transcription at the RNA level of any one gene in either the in vitro infected cells or fresh leukemic cells. However, a number of the in vitro transformed cell lines do express c-sis, a gene not normally expressed in hematopoietic cells except megakaryocytes. It is not known whether activation of c-sis, which codes for one of two subunits of platelet-derived growth factor (PDGF), contributes to transformation by HTLV in these cases. The fact that the transformed cells neither express PDGF receptors nor respond to PDGF for proliferation (our unpublished data with J. Huang and T. Deuel) makes it an unlikely contributory factor.

Other genes that are of obvious interest are those involved in T cell proliferation and activation. One gene that is clearly activated upon HTLV infection is the receptor for TCGF. However, this abundant expression of the TCGF receptor is not coupled to expression of TCGF itself, thus ruling out a simple autostimulation model. The ability of the HTLV-transformed cells to grow in the absence of endogenous or exogenous TCGF may be due to intrinsic activation of the receptor by a mechanism yet to be defined. Finally, several other genes that are turned on in mitogen-activated T cells are also turned on by HTLV infection. It would be of interest to know whether a common mechanism underlies the activation of all of these T cell-specific genes. A trans-acting mechanism, eg, via ltr, could theoretically provide this link.

THE CYTOPATHIC, NONTRANSFORMING VARIANT, HTLV-III, AND ITS ASSOCIATION WITH AIDS

AIDS is a recently recognized disease manifested by opportunistic infections as a result of severe immunosuppression and OKT4^+ helper T cell depletion (reviewed). Although there were numerous ideas regarding the cause of AIDS, the epidemiologic data strongly suggested a transmissible agent in this disease. We initially proposed that this was likely to be a virus since viruses are known to be more specifically tropic for lymphocytes than bacteria or fungi and since filtered factor VIII, which was known to induce AIDS, should not contain bacteria or fungi but could contain viruses. Since the apparent target cell appears to be an OKT4^+ lymphocyte, since some retroviruses such as feline leukemia virus can cause both leukemia and an AIDS-like disease in animals, and since Africa was the likely origin of HTLV as well as AIDS, we and M. Essex and his colleagues first proposed that a virus of the HTLV family might be the etiologic agent of AIDS. However, isolation of the then-known subgroups of HTLV-I and HTLV-II were sporadic and infrequent, and only 20% of the AIDS patients have demonstrable antibodies against purified core antigens of these viruses. Therefore, one was left with the uncertainty whether HTLV-I and HTLV-II infections were merely opportunistic in AIDS patients or whether they have any etiologic significance.

In support of the involvement of a virus of the HTLV family in the etiology of AIDS was the demonstration by Essex and colleagues that up to 80% of AIDS patients have serum antibodies that react with the envelope protein of HTLV-I. At the same time, retrovirus particles not closely related to HTLV-I and HTLV-II were frequently detected by electron microscopy and reverse transcriptase activity in short-term cultured cells of AIDS patients in our laboratory. Such observations of one patient with lymphadenopathy had been reported by Barré-Sinoussi et al. Although similar particles were detected in our laboratory as early as November 1982, the transient nature of their expression and the early inability of our group or others to transmit the viruses to established cell lines had frustrated their further characterization beyond the fact that they were not closely related to HTLV-I or HTLV-II. Isolation of these viruses was difficult because they lack immortalizing capacity and are highly cytopathic. However, this problem was solved when our laboratory first transmitted these viruses to clones of a previously immortalized mature T cell line that was relatively resistant to the cytopathic effects. Continuous production of high titers of these viruses was attained by cloning the infected cells. This advance made detailed biochemical, serologic, and molecular characterization of the viruses possible. The results indicate that the different AIDS virus isolates are indistinguishable from each other at the protein level and thus represent one virus entity. Furthermore, these studies led to two major conclusions: (1) This virus is the etiologic agent of AIDS (a major practical advance is the feasibility of development of a blood bank assay). (2) This virus is T lymphotropic, and shares many biochemical and structural features with HTLV-I and HTLV-II. In accordance with the agreement about nomenclature for human retroviruses at a Cold Spring Harbor, New York, meeting in 1983, we call the AIDS viruses HTLV-III.

HTLV-III has been repeatedly obtained from patients with AIDS or pre-AIDS. It preferentially
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infects human T4 lymphocytes. When transmitted to fresh lymphocytes, it is replicated transiently (for two to three weeks) until the infected cells are gradually depleted. Electron micrographs of primary or infected cultures show many retrovirus particles budding off the membrane of a small percentage of cells. The mature virions often display a cylindrical core (Fig 5). A similar structural feature has been observed with other type-C viruses.

Many established cell lines were tested for transmission of this virus. A human mature T cell line called HT was highly permissive for virus replication and was resistant to the cytopathic effect. Two HT cell clones (H4 and H9) were found to be optimal targets for infection and continuous production of high-titer virus. Lysates of purified HTLV-III in an enzyme-linked immunosorbent assay (ELISA) or Western blot assay reacted specifically with sera from >90% of patients with AIDS and pre-AIDS and from a significant number of high-risk homosexuals, intravenous drug users, and hemophiliacs. More interestingly, each of ten blood donors whose recipients subsequently developed AIDS scored positive in a double-blind assay of hundreds of samples (M.G. Sarngadharan and R.C. Gallo, unpublished observations). Therefore, nature has indirectly fulfilled one of Koch's postulates by documenting transmission of the agent prior to development of the disease. A direct animal model was provided when chimpanzees were infected experimentally with HTLV-III. Theses animals mounted an immune response to the virus, and some developed enlarged lymph nodes analogous to the lymphadenopathy syndrome that often precedes AIDS. In our view, the seroepidemiologic data, the biological properties of this virus in vivo and in vitro, and the blood transfusion results unambiguously establish HTLV-III as the etiologic agent of AIDS.

The AIDS agent has several biological and biochemical properties in common with HTLV-I and HTLV-II: the T cell tropism, cytopathic effect, ability to induce syncytia and formation of multinucleated giant cells, a high molecular weight, Mg⁺ preferring reverse transcriptase, and a p24 core antigen. However, direct evidence that plants this virus firmly in the HTLV family came from the structural and functional conservation of some of the viral proteins, and distant
nucleic acid homology of its genomes to HTLV-I and HTLV-II. Antisera raised against HTLV-I or HTLV-II p24 react weakly with HTLV-III p24, and patient sera that recognize the gp65 env precursor of HTLV-III also recognize the gp61-65 of HTLV-I or HTLV-II (M.G. Sarngadharan, J. Schupbach, and R.C. Gallo, unpublished observations). Furthermore, HTLV-III nucleic acid probes detect distantly homologous sequences within cloned HTLV-I and HTLV-II genomes. Nucleotide sequence analysis of a cloned genome of HTLV-III suggests that it also has a \( \text{lor} \) sequence (our unpublished observations). Furthermore, transcription initiated within HTLV-II LTR is also greatly enhanced by a trans-acting viral product, presumably \( \text{lor} \). These properties characterize members of the HTLV family, and related viruses such as the simian T cell leukemia viruses and bovine leukemia virus.

**ORIGIN OF HTLV**

The HTLV family itself belongs to a superfamily of lymphotropic retroviruses that includes to date a simian T cell leukemia virus (PTLV)\(^{74,75} \) and bovine leukemia virus (BLV). STLV is highly related to but distinguishable from HTLV-I\(^{78} \) and BLV is only distantly homologous to HTLV.\(^{77} \) All of these viruses probably have a common ancestor, although the exact evolutionary lineage is not known. STLV is prevalent among Old World monkeys in Africa and Asia but to date has not been found in New World monkeys.\(^{73,74} \) Similarly, HTLV-I infection is notably absent in the vast majority of Western Europe and American Caucasians, but the virus is endemic in Africa, Central and South America, the Caribbean, and restricted coastal regions of southwestern Japan. Identification of areas endemic for HTLV-II must await studies using a simple, specific test that distinguishes HTLV-I and HTLV-II, such as the neutralization assay described by Clapham et al.\(^{33} \) Although extensive seroepidemiology for HTLV-III has not been completed, the prevalence of HTLV-III in Africa and the Caribbean is implied, since the AIDS disease is endemic in Zaire and Haiti.\(^{62} \) We had previously proposed that HTLV-I originated in Africa\(^{77} \) and would now extend this idea to include all of the HTLV family members. HTLV-I was probably brought to the Americas through the slave trade, and to the southern islands of Japan by European seamen (mainly Portuguese and Dutch) involved in trade with Africa and Japan, with specific contact with the southern islands of Japan.\(^{78} \) HTLV-III may have entered Haiti with the immigrant black population from Africa, and more recently into North America and Europe through homosexual contacts of infected individuals from the Caribbean or Africa.

**PERSPECTIVES**

During the past five years, the cause of two complex human diseases involving the proliferation of human T cells has been clarified. Adult T cell leukemia (and certain related non-Hodgkin’s T cell lymphomas) and AIDS have been shown to be due to related human retroviruses, HTLV-I and HTLV-III, respectively. These viruses and the related HTLV-II (which has not yet been clearly linked to the cause of any human disease) belong to an expanding family of human T-lymphotropic retroviruses known as HTLV, and are the only known class of human retroviruses. These disease systems, as well as the possibility of reproducing some aspects of the diseases in vitro, provide an unprecedented opportunity to learn basic mechanisms involved in the control of growth of a human cell. The recent data implying a trans-acting transcriptional activation of T cell proliferation genes by the product (pX or \( \text{lor} \)) at the 3' end of HTLV-I and HTLV-II have already opened up a new mechanism of retrovirus-induced leukemia and provided a basis for the definition of a new category of leukemia viruses, which also includes bovine leukemia virus. We propose to call these trans-activating or type T leukemia viruses. From nucleotide sequence analysis of the HTLV-III genome, we know that it too has a pX (\( \text{lor} \)) region. It would be important to determine whether the expression of this protein is central to the killing effect of HTLV-III–infected T cells. Alternatively, the \( \text{lor} \) product of HTLV-III may function similarly as related products of HTLV-I and HTLV-II, but the immortalizing activity of HTLV-III is masked by the cytopathic effect of the virus.

There are increasing suggestions that HTLV-I may be indirectly involved in the origin of some B cell lymphoid malignancies. Further studies should be directed to the elucidation of the mechanism of this effect. Current work by Dean Mann in collaboration with our laboratory indicates that B cell proliferation in some CLL patients may be a monoclonal response to antigens expressed in their HTLV-I–infected “normal” T cells. This exciting finding could also explain the origin of B cell lymphomas in AIDS patients (HTLV-III–infected T cells), but studies of this kind in AIDS with lymphomas have not yet been conducted.

Much more work is needed on HTLV-II. We still have no convincing evidence that this virus causes human disease and no idea where and how the few known infected people acquired the virus. Therefore, further studies of HTLV-II will be focused on seroepidemiology.

Our thoughts about future work on HTLV-III and AIDS in some respects parallel those concerning
HTLV-III can induce lymphadenopathy and immune abnormalities in chimpanzees. If a vaccine meets the criteria described above, other critical questions will relate to decisions as to which groups should be vaccinated. Because it is possible that a vaccine may not work and because there are people already ill, new approaches to therapy are urgently needed. Collaborative studies with Samuel Broder at NCI are directed to using relatively specific inhibitors of HTLV-III reverse transcriptase. The goal is to inactivate cellular replication of virus. We hope to combine this with approaches designed to kill viral-positive "reservoir" cells and to reconstitute normal T cells by transplantation and the selective use of TGF (IL-2).

What about other retroviruses (HTLV-IV, etc) in association with other lymphoid malignancies? There is no way to answer this question, but we think that the greatest chance to find leads will come from studies of African patients and normal volunteers, because we have reason to believe that viruses of this family are prevalent in much of Africa, and we have serologic results suggestive of retroviruses yet to be discovered.

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The family of human T-lymphotropic leukemia viruses: HTLV-I as the cause of adult T cell leukemia and HTLV-III as the cause of acquired immunodeficiency syndrome

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