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Molecular Studies of Human T Cell Leukemia/Lymphotropic Retroviruses

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A. Introduction

Human T cell leukemia/lymphoma virus (HTLV), a name originally used to designate a retrovirus associated with a form of mature T cell malignancy, namely adult T cell leukemia (ATL), now stands for a family of human retroviruses isolated in nature, which preferentially infect a subset of T lymphocytes [20]. In addition to their common cell tropism, viruses of this family share other biological, physiochemical, and biochemical properties. However, one major difference in the biological properties of these viruses is directly reflected in their different disease spectrums and transforming capabilities. The virus associated with ATL, renamed HTLV-I, transforms T cells efficiently in culture. A second virus, HTLV-II, which was obtained initially from culture T cells of a patient with hairy cell leukemia, also has the capacity to transform T cells in vitro. A third virus subgroup, HTLV-III, lacks transforming activity but is instead highly cytopathic. HTLV-III has been unambiguously shown to be the etiological agent of the acquired immunodeficiency syndrome (AIDS) [4, 9, 11, 12].

Almost all exogenous, pathogenic animal retroviruses 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*) that are necessary for virus replication. 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 which codes for a product necessary for the initiation and usually also maintenance of the transformed phenotype. Members of the HTLV family form a third distinct category. They are replication competent, their genomes containing all three replicative genes. In addition, HTLV-I and HTLV-II contain nucleotide sequences between the *env* gene and the 3′ LTR which contain a long open reading frame (LOR). The LOR sequences are not derived from conserved cellular genes, in contrast to retroviral oncogenes. Even though HTLV-I appears to induce ATL only after long latency periods, along with HTLV-II it can transfrom T lymphocytes rapidly and efficiently in vitro. The LOR product of HTLV-I and HTLV-II is believed to be important in initiating transformation by these viruses. The definition of a LOR gene of HTLV-III is less clear. Other retroviruses that fit into this category are the distantly related bovine leukemia virus (BLV) and a simian retrovirus (STLV-I), which is closely related to HTLV-I. Table 1 summarizes comparisons of leukemogenic/transforming properties of viruses in these three categories.

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Table 1. Properties of the three classes of leukemia viruses

	Acute	Chronic	(HTLV/BLV)
Disease induction	Rapid	Slow	Slow
Presence of *onc* genes	Yes	No	No
Presence of gene coding for nonvirion structural protein	Yes	No	Yes
Clonality of tumor cells	Polyclonal	Monoclonal	Monoclonal
Provirus integration site	Random	Specific	Random
Transformation in vitro	Yes	No	Yes
Mechanism of action	*trans*	*cis*	(*trans*)

B. The Genomes of HTLV-I and HTLV-II

The complete nucleotide sequence of one isolate of HTLV-I [13] and partial sequences of other HTLV-I isolates (unpublished) as well as one of HTLV-II [7, 16, 18] have been determined. Some unusual features in common among HTLV-II and the several nearly identical isolates of HTLV-I have been revealed by these studies. First, the long terminal repeat (LTR) is unusually long compared with other retroviruses, with the extra length attributed to the R and U5 sequences. Second, a long, noncoding sequence with multiple-stop codons separates the *gag* and *pol* genes. Third, there is a region of about 1.6 kb between the carboxy terminus of the *env* gene and 3′ LTR, which contains several open reading frames. This region, initially referred to as the pX region [13], is not closely homologous to cellular sequences of vertebrate cells [1] and therefore is not a typical retroviral oncogene. Deletions and substitutions within the first 0.6 kb of pX found in a variant, HTLV-Ib, obtained from an African ATL patient did not affect the transforming capacity of this virus [6a] (unpublished work with L. Ratner). Comparison of the pX of HTLV-I and that of HTLV-II revealed that the first 0.6 kb is nonconserved, but the following 1.0 kb is highly conserved, and contains a single long open reading frame, LOR [7, 14]. Splice acceptor consensus sequences are present 5′ to LOR, which does not initiate with the ATG codon [7]. Therefore, the LOR protein product is likely to be a fused protein containing amino acid residues coded by *gag* or *env*. The HTLV-I LOR sequence predicts a protein product of at least 38 kd. Sera of patients positive for HTLV-I recognize a 40- to 42-kd protein expressed in all HTLV-transformed cells, including nonproducer cells that do not synthesize virus or virion structural proteins. Amino acid analysis of cyanogen bromide cleavage fragments of this protein suggests that it is coded at least in part by the LOR sequences [8]. Sequence analysis of the BLV genome also reveals the presence of an open reading frame similar in size to HTLV-I LOR in the corresponding region [10].

C. Possible Mechanism of Transformation and Leukemogenesis by HTLV-I and HTLV-II

Cells infected by HTLV-I and HTLV-II in vitro are initially polyclonal and go through a growth crisis within 4–6 weeks. Predominantly clonal cells then emerge as immortalized cells (unpublished data). Often, these cells become independent of T cell growth factor (TCGF) and their morphology resembles that of the primary leukemic cells [3]. Although the transformed cells are clonal, the site of provirus integration is variable in different clones. The lack of a conserved integration site suggests that these viruses act by a *trans*-mechanism in cellular transformation, i.e., a diffusible viral protein product is directly involved. By analyzing expression of different viral structural proteins at different time intervals after infection and clones of trans-

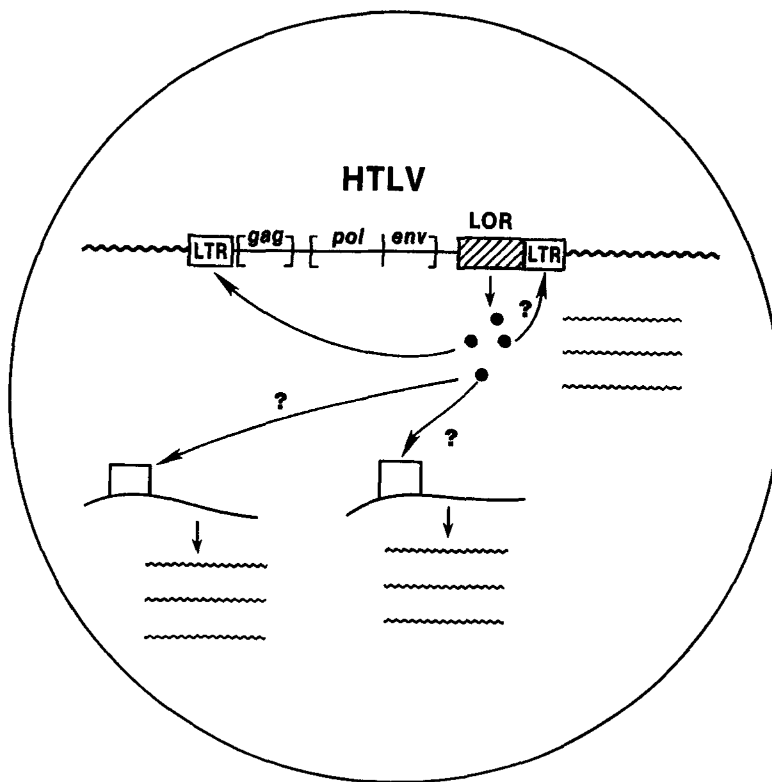

Fig. 1. Transcriptional activation of viral and cellular genes by the LOR protein: A model for the mechanism of transformation by HTLV

formed cells containing defective proviruses, we have ruled out the expression of *gag, pol,* and *env* proteins as a prerequisite for the initiation of transformation (unpublished data). The only remaining candidate is the LOR protein, especially in view of the high degree of conservation of this gene between the two transforming HTLVs. Studies on the promoter-enhancer activity of LTRs of HTLV-I and HTLV-II showed that transcription of LTR-linked genes is elevated manifold in infected cells, indicating a *trans*-acting transcriptional activation phenomenon via a viral protein [17]. There is indirect evidence that the LOR protein is responsible for this enhancing activity. Based on these studies, one can construct a model in which the LOR protein can also regulate cellular promoter-enhancer function at distant sites and the cellular genes regulated are associated with T cell proliferation. Therefore, the mechanism of transformation by HTLV is more similar to certain DNA tumor viruses than to other retroviruses. Several genes have already been identified that are activated by infection with HTLV-I and HTLV-II, and all these have been linked to T cell proliferation (Fig. 1). Therefore, it is possible to test directly whether the LOR protein binds to the enhancer-promoter sequences of these genes.

Unlike the in vitro-transformed cells, fresh ATL cells frequently contain no detectable viral mRNA or proteins, including LOR [2]. In addition, although each of these ATL cell samples is monoclonal, the integration sites of these proviruses vary. So the virus does not seem to function in *cis* or *trans* in maintaining the leukemic state. We speculate a two-stage leukemogenesis event, with the early stage being analogous to the in vitro transformation process, resulting in immortalization and enhanced proliferation of the infected cells. This in turn increases the chance of a mutation or gene rearrangement which leads to the progression of the disease. At this late stage, the virus has done its damage and is no longer needed.

D. Molecular Biology of the HTLV-III, the Etiological Agent of AIDS

A novel member of the HTLV family designated as HTLV-III has recently been identified as the etiological agent of AIDS, a disease characterized by depletion of the OKT4+ helper T cells (Gallo et al., this

volume). We have cloned the genomes of several isolates of HTLV-III [5, 15]. Nucleotide sequence analysis of two of these clones revealed many structural similarities to HTLV-I and HTLV-II, particularly in the presence of extra, non-cell derived genes. Recent experiments have indicated that HTLV-III infected cells contained factors that activate transcription of HTLV-III LTR-linked genes [19], suggesting that the HTLV-III has a similar gene in *trans*-acting transcriptional activation. Either HTLV-III "LOR" activates a different set of cellular genes from LOR of HTLV-I or -II to account for the cytopathic, rather than immortalizing, effect of the virus, or HTLV-III has a transforming activity which is masked by its profound cytopathic effect.

The HTLV-III genome is completely exogenous and does not carry a conserved cellular gene. Integrated provirus can be detected in infected cell cultures as well as peripheral blood lymphocytes and lymph node tissue of AIDS and pre-AIDS patients [15]. The infected cells are polyclonal with respect to provirus integration, as expected for a virus that induces cell death rather than clonal expansion of the target cells. Furthermore, a large amount of unintegrated viral DNA persists in both the long-term cultured cells and patient tissues [15], a feature that seems to be characteristic of other cytopathic retroviruses. We also found that very few (less than 1 in 100) cells were infected in a population of lymphocytes from AIDS and pre-AIDS patients, and virus information is not detectable in several Kaposi tumor tissues examined. These results suggest that HTLV-III does not have a direct role in the proliferation of lymphocytes in lymphadenopathy of pre-AIDS patients or in the clonal expansion of endothelial cells of Kaposi tumors. These are secondary syndromes that develop as a consequence of the depletion or impaired function of the helper-inducer T-lymphocytes infected by HTLV-III.

One salient feature emerging from analyses of the genomes of different HTLV-III isolates is the extent of polymorphism among different isolates. So far, all isolates can be distinguishable from each other based on the analyses with five or six restriction enzymes. There is a whole spectrum with respect to the degree of similarity among different isolates. An isolate from a Haitian patient has practically no correspondence of restriction enzyme sites except in the LTR regions [6 b] and probably represents one end of the spectrum. This degree of genetic diversity is not seen with different HTLV-I and HTLV-II isolates, and may be due to immunoselection or to the highly replicative nature of HTLV-III as opposed to the cryptic state of the HTLV-I and HTLV-II proviruses in vivo. It will be important to determine whether changes in the HTLV-III genomes cluster in any particular regions and whether they are significant enough to alter the immunological reactivities of the different isolates.

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