

# Human T-Cell Leukemia Virus Type I at Age 25: A Progress Report

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## Abstract

**It has been 25 years since the discovery of human T-cell leukemia virus type I (HTLV-I) and its role in adult T-cell leukemia. Here, in brief, we review the current state of our understanding of HTLV-I epidemiology, viral biology, pathogenesis, and treatment. We discuss how HTLV-I may transform cells through destabilization of cellular genomic integrity and induction of cellular tolerance for chromosomal errors.** (Cancer Res 2005; 65(11): 4467-70)

In 1980, Poiesz et al. (1) published a seminal article establishing for the first time a link between a retrovirus and a human cancer in a patient with cutaneous T-cell lymphoma. Several years earlier, a clinical entity called adult T-cell leukemia was described in Japan by Uchiyama et al. (2). Contemporaneous research on adult T-cell leukemia also led to the isolation of a retrovirus named adult T-cell leukemia virus by Hinuma et al. (3) and Yoshida et al. (4). Because adult T-cell leukemia virus and the virus isolated by Poiesz et al. were later shown to be identical, a single name, human T-cell leukemia virus type I (HTLV-I), was adopted. Twenty-five years after the discovery of HTLV-I, we briefly review current progress in our understanding of this transforming virus.

## Epidemiology

Approximately 10 to 20 million individuals are estimated to be infected with HTLV-I worldwide. The virus is endemic in southwest Japan, the Caribbean islands, countries surrounding the Caribbean basin, parts of Central Africa, and South America. In addition, epidemiologic studies of HTLV-I have revealed high seroprevalence rates in Melanesia, Papua New Guinea, the Solomon Islands, and among Australian aborigines. In Japan, ~1.2 million individuals are estimated to be infected by HTLV-I, and more than 800 cases of adult T-cell leukemia are diagnosed each year. The cumulative risk of adult T-cell leukemia among HTLV-I carriers in Japan is estimated to be about 6.6% for men and 2.1% for women, indicating that most of HTLV-I carriers are asymptomatic throughout their life (5). Analysis of naive individuals who seroconvert after marrying an HTLV-I seropositive spouse showed that the proviral gp46 sequences are identical among married couples. This finding verified that HTLV-I is transmitted from a seropositive individual to the uninfected spouse. Interestingly, proviral loads between couples are frequently different despite infection by the same HTLV-I virus, suggesting a significant contributory role of host factors to viral replication (6).

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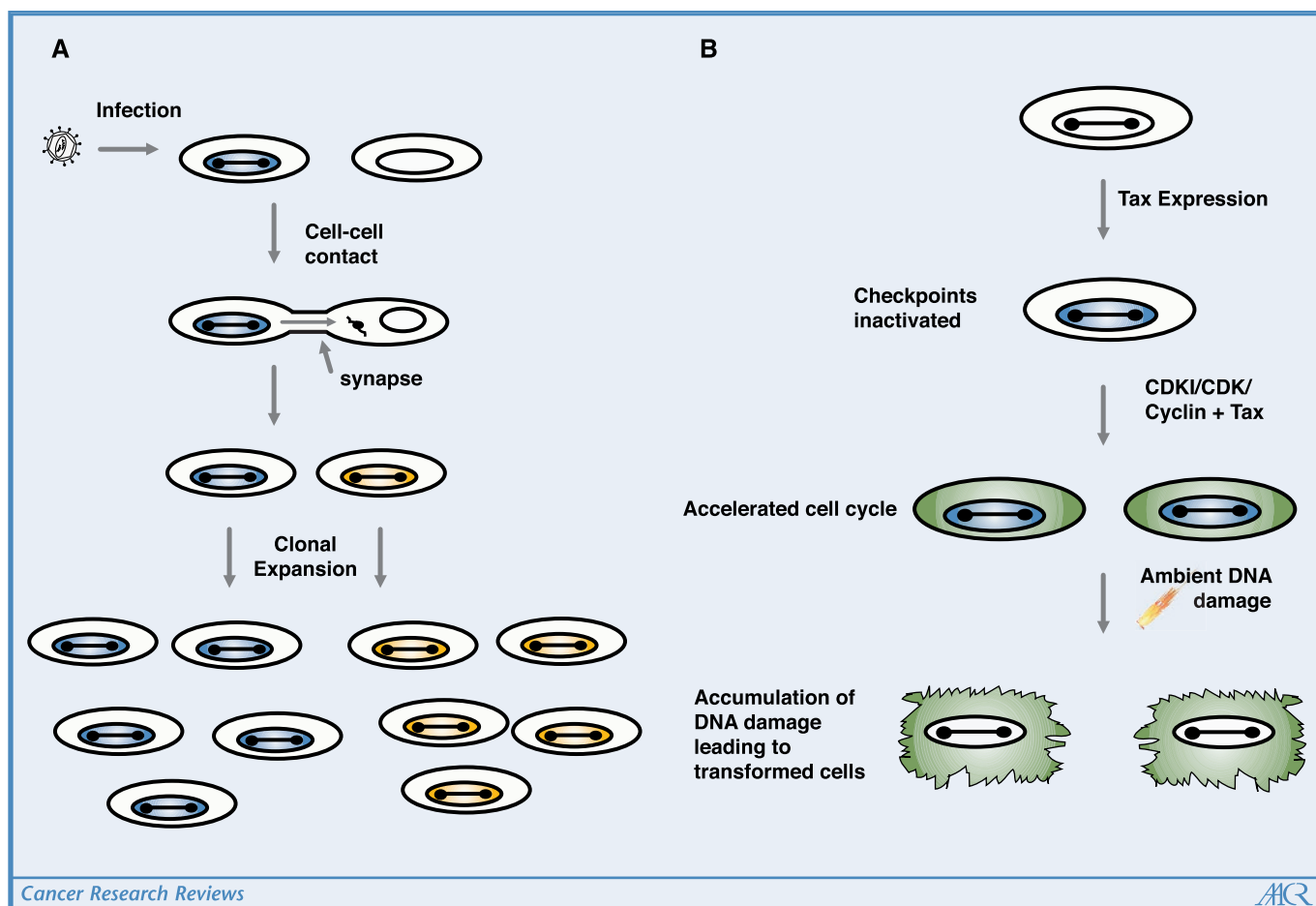
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## The Biology of Human T-Cell Leukemia Virus Type I

HTLV-I is a *Deltaretrovirus* of 9 kb in size with a unique transmission strategy. *In vivo* cell-free virions are not detected in the sera of HTLV-I-infected individuals. *In vitro* infectivity by virions in tissue culture is much lower than that achieved by cell-to-cell transmission. These findings suggest that optimal HTLV-I spread is cell to cell. Accordingly, when infected cells contact uninfected counterparts, "virological synapses" are formed and Gag-viral genomic RNA complexes are transferred from the infected into the uninfected cells (Fig. 1A; ref. 7). Moreover, because the virus can infect many types of cells (e.g., B-lymphocytes, monocytes, and fibroblasts), expression of the HTLV-I receptor is predicted to be ubiquitous. Indeed, Manel et al. (8) and Kim et al. (9) recently identified the commonly expressed glucose transporter 1 as the HTLV-I receptor, thereby explaining how the virus can infect a myriad of different cell types.

The mode of transmission of HTLV-I suggests that the virus benefits from a net increase in the reproductive rate of infected cells (Fig. 1A). Like other retroviruses, HTLV-I integrates as a provirus into the cellular chromosome. After integration, viral transcription proceeds in two ways. Basal transcription is first dictated by the amount of cellular cyclic-AMP responsive [e.g., cyclic AMP-responsive element binding protein (CREB), CREB-binding protein, p300, and p300/CREB-binding protein-associated factor] factors (10–12). After synthesis of viral proteins, activated transcription is then guided by the HTLV-I Tax protein (13–15). Tax activates not only the viral long terminal repeat (LTR) but also a large array of cellular genes (16, 17) via four cellular signal transduction pathways [i.e., CREB/activating transcription factor, nuclear factor- $\kappa$ B (NF- $\kappa$ B), activator protein, and serum response factor; refs. 18, 19]. Tax is also the HTLV-I-encoded cell transforming protein which serves to increase proliferation and clonal expansion of virus-infected cells (13, 20–22). In addition to Tax, the viral Rex protein (23), and other viral accessory proteins (24) also contribute to HTLV-I replication.

Progress has been made in our understanding as to how HTLV-I increases net proliferation of infected cells. A current model for cellular transformation (Fig. 1B), which does not exclude others, is that two types of events mediated by Tax occur after HTLV-I infects cells. First, because cancer is fundamentally a disease of genetic mistakes (25), the virus must confer to the infected normal cell a tolerance for chromosomal changes/damages. In HTLV-I cells, this occurs through the abrogation by Tax of several cellular checks (26–28), which normally sense or repair DNA mistakes and reject the propagation of uncorrected genetic errors by sending cells to apoptosis. Nevertheless, tolerance for genetic instability through checkpoint inactivation in itself neither creates DNA damage nor transforms cells. Hence, a second event, Tax interaction with cyclins, cyclin-dependent kinases (cdk), and cdk inhibitors (10), enhances genetic errors via unchecked acceleration



**Figure 1.** Cell-to-cell spread by HTLV-I and cellular transformation by Tax. **A**, HTLV-I virus infects the cell and establishes a provirus. The most efficient route of HTLV-I spread is then via cell-to-cell contact through a synapse. Once spread of infection is established, clonal expansion of infected cells facilitates future cell-to-cell spread. **B**, a model for stepwise transformation of cells by Tax. Initially, Tax (blue) induces tolerance by the cell of aneuploidogenic and clastogenic DNA damage through inactivation of several cellular checkpoints such as p53/retinoblastoma, spindle assembly checkpoint, and G<sub>2</sub>-M checkpoint. Then, via interactions with cyclins, cdk, and cdk inhibitors, Tax accelerates cell cycle progression (green) so that cells are deprived of sufficient time to adequately correct ambient DNA damage, and genetic lesions become fixed. Accumulation of damaged DNA ultimately converts the cell to a transformed phenotype (ruffled cells). Once transformation is achieved, Tax is no longer required (clear nuclei), and its expression may be shut off through either genetic or epigenetic means (see text).

through all phases of the cell cycle (29–31). In settings whereby the actions of checkpoints are inactivated, hastening cell cycle progression increases the ambient amount of cellular genetic mistakes (10, 32).

Tax expression is frequently impaired in adult T-cell leukemia cells through genetic and epigenetic mechanisms (33) such as through mutation, insertions, and deletion of *tax* gene (34); deletion of 5′-LTR (35); or DNA methylation of 5′-LTR (36). *Tax* gene transcription was undetectable in about two thirds of adult T-cell leukemia cases by reverse transcription-PCR. These findings indicate that whereas Tax may be needed to initiate transformation, it is not always required for the maintenance of a leukemic state. Hence, whereas Tax promotes the proliferation of infected cells and inhibits their apoptosis in an early phase of transformation, once a leukemic state is achieved cells acquire the ability to proliferate without Tax expression. Loss of Tax expression seen in some adult T-cell leukemia cells suggests that genetic and epigenetic alterations are implicated when adult T-cell leukemia cells transit to a Tax-independent phase of growth. Analyses of cellular DNA changes have revealed hyper- and hypomethylated genes in adult T-cell leukemia cells (37, 38). For example, early

growth response 3 (*EGR3*) gene, which is a critical transcriptional factor for induction of Fas ligand, is hypermethylated in adult T-cell leukemia cells. Accordingly, although adult T-cell leukemia cells highly express Fas antigen on their surfaces, they do not produce Fas ligand. Thus, through suppression of *EGR3*, adult T-cell leukemia cells escape Fas ligand activation-induced cell death. These findings suggest that HTLV-I-infected cells can use both genetic and epigenetic means to acquire malignant phenotypes during their long latency periods.

### Pathogenesis of Human T-Cell Leukemia Virus Type I

Because adult T-cell leukemia cells are derived from activated helper T-lymphocytes, which play a central role in the immune system, their phenotype of cytokine production can influence the diverse symptoms and complications observed in patients. Approximately 70% of the time, hypercalcemia complicates adult T-cell leukemia. In adult T-cell leukemia, parathyroid hormone-related peptide has reportedly been implicated in hypercalcemia; however, the level of parathyroid hormone-related peptide does not always strictly correlate with the extent of hypercalcemia. On

the other hand, adult T-cell leukemia patients often express receptor activator of NF- $\kappa$ B ligand, which cooperates with macrophage colony-stimulating factor to induce the differentiation of hematopoietic precursors into osteoclasts (39); and this may account for the observed hypercalcemia. Adult T-cell leukemia patients also frequently have opportunistic fungal, protozoal, and/or other viral infections; and these concurrent pathogens may influence disease outcome. Inevitably, some impairment in T-cell function and immunodeficiency occur in adult T-cell leukemia. One contribution to this impairment may be due to decreased numbers of naive T-lymphocytes in HTLV-I-infected individuals (40). Previously, the *FOXP3* gene was identified as a master gene that controls the phenotype of immunoregulatory T-cells. *FOXP3* induces the expression of immunoregulatory surface molecules, which suppress the proliferation of T-lymphocytes. Intriguingly, *FOXP3* gene transcription is reportedly up-regulated in some adult T-cell leukemia cases [10 of 17 (59%); ref. 41], which might mechanistically explain the occurrence of immunodeficiencies.

A very small proportion of HTLV-I-infected individuals (0.1-2%) develop a separate clinically distinct neurologic disease known as HTLV-I-associated myelopathy (HAM) or tropical spastic paraparesis (TSP; refs. 42, 43). HAM/TSP is a progressive myelopathy with weakness and spasticity of the extremities, urinary and bowel incontinence, and loss of peripheral nerve function. Patients with HAM/TSP, unlike those with adult T-cell leukemia, have high anti-HTLV-I CTL responses (44). Moreover, unlike in adult T-cell leukemia, the proliferation of HTLV-I-infected cells is polyclonal in HAM/TSP patients; this finding is consistent with a disease that does not develop from monoclonal expansion of malignant cell(s). The pathologic *raison d'être* for HAM/TSP seems not to be a direct effect of the virus as much as an indirect consequence of an overly vibrant immune response by the host to the virus (43).

## Treatment

Current chemotherapeutic regimens, regardless of treatment intensity, fail to improve the survival of adult T-cell leukemia patients (45). By contrast, remarkable progress in adult T-cell leukemia treatment has been observed using allogeneic hematopoietic stem cell transplantation (46). In one study, 10 patients with adult T-cell leukemia were treated with allogeneic hematopoietic stem cell transplantation, and median leukemia-free survival after allogeneic hematopoietic stem cell transplantation was 17.5 months. In a second study, 16 patients with adult T-cell leukemia were treated with reduced conditioning intensity allogeneic stem cell transplantation from human leukocyte antigen-matched sibling donors. Provirus load became undetectable in eight patients (47). Anti-Tax CTL, as measured by the tetramer assay, was markedly increased after stem cell transplantation, indicating that enhanced CTL function can effectively combat HTLV-I-infected cells. As a further measure of efficacy, Tax-specific CTLs were induced only in patients who maintained remission from disease (48).

On the horizon, new applications against adult T-cell leukemia are being investigated. Proteasome inhibitors can suppress NF- $\kappa$ B activity, which plays a central role in adult T-cell leukemia. Bortezomib (a proteasome inhibitor formerly known as PS-341) has been shown to exhibit anti-adult T-cell leukemia effect *in vitro* and *in vivo* (49-51). In addition, monoclonal antibodies against adult T-cell leukemia cells, such as anti-CD25 (52), anti-CD52, anti-CD2, and anti-CCR4 (53), are being developed to treat adult T-cell leukemia.

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