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Pathogenesis and Treatment of Human T-Cell Leukemia Virus Infection

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Abstract

The pathogenesis of human T-cell leukemia virus (HTLV)–induced adult T-cell leukemia-lymphoma (ATLL) was explored using an infectious molecular viral clone and a transgenic mouse model. Activation of nuclear factor- κ B by the HTLV transcriptional trans-activator protein Tax was found to be important for lymphocyte immortalization and tumorigenesis. Interferon- γ regulates tumor development owing primarily to angiostatic effects. Translational clinical studies of chemotherapy, interferon- α , and nucleoside reverse transcriptase inhibitors have also assisted in identifying the pathogenic features of ATLL.

Keywords

Human T-cell leukemia virus; Tax; Interferon- γ ; Adult T-cell leukemia-lymphoma; Immortalization; Lymphocytes

Introduction

Human T-cell leukemia virus type 1 (HTLV-1) is a member of the δ retrovirus family, and the etiologic agent of a form of lymphoma designated adult T-cell leukemia-lymphoma (ATLL) (1). It is also the etiologic agent of a neurologic disease designated tropical spastic paraparesis or HTLV-associated myelopathy (HAM). ATLL is usually a CD4⁺ lymphocytic lymphoma, with frequent involvement of the blood, central nervous system, bone, and other visceral sites, with associated hypercalcemia (2). The neoplasm is poorly responsive to aggressive forms of chemotherapy, and 4-yr survival is <5%. HAM is a slowly progressive neurodegenerative disorder characterized by lower limb spastic paraparesis, which responds transiently to glucocorticoids or other immuno-suppressants (3).

Ten to 20 million individuals are infected with HTLV-1 worldwide, with endemic sites of infection in southern Japan, the Caribbean, Central and South America, and parts of Africa and the Middle East (4). The virus may be acquired by breast feeding, sexual activity, or exposure to infected blood samples. A critical viral gene associated with the development of both ATLL and HAM encodes the transcriptional transactivator Tax (5). Immortalization of CD4⁺ lymphocytes, resistance to apoptosis, and induction of angiogenesis are activities of Tax that contribute to the development of ATLL. Induction of aberrant immune responses by Tax, mediated by cytotoxic T-lymphocytes and cytokine release, contributes to the development of HAM (3). Further understanding of the variable clinical responses to HTLV-1 infection

depends on analysis of appropriate tissue culture and animal model systems, and on translational studies associated with clinical trials.

There are several pieces of evidence that Tax is the critical oncoprotein of HTLV-1 (5). Although deletions occur in various locations in the provirus of ATLL patients, the tax open reading frame (ORF) remains intact. Expression of Tax is sufficient for transformation of NIH 3T3 cells and Rat-1 or Rat-2 cells. A herpesvirus expressing Tax results in immortalized CD4⁺ lymphocytes.

Tax is a 40-kDa nuclear protein that has transcriptional and posttranscriptional effects (Fig. 1) (5). Tax activates transcription of the viral promoter through the cyclic adenosine monophosphate–response enhancer binding protein (CREB)/activated in transcription factor (ATF) pathway. Tax also activates expression of the nuclear factor- κ B (NF- κ B) pathway resulting in overexpression of cellular genes, including interleukin-2 (IL-2), IL-3, IL-4, IL-6, IL-10, and IL-15; IL-2 receptor α -chain; interferon- γ (IFN- γ); and granulocyte macrophage colony-stimulating factor (GM-CSF). In addition, Tax activates p67 serum response factor to activate the basal level of viral expression and enhance expression of the cellular genes *c-fos* and *c-jun*. Furthermore, Tax activates zinc-finger transcription factors Sp1 and early growth response factor-1 (Egr-1) to activate parathyroid-related protein (PTHrP) expression and platelet-derived growth factor gene. Tax also transrepresses basic helix-loop-helix (bHLH) transcription factors to downregulate DNA polymerase β , Bcl-2 apoptosis family member Bax, tumor suppressor protein p53, Src tyrosine kinase family member Lck, and alternate reading frame protein.

Posttranscriptional effects of Tax may occur through physical interactions with other cellular proteins (1). For example, Tax binds p16INK4a, to block its repressive effects on Rb; cyclin-dependent kinase 4 (Cdk4) and Cdk6, to upregulate their activities; mitotic arrest defect protein MAD-1, to inhibit cell-cycle checkpoint regulation; and Smad proteins that block the tumor suppressive effects of transforming growth factor- β .

Infectious HTLV-1 Molecular Clone

A full-length infectious molecular clone of HTLV-1, designated ACH, was constructed for a molecular dissection of biologic activities of the virus (6). Transfection of pACH into human embryonic kidney 293T cells or primary human lymphocytes gave rise to infectious, replication-competent virus particles, capable of immortalizing T-lymphocytes, and productively infecting rabbits (7).

Mutations were made in the Tax protein that selectively affected its ability to activate transcription through the NF- κ B or CREB/ATF pathways (8,9). Tax activation of NF- κ B occurs through binding and activation of inhibitor κ B kinase (IKK) kinases, such as mitogen-activated kinase kinase-1; the regulatory γ -subunit of IKK; and the precursor of NF- κ B isoform p50, p105 (10). Tax mutants that selectively repressed its ability to activate NF- κ B did not affect virus replication but lacked immortalization activity.

Tax activation of CREB occurs through physical interactions with CREB, or CREB-binding proteins (CBPs or p300) (10). ACH viral strains expressing Tax mutants that selectively affected CREB activation (M47 and V89A) had markedly reduced virus production compared to ACH but were still capable of T-cell immortalization, suggesting that virus replication and immortalization are separate activities (8,9). The parental and Tax mutant ACH viruses were capable of immortalizing purified CD4⁺ or CD8⁺ lymphocytes. However, in a mixed cell population, parental ACH virus preferentially immortalized CD4⁺ lymphocytes, whereas M47 mutant ACH virus preferentially immortalized CD8⁺ lymphocytes (8). The preference of HTLV-1 for immortalization of CD4⁺ lymphocytes recapitulates in vivo observations that

ATLL is generally derived from mature CD4⁺ lymphoid cells. These findings suggest that HTLV-1 selectivity for CD4⁺ lymphocytes may occur at the transcriptional level. Further studies are required to determine whether the effects are owing to higher levels of virus expression in CD4⁺ lymphocytes or direct effects on CREB-responsive cellular genes on CD4⁺ lymphocyte proliferation or resistance to apoptosis.

The envelope protein is expressed on the surface of virions and infected cells and mediates binding to the viral receptor. However, additional roles of other retroviral envelope proteins have been identified. For example, murine spleen focus-forming virus envelope can interact with the erythropoietin receptor and induce erythropoietin-independent growth of erythroid cells (11). Avian erythroblastosis virus S13 encodes an envelope protein fused to v-Sea capable of transforming fibroblasts and erythroblasts (12). Sheep Jaagsiekte respiratory retrovirus envelope activates the cellular Akt pathway resulting from phosphatidylinositol turnover resulting in bronchioalveolar carcinoma (13). Mutations were made in the envelope protein encoded by pACH to assess the effects on virus replication and immortalization (14). Mutations in several conserved residues within the surface envelope protein had no effects on either biologic activity, whereas a mutant at residue 101 resulted in a virus with reduced virus entry, replication, and immortalization. In addition, mutants of pACH lacking the C-terminal cytoplasmic tail of the envelope glycoprotein, or a pACH mutant lacking envelope altogether, were neither replication competent nor capable of immortalization. These findings indicated that HTLV-1 cell-to-cell spread is required for immortalization.

Mutations were also made in the accessory gene products of ACH, designated p12, p13, p30, and HTLV-1 basic leucine zipper protein (HBZ) (Fig. 1). The p12 gene product is a 99 amino acid endoplasmic reticulum protein that interacts with vacuolar H⁺ adenosine triphosphatase, and β - and γ -chains of IL-2 receptor (15). The ACH p12 mutant virus showed no defect in replication in proliferating human or rabbit T-cells, full T-cell immortalization activity, and cell growth requirements for IL-2 similar to those of the parental ACH virus (16,17). However, the ACH p12 mutant virus was markedly impaired in its ability to establish infection in rabbits (18). Moreover, studies with quiescent human or rabbit lymphocytes in culture also demonstrated a profound defect of the ACH p12 mutant for replication in culture (19,20). In following up on these observations, it was found that p12 is capable of enhancing cytoplasmic calcium release and activation of nuclear factor of activated T-cells (21). These activities of HTLV-1 p12 are analogous to those of the human immunodeficiency virus-1 Nef protein (22).

A single ORF of HTLV-1 is responsible for expression of p30 and p13, by use of the first or second initiator methionine codon (16). The p30 protein is a nucleolar protein with distant homology to transcription factors Oct-1 and Oct-2, and POU-M1 (15). The p30 protein was shown to regulate differentially CREB-response element- and Tax-responsive element-mediated transcription through interaction with CBP or p300. By contrast, the p13 protein is a mitochondrial protein. It is associated with mitochondrial clustering and alteration of respiratory activity resulting in a permeability transition. Mutations in p30 or p13 ORFs did not affect virus replication or immortalization in tissue culture (16). However, attenuated replication in rabbits was seen with these mutants, as well as reversions that corrected the defects (23,24).

A protein designated HBZ is encoded from the negative proviral strand (25). It forms heterodimers with CREB-2 to inhibit viral *trans*-activation. Moreover, it binds c-Jun and JunB, to block activator protein-1-induced gene expression. Our ongoing studies of ACH mutants encoding mutations in HBZ should be informative in defining the role of HBZ in virus-infected cells in culture and in vivo.

These examples provide lessons in how the infectious molecular clone can provide new insights into HTLV-1 biology.

Tax Transgenic Mice

To address determinants for lymphoma development in vivo, we have developed a novel transgenic mouse model using the HTLV-1 tax gene. Previous transgenic models have used various promoters, including the HTLV-1, mouse mammary tumor virus, or simian virus 40 promoters, or the promoter from the Thy1.2 or CD4 genes, which resulted in various nonmalignant and malignant disorders, but no hematopoietic malignancies (26). To restrict Tax expression to the lymphoid compartment, we expressed Tax under the regulation of the granzyme B transcriptional regulatory region (27). Three independent founder mice and all of their progeny developed a similar hematopoietic neoplasm, composed of either natural killer cells or CD8⁺ lymphocytes. Tumors arose on extremities, including the tail, ears, limbs, or nose, at a median time of 8 mo in males and 12 mo in females, and disseminated to the liver, lung, and mesenteric lymph nodes. Bone marrow and blood involvement was evident in the majority of animals, and more than 80% of the mice developed bone metastases.

Tumor cells and cell lines expressed high levels of NF- κ B (28). Tumor cells proliferated spontaneously in culture, and these cells were resistant to γ irradiation-induced apoptosis. Inhibitors of NF- κ B activation, including sodium salicylate and cyclopentenone prostaglandins, blocked tumor cell proliferation in culture and sensitized the cells to γ irradiation. A proteasome inhibitor, PS-341 or bortezomib (Velcade), which blocks degradation of the inhibitor of NF- κ B, also inhibited tumor cell proliferation in culture and sensitized cells to γ irradiation-induced apoptosis (29). Moreover, PS-341 inhibited tumor cell proliferation in vivo in transgenic mice and mice transplanted with Tax transgenic tumor cells. These findings further substantiate the critical role of NF- κ B for tumorigenesis.

Several NF- κ B-inducible cellular genes were expressed in Tax transgenic tumors and cell lines, including IL-6, IL-10, and IL-15, as well as IFN- γ (30). Breeding Tax transgenic animals with IFN- γ homozygous knockout (KO) animals resulted in transgenic homozygous KO animals with enhanced tumorigenesis (31). In these animals, tumors arose more rapidly, and visceral involvement and death occurred earlier than in Tax transgenic animals that were heterozygous or homozygous for IFN- γ gene. Furthermore, a more rapid appearance and growth of Tax transgenic tumor cells transplanted into animals was evident in mice with homozygous deletion of the IFN- γ gene than in normal mice of the same strain. These effects did not appear to be owing to differences in cellular immune responses to Tax transgenic tumors. However, tumors in mice with homozygous deletion of the IFN- γ gene showed enhanced angiogenesis compared with tumors in normal mice. These findings are consistent with the known antiangiogenesis activity of IFN- γ . Gene array studies demonstrated enhanced expression of vascular endothelial growth factor and tenascin C and reduced expression of tissue inhibitor of metalloproteinase-1 and tumor necrosis factor in mice with homozygous deletion of the IFN- γ gene compared with normal mice.

Other Model Systems

Studies of the infectious molecular clone and Tax transgenic mice provide two very useful model systems for studies of HTLV-1 biology, but these approaches are imperfect model systems for ATLL. Although HTLV-1 replicates in rabbits, no pathology has been reproducibly seen (7). Moreover, HTLV-1 infects and replicates to a very restricted extent in immunocompetent or immunodeficient mice (32). Cell lines from highly selected ATLL cell lines produce T-cell malignancies in immunodeficient mice, but ACH immortalized cells, and most HTLV-1-infected cell lines lack this activity. Thus, efforts to overcome these limitations in several different ways are ongoing. For this purpose, we are passaging HTLV-1 through

rabbits to select for a variant with high replication potential and pathogenic properties. Alternatively, we are attempting to adapt HTLV-1 for replication in mice by selection of variants that replicate in murine cells or by substitution of the HTLV-1 env gene with that from murine leukemia virus (33). Alternatively, studies are under way to examine replication of ACH virus and infected cells transplanted into nonobese diabetic/severe combined immunodeficiency mice with homozygous deletion of the $\beta 2$ microglobulin gene (34). Finally, efforts are under way to clone the HTLV-1 receptor, to enhance virus entry into murine cells (35).

Chemoantiretroviral Therapy for ATLL

Translational research studies have also been undertaken in HTLV-1-infected patients with ATLL (2). In our current study, ATLL patients receive infusional combination chemotherapy as initial treatment for the malignancy, as cytotoxic therapy for infected, malignant cells, in an attempt to improve on previously reported results obtained with bolus combination chemotherapy regimens. This is followed by antiretroviral therapy with IFN- α , and a combination of nucleoside reverse transcriptase inhibitors, zidovudine (azidothymidine), and lamivudine (3-thiocyridine), in order to prevent rebound virus replication and spread.

Studies of the action of IFN- α on HTLV-1 replication demonstrated a selective effect on virus assembly, through inhibition of Gag protein targeting to lipid rafts (36). Ongoing studies will determine whether the primary target in rafts is a membrane lipid or protein, and whether HTLV-1 modulates IFN- α production and sensitivity. Furthermore, studies of IFN- α and nucleoside analogs include studies of selection of resistant viral variants in culture or patients, and examination of the mechanism of altered antiviral sensitivity of these variants.

Conclusion

HTLV-1-induced lymphoid malignancies provide an excellent example of a virus-induced malignancy in which individual steps in oncogenesis can be identified. An infectious molecular clone of HTLV-1 has identified Tax activation of NF- κ B as a critical step in T-cell immortalization. Tax transgenic mice also exhibit NF- κ B activation in tumor cells, and inhibition of this pathway results in anti-tumor activity in culture and in vivo. Ongoing clinical trials provide unique translational research opportunities to assess further the roles of virus replication and T-cell immortalization resulting in ATLL. HTLV-1-induced ATLL provides a paradigm to study oncogenic pathways central to a wide range of malignant processes.

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Fig. 1.

The HTLV genome is shown with the functions of the viral gene products indicated. The Tax protein is the principal oncogenic protein of the virus, which functions as a transcriptional *trans* activator of the indicated target genes, via interactions with the cellular transcription factors CREB/ATF, SRF, NF-κB, SP1, EGR-1 and bHLH proteins.