

Human T Lymphotropic Virus Type-I and Adult T-Cell Leukemia in Japan

Kazunari Yamaguchi, Toshiki Watanabe

*Kumamoto University School of Medicine, Kumamoto and Division of Pathology,
Department of Cancer Research, Institute of Medical Science, University of Tokyo,
Tokyo, Japan*

Abstract

HTLV-I is the first retrovirus to be associated directly with human malignancy. In ATL-endemic areas, the rate of HTLV-I carriers is high. Both HTLV-I and ATL have been shown to be endemic in some regions of the world, especially in southwest Japan, the Caribbean islands, South Americas, and parts of Central Africa. Antibodies against HTLV-I have been found in over one million individuals, and more than 700 cases of ATL have been diagnosed each year in Japan alone. The cumulative incidence of ATL among HTLV-I carriers in Japan is estimated at 2.5% (3-5% in males, 1-2% in females). In endemic areas, HTLV-I Ab were found in the sera of 6 to 37 percent of healthy adults over 40 years of age. This clustering is thought to be due to the limited transmission of virus between socially isolated populations. The diagnostic criteria for HTLV-I associated ATL have been defined as follows. 1) Histologically and/or cytologically proven lymphoid malignancy with T cell antigens. 2) Abnormal T-lymphocytes present in the peripheral blood, except in the lymphoma type. 3) Serum specimens for all patients with ATL have HTLV-I Ab. 4) Demonstration of clonality of HTLV-I proviral DNA is a definite diagnosis of ATL. ATL shows diverse clinical features but can be divided into four subtypes: acute, chronic, smoldering, and lymphoma type. The pattern of HTLV-I transmission is through one of three different modes. Infected mothers can transmit the virus to newborns mainly via breast milk. The virus also can be transmitted from male to female by sexual intercourse, and through blood transfusion. Chemotherapy is not effective; the acute and lymphoma types have a poor prognosis. ATL is generally treated with curative intent using combination chemotherapy, although long-term success has been very limited. Unfortunately that advance did not translate into an improvement in the overall survival; the median remain 10 months. In contrast, smoldering ATL, or some cases of chronic ATL, may have a more protracted natural course, which may be compromised by aggressive chemotherapy. Alternative strategies for both acute and chronic forms are clearly needed. After infection of HTLV-I, there is a long latent period before onset of ATL. Analyses by PCR showed that clearly proliferation occurred in intermediate state or even carriers with high virus load. Such clonal proliferation might be preleukemic stage, which suggested that carriers with high virus load should be risk group to have ATL.

1. Abstract

HTLV-I infection is causally associated with a variety of human diseases including leukemia/lymphoma, myelopathy, and uveitis. Tax protein of HTLV-I, which is considered oncogenic, binds to transcription factors or other cytoplasmic cellular molecules involved in the fundamental cell function and thereby induces cellular changes. The interaction between HTLV-I infected cells with dys-

regulated function and different kinds of cells in the host, such as lymphocytes and vascular endothelial cells through viral peptides, antigen receptors, cell adhesion molecules, and cytokines, appears to be one of the basic mechanisms underlying the development of HTLV-I-associated diseases. This interaction may play a major role in determining tumorigenicity and in forming clinical features of the disease.

2. Introduction

Discovery of HTLV-I subsequently extended the spectrum of diseases associated with HTLV-I infection, such as HTLV-I associated myelopathy (HAM)/tropical spastic paraparesis (TSP), HTLV-1 uveitis (HU), and others. The interaction between HTLV-I and cellular molecules produces the key initial change that may induce subsequent changes in the virus-infected individual and eventually lead to the development of HTLV-I associated diseases.

3. Human T-Lymphotropic Virus Type-I (HTLV-I)

3.1. Genome Structure and Protein Products of HTLV-I

HTLV-I is the first retrovirus to be associated directly with human malignancy. The HTLV-I provirus genome is 9032 bp long and contains gag, pol, and env genes that encode the viral matrix, capsid and nucleocapsid proteins, enzymes such as reverse transcriptase, integrase, and protease, and envelope protein consisting of a surface glycoprotein and a transmembrane protein. In addition to the viral structural genes, the HTLV-I genome contains unique genes principally encoded by sequences at the 3' end of the genome that are called pX. The pX region is not common to other replication-competent retroviruses. The proteins encoded by these genes are not viral structural proteins but regulatory proteins called Tax, Rex and p21^{rexIII}. Other proteins such as p12I, p13II, and p30II reportedly were also encoded from the X region located between the end of the envelope gene and the beginning of tax/rex. Three different species of mRNA are detectable: full-sized genomic RNA for the Gag and Gag/Pol fusion proteins, single-spliced mRNA for envelope protein, and double-spliced mRNA for three regulatory proteins, Tax, Rex, and p21^{rexIII}. These three different mRNA species in the cytoplasm control the expression of viral proteins.

Tax is a 40-kDa nuclear phosphoprotein that transactivates the transcription of HTLV-I and a variety of cellular genes such as those for IL-1, IL-2R α chain, c-fos, and parathyroid hormone-related peptide (PTHrP). No homology exists between tax and any known oncogene. The tax gene product is responsible for enhancement of transcription of viral and cellular genes. The tax protein has been thought to play a critical role in leukemogenesis.

3.2. Interaction between Tax Protein and Various Cellular Molecules

The direct repeats of the 21-bp enhancer in the LTR are needed for Tax to exert its transactivation activity. However, Tax protein does not directly bind to the enhancer elements; it rather shows its effect through interacting with transcription factors that can bind the

sites contained in the three repeats of the enhancer elements in the LTR. Tax can bind to the members of CREB (c-AMP responsive element binding proteins)/ATF (activating transcription factor) family, which form homodimers or heterodimers and bind to CRE (C-AMP responsive element) or to the 21-bp enhancer. Tax interacts with the basic region-leucine zipper (bZip) DNA-binding domain of cellular transcription factors that bind to the CRE target sites to promote peptide dimerization.

Furthermore, Tax binds to cytoplasmic inhibitor of NF- κ B proteins or NF- κ B precursor proteins and promotes nuclear translocation of NF- κ B and precursor proteins, which another mechanism of transactivation by Tax. In a similar way, Tax reportedly prevents formation of p16INK4A/cyclin-dependent kinase 4 (CDK4) complex by interacting with cyclin-dependent kinase inhibitor p16INK4A, possibly through the ankyrin motifs, resulting in CDK4 activation.

Dysregulated expression of variety of cellular genes in HTLV-I infected cells may play a key role in the development of HTLV-I associated diseases and may be responsible for some characteristic clinical features of the diseases. The enhanced expression of PTHrP appears to be a principal mechanism of hypercalcemia, which is often observed in ATL cases. IL-1 and GM-CSF may contribute to the neutrophilia in ATL. Increased production of cytokines such as TNF- α , IL-1, IL-2, and IL-6 in virus-infected cells in the central nervous system (CNS) may be one of the mechanisms responsible for the development of HAM/TSP.

4. Adult T-Cell Leukemia (ATL)

ATL develops in a small proportion of HTLV-I infected people (1 in 1,000-2,000 seropositive individuals per year) after a long latent period. ATL was first reported in Japan, where it has a high incidence in the southwestern region. HTLV-I was first isolated by Gallo's group from cultured cells from one patient with an aggressive variant of mycosis fungoides and one with Sezary syndrome. Although both patients were thought to have cutaneous T-cell lymphoma, they had several unusual features that suggest the disorder now called ATL. A definite diagnosis of ATL is based on the presence of HTLV-I proviral DNA in the tumor cell DNA. ATL cells originate from the CD4 subset of peripheral T cells. ATL shows diverse clinical features but can be divided into four subtypes: acute, chronic, smoldering, and lymphoma type. HTLV-I infection is caused by transmission of live infected lymphocytes from mother to child, from man to woman, or by blood transfusion.

4.1. Diagnosis: The Diagnostic Criteria for HTLV-I Associated ATL have been Defined as Follows

- 1) Histologically and/or cytologically proven lymphoid malignancy with T cell surface antigens.
- 2) Abnormal T-lymphocytes consistently present in the peripheral blood, except in the lymphoma type. These abnormal T lymphocytes include not only typical ATL

cells, the so-called flower cells, but also the small mature T-lymphocytes with incised or lobulated nuclei that are characteristic of the chronic or smoldering type.

3) Serum specimens for all patients with ATL have anti-HTLV-I antibodies. There is no difference in the pattern of serum antibodies between ATL patients and HTLV-I can be demonstrated by indirect immunofluorescence, enzyme-linked immunosorbance, passive hemagglutination, and by Western blotting methods.

4) Demonstration of clonality of HTLV-I proviral DNA is essential to diagnosis of ATL. Southern blot analysis was used for this purpose. We developed the novel method using inverse polymerase chain reaction to detect the clonality of HTLV-I proviral DNA. Diagnosis could be made within 3 days using this method. It enabled us to detect specifically the presence of minimal numbers of ATL cells with high sensitivity.

4.2. Classification of ATL

Clinical manifestations of acute type ATL are acute presentation of symptoms signs such as general malaise, fever, cough, dyspnea, abdominal fullness, thirst, drowsiness, lymph node enlargement, hepatosplenomegaly and jaundice, and abnormal laboratory findings including a marked leukocytosis, hypercalcemia, high serum level of LDH, and a soluble form of interleukin receptor (IL-2R) α chain, and the appearance of characteristic leukemic cells with deeply convoluted or lobulated nuclei. Chronic type ATL is characterized by mild symptoms and signs and a longer clinical course. Patients with smoldering type ATL have a few leukemic cells in their peripheral blood and frequently present skin lesions such as papules, nodules, and erythema; lymph node enlargement and splenomegaly are minimal, and serum LDH level is either slightly elevated or normal. The predominant feature of lymphoma type ATL is lymph node enlargement but not leukemic manifestation. Major complications of ATL are hypercalcemia and serious infections by bacteria, fungi, protozoa, and viruses. Common infections are *Pneumocystis carini* pneumonia, aspergillosis or candidiasis, and cytomegalovirus pneumonia. Combination chemotherapy using various anti-cancer drugs often produces partial or complete remission of the disease; the remission, however, is transient. The prognosis of ATL is usually very poor. Median survival time is 8 months for the acute type, 12 months for the lymphoma type, and 24 months for the chronic type. The four-year survival rates are 5.0% for the acute type, 5.7% for the lymphoma type, 26.9% for the chronic type, and 62.8% for the smoldering type. The relative percentage of each type of ATL case is roughly: 55% acute type; 20% lymphoma type; 20% chronic type; and 5% smoldering type.

4.3. Treatment

ATL is generally treated with curative intent using combination chemotherapy, although long-term success has been very limited. The acute form, with hyper-

calcemia, high LDH levels and an elevated white blood cell count carries a particular poor prognosis. Sequential trials in Japan have resulted in the complete remission rate being increased from 16% with a four drug combination to 43% with eight drugs. Unfortunately that advance did not translate into an improvement in the overall survival; the median remain 8 months, with deaths usually the result of severe respiratory infection or hypercalcemia, often associated with drug resistance. In contrast, smoldering ATL, or some cases of chronic ATL, may have a more protracted natural course, which may be compromised by aggressive chemotherapy. Alternative strategies for both acute and chronic forms are clearly needed.

4.4. Natural Course of HTLV-I Infection

After infection of HTLV-I, there is a long latent period before onset of ATL. Although the exact natural course to onset of ATL is still unknown, there are many clues to clarify the natural course. Intermediate state of HTLV-I infection was defined as condition with increased number of HTLV-I infected cells, in which integration of HTLV-I provirus is random. This condition is associated with immunodeficient state. Analyses by inverse PCR showed that clonal proliferation occurred in this condition or even HTLV-I carriers with high virus load. Such clonal proliferation might be pre-leukemic stage, which suggested that HTLV-I carriers with high virus load should be risk group to have ATL.

5. Epidemiology

Both HTLV-I and ATL have been shown to be endemic in some regions of the world, especially in southwest Japan, the Caribbean islands, the countries surrounding the Caribbean basin, and parts of Central Africa. An unexpected seroprevalence of HTLV-I was found in Melanesia, especially in Papua New Guinea. Seropositivity was also detected in Australian Aborigines and in some areas of the Solomon Islands. Antibodies against HTLV-I have been found in over one million individuals, and more than 700 cases of ATL have been diagnosed each year in Japan alone. The cumulative (life span of 70 years) incidence of ATL among HTLV-I carriers in Japan is estimated at 2.5% (3-5% in males and 1-2% in females) if competing risks for other diseases are disregarded. Seroepidemiologic studies in Japan have indicated that the incidence of HTLV-I infection varies among cities within the endemic region. This clustering is thought to be due to the limited transmission of virus between socially isolated populations. In endemic areas of Japan, anti-HTLV-I antibodies were found in the sera of 6 to 37 percent of healthy adults over 40 years of age. The pattern of HTLV-I transmission is through one of three different modes. HTLV-I infected mothers can transmit the virus to newborns mainly via breast milk. The virus also can be transmitted from male to female by sexual intercourse, and through blood transfusion.

6. Declining Trends in HTLV-I Prevalence Among Blood Donors in Japan

The pattern of HTLV-I transmission is through one of three modes. HTLV-I infected mothers can transmit the virus to newborns mainly via breast milk. The virus also can be transmitted through blood transfusion and from male to female by sexual intercourse. Routine screening of donated blood for HTLV-I by gelatin particle agglutination assay has been conducted in all blood centers in Japan since 1986. Inaba et al reported the effectiveness of screening in preventing transmission of HTLV-I through blood transfusion and the current status of patients with confirmed seroconversion due to transfusions given before the implementation of screening. Their study confirmed that the present donor screening program for HTLV-I can almost completely prevent virus transmission by transfusion and complications of HTLV-I transmission were at lower rates than expected.

Blood donations in the United States have also been screened for antibody to HTLV-I/II since November 1988. Antibodies to HTLV-I and II were measured in 1.7 million donors at five US blood centers during 1991-1995. Among those tested, 156 were HTLV-I seropositive and 384 were HTLV-II seropositive. In contrast to monotonously increasing age-specific HTLV-I seroprevalence, HTLV-II prevalence rose until age 40-49 years and declined thereafter, suggesting a birth cohort effect. From January 1989 to December 1996, 59,426 blood donors in Guadeloupe (French West Indies) were screened for antibodies to HTLV-I. Of the screened blood donors, 195 were confirmed as seropositive, for an overall HTLV-I prevalence of 0.33 percent. A marked decrease in overall HTLV-I prevalence with time (from 0.47% in 1989 to 0.13% in 1996) was observed, which can be explained mainly by the decreasing percentage of recruited new donors during the study period.

In Japan, we had followed gender- and age-specific cross-sectional HTLV-I seroprevalence among blood donors in Kumamoto Prefecture, which includes moderately prevalent areas of HTLV-I. The data showed that 16 to 19 year olds in 1986 and 20 to 29 year olds in 1993 represented nearly the same cohort, because the median age in both groups was 24.5 years in 1993. Therefore, comparison of the HTLV-I positive rate for the two groups gave an estimate of the change in the rate over 7 years within the cohort. In males, 265 of 22,143 donors (1.20%) were seropositive for HTLV-I among 16 to 19 year olds in 1986, and 214 were seropositive among 20,076 (1.07%) donors in 20 to 29 year olds in 1993. In females, the seropositivity rates were 0.98% in 16 to 19 year olds in 1986, and 0.83% in 20 to 29 year olds in 1993. Thus, the seropositive rates declined in both sexes. However, the average annual rate of immigration to Kumamoto was 2.37%. If seropositive rates for 20 to 29 year olds in 1993 are adjusted for the dilution effect due to immigration, the adjusted carrier rate for males is 1.26% and that for females is 0.98%. The adjusted carrier rates for both sexes are

almost the same as those for 16 to 19 year olds in 1986. This indicates that horizontal transmission was negligible for those in the cohort who were in their early reproductive period. The best goodness of fit model indicated that the HTLV-I carrier rate will decline exponentially, and that the rate will decrease by 50% approximately every 10 years for both sexes. It is probable that in recent years southwest Japan has lost the conditions that are favorable for HTLV-I endemicity and the virus will be virtually non endemic within a few generations.

The annual age- and sex-specific HTLV-I carrier rate of blood donors in Kumamoto revealed that the carrier rates of all the age groups below 50 years declined linearly in both sexes. Although several factors, such as a notification program at obstetric clinics, methodological and technical improvement of the assays, wider knowledge of HTLV-I infection, and immigration of individuals from a non endemic area, might cause an absolute decline of the carrier rate of the blood donors, these factors could not explain the acceleration of the relative declining rate among younger donors. Recent drastic ameliorations of sanitary environment and changes in lifestyle in Japan must in part have altered the HTLV-I prevalence, as is seen in the hepatitis B virus infection rate. Above all, the shortening in the period of breast feeding is regarded as a critical factor in the account for the unvarying downward trends in the HTLV-I seropositivity. A decrease in the birth rate, which parallels the declining number of infected babies born to mothers who eluded vertical transmission but would later acquire infection from their carrier husband, may be another factor. A reduction in horizontal transmission from males to females by common use of condoms for contraception may play a supplemental role. Thus, these data are consistent with the hypothesis that there exists a birth cohort effect in the prevalence of HTLV-I.

We quantified HTLV-I provirus load among blood donors using the Amplisensor system, which utilizes fluorescence to measure PCR products. Samples from 256 donors were analyzed, showing that provirus load ranged from less than 0.1% to 56% among carriers. Among 18 donors with high provirus load (more than 10%), Southern blotting detected monoclonal integration of HTLV-I in infected cells in 2 cases, both of them showing high soluble interleukin-2 receptor levels. It will be important to carefully follow these donors with a high HTLV-I provirus load. Blood donors notified of HTLV infection report negative psychological and social effects following notification in the United States.

7. Characteristics of HTLV-I Infected T Cells

Cell surface analysis of leukemic cells from ATL patients using a series of monoclonal antibodies against various cell surface molecules has demonstrated that leukemic cells are CD3+, 4+, 8- in the majority (80-90%) of ATL cases and either CD3+, 4+, 8+, CD3+, 4-, 8+, or CD3+, 4-, 8- in the remaining 10-20% of ATL cases. The latter unusual cell surface

phenotype has been associated with poor prognostic signs such as a marked organomegaly and bulky tumor mass. The expression of so-called T cell-activation antigens including Ki67, transferrin receptor, IL-2R α chain, and HLA-DR is common in leukemic cells. The T cell receptor expressed on leukemic cells is usually a heterodimer of α and β chains. Cell surface expression of CD3 complex composed of six peptide chains is usually downregulated both in fresh leukemia cells and HTLV-I-infected cell line cells, although the amount of expressed mRNA of T cell receptor α and β chain genes and the CD3 subunit genes is not reduced but rather increased.

Many studies reported that various cytokines were produced in leukemic cells from ATL patients, HTLV-I-infected lymphocytes from HAM patients, and HTLV-I-infected cell lines; this suggested a potential role of overexpressed cytokines in the development and/or pathogenesis of the diseases caused by HTLV-I infection. The cytokines include IL-1, IL-2, IL-6, TGF β , TNF α , β , GM-CSF, and gp34. Upregulated cytokine receptors such as IL-2R α chain, Fas/Apo 1, and OX40 have also been reported.

In addition to the membrane form IL-2R α chain, soluble form receptors were detected by a sandwich enzyme-linked immunoassay. Subsequently the serum levels of the soluble IL-2R α chain were markedly elevated in ATL patients and could be used as a rough indicator of the total amount of tumor cells.

8. Cell Growth of HTLV-I Infected T Cells in Vitro

Spontaneous ³H-thymidine uptake by fresh peripheral blood leukemic cells from ATL patients is usually very low, indicating that most are not proliferating. We do not have any direct evidence that clearly demonstrates the site where leukemic cells are proliferating in vivo, although lymph nodes are likely organs. Recently, abnormal findings on the molecules involved in the cell cycle regulation have been presented. One of such is that 10 of 37 ATL samples had loss of p15 and/or p16INK4A genes. p15 and p16INK4A inhibit activity of CDK4 and CDK6 that inactivate retinoblastoma protein by converting it into a hyperphosphorylated form together with cyclin D in G1-to S phase transition.

The oncogenic potential of Tax has also been demonstrated in tax transgenic mice, which revealed different phenotypes including mesenchymal tumors, Sjogren syndrome, brain tumors, lymphoid tumors, and large granular lymphocyte leukemia. These different phenotypes may be due to the different promoters used and different levels of tax expression in various cells. In summary, the tax gene is oncogenic both in vitro and in vivo, although it is still unknown whether Tax expression alone prompts the development of ATL or responsible for the neoplastic cell growth of ATL cells.

9. Tumorigenicity of Htlv-I Infected Cells

Accumulating data show that Tax plays a critically important role in the development of HTLV-I-associated diseases, especially in the leukemogenesis of ATL. One of the puzzling observations, however, is that the expression of HTLV-I including Tax is not always detectable in tumorigenic cell line cells and is usually undetectable or appears at very low levels in leukemic cells of ATL patients. Our conclusion is that, at least in this SCID mouse model, HTLV-I expression is not required for cell growth of tumorigenic cell lines and leukemic cells from ATL patients. Taking these results together with the epidemiological fact that ATL develops after a long latency period at low incidence rate, a multistep oncogenesis model may be applied to the development of ATL. Indeed, the stochastic analysis of the age-specific occurrence of ATL, using 357 cases, suggested the presence of age-dependent accumulation of leukemogenic events, estimated to be five within HTLV-I-infected T cells prior to the development of ATL. In addition, the expansion of a population of lymphocytes with monoclonal HTLV-I provirus integration was detectable in carriers that were asymptomatic or in a pre-leukemic state, and the pX region was generally preserved in most of the cases that showed deleted or defective provirus integration.

10. Immune Response to HTLV-I

HTLV-I-infected individuals produce antibodies against protein products of viral env, gag, and tax gene. Serum titers of anti-HTLV-I antibodies are usually higher in HAM patients than in asymptomatic carriers and ATL patients. Neutralizing antibodies that inhibit cell fusion or infection of the pseudotype virus were demonstrated in HTLV-I-infected individuals, and neutralization epitopes has been localized on viral envelope protein gp46.

HTLV-I-specific cytotoxic T cells (CTL) have also been demonstrated in patients with HAM/TSP, ATL, and HU, as well as in asymptomatic carriers, and these cells appear to play a role in controlling viral replication in vivo. It is, therefore, still an open question whether high immune responses in HAM/TSP patients are responsible for the development of the disease or merely reflect one aspect of the disease.

11. Conclusion

During the past two decades since the discovery of ATL, accumulating evidence has helped us to get deep insights in to the clinical features of the diseases, virological properties of HTLV-I, and the interaction between viral products and cellular molecules. However, the precise mechanism of the development and pathogenesis of HTLV-I-associated diseases still remains unknown. The goal of future studies should be to fully and precisely understand the diseases, based on the increasing knowledge of molecular events that occur dur-

ing their development in HTLV-I-infected people, and to develop an effective treatment.

References

1. Yamaguchi K. Human T-lymphotropic virus type I in Japan. *Lancet*. 1994;343:213-216.
2. Inaba S, Okochi K, Sato H, et al. Efficacy of donor screening for HTLV-I and the natural history of transfusion-transmitted infection. *Transfusion*. 1999;39:1104-1110.
3. Murphy EL, Glynn SA, Frیدی J, et al. Retrovirus Epidemiology Donor Study (REDS) Study Group. Increased prevalence of infectious diseases and other adverse outcomes in human T-lymphotropic virus types I- and II-infected blood donors. *J Infect Dis*. 1997;176:1468-1475.
4. Oguma S, Imamura Y, Kusumoto Y, et al. Stable human T-lymphotropic virus type I carrier rates for 7 years among a teenaged blood donor cohort of 1986 in Kumamoto, Japan. *Leuk Res*. 1995;19:567-571.
5. Etoh K, Yamaguchi K, Tokudome S, et al. Rapid quantification of HTLV-I provirus load: detection of monoclonal proliferation of HTLV-I-infected cells among blood donors. *Int J Cancer*. 1999;81:859-864.