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Human T-cell leukemia virus type I (HTLV-I) infection and the onset of adult T-cell leukemia (ATL)

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Abstract

The clinical entity of adult T-cell leukemia (ATL) was established around 1977, and human T-cell leukemia virus type I (HTLV-I) was subsequently identified in 1980. In the 25 years since the discovery of HTLV-I, HTLV-I infection and its associated diseases have been extensively studied, and many of their aspects have been clarified. However, the detailed mechanism of leukemogenesis remains unsolved yet, and the prognosis of ATL patients still poor because of its resistance to chemotherapy and immunodeficiency. In this review, I highlight the recent progress and remaining enigmas in HTLV-I infection and its associated diseases, especially ATL.

Background

In 1977, Takatsuki et al. reported adult T-cell leukemia (ATL) as a distinct clinical entity [1-3]. This disease is characterized by its aggressive clinical course, infiltrations into skin, liver, gastrointestinal tract and lung, hypercalcemia and the presence of leukemic cells with multilobulated nuclei (flower cell) (Figure 1). In 1980, Poiesz et al. discovered a human retrovirus in a cell line derived from a patient with ATL, and designated it human T-cell leukemia virus type I (HTLV-I) [4,5]. The linkage between ATL and HTLV-I was proven by Hinuma et al., who demonstrated the presence of an antibody against HTLV-I in patient sera [6]. Thereafter, Seiki et al. determined the whole sequence of HTLV-I and revealed the presence of a unique region, designated pX [7]. The pX region encodes several accessory genes, which control viral replication and the proliferation of infected cells [8]. In this review, I describe the recent advances in the field of HTLV-I and ATL research, with particular focus on the mechanism of leukemogenesis and therapeutic aspects.

1. History of humans and HTLV-I

HTLV-I is a member of the Deltaretroviruses, which include HTLV-II, bovine leukemia virus and simian T-cell leukemia virus (STLV). The latter two viruses also cause lymphoid malignancies in the host, similar to the case with HTLV-I. HTLV and STLV are thought to originate from common ancestors, and share molecular, virological and epidemiological features. Therefore, they have been designated primate T-cell leukemia viruses (PTLVs). Phylogenetical analyses have revealed that HTLV-Ic first diverged from simian leukemia virus around $50,000 \pm 10,000$ years ago, while the spread of PTLV-I in Africa is estimated to have occurred at least $27,300 \pm 8,200$ years ago. Subsequently, HTLV-Ia, which is the most common subtype in Japan, diverged from the African strain $12,300 \pm 4,900$ years ago [9]. Thus, these viruses have had a long history with humans after the interspecies transmission. In contrast, human immunodeficiency virus type 1 (HIV-1) is thought to originate from simian immunodeficiency virus in chimpanzees (SIV_{CPZ}) [10], and the interspecies

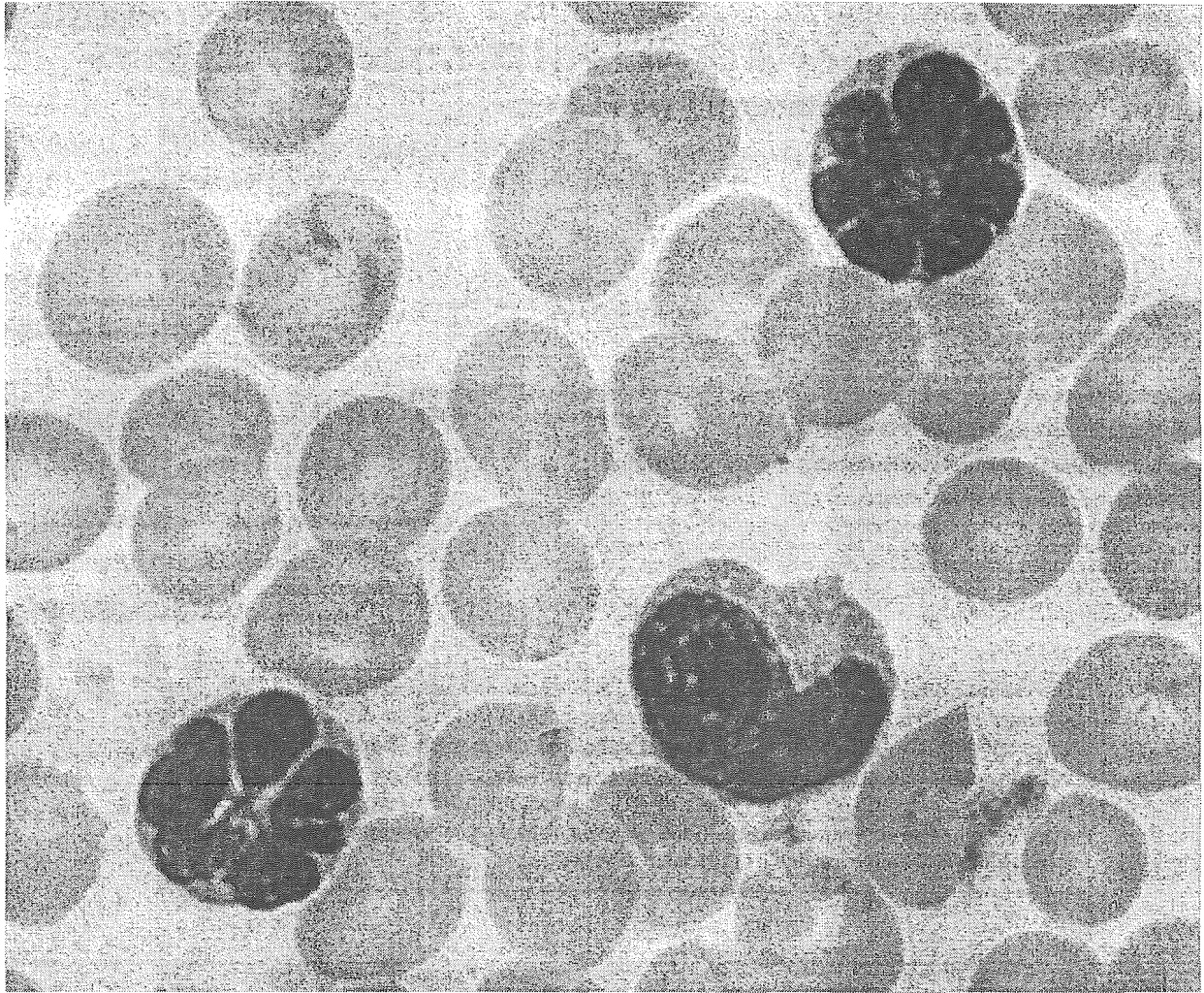


Figure 1

Typical "flower cell" in the peripheral blood of an acute ATL patient. In the peripheral blood of an acute ATL patient, leukemic cells with multilobulated nuclei.

transmission to humans is estimated to have occurred recently.

2. How does HTLV-I spread in humans?

There are approximately 10–20 million HTLV-I carriers in the world [11]. In particular, HTLV-I is endemic in Japan, parts of central Africa, the Caribbean basin and South America. In addition, epidemiological studies of HTLV-I have revealed high seroprevalence rates in Melanesia, Papua New Guinea and the Solomon islands, as well as among Australian aborigines [12]. In Japan, approximately 1.2 million individuals are estimated to be infected by HTLV-I, and more than 800 cases of ATL are

diagnosed each year [13]. Moreover, this virus also causes the neurodegenerative disease, HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [14,15]. The cumulative risks of ATL among HTLV-I carriers in Japan are estimated to be about 6.6% for men and 2.1% for women, indicating that most HTLV-I carriers remain asymptomatic throughout their lives [16].

3. How does HTLV-I replicate and increase its copy number?

The HTLV-I provirus has a similar structure to other retroviruses: a long terminal repeat (LTR) at both ends and internal sequences such as the *gag*, *pol* and *env* genes. A

characteristic of HTLV-I is the presence of the pX region, which exists between *env* and the 3'-LTR. This region encodes several accessory genes, which include the *tax*, *rex*, *p12*, *p21*, *p30*, *p13* and *HBZ* genes. Among these, the *tax* gene plays central roles in viral gene transcription, viral replication and the proliferation of HTLV-I-infected cells. Tax enhances viral gene transcription from the 5'-LTR via interaction with cyclic AMP responsive element binding protein (CREB). Tax also interacts with cellular factors and activates transcriptional pathways, such as NF- κ B, AP-1 and SRF [8,17-20]. For example, activation of NF- κ B induces the transcription of various cytokines and their receptor genes, as well as anti-apoptotic genes such as *bcl-xL* and *survivin* [21-23]. The activation of NF- κ B has been demonstrated to be critical for tumorigenesis both *in vitro* and *in vivo* [24,25]. On the other hand, Tax variant without activation of NF- κ B has also been reported to immortalize primary T-lymphocytes *in vitro* [26], suggesting that mechanisms of immortalization are complex. In addition to NF- κ B, activation of other transcriptional pathways such as CREB by Tax should be implicated in the immortalization and leukemogenesis.

Tax also interferes with the functions of p53, p16 and MAD1 [27-30]. These interactions enable HTLV-I-infected cells to escape from apoptosis, and also induce genetic instability. Although inactivation of p53 function by Tax is reported to be mediated by p300/CBP [27,28,31] or NF- κ B activation [32], Tax can still repress p53's activity in spite of loss of p300/CBP binding or in cells lacking NF- κ B activation [33], indicating the mechanism of p53 inactivation by Tax needs further investigation.

Although Tax promotes the proliferation of infected cells, it is also the major target of cytotoxic T-lymphocytes (CTLs) *in vivo*. Moreover, excess expression of Tax protein is considered to be harmful to infected cells. Therefore, HTLV-I has redundant mechanisms to suppress Tax expression. Rex binds to Rex-responsive element (RxRE) in the U3 and R regions of the 3'-LTR, and enhances the transport of the unspliced *gag/pol* and the singly spliced *env* transcripts. By this mechanism, double-spliced *tax/rex* mRNA decreases, resulting in suppressed expression of Tax [34]. On the other hand, p30 binds to *tax/rex* transcripts, and retains them in the nucleus [35]. The *HBZ* gene is encoded by the complementary strand of HTLV-I, and contains a leucine zipper domain. HBZ directly interacts with c-Jun or JunB [36], or enhances their degradation [37], resulting in the suppression of Tax-mediated viral transcription from the LTR.

Transforming growth factor- β (TGF- β) is an inhibitory cytokine that plays important roles in development, the immune system and oncogenesis. Since TGF- β generally suppresses the growth of tumor cells, most tumor cells

acquire escape mechanisms that inhibit TGF- β signaling, including mutations in its receptor and in the Smad molecules that transduce the signal from the receptor. Tax has also been reported to inhibit TGF- β signaling by binding to Smad2, 3 and 4 or CBP/p300 [38,39]. Inhibition of TGF- β signaling enables HTLV-I-infected cells to escape TGF- β -mediated growth inhibition.

ATL cells have been reported to show remarkable chromosomal abnormalities [40], which should be implicated in the disease progression. Tax has been reported to interact with the checkpoint protein MAD1, which forms a complex with MAD2 and controls the mitotic checkpoint. This functional hindrance of MAD1 by Tax protein causes chromosomal instability, suggesting the involvement of this mechanism in oncogenesis [30]. Recently, Tax has been reported to interact with Cdc20 and activate Cdc20-associated anaphase-promoting complex, an E3 ubiquitin ligase that controls the metaphase-to-anaphase transition, thereby resulting in mitotic abnormalities [41].

In contrast to HTLV-I, HTLV-II promotes the proliferation of CD8-positive T-lymphocytes *in vivo*. Although it was first discovered in a patient with variant hairy cell leukemia, HTLV-II is less likely to have oncogenic properties since there is no obvious association between HTLV-II infections and cancers. Regardless of the homology of their *tax* sequences, the oncogenic potential of Tax1 (HTLV-I Tax) is more prominent than that of Tax2 (HTLV-II Tax). The most striking difference is that Tax2 lacks the binding motif at C-terminal end to PDZ domain proteins, while Tax 1 retains it [42]. When the PDZ domain of Tax1 is added to Tax2, the latter acquires oncogenic properties in the rat fibroblast cell line Rat-1, indicating that this domain is responsible for the transforming activity of HTLV-I [43].

To understand the pleiotropic actions of Tax protein more clearly, transcriptome analyses are essential. The transcriptional changes induced by Tax expression have been studied using DNA microarrays, which revealed that Tax upregulated the expression of the mixed-lineage kinase MLK3. MLK3 is involved in NF- κ B activation by Tax as well as NIK and MEKK1 [44]. In addition to transcriptional changes, Tax is also well known to interact with cellular proteins and impair or alter their functions. For example, proteomic analyses of Tax-associated complexes showed that Tax could interact with cellular proteins, including the active forms of small GTPases, such as Cdc42, RhoA and Rac1, which should be implicated in the migration, invasion and adhesion of T-cells, as well as in the activation of the JNK pathway [45].

4. How does HTLV-I transmit and replicate in vivo?

Receptor and transmission of HTLV-I

HTLV-I can infect various types of cells, such as T-lymphocytes, B-lymphocytes, monocytes and fibroblasts [46]. Glucose transporter 1 (GLUT-1) has been identified as a receptor for HTLV-I and this receptor is ubiquitously expressed on cell surfaces [47]. However, the HTLV-I provirus is mainly detected in CD4-positive lymphocytes, with about 10% in CD8-positive T-lymphocytes [48]. This situation possibly arises because Tax mainly induces the increase of CD4-positive T-lymphocytes *in vivo* by enhanced proliferation and suppressed apoptosis.

In HTLV-I-infected individuals, no virions are detected in the serum. In addition, the infectivity of free virions is very poor compared with that of infected cells. These findings suggest that HTLV-I is spread by cell-to-cell transmission, rather than by free virions. *In vitro* analyses of HTLV-I-infected cells revealed that HTLV-I-infected cells form "virological synapses" with uninfected cells. Contact between an infected cell and a target cell induces the accumulation of the viral proteins Gag and Env, viral RNA and microtubules, and the viral complex subsequently transfers into the target cell [49]. HTLV-I also spreads in a cell-to-cell manner via such virological synapses *in vivo*.

HTLV-I is mainly transmitted via three routes: 1) mother-to-infant transmission (mainly through breast feeding) [50]; 2) sexual transmission (mainly from male-to-female); and 3) parenteral transmission (blood transfusion or intravenous drug use) [12]. In either route, HTLV-I-infected cells are essential for transmission. This was supported by the findings that fresh frozen plasma from carriers did not cause transmission [51] and freeze-thawing of breast milk reduced vertical transmission [52].

Provirus load and transmission

The provirus load varies more than 1000-fold among asymptomatic carriers [53]. Since most infected cells are considered to have one copy of the provirus, the provirus load indicates the percentage of infected cells among lymphocytes. The provirus load is relatively constant during the latent period [53]. Analysis of naive individuals who seroconvert after marrying an HTLV-I-seropositive spouse demonstrated that the proviral gp46 sequences are identical among married couples. This finding confirmed that HTLV-I is transmitted from a seropositive individual to an uninfected spouse. The provirus loads frequently differ between couples despite infection by the same HTLV-I virus, indicating that the number of infected cells is determined by host factors rather than virus itself [54].

Why does HTLV-I increase the number of infected cells by the pleiotropic actions of Tax? The provirus load in peripheral blood mononuclear cells (PBMCs) is well cor-

related with that in breast milk, and a higher provirus load in breast milk increases the risk of vertical transmission of HTLV-I [55,56]. Similarly, a higher provirus load in PBMCs may be associated with a higher risk of sexual transmission. Thus, an increase in the number of infected cells by the actions of accessory genes, especially *tax*, facilitates transmission. Therefore, HTLV-I has strategies that increase the number of HTLV-I-infected cells via the action of accessory gene products, thereby increasing the chance of transmission.

Clonal expansion of HTLV-I-infected cells

After HTLV-I infection, viral proteins such as Tax protein promote the proliferation of infected cells and also inhibit apoptosis by their pleiotropic actions. Since the HTLV-I provirus is randomly integrated into the host genome, the identification of integration sites enables to identify each infected clone, and to trace the kinetics of infected cells *in vivo*. Analyses using inverse PCR, which can identify the integration sites of the HTLV-I provirus, revealed that the proliferation of infected cells is oligoclonal, and that infected cells persistently survive *in vivo* [57-59]. Importantly, such clonal expansion in carriers is directly associated with the onset of ATL [60]. Thus, the viral strategies to increase the number of HTLV-I-infected cells work efficiently in most carriers without any adverse effects. However, the increased number of infected cells causes an excess immune reaction, leading to inflammatory diseases, HAM/TSP, infective dermatitis [61] or HTLV-I-associated uveitis [62]. Moreover, such prolonged proliferation of infected CD4-positive T-lymphocytes results in the onset of ATL in some carriers after a long latent period.

Inactivation of Tax expression in ATL cells

As mentioned above, Tax expression confers advantages and disadvantages on HTLV-I-infected cells. Although the proliferation of infected cells is promoted by Tax expression, CTLs attack the Tax-expressing cells since Tax is their major target [63]. In HTLV-I-infected cells, Rex, p30 and HBZ suppress Tax expression. On the other hand, loss of Tax expression is frequently observed in leukemic cells. Three mechanisms have been identified for inactivation of Tax expression: 1) genetic changes of the *tax* gene (nonsense mutations, deletions or insertions) [64,65]; 2) DNA methylation of the 5'-LTR [65,66]; and 3) deletion of the 5'-LTR (Figure 2) [67]. Among fresh leukemic cells isolated from ATL patients, about 60% of cases do not express the *tax* gene transcript. Interestingly, ATL cells with genetic changes of the *tax* gene expressed its transcripts, suggesting that ATL cells do not silence the transcription when the *tax* gene is abortive [65]. Loss of Tax expression gives ATL cells advantage for their survival since they can escape from CTLs.

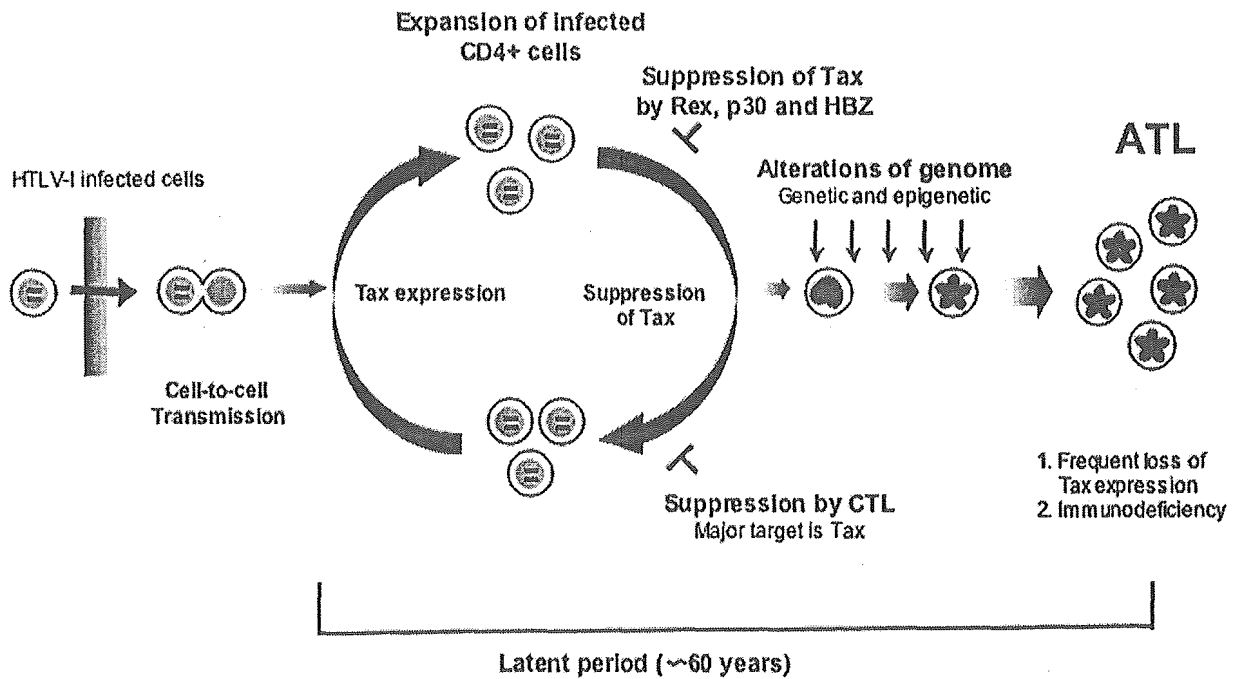


Figure 2

Natural course of HTLV-I infection to onset of ATL. HTLV-I is transmitted via three routes, and infected cells are necessary in all three. After infection, HTLV-I promotes clonal proliferation of infected cells by pleiotropic actions of Tax. Tax expression is suppressed by viral accessory gene products, such as Rex, p30 and HBZ proteins. Proliferation of HTLV-I infected cells is controlled by cytotoxic T-cells *in vivo*. After a long latent period, ATL develops in about 5% of asymptomatic carriers. The expression of Tax is inactivated by several mechanisms, suggesting that Tax is not necessary in this stage. Alternatively, alternations in the host genome accumulate during the latent period, finally leading to onset of ATL.

Longer lifespan of HTLV-I-infected cells and cancer

Lymphoid malignancy with a T-cell origin is rare compared with B-cell malignancy. ATL shares hematological, pathological and immunological features with cutaneous T-cell lymphoma (CTCL; Sezary syndrome and Mycosis fungoides). The frequency of CTCL in Japan is estimated to be one/million/year. On the other hand, the frequency of ATL among carriers is estimated to be 1000/million/year. From these data, HTLV-I infection is estimated to increase the risk of T-cell malignancy by up to 1000-fold in carriers.

HTLV-I infection confers a long lifespan on the infected cells due to the pleiotropic actions of Tax, resulting in increased numbers of infected cells. Such infected cells are essential for the transmission of HTLV-I. This strategy to increase the number of infected cells *in vivo* is thought to increase the incidence of cancer in T-cells. What is the mechanism for this oncogenesis? DNA methylation is known to be associated with aging. Some genes are hypermethylated in older people, indicating that DNA hypermethylation is a physiological phenomenon in some genes. Under normal circumstances, T-lymphocytes

survive for several years, and long-lived T-lymphocytes with disordered methylation should be replaced. However, HTLV-I-infected T-cells are considered to survive and accumulate abnormal methylation. The process of oncogenesis is similar to that of evolution [68]. The infected cells that are suitable for survival should be selected *in vivo*, and epigenetic and genetic changes of the genome play critical roles in this selection. Accumulating alterations of the host genome transform the HTLV-I-infected cells into ATL cells, and also enable ATL cells to proliferate in the absence of Tax expression (Figure 2). In the provirus, DNA methylation of the 5'-LTR silences viral transcription in leukemic cells, which facilitates the escape of ATL cells from the host immune system [65].

5. Somatic alterations in ATL cells

As described, some ATL cells can proliferate without functional Tax protein, suggesting that somatic (genetic and epigenetic) alterations cause transcriptional or functional changes to the host genes. The *p53* gene is frequently mutated in various cancers, and these mutations are associated with disease progression and a poor prognosis. The mutation rate of the *p53* gene in ATL cells has been reported to be 36% (4/11) and 30% (3/10) [69-71]. The *p16* gene is an inhibitor of cyclin-dependent kinase 4/6, and blocks the cell cycle. Genetic changes in this gene (deletion in most cases) have been described in many types of cancer cells. Deletion of the *p16* gene has also been reported in ATL cells [72]. Moreover, DNA methylation of the promoter region of the *p16* gene is also implicated in the suppression of *p16* [73]. In addition, genetic changes in the *p27^{KIP1}*, *RB1/p105* and *RB2/p130* genes have been reported in ATL, although they are relatively rare: 2/42 (4.8%) for the *p27^{KIP1}* gene; 2/40 (5%) for the *RB1/p105* gene; and 1/41 (2.4%) for the *RB2/p130* gene [74]. The fact that higher frequencies of genetic changes in these tumor suppressor genes are observed among aggressive forms of ATL suggests that such genetic changes are implicated in disease progression.

Fas antigen was the first identified death receptor. It transduces the death signal by binding of its ligand, Fas ligand (FasL). ATL cells highly express Fas antigen on their cell surface [75], and are highly susceptible to death signals mediated by agonistic antibodies to Fas antigen, such as CH-11. Genetic changes of *Fas* gene in ATL cells, which confer resistance to the Fas-mediated signal, have been reported [76,77]. Normal activated T-lymphocytes express FasL as well as Fas antigen. Apoptosis induced by auto-crine mechanisms is designated activation-induced cell death (AICD) and this controls the immune response [78]. Although ATL cells express Fas antigen, they do not produce FasL, thereby enabling ATL cells to escape from AICD. Attempts to isolate hypermethylated genes from ATL cells identified the *EGR3* gene as a hypermethylated

gene compared to PBMCs from carriers [79]. *EGR3* is a transcriptional factor with a zinc finger domain, that is essential for transcription of the *FasL* gene [80]. The finding that *EGR3* gene transcription is silenced in ATL cells could account for the loss of FasL expression, and the escape of ATL cells from AICD. Thus, alterations of the *Fas* (genetic) and *EGR3* (epigenetic) genes are examples of ATL cell evolution *in vivo*.

Disordered DNA methylation has been identified in the genome of ATL cells compared with that of PBMCs from carriers: hypomethylation is associated with aberrant expression of the *MEL1S* gene [81], while hypermethylation silences transcription of the *p16* [73], *EGR3* and *KLF4* genes as well as many others [79]. It is reasonable to consider that other currently unidentified genes are involved in such alterations of the genome in ATL cells, and play roles in leukemogenesis.

Transcriptome analyses using DNA microarrays have revealed transcriptional changes that are specific to ATL cells. Among 192 up-regulated genes, the expressions of the *tumor suppressor in lung cancer 1 (TSLC1)*, *caveolin 1* and *prostaglandin D2 synthase* genes were increased more than 30-fold in fresh ATL cells compared with normal CD4+ and CD4+, CD45RO+ T-cells [82]. *TSLC1* is a cell adhesion molecule that acts as a tumor suppressor in lung cancer. Although *TSLC1* is not expressed on normal T-lymphocytes, all acute ATL cells show ectopic *TSLC1* expression. Enforced expression of *TSLC1* enhances both the self-aggregation and adhesion abilities to vascular endothelial cells in ATL cells. Thus, *TSLC1* expression is implicated in the adhesion or infiltration of ATL cells. By screening a retrovirus cDNA library from ATL cells, a gene with oncogenic potency was identified in NIH3T3 cells, and designated the *Tgat* gene [83]. Ectopic expression of the *Tgat* gene is observed in aggressive forms of ATL, and *in vitro* experiments showed that its expression is associated with an invasive phenotype.

6. Immune control of HTLV-I infection

The host immune system, especially the cellular response, against HTLV-I exerts critical control over virus replication and the proliferation of infected cells [84]. CTLs against the virus have been extensively studied, and Tax protein was found to be the dominant antigen recognized by CTLs *in vivo* [63]. HTLV-I-specific CD8-positive CTLs are abundant and chronically activated. The paradox is that the frequency of Tax-specific CTLs is much higher in HAM/TSP patients than in carriers. Since the provirus load is higher in HAM/TSP patients, this finding suggests that the CTLs in HAM/TSP cannot control the number of infected cells. One explanation for this is that the CTLs in HAM/TSP patients show less efficient cytolytic activity toward infected cells, whereas CTLs in carriers can suppress the

proliferation of infected cells [85]. Hence, the gene expression profiles of circulating CD4+ and CD8+ lymphocytes were compared between carriers with high and low provirus loads. The results revealed that CD8+ lymphocytes from individuals with a low HTLV-1 provirus load show higher expressions of genes associated with cytolytic activities or antigen recognition than those from carriers with a high provirus load [86]. Thus, CD8+ T-lymphocytes in individuals with a low provirus load successfully control the number of HTLV-I-infected cells due to their higher CTL activities. Thus, the major determinant of the provirus load is thought to be the CTL response to HTLV-I.

As mentioned above, the provirus load is considered to be controlled by host factors. Considering that the cellular immune responses are critically implicated in the control of HTLV-I infection, human leukocyte antigen (HLA) should be a candidate for such a host genetic factor. From analyses of HAM/TSP patients and asymptomatic carriers, HLA-A02, and Cw08 are independently associated with a lower provirus load and a lower risk of HAM/TSP. In addition, polymorphisms of other genes (*TNF- α* , *SDF-1*, *HLA-B54*, *HLA-DRB-10101* and *IL-15*) are also associated with the provirus load, although their associations are not as significant compared with HLA-A02, and Cw08 [87,88]. Regarding the onset of ATL, only a polymorphism of *TNF- α* gene was reported to show an association [89]. However, familial clustering of ATL cases is a well-known phenomenon, strongly suggesting that genetic factors are implicated in the onset of ATL [90-92].

Spontaneous remission is more frequently observed in patients with ATL than those with other hematological malignancies [90,93]. Usually, this phenomenon is associated with infectious diseases, suggesting that immune activation of the host enhances the immune response against ATL cells. If the immune response against HTLV-I is implicated in spontaneous remission, this suggests the possibility of immunotherapy for ATL patients by the induction of an immune response to HTLV-I [94], for example via antigen-stimulated dendritic cells.

Immunodeficiency in ATL patients is pronounced, and results in frequent opportunistic infections by various pathogens, including *Pneumocystis carinii*, cytomegalovirus, fungus, *Strongyloides* and bacteria, due to the inevitable impairment of the T-cell functions [95]. To a lesser extent, impaired cell-mediated immunity has also been demonstrated in HTLV-I carriers [96]. Such immunodeficiency in the carrier state may be associated with the leukemogenesis of ATL by allowing the proliferation of HTLV-I-infected cells. A prospective study of HTLV-I-infected individuals found that carriers who later develop ATL have a higher anti-HTLV-I antibody and a low anti-Tax antibody level for up to 10 years preceding their diag-

nosis. This finding indicates that HTLV-I carriers with a higher anti-HTLV-I titer, which is roughly correlated with the HTLV-I provirus load, and a lower anti-Tax reactivity may be at the greatest risk of developing ATL [97]. The anti-HTLV-I antibody and soluble IL-2 receptor (sIL-2R) levels are correlated with the HTLV-I provirus load [53], and a high antibody titer and high sIL-2R level are risk factors for developing ATL among carriers [98]. Taken together, these findings suggest that a higher proliferation of HTLV-I-infected cells and a low immune response against Tax may be associated with the onset of ATL. Given these findings, potentiation of CTLs against Tax via a vaccine strategy may be useful for preventing the onset of ATL [99].

EBV-associated lymphomas frequently develop in individuals with an immunodeficient state associated with transplantation or AIDS. This has also been reported in an ATL patient [100]. Does such an immunodeficient state influence the onset of ATL? Among 24 patients with post-transplantation lymphoproliferative disorders (PT-LPDs) after renal transplantation in Japan, 5 cases of ATL have been reported. Considering that most PT-LPDs are of B-cell origin in Western countries, this frequency of ATL in Japan is quite high. Although the high HTLV-I seroprevalence is due to blood transfusion during hemodialysis, the immunodeficient state during renal transplantation apparently promotes the onset of ATL [101]. In addition, when experimental allogeneic transplantation was performed to 12 rhesus monkeys and immunosuppressive agents (cyclosporine, prednisolone or lymphocyte-specific monoclonal antibodies) were administered to prevent rejection, 4 of the 7 monkeys that died during the experiment showed PT-LPDs. Importantly, the STLV provirus was detected in all PT-LPD samples [102]. These observations emphasize that transplantation into HTLV-I-infected individuals or from HTLV-I positive donors require special attention.

Although the mechanism of immunodeficiency remains unknown, some previous reports have provided important clues. One mechanism for immunodeficiency is that HTLV-I infects CD8-positive T-lymphocytes, which may impair their functions [48]. Indeed, the immune response against Tax via HTLV-I-infected CD8-positive T-cells renders these cells susceptible to fratricide mediated by autologous HTLV-I-specific CD8-positive T-lymphocytes [103]. Fratricide among virus-specific CTLs could impair the immune control of HTLV-I. Another mechanism for immunodeficiency is based on the observation that the number of naive T-cells decreases in individuals infected with HTLV-I via decreased thymopoiesis [48]. In addition, CD4+ and CD25+ T-lymphocytes are classified as immunoregulatory T-cells that control the host immune system. Regulatory T-cells suppress the immune reaction via the

expression of immunoregulatory molecules on their surfaces. The *FOXP3* gene has been identified as a master gene that controls gene expressions specific to regulatory T-cells. *FOXP3* gene transcription can be detected in some ATL cases (10/17; 59%) [104]. Such ATL cells are thought to suppress the immune response via expression of immunoregulatory molecules on their surfaces, and production of immunosuppressive cytokines.

6. Pathogenesis of HTLV-1 infection

ATL cells are derived from activated helper T-lymphocytes, which play central roles in the immune system by elaborating cytokines and expressing immunoregulatory molecules. ATL cells are known to retain such features, and this cytokine production or surface molecule expression may modify the pathogenesis.

ATL is well known to infiltrate various organs and tissues, such as the skin, lungs, liver, gastrointestinal tract, central nervous system and bone [95]. This infiltrative tendency of leukemic cells is possibly attributable to the expressions of various surface molecules, such as chemokine receptors and adhesion molecules. Skin-homing memory T-cells uniformly express CCR4, and its ligands are thymus and activation-regulated chemokine (TARC) and macrophage-derived chemokine (MDC). CCR4 is expressed on most ATL cells. In addition, TARC and MDC are expressed in skin lesions in ATL patients. Thus, CCR4 expression should be implicated in the skin infiltration [105]. On the other hand, CCR7 expression is associated with lymph node involvement [106]. OX40 is a member of the tumor necrosis factor family, and was reported to be expressed on ATL cells [107]. It was also identified as a gene associated with the adhesion of ATL cells to endothelial cells by a functional cloning system using a monoclonal antibody that inhibited the attachment of ATL cells [108]. Thus, OX40 is also implicated in the cell adhesion and infiltration of ATL cells.

Hypercalcemia is frequently complicated in patients with acute ATL (more than 70% during the whole clinical course) [109]. In hypercalcemic patients, the number of osteoclasts increases in the bone (Figure 3). RANK ligand, which is expressed on osteoblasts, and M-CSF act synergistically on hematopoietic precursor cells, and induce the differentiation into osteoclasts [110]. ATL cells from hypercalcemic ATL patients express RANK ligand, and induced the differentiation of hematopoietic stem cells into osteoclasts when ATL cells were co-cultured with hematopoietic stem cells [111]. In addition, the serum level of parathyroid hormone-related peptide (PTH-rP) is also elevated in most of hypercalcemic ATL patients. PTH-rP indirectly increases the number of osteoclasts, as well as activating them [112,113], which is also implicated in mechanisms of hypercalcemia.

7. Treatment of ATL – the remaining mission and challenges

Regardless of intensive chemotherapies, the prognosis of ATL patients has not so improved. The median survival time of acute or lymphoma-type ATL was reported to be 13 months with the most intensive chemotherapy [114]. Such a poor prognosis might be due to: 1) the resistance of ATL cells to anti-cancer drugs; and 2) the immunodeficient state and complicated opportunistic infections as described above. Regarding the resistance to anti-cancer drugs, one mechanism is the activated NF- κ B pathway in ATL cells [115], which increases the transcription of anti-apoptotic genes such as *bcl-xL* and *survivin*. A proteasome inhibitor, bortezomib, is currently used for the treatment of multiple myeloma. One of its mechanisms is suppression of the NF- κ B pathway by inhibiting the proteasomal degradation of I κ B protein. Several groups have shown that bortezomib is effective against ATL cells both *in vitro* and *in vivo* [116-119]. Since the sensitivity to bortezomib is well correlated with the extent of NF- κ B activation, the major mechanism of the anti-ATL effect is speculated to be inhibition of NF- κ B. In addition, an NF- κ B inhibitor has also been demonstrated to be effective against ATL cells [120].

During chemotherapy for ATL, chemotherapeutic agents worsen the immunodeficient state of ATL patients. In this regard, antibody therapy against ATL cells has advantages due to its decreased adverse effects. A humanized monoclonal antibody to CD25 has been clinically administered to patients with ATL [121,122]. In addition, a monoclonal antibody to CD2 is at the preclinical stage [123]. As described above, most ATL cells express CCR4 antigen on their surfaces, and a humanized antibody against CCR4 is being developed as an anti-ATL agent [124].

Advances in the treatment of ATL were brought about by allogeneic bone marrow or stem cell transplantation [125,126]. Absence of graft-versus-host disease (GVHD) was linked with relapse of ATL, suggesting that GVHD or graft-versus-ATL may be implicated in the clinical effects of allogeneic stem cell transplantation [125]. Furthermore, 16 patients with ATL, who were over 50 years of age, were treated with allogeneic stem cell transplantation with reduced conditioning intensity (RIST) from HLA-matched sibling donors [127]. Among 9 patients in whom ATL relapsed after transplantation, 3 achieved a second complete remission after rapid discontinuation of cyclosporine A. This finding strongly suggests the presence of a graft-versus-ATL effect in these patients. In addition, Tax peptide-recognizing cells were detected by a tetramer assay (HLA-A2/Tax 11-19 or HLA-A24/Tax 301-309) in patients after allogeneic stem cell transplantation [128]. In 8 patients, the provirus became undetectable by real-time PCR. Among these, 2 patients who received grafts

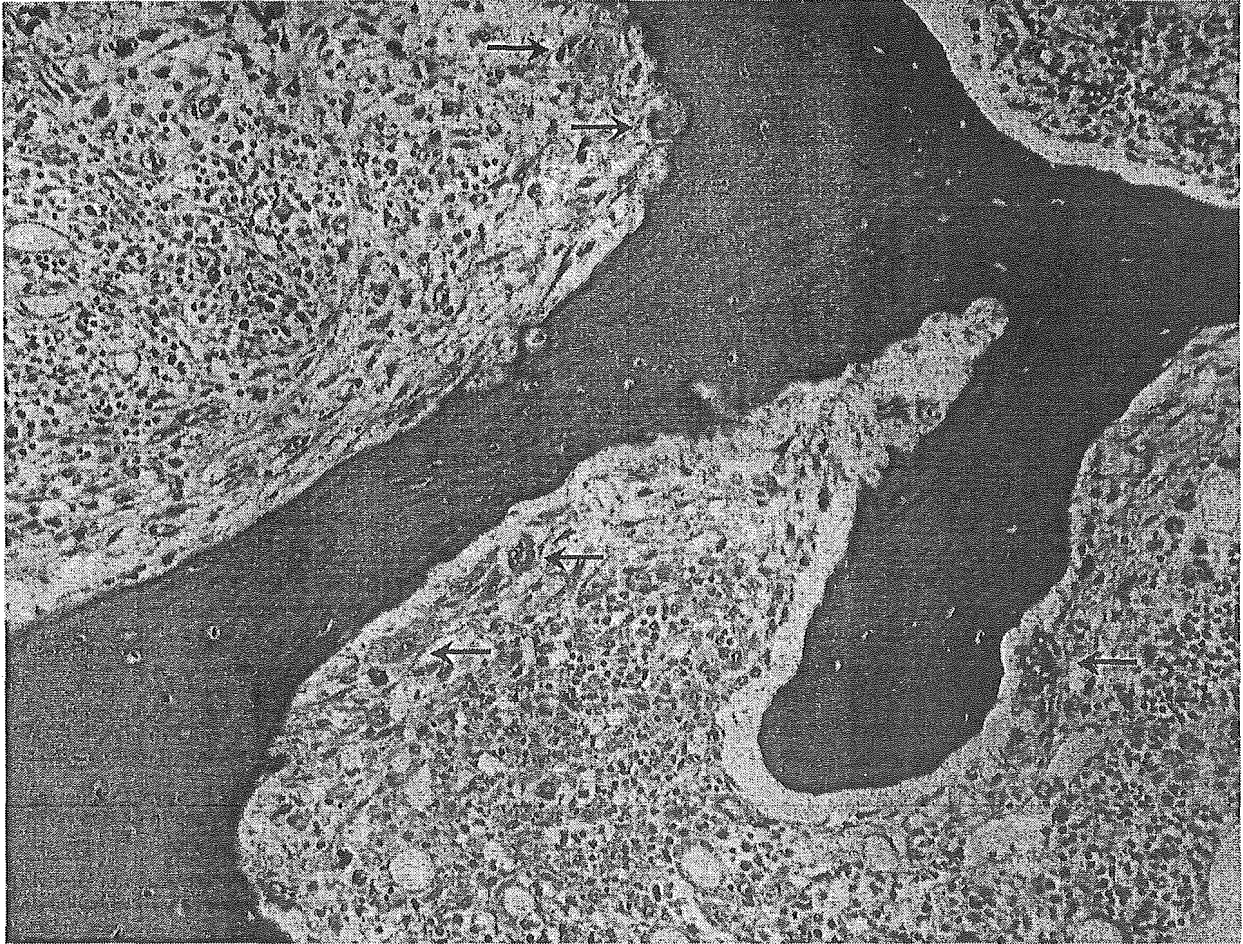


Figure 3

Increased number of osteoclasts in the bone of a hypercalcemic ATL patient. In a hypercalcemic patient, the number of osteoclast (arrows) increased in the bone, which accelerated bone resorption.

from HTLV-I-positive donors also became provirus-negative by real-time PCR after RIST. Since the provirus load is relatively constant in HTLV-I-infected individuals [53], this finding indicates an enhanced immune response against HTLV-I after RIST, which suppresses the provirus load. This may account for the effectiveness of allogeneic stem cell transplantation to ATL. However, Tax expression is frequently lost in ATL cells as described above. Many questions arise, such as whether the *tax* gene status is correlated with the effect of allogeneic stem cell transplantation, and whether the effectiveness of the anti-HTLV-I immune response is against leukemic cells or non-leukemic HTLV-I-infected cells. Nevertheless, these data suggest that potentiation of the immune response against viral proteins such as Tax may be an attractive way to treat ATL

patients [94]. Such strategies may enable preventive treatment of high-risk HTLV-I carriers, such as those with familial ATL history, predisposing genetic factors to ATL, a higher provirus load, etc.

8. Two human retroviruses – HTLV-I and HIV-1

As described in the first section, HTLV-I has resided in humans for a long time. On the other hand, HIV-1 has only been recently transmitted to humans, probably from chimpanzees. Due to the comparatively small genomic differences between humans and chimpanzees, this virus can quickly adapt to human cells. These two human retroviruses are opposite in many aspects. HIV-1 vigorously replicates *in vivo*, and the maximum production of HIV-1 virions in the body can reach 10^{10} per day. Since reverse

transcriptase is an error-prone enzyme due to its lack of proof-reading activity, it produces about one mistake per replication, resulting in tremendous errors in the proviral sequence during replication. Although most of these variations ruin the virus replication due to nonsense mutations or impairment of viral gene functions, some become capable of replicating under different circumstances such as the presence of anti-HIV drugs and activation of the host immune system. This can account for why HIV-1 acquires resistance against anti-HIV drugs, and escape from CTLs. On the other hand, HTLV-I increases its copy number in two ways, namely replication of HTLV-I itself and the proliferation of HTLV-I-infected cells *in vivo*. Although immune responses (antibodies, CTLs) against viral proteins suggest the presence of active viral replication *in vivo*, most of increased HTLV-I provirus load (the number of infected cells) is considered to be due to proliferation of infected cells since CTLs efficiently eliminate virus-expressing cells. Therefore, there is much less variation in the HTLV-I provirus sequence compared with HIV-1 [129]. However, this strategy by which HTLV-I increases the number of infected cells due to clonal expansion generates unfortunate side effects for both the host and the virus, namely oncogenesis of CD4-positive T-lymphocytes and the development of ATL.

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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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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- κ B (NF- κ 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

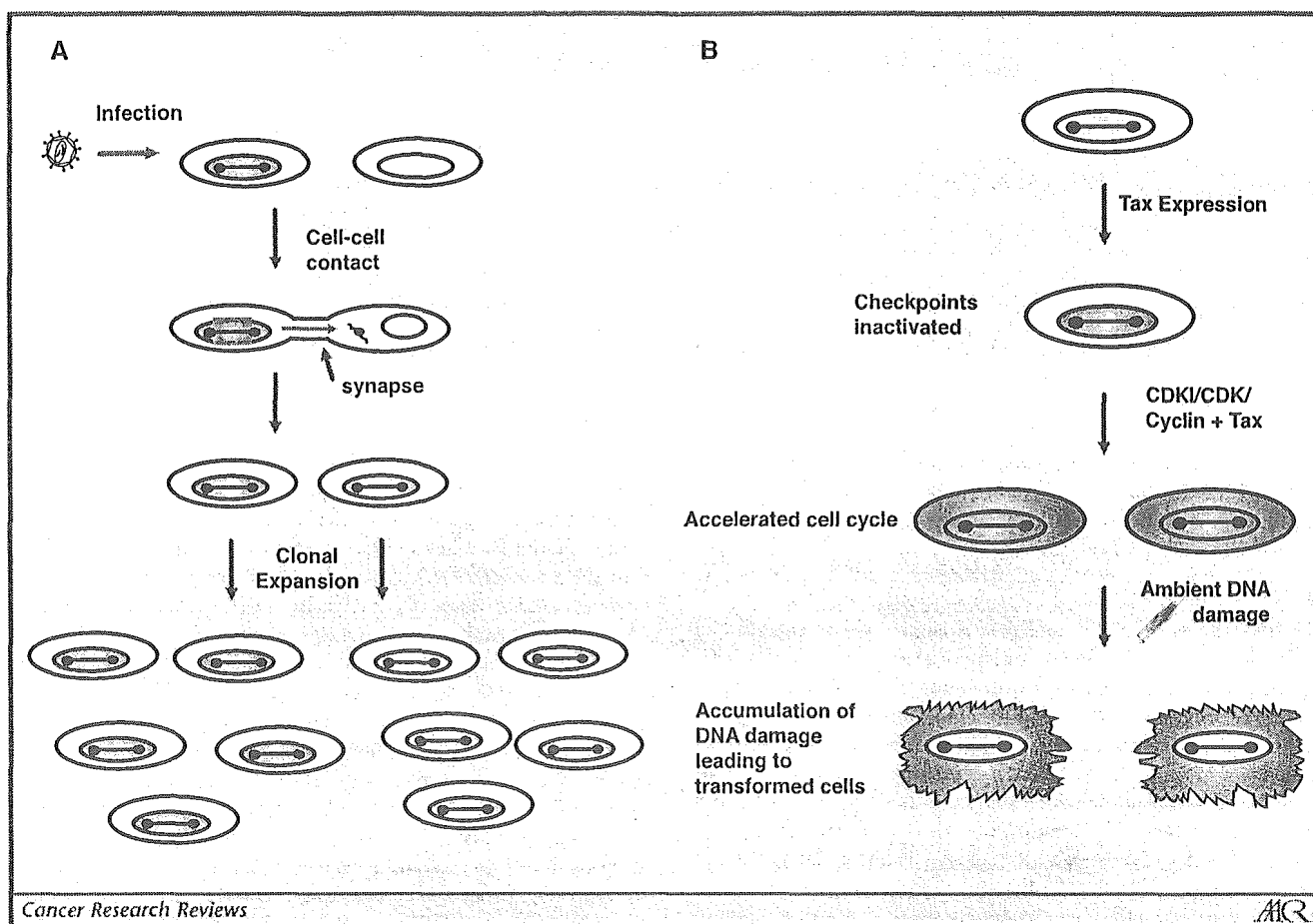


Figure 1. Cell-to-cell spread by HTLV-I and cellular transformation by Tax. **A**, HTLV-I virus infects the cell and establishes a provirus. The most efficient route of HTLV-I spread is then via cell-to-cell contact through a synapse. Once spread of infection is established, clonal expansion of infected 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 G₂-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- κ 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- κ 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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Natural history of adult T-cell leukemia/lymphoma and approaches to therapy

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After cell-to-cell transmission, HTLV-I increases its viral genome by *de novo* infection and proliferation of infected cells. Proliferation of infected cells is clonal and persistent *in vivo*. During the carrier state, infected cells are selected *in vivo* by the host's immune system, the genetic and epigenetic environment of proviral integration sites, and other factors. In leukemic cells, *tax* gene expression is frequently impaired by genetic and epigenetic mechanisms. Such loss of Tax expression enables ATL cells to escape the host immune system. On the other hand, ATL cells acquire the ability to proliferate without Tax by intracellular genetic and epigenetic changes. Despite advances in support and the development of novel treatment agents, the prognosis for ATLL remains poor. A number of therapies, however, do appear to improve prognosis compared to CHOP (VEPA). These include interferon- α plus zidovudine (probably after 1–2 cycles of CHOP), intensive chemotherapy as in LSG-15 with G-CSF support and Allo-SCT (which includes the potential for cure). Emerging novel approaches include HDAC inhibitors, monoclonal antibodies, and proteasome inhibitors. Comparison between different therapeutic approaches is complicated by the range of natural history of ATLL, different recruitments of naïve-to-therapy, refractory or relapsed patients, and variations in the reporting of outcome that frequently excludes difficult-to-evaluate patients. Moreover, results from relatively small proof-of-principle studies have not been extended with randomized, controlled trials. As a result, currently, there is no clear evidence to support the value of any particular treatment approach over others. To avoid further unnecessary patient suffering and to identify optimal therapy as rapidly as possible, large randomized, controlled trials encompassing multicenter, international collaborations will be necessary.

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Disease course from HTLV-I infection to onset of ATL

After infection by HTLV-I, a subpopulation of carriers (6% male and 2% female subjects) develops ATL after a long latent period. Although genetic, environmental, and viral factors in addition to the host immune response should be implicated in leukemogenesis, the exact mechanism remains to be elucidated. Below, we highlight first the current understanding of the disease course leading to the onset of ATL.

Transmission of HTLV-I

HTLV-I can infect various cell types, including T cells, B cells, and synovial cells, suggesting that its receptor is ubiquitously expressed. The receptor for HTLV-I has been identified as glucose transporter type 1 (GLUT1) (Manel *et al.*, 2003). Its expression on T lymphocytes is enhanced by mitogen or TGF- β (Jones *et al.*, 2005), which has been shown to increase the infectivity of HTLV-I. *In vitro* experiment shows that transmission by free virion is very inefficient, whereas transmission by infected cells is much more efficient. This is because HTLV-I transmits naturally in a cell-to-cell fashion. When an HTLV-I-infected cell attaches to uninfected cells, the HTLV-I-infected cells form 'virological synapses' with uninfected cells. Contact between an infected cell and a target cell polarizes the microbutule-organizing center (MTOC) at the cell–cell junction, and then viral proteins, such as Gag and viral genome RNA, accumulate at this junction and the viral complex subsequently transfers into the target cell (Igakura *et al.*, 2003). In HTLV-I-infected cells, expression of ICAM-1 is upregulated, and antibody to ICAM-1 induces MTOC polarization in HTLV-I-infected cells, suggesting that increased expression of ICAM-1 facilitates cell-to-cell transmission of HTLV-I (Barnard *et al.*, 2005). Thus, transmission of HTLV-I needs living infected cells.

HTLV-I-infected cells enter into the human body via three major routes: (1) mother-to-infant transmission (mainly through breast-feeding), (2) sexual transmission, and (3) parenteral transmission. Of note, fresh frozen plasma from seropositive donors did not transmit HTLV-I (Okochi *et al.*, 1984). Hence, if living cells can be eliminated by freeze and thawing, then feeding

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the mother's breast milk to infants will not increase viral transmission (Ando *et al.*, 2004). These findings clearly show that physiological transmission requires live HTLV-I-infected cells. To facilitate its transmission, HTLV-I increases clonally the population of infected cells by the pleiotropic actions of viral proteins, especially Tax (Yoshida, 2001; Gatza *et al.*, 2003; Jeang *et al.*, 2004; Matsuoka and Jeang, 2005).

HTLV-I-infected cells in vivo

In HTLV-I carriers and ATL patients, no free virion has been demonstrated *in vivo*. The presence of antibody and provirus are evidence for HTLV-I infection. After transmission of HTLV-I, reverse transcriptase generates proviral DNA from the genomic viral RNA, and the provirus is integrated into the host genome by viral integrase. Since most ATL cells contain one copy of HTLV-I provirus, and ATL cells are derived from HTLV-I-infected cells, it is reasonable to consider that most of the HTLV-I-infected cells have one copy of the provirus. Therefore, quantification of provirus is thought to reflect the number of HTLV-I-infected cells *in vivo*. Quantification of HTLV-I provirus among infected individuals demonstrates that provirus load differs more than 1000-fold among asymptomatic carriers (Etoh *et al.*, 1999). Since living HTLV-I-infected cells are essential for transmission as mentioned above, an increased number of HTLV-I-infected cells is thought to facilitate transmission. Indeed, higher provirus load in the breast milk is correlated with an increased risk of vertical transmission from seropositive mothers (Li *et al.*, 2004). In this regard, the mechanism to increase HTLV-I-infected cells by actions of accessory genes, especially *tax*, provides the rationale for enhancing infectivity.

Clonal proliferation of HTLV-I-infected cells

After transmission, HTLV-I increases viral copy number both by *de novo* infection and clonal proliferation of infected cells. In this strategy, Tax plays a central role in increasing the number of HTLV-I-infected cells by promoting proliferation and inhibiting apoptosis. Since the integration sites of HTLV-I provirus are random, the demonstration of discrete integration sites can be employed to identify individual HTLV-I-infected clones. Inverse PCR or linker-mediated PCR was utilized to characterize integration sites, and revealed that proliferation of HTLV-I-infected cells was oligoclonal (Figure 1) (Etoh *et al.*, 1997). Importantly, these clonal proliferations of HTLV-I-infected cells are persistent since the same clones can be detected at the different time points (Etoh *et al.*, 1997; Cavrois *et al.*, 1998). As an example, a HAM/TSP patient developed lymphoma-type ATL. The ATL clone was identified in a blood sample obtained before the onset of ATL, which showed that the same clone was already present during HAM/TSP (Tamiya *et al.*, 1995). In addition, the prospective study of the Miyazaki cohort identified carriers who developed ATL during follow-up. The presence of

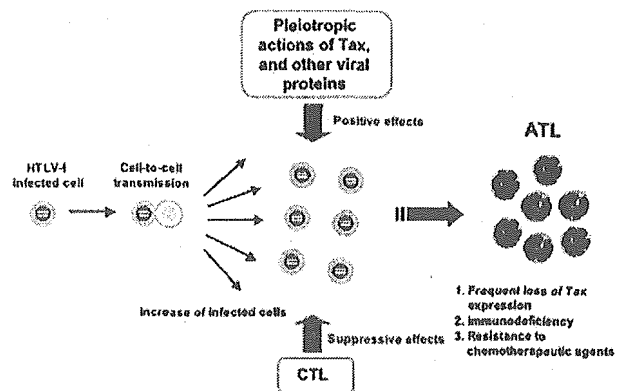


Figure 1 Natural course from HTLV-I infection to onset of ATL. HTLV-I is transmitted in a cell-to-cell fashion. After infection, HTLV-I promotes clonal proliferation of infected cells by pleiotropic actions of Tax and other viral proteins. Proliferation of HTLV-I-infected cells is controlled by cytotoxic T cells *in vivo*. After a long latent period, ATL develops in about 5% of asymptomatic carriers. The expression of Tax is inactivated by several mechanisms, suggesting that Tax is not necessary in this stage. Alternatively, alterations and errors in the host genome accumulate progressively during the latent period, finally leading to onset of ATL

leukemic clones was detected during the carrier state by inverse PCR method. Such clonal proliferation is directly associated with the onset of ATL (Okayama *et al.*, 2004), and these studies clearly illustrate that HTLV-I-infected clones can transform to malignancy during the carrier state.

HTLV-I clonal cells are more heterogeneous and less stable during seroconversion than in long-term carriers (Tanaka *et al.*, 2005). This finding suggests that during seroconversion, HTLV-I-infected cells are actively selected *in vivo*, and thereafter, selected clones predominate in long-term carriers. Such selection may be due to several factors, including the immune pressure exerted by cell-mediated immunity (Bangham, 2003), the productivity of viral proteins, and the characteristics of the integration sites which influence viral transcription.

Integration sites of HTLV-I provirus

To clarify the significance of integrated genome positions, integration sites of HTLV-I provirus are determined by inverse PCR in carrier states and ATL. The frequencies of HTLV-I provirus integration into transcription units (from the first exon to the last exon) are 26.8% (15/56) in carriers and 33.9% (20/59) in ATL. These are equivalent to the frequency calculated based on random integration (33.2%) (Doi *et al.*, 2005). By contrast, HIV-1, another human retrovirus, integrates predominantly (91%) into actively transcribed genes of T lymphocytes (Han *et al.*, 2004), and integration sites are evenly distributed within the transcription units. Additionally, the frequency of integration into transcription units is 34.2% for murine leukemia virus (MLV), and these sites are clustered near the transcriptional

start sites (Wu *et al.*, 2003). Similarly, HTLV-I provirus is prone to integration near the transcriptional start sites in leukemic cells ($P=0.006$). Thus, integration pattern of HTLV-I is similar to MLV rather than HIV-1. HIV-1 can infect nondividing cells by passing through the nuclear pore, whereas MLV can infect only dividing cells. These data suggest that HTLV-I may infect only dividing cells.

In addition, HTLV-I integration sites in the carriers favor the alphoid repetitive sequences (11/56: 20%) whereas in leukemic cells they disfavored these sequences (2/59: 3.4%). Alphoid repeats are a component of centromeric heterochromatin and consist of monomeric 171 bp repeats. When HIV-1 is integrated into alphoid repetitive sequences, it adopts a latent state due to the influence of the surrounding heterochromatin (Jordan *et al.*, 2003). Thus, genome surrounding integration sites influences the transcription of viral genes. HTLV-I-infected cells in which the provirus is integrated into alphoid repetitive sequence are considered to be in the latent state. Such infected cells are enriched in carrier state, indicating that infected cells with less viral gene expression are favored since such cells can escape from the host's cytotoxic T lymphocytes (Bangham, 2003). However, integration into alphoid sequences is infrequent in leukemic cells, indicating that among surviving HTLV-I-infected cells those with higher production of viral proteins are more likely to transform into malignant cells.

HTLV-I provirus in ATL cells

The *tax* gene plays a central role by its pleiotropic actions in the proliferation and leukemogenesis of HTLV-I-infected cells *in vivo* (Franchini *et al.*, 2003). However, its transcription is detected in only 34% of ATL cases by RT-PCR (Takeda *et al.*, 2004). Tax production is impaired by several mechanisms: (1) genetic changes (nonsense mutation, insertion, and deletion) of *tax* gene, (2) deletion of 5'-long terminal repeat (LTR), and (3) DNA methylation of 5'-LTR. Genetic changes of *tax* gene were observed in five of 47 cases (11%) (Furukawa *et al.*, 2001; Takeda *et al.*, 2004). Deletion of 5'-LTR, which is the promoter/enhancer for viral gene transcription, was observed in 14 of 47 cases (30%). This type of defective provirus designated type 2 defective provirus (Tamiya *et al.*, 1996) lacks 5'-LTR and internal sequences, such as *gag*, *pol*, and *env* genes. The frequency of type 2 defective provirus was much higher in acute and lymphoma-type ATL than in chronic type ATL, suggesting a close association with disease progression. Heavy methylation of the 5'-LTR region was also associated with silencing of viral gene transcription (Koiwa *et al.*, 2002; Takeda *et al.*, 2004). Tax expression is absent or severely impaired in ATL cells with heavily methylated 5'-LTR. Such changes are predominantly observed in aggressive subtypes of ATL, which suggests that at this stage, ATL cells acquire the ability to proliferate without Tax expression. In turn, such changes enable ATL cells to escape from the host immune system by

loss of Tax expression (Figure 1). This finding reveals a duality in the Tax protein: its expression induces proliferation and inhibits apoptosis of HTLV-I-infected cells, and also evokes the host's immune response including cytotoxic T cells to kill virus-infected cells.

Somatic changes in ATL cells

As described above, ATL cells do not always need Tax expression in the later stage of leukemogenesis. Genetic and epigenetic changes imprinted into the genome should be implicated in such multistep leukemogenesis. Regarding genetic changes, mutation of p53, and deletion of p16 have been reported in ATL. Usually, ATL cases with genetic changes in p53 and p16 have a poor prognosis (Yamada *et al.*, 1997). Therefore, these genetic changes are thought to be associated with disease progression. A transcriptional profile of ATL cells by DNA chip analysis identified aberrantly transcribed genes (Sasaki *et al.*, 2005). Among them, the expression of *tumor suppressor in lung cancer 1 (TSLC1)* gene is upregulated in ATL cells. *TSLC1* gene was identified as a tumor suppressor gene in lung cancer cells. Since this molecule is associated with cell adhesion, loss of its expression is associated with the invasive phenotype of lung cancer cells (Kuramochi *et al.*, 2001). However, its ectopic expression is associated with leukemogenesis possibly due to the conferring of an adhesive phenotype to ATL cells.

Epigenetic changes are recognized as mechanisms implicated in oncogenesis as well as genetic changes. Since genetic changes in specific genes in ATL cells have not been identified except for p53 and p16, and there is no consistent chromosomal change, it is possible that epigenetic changes such as DNA methylation play an important role in leukemogenesis by inhibiting the transcription of tumor suppressor genes or inducing aberrant expression of oncogenes. Aberrantly methylated DNA regions were identified by a methylated CpG-island amplification/representational difference analysis method (Toyota *et al.*, 1999). *MELIS* gene was hypomethylated and aberrantly expressed in ATL cells (Yoshida *et al.*, 2004). Since such expression conferred the resistance against TGF- β , the *MELIS* gene expression is implicated in resistance of ATL cells to TGF- β . On the other hand, *EGR3* gene has been demonstrated to be hypermethylated in ATL cells (Yasunaga *et al.*, 2004). *EGR3* is a transcriptional factor, which is essential for transcription of the *FasL* gene (Mittelstadt and Ashwell, 1998). Normal activated T lymphocytes express FasL as well as Fas antigen. Apoptosis induced by autocrine mechanisms is designated activation-induced cell death (AICD) and this controls the immune response (Krueger *et al.*, 2003). Although ATL cells express Fas antigen, they do not produce FasL, thereby enabling ATL cells to escape from AICD. Suppressed transcription of *EGR3* gene might be the mechanism allowing ATL cells to escape from AICD. Thus, epigenetic changes of the genome play an important role in oncogenesis of ATL.