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成人T細胞性白血病（ATL）をモデルとしたウイルス感染関連がんに対する革新的治療法の開発に関する研究（若手医師・協力者活用に要する研究）

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総括研究報告書

成人T細胞性白血病(ATL)をモデルとしたウイルス感染関連がんに対する革新的治療法の開発(若手医師・協力者活用に要する研究)に関する研究

主任研究者:松岡 雅雄　京都大学ウイルス研究所 教授

研究要旨

ヒトT細胞白血病ウイルスI型は長い潜伏期間の後に一部のキャリアに成人T細胞白血病(ATL)を発症させるが、ATLに対しては強力な化学療法によっても平均生存期間は1年に過ぎない。ATL に対する移植療法の有効性が明らかになりつつあり新たな展開がもたらされている。本研究ではATLに対する骨髄非破壊的移植療法(ミニ移植)の有効性との関連で ATL 細胞の分子生物学的解析を行い、ミニ移植有効性の分子基盤を明らかにすることを目的した。ゲノムに組み込まれたHTLV-Iプロウイルスは ATL 細胞に残る唯一の感染の証拠であり、HTLV-Iプロウイルスの解析は残存ATL細胞の検出のみならずウイルス遺伝子と移植療法の関連を明らかにすることが期待される。今回、移植後にドナーHTLV-I感染細胞に由来するATLが発症した症例の解析から、宿主免疫能の重要性、tax 遺伝子発現の意義が示された。またミニ移植症例の解析から tax 遺伝子の発現が障害されている症例で移植後に再発が認められており移植後の抗ウイルス免疫の重要性が示唆された。

A. 採択された研究事業での研究概要

本研究では難治性血液悪性腫瘍である成人 T 細胞白血病(adult T-cell leukemia: ATL)に対して骨髄非破壊的移植療法(ミニ移植)の有効性を明らかにすると共にヒト T 細胞白血病ウイルス I 型(human T-cell leukemia virus type I: HTLV-I)の分子生物学的解析、ウイルスに対する宿主の免疫反応の解析を行うことによって移植療法有効性の分子基盤を解明し、この研究をウイルスによる悪性腫瘍に対する治療法開発のモデルとすることを目的とする。京都大学ウイルス研究所ではATL細胞におけるHTLV-Iプロウイルスの分子生物学的解析を行うと共に残存腫瘍細胞検出系を開発し、ATLの治療法の確立に向けた臨床研究を研究班内の研究グループと共に展開する。本研究班は、ATL の多発地域である鹿児島、長崎、沖縄など九州の主要施設に、国立がんセンター、東京医科歯科大学、京都大学の研究者などを加えた全国的かつ特徴的な組織であり、エビデンスに基づく ATL 治療法の確立と標準化を図る。ATL の分子生物学的解析を行い、ミニ移植の有効性の分子基盤を明らかにする臨床研究である。移植後にドナーのHTLV-I感染細胞に由来するATLが発症した症例の解析から、宿主免疫能の重要性、tax 遺伝子発現の意義が示された。また移植症例の解析から tax 遺伝子の発現が障害されている症例で移植後に再発が認められ、移植後の抗ウイルス免疫の重要性が示唆された。

B. 採択された研究事業での研究実績

1)移植後、早期にドナー由来ATLが発症した症例の解析:再発したリンパ腫型 ATL 患者に HLA 一致同胞 (HTLV-I 陽性者)からの移植を行った。移植後に ATL が再発したが、この時の T リンパ球のキメリズム解析でドナー型であることが示された。この ATL 細胞における HTLV-I プロウイルスを解析したところ、完全なHTLV-Iプロウイルス1コピーがゲノムに組み込まれていた。組み込み部位を使った腫瘍特異的PCRにより腫瘍細胞の検出を行った所、移植後3週間でATL細胞が出現していることが示された。また、ドナー体内におけるこの HTLV-I 感染クローンの存在を解析したところドナー末梢血単核球中に、このクローンの存在が証明された。この結果より、移植患者でATLとなった HTLV-I 感染細胞はドナー体内

に存在しており、移植に伴いレシピエント体内に移入されATLとなったことが示された。また、このATL細胞のプロウイルスDNAメチル化の解析からプロウイルス内部にDNAメチル化の蓄積を認めた。このため移植後に新規感染によりドナー由来リンパ球に感染が起こったのではなく、ドナー体内にある期間存在していたHTLV-I感染細胞が移植後、早期にレシピエント体内でATLとなったことが示唆された。

2）移植症例の解析：移植症例の内、5'側LTRの欠損した2型欠損型プロウイルスを有するものを同定した。このような場合、組み込み部位によってはゲノムに存在する細胞側遺伝子プロモーターをプロウイルスがトラップしている可能性がある。解析できたケースでは、プロウイルスは細胞側プロモーターをトラップしていなかった。このため本症例ではtax遺伝子の発現はないことが予想された。本症例は移植後にATLを再発していた。

（倫理面への配慮）

本研究は各施設の倫理委員会、京都大学「医の倫理委員会」の承認の下に行われている。

C. 考察

ウイルス発がんと宿主免疫は密接な関係があり、移植に伴う免疫抑制によりウイルス発がんが促進されることはEpstein-Barr virus (EBV), Kaposi-sarcoma associated herpesvirus（KSHV）などで報告されている。また後天性免疫不全症候群では、EBV, KSHVによる発がんだけでなくHPVによる子宮頸癌などの発症促進も報告されている。これは免疫系破壊により腫瘍細胞の増殖抑制が無くなることが大きな原因として考えられている。慢性腎不全のHTLV-Iキャリアに腎臓移植を行った後にATL発症促進が報告されている。本症例はHTLV-Iキャリアドナーからの移植でドナー感染細胞が短期間に腫瘍となっている。ドナーにはATLの発症は認められず、ドナー体内では免疫系により増殖がコントロールされていたHTLV-I感染細胞クローンが移植という免疫抑制状態で短期間に悪性化したものと理解される。このような免疫抑制状態では免疫原性の高いTaxの発現が抑制を受けないことが予測される。本症例のATL細胞はtax遺伝子の発現が認められており、この仮定と一致する所見である。

移植ATL症例での解析から、殆どの症例ではtax遺伝子を発現できるプロウイルスを有していた。しかし、5'側LTRが欠損するプロウイルス（2型欠損型）を有している症例も存在した。この内、1例で組み込み部位の同定でtax遺伝子の発現ができない構造であることが確認された。この症例は、移植後にATLが再発しており、抗ウイルス免疫反応がATL再発を抑制していることを示しているのかもしれない。今後、症例の蓄積によりウイルス遺伝子発現と移植療法の関連が明らかになることが期待される。

D. 健康危険情報

特になし

E. その他実施した臨床研究・治験の概要及び実績

なし

研究成果の刊行に関する一覧表

主任研究者：京都大学ウイルス研究所　松岡　雅雄

書籍

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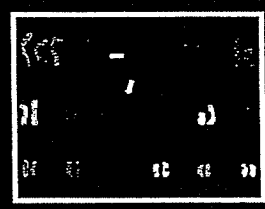
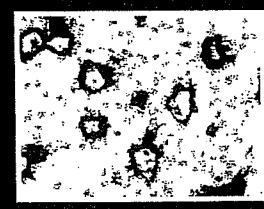
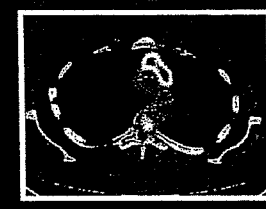




THE Lymphomas

SECOND EDITION

GEORGE P. CANELLOS

T. ANDREW LISTER

BRYAN YOUNG

SAUNDERS

ELSEVIER

29

Human T-Cell Leukemia Virus Type I

Masao Matsuoka, M.D., Ph.D.

Human T-cell leukemia virus type I (HTLV-I) is the first human retrovirus shown to be linked with human disease. In 1977, adult T-cell leukemia/lymphoma (ATL) was proposed as a distinct clinical entity from its unique geographic distribution and clinical features.[1] Then, its causative retrovirus, HTLV-I, was identified in the human T-cell line,[2] which finally clarified that HTLV-I was the causative virus of ATL.[3] The discovery of HTLV-I leads to further characterization of ATL and HTLV-I infection, and discloses the virologic mechanism of transformation.

VIROLOGY OF HTLV-I

HTLV-I belongs to the delta-type retroviruses, which also include bovine leukemia virus (BLV), human T-cell leukemia virus type II (HTLV-II), and simian T-cell leukemia virus (STLV).[4] BLV and STLV have also been associated with neoplastic diseases as well as HTLV-I. The structure of HTLV-I provirus is similar to other retroviruses that contain *gag*, *pol*, and *env* genes flanked by long terminal repeat (LTR) sequences at both ends.[5] A unique structure was found between *env* and the 3'-LTR, denoted the pX region, which encodes the regulatory proteins, $p40^{tax}$ (Tax), $p27^{rex}$ (Rex), p12, p13, p30, p21, and HBZ (Fig. 29–1). The presence of accessory genes is the characteristics of delta-type retroviruses as well as foamy virus and lentivirus including human immunodeficiency virus type 1 (HIV-1).

Function of Tax

Among these accessory proteins encoded by HTLV-I, Tax protein plays a central role in the proliferation of infected cells and the leukemogenesis because of its pleiotropic actions (Fig. 29–2).[6] Tax potently increases the expression of viral genes through the viral LTR and stimulates the transcription of cellular genes through cellular signaling pathways of NF-κB, CREB, SRF, and AP-1. Tax does not bind to promoter or enhancer sequences by itself, but it interacts with cellular proteins that are transcriptional factors or modulators of cellular functions.

Transcriptional Activation

Tax can activate the NF-κB pathway by interacting with IKKγ. IKKα, β, and γ form a 700-kDa complex in which IKKγ functionally adapts Tax into this large complex.[7] The activated complex phosphorylates IκB, which detach NF-κB, result in activation of NF-κB. Among the various functions of the Tax protein, activation of NF-κB has been shown to be essential to transformation by HTLV-I.[8] Activation of the NF-κB pathway induces transcription of various cytokines and their receptor genes as well as numerous genes associated with apoptosis and the cell cycle. For example, Tax can activate the transcription of interleukin (IL)-2Rα and IL-2 genes through the NF-κB pathway. In addition, the transcription of IL-6, IL-15, and GM-CSF genes can be activated by the Tax protein via NF-κB. Such activation of genes associated with cell proliferation seems to be involved in the growth of HTLV-I–infected cells both in vitro and in vivo. Tax can also induce expression of Bcl-xL via activation of NF-κB, which renders ATL cells apoptosis-resistant.[9] As well as HTLV-I–transformed cell lines, increased expression of Bcl-xL was observed in fresh ATL cells,[10] which may account for the resistance of ATL cells to chemotherapy.

For activation of the viral LTR, Tax requires at least two 21-bp enhancers containing an imperfect cAMP-responsive element bound to a cyclicAMP response element binding protein (CREB).[11] Tax can bind to both CREB- and CREB-binding protein (CBP), the latter of which is a transcriptional co-activator.[12] Under physiologic conditions, only phosphorylated CREB induced by stimulation can bind to CBP. Tax shunts this pathway, resulting in stimulation-independent activation of the CREB pathway. CBP acetylates histone and opens the nucleosome structure around the transcriptional site.

Transcriptional Repression

Conversely, Tax can trans-repress transcription of certain genes, such as DNA polymerase β, *lck*, *p18*, and *p53* genes. For trans-repression of *p18* gene transcription, the E-box, which binds to transcriptional factor E47, is critical. Tax protein itself could not bind to E-box or E47, but interferes with binding of E47 to the transcriptional co-activator, p300, resulting in repression of transcription.[13] p53-dependent transcription is also repressed by Tax protein. Similar to trans-repression of p18, Tax does not bind to the p53- or p53-binding site, but rather inhibits the recruitment of CBP to p53 on the p53-binding sites.[13] This mechanism of trans-repression contrasts with that of trans-activation of the CREB pathway by Tax protein. Although both mechanisms depend on binding the Tax protein to the transcriptional activator, CBP/p300, their effect on transcription is quite different.[6]

Functional Inhibition

Apart from transcriptional regulation, Tax can influence the function of cellular factors. Tax protein can interact with a negative inhibitor of cyclin-dependent kinase (CDK) 4, $p16^{INK4a}$, via its ankyrin motif, and impair its function.[14] Since $p16^{INK4a}$ is an inhibitor of CDK4, its functional inactivation leads to activation of CDK4/6, phosphorylation of Rb, and finally G1/S transition.

Transforming growth factor β (TGF-β) is an inhibitory cytokine that plays important roles in development, the

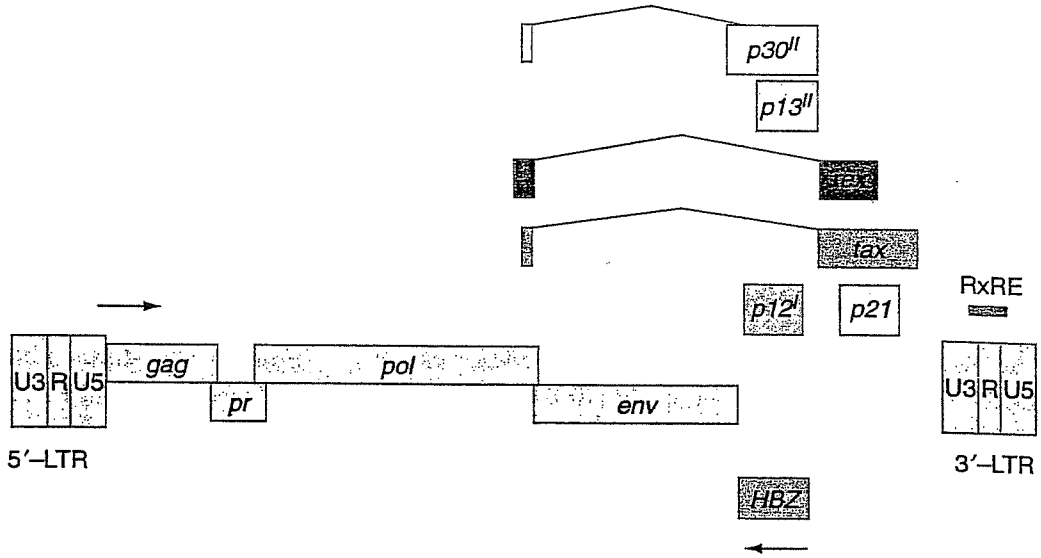


Figure 29–1. Structure of HTLV-I provirus and its encoding genes. The structure of HTLV-I provirus genome, and its encoded accessory gene have been shown. *Arrows* indicate orientation of viral gene transcription. Pr, protease.

immune system, and oncogenesis. Since TGF-β generally suppresses the growth of tumor cells, most tumor cells acquire escape mechanisms to inhibit signaling from TGF-β, which include mutation of its receptor and mutation of Smad molecules that transduce the signal from the receptor. Tax is also reported to inhibit the signal of TGF-β by its binding to Smad2, 3, and 4, or CBP/p300.[15,16] Inhibition of TGF-β signaling enables HTLV-I–infected cells to escape TGF-β–mediated growth inhibition.

ATL cells are well known to show remarkable chromosomal abnormalities, which are thought to reflect chromosomal instability. Tax has been reported to interact with the checkpoint protein, MAD1, which forms a complex with MAD2 and controls the mitotic checkpoint. The functional hindrance of MAD1 by Tax protein causes chromosomal instability, suggesting the involvement of this mechanism in oncogenesis.[17]

Rex

Rex acts at the post-transcriptional level to regulate viral gene expression, which enhances the expression of the unspliced *gag/pol* and singly spliced *env* transcripts, and decreases *tax/rex* mRNAs.[6] Therefore, Rex is a negative regulator for viral transcription by inhibiting the expression of the *tax* gene. Rex binds to the Rex-responsive elements (RxRE), which is located in the 3'-LTR (R/U3 region) (Fig. 29–1), which mediates the nuclear transport of unspliced viral mRNA, and also regulates RNA processing.

p30

p30 interacts with the transcriptional co-activator, CBP/p300, which competes with Tax at 5'-LTR, resulting in reduced viral gene expression. In addition, p30 specifically binds to *tax/rex* mRMA, and retains it in the nucleus. Thus,

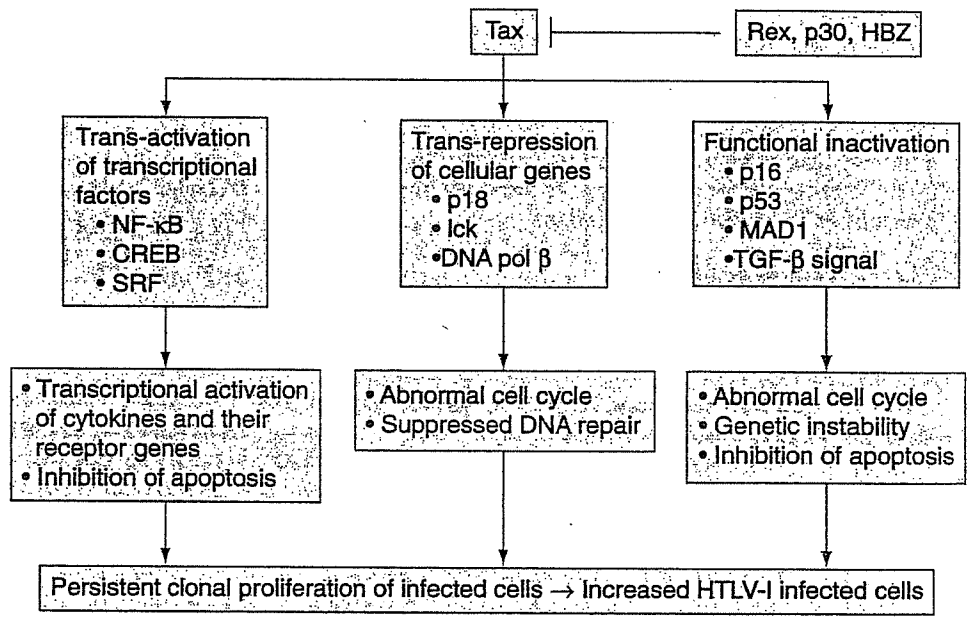


Figure 29–2. Pleiotropic actions of Tax. Pleiotropic actions of Tax proteins are summarized. Expression of Tax is also down-regulated by Rex, p30, and HBZ.

p30 reduces the production of Tax protein by a post-transcriptional mechanism, leading to suppression of viral gene transcription.[18]

HBZ

HBZ, which is encoded by the complementary strand of HTLV-I (Fig. 29–1), binds to c-Jun and JunB, and decreases the DNA-binding activity of these transcriptional factors,[19] which in turn reduces the viral transcription from LTR. This finding shows that HBZ is another negative regulator for virus gene expression.

p12

Open reading frame (ORF) 1 of the pX region encodes p12 (Fig. 29–1), which is present in the endoplastimic reticulum and Golgi apparatus. p12 has been shown to play an important role in establishment of HTLV-I infection and optimal viral infectivity in vivo and quiescent primary lymphocytes.[20,21] As a mechanism, p12 has been shown to interact with calreticulin and calnexin, and increase cytoplasmic calcium, leading to NFAT activation in T lymphocytes.[22] With this action, p12 facilitates host cell activation and establishment of persistent infection. Although the expression of p12 protein in vivo has remained obscure, cytotoxic T lymphocytes (CTLs) against p12 have been demonstrated in individuals infected with HTLV-I, indicating that p12 protein is expressed in vivo.[23]

p13

p13 is gene product encoded by ORFII of pX region, and selectively targeted to mitochondria, which is inserted in the inner mitochondrial membrane.[24] The presence of cytotoxic T cells against p13 in the carriers shows its expression in vivo.[23] Expression of p13 suppresses the growth of tumor cells and also increases the sensitivity to Ca2+-mediated stimuli.[25] The function of p13 in viral replication and cell proliferation needs further study.

Thus, HTLV-I has redundant strategies to increase infected cells through its encoded proteins, Tax and p12, which promote cell proliferation, inhibit apoptosis, and increase genetic and chromosomal instability (Fig. 29–2). With these strategies, HTLV-I increases its copies in vivo by promoting the proliferation and survival of infected cells, and causes ATL as a consequence of the strategies. On the other hand, Tax is also a major target recognized by cytotoxic T lymphocytes (CTL) in vivo. To suppress the *tax* gene expression, HTLV-I also has redundant mechanisms to suppress the expression and function of Tax by Rex, p30, and HBZ as described above, which reduce viral gene expression and virus production. Such mechanism enables HTLV-I–infected cells to suppress viral gene expression and escape from the host immune system.

EPIDEMIOLOGY OF HTLV-I

On a global basis, 10 million to 20 million people are estimated to be infected with HTLV-I. HTLV-I 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 revealed high seroprevalence rates in Melanesia, Papua New Guinea, and the Solomon Islands, and among Australian aborigines.[26] In Japan, approximately 1.2 million individuals were estimated to be infected by HTLV-I, and more than 800 cases of ATL are diagnosed each year.[27] The cumulative risk of ATL among HTLV-I carriers in Japan was estimated at about 6.6% for men and 2.1% for women, indicating that most of HTLV-I carriers are asymptomatic throughout their lives.[28]

TRANSMISSION OF HTLV-I

HTLV-I is transmitted by three routes: (1) mother to infant (via breast milk); (2) horizontal (sexual); and (3) parenteral (blood transfusion or intravenous drug use). Infected cells are essential for transmission of HTLV-I via any of these routes, which has been demonstrated by the absence of seroconvertors among recipients of fresh frozen plasma transfusions.[29] The transmission efficiency of free virion is estimated to be 1 in 10^5 to 10^6 virion, whereas cell-to-cell transmission is much more efficient.[30] Thus, transmission of HTLV-I requires cell-to-cell contact. Such contact induces polarizations of the cytoskeleton of an infected cell to the cell-to-cell junction (virologic synapse), and then Gag protein complexes containing the virus genome accumulate at this junction, finally leading to the transfer of these complexes to uninfected cells.[31] This mechanism can explain why cell-to-cell contact is essential to transmission of HTLV-I.

HTLV-I can infect various cell types, including B lymphocytes, dendritic cells, fibroblast, rat cells, and mouse cells, indicating that its receptor is ubiquitously expressed on cell surfaces. Glucose transporter, GLUT-1, has been identified as a receptor of HTLV-I,[32] which also proved that the receptor for HTLV-I is ubiquitously expressed. However, after transmission in vivo, HTLV-I provirus was predominantly found in the CD4-positive T lymphocytes.[33] This suggests that HTLV-I could increase the number of infected CD4-positive T lymphocytes in vivo after infection, coinciding with the finding that HTLV-I can transform only T lymphocytes in vitro, most of which were CD4-positive. Even a retrovirus vector expressing only Tax could transform CD4-positive T lymphocytes in vitro.[34] Taken together, these findings suggest that Tax promotes proliferation, and inhibits apoptosis, of HTLV-I–infected CD4-positive T lymphocytes in vivo.

IMMUNOLOGICAL CONTROLS OF HTLV-I INFECTION

Although HTLV-I can promote the proliferation of infected cells by viral gene products such as Tax, it also induces the immune response to HTLD-1 to eliminate the infected cells (Fig. 29–3).

Immune Response Against Tax Protein

Among viral proteins, Tax has been shown to be a major target of CTLs in vivo.[35] Therefore, Tax-expressing cells are considered to be eliminated in vivo. Indeed, depletion of CD8-positive T lymphocytes from the peripheral blood

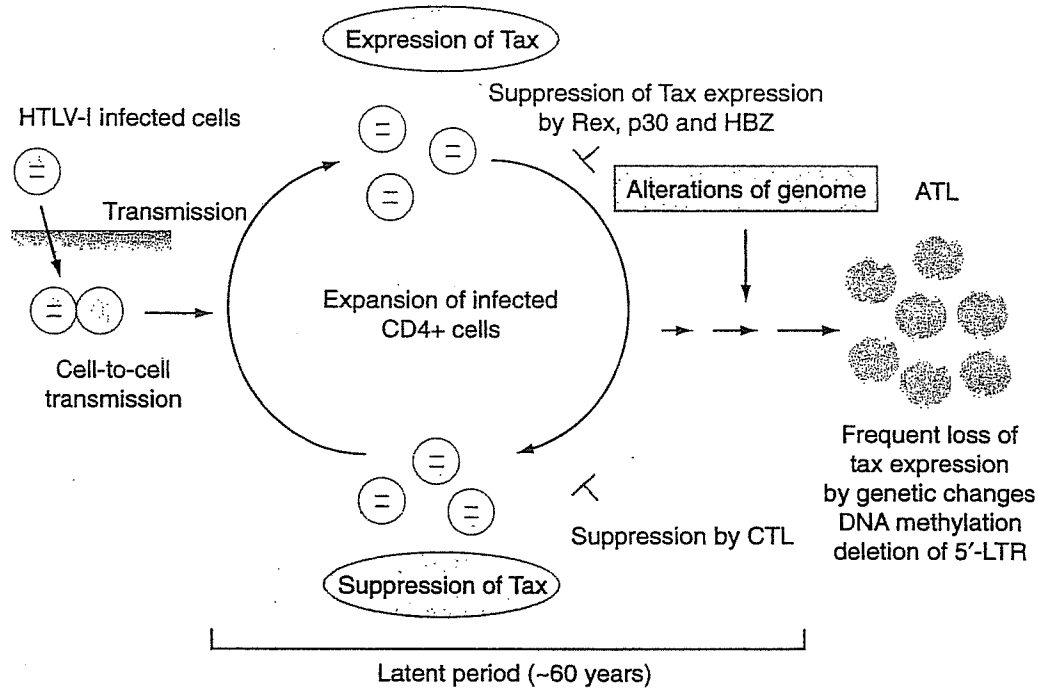


Figure 29–3. Natural course from the infection of HTLV-I to onset of ATL. HTLV-I is transmitted via three routes, and infected cells are necessary in all three. After infection, HTLV-I promotes clonal proliferation of infected cells by pleiotropic actions of Tax. Proliferation of HTLV-I–infected cells 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.

mononuclear cells of HTLV-I–infected individuals in vitro promoted Tax expression in the CD4-positive subpopulation, indicating that CD8-positive CTLs suppressed Tax expression in vivo.[36] Thus, the survival of HTLV-I–infected cells depends on the balance of proliferative actions of Tax and the host immune system. These findings suggest that in asymptomatic carriers, CTLs against HTLV-I can control the growth of cells carrying the HTLV-I provirus, resulting in preventing the development of ATL.

Immunodeficiency and Development of ATL

Opportunistic infections such as fungal, protozoal, and viral infections are common in patients with ATL due to the inevitable impairment of T-cell function. To a lesser extent, impaired cell-mediated immunity has also been demonstrated in HTLV-I carriers. Such immunodeficiency in the carrier state might be associated with leukemogenesis of ATL by allowing proliferation of HTLV-I–infected cells. A prospective study of HTLV-I–infected individuals identified carriers who later developed ATL and showed that the anti-Tax antibody level was low in all ATL cases for up to 10 years preceding their diagnosis. This finding indicates that HTLV-I carriers with a higher anti–HTLV-I titer, which is roughly correlated with HTLV-I provirus load, and a lower anti-Tax reactivity may be at greatest risk of ATL.[37] The levels of anti–HTLV-I antibody and soluble IL-2 receptor (sIL-2R) have been shown to be correlated with HTLV-I provirus load,[38] and high antibody titers, and high sIL-2R were risk factors for developing ATL among carriers.[39] Taken together, these findings suggest that a higher proliferation of HTLV-I–infected cells, and a low immune response against Tax, might be associated with the onset of ATL. Given this finding, potentiation of CTLs against Tax by a vaccine strategy might be useful in preventing the onset of ATL.[40]

EBV-associated lymphomas frequently develop in individuals with an immunodeficient state associated with transplantation or AIDS. Does such an immunodeficient state, which abrogates the immune function suppressing HTLV-I–infected cells, affect the onset of ATL? Among 24 patients with post-transplantation lymphoproliferative disorders (PT-LPD) after renal transplantation in Japan, five cases of ATL have been reported. Considering that most of PT-LPD is of B-cell origin in Western countries, this frequency of ATL was quite high. Although high seroprevalence of HTLV-I is due to blood transfusion during hemodialysis, the immunodeficient state during renal transplantation apparently promotes the onset of ATL.[41]

Impaired cell-mediated immunity, such as suppressed T-cell response to EBV, and seronegativity against purified protein derivative, has been reported in HTLV-I carriers, also indicating relationship to immunodeficiency. One mechanism of immunodeficiency is that HTLV-I infects CD8-positive T lymphocytes, which might impair the function of CD8-positive T lymphocytes.[33] 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.[42] Fratricide among virus-specific CTLs could impair the immune control of HTLV-I. Another mechanism of immunodeficiency is based on the observation that the number of naive T cells decreased in individuals infected with HTLV-I via decreased thymopoiesis.[33]

HTLV-I–INFECTED CELLS IN VIVO

HTLV-I provirus load, which is correlated with the number of HTLV-I–infected cells, varied by more than 100-fold among HTLV-I carriers. When the sequential DNA samples from peripheral blood mononuclear cells of the same HTLV-I carriers who were followed in a cohort study were analyzed, provirus loads fluctuated only 2- to 4-fold in most

carriers, showing that provirus loads were relatively constant over time for up to 7 years in individual carriers.[38] Analyses of HTLV-I provirus load in seroconverters showed that the same virus sequences were identified in the married couples (infected persons transmitted virus to partners); however, their provirus loads were quite different, suggesting that provirus load was determined not by HTLV-I itself, but rather by host factors.[43] One of the candidates for such host factors is the immune response, especially CTLs against HTLV-I, which control the number of HTLV-I–infected cells (Fig. 29–3).[44]

Clonal Expansion of HTLV-I–Infected Cells

Since HTLV-I provirus is randomly integrated in the host genome,[45] the integration site is specific to each HTLV-I–infected cell. When integration sites of HTLV-I provirus were identified in infected cells by inverse PCR, HTLV-I–infected cells clonally proliferated in carriers, some clones were shown to persist for a long time in the same individuals.[46,47] Most of these persistent clones were CD4-positive lymphocytes, which is consistent with the finding that HTLV-I predominantly immortalizes CD4-positive T lymphocytes in vitro.

The HTLV-I provirus is genetically stable, especially compared with the other major human retrovirus, human immunodeficiency virus (HIV). It has been postulated that increased HTLV-I load is achieved not by virus replication, but by clonal proliferation of infected cells. Since reverse transcriptase is an error-prone DNA polymerase, a higher replication rate generates the vast diversity in the virus genome. In HIV, a higher rate of mutation generated in the viral replication results in acquisition of drug resistance, and escape from the host immune system. On the other hand, HTLV-I increases its copies mainly by proliferation of HTLV-I–infected cells. In such situation, HTLV-I provirus in the host cells is replicated by cellular DNA polymerase with proofreading activity. Therefore, HTLV-I provirus is genetically stable in striking contrast to HIV-I. Such clonal expansion of HTLV-I–infected cells is directly associated with the onset of ATL (Fig. 29–3).

However, the reverse transcriptase (RT) inhibitor inhibits the replication of HTLV-I in vitro,[48] and in addition, in vivo administration of RT inhibitor also suppresses the provirus load.[49] This finding shows the possibility that HTLV-I replicates in vivo, although it remains unknown how much such replication can account for provirus load in vivo.

GENETIC FACTORS AFFECTING PROVIRUS LOAD AND SUSCEPTIBILITY TO HTLV-I–ASSOCIATED DISEASES

Cellular immunity, including MHC molecules, influences the provirus load in HTLV-I–infected individuals. In addition, other polymorphisms of genes might influence the provirus load. For example, although the higher provirus load is associated with the risk of HAM/TSP, polymorphism of the TNF gene (*TNF-863A*) increases susceptibility to the disease, whereas SDF-1 (*SDF-1 +801A* 3'UTR), and IL-15 (*IL-15 191C*) gene polymorphism are protective at onset.[50] Among these polymorphisms, *IL-15 191C* has been implicated in reducing the provirus load. Familial clustering of ATL patients has been reported, suggesting predisposing genetic factors. Polymorphism of tumor necrosis factor α (TNF-α) has been shown to be associated with ATL in comparison with asymptomatic carriers, suggesting that genetic polymorphism, which increases the production of TNF-α, is associated with susceptibility to ATL.[51] Such genetic analysis utilizing rapidly accumulating knowledge and developing technology will clarify the genetic basis of the familial clustering of ATL patients.

ADULT T-CELL LEUKEMIA/LYMPHOMA

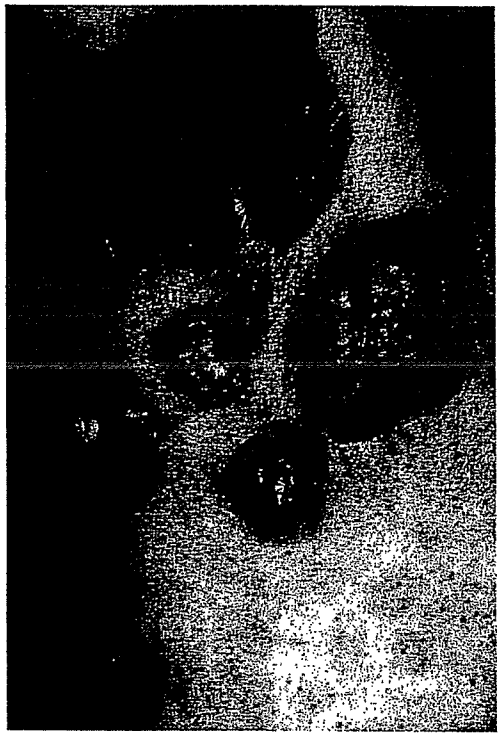
Clinical Features of ATL

The onset of ATL is slightly more common in males (male-to-female ratio is 1.16:1). However, since female carriers are predominant in HTLV-I infection, the risk for developing ATL is three times higher for males. Average age at the onset of ATL is 60 years in Japan. On the other hand, the average age of Caribbean and African ATL patients is about 43 years, suggesting that the onset is influenced by genetic or environmental factors.[52]

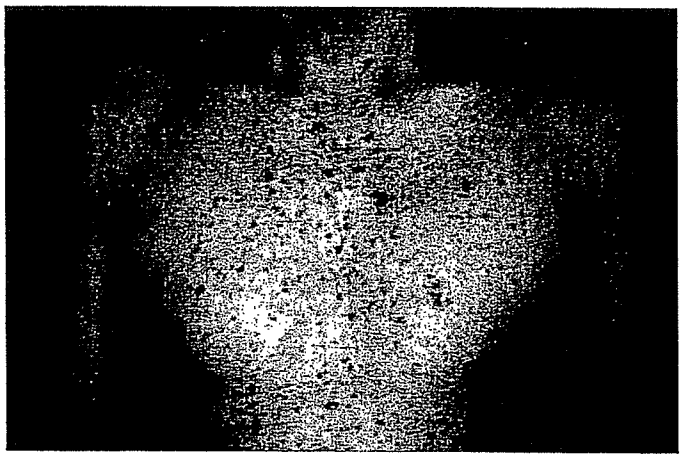
The findings at onset are abdominal pain, diarrhea, pleural effusion, ascites, cough, sputum, and an abnormal shadow on chest x-ray films. Predominant physical findings are peripheral lymph node enlargement (72%), hepatomegaly (47%), splenomegaly (25%), and skin lesions (53%).[52] ATL cells tend to infiltrate into various organs/tissues, including skin, liver, lung, gastrointestinal tract, central nervous system, and bone. Various skin lesions, such as papules, erythema, and nodules are frequently observed in ATL patients (Fig. 29–4). In the skin, ATL cells densely infiltrate the dermis and epidermis, forming Pautrier's microabscesses in the epidermis (Fig. 29–5). In the bone, infiltration of ATL cells causes punched-out lesions (Fig. 29–6). Pulmonary complication is also frequently observed in patients with ATL, which includes leukemic infiltration (Fig. 29–7) and pulmonary infections. The high frequency of hypercalcemia is the most striking feature of ATL; about 70% of ATL patients have high serum Ca2+ levels during the clinical course of the disease, particularly during the aggressive stage of ATL. In the bone of ATL patients with hypercalcemia, the number of activated osteoclasts increase (Fig. 29–8).

White blood cell (WBC) count ranges from normal to 500×10^9/L. Blood involvement is frequently observed in patients with ATL, and leukemic cells in peripheral blood resemble Sézary cells, having indented or lobulated nuclei (Fig. 29–9). Since infiltration of ATL cells into bone marrow is usually not so dense, anemia and thrombocytopenia are rare. Eosinophilia is frequently observed in ATL patients, as well as other T-cell malignancies.

Serum lactate dehydrogenase (LDH) is elevated in most ATL patients, and higher LDH levels indicate an advanced or aggressive disease state. Thus, serum calcium and LDH levels reflect the extent of disease, and are useful for monitoring the remaining tumor or disease activity. Hyper-



A



B

Figure 29–4. Skin lesions in ATL patients. Skin lesions observed in ATL patients are variable. Tumor formation is common among skin involvement in ATL patients (A). Papule is also observed in a patient with acute ATL (B). (See color insert.)

bilirubinemia, observed when ATL cells infiltrate the liver, indicates a poor prognosis. Hypergammaglobulinemia is very rare in ATL, which is consistent with the fact that ATL cells have suppressor-inducer activity to immunoglobulin synthesis in vitro. ATL cells are known to express interleukin 2 (IL-2) receptor alpha chain on their surfaces, and also secrete its soluble forms. Therefore, levels of SIL-2R are elevated in the sera of patients with ATL, the levels of SIL-2R being correlated with the tumor mass and clinical course.

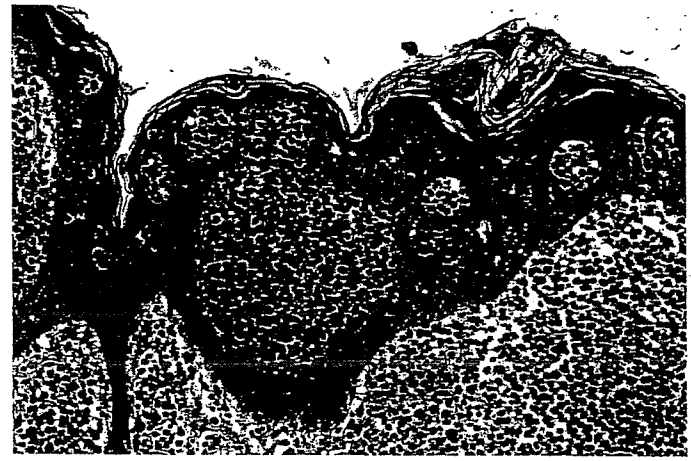


Figure 29–5. Skin involvement of ATL. ATL cells infiltrate into epidermis and form Pautrier's microabscesses. (See color insert.)

Pathogenesis of ATL

ATL is a neoplasm of activated helper T lymphocytes, which elaborate various cytokines, and express the immunoregulatory molecules on the surface. Such cytokines produced by ATL cells influence the pathophysiology of ATL. Eosinophilia is frequently observed in patients with ATL, which is caused by elevated IL-5. In addition, elaborated parathyroid hormone–related protein (PTH-rP) from ATL cells activates osteoclasts and promotes bone resorption, which is implicated in hypercalcemia. ATL cells produce

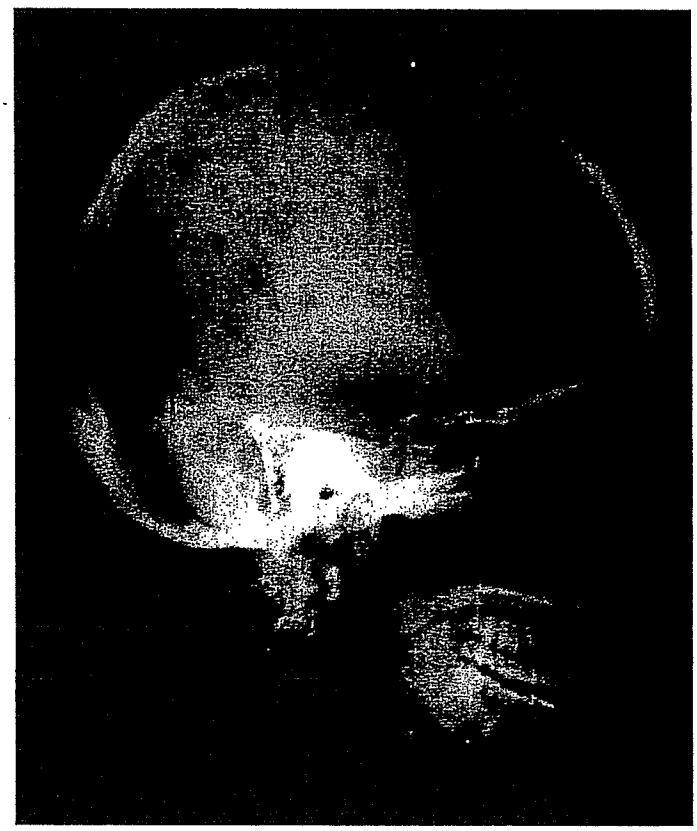


Figure 29–6. Punched-out lesions in the skull of an acute ATL patient.

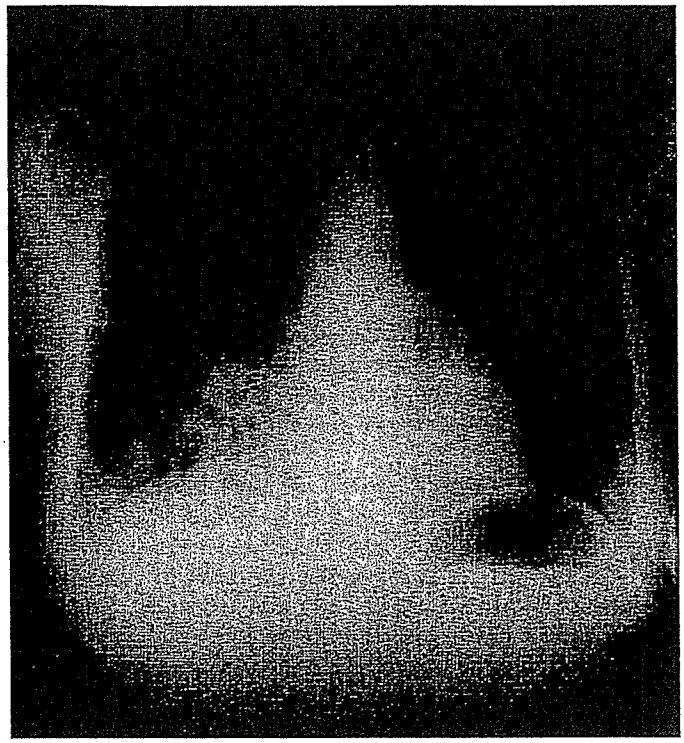


Figure 29–7. Pulmonary infiltration of ATL cells.

other cytokines, including TGF-β, IL-10, IL-8, and M-CSF, which modify the pathogenesis of ATL.

Hypercalcemia is more severe in patients with ATL than those with other hematologic malignancies. Several pathologic studies of ATL patients with hypercalcemia have indicated that high serum Ca2+ levels are due to an increased number of osteoclasts and accelerated bone resorption (Fig. 29–8). Bone is constitutively remodeled by osteoblasts (matrix synthesis) and osteoclasts (bone resorption). Osteoclasts are derived from hematopoietic precursor cells and belong to the monocyte macrophage lineage. During differentiation of osteoclasts, precursor cells sequentially express c-Fms (receptor of M-CSF) followed by receptor activator nuclear factor κB (RANK).[53] M-CSF and RANK

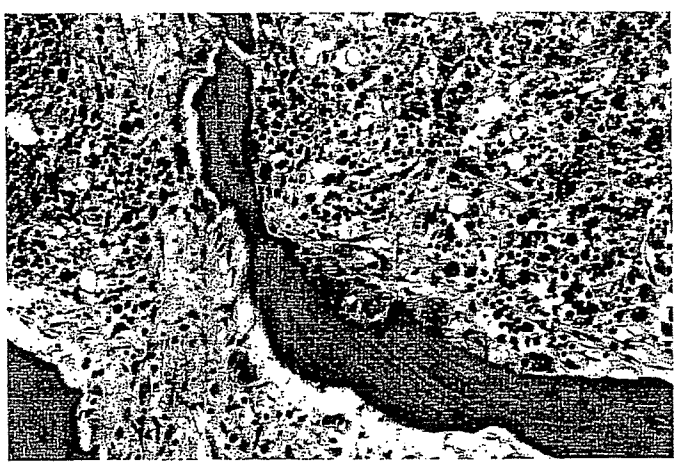


Figure 29–8. Increased osteoclasts in a hypercalcemic ATL patient. In a hypercalcemic patient, the number of osteoclast increased in the bone, which accelerated bone resorption. (See color insert.)

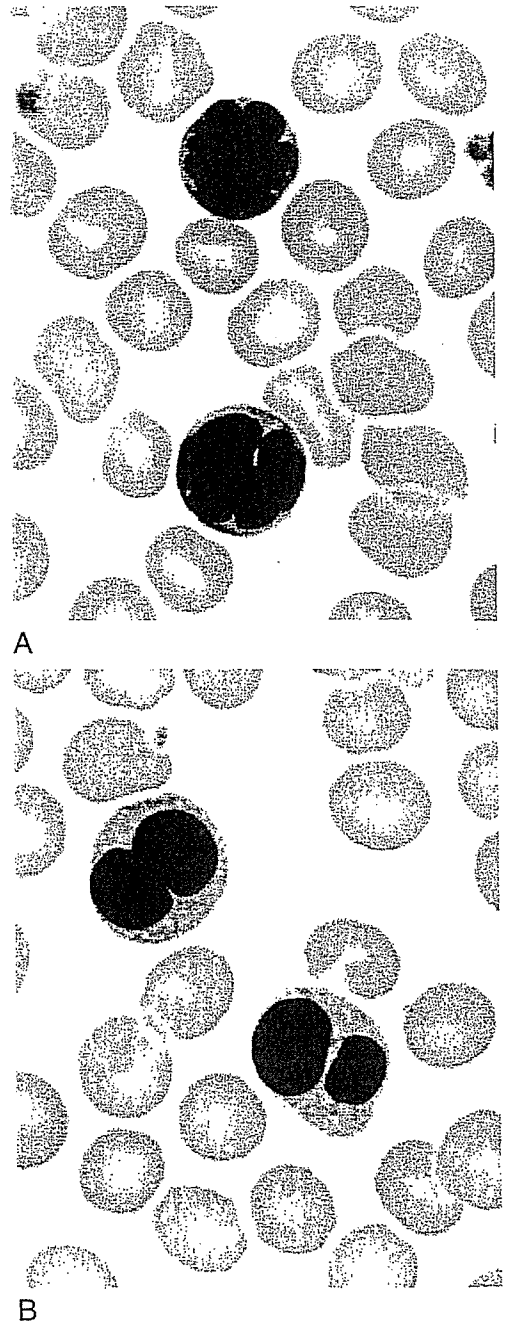


Figure 29–9. Cell morphology of ATL cells. ATL cells from an acute ATL (A) and a chronic ATL (B) are shown. (See color insert.)

ligand (RANKL) have been shown to be critical factors for the differentiation of osteoclasts, which are physiologically produced by stromal cells and osteoblasts. ATL cells from patients with hypercalcemia, which highly expressed the transcripts of the RANKL gene, induced the differentiation of hematopoietic precursor cells into osteoclasts in vitro in the presence of M-CSF.[54] It showed that RANKL expressed on ATL cells induced the differentiation into osteoclasts, resulting in increased number of osteoclasts and hypercalcemia in cooperation with PTH-rP. Thus, the immunoregulatory molecules on the ATL cells modify the pathogenesis.

Chemokines and their receptors have been implicated in the migration and tissue localization of lymphocytes. ATL

cells are well known to infiltrate into various organs or tissues, frequently invading skin or lymphoid tissues, which suggests that the differential expression of chemokine receptors might determine the migration of ATL cells. Analysis of chemokine receptor expression revealed that CCR4 was frequently expressed on HTLV-I–transformed cell lines and fresh ATL cells.[55] CCR4-positive T lymphocytes contain skin-seeking memory T cells, suggesting that expression of CCR4 accounts for frequent infiltration of ATL cells into skin. On the other hand, expression of CCR7 was reported to be associated with involvement of lymphoid tissues and lymph node enlargement.[56]

Diagnosis of ATL

The diagnostic criteria for ATL have been defined as follows: (1) histologically and/or cytologically proven lymphoid malignancy with T-cell surface antigens; and (2) abnormal T lymphocytes present in the peripheral blood except for the lymphoma-type. These abnormal T lymphocytes include not only typical ATL cells, the so-called flower cells, but also the small and mature T lymphocytes with incised or lobulated nuclei that are characteristic of the chronic or smoldering type. Additional criteria follow: (3) antibody to HTLV-I present in the sera at diagnosis; and (4) demonstration of monoclonal integration of HTLV-I provirus by the Southern blot method.

Morphology of ATL Cells

Most ATL cells characteristically exhibit lobular division of their nuclei; most are bi- or multi-foliate, and are separated by deep indentations. Cells with such a nuclear configuration are designated as "flower cells" (Fig. 29–9A). Cells from chronic ATL are relatively uniform in size and nuclear configuration, and smaller than those seen in either acute or smoldering ATL (Fig. 29–9B). Cells in this type also exhibit lobular division of their nuclei. However, most of the lobulated nuclei are bi- or tri-foliate. Cells from the smoldering type are relatively large and lack cytoplasmic granules or vacuoles. The nuclear chromatin is in coarse strands and is deeply stained.

Serology of HTLV-I

Anti-HTLV-I antibodies are positive in almost all patients with ATL, although seronegative ATL cases have been rarely reported.[52] The presence of serum antibodies against HTLV-I can be demonstrated by enzyme-linked immunosorbence, gelatin particle hemagglutination, indirect immunofluorescence, and the Western blot method.

Immunologic Characterization of ATL Cells

Surface phenotype of typical ATL cells is positive for CD2, 3, 4, 25, and HLA-DR, and is negative for CD7 and 8, indicating that ATL cells are derived from activated helper T lymphocytes. A characteristic feature of ATL cells is the decreased expression of T-cell receptor (TCR)/CD3 complex on their surfaces. There are several reports of ATL cases with different leukemic cell phenotypes, such as CD4+ and CD8+, CD4− and CD8−, and CD4− and CD8+.[29] Most of these variant forms are observed in patients with acute ATL and indicate poor prognosis. CD95 (Fas/APO-1) antigen is highly expressed on the leukemic cells in most patients with ATL, and such ATL cells are highly susceptible to antibody against Fas antigen. On the other hand, Fas-negative ATL cases were also reported and underlying mutations of the Fas gene were identified.[52]

HTLV-I Provirus Genome in Leukemic Cells of ATL

Definitive diagnosis of ATL requires detection of monoclonal integration of HTLV-I provirus in genomic DNA from tumor cells, especially in endemic areas. Monoclonal integration of HTLV-I provirus can be detected by the Southern blot method.

Tax plays a critical role in the proliferation of HTLV-I–infected cells and leukemogenesis. However, it is a major target of cytotoxic T cells. Thus, the presence of Tax in HTLV-I–infected cells provides advantages and disadvantages for survival of HTLV-I–infected cells. Since Tax expression is not frequently detected in ATL cells,[58] they presumably acquire the ability to proliferate independent of Tax function during the leukemogenesis. ATL cells frequently lose Tax expression by several mechanisms. Although the 5′-LTR is a viral promoter for transcription of viral genes, it was reported that 5′-LTR of HTLV-I provirus was deleted in 39% of cases examined, indicating that the viral gene transcription was impaired in ATL cells with such a provirus.[59] The second mechanism is the nonsense or missense mutation of the *tax* gene in fresh ATL cells. It is noteworthy that ATL cells in some cases had mutations in the Class I MHC recognition site of the Tax protein, resulting in escape from immune recognition.[60] The third mechanism is epigenetic change of the 5′-LTR: the 5′-LTR was selectively methylated, which silenced the transcription of viral genes.[58,61] With these mechanisms, ATL cells lost Tax expression, and could escape from the host immune surveillance system. It is speculated that Tax plays an important role in persistent proliferation of HTLV-I–infected cells during the carrier state, and then genetic and epigenetic changes accumulate in the host genome mediated by mutator phenotype of Tax,[62] which finally leads to Tax-independent proliferation and escape from the host immune system by inactivation of the *tax* gene.[4]

Chromosomal Abnormalities of ATL Cells

Karyotype analyses of 107 patients with ATL revealed several chromosomal abnormalities[63]: trisomies of chromosome 3 (21%), 7 (10%), and 21 (9%); monosomy of the X chromosome (38%) in females; loss of a Y chromosome (17%) in males; structural abnormalities, including translocations involving 14q32 (28%) or 14q11 (14%), and deletion of 6q (23%). Although there is no specific chromosomal abnormality for ATL, chromosomal abnormalities accumulate during disease progression. Comparative genomic hybridization (CGH) analyses of ATL revealed gains at chromosome 14q, 7q, and 3p. Genomic alterations increase in aggressive ATL compared with indolent forms (chronic and smoldering), which indicate the marked chromosomal instability in ATL cells.[64]

Classification of ATL

Clinical features of ATL vary among patients. Therefore, ATL patients can be classified into four clinical subtypes according to the clinical features: acute, chronic, smoldering, and lymphoma type. The Lymphoma Study Group (1984–1987) in Japan proposed the following diagnostic criteria for classifying ATL into the following four subtypes[65]:

1. *Smoldering type.* Five percent or more abnormal lymphocytes of T-cell nature in peripheral blood (PB); normal lymphocyte level (<4×10⁹/L); no hypercalcemia; LDH value of up to 1.5 times the normal upper limit; no lymphadenopathy; no involvement of liver, spleen, central nervous system (CNS), bone, or gastrointestinal tract; and neither ascites nor pleural effusion. Skin and pulmonary lesion(s) may be present. In patients with less than 5% abnormal T lymphocytes in PB, at least one histologically proven skin or pulmonary lesion should be present.
2. *Chronic type.* Absolute lymphocytosis of more than 3.5×10⁹/L; LDH value up to twice the normal upper limit; no hypercalcemia; no involvement of CNS, bone, or gastrointestinal tract; and neither ascites nor pleural effusion. There may be histologically proven lymphadenopathy with or without extranodal lesions, and there may be involvement of liver, spleen, skin, and lung, and 5% or more abnormal lymphocytes.
3. *Lymphoma type.* No lymphocytosis, 1% or less abnormal lymphocytes; histologically proven lymphadenopathy with or without extranodal lesions.
4. *Acute type.* The most common subtype, highly aggressive malignancy that shows lymphadenopathy, hepatosplenomegaly, and skin lesions, but does not meet the criteria of the other types.

Treatment and Prevention of ATL

Aggressive forms of ATL (acute and lymphoma-type) are generally treated with combination chemotherapy, although long-term success has been very limited. The acute form, with hypercalcemia, high LDH levels, and an elevated white blood cell count, carries a particularly 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.[66] In the recent study by Lymphoma Study Group in Japan, the improved survival of ATL patients has been reported by a granulocyte colony–stimulating factor–supported multiagent chemotherapy protocol. Although the median survival remains 13 months in this study, the estimated 2-year survival was 31.3%.[67] 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.

The major obstacles in the treatment of patients with ATL include drug resistance and development of opportunistic infections caused by various organisms, such as *Pneumocystis jiroveci*, cytomegalovirus, *Strongyloidiasis*, and a variety of fungi, indicating that cell-mediated immunity is severely impaired in these patients.[52] The prophylactic measures should be taken for patients with ATL. The mechanism of drug resistance in ATL cells has been studied. One mechanism is that ATL cells show elevated NF-κB activity, which induces antiapoptotic genes such as *bcl-xL* and *inhibitor of apoptosis protein*.[9] To overwhelm the drug resistance mediated by NF-κB, the proteasome inhibitor, PS-341, is a candidate for treatment.[68,69]

Although the frequency of opportunistic infections is much higher in patients with ATL than in those with other hematologic malignancies, the underlying mechanism(s) remained unsolved. Opportunistic malignancies, Kaposi's sarcoma,[70] and Epstein–Barr virus (EBV)–associated lymphoma[71] have also been reported in patients with ATL, which also indicates a state of immunodeficiency in these patients.

α-Interferon (α-IFN) combined with azidothymidine (AZT) was administered to 19 patients with ATL, and major responses (complete plus partial remissions) were achieved in 58% of the patients (11 of 19), including complete remission in 26% (5 of 19).[72] The mechanism of this combination therapy remains unknown since these drugs have no effect in vitro. In primary effusion lymphoma (PEL), α-IFN induced the expression of tumor necrosis factor–related apoptosis-inducing ligand and AZT suppressed NF-κB activity.[73] Both effects are considered to synergistically induce the apoptosis of PEL cells. Humanized monoclonal antibody against IL-2 receptor was also used for patients with ATL,[74] although its effect was limited.

Allogeneic stem cell transplantation (allo-SCT) for patients with ATL has been reported.[75] Median leukemia-free survival after allo-SCT was more than 17.5 months. Since autologous transplantation has been shown to be ineffective for ATL, graft versus leukemia should play an important role in anti-ATL effect. In this regard, it is noteworthy that two patients without graft-versus-host disease relapsed with ATL. The immune response by cytotoxic T lymphocytes against Tax protein has been augmented after BMT, indicating that such anti-Tax cytotoxic T cells exert an antitumor effect on ATL cells.[76]

It is obvious that the reduced transmission of HTLV-I directly leads to prevention of ATL. Since the breast feeding is thought to be major route of vertical transmission, bottle feeding is recommended for seropositive mothers instead of breastfeeding in Japan. About 18% of infants have been seroconverted by breast feeding from seropositive mothers. Refraining from breastfeeding could reduce the seroconversion rate to about 3%.[77] The transmission route of HTLV in seroconverted children in spite of complete bottle feeding remains unknown, although intrapartum transmission is suspected.

For HTLV-I transmission by blood transfusion, all donated blood at blood centers were subjected to HTLV-I antibody testing beginning in November 1986 in Japan. After this, none of the recipients, even patients with hematologic disorders who received multiple transfusions, were seroconverted. In Japan, absolute decline of the carrier rate among the young generation was observed, possibly due to complete achieving of blood donor screening, and success in preventing most maternal transmission by refraining from breastfeeding.

In HIV-1, the prophylactic administration of antiviral drugs to individuals who had accidental exposure to

infected blood can reduce the transmission. Since nucleoside reverse transcriptase inhibitors such as azidothymidine can block the replication of HTLV-I,[48] prophylactic administration should be considered after accidental exposure to blood of HTLV-I–infected individuals.

HTLV-I–RELATED DISORDERS

HTLV-I is the causative virus of not only ATL, but also other inflammatory diseases, such as HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP), chronic lung diseases, infective dermatitis, arthropathy, and uveitis. In patients with HAM/TSP and HTLV-I uveitis, an increased number of HTLV-I–infected cells has been reported, suggesting that inflammatory reactions, which include excessive production of cytokines and increased expression of adhesion molecules in HTLV-I–infected cells, and host immune response against infected cells, play a critical roles in the pathogenesis of these diseases.

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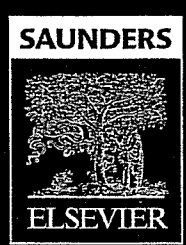

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HTLV-I basic leucine zipper factor gene mRNA supports proliferation of adult T cell leukemia cells

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Human T cell leukemia virus type I (HTLV-I) causes adult T cell leukemia (ATL) in 2–5% of carriers after a long latent period. An HTLV-I encoded protein, Tax, induces proliferation and inhibits apoptosis, resulting in clonal proliferation of infected cells. However, *tax* gene expression in ATL cells is disrupted by several mechanisms, including genetic changes in the *tax* gene and DNA methylation/deletion of the 5′ long terminal repeat (LTR). Because Tax is the major target of cytotoxic T-lymphocytes *in vivo*, loss of Tax expression should enable ATL cells to escape the host immune system. The 5′ LTR of HTLV-I is frequently hypermethylated or deleted in ATL cells, whereas the 3′ LTR remains unmethylated and intact, suggesting the involvement of the 3′ LTR in leukemogenesis. Here we show that a gene encoded by the minus strand of the HTLV-I proviral genome, *HTLV-I basic leucine zipper factor* (*HBZ*), is transcribed from 3′-LTR in all ATL cells. Suppression of *HBZ* gene transcription by short interfering RNA inhibits proliferation of ATL cells. In addition, *HBZ* gene expression promotes proliferation of a human T cell line. Analyses of T cell lines transfected with mutated *HBZ* genes showed that *HBZ* promotes T cell proliferation in its RNA form, whereas HBZ protein suppresses Tax-mediated viral transcription through the 5′ LTR. Thus, the single *HBZ* gene has bimodal functions in two different molecular forms. The growth-promoting activity of *HBZ* RNA likely plays an important role in oncogenesis by HTLV-I.

oncogenesis | retrovirus | bimodal function

Human retroviruses use their genomes very efficiently because of their limited genome size. Their accessory genes elaborately control replication of genome copy (1). The HIV vigorously replicates to yield progeny virus, whereas human T cell leukemia virus type I (HTLV-I) increases the number of infected cells by the activity of accessory genes (2, 3). HTLV-I was the first human retrovirus associated with human disease (4, 5). After transmission of HTLV-I, 2–5% of carriers are likely to develop adult T cell leukemia (ATL) after a long latent period (6). HTLV-I belongs to the δ-retrovirus group, which includes bovine leukemia virus and simian T-cell leukemia virus. In contrast to HIV, HTLV-I is transmitted in a cell-to-cell fashion requiring transfer of infected cells (7). To facilitate transmission, HTLV-I increases the number of infected cells through the activity of accessory genes, which are encoded by the pX region located between *env* and the 3′ long terminal repeat (LTR). These genes include *tax*, *rex*, *p30*, *p12*, *p13*, and *HTLV-I basic leucine zipper factor* (*HBZ*) (3, 8). Among them, *tax* is thought to play a central role in increasing the number of infected cells. Tax activates transcriptional pathways, including nuclear factor κ-B, cAMP response element-binding protein, activator protein-1, and serum responsive factor (2, 3). In addition, Tax can functionally inactivate p53 (9), resulting in inhibition of apoptosis. Thus, Tax promotes proliferation and suppresses apoptosis of infected cells, leading to clonal proliferation (10–12). As a consequence, HTLV-I causes ATL, a fatal neoplastic disease of CD4-positive T-lymphocytes.

Despite its critical role in proliferation of infected cells, Tax expression in ATL cells is disrupted by several mechanisms, including genetic changes in the *tax* gene (13), DNA methylation (14, 15), or deletion of the 5′ LTR (16). Because Tax is the major target of cytotoxic T-lymphocytes (17), Tax-expressing cells are rapidly eliminated *in vivo*. Therefore, loss of Tax expression could enable ATL cells to evade the host immune system. On the other hand, the role of HTLV-I-encoded viral genes has not yet been determined in ATL cells that lack Tax expression. In ATL cells, the HTLV-I 3′ LTR remains unmethylated and intact (18), whereas the 5′ LTR is frequently hypermethylated or deleted. Based on these observations, we hypothesized that promoter/enhancer activity of the HTLV-I 3′ LTR was essential for proliferation and survival of ATL cells. Transcription from the minus strand of HTLV-I has been reported (19), and the HBZ was subsequently found to inhibit Tax-mediated transactivation of viral gene transcription from the 5′ LTR by heterodimerizing with either cAMP response element-binding protein 2, c-Jun or JunB (20, 21).

In this study, we found that the *HBZ* gene was expressed in all ATL cells and that suppression of *HBZ* transcription by short interfering RNA (siRNA) decreased ATL cell proliferation. Mutant analyses showed that the *HBZ* gene promoted proliferation of T cells in its RNA form, whereas HBZ protein inhibited Tax-mediated transactivation through the HTLV-I LTR. These findings suggest that *HBZ* plays an important role in oncogenesis by HTLV-I.

Results

The Spliced Form of *HBZ* Is Expressed in All ATL Cells. We first determined the transcription start site of *HBZ* by using 5′ RACE (Fig. 1*A*). Contrary to a previous report (20), the *HBZ* gene was spliced and transcriptional start sites were identified in the R and U5 region of the 3′ LTR. The first 4 amino acids of the predicted HBZ protein differed from the previously reported sequence (Fig. 1*B*). The 3′ end of the transcript was also determined by 3′ RACE (Fig. 1*A*). A polyadenylation signal was found in 3′ untranslated region of *HBZ*. The spliced *HBZ* gene does not overlap with the *tax*-encoding region, indicating that an antisense RNA to *tax* is not generated. We next analyzed *HBZ* transcription in ATL cell lines, fresh ATL cells, and peripheral blood mononuclear cells from HTLV-I carriers by RT-PCR. In three ATL cell lines (ED, ATL-43T, and TL-Oml), *tax* gene transcription was silenced, whereas *HBZ* transcription was detected in all cell lines (Fig. 1*C*). *HBZ* was transcribed in all seven fresh ATL cell samples, whereas *tax* gene transcription was observed in two cases (Fig. 1*D*). Furthermore, *HBZ* gene transcription was detected in two of three carriers. Although genetic changes (nonsense mutations, insertions, and deletions) in *tax* have been reported in refs. 13 and 15, *HBZ* sequences did not contain

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Conflict of interest statement: No conflicts declared.

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Abbreviations: ATL, adult T cell leukemia; HBZ, HTLV-I basic leucine zipper factor; HTLV-I, human T cell leukemia virus type I; siRNA, short interfering RNA; SM HBZ, *HBZ* gene with silent mutations.

Data deposition: The spliced *HBZ* sequence reported in this paper has been deposited in the GenBank database (accession no. DQ273132).

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