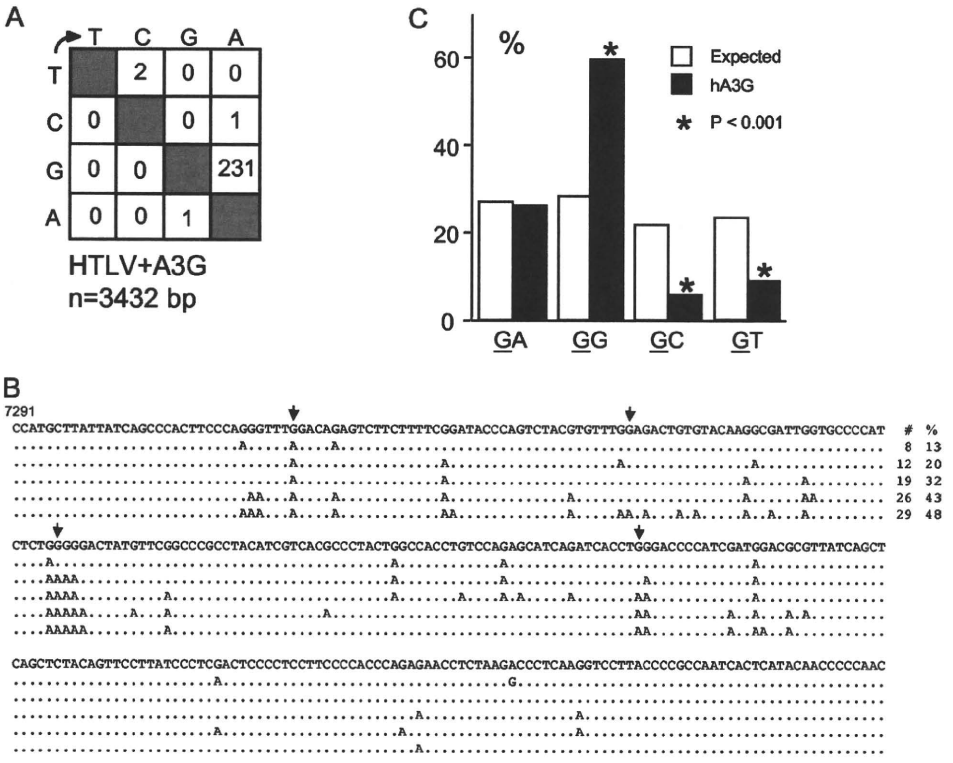

FIG. 5. *In vitro* editing of the HTLV-1 genome by hA3G. (A) Mutation matrix for hyperedited *tax* gene sequences derived from cloned 86°C 3D PCR products. The number of bases sequenced (n) is shown. (B) Hypermutated *tax* sequences from five representative PCR clones. The sequence is given with respect to the viral plus strand. Only differences from the viral plus strand are shown. The number (#) and percentage (%) of G-to-A mutations per clone are shown beside each sequence. The base composition of the sequence is 75 T (24%), 113 C (36.2%), 60 G (19.2%), and 64 A (20.5%). The position number of the first base is indicated above the base. Arrows indicate positions where G-to-A-mediated nonsense mutations were detected in ATL cases. (C) Bulk dinucleotide context of HTLV *tax* region DNA strand by hA3G. The substitution frequency as a function of the 3′ nucleotide is shown on the *y* axis. Mutated G residues are underlined. A χ^2 analysis showed that the observed frequencies by hA3G deviated significantly from the expected values ($P < 0.001$).

mutations at two sites where nonsense mutations were more frequently detected in ATL cases were also frequent among 3D PCR clones. As expected, a preference for GG dinucleotide context in hA3G-mediated G-to-A editing was observed (Fig. 5C). Our data suggested that hA3G is indeed able to actively deaminate the HTLV-1 genome during reverse transcription although with low editing frequency, leading to G-to-A mutations in the plus strand of the provirus.

DISCUSSION

In this study, in order to acquire a full mutation spectrum of HTLV-1 virus, the sequences of proviral genomes were determined in 60 ATL cases as well as 10 asymptomatic carriers. In addition to deletions and insertions, we identified base substitutions and found that G-to-A mutations were the most prevalent. In addition to the observation that G-to-A mutations in ATL patients and carriers occurred at the preferred target sequence of hA3G, we also experimentally confirmed that hA3G actively introduced G-to-A mutations to the HTLV-1 viral genome. It has been reported that hypermutation in the HTLV-1 genome was a rare event (35) and that HTLV-1 was relatively resistant to the antiviral activity of hA3G (33). Unlike HIV, which counteracts hA3G by producing Vif, HTLV-1 does not express an accessory protein that interferes with hA3G. A peptide motif in the C terminus of the HTLV-1 nucleocapsid has been shown to inhibit hA3G packaging into nascent virions (4). However, inhibition of hA3G-induced G-to-A mutations by HTLV-1 was partial (37). Previous studies analyzed small regions of provirus amplified by PCR in carriers or HAM/TSP patients, while proviral sequences of ATL cells were determined by direct sequencing. Since previous studies used error-prone *Taq* polymerase, it was very difficult to identify mutations after cloning (36). The number of ATL cases was so limited in the previous studies that mutations could not be distinguished from polymorphisms (34). This study first analyzed the entire sequence of the HTLV-1 provirus from 60 ATL cases by direct sequencing and also determined the proviral sequences of clones derived from HTLV-1 carriers using a precise DNA polymerase, which enabled us to identify mutations in HTLV-1 genomes. Consistent with previous studies (34, 35), our results showed that mutations were relatively rare. However, among the rare mutations in HTLV-1 genomes, G-to-A mutations in the target sequences of hA3G were predominant. In addition to G-to-A mutations, other mutations were also observed in ATL patients and carriers, indicating the role of other deaminases including AID and error-prone reverse transcriptase (27).

This study demonstrated important roles for cytidine deami-

nase(s) in generating mutations in HTLV-1 proviruses. So far, at least 12 cytidine deaminases have been identified, many of which share significant sequence homology. They are thought to have evolved through gene transpositions and duplications (45). Although some cytidine deaminases, including APOBEC1 and AID, target cellular genes, most human APOBEC proteins are thought to defend the host against retroviruses. Our study suggests that hA3G is responsible for G-to-A mutations in HTLV-1. hA3G binds to single-stranded DNA and preferentially deaminates CCCA and CCCG sequences during reverse transcription (48). This strategy of hA3G can induce nonsense mutations in the plus-strand coding sequence, since TGG is a target of hA3G, resulting in nonsense mutations like TAG or TGA. However, the *HBZ* gene is encoded by the minus strand of the provirus. The *HBZ* gene is therefore much less susceptible to nonsense mutations generated by hA3G. Furthermore, there are few target sequences for hA3G in the plus strand of the *HBZ* coding RNA. In addition, the coding sequence of the *HBZ* gene overlaps that of the *p12* gene. Thus, HBZ further avoids missense mutation in the minus strand.

The frequencies of G-to-A changes in the HTLV-1 provirus (0.21% for the *pol* gene and 0.11% for the *tax* gene) in the carriers are slightly lower than that for Vif-positive HIV-1 (0.57%) (48), indicating that HTLV-1 is resistant to hA3G during reverse transcription. Why are the few nonsense mutations that do occur retained in the HTLV-1 provirus? The difference between HTLV-1 and HIV-1 is related to their distinct strategies of propagation. HIV-1 replicates vigorously *in vivo*, producing tremendous numbers of viral particles. Viruses with nonsense mutations cannot replicate and thereby disappear *in vivo*. On the other hand, HTLV-1 promotes the proliferation of the infected cells themselves by the action of its regulatory and accessory genes. Therefore, HTLV-1-infected cells can proliferate despite nonsense mutations occurring in most of the viral genes, provided the cells retain the minimum set of viral genes that relate to proliferation. This might be a reason why so many nonsense mutations in various viral genes remain in the provirus. The only gene with no nonsense mutations is *HBZ*. It is likely that the *HBZ* gene is indispensable for proliferation of ATL cells, as our previous study reported (38).

Previous studies suggest that Tax is critical for proliferation of HTLV-1-infected cells and oncogenesis. However, we reported that Tax expression is frequently disrupted by three mechanisms (29). It has been speculated that Tax expression is not necessary in the late stages of ATL. Rather, since Tax is a major target of CTLs, ATL cells without Tax expression are selected during leukemogenesis. However, as shown in this study, nonsense mutations were likely generated by hA3G during reverse transcription, indicating that Tax expression was not necessary even in the carrier state. In one carrier, 9 of 21 clones shared a nonsense mutation in the *tax* gene, demonstrating clonal expansion of HTLV-1-infected cells with this mutation (see Table S5 in the supplemental material). Furthermore, 7 of 60 ATL cases contained nonsense mutations in the *tax* gene. HTLV-1-infected cells with nonsense mutations could proliferate *in vivo* and be transformed to ATL cells. A nonsense mutation in the *tax* gene has been detected in asymptomatic carriers (8). This study suggests that this nonsense mutation was generated by hA3G during reverse transcription. Another explanation is that loss of the expression of some viral gene(s) by nonsense mutations benefits the cells. Tax generates DNA damage that activates checkpoints (16, 22). In addition, it has been reported that HTLV-1-infected cells become cell cycle arrested due to the activity of Tax (19, 23). Therefore, HTLV-1-infected cells with nonsense mutations in the *tax* gene might have a growth advantage by losing Tax expression. We previously reported that HTLV-1 provirus lacking the 5′LTR and the second exon of the *tax* gene was detected in ATL cases. By sequencing the integration sites in such defective provirus, we found short 6-bp repeats generated by integrase. This finding showed that defective proviruses that could not produce Tax were generated before integration (31). Taken together, these findings indicate that Tax is not necessary for oncogenesis at least in some ATL cases.

This study suggests that nonsense mutations in the HTLV-1 provirus are generated by hA3G in both ATL cases and HTLV-1 carriers. The fact that hA3G targets the minus strand during reverse transcription explains why the *HBZ* gene is not susceptible to such nonsense mutations. In contrast, HTLV-1-infected cells take advantage of hA3G to escape from the host immune system by losing expression of other viral proteins, while the *HBZ* gene remains intact.

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Review Article

HTLV-1 and the Host Immune System : How the Virus Disrupts Immune Regulation, Leading to HTLV-1 Associated Diseases

Yorifumi Satou and Masao Matsuoka

Human T-cell leukemia virus type 1 (HTLV-1) was the first retrovirus shown to cause human diseases, such as adult T-cell leukemia (ATL) and HTLV-1 associated myelopathy/tropic spastic paraparesis (HAM/TSP). Despite extensive study for three decades, it remains elusive how HTLV-1 induces these diseases. HTLV-1 mainly infects CD4 T cells, inducing dysregulation of the host immune system. Recent studies have uncovered the mechanisms of differentiation and function of CD4 T cells at the cellular and molecular levels, extending our understanding of the pathological conditions associated with HTLV-1 infection. This review focuses on recent advances in our understanding of the interaction between HTLV-1 and the host immune system, which should provide us a clue to the mechanisms of HTLV-1 mediated pathogenesis. [*J Clin Exp Hematopathol 50(1) : 1-8, 2010*]

Keywords: human T-cell leukemia virus type 1, adult T-cell leukemia, human T-cell leukemia virus type 1 bZIP factor, chronic viral infection, regulatory T cells

INTRODUCTION

Human T-cell leukemia virus type 1 (HTLV-1) is a complex retrovirus that may have been transmitted to humans from monkeys more than ten thousands years ago.[1] The human host has several immune mechanisms that eliminate foreign pathogens, and like other successful pathogens, HTLV-1 must have strategies for evading the host immune response. Like human immunodeficiency virus (HIV), HTLV-1 mainly infects CD4 T cells, which are the central regulators of the acquired immune response. To establish persistent infection, HTLV-1 perturbs the regulation of CD4 T cells, sometimes leading to adult T-cell leukemia (ATL)[2,3] (Fig. 1) or sometimes leading to chronic inflammatory diseases such as HTLV-1 associated myelopathy/tropic spastic paraparesis (HAM/TSP),[4] uveitis,[5] arthritis,[6] and alveolitis.[7]

Since the discovery of HTLV-1, extensive studies have been performed using various experimental approaches. However, the nature of HTLV-1 pathogenesis still remains elusive. This problem is a serious obstacle to establishing effective therapies for HTLV-1 associated diseases.

Precise insight into HTLV-1 mediated pathogenesis requires careful consideration of the host cells and the effect HTLV-1 has on them. Thus in this review, we focus on the interaction between HTLV-1 and the host immune system. We believe that understanding this interaction will be helpful for understanding the pathogenesis of HTLV-1 associated diseases.

Preferential expansion of HTLV-1-infected CD 4 T cells

After the entry of a retrovirus into a host cell, the viral genomic RNA is reverse transcribed into a double strand DNA form and integrated into the host chromosomal DNA. The integrated virus, known as a provirus, expresses viral genes to achieve further transmission. HTLV-1 is widely believed to replicate primarily not as free viral particles, but as provirus, by inducing the proliferation of infected host cells.[3,8] Although HTLV-1 can infect various kinds of cells, such as dendritic cells, B cells, macrophages, and T cells,[9] the virus preferentially induces the clonal expansion of CD4 T cells.[10,11] This clonal expansion is presumed to be related to the transformation of infected CD4 T cells in some carriers.

The proliferation of infected host CD4 T cells is thought to be induced by viral accessory or regulatory proteins (Fig. 2). Many studies have focused on a viral protein, Tax, and much has been learned about its functions.[12,13] Tax is a transcriptional co-factor, and hijacks many signaling pathways

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Laboratory of Virus Control, Institute for Virus Research, Kyoto University, Kyoto, Japan
Address correspondence and reprint request to Yorifumi Satou
Laboratory of Virus Control, Institute for Virus Research, Kyoto University, 53 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan
e-mail : ysatou@virus.kyoto-u.ac.jp

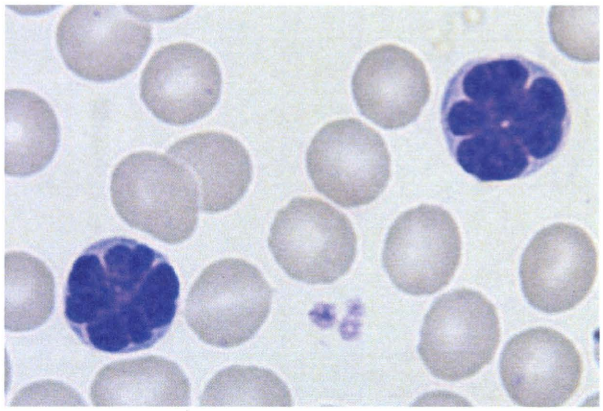

Fig. 1. Morphological findings of typical ATL cells. ATL cells with lobulated nuclei are called as "flower cells".

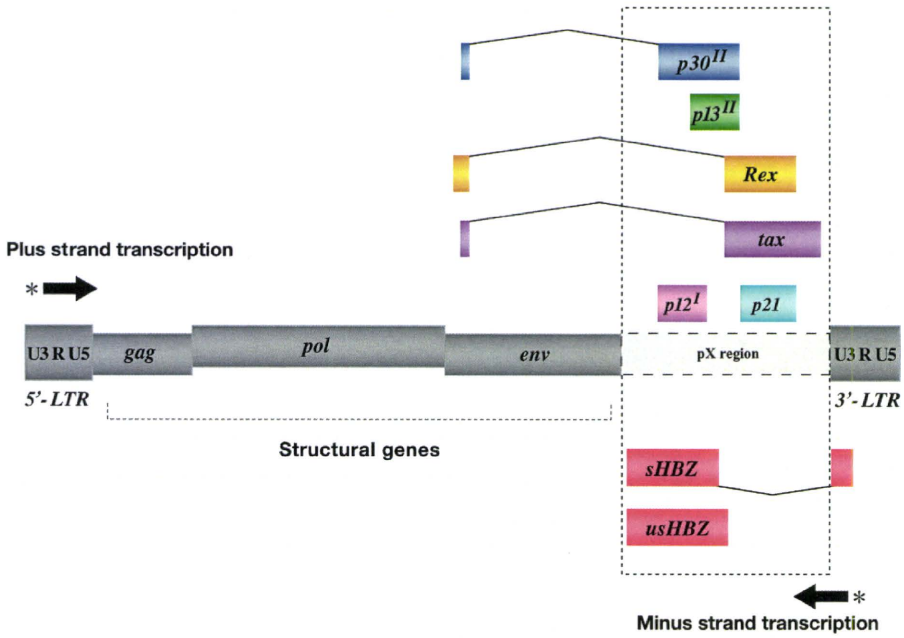

Fig. 2. The structure of HTLV-1. HTLV-1 encodes accessory and regulatory genes in the pX region as well as viral structural genes.

related to anti-apoptosis or cell proliferation (Fig. 3). Therefore Tax is widely considered a major player in inducing the proliferation of infected cells as well as in the transforming activity of HTLV-1. But at the same time, Tax is known as a major target of the host immune system.[14,15] The expression of Tax in the host cell induces attack by cytotoxic T lymphocyte (CTLs), resulting in the elimination of the infected cell. In line with this notion, the expression of Tax seems to be reduced during the process of the leukemogenesis,[16] suggesting that Tax expression is disadvantage for the survival of infected cells, at least in immune competent individuals.

Another viral gene encoding the HTLV-1 bZIP factor (HBZ) was recently identified,[17] and subsequently a novel splice isoform was identified by three different groups independently.[18-20] More recent reports showed that the new splice isoform of the transcript is not only more abundant but also functionally more important than the other type of transcript.[21,22] HBZ is reported to have an effect on the increased proliferation of infected cells.[20,23] However, HBZ has been shown to suppress the transcriptional activity of c-Jun[24,25] and the classical pathway of NF-κB[26] *in vitro*, indicat-

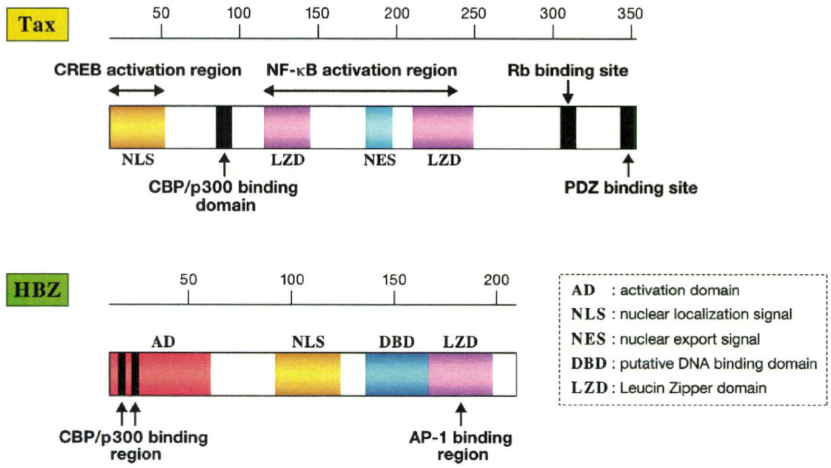

Fig. 3. The schematic structure of viral proteins, Tax and HBZ. Both Tax and HBZ play crucial roles in the HTLV-1 pathogenesis by interacting cellular factor as shown in this figure.

ing that HBZ protein is unlikely to stimulate cell proliferation *in vitro* (Fig. 3). Consistent with this notion, the deletion of the HBZ gene in a molecular clone of HTLV-1 had no influence on its *in vitro* transforming activity.[27] On the other hand, HBZ RNA stimulates cell proliferation when over-expressed *in vitro*.[20] The fact that HBZ is a constitutively expressed viral gene suggests that it has a role indispensable to the survival of HTLV-1 *in vivo*,[20,21] yet further experiments are required to elucidate the molecular function of HBZ or its role in HTLV-1 related pathogenesis. At the present time, it is controversial how HTLV-1 specifically induces the clonal expansion of CD4 T cells, but this CD4 specific function of HTLV-1 must be an important clue to the pathogenesis of ATL and HTLV-1 related chronic inflammatory diseases.

HTLV-1 and CD 4 T cell subsets including Foxp3⁺regulatory T (Treg) cells

When ATL was established as a distinct clinical entity, there was little information available about the host cells beyond the fact that they were T cells.[28] Progress in immunology has now led to a more detailed understanding of T-cell subsets. Furthermore, the mechanisms regulating T-cell differentiation, activation, and function have now been better elucidated. Thus it is time for us to re-evaluate the influence of HTLV-1 infection on CD4 T cells.

ATL cells are typically $CD4^+CD25^+$ T cells, a fact which initially suggested that ATL cells was derived from activated T cells.[29] Therefore, in addition to resting peripheral blood mononuclear cells (PBMCs), phytohemagglutinin (PHA) stimulated cells have been used as normal counterparts of ATL cells for various experiments. Later, $CD4^+CD25^+$ T cells were also considered to act as regulatory T cells (T_{reg} cells) that function to suppress excessive immune responses.[30] Within the $CD4^+CD25^+$ T cell subset, it was impossible to distinguish T_{reg} cells from activated T cells until the identification of Foxp3 as a T_{reg} "master switch." Several groups independently reported that Foxp3 plays crucial roles in the differentiation, function, and homeostasis of T_{reg} cells.[31-33] Most ATL cells are $CD4^+CD25^+Foxp3^+$, indicating that they may be derived from T_{reg} cells.[34,35] The accumulation of $Foxp3^+$ATL cells could be a possible reason for the immune compromised status frequently observed in ATL cases,[36] yet some ATL cells lost regulatory functions.[37-39] As Foxp3 has important roles for T_{reg} cell function, a report that $Foxp3^+$ ATL cells had lower Foxp3 expression levels when compared with normal $Foxp3^+$ T_{reg} cells indicates that the suppressive function is impaired in such ATL cells.[35] To further complicate the picture, the stimulation of naïve human CD4 T cells transiently induces the expression of Foxp3,[40] indicating that the $Foxp3^+$ ATL cells could possibly be derived from such an activated cell population. Conversely, a recent study indicated that $Foxp3^+$ T_{reg} cells have plasticity : thus $Foxp3^+$ T_{reg} cells can convert to Foxp3 negative cells.[41] This report indicates the possibility that Foxp3 negative ATL cells might come from T_{reg} cells that formerly expressed Foxp3. The immunohistochemical finding that Foxp3 expression is heterogeneous in some ATL lymph nodes may reflect this plasticity of Foxp3 expression in T_{reg} cells.[36]

The HTLV-1 viral protein Tax is reported to suppress Foxp3 expression at the transcriptional level when over-expressed in primary human CD4 T cells.[42] This indicates that Tax can influence the expression of Foxp3 in HTLV-1 infected T_{reg} cells. These data collectively suggest that under-

standing Foxp3$^+$ T_{reg} cells as an important subpopulation of CD4 T cells is important for elucidating the leukemogenesis of ATL by HTLV-1, yet further experiments are required to address precisely how HTLV-1 disturbs the homeostasis of T_{reg} cells.

To understand the pro-inflammatory properties of HTLV-1, it is important to consider another subset of the T cell population : inflammatory effector T cells. Previous reports suggest that HTLV-1 infection reduces the fraction of naïve T cells,[43] whereas HTLV-1 is enriched in CD4$^+$CD45RO$^+$ effector/memory CD4 T cells.[11] It remains unknown whether the shift from naïve to effector/memory T cells results from systemic pro-inflammatory circumstances or a CD4 T-cell intrinsic effect of HTLV-1 infection. HTLV-1 infection enhances not only the generation of effector/memory CD4 T cells but also increase the proliferation of the CD4 T cell subset,[44] indicating that CD4 T cells are continuously activated *in vivo*. Such activated CD4 effector T cells may migrate into the tissues, i.e., central nervous system, joints, lung, or uvea, in genetically susceptible individuals, leading to inflammation like that seen with certain autoimmune diseases.[45] Since CD4 T cells are the predominant cells detected in early inflammatory lesions,[46] activation of CD4 T cells is likely one of major contributors to HTLV-1-induced inflammation. Among the various effector functions of CD4 T cells, hyper-production of IFN-γ is widely believed to contribute to the onset of HTLV-1 mediated chronic inflammation.[47,48] Th_1 cells, a major subset of CD4 T cells, are characterized by their ability to produce IFN-γ. In addition to Th_1 cells, a recently identified Foxp3$^+$ subset, CD4$^+$CD45RA$^-$Foxp3^{low} non-T_{reg} cells, also have the potential to produce various inflammatory cytokines, such as IFN-γ, IL-4, and IL-17.[49,50] HTLV-1 may disturb the infected CD4 T-cell differentiation and function in cell-intrinsic manner (Fig. 4). Unraveling the picture require a two-pronged approach : learning more *in vivo* about the status and functioning of CD4 T cells during HTLV-1 infection, and ascertaining *in vitro* the molecular mechanisms by which HTLV-1 disturbs their differentiation, function, and homeostasis. A better understanding of how HTLV-1 causes inflammation should help us to establish therapeutic or preventive procedures for HTLV-1 associated diseases.

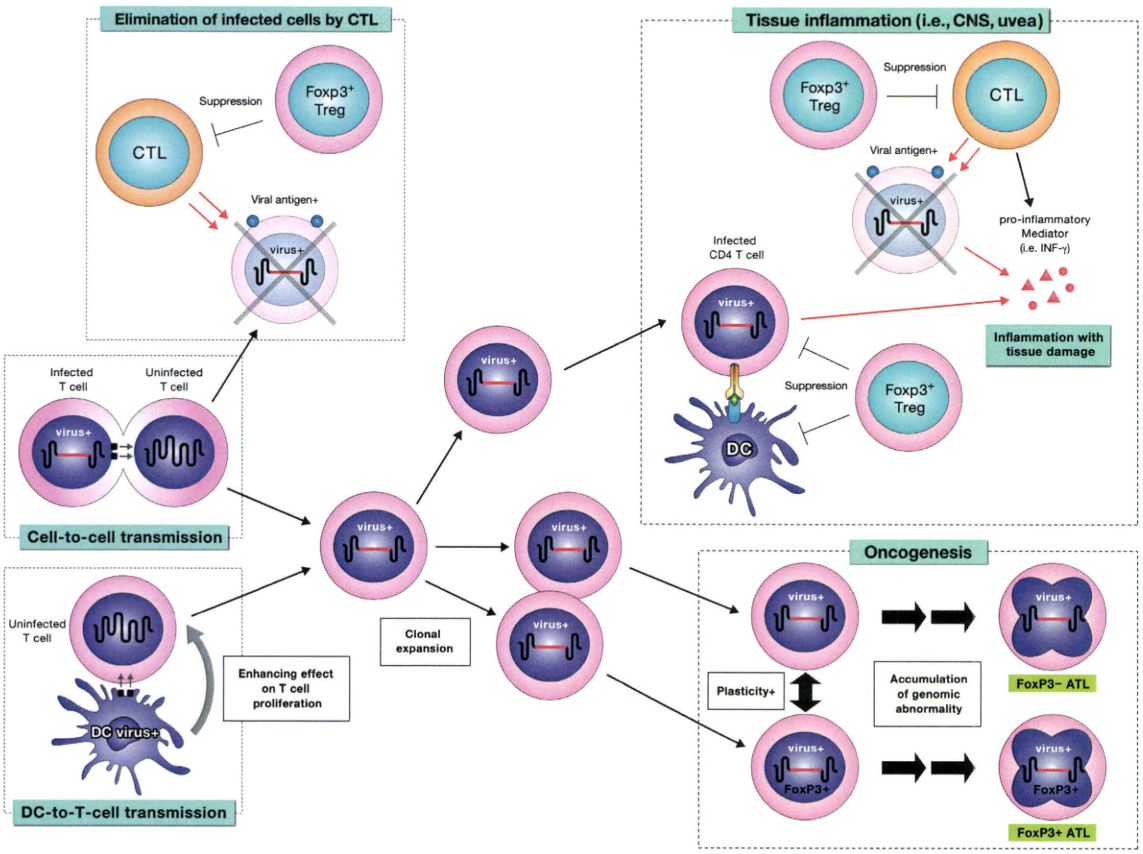

Fig. 4. The schematic figure of HTLV-1 infection in the host immune system. HTLV-1 enters and dysregulates the host immune system, resulting in chronic inflammation or transformation of infected cells. CTL, cytotoxic T cell ; DC, dendritic cell.

Antigen presenting cells (APCs) and T cell interaction

The most specialized machinery for T cells is obviously the T cell receptor and its signaling pathway. By using this system, naïve T cells recognize a cognate antigen, and then are activated either to differentiate or to undergo apoptosis. To understand the T-cell abnormalities induced by HTLV-1, we need to take into account APCs as crucial partners that regulate the fates of T cells *in vivo*. It is difficult for *in vitro* experiments to elucidate this aspect of HTLV-1 infection ; thus we need to establish useful *in vivo* systems of HTLV-1 infection. One of the most famous animal models is the tax transgenic mouse, which clearly demonstrated that tax potentially induces T-cell lymphoma[51,52] and chronic inflammatory diseases[53] *in vivo*, yet the process of the leukemogenesis and the detailed immunological status in that model still remain elusive. In addition, this transgenic system has a limitation in that we cannot study the immune response against the viral antigen. Several animal models, such as rabbits, rats, non-human primates, or humanized mice, can be infected with HTLV-1 and used as candidate hosts to study the effect of HTLV-1 on the interaction between T cells and APCs.[54]

Dendritic cells (DCs) are susceptible to HTLV-1 infection, and HTLV-1 infected DCs stimulate autologous lymphocyte proliferation of CD4 and CD8 T cells.[55,56] A recent study also clearly demonstrated that cell-free HTLV-1 efficiently infects DCs, and the infected DCs promote *de novo* infection of CD4 T cells (Fig. 4).[57] As with HIV, this transmission occurs in a biphasic manner. The early phase, called trans infection, occurs when DCs capture and transfer virions to CD4 T cells. The later phase, termed cis infection, occurs when virus produced *de novo* from infected DCs is transmitted to CD4 T cells. Cis infection should play a critical role in spreading the virus *in vivo*, because HTLV-1 is thought to be poorly infectious as a free virus and to spread primarily in a cell-to-cell manner. This study also indicated that the DC-T cell interaction induces activation of the T cells via the recognition of the antigen on the DCs. Thus the T cell's future differentiation status may be determined not only by the provirus within it, but by its interaction with the DC.

The cytotoxic T lymphocyte (CTL) response to HTLV-1

HTLV-1 is recognized as a foreign pathogen in infected individuals, and a virus-specific CTL response is found in the majority of carriers.[14,15] The CTL response is a critical component of the host immune response against HTLV-1. CTLs predominantly recognize the viral antigen Tax and contribute to the pathogenesis of chronic inflammatory diseases.[14,15] The level of CTL response differs among HTLV-1 carriers, and influences the 'set point' of proviral load. The frequency of HTLV-1 specific CTLs alone does not reflect the efficacy of the CTL response in chronically infected individuals, because antigenic stimulus fluctuates depending on the viral load.[58] If the immune response is efficient, the viral load decreases, which reduces the frequency of virus-specific CTLs. Rather, the overall lytic efficiency of the CTL population — which may reflect the frequency, specificity, and/or activation status of CTLs — must be evaluated by *ex vivo* culture of the CTLs together with HTLV-1-infected CD4 T cells from the same donor, and is well correlated with the proviral load.[59]

Since the genetic variation of HTLV-1 itself is quite limited, the level of CTL response must be determined by host factors. Virus-specific CTLs are activated via recognition of viral antigen presented on the APC. Since antigen presentation depends on HLA class I molecules, the HLA class I genotype influences the CTL response. Previous studies demonstrated that HLA-A02 and HLA Cw 08 are associated with low proviral load and low prevalence of HAM/TSP.[60,61]

Another factor that may influence the CTL response is the activity of $Foxp3^+$ T_{reg} cells. T_{reg} cells play a crucial role in controlling the CTL response through the direct suppression of APCs or CTLs via two mechanisms : one dependent on cell contact, i.e., CTLA-4, and another dependent on the secretion of inhibitory cytokines, such as TGF-β or IL-10.[62] There is a strong negative correlation between the frequency of CD4 $Foxp3^+$ Tax negative T_{reg} cells and the rate of CTL-mediated lysis of autologous HTLV-1 infected cells *ex vivo*.[63] This result suggests that T_{reg} cells indeed suppress the CTL response in HTLV-1 infected individuals.

A portion of CTLs are themselves infected with HTLV-1.[64] HTLV-1 specific CTLs are more susceptible to HTLV-1 infection compared with EBV-specific CTLs, indicating that cell contact between CTLs and APCs promotes the spread of HTLV-1 *in vivo* when HTLV-1 specific T cells encounter their antigens presented by APCs.

The CTL response is important not only for the understanding of the pathogenesis of inflammatory diseases but also for the treatment of ATL. An allogeneic bone marrow transplant,[65,66] which so far has been the only therapeutic procedure to achieve long term survival, increases the CTL response against the viral Tax antigen.[67] This observation suggests that enhancing the CTL response to viral antigens may be an effective therapeutic approach.

Concluding remarks

Since the identification of ATL as a distinct clinical entity, some progress has been made in preventing and treating the disease. In particular, the identification of a transmission route from the mother to her child through breast milk enables us to reduce *de novo* HTLV-1 infection.[68] In addition, recent approaches using allogeneic bone marrow transplantation have significantly improved the prognosis of ATL patients,[65,66] suggesting that enhancement of the immune response to HTLV-1

is a possible strategy for treatment of HTLV-1 associated human diseases. A better understanding of the interactions between HTLV-1 and the host immune system should provide us additional clues to effective therapies for HTLV-1-associated diseases.

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Review

HTLV-1 bZIP factor gene: Its roles in HTLV-1 pathogenesis

Masao Matsuoka

Institute for Virus Research, Kyoto University, 53 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606-8507, Japan

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ABSTRACT

The HTLV-1 bZIP factor (HBZ) gene is transcribed as an anti-sense transcript of HTLV-1 from the 3′ long terminal repeat (LTR). Recent studies showed that the *HBZ* gene was expressed in all ATL cases, suggesting its critical role in leukemogenesis. In addition, only the *HBZ* gene sequence remains intact, unaffected by nonsense mutations and deletion. HBZ mRNA promotes proliferation of adult T-cell leukemia (ATL) cells. The HBZ protein has three domains: activation, central, and bZIP domains. HBZ interacts with a variety of cellular factors, and modulates not only cellular functions, but also viral gene transcription from 5′LTR. The complex functions of HBZ modulate T-cells, and promote their proliferation, which is likely indispensable for leukemogenesis by HTLV-1.

Contents

1. Introduction

Human T-cell leukemia virus type 1 (HTLV-1) was first reported in 1980 (Poiesz et al., 1980). Thereafter, its causative association with adult T-cell leukemia (ATL) was clarified (Hinuma et al., 1981). The whole sequence was reported by Yoshida's group (Seiki et al., 1983), who revealed the unique region between env and 3′ long terminal repeat (LTR), designated the pX region. The pX region encodes regulatory genes, *tax* and *rex*, in addition to accessory genes, *p12*, *p13*, and

E-mail address: mmatsuok@virus.kyoto-u.ac.jp

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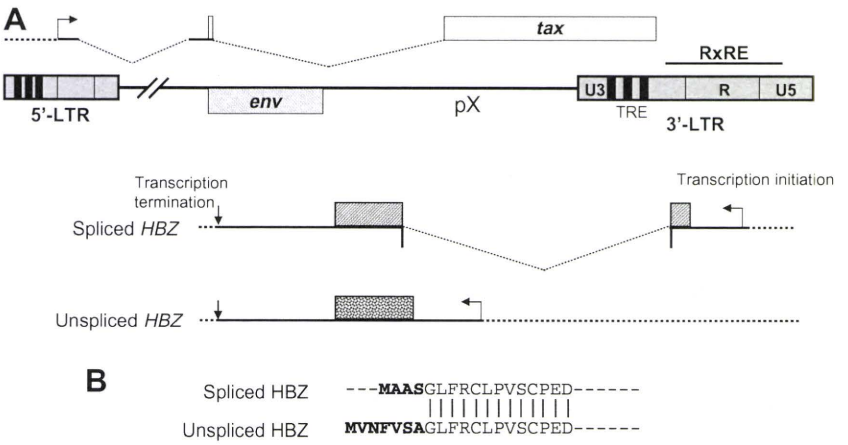


Fig. 1. Spliced and unspliced *HBZ* genes. Schema of the spliced and unspliced *HBZ* genes is shown (A). Structure of *tax* gene is also shown. The coding sequences of the *HBZ* and *tax* gene do not overlap. The N-terminal region of predicted amino acid sequences are shown in (B).

p30. At that time, researchers of HTLV-1 noticed an open reading frame in the minus strand. The presence of an anti-sense transcript of HTLV-1 was first detected by Northern blot in HTLV-1-infected cell lines (Larocca et al., 1989). However, the function and exact structure of this gene product remained unknown. This is because researchers naturally focused on Tax, a fascinating viral protein with pleiotropic actions, and the concept of anti-sense transcription was not so common at that time. In 2002, a viral protein that binds to CREB-2 was found by yeast two-hybrid screening, and named HTLV-1 bZIP factor (HBZ). HBZ was first found to inhibit viral gene transcription of the sense strand (Gaudray et al., 2002). In a separate study, we analyzed HTLV-1 provirus in ATL cells, and reported that Tax expression was frequently disrupted by several mechanisms (Matsuoka and Jeang, 2007). However, the *HBZ* gene sequence, as well as the 3′LTR that is its promoter, remained intact (Fan et al., 2010; Yoshida et al., 2008). In addition, the spliced form of the *HBZ* gene transcript was identified and was expressed in all ATL cases. Importantly, *HBZ* gene expression promoted proliferation of ATL cells (Satou et al., 2006). Thus, HBZ is now recognized as a critical gene for ATL cells. In this review, recent findings on HBZ are summarized, and its roles in HTLV-1 infectivity and oncogenesis discussed.

2. Structure and transcription of the *HBZ* gene

Transcription start sites of the *HBZ* gene were identified by 5′ RACE (Cavanagh et al., 2006; Murata et al., 2006; Satou et al., 2006). These studies revealed different transcripts of the *HBZ* gene: a spliced form (sHBZ) and an unspliced form (usHBZ) (Fig. 1). The first exon of the s*HBZ* gene transcript is present in U3 and R regions of the 3′LTR. The difference between sHBZ and usHBZ is only a few amino acids, as shown in Fig. 1B. Transcription start sites of the spliced *HBZ* gene were scattered in the U5 and R regions of the 3′LTR, which is consistent with the finding that the predicted promoter was TATA-less (Yoshida et al., 2008). Analyses of transcription factor binding sites of the promoter region showed that three Sp1 binding sites were critical for transcription of the *HBZ* gene while the third Sp1 site was most important.

Binding of Sp1 to this site was proved by chromatin immunoprecipitation. It has been reported that Sp1 is critical for many TATA-less promoters (Boam et al., 1995; Liu and Cowell, 2000). Since Sp1 is a well-known regulator of housekeeping genes, transcription of the s*HBZ* gene may be relatively constant. Taken together, Sp1 is critical for transcription of the *HBZ* gene. As described below, the *HBZ* gene transcript is better correlated with provirus load than the *tax* gene transcript (Saito et al., 2009), indicating that the *HBZ* gene is constantly expressed in HTLV-1 infected cells. Thus, transcription from the minus strand contrasts with that from the 5′LTR on the plus strand, which is highly inducible by Tax. Viral genes other than the *HBZ* gene are transcribed by the 5′LTR and HBZ suppresses transcription from the 5′LTR. This is an ingenious mechanism to control expression of viral proteins. Tax can activate transcription of the *HBZ* gene through a Tax responsive element (TRE) (Landry et al., 2009; Yoshida et al., 2008). However, its enhancement is not so significant, and Tax expression is usually controlled *in vivo*. Therefore, the significance of Tax mediated upregulation of the *HBZ* gene transcription remains to be determined.

Quantitative analyses of the *HBZ* gene transcripts were reported by two groups (Saito et al., 2009; Usui et al., 2008). Transcripts of the spliced form of the *HBZ* gene were fourfold more abundant than those of the *usHBZ* gene (Usui et al., 2008). This observation correlates with the finding that the promoter activity of the s*HBZ* gene was much higher than that of the us*HBZ* gene (Yoshida et al., 2008). Relative expression level of the *HBZ* gene that was adjusted by provirus load was almost equivalent among HTLV-1 carriers, HAM/TSP patients, and ATL patients (Saito et al., 2009). A previous study reported that HTLV-1 provirus load was correlated with the expression level of the *tax* gene (Yamano et al., 2002). However, the *HBZ* gene transcript was more closely correlated with provirus load than was the level of the *tax* gene transcripts (Saito et al., 2009). Kinetic study of the s*HBZ* gene transcripts in rabbits shows that s*HBZ* gene transcription was detected one week post-infection and

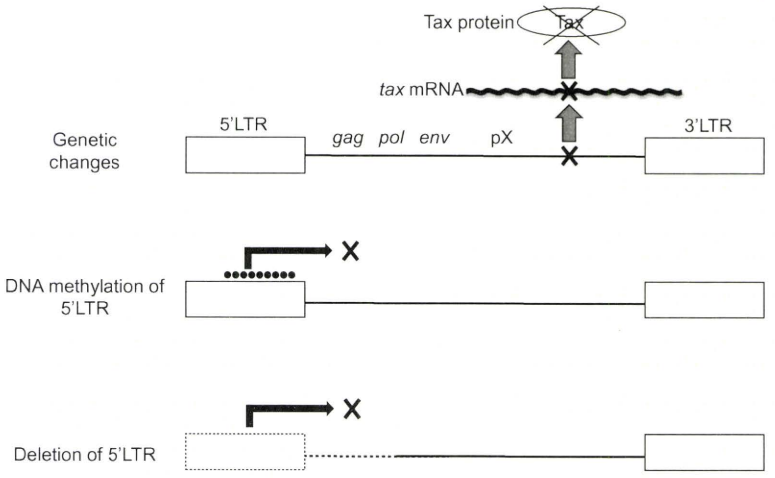


Fig. 2. Three mechanisms to inactivate Tax expression in ATL cells. (1) Genetic changes of the *tax* gene (nonsense mutation, deletion and insertion); (2) DNA methylation of 5′LTR silences transcription of the *tax* gene; and (3) deletion of 5′LTR, which is the promoter/enhancer of the *tax* gene transcription.

increased and stabilized, while other viral genes were at or below the limit of detection (Li et al., 2009). This finding supports a correlation between s*HBZ* gene expression, provirus load, and survival of HTLV-1 infected cells.

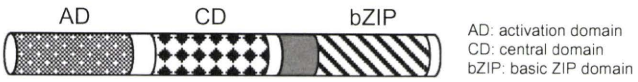
It is noteworthy that the *HBZ* mRNA was well correlated with disease severity in HAM/TSP patients. In addition, neopterin in the cerebrospinal cord fluid, which reflects cellular immune responses, was positively correlated with the level of *HBZ* mRNA, but not with *tax* mRNA. Taken together, these results show that the *HBZ* gene expression is closely linked with disease severity of HAM/TSP, suggesting that the *HBZ* gene expression plays critical roles in proliferation of HTLV-1 infected cells and pathogenesis.

3. The *HBZ* gene in ATL cells

HTLV-1 provirus is the only evidence of HTLV-1 infection in ATL cells, and analysis of provirus is expected to provide clues on leukemogenesis, and on which viral gene is critical for ATL cells or preferential integration sites of HTLV-1 provirus in leukemic cells. It has been thought that Tax is critical for leukemogenesis due to the abundance of reports on its potent effect on cell proliferation, genetic instability, and dysregulation of the cell cycle (Grassmann et al., 2005). Tax expression in transgenic animals induces cancers depending on the promoter used (Lairmore et al., 2005). For example, transgenic mice that expressed Tax under control of the human granzyme B gene promoter developed natural killer cell leukemia while salivary and mammary adenomas were found in the transgenic mice expressing Tax under the CD3-epsilon promoter–enhancer. *In vitro* expression of Tax could immortalize human T-lymphocytes although Tax expression could not transform T-lymphocytes (Akagi et al., 1995).

However, Tax expression was not detected in about 60% of freshly isolated samples from ATL cases (Takeda et al., 2004), indicating that Tax expression is not always necessary for ATL. Detailed analyses of HTLV-1 provirus showed three mechanisms to disrupt Tax expression in ATL cells (Fig. 2). First, the 5′LTR, which is a promoter/enhancer of viral gene transcription of the plus strand, was deleted (Miyazaki et al., 2007; Tamiya et al., 1996). Second, CpG sites of the 5′LTR were hypermethylated, which lead to silencing of plus strand viral gene transcription (Koiwa et al., 2002; Takeda et al., 2004; Taniguchi et al., 2005). Finally, genetic changes (nonsense mutations, deletions, and insertions) were found in the *tax* gene itself (Furukawa et al., 2001; Takeda et al., 2004). These findings suggest that Tax expression is not necessary in the late steps of the leukemogenic process although its expression is required at an early stage of ATL or in the carrier state. However, an analysis of defective provirus without a 5′LTR showed that 8 of 12 cases retained 6-bp short repeats at both ends of the LTR, while four cases lacked these sequences. Since these short repeats are generated during integration, the defective provirus with the 6-bp short repeat is formed before integration. Importantly, defective proviruses in two of eight cases lacked the second exon of the *tax* gene (Miyazaki et al., 2007), indicating that Tax was not expressed in these cases prior to development of leukemia. Nonsense mutations in the *tax* gene by APOBEC3G were found in 7 of 60 ATL cases (Fan et al., 2010). Similar nonsense mutations of the *tax* gene were also found in the carrier state. Thus, Tax expression is not necessary at least for these cases. Conversely, nonsense mutations were not found in the *HBZ* gene in both ATL cases and HTLV-1 carriers, suggesting that HBZ expression is indispensable for proliferation and survival of ATL cells and HTLV-1 infected cells.

As described, three mechanisms prevent Tax expression in ATL cells. However, the 3′LTR remained unmethylated and intact in all of the ATL cases, indicating that the 3′LTR was critical for ATL cells. Since viral genes other than the *HBZ* gene are transcribed from the 5′LTR that is frequently silenced or lost, we expected *HBZ* to be the only viral gene that is consistently expressed in ATL cells. In fact, the *HBZ* gene was transcribed in all ATL cases while transcripts of the *tax* gene were detected in



AD	*Binding with 26S proteasome (usHBZ) Degradation of c-Jun *Interaction with p300
CD	*Nuclear localization
AD+bZIP	*Binding with p65, inhibition of classical NF-κB pathway *Increase of hTERT promoter activity *Activation of JunD
bZIP	*Interaction and inhibition of c-Jun, Jun B, CREB, CREB2

Fig. 3. Functional domains of HBZ. HBZ has three domains: activation, central, and bZIP. Functions of each domain are summarized.

only a limited number of cases (Satou et al., 2006). Suppressing expression of the *HBZ* gene inhibited proliferation of ATL cells (Satou et al., 2006), indicating that the *HBZ* gene has growth-promoting activity in ATL.

4. Property and function of HBZ protein

The spliced transcript of HBZ is translated into a polypeptide of 206 amino acids, while the protein product of unspliced HBZ is a polypeptide of 209 amino acids. HBZ has three domains; the activation, central, and bZIP domains (Fig. 3). HBZ protein is localized in the nucleus with a speckled pattern. Three regions are associated with nuclear localization: two regions rich in basic amino acids and a DNA binding domain (Fig. 3) (Hivin et al., 2005). In addition, the integrity of the HBZ amino acid sequence is necessary for the speckled distribution in the nucleus. HBZ is localized in heterochromatin, consistent with its association with transcriptional inhibition (Hivin et al., 2005). Furthermore, HBZ has been shown to sequester JunB to nuclear bodies, thus suppressing JunB-dependent transcriptional activity (Hivin et al., 2007).

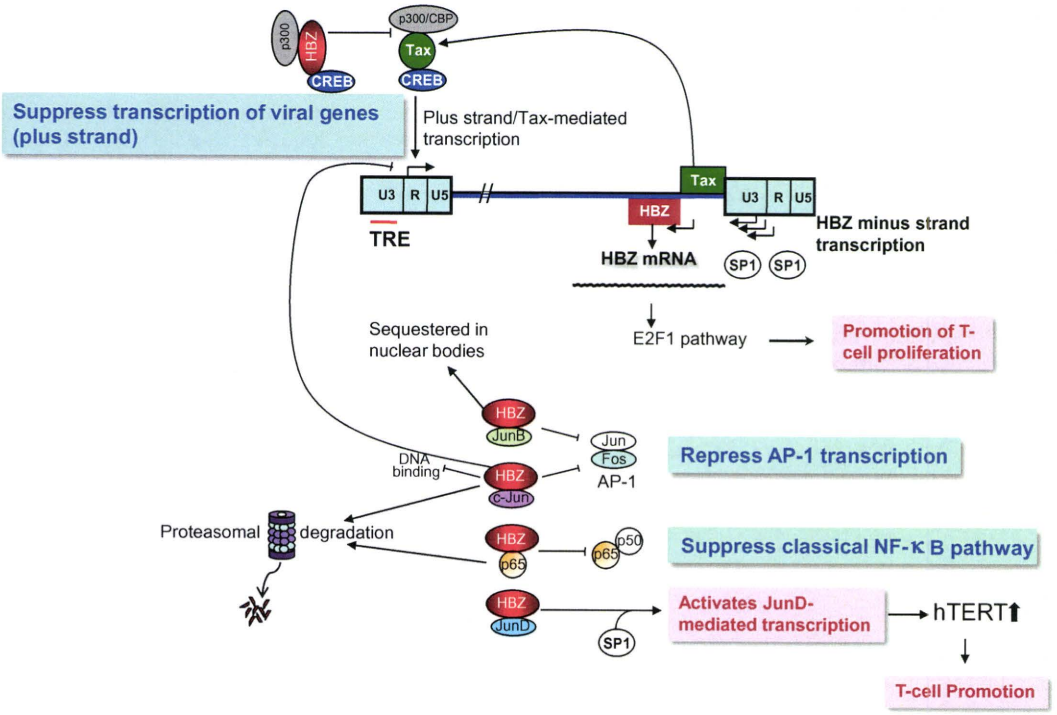


Fig. 4. Functions of HBZ. HBZ protein interacts with c-Jun or CREB and suppresses viral transcription from the 5′LTR. *HBZ* mRNA promotes T-cell proliferation. A transcription factor, SP1, plays a critical role in the *HBZ* gene transcription. Detailed description can be found in the text.

HBZ is not necessary for *in vitro* transformation of T-cells although mutation of the *HBZ* gene resulted in decreased proliferation of infected cells *in vivo* (Arnold et al., 2006), implicating HBZ in infectivity and viral persistence.

4.1. Suppression of Tax mediated viral gene transcription

HBZ was first found as a viral protein that binds to CREB-2 (ATF-4) (Gaudray et al., 2002). Through bZIP domain interaction, HBZ abolished the ability of CREB-2 to bind to the Tax responsive element (TxRE) in the HTLV-1 LTR, resulting in the suppression of transcription from the 5′LTR by Tax (Fig. 4). Moreover, HBZ interacts with cellular coactivators CBP/p300, via LXXLL-like motifs in its N-terminal region, leading to suppression of viral transcription by inhibiting the recruitment of CBP/p300 to the HTLV-1 promoter (Clerc et al., 2008). So far, there is no report that HBZ has the capacity to associate with DNA directly. It is apparent that HBZ exerts its suppressive effect on HTLV-1 transcription mainly by interacting with cellular proteins on the HTLV-1 promoter.

4.2. Interaction of HBZ with cellular factors with bZIP domains

The identification of other cellular factors that interact with HBZ was undertaken on an individual basis after analyses of the factors suspected to be involved in the signal pathway of ATL. HBZ, via its bZIP domain, reportedly forms heterodimers with several AP-1 transcriptional family members, such as c-Jun, JunB, and JunD, but not c-Fos, and modulates their activity (Fig. 4) (Basbous et al., 2003; Thebault et al., 2004). Analysis revealed that HBZ decreases the DNA binding capability of c-Jun and JunB, as is the case for ATF-4 and CREB. JunB was sequestrated into nuclear bodies through interaction with HBZ (Hivin et al., 2007). For c-Jun, HBZ enhances degradation of c-Jun, as described later, and inhibits its DNA binding (Fig. 4). Interactions between HBZ and other bZIP factors, such as ATF1 and ATF2, have also been shown (Isono et al., 2008; Lemasson et al., 2007). Recent study using coiled-coil arrays revealed binding of factors containing bZIP domains with HBZ (Reinke et al., 2010). The function of such associations remains to be elucidated.

4.3. Activation of transcription by interacting with JunD

HBZ suppresses transcription mediated by c-Jun and JunB. However, HBZ can also activate transcription mediated by JunD, another member of the AP-1 family. HBZ forms heterodimers with JunD via its bZIP domain, and the activation domain of HBZ is necessary for this activation (Thebault et al., 2004). In addition to this activity, HBZ–JunD heterodimers interact with Sp1 and activate transcription of the human telomerase catalytic subunit (hTERT) (Fig. 4) (Kuhlmann et al., 2007).

4.4. Inhibition of the classical NF-κB pathway

NF-κB is activated in HTLV-1 infected cells and ATL cells. Activated NF-κB plays important roles in the proliferation of ATL cells and inhibition of apoptosis (Sun and Yamaoka, 2005). Tax can activate both the classical and alternative pathways of NF-κB. Tax binds to IκB, resulting in dissociation of the IκB/NF-κB complex and promoting nuclear translocation of NF-κB (Suzuki et al., 1995). A further mechanism is Tax enhancing IκBα degradation by interacting with IKKγ, leading to subsequent activation of NF-κB (Jin et al., 1999). In contrast, HBZ inhibits only the classical NF-κB pathway, doing so by two mechanisms (Fig. 4). One mechanism is that HBZ inhibits DNA binding of p65. Another is that HBZ increases the expression of PDLIM2, the E3 ubiquitin ligase of p65, leading to increased ubiquitination and enhanced degradation of p65 (Zhao et al., 2009).

Many viruses have developed strategies to manipulate NF-κB signaling through the use of multifunctional viral proteins. HIV-1 Nef induces the expression of the NF-κB inhibitor IκBα, to suppress this pathway (Qiao et al., 2006). In Epstein–Barr virus, the LMP-1 viral protein activates the NF-κB pathway by recruiting cellular adaptor proteins, TNF receptor-associated factor families and TNF receptor-associated death domain, to its C-terminal domain. Like HBZ, the EBV bZIP protein, BZLF1, suppresses the classical NF-κB pathway by inhibiting DNA binding of p65 (Morrison and Kenney, 2004). Similar suppression of NF-κB has been reported for other viruses, including African swine fever virus, and human herpesvirus-8 (HHV-8). In the case of HHV-8, vFLIP activates both the classical and alternative pathways while viral interferon regulatory factor 3 (vIRF3: LANA2) inhibits NF-κB by binding to IKKβ. These findings show that NF-κB suppressive activities are common among different viruses, suggesting that these suppressive activities are important for viral infection. A virus might escape from the host immune system by suppressing the classical NF-κB pathway.

5. The proteasome and HBZ

One mechanism by which HBZ inhibits transcriptional activation by c-Jun and JunB (Basbous et al., 2003) is by forming heterodimers, and thereby inhibiting their DNA binding. HBZ further inhibits c-Jun by promoting the degradation of its protein product (Fig. 4) (Matsumoto et al., 2005). Since it can be blocked by a proteasome inhibitor, this effect relies on a proteasome-mediated pathway. Degradation of c-Jun does not, however, depend on ubiquitination (Isono et al., 2008). usHBZ protein directly interacts with both the 26S proteasome and c-Jun, which results in the delivery of c-Jun to the proteasome.

Thus, HBZ suppresses c-Jun by three mechanisms; (1) inhibition of DNA binding, (2) ubiquitin-independent degradation of c-Jun, and (3) sequestration of c-Jun in nuclear bodies.

It has been reported that degradation of c-Jun by sHBZ is much weaker than that by usHBZ (Isono et al., 2008). However, inhibition of AP-1 mediated transcription by sHBZ was much stronger than that of usHBZ (Yoshida et al., 2008). For the sHBZ protein, the inhibition of DNA binding by c-Jun or sequestration in nuclear bodies might be the predominant mechanism of transcriptional suppression. Further, the expression level of sHBZ RNA is much higher than that of usHBZ RNA, and the half-life of sHBZ protein is much longer than that of usHBZ. Only one HBZ protein was detected in ATL cell lines (Arnold et al., 2008). Taken together, sHBZ expression is much higher than that of usHBZ, and only sHBZ RNA has growth-promoting activity as described later, indicating that sHBZ is more important than usHBZ for HTLV-1 infected cells and ATL cells.

6. Function of the *HBZ* gene as RNA

Suppressed expression of the *HBZ* gene by shRNA leads to decreased proliferation of ATL cell lines (Arnold et al., 2008; Satou et al., 2006). Expression of HBZ in transgenic mice increases the number of T-cells (Satou et al., 2006), and tumor formation and infiltration of ATL cells is decreased by suppressed HBZ expression (Arnold et al., 2008). Thus, HBZ expression is associated with proliferation of ATL cells *in vivo* and *in vitro*. Mutation analyses of the *HBZ* gene showed that HBZ RNA, rather than HBZ protein, has a growth-promoting effect on T-cells (Fig. 4) (Satou et al., 2006). The coding sequence of the *HBZ* gene was replaced with silent mutations, which could produce the same protein while the RNA structure was completely altered. This mutant did not have a growth-promoting activity. Analysis of the transcription profile using DNAchip demonstrated that expression of the *HBZ* gene upregulates *E2F1* gene transcription. Only sHBZ RNA, not usHBZ RNA, promotes proliferation of T-cells, indicating that the first exon of the sHBZ transcript is critical for this activity (Yoshida et al., 2008). This exon overlaps with the Rex responsive element (RxRE) in the R region of 3′LTR. The RxRE region forms a stem-loop structure that binds to Rex. Rex promotes the export of viral RNA with a RxRE region, Thus, sHBZ RNA functions to promote proliferation of T-cells by a region containing the first exon, which can form a strong stem-loop structure. Further details of how sHBZ RNA promotes proliferation remain to be elucidated.

7. Anti-sense transcript of HTLV-2

HTLV-2 is similar to HTLV-1: both retroviruses target T-lymphocytes, and induce their proliferation. However, HTLV-2 tends to infect $CD8^+$ T-cells instead of $CD4^+$ T-cells (Kwaan et al., 2006), and induces cancer in humans only in very rare cases. Following the discovery of HBZ, an anti-sense transcript was identified in HTLV-2. Its product was named anti-sense protein of HTLV-2 (APH-2) (Halin et al., 2009). APH-2 mRNA is transcribed from the 3′LTR, and spliced and polyadenylated like the *sHBZ* gene. Although APH-2 does not have a basic leucine zipper domain, it interacts with CREB and suppresses Tax2-mediated transcription. Thus, APH-2 has similar structure and functions to sHBZ. However, it remains to be elucidated whether APH-2 induces proliferation of infected lymphocytes *in vivo*.

8. *HBZ* gene transcript and diseases

The *HBZ* gene is the only viral gene that is consistently expressed in HTLV-1 infected cells and ATL cells, indicating that HBZ plays a critical role in both infected cells and leukemic cells. For ATL cells, HBZ RNA has growth-promoting activity. ATL cells frequently express Foxp3, a master factor of regulatory T-cells. HBZ expression is associated with a phenotype of regulatory T-cells (our unpublished data). Thus, HBZ not only promotes proliferation of ATL cells, but also modulates the phenotype of HTLV-1 infected cells.

The expression level of *HBZ* transcripts is closely correlated with provirus load and the disease severity of HAM/TSP (Saito et al., 2009). Increased infected cells are also implicated in the pathogenesis of HAM/TSP. There are two scenarios for the role of HBZ: (1) HBZ is important for the survival of HTLV-1 infected cells *in vivo*, (2) HBZ expression confers infiltrative phenotypes on infected cells, and causes dysregulation of cytokine production. The role of *HBZ* gene in HAM/TSP should be studied in the future.

9. Perspectives

A limited number of viral genes are implicated in virus-induced oncogenesis. Both E6 and E7 are critical for human papilloma virus (HPV)-induced cervical cancer. In hepatitis B virus-induced hepatoma, HBx protein is a critical viral factor for oncogenesis. The pleiotropic functions of Tax are thought to play important roles in HTLV-1, however, this does not explain the frequent loss of Tax expression from ATL. It is hypothesized that Tax expression is necessary in the early stages of leukemia, but not required in late stages. However, HBZ is expressed in all ATL cases, and promotes proliferation of ATL cells. This indicates that HBZ is a critical viral gene in oncogenesis by HTLV-1. A few ATL cases had HTLV-1 provirus that could not produce Tax before its integration (Miyazaki et al., 2007; Tamiya et al., 1996). In addition, nonsense mutations of viral genes except the *HBZ* gene are generated by APOBEC3G before integration of provirus (Fan et al., 2010). These findings suggest that the *HBZ* gene is responsible for leukemogenesis by HTLV-1. Like other viral proteins of oncogenic viruses, recent studies

show the multiple functions of HBZ. Further studies of *HBZ* gene will shed light on leukemogenesis by HTLV-1. Further, HBZ might be a novel target for prevention of, and therapies for, HTLV-1-associated diseases.

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HTLV-1 bZIP Factor Induces T-Cell Lymphoma and Systemic Inflammation *In Vivo*

Yorifumi Satou[1], Jun-ichirou Yasunaga[1], Tiejun Zhao[1], Mika Yoshida[1], Paola Miyazato[1], Ken Takai[1], Kei Shimizu[2], Koichi Ohshima[2], Patrick L. Green[3], Naganari Ohkura[4], Tomoyuki Yamaguchi[4], Masahiro Ono[4], Shimon Sakaguchi[4], Masao Matsuoka[1]*

1 Laboratory of Virus Control, Institute for Virus Research, Kyoto University, Kyoto, Japan, **2** Department of Pathology, Kurume University School of Medicine, Kurume, Japan, **3** Center for Retrovirus Research and Departments of Veterinary Biosciences and Molecular Virology, Immunology and Medical Genetics, The Ohio State University, Columbus, Ohio, United States of America, **4** Department of Experimental Pathology, Institute for Frontier Medical Sciences, Kyoto University, Kyoto, Japan

Abstract

Human T-cell leukemia virus type 1 (HTLV-1) is the causal agent of a neoplastic disease of $CD4^+$ T cells, adult T-cell leukemia (ATL), and inflammatory diseases including HTLV-1 associated myelopathy/tropical spastic paraparesis, dermatitis, and inflammatory lung diseases. ATL cells, which constitutively express CD25, resemble $CD25^+CD4^+$ regulatory T cells (T_{reg}). Approximately 60% of ATL cases indeed harbor leukemic cells that express FoxP3, a key transcription factor for T_{reg} cells. HTLV-1 encodes an antisense transcript, *HTLV-1 bZIP factor* (*HBZ*), which is expressed in all ATL cases. In this study, we show that transgenic expression of HBZ in $CD4^+$ T cells induced T-cell lymphomas and systemic inflammation in mice, resembling diseases observed in HTLV-1 infected individuals. In *HBZ*-transgenic mice, $CD4^+Foxp3^+$ T_{reg} cells and effector/memory $CD4^+$ T cells increased *in vivo*. As a mechanism of increased T_{reg} cells, HBZ expression directly induced *Foxp3* gene transcription in T cells. The increased $CD4^+Foxp3^+$ T_{reg} cells in HBZ transgenic mice were functionally impaired while their proliferation was enhanced. HBZ could physically interact with Foxp3 and NFAT, thereby impairing the suppressive function of T_{reg} cells. Thus, the expression of HBZ in $CD4^+$ T cells is a key mechanism of HTLV-1-induced neoplastic and inflammatory diseases.

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* E-mail: mmatsuok@virus.kyoto-u.ac.jp

Introduction

Human T-cell leukemia virus type 1 (HTLV-1) was the first human retrovirus associated with human diseases including adult T-cell leukemia (ATL) [1,2] and HTLV-1 associated myelopathy/tropical spastic paraparesis (HAM/TSP)[3,4]. One of the virological attributes of HTLV-1 is that it transmits mainly by cell-to-cell contact [5,6]. Therefore, HTLV-1 induces the proliferation of infected $CD4^+$ T cells to increase further transmission [7]. HTLV-1 encodes several regulatory and accessory genes in the pX region located between the *env* gene and the 3′ LTR [7,8]. Among the viral genes, *tax* possesses *in vitro* transforming activity and can induce cancers in transgenic (Tg) animals via its pleiotropic actions [9,10]. Yet the expression of Tax is frequently disrupted in ATL [7]. In contrast, the *HTLV-1 bZIP factor* (*HBZ*) gene, which is encoded in the minus strand of the HTLV-1 genome [11,12], is transcribed in all ATL cases [13]. Recently, it has been reported that APOBEC3G generates nonsense mutations in all HTLV-1 genes except *HBZ* [14], suggesting that the *HBZ* gene is indispensable for the growth and/or survival of ATL cells and HTLV-1 infected cells. The *HBZ* gene product promotes the proliferation of ATL cells [13,15]. Further, *HBZ* mRNA expression in HAM/TSP patients was well correlated with disease severity [16]. These findings suggest that *HBZ* has a critical role in the development of ATL and HAM/TSP.

It has been shown that ATL cells functionally and phenotypically resemble $Foxp3^+$ $CD25^+CD4^+$ regulatory T (T_{reg}) cells, which control immune responses against self- and non-self-antigen [17]. ATL cells constitutively express CD25 and scarcely produce interleukin-2 (IL-2)[18,19]. Furthermore, two thirds of ATL cases harbor leukemic cells expressing FoxP3 [20,21], a key transcription factor for the generation and function of T_{reg} cells [22,23,24]. In HTLV-1 carriers, HTLV-1 provirus is detected mainly in $CD4^+$ effector/memory T cells and T_{reg} cells [25,26,27]. Thus, HTLV-1 favors T_{reg} cells and effector/memory T cells *in vivo*, and transforms them. However, how HTLV-1 targets these T cell subpopulations remains to be elucidated.

In this study, we show that transgenic expression of HBZ increases $Foxp3^+$ T_{reg} cells and effector/memory T cells, leading to development of T-cell lymphomas and systemic inflammatory diseases. In addition, the suppressive function of T_{reg} cells is severely impaired in HBZ transgenic mice. At the molecular level, we show that HBZ interacts with Foxp3 and NFAT, interrupting the function of each molecule, and leading to the deregulation of Foxp3-mediated transcriptional control of the genes associated