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HTLV-1's Foxy Strategy for Survival and Transmission

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Human T-cell leukemia virus type 1 (HTLV-1) is the causative agent of adult T-cell leukemia-lymphoma (ATL) and inflammatory diseases including HTLV-1-associated myelopathy (HAM). A remarkable feature of HTLV-1 is that this virus transmits primarily through cell-to-cell contact. HTLV-1 increases the number of infected cells *in vivo* to ensure its survival and transmission. Therefore, survival of HTLV-1-infected cells *in vivo* is very critical for transmission under the host immune surveillance. HTLV-1 possesses multiple strategies to evade host immune responses. Among viral genes, Tax and HTLV-1 bZIP factor (HBZ) play crucial roles in the proliferation of infected cells and the subsequent development of ATL. Although Tax strongly activates the NF-kB pathway, the immunogenicity of Tax is very high; it is a major target of cytotoxic T lymphocytes. Therefore, the virus minimizes Tax production, expressing it only intermittently *in vivo*. On the other hand, the immunogenicity of HBZ is low, and its expression is maintained in all ATL cases. HBZ transforms the immunophenotype of infected cells into regulatory T cell-like (CD4+ CD25+ CCR4+ TIGIT+ Foxp3+), and promotes the production of immunosuppressive cytokines. Furthermore, HBZ mRNA not only encodes the protein but also functions itself like long non-coding RNA. As a result, Tax and HBZ enable long-term escape from host immunity, persistent infection, and proliferation of infected cells. Here, we review the viral strategies to counteract to host immune surveillance system.

Keywords: HTLV-1, HBZ, tax, regulatory T cell, IL-10

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INTRODUCTION

Human T-cell leukemia virus type 1 (HTLV-1) causes the neoplastic disease, adult T-cell leukemia-lymphoma (ATL), and various inflammatory diseases including HTLV-1 associated myelopathy (HAM) and uveitis (HU) (1). A part of HTLV-1 carriers (∼5% in Japan) is estimated to develop ATL after a long latent period (2). HTLV-1 is derived from simian T-cell leukemia virus type 1 (STLV-1) (3). Interspecies transmission from monkeys to humans is estimated to have occurred ∼ 50,000–20,000 years ago (4). Thus, this virus has survived for a long time in monkeys and humans. Since this virus causes persistent infection in the host, it must have strategies to survive *in vivo* and to enable its transmission to new hosts. To achieve these ends, the virus modulates the character of infected cells to make them resistant to host immune responses and advantageous for viral transmission. This article reviews these viral strategies, which are closely linked to the pathogenesis of HTLV-1.

HTLV-1 CAUSES THE PROLIFERATION OF INFECTED CELLS

An important attribute of HTLV-1 is that this virus transmits primarily through cell-to-cell contact (5). Cell-free virions have very poor infectivity even in *in vitro* culture (6). To facilitate viral transmission, HTLV-1 increases the number of infected T cells *in vivo* by causing them to proliferate (7). HTLV-1 transmits via three main routes: (1) mother-to-infant transmission through breast feeding, (2) sexual transmission, primarily male-to-female, and (3) blood transfusion and needle sharing. For transmission via breast-feeding and sexual contact, HTLV-1 infected cells must migrate into semen and breast milk. Viral genes must enable infected cells to have such attributes. T cells in the breast milk and semen have effector/memory phenotype (8). In HBZ transgenic (HBZ-Tg) mice, HBZ expressing T cells show effector/memory T-cell phenotype (9), indicating that HBZ coverts expressing T cells to effector/memory phenotype. Thus, the immunophenotype of infected cells is determined by HBZ (10). After entering into new host, Tax is essential for *de novo* infection (8).

The HTLV-1 provirus encodes structural genes (*gag*, *pol*, and *env*), regulatory genes (*tax* and *rex*) and accessory genes [*p12*, *p13*, *p30* and *HTLV-1 bZIP factor* (*HBZ*)] (7). The *HBZ* gene is encoded in the minus strand of the provirus, and expressed as anti-sense transcripts (11), whereas all other viral genes are transcribed from the plus strand. Sense and anti-sense transcription of viral genes *in vivo* are differentially regulated and have different functions (**Figure 1**). Transcription of the sense strand genes depends on Tax. Tax trans-activates plus-strand transcription of HTLV-1 through Tax-responsive elements in long terminal repeat (LTR). Sense-strand genes encode Gag, Pol, and Env, which are essential for the formation of viral particles. Thus, sense-strand transcription is necessary for *de novo* infection. Tax mediated activation of sense strand genes also increases Rex expression, which inhibits splicing of viral genes, resulting in suppressed Tax expression. In contrast, the anti-sense transcript, HBZ, is not needed for *de novo* infection, but is critical for clonal proliferation of infected cells *in vivo* (12). Thus, Tax and HBZ have different roles in the life cycle of this virus.

HTLV-1 is susceptible to APOBEC3G (A3G). Non-sense mutations caused by A3G are frequently observed in the *tax* gene (13, 14). HTLV-1 infected cells and ATL cases with mutated *tax* genes were also reported (14). Clonal proliferation of infected T cells with non-sense mutations of *tax* is found in carriers and ATL cases (15–17). These findings indicate that HBZ can induce clonal proliferation of HTLV-1 infected cells and cause ATL even without Tax (18). HBZ promotes proliferation of T cells *in vitro* and *in vivo* (19). Conversely, a burst of Tax expression (see below) suppresses cell cycling of T cells rather than inducing their proliferation (20).

TRANSIENT EXPRESSION OF TAX: TO EXPRESS OR NOT TO EXPRESS

Tax is essential for *de novo* infection by HTLV-1 (5). However, Tax is a highly immunogenic viral protein (21, 22). Cytotoxic T lymphocytes (CTLs) against Tax are frequently detected in HTLV-1 infected individuals (23). Tax expression is thus the Achilles heel of HTLV-1: it is necessary for transmission, but it renders the expressing cells vulnerable to the host immune response. *Ex vivo* culture of peripheral blood mononuclear cells (PBMCs) induces Tax expression, indicating that Tax expression is largely suppressed *in vivo* (24). HTLV-1 minimizes Tax expression by intermittent transcription (Tax burst) (20, 25). Stresses like low pH or oxidative stress can induce Tax expression (20). This Tax burst is strongly associated with the activation of p38 MAP kinase (p38 MAPK). p38 MAPKs sense extracellular stress *in vivo*, including heat shock, ultraviolet light, and hypoxia (5). Activation of plus-strand viral transcription is associated with an increase in the tri-methylation at the 4th lysine residue of the histone H3 protein (H3K4me3) at the HTLV-1 5′LTR promoter, and reduced levels of histone H2A monoubiquitylated at lysine 119 (H2AK119ub1) (26). The duration of Tax expression in PBMCs from an ATL patient with one dominant clone is estimated to be <1 h using single-molecule RNA FISH (27).

Transient Tax expression induces dramatic changes in the transcriptome of expressing cells. In particular, NFkB is strongly activated and anti-apoptotic genes are upregulated by the Tax burst (20). Transient Tax expression generates vigorously proliferating cells, and may be a viral mechanism for maintaining the infected cell population. This type of Tax expression is observed in about half of ATL cases (18).

FUNCTION OF HBZ

In contrast to Tax, HBZ is constantly expressed in ATL cells and HTLV-1 infected cells (19, 28). Transcription of *HBZ* is driven by the cellular transcription factor SP1 (29). Since the immunogenicity of HBZ protein is low (30), the CTL response to HBZ is weak *in vivo*: although CTLs to HBZ are critical for determining the provirus load in HTLV-1 carriers (31). This is a reason why infected cells and ATL cells can express HBZ *in vivo*. Such low immunogenicity of viral proteins is observed in other oncogenic viruses including Epstein-Barr virus (EBV) and human papilloma virus (HPV). The necessity for persistent expression selects for low immunogenicity of these viral proteins (32).

HBZ expression affects the host cell in myriad ways, some of which are summarized in **Figure 2**. Of particular interest is the fact that HBZ induces transcription of the *Foxp3* gene by activating the TGF-b/Smad pathway (33). Indeed, most ATL cells express Foxp3 and ∼30–40% of infected T cells express Foxp3 (34). Foxp3 is the master gene of regulatory T (Treg) cells for their differentiation and functions. Therefore, HBZ-expressing T cells acquire Treg-like immunophenotypes. Furthermore, HBZ induces the expression of other Treg-associated molecules, including CCR4 and T cell immunoglobulin and ITIM domain (TIGIT) (35, 36). Treg cells express immunosuppressive molecules on their surfaces and produce immunoinhibitory cytokines like TGF-b and IL-10. These attributes of Treg cells benefit the survival of infected cells *in vivo*.

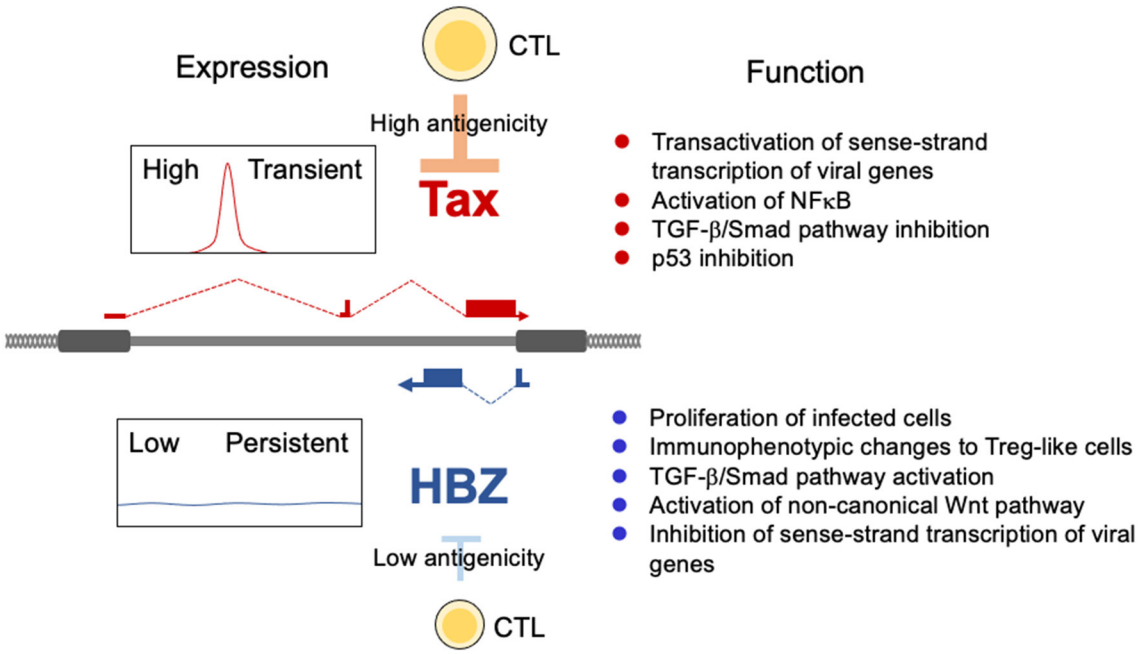

FIGURE 1 | Expression patterns and functions of *tax* and *HBZ*. Tax is intermittently transcribed from the 5′LTR, whereas HBZ is constantly expressed from the 3′LTR. Tax function is essential for *de novo* infection. HBZ promotes the proliferation of expressing T cells and drives infected cells toward a Treg-like immunophenotype.

HBZ contains bZIP domain that is similar to that of c-Fos (12). Therefore, HBZ interacts with the transcription factors of AP-1 family, such as c-Jun, JunD and ATF3 (37, 38).

Interaction between JunD and HBZ promotes proliferation of ATL cells by the following mechanism (38, 39). JunD mRNA produces two protein isoforms using alternative translation initiation sites: full-length JunD (JunD-FL) and Δ-JunD that is an N-terminal truncated form of JunD-FL. HBZ promotes translation of Δ-JunD by depleting the ribosomal protein S25 (39), which is unable to bind to a tumor suppressor, menin. Thus, enhanced Δ-JunD expression by HBZ results in promoted proliferation. In addition, HBZ protein interacts with Rb/E2F1 complex and activates the transcription of E2F-target genes associated with cell cycle progression (40).

HBZ strongly inhibits canonical Wnt pathway by interacting with lymphoid enhancer-binding factor 1 (LEF-1), and upregulates expression of non-canonical Wnt ligand, Wnt5a (41). Since knocking down of Wnt5a in ATL cells repressed cellular proliferation, activated non-canonical Wnt pathway by HBZ plays an important role in the pathogenesis of ATL.

Different from protein, RNA itself is not recognized by CTLs. Therefore, functional RNAs are of advantage for viral replication and survival of infected cells. Epstein-Barr virus (EBV) and Kaposi sarcoma herpes virus (KSHV) encode viral microRNAs (42, 43). In addition, viral microRNAs of bovine leukemia virus (BLV) are critical for proliferation of infected cells and oncogenesis (44, 45). HBZ is a unique viral gene in that HBZ mRNA functions not only to produce the protein but also as mRNA itself, in a manner resembling that of long non-coding RNAs (19, 46). Such mRNAs are named coding non-coding RNAs (cncRNAs) or bifunctional RNAs (47). Using RNA FISH, HBZ mRNA is found to be mainly present in the nucleus. When HBZ is expressed by its native promoter, the 3′LTR, HBZ mRNA is mainly present in the nucleus, but it resides in the cytoplasm when expressed by the exogenous strong promoter. The difference between the HBZ mRNAs in these two scenarios is the length of the poly A tail: poor polyadenylation is the cause of the nuclear localization of HBZ mRNA (48). HBZ mRNA expressed by the 3′LTR can promote the proliferation of T cells, whereas HBZ mRNA expressed by a strong promoter did not promote T-cell proliferation, indicating that nuclear localization is involved in this function. Interestingly, the anti-sense transcript of human immunodeficiency virus type 1 (HIV-1), *ASP*, is also chiefly localized in the nucleus with poor polyadenylation, indicating that this nuclear localization is common to anti-sense transcripts of the retrovirus.

The 5′ region of HBZ mRNA is responsible for its functions in the nucleus (46). This region forms a strong stem-loop structure, which is likely involved in interaction of *HBZ* mRNA with cellular factors. *HBZ* mRNA promotes the proliferation of T cells and enhances transcription of anti-apoptotic genes including *survivin* (46). Furthermore, HBZ mRNA interferes with the basal transcription machinery, leading to suppression of sense-transcription from the LTR (49). It is reported that HBZ protein also suppresses sense-transcription from the LTR (11). Thus, both HBZ mRNA and protein are involved in the suppression of sense transcription of viral genes (11, 49). Viral proteins that are encoded in the plus strand are well-recognized by CTLs. Silencing of transcription of sense-strand viral genes helps infected cells to escape from the host immune response.

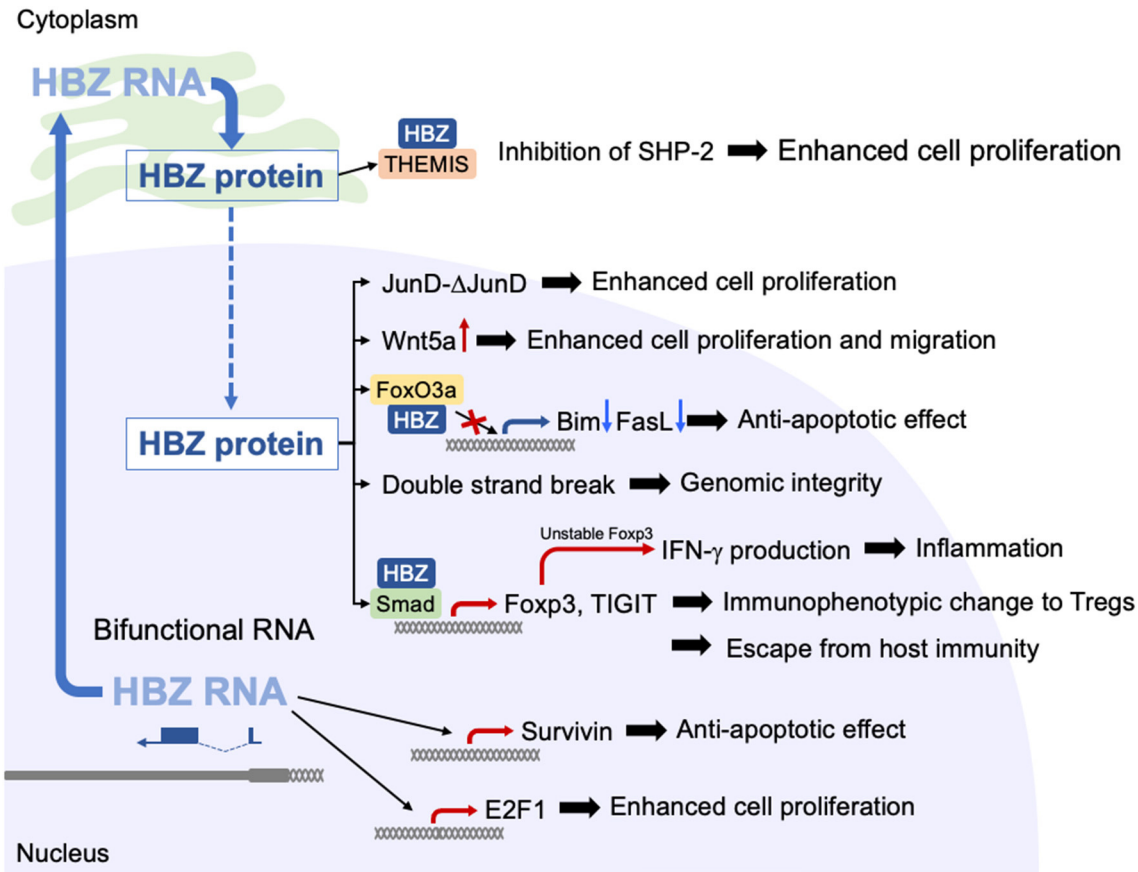

FIGURE 2 | Functions of HBZ protein and *HBZ* RNA. HBZ protein and *HBZ* RNA have a variety of functions in expressing T cells. HBZ RNA is mainly present in the nucleus, where it promotes the proliferation of T cells and suppresses apoptosis. HBZ protein induces transcription of the *Foxp3* gene by activation of TGF-b/Smad pathway.

Since HBZ is critical for survival of ATL cells, its knockdown strongly suppresses proliferation of ATL cells (19). Therefore, HBZ is the ideal therapeutic target of ATL. HBZ functions as RNA and protein. Targeting HBZ RNA is the best choice although delivery to ATL cells is very difficult. Immunization by HBZ protein can suppress ATL cells (50) although method of strong immunization with adjuvants or mRNA should be established to overcome low immunogenicity of HBZ protein.

HOW INFECTED CELLS EVADE HOST IMMUNOSURVEILLANCE

HTLV-1 largely depends on clonal proliferation of infected cells to persist *in vivo*. It is critical for infected cells to evade the host immune system. One mechanism for this evasion is that HBZ causes infected cells to acquire a Treg-like phenotype. Treg cells express immunosuppressive surface molecules and produce immunosuppressive cytokines like TGF-b and IL-10, which enable the virus to evade host immunosurveillance. IL-10 secretion is elevated in HTLV-1 infected cells of carriers and ATL patients (51). IL-10 is an immunomodulating cytokine that is critical for suppressing excessive immune activation and consequent tissue damage (52). IL-10 suppresses the antigen presenting capacity of dendritic cells (DCs) and leads to the exhaustion of T cells, which allows viruses to persist (52, 53). Several viruses utilize the immunosuppressive function of IL-10 to establish persistent infection (54). For HTLV-1, HBZ-mediated enhanced expression of the co-inhibitory receptor TIGIT is thought to be a mechanism of increased IL-10 production (**Figure 3**) (36). TIGIT-mediated signaling increases IL-10 production from not only DCs but also T cells. Since TIGIT is a co-inhibitory receptor, its signaling normally inhibits the proliferation of T cells. However, HBZ impairs this inhibitory signaling from TIGIT via interaction with THEMIS, which forms a complex with Grb2 and SHP-2 (55). Thus, HBZ induces TIGIT expression but impairs its inhibitory function within infected cells.

IL-10 does not promote the proliferation of normal T cells. However, it is reported to promote the proliferation of ATL cells (56). This difference is thought to be due to another activity of HBZ: HBZ modulates intracellular signaling from the IL-10 receptor by interacting with STAT1 and STAT3 (**Figure 3**) (34). Combining HBZ mediated enhancement of IL-10 production

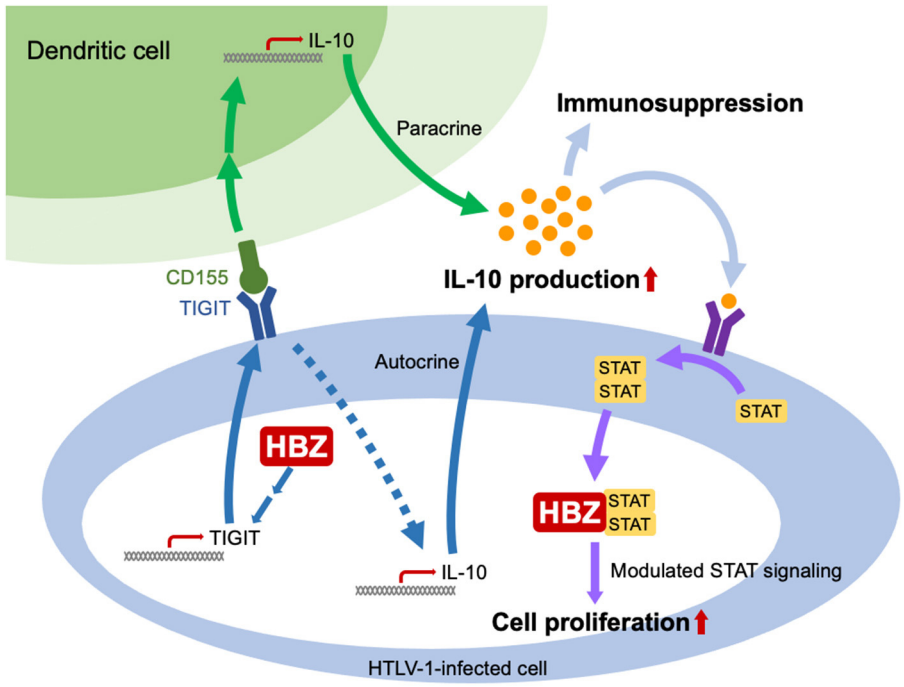

FIGURE 3 | HBZ increases production of IL-10 and alters intracellular signaling from the IL-10 receptor. HBZ induces expression of TIGIT. When TIGIT binds to its ligand, CD155, IL-10 production by DC's is enhanced. In addition, the reverse signal from TIGIT to the infected T cell increases its IL-10 production. TGF-b production is also enhanced by HBZ (not shown). Thus, the immunosuppressive cytokines TGF-b and IL-10 inhibit the host immune response. HBZ also binds to STAT1 and 3 and modulates intracellular signaling from the IL-10 receptor, enhancing proliferation of the infected cell even while the response of neighboring uninfected cells is suppressed.

with modulated signaling from the IL-10 receptor seems to be a clever strategy of HTLV-1 – a strategy that enables both proliferation of infected T cells and suppression of host immune responses.

An accessory protein p12 interferes with the intracellular trafficking of major histocompatibility complex class I heavy chain (MHC-I-Hc) of HLA-A2, -B7, and -Cw4, resulting in downmodulates its cell surface expression (57). Down-modulated MHC-I-Hc impairs recognition of HTLV-1 infected cells by CTLs. It is noteworthy that p12 is expressed from the plus-strand of the provirus by Tax-dependent transcription. Immunosuppressive effect of p12 enables Tax expressing cells to escape from CTLs. Loss of MHC-I allows attack from natural killer (NK) cells. However, p12 also down-modulates expression of intercellular adhesion molecule 1 (ICAM-1) and ICAM-2, and K cell activating receptors, NCR and NKG2D (58), which confers resistance of HTLV-1 infected cells to NK cells.

HTLV-1 INFECTION IN HEMATOPOIETIC STEM CELLS

High throughput sequencing enables us to identify a wide variety of HTLV-1 provirus integration sites (17). The presence of identical integration sites among cells of different hematopoietic lineages (CD4 T cells, CD8 T cells, B cells, monocytes and neutrophils) in the same HTLV-1 infected individuals (59). This is also demonstrated by the report of two cases with ATL clones that had different T-cell receptor gene rearrangements and identical proviral integration sites (60). These data suggest that HTLV-1 infects hematopoietic stem cells and that infected cells differentiate *in vivo*. It is possible that HBZ directs the differentiation toward Treg cells. What is the advantage for HTLV-1 to infect hematopoietic stem cells? Since the bone marrow (BM) is under hypoxic conditions, immune responses are suppressed (61), which likely allows infected cells to express Tax. Indeed, an unexpectedly high frequency of *tax* mRNA-expressing cells was reported in the BM of HAM patients (62). Newly infected hematopoietic stem cells at the BM can differentiate without Tax expression. HBZ directs differentiation of infected cells to Treg cells and promotes their proliferation. Since the actions of HBZ are specialized to Treg cells, it is unlikely that HBZ increases the number of other hematopoietic cells. Thus, most infected cells only have to express the *HBZ* gene and the necessity to express more immunogenic Tax is not essential in the periphery.

DIFFERENT SUBTYPES IN ATL CASES

Latently infected cells lead to development of ATL in some HTLV-1 carriers. Although ATL is caused by HTLV-1, the requirements of ATL cells for viral genes are not uniform. Tax is not expressed in approximately half of ATL cases, whereas HBZ

is expressed in all (63). Importantly, mutations that abrogate Tax expression can occur very early, before proviral integration, and ATL can still develop. Non-sense mutations of the *tax* gene are formed by APOBEC3G, which means that they are generated before the proviral integration (14). Furthermore, deletion of the 5′LTR also occurs before the integration of HTLV-1 provirus, since the genomic regions adjacent to the LTR retain six bp repeats (64). Since Tax is not expressed before proviral integration, these findings indicate that leukemogenesis of these ATL cases depends on HBZ alone (18).

In the other half of ATL cases, the HTLV-1 provirus retains the structure to express Tax (intact *tax* gene and 5′LTR, unmethylated 5′LTR). In these cases, the level of *tax* transcription is low, indicating that ATL cells in these cases are similar to the MT-1 cell line, which expresses Tax intermittently (20). It is noteworthy that only *in vitro* cultured HTLV-1 infected cell lines produce abundant Tax. Most of these cell lines are established only *in vitro* and do not reflect ATL cells *in vivo*.

CONCLUDING REMARKS

HTLV-1 has existed in humans for a long time since its interspecies transmission from monkeys. STLV-1 has existed in monkeys for even longer. These viruses have acquired strategies to evade the host immune response and to increase their chances of transmission. Treg like cells are suitable resident cells for HTLV-1 to escape from CTLs. Treatments that intervene in these strategies could be useful in preventing the development of ATL.

AUTHOR CONTRIBUTIONS

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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