TCF1 and LEF1 act as T-cell intrinsic HTLV-1 antagonists by targeting Tax

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Human T-cell leukemia virus type 1 (HTLV-1) is a delta-type retrovirus that induces malignant and inflammatory diseases during its long persistence in vivo. HTLV-1 can infect various kinds of cells; however, HTLV-1 provirus is predominantly found in peripheral CD4 T cells in vivo. Here we find that TCF1 and LEF1, two Wnt transcription factors that are specifically expressed in T cells, inhibit viral replication through antagonizing Tax functions. TCF1 and LEF1 can each interact with Tax and inhibit Tax-dependent viral expression and activation of NF-κB and AP-1. As a result, HTLV-1 replication is suppressed in the presence of either TCF1 or LEF1. On the other hand, T-cell activation suppresses the expression of both TCF1 and LEF1, and this suppression enables Tax to function as an activator. We analyzed the thymus of a simian T-cell leukemia virus type 1 (STLV-1) infected Japanese macaque, and found a negative correlation between proviral load and TCF1/LEF1 expression in various T-cell subsets, supporting the idea that TCF1 and LEF1 negatively regulate HTLV-1 replication and the proliferation of infected cells. Thus, this study identified TCF1 and LEF1 as Tax antagonistic factors in vivo, a fact which may critically influence the peripheral T-cell tropism of this virus.

HTLV-1 | Tax | TCF1 | LEF1

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uman T-cell leukemia virus type 1 (HTLV-1) causes a malignancy named adult T-cell leukemia (ATL) and several inflammatory diseases including HTLV-1–associated myelopathy/ tropical spastic paraparesis (HAM/TSP) (1, 2). HTLV-1 encodes a critical transactivator, Tax, that induces the activation and subsequent clonal expansion of infected T cells in vivo (2, 3). Tax is transcribed from the viral promoter 5′ long terminal repeat (LTR), where it further enhances HTLV-1 viral transcription by recruiting cellular CREB protein to Tax-responsive elements (TRE). However, Tax expression is frequently silenced in ATL cells due to genetic and epigenetic changes in the viral 5′LTR. On the other hand, Tax expression is induced in ATL cells due to genetic and epigenetic changes in the viral 5′LTR and the tax gene (4–7), a possible consequence of host immune surveillance (8). On the other hand, Tax is able to down-regulate TCF1/LEF1 expression and activation of NF-κB and AP-1. As a result, HTLV-1 replication is suppressed in the presence of either TCF1 or LEF1. On the other hand, T-cell activation suppresses the expression of both TCF1 and LEF1, and this suppression enables Tax to function as an activator. We analyzed the thymus of a simian T-cell leukemia virus type 1 (STLV-1) infected Japanese macaque, and found a negative correlation between proviral load and TCF1/LEF1 expression in various T-cell subsets, supporting the idea that TCF1 and LEF1 negatively regulate HTLV-1 replication and the proliferation of infected cells. Thus, this study identified TCF1 and LEF1 as Tax antagonistic factors in vivo, a fact which may critically influence the peripheral T-cell tropism of this virus.

TCF1/LEF1 Are Expressed at Low Levels in HTLV-1–Infected T Cells. Previously we reported that HBZ impaired the DNA-binding ability of TCF1/LEF1 and thereby suppressed the canonical Wnt pathway, shaping an HTLV-1–favorable host environment (16). Interestingly, upon further study, we found that TCF1 and LEF1 mRNA and protein levels were invariably low in HTLV-1–infected cell lines, in contrast to most HTLV-1–negative T-cell lines except Kit225 (Fig. 1 A and B). Fresh ATL cells exhibited reduced expression of TCF1 and LEF1 compared with CD4 T cells from a healthy donor (Fig. 1 C). Moreover, by analyzing microarray data of HTLV-1–infected individuals including asymptomatic carriers (AC), HAM/TSP, and ATL patients (GSE19080 and GSE33615), we observed similar down-regulation of TCF1 and LEF1 (Fig. S1 A and B).

Significance

HTLV-1 is a peripheral T-cell tropic virus and induces proliferation of CD4+ T cells, resulting in T-cell malignancy and inflammatory diseases. Recent studies demonstrated that several restriction factors inhibiting HIV are also inhibitory to HTLV-1. We identified two T-cell–specific proteins, TCF1 and LEF1, as HTLV-1 restriction factors that determine the peripheral T-cell tropism of this virus by targeting Tax. They are highly expressed in immature thymocytes and thereby become a natural inhibitor barrier for HTLV-1 replication in the thymus. However, their expression can be down-regulated by Tax, as well as by activation and differentiation of T cells. These findings provide a mechanistic understanding of how HTLV-1 induces T-cell malignancies in the periphery but never in the thymus.

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TCF1 and LEF1 Inhibit HTLV-1 Replication by Antagonizing Tax. Next we examined the biological effects of this antagonism on Tax. HTLV-1 replication depends on Tax-driven transcription from the 5' LTR. To address whether TCF1 and LEF1 are detrimental to HTLV-1 replication, we used an infectious clone of HTLV-1, pXIMT-M (18). HTLV-1 virus production measured by p19 ELISA was inhibited by TCF1 or LEF1 in a dose-dependent manner (Fig. 3A). Furthermore, expression of viral proteins that rely on Tax, such as gp46, p19, p24, and even Tax itself, was suppressed by TCF1 or LEF1 (Fig. 3B). We also found that endogenous TCF1 or LEF1 is also able to suppress HTLV-1 replication (Fig. S4).

On the other hand, HBZ transcription, which is initiated from viral 3' LTR and slightly enhanced by Tax (19), was not suppressed but rather enhanced by TCF1 or LEF1 (Fig. 3B), in sharp contrast to Tax (Fig. 3B). To see whether this was associated with differential regulation of the HTLV-1 5' and 3' LTRs domain of TCF1 was indispensable for binding to Tax whereas all three domains were required for LEF1 to bind to Tax properly (Fig. S3A). Reporter assays with WT-Luc also functionally verified this result (Fig. S3B).

Nevertheless, due to their broad-spectrum antagonism of Tax, we suspected TCF1 and LEF1 might competitively bind to Tax over other host factors that are hijacked by Tax for transcription of the viral LTR. CREB is recruited by Tax for its activation of the HTLV-1 5' LTR (3). We found that TCF1 or LEF1 dose-dependently displaced CREB from Tax (Fig. 2D), which suggests that TCF1 and LEF1 each hinder the interaction between Tax and CREB. Thus, these data demonstrate that TCF1 and LEF1 are Tax antagonists that likely execute their inhibition via direct interaction with Tax.

**Fig. 1.** TCF1 and LEF1 are expressed at low levels in HTLV-1–infected T cells. (A) TCF1 and LEF1 mRNA expression is invariably low in HTLV-1–infected cell lines. Total RNA was extracted for each cell line and subjected to quantitative real-time PCR (qPCR) analysis. Results are shown as relative mRNA expression of TCF1 or LEF1 normalized to that of 18S rRNA. (B) TCF1 and LEF1 protein expression of cell lines used in A. β-tubulin expression was used as a control. (C) TCF1 and LEF1 mRNA expression is lower in fresh ATL cases. Peripheral CD4 T cells from a healthy donor (HD) and four ATL patients were subjected to RNA extraction and following qPCR analysis. Results are shown as relative mRNA expression of TCF1 or LEF1 normalized to that of 18S rRNA. “Fold exp.” indicates fold expression of normalized mRNA level of TCF1 or LEF1.

TCF1 and LEF1 Interact with Tax and Impair its Transactivating Ability. TCF family members have been recently reported to inhibit HIV type 1 (HIV-1) basal transcription (17). Therefore, we analyzed effects of TCF1 and LEF1 on transcription from the HTLV-1 LTR. As observed in HIV-1, we found that Tax-mediated activation of WT-Luc, which contains five tandem repeats of the TRE from HTLV-1 5' LTR, was inhibited by TCF1 or LEF1 (Fig. 2A). Moreover, activation of the NFκB and AP1 pathways by Tax was also suppressed by TCF1 or LEF1 (Fig. 2A). Neither the activator of the Wnt pathway β-catenin nor the inhibitor Axin2 had such effects (Fig. S2A), indicating that the effects of TCF1 and LEF1 were mediated in a Wnt-independent manner. Furthermore, neither TCF1 nor LEF1 could inhibit the activation of these reporters by other transcription factors (Fig. S2B), suggesting that TCF1 and LEF1 specifically impair Tax function. We performed coimmunoprecipitation (co-IP) and found that TCF1 and LEF1 could each associate physically with Tax in vivo (Fig. 2B). Using a series of deletion mutants of Tax, we found that TCF1 and LEF1 predominantly bound to the C-terminal region of Tax (Fig. S2C). The PDZ-binding motif (PBM) is known to be localized in the C-terminal end of Tax (3). We found that removal of the PBM greatly impaired Tax binding to TCF1 or LEF1 (Fig. 2C), indicating that the PBM of Tax is critical for its binding with TCF1/LEF1. However, Tax bound to distinct regions of TCF1 and LEF1. The central regulation
Higher Expression of TCF1 and LEF1 Is Associated with Low STLV-1 Proviral Load in Vivo. The above results suggest that Tax function and HTLV-1 replication are impaired in TCF1/LEF1-expressing cells, most likely in thymocytes that express higher levels of TCF1 and LEF1. To analyze the relationship between TCF1/LEF1 expression and proviral load (PVL) in vivo, a model of HTLV-1 infection was required. We have reported that by TCF1/LEF1, we performed reporter assays with the complete 5′ and 3′ LTR sequences. Tax mildly activated the 3′ LTR, and this activation was enhanced by TCF1 or LEF1 (Fig. 3C). This observation explains why HBZ transcription increased in the presence of TCF1/LEF1 (Fig. 3B). Consistent with the WT-Luc result (Fig. 2A), TCF1 or LEF1 significantly suppressed Tax-induced 5′ LTR activation (Fig. 3C). To evaluate the effect of TCF1/LEF1 upon HTLV-1 de novo infection, we cocultivated Jurkat or normal CD4 T cells with lethally irradiated MT-2 cells.

Tax expression was detected predominantly in the TCF1/LEF1 low-expressing fraction (Fig. 3D), suggesting that TCF1/LEF1 restricts HTLV-1 de novo viral expression and its replication.

Tax Down-Regulates TCF1 and LEF1 via STAT5a. Antigen encounter or T-cell activation were reported to trigger TCF1/LEF1 down-regulation (15). We confirmed that phosphor myristate acetate (PMA)/ionomycin (P/I) stimulation down-regulate TCF1 and LEF1 in Jurkat and primary CD4 T cells (Fig. 4A and Fig. S5A).

Therefore, we suspected that reduced expression of TCF1 and LEF1 in HTLV-1–infected cells is also caused by Tax, which is known to activate T cells (3). As expected, Tax induced the expression of the same activation markers as P/I stimulation (Fig. S5B), and suppressed the expression of TCF1 and LEF1 in Jurkat cells (Fig. 4A). Furthermore, cadmium-induced Tax expression in JX9-9, a modified Jurkat line that expresses Tax under a metallothionein promoter (20), also down-regulated TCF1 and LEF1 (Fig. 4B). However, Tax (Fig. S5C) did not inhibit transcription from the TCF1 and LEF1 promoters. To see whether the NFκB, NFAT, or AP1 pathways, the three major TCR downstream pathways, are involved in TCF1/LEF1 down-regulation (21), we activated them by electroporation of the corresponding transcription factors into Jurkat (Fig. S5D). However, neither single nor combined activation of these pathways clearly suppressed TCF1 or LEF1 expression (Fig. S5E).

JAK/STAT signaling, a major cytokine pathway of T cells that becomes active following T-cell activation (22), has been found to be constitutively active in HTLV-1–infected T cells (23). Because STAT proteins are transcription factors that activate this pathway (24), we examined the effect of STAT5a, which is reported to be a target of Tax (25). First, we confirmed that STAT5a expression was induced upon P/I stimulation and Tax expression (Fig. 4C). Then we overexpressed either the wild type or the constitutively active form of STAT5a in Jurkat cells, and found significantly decreased expression of TCF1 and LEF1 (Fig. 4D).

Fig. 3. TCF1 and LEF1 each inhibit HTLV-1 replication by antagonizing Tax. (A) TCF1 and LEF1 each inhibits HTLV-1 production (Upper) and protein expression (Lower). pX1MT-M (0.5 μg) was transfected with or without TCF1 or LEF1 into 293FT cells. 48 h later, supernatants were collected for p19 ELISA and cells were lysed for Western blot. (B) TCF1 and LEF1 each inhibit Tax transcription (Lower) but not HBZ transcription (Upper). pX1MT-M (0.5 μg) was transfected with or without TCF1 or LEF1 into 293FT cells. 44 h later, RNA was extracted for qPCR analysis. (C) TCF1 and LEF1 each slightly enhance Tax-mediated 3′ LTR-Luc (Left) activation, whereas they significantly suppress 5′ LTR activation (Right). Reporter assays were performed in Jurkat cells. (D) Jurkat or normal human CD4 T cells were either cultured alone (Upper) or cocultivated with lethally irradiated (150 Gy) MT-2 cells (Lower) at a 2:1 ratio. 48 h later (when MT-2 cells were all dead), cells were stained for intracellular Tax and TCF1 or LEF1. Numerals indicate percentages of gated populations. Fold exp. indicates fold expression.

Fig. 4. Tax down-regulates the expression of TCF1 and LEF1 via STAT5a. (A) P/I stimulation (Upper) or Tax overexpression (Lower) inhibits TCF1/LEF1 transcription in Jurkat. For P/I stimulation, cells were treated with 50 ng/mL of PMA and 500 ng/mL of ionomycin (P/I) for 5 h and then subjected to RNA extraction and qPCR analysis. Overexpression of Tax was achieved by electroporation and 24 h later, RNA was extracted for qPCR. (B) Tax induction in JX9-9 down-regulates the expression of TCF1 and LEF1. JX9-9 was cultured in RPMI supplemented with 20 μM of cadmium (Cd) to induce Tax expression. At indicated time points, cells were lysed for Western blot analysis. (C) P/I stimulation or Tax overexpression induces STAT5a expression in Jurkat. P/I stimulation and Tax overexpression were performed as in A. (D) Overexpression of STAT5a down-regulates TCF1 and LEF1. Jurkat was transfected with wild type (WT) or constitutively active (CA) STAT5a by electroporation. 24 h later, RNA was extracted for qPCR. Fold exp. indicates fold expression.
STLV-1 encoded Tax and STLV-1 bZIP factor (SBZ) possess functions similar to those of HTLV-1 Tax and HBZ, and an STLV-1–infected Japanese macaque developed T-cell lymphoma (26), indicating that STLV-1–infected Japanese macaques can serve as a suitable model of HTLV-1 infection. STLV-1 Tax is highly homologous to HTLV-1 Tax (26). Similar to HTLV-1 Tax, it also has a typical PDZ-binding motif (ETDV) in its C-terminal end. We sorted various T-cell subsets from an STLV-1–infected Japanese macaque (Fig. 5A) and found that CD4+CD8+ thymocytes (T-CD8) showed the highest expression levels of TCF1 and LEF1 (Fig. 5A) whereas their PVL was the lowest (Fig. 5B). This result is consistent with our hypothesis that TCF1 and LEF1 inhibit viral expansion through impairing both the function and expression of Tax (Fig. 2 and 3). CD4+ thymocytes (T-CD4) were about twofold higher in TCF1/LEF1 expression (Fig. 5A) than their counterparts in the periphery (P-CD4). However, the PVL of P-CD4 T cells was 10-fold higher than that of T-CD4 T cells (Fig. 5B). Similar measurements were made in thymic (T-CD8) and peripheral CD8 T cells (P-CD8) (Fig. 5A and B). Interestingly, only a 1.3-fold increase of PVL in P-CD8 over T-CD8 was observed, in contrast to a 10-fold increase in P-CD4 over T-CD4 (Fig. 5B). Along with the fact that thymic CD8 and CD4 T cells had similar PVLs, this observation implies a much smaller expansion of infected CD8 T cells in the periphery than of CD4 T cells, an observation in agreement with a previous report showing that HTLV-1’s in vivo tropism is a consequence of predominant expansion of peripheral CD4 over CD8 T cells (27).

Next we compared the levels of transcriptional activity from the 5’ and 3’ LTRs of the provirus in STLV-1–infected cells. We did this by normalizing either Tax or SBZ transcription to PVL. Recall that TCF1/LEF1 regulate transcription of these genes in opposing manners (Fig. 3B). The 5’ LTR was clearly more active in peripheral CD4 or CD8 T cells than their thymic counterparts (Fig. 5C). In contrast, transcription from the 3’ LTR was more active in thymocytes, although the differences were not so big as with the 5’LTR (Fig. 5D). Memory (CD45RA+) CD4 T cells from the spleen of the STLV-1–infected Japanese macaque showed lower TCF1 and LEF1 expression but much higher PVL than naive (CD45RA+) CD4 T cells (Fig. 5 E and F), which is in agreement with the fact that HTLV-1–infected cells have mostly a memory phenotype (28).

Discussion

During coevolution between virus and the host, host cells acquire many restriction factors that suppress viral replication (29, 30). HTLV-1 is derived from STLV-1 in monkeys, just like HIV-1 is derived from SIV. Many restriction factors have been reported for HIV-1 (31). However, restriction factors for HTLV-1 have not been studied extensively. It has been reported that APO-BEC3G suppresses replication of HTLV-1 whereas Gag protein inhibits incorporation of APOBEC3G into the virion (32). Recently, SAMHD1 has been reported to suppress replication of HTLV-1 in monocytes (33). Tax is indispensable for HTLV-1 replication because expression of most viral genes, including all HTLV-1 structural genes, depends on transcription from the 5’ LTR that is activated by Tax. Moreover, Tax also plays a key role in dysregulating the cellular environment toward one which favors viral propagation, such by activation and transformation of an infected T cell (2). It is presumed that the T-cell tropism of HTLV-1 is more likely determined by postinfection events triggered by the virus because viral receptors are expressed in a wide variety of host cells (34). This study suggests that TCF1 and LEF1 are factors that restrict the tropism of this virus to peripheral T cells. In thymocytes expressing high levels of TCF1 and LEF1, these factors impair the functions of Tax, likely hindering not only viral replication but also the proliferation of the infected cells.

Restriction of tropism to peripheral T cells is likely a useful adaptation for HTLV-1. If HTLV-1 could replicate efficiently in the thymus, it might cause serious damage to the host immune system and thus the host. Furthermore, this virus is transmitted via breast-feeding or sexual transmission through infected T cells, so infected T cells must enter breast milk or semen. Most T cells in breast milk are peripheral T cells with an effector/memory phenotype (35). Restriction by TCF1/LEF1 would explain viral tropism to peripheral T cells and facilitate transmission of the virus.

Neoplasms of immature T cells have not been reported in HTLV-1–infected individuals. However, transgenic expression of Tax in the thymus induced immature T-cell lymphomas (36). These findings suggest that overexpression of Tax is oncogenic even for thymocytes, but that Tax expression or functions are normally impaired in the thymus of infected individuals. This study presents a mechanism for how thymocytes are relatively resistant to HTLV-1 infection and leukemogenesis in vivo, by identifying TCF1 and LEF1 as antagonists for Tax. We discovered an unexpected Wnt-independent role of TCF1 and LEF1 as Tax antagonists and demonstrated that this antagonism renders thymocytes less permissive for HTLV-1 replication compared with peripheral T cells.

The roles of TCF1/LEF1 have been well established in the thymus; they are indispensable in driving T-cell development (37). Nevertheless, their functions in the periphery remain
unknown. Recent studies showed that down-regulation of TCF1/LEF1 always occurs in activated or differentiated peripheral T cells (38). HTLV-1 may exploit this down-regulation to achieve its expansion, because down-regulation of TCF1/LEF1 allows Tax to execute its functions. A previous report also indicated that preactivated primary T cells are easier to transform by HTLV-1 (39). Down-regulation of TCF1/LEF1 upon T-cell activation/differentiation would allow Tax expression and subsequent HTLV-1 expansion.

Down-regulation of TCF1/LEF1 also occurs as T cells develop or differentiate, from DP to SP in the thymus (Fig. 5A), or from naive to memory in the periphery (Fig. 5E). Therefore, our results also imply an interesting possibility that HTLV-1 might achieve its expansion as infected T cells differentiate or even by driving differentiation of infected T cells to reduce TCF1/LEF1 expression. Indeed, a recent report using humanized mice showed altered T-cell development upon HTLV-1 infection in that the mature SP population, instead of immature DN or DP, becomes dominant in the thymus (40). This finding suggests that thymocytes are propelled to develop by HTLV-1 or the virus selectively expands in the more differentiated subsets. Similarly, in a previous study of peripheral T cells, we demonstrated that HTLV-1–infected T cells were mostly memory cells and the number of naive cells was significantly decreased (28). Our current results also reveal the preferential infection of CD4 effector/memory T cells by STLV-1. However, to clarify the roles of T-cell development/differentiation in contributions of HTLV-1 expansion, further studies are needed.

STLV-1–infected Japanese macaque has been demonstrated to be a suitable model for HTLV-1 infection (26). It also served as an ideal model to analyze the impact of the antagonism of TCF1/LEF1 against Tax in vivo. However, due to the complexity of viral infections in vivo, other factors such as the susceptibility to viral infections, postinfection mitotic potential and cytotoxic T-cell killing efficiency might affect the consequence of an infection in a specific T-cell subset. Indeed, the tropism of the virus for peripheral CD4 T cells over peripheral CD8 T cells does not appear to be explained by TCF1/LEF1 levels. More detailed investigations in STLV-1–infected Japanese macaques are expected to clarify these points in the future.

TCF1/LEF1 regulate the HTLV-1 5′ and 3′ LTR activities in opposition to their interaction with Tax (Fig. 3C). This may result in distinct expression levels of Tax and HBZ in vivo in different T-cell subsets or during various stages of infection. Interestingly, valproate, a histone deacetylase inhibitor, was reported to induce Tax expression while suppressing that of HBZ (41). These intriguing observations that the HTLV-1 5′ and 3′ LTR are regulated in opposite ways by multiple mechanisms, in addition to frequently observed contradictory functions of Tax and HBZ, may suggest a complex but fine-tuned viral pathogenesis. For instance, although activation of NF-κB pathway has been considered a critical function of Tax for cellular transformation (2, 3), the recent studies have reported that hyperactivation of NF-κB pathway induces cellular senescence whereas HBZ suppresses this action of Tax, thereby enabling clonal expansion (42). This study shows that TCF1/LEF1 inhibit Tax-mediated NF-κB activation by direct binding to Tax. Furthermore, TCF1/LEF1 inhibit various functions of Tax, whereas HBZ selectively modulates signaling pathways (43, 44). Thus, Tax and HBZ collaboratively function for clonal expansion and viral replication, whereas TCF1/LEF1 inhibit functions of Tax by direct interaction, which leads to suppression of viral replication and proliferation of infected cells.

In summary, we here identify TCF1 and LEF1 as previously unidentified Tax antagonists that likely restrict viral expansion in the thymus. The critical interplay of TCF1 and LEF1 with Tax during HTLV-1 infection may shed light on how HTLV-1 achieves its tropism and persistence in peripheral T cells in vivo.

Materials and Methods

Primary Samples Ethics Statement. The experiments using primary samples in this study were conducted according to the principles expressed in the Declaration of Helsinki. This study was approved by the Institutional Review Board of Kyoto University (approval numbers G310 and E2005). All ATL patients and healthy individuals provided written informed consent for the collection of samples and subsequent analysis. A Japanese macaque used in this study was 3 y old and naturally infected with STLV-1. The monkey was reared in the Primate Research Institute, Kyoto University. All animal studies were conducted in accordance with the protocols of experimental procedures approved by the Animal Welfare and Animal Care Committee of the Primate Research Institute (approval number 2011-095).

Cell Lines. ATL-derived T-cell lines (HPB-ATL-2, HPB-ATL-T, ATL-43T+, ATL-55T+, ED, MT-1, and TL-Om1), HTLV-1–transformed T-cell lines (ATL-35T, Hut102, MT-2, and MT-4) were used in this study. Jurkat, CEM, Hut78, SupT1, Molt4, and Ki225 are HTLV-1–negative T-cell lines. All T-cell lines were maintained in RPMI supplemented with 10% (vol/vol) FBS, whereas Ki225, ATL-43T+, and ATL-55T+ were maintained in the media supplemented with 100 U/ml of recombinant IL-2. 293FT (Life Technologies) is a subline of HEK293, which originated from a human embryonic kidney cell.

Plasmids. Expression vectors for TCF1, LEF1, and Tax were described (16, 45). Flag-CREB was made by subcloning the CREB coding sequence into pCAG-Flag. WT-Luc and 5′ LTR-Luc were kind gifts from J. Fujisawa, Kansai Medical University, Osaka, pX1MT-M was a generous gift from D. Derse, National Cancer Institute, Frederick, MD. NF-κB-Luc and AP1-Luc were purchased from Stratagene. 3′ LTR-Luc was described (19).

Antibodies. Rabbit monoclonal antibodies for TCF1 (C63D9) and LEF1 (C12A5) were purchased from Cell Signaling Technology. HRP conjugated mouse anti-FLAG (M2), Myc (9E10), α-tubulin (DM1A), and Tax (MI73) were described (16). For flow cytometric analysis of cell surface markers, APC-Cy7 anti-CD3 (SP34-2), PerCP-Cy5.5 anti-CD4 (OKT4), V500 anti-CD8 (RPA-T8), and PE anti-CD45RA (SH9) were used. PerCP-Cy5.5 anti-CD4 (OKT4) was purchased from Biolegend, whereas the others were from BD.

Detection of Tax and TCF1/LEF1 by Flow Cytometry. Intracellular staining for Tax and TCF1/LEF1 was performed using the kit from eBioscience. DyLight 649 conjugated donkey anti-rabbit IgG and FITC conjugated goat anti-mouse IgG were purchased from Biolegend. Normal mouse IgG was purchased from Santa Cruz and used for blocking nonspecific binding.

ELISA. Supernatants from cultured cells were centrifuged at 1,710 × g for 5 min to remove debris and then diluted and quantified for p19 by ELISA. Supernatants from cultured cells were centrifuged at 1,710 × g for 5 min to remove debris and then diluted and quantified for p19 by ELISA (Zetaplex) according to manufacturer’s instructions.

Sorting by FACS Aria II. See Fig. S5 for details.

Electroporation, real-time PCR, knockdown, Western blot, commuino-precipitation, and reporter assays were performed as described (16).

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Supporting Information

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**SI Materials and Methods**

**Primer Information.** Human TCF1 (forward): 5′-AGGGAAGCAGGAGCTGCAGC
Human TCF1 (reverse): 5′-GCACCTGCAATGACCTTGGC
Human/monkey LEF1 (forward): 5′-CTCAGGAGGCTACACGC
Human/monkey LEF1 (reverse): 5′-GCACTCTGCAATGACCTTGGC
Monkey TCF1 (forward): 5′-GTCTCTCTCTCTACGAC
Monkey TCF1 (reverse): 5′-CTGAGGTCAGGGAGTAGAA
Human/monkey 18s rRNA (forward): 5′-AACCCGTTGAACTCCATT
Human/monkey 18s rRNA (reverse): 5′-CCATCCAATCGGTAGTAGCG
Human/monkey RAG1 (forward): 5′-CCCACCTTGAGACCTAC
Human/monkey RAG1 (reverse): 5′-CACCCGGAACAGCTTAATT
Probe for RAG1: 5′-CCCCAGATGAAATTCAGCACCTATA
HTLV-1 Tax (forward): 5′-ATGGCCCACTTCCCAGGGTT
HTLV-1 Tax (reverse): 5′-CCGAACATAGTCCCCCAGAG
HTLV-1 HBZ (forward): 5′-ATGGCGGCCTCAGG
HTLV-1 HBZ (reverse): 5′-GCTTTTCCTCCCTGGAGGCGC
STLV-1 Tax (forward): 5′-CTACCTATTCCAGGACCACAG
STLV-1 Tax (reverse): 5′-CGTGCCAATCGGTAAATGTCC
 Probe for STLV-1 Tax: 5′-CCCGCCACGCTGACAGCCTGGCAG
 STLV-1 SBZ (forward): 5′-CAGACCGGGAGACTCCCTGCTC
 STLV-1 SBZ (reverse): 5′-CTTGTGGTGTCCCTCCAGAG
 Human CD25 (forward): 5′-AGTCAGTTTCCAGGTGAAGA
 Human CD25 (reverse): 5′-TCTCCATGGTGCAGCCATT
 Human CD44 (forward): 5′-GCCCTTCCATAGCCTAATCC
 Human CD44 (reverse): 5′-CTTTGGCTGTCGTCACCTCTCCAGAAGC
 Human CD69 (forward): 5′-CCCTATCAGTGCGGCAATAC
 Human CD69 (reverse): 5′-TGTTAGCCACACAGCTTCT

**Target Sequences of shRNAs.** Negative control shRNA (shNC): 5′-GCGCGATAGCGCTAATAATTT
TCF1-sh1: 5′-CAACTCTCTCTCTACGACTT
TCF1-sh2: 5′-CTCCTCTCTCTCTCTCTCTCTC
LEF1-sh8: 5′-CCACACTGACAGTGACCTAAT
LEF1-sh10: 5′-CCCATCAGATGTCACCTC

**Fig. S1.** (A) Relative TCF1/LEF1 expression in healthy donors (HD), asymptomatic carriers (AC), and HAM/TSP patients (HAM). Data (GSE19080) were obtained from Gene Expression Omnibus website (www.ncbi.nlm.nih.gov/geo). (B) Relative TCF1/LEF1 expression in healthy donors (HD) and ATL patients. Data (GSE33615) were obtained from Gene Expression Omnibus website (www.ncbi.nlm.nih.gov/geo). Statistical analysis was performed with nonparametric Mann–Whitney u test for A and B.
Fig. S2.  (A) β-catenin or Axin2 expression vector was cotransfected along with pCG-Tax in Jurkat and 24 h later reporter assay was performed. The amount of β-catenin or Axin2 transfected had been adjusted to induce a similar change of Wnt activity as TCF1 or LEF1 did in Fig. 2A. (B) Reporter assays were performed using respective transcription factors for each reporter (CREB for WT-Luc, p65 for NFκB-Luc, and c-Jun for AP1-Luc). (C) Physical interactions between TCF1 (Upper) or LEF1 (Lower) and truncated mutants of Tax indicate C-terminal region of Tax is indispensible for Tax interacting with TCF1 or LEF1. Scheme of truncated mutants is shown above the results.
Fig. S3. (A) Physical interactions between Tax and truncated mutants of TCF1 (Upper) or LEF1 (Lower). Co-IP was performed in 293FT. (B) Effects of WT and truncated mutants of TCF1 (Upper) or LEF1 (Lower) on Tax-mediated WT-Luc activation. Reporter assays were performed in Jurkat.

Fig. S4. Knockdown of endogenous TCF1 or LEF1 enhances HTLV-1 production. Vectors encoding negative control shRNA (shNC) or shRNAs specific for TCF1 (sh1 and sh2) or LEF1 (sh8 and sh10) were cotransfected with pX1MT-M in 293FT. 48 h later, supernatants were collected for p19 ELISA (Lower). Cells were prepared for qPCR to confirm the knockdown efficiency (Upper).
Fig. S5. (A) T-cell activation by P/I stimulation down-regulated TCF1/LEF1 expression in normal human CD4 T cells. Cells were isolated by negative selection from a healthy donor and stimulated by PMA (50 ng/mL) and ionomycin (500 ng/mL) for 5 h before harvesting for RNA extraction. (B) Both P/I stimulation and Tax expression induced T-cell activation markers including CD25, CD44, and CD69 in Jurkat. (C) Tax did not suppress TCF1/LEF1 promoter activity. The promoter region of TCF1, which spans −1347 to +212 bp relative to the transcription start site, and that of LEF1 spanning positions −521 to +725 bp were amplified and cloned into pGL4.10. Luciferase assays were performed by transfecting those reporter constructs with or without Tax-expressing vector to Jurkat. All luciferase values were normalized with the activity of Renilla luciferase. (D) p65, NFATc1, and c-Jun activated respective reporters as P/I stimulation. Reporter assays were performed in either P/I stimulated (Upper) or transcription factors-transfected (Lower) Jurkat. (E) Single or combined activations of three major pathways downstream TCR did not down-regulate TCF1/LEF1. Transfections in B and E were done by electroporation and, 24 h later, cells were collected for RNA extraction and subsequent qPCR analysis.
Fig. S6. Sorting strategies of thymocytes shown in Fig. 5. Thymocytes of the Japanese macaque were stained by PerCpCy5.5 anti-CD4 and V500 anti-CD8. PBMCs were purified from blood of the monkey and stained by APC-Cy7 anti-CD3, PerCpCy5.5 anti-CD4, and V500 anti-CD8. Splenocytes of the monkey were stained by APC-Cy7 anti-CD3, PerCpCy5.5 anti-CD4, and PE anti-CD45RA. Cells were subjected to sorting by FACS Aria II. All seven sorted populations were denoted in red.