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HTLV-1 bZIP factor impairs cell-mediated immunity by suppressing production of Th1 cytokines

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HTLV-1 bZIP factor impairs cell-mediated immunity by suppressing production of Th1 cytokines

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Adult T-cell leukemia (ATL) patients and human T-cell leukemia virus-1 (HTLV-1) infected individuals succumb to opportunistic infections. Cell mediated immunity is impaired, yet the mechanism of this impairment has remained elusive. The *HTLV-1 basic leucine zipper factor* (*HBZ*) gene is encoded in the minus strand of the viral DNA and is constitutively expressed in infected cells and ATL cells. To test the hypothesis that HBZ contributes to HTLV-1–associated immunodeficiency, we challenged transgenic mice that express the *HBZ* gene in CD4 T cells (HBZ-Tg mice) with herpes simplex virus type 2 or *Listeria monocytogenes*, and evaluated cellular immunity to these pathogens. HBZ-Tg mice were more vulnerable to both infections than non-Tg mice. The acquired immune response phase was specifically suppressed, indicating that cellular immunity was impaired in HBZ-Tg mice. In particular, production of IFN-γ by CD4 T cells was suppressed in HBZ-Tg mice. HBZ suppressed transcription from the *IFN-γ* gene promoter in a CD4 T cell–intrinsic manner by inhibiting nuclear factor of activated T cells and the activator protein 1 signaling pathway. This study shows that HBZ inhibits CD4 T-cell responses by directly interfering with the host cell-signaling pathway, resulting in impaired cell-mediated immunity in vivo. (*Blood*. 2012;119(2):434-444)

Introduction

Human T-cell leukemia virus type 1 (HTLV-1) is a retrovirus that mainly infects CD4 T cells,[1] a critical cell population for the host defense against foreign pathogens. HTLV-1 is known as the causal agent of adult T-cell leukemia (ATL),[2-4] a leukemia derived from CD4 T cells, and chronic inflammatory diseases, including HTLV-1-associated myelopathy/tropical spastic paraparesis,[5,6] alveolitis,[7] and uveitis. It has also been recognized that HTLV-1 infection is complicated by opportunistic infections caused by *Pneumocystis jiroveci*, herpes zoster virus, cytomegalovirus, or *Strongyloides stercoralis*.[8] However, the mechanism by which HTLV-1 causes immune deficiency has remained unknown.

Another human pathogenic retrovirus, HIV, replicates vigorously in vivo and produces a large number of virions. As a result of abundant viral production, HIV-infected CD4 T cells proceed to apoptosis, a phenomenon that eventually results in AIDS. In contrast, HTLV-1 increases its copy number primarily in the form of a provirus, by promoting the clonal proliferation of infected host CD4 T cells.[9,10] Despite this opposite effect on CD4 T-cell homeostasis compared with HIV, HTLV-1 infection and ATL are frequently accompanied by a deficiency of cellular immunity resembling that seen with AIDS.

HTLV-1 encodes several regulatory and accessory genes in the viral genome.[1,11] The viral proteins expressed by the integrated provirus control viral gene transcription and induce host cell proliferation, enabling HTLV-1 to achieve persistent infection. Among the viral genes of HTLV-1, *HTLV-1 bZIP factor* (*HBZ*), which is encoded in the minus strand,[12] is a constitutively expressed viral gene.[13] It has been reported that there are 2 major transcripts of the *HBZ* gene: spliced HBZ (sHBZ) and unspliced HBZ (usHBZ).[14] Based on the findings that sHBZ is more abundantly expressed than usHBZ[15] and that sHBZ has a functionally stronger effect than usHBZ,[16] we focused on sHBZ in this study.

Recently, we have reported that sHBZ expression increases the number of regulatory T cells (Tregs) by inducing transcription of the *Foxp3* gene in transgenic mice that express the *HBZ* gene in CD4 T cells (HBZ-Tg mice).[17] An increase in Tregs might be implicated in the immunodeficiency observed in ATL patients. Furthermore, previous studies have reported that HBZ suppresses host cell-signaling pathways that are critical for T-cell receptor signaling in the immune response, such as the NF-κB[18] and AP-1 pathways.[19] These findings led us to hypothesize that HBZ might have important roles in the dysregulation of cellular immunity associated with HTLV-1 infection.

To verify this hypothesis, we used HBZ-Tg mice that express sHBZ in CD4 T cells and studied well-established infection models of 2 pathogens. The first model involves intravaginal viral infection with herpes simplex virus type-2 (HSV-2). IFN-γ production by CD4 T cells is critical for the exclusion of HSV-2 from the host.[20,21] The other model involves infection with the Gram-positive intracellular bacterium, *Listeria monocytogenes* (LM), which is known as an opportunistic pathogen. In LM infection, CD4 T cells play pivotal roles in the acquired immune response by producing IFN-γ and inducing the activation of macrophages, which eliminate LM

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by phagocytosis and subsequent bactericidal activity.[22,23] Indeed, previous reports have shown that some ATL patients are infected with these 2 pathogens.[24,25] Using these 2 infection models, we demonstrated that sHBZ suppresses cell-mediated immunity. Furthermore, we determined the molecular mechanism of this HBZ-mediated immune suppression.

Methods

Mice

Wild-type C57BL/6J mice were purchased from CREA Japan. Transgenic mice expressing the *sHBZ* gene under control of the CD4 promoter/enhancer/silencer have been described previously.[13] All HBZ-Tg mice were heterozygotes for the transgene. All mice used in this study were maintained in a specific pathogen-free facility and handled according to protocols approved by Kyoto University.

Herpes simplex virus type 2 infection

The HSV-2 wild-type strain UW268 and thymidine kinase (TK)-negative strain UWTK (a gift from T. Suzutani, Fukushima Medical University) used in this study were propagated and titrated on Vero cells.[26] Acyclovir was used for propagation of UWTK to block emergence of TK^+ revertant. To increase their susceptibility to HSV-2, we injected mice subcutaneously with medroxyprogesterone acetate, Depo-provera (Sigma-Aldrich), (2 mg/mouse). Five days after this hormone injection, mice were anesthetized using Avertin (Sigma-Aldrich), preswabbed with a type 2 Calgiswab (Puritan), and inoculated intravaginally with 10^3 or 10^4 plaque-forming units (PFU) of UW268. For studies of secondary infection, mice were first immunized intravaginally with 10^6 PFU of UWTK, and 4 weeks later, they were inoculated intravaginally with 10^5 PFU of UW268. Vaginal secretions were collected by 3 pipettings with 15 μL of PBS, swabbed with a Calgiswab, and added to 955 μL of 5% FCS-DMEM and stored at −80°C. HSV-2 titers were determined by plaque assay on Vero cells. Five days after primary infection, lavage fluid from the vaginal tract was harvested similarly by 3 pipettings with 20 μL of PBS.

At 6 days after infection, the vaginal tissues of infected mice were fixed in 10% formalin in phosphate buffer and embedded in paraffin. H&E staining was performed according to standard procedures. The presence of HSV-2 antigen in tissues was detected using rabbit polyclonal anti–herpes simplex virus type 2 (Dako North America). Images were captured using a Provis AX80 microscope (Olympus) equipped with OLYMPUS DP70 digital camera, and detected using a DP manager system (Olympus; original total magnification ×200).

Splenic CD4 T cells from HSV-2 primary-infected mice were stimulated in a 96-well plate coated with CD3 mAb (1 μg/mL) and CD28 mAb (1 μg/mL) for 24 hours. For antigen specific stimulation, CD4 T cells were cocultured for 48 hours in the presence of irradiated T cell–depleted splenocytes as antigen-presenting cell (APC) and heat-inactivated HSV-2 (heat inactivated at 56°C for 2 hours) at a multiplicity of infection of 1. Supernatant was collected and stored at −20°C until assay.

Evaluation of resistance and immune response to LM in mice

Wild-type LM strain EGD was used in this study. The bacterial suspension was prepared as described previously.[27] For primary infection, mice were inoculated intravenously with 10^3 colony-forming units (CFUs) of LM and the bacterial burden in the spleen was determined on day 2 or 5 after infection.

For studies of secondary infection, mice were immunized intravenously with 10^3 CFUs of LM. From day 3 through day 6.5 after immunization, the drinking water supplemented with ampicillin (2 mg/mL) was given to clear any remaining LM. On day 7, mice were challenged with 10^6 CFUs of LM, and the spleens and sera were harvested after 3 or 12 hours. Spleens were homogenized in PBS, and the number of viable bacteria was determined by plating 10-fold serial dilutions on tryptic soy agar plates and counting the CFUs.

For cytometric assays, immunized mice were re-inoculated with 10^7 CFUs of LM. Splenocytes were harvested after 12 hours, cultured in the presence of protein transport inhibitor for 6 hours, and evaluated by the FACSCanto II (BD Biosciences) for cell surface and intracellular markers.

To determine the functional development of CD4 T cells in immunized mice, we purified splenic CD4 T cells and then stimulated them in a 96-well plate coated with CD3 mAb and CD28 mAb. For LM specific stimulation, CD4 T cells were cocultured with mouse bone marrow–derived macrophages (BMDMs) differentiated in the presence of 100 ng/mL of M-CSF and pulsed with viable LM at a multiplicity of infection of 10. Supernatant after stimulation for 24 hours was collected and stored at −20°C until assay.

Analysis of virus vector-transduced CD4 T cells

Retroviral transduction was performed as described previously.[17] The spliced HBZ gene was cloned into a retroviral vector, pMXs-Ig (a gift from T. Kitamura, The University of Tokyo), to generate pMXs-Ig-HBZ. This plasmid DNA was transfected into the packaging cell line, Plat-E. For retroviral transduction, $CD25^-CD4^+$ cells were enriched by a CD4 enrichment kit (BD Biosciences PharMingen) and were activated by anti-CD3 Ab (0.5 μg/mL) and rIL-2 (50 U/mL) in the presence of T cell–depleted and x-irradiated (20 Gy) C57BL/6J splenocytes as APCs in 12-well plates. After 16 hours, activated T cells were transduced with viral supernatant in the presence of 4 μg/mL polybrene and centrifuged at 1700*g* for 60 minutes. Then, transduced CD4 T cells were stimulated by phorbol 12-myristate 13-acetate (PMA; 50 ng/mL) and ionomycin (1 μg/mL) or plate-coated CD3 mAb (1 μg/mL) and CD28 mAb (1 μg/mL) in the presence of protein transport inhibitor and analyzed by a flow cytometry as shown in Figure 3. Dead cells were excluded using forward and side scatter and LIVE/DEAD Fixable Dead Cell Stain Kit (Invitrogen) by flow cytometry. Thereafter, intracellular cytokines were measured.

For generation of the lentivirus vector, sHBZ cDNA was cloned into pCS2-EF-GFP (a gift from H. Miyoshi, RIKEN BioResource Center) as previously described.[13] In brief, 293FT cells were cotransfected with the lentivirus vector, pCMV-Δ8/9 and pVSVG and supernatant containing virus was used for transduction. The lentivirus titer was determined on 293FT cells.

Empty vectors that express only GFP were used as controls for retroviral and lentiviral transductions.

IFN-γ promoter assay

Nucleotides −670 to +64 of the IFN-γ promoter region were amplified by PCR using human genomic DNA as a template, and cloned into pGL4.22 (Promega). The PathDetect pAP-1-Luc and pNFAT-Luc Cis-Reporter Plasmids were purchased from Promega. Transfection and luciferase assay were performed according to supplemental Methods (available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article).

ChIP assay

sHBZ-expressing Jurkat cells were stimulated with PMA and ionomycin. ChIP assay was performed as reported previously.[28] ChIP DNA samples were subjected to the StepOnePlus real-time PCR system using Power SYBR Green PCR Master Mix (Applied Biosystems). The sequences of the primers for the human IFN-γ promoter were: 5′-TACCAGGGC-GAAGTGGGGAG-3′ (sense) and 5′-GGTTTTGTGGCATTTGGGTG-3′ (anti-sense).

Statistical analysis

For in vitro and in vivo experiments, multiple data comparisons were performed using the Student unpaired *t* test.

Results

High susceptibility of HBZ-Tg mice to HSV-2 infection

We first evaluated the susceptibility of HBZ-Tg mice to HSV-2 infection. Recently, we reported that HBZ-Tg mice frequently develop T-cell lymphoma and dermatitis after 10 weeks.[17] Therefore, HBZ-Tg mice without skin symptoms at 7 to 10 weeks of age were used in this study. It has been reported that the host immune response against primary HSV-2 infection can be divided into 2 stages: the innate immune response plays a dominant role by day 2 after infection, whereas cellular immunity plays an important role later, after day 5 after infection.[29] IFN-γ production by CD4 T cells is known as a critical factor in the cellular immune response against pathogens.[29] To determine whether cellular immunity is impaired in HBZ-Tg mice, we pretreated HBZ-Tg and non-Tg mice with Depo-provera for efficient infection and inoculated them with HSV-2 through the vaginal route.[30] The viral titer of HSV-2 in the lesion was measured. In this primary infection assay, there was no significant difference in the viral titers between non-Tg and HBZ-Tg mice at day 2 after inoculation (Figure 1A), when innate immunity is responsible for the host defense. In contrast, at day 6 after infection, when acquired immunity becomes important, HBZ-Tg mice showed significantly higher viral titers of HSV-2 than non-Tg mice (Figure 1A). Immunohistochemical analysis revealed that abundant viral antigens were detected in the vaginal epithelial cells and ganglia of HSV-2 challenged HBZ-Tg mice but not in non-Tg mice (Figure 1B).

To explore the mechanism of this immune deficiency, we examined cytokine production by CD4 T cells stimulated with antibodies to CD3 and CD28 or with heat-inactivated HSV-2 and APC. On day 6 after infection, the production of Th1 effector cytokines, including IFN-γ, IL-2, and TNF-α, was significantly reduced in CD4 T cells from HBZ-Tg mice compared with non-Tg mice (Figure 1C). Furthermore, IFN-γ concentration in vaginal wash fluids at day 5 after infection was significantly suppressed in HBZ-Tg compared with non-Tg mice (Figure 1D). When we challenged mice with a 50% lethal dose of HSV-2, the survival rate of non-Tg mice at day 20 after infection was 53%. In contrast, HBZ-Tg mice could not survive a viral challenge at the same dose (Figure 1E).

To study acquired immunity against HSV-2, we immunized and challenged mice as shown in Figure 1F. First, mice were immunized by TK-negative HSV-2 strain, the attenuated mutant of HSV-2, and then they were challenged with wild-type HSV-2. The vaginal virus titer in HBZ-Tg mice at day 3 after challenge was similar to that in nonimmune non-Tg mice (Figure 1F), whereas HSV-2 was not detected in immune non-Tg mice. The difference in viral titer between non-Tg and HBZ-Tg mice was much more remarkable in these secondary infection experiments than in the previous primary infection experiments, implicating impaired acquired immunity in HBZ-Tg mice. These results demonstrate that expression of sHBZ in CD4 T cells induces a deficiency in the immune response against HSV-2 and impairs the production of IFN-γ, IL-2, and TNF-α.

HBZ-Tg mice have an impaired T cell–dependent immune response to LM

We next evaluated the susceptibility of HBZ-Tg mice to infection with LM via an intravenous route. As with HSV-2 infection, production of IFN-γ by CD4 T cells plays a crucial role in the growth inhibition and elimination of LM in vivo.[31,32] On day 2 or 5 after primary infection with LM, we removed spleens and evaluated the bacterial burdens in the organs. The number of LM recovered from HBZ-Tg spleen on day 2 was comparable to that from non-Tg mice, yet the bacterial burden in HBZ-Tg mice at day 5 was higher than that in non-Tg mice (Figure 2A), suggesting a reduced protection in HBZ-Tg mice against LM, especially when acquired immunity is being established. We next a performed secondary infection experiment to evaluate the T cell–dependent immunity that developed after primary infection. Non-Tg mice immunized with a small dose of LM and later challenged with a high dose exhibited a significant level of bacterial elimination 12 hours after challenge compared with nonimmunized mice (Figure 2B). By contrast, such a significant level of bacterial elimination was not observed in immunized HBZ-Tg mice (Figure 2B), indicating that acquired LM-specific immunity is impaired in HBZ-Tg mice.

Characterization of cytokine production in the LM-infected mice

We next measured the concentration of several cytokines in the sera and homogenized spleen supernatant of HBZ-Tg and non-Tg mice during secondary infection with LM. IFN-γ, TNF-α, IL-2, IL-6, and IL-10 were decreased in HBZ-Tg mice (Figure 2C) compared with non-Tg mice. On the other hand, IL-12, which is mainly secreted by APCs, was increased in HBZ-Tg at 12 hours. To explore whether impaired production of Th1 cytokines by CD4 T cells is responsible for the decrease in levels of IFN-γ, TNF-α, and IL-2 in the serum, we enriched CD4 T cells from the spleens of immunized mice and then stimulated the cells ex vivo nonspecifically (with mAbs to CD3 and CD28) or specifically (with BMDMs pulsed with viable LM). The ability of CD4 T cells from HBZ-Tg mice to produce IFN-γ and IL-2 in response to either kind of stimulation was markedly impaired compared with that of cells from non-Tg mice (Figure 2D). In contrast, a considerable amount of TNF-α production was detected in tests of both HBZ-Tg and non-Tg CD4 T cells after stimulation with LM-pulsed BMDMs. However, this level of TNF-α was almost comparable with that observed in the culture of LM-pulsed BMDMs alone (Figure 2D). Therefore, the TNF-α detected in this experiment was probably produced by the macrophages, not by the CD4 T cells. These results strongly suggest that the ability of CD4 T cells to produce Th1 cytokines is impaired in HBZ-Tg mice.

Because IFN-γ is reported to play a pivotal role in the acquired protection of mice against LM,[22,23] we focused on IFN-γ production by LM-specific CD4 T cells. Splenic cell suspensions were prepared from 2 groups of mice immunized and challenged according to the protocol shown in Figure 2B. Cells were cultured for 6 hours in the presence of protein transport inhibitor and then subjected to flow cytometric analysis for IFN-γ production by intracellular cytokine staining. The number of IFN-γ–producing CD4 T cells in HBZ-Tg mice was remarkably reduced compared with that in non-Tg mice (Figure 3A). In contrast, IFN-γ production by CD8 T cells showed no significant difference between non-Tg and HBZ-Tg mice (Figure 3A). In addition, there were no differences between HBZ-Tg mice and control littermates in both total and $CD4^+$ splenocytes (supplemental Figure 1).

We recently reported that the proportion of $Foxp3^+$ $CD4^-$ T cells is increased in HBZ-Tg mice.[17] A previous study reported that Foxp3 expression inhibits the production of IFN-γ,[33] suggesting that a decreased proportion of effector T cells in HBZ-Tg mice might be responsible for the low number of IFN-γ–producing CD4

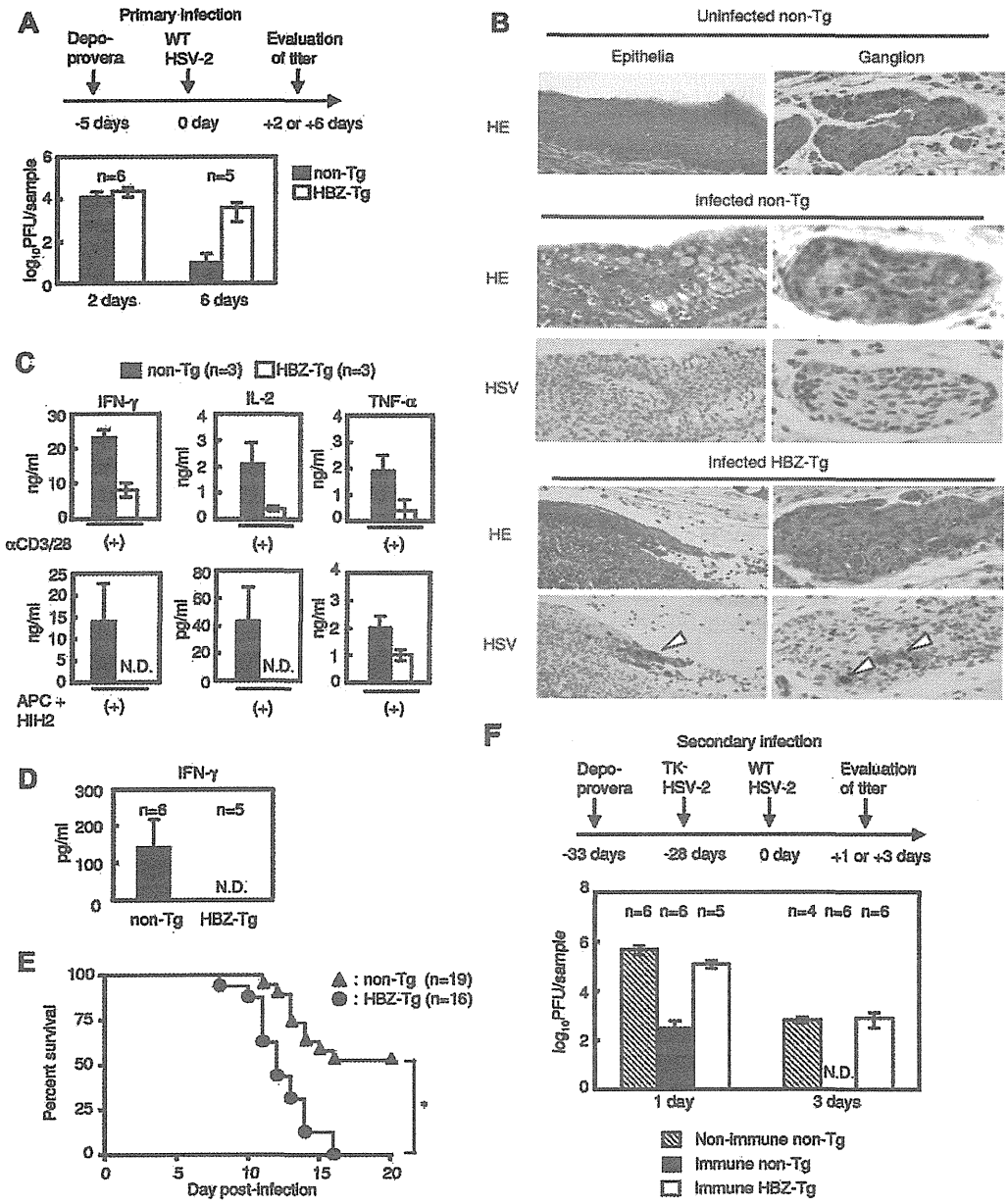

Figure 1. Transgenic mice expressing sHBZ in CD4 T cells are highly susceptible to intravaginal infection with HSV-2. (A) Virus titer in vaginal washes in primary infection. (B) Histologic analysis of epithelia and ganglion in vaginal tissue from mice infected with HSV-2. Uninfected vaginal tissues are presented as controls. HE indicates H&E stain; and HSV, immunohistochemical analysis for the viral antigen. Arrowheads indicate HSV-2–positive cells. (C) Cytokine production by splenic CD4 T cells from mice infected with 10^4 plaque-forming units (PFU) of HSV-2. Cells were stimulated with mAbs to CD3 and CD28 or APC plus heat-inactivated HSV-2 (HIH2) in ex vivo culture. (D) IFN-γ concentration in vaginal wash fluid harvested at day 5 after infection. (E) Survival curve of non-Tg or HBZ-Tg mice infected with 10^3 PFU of HSV-2. $^*P < .05$ (log-rank test). (F) Viral titer in vaginal washes during HSV-2 secondary infection. To evaluate adaptive immunity against HSV-2 infection, mice were immunized and infected with the virus as shown in the upper panel. Bars represent the mean ± SD of all mice per genotype. Two or 3 independent experiments have been performed. N.D. indicates not detected.

T cells. However, the impairment of IFN-γ production was still observed in the Foxp3-negative effector CD4 T-cell population (Figure 3B), indicating that the reduction in IFN-γ production is independent of Foxp3 expression. These results collectively indicate that transgenic expression of sHBZ in CD4 T cells results in a reduction in effector cytokine production by CD4 T cells.

sHBZ directly inhibits IFN-γ production in a CD4 T cell–intrinsic manner

To determine whether sHBZ-mediated IFN-γ suppression was induced by a cell-intrinsic effect of sHBZ in CD4 T cells or by a dysregulated immunologic status in vivo indirectly caused by sHBZ expression, we used a retrovirus vector to express sHBZ in naive CD4 T cells. Wild-type CD4 T cells transduced with sHBZ showed lower IFN-γ production than empty vector-transduced cells (Figure 4A-B), demonstrating that sHBZ directly suppresses IFN-γ production in CD4 T cells. It is noteworthy that sHBZ suppressed IFN-γ production in human CD4 T cells as well as mouse T cells. This suppression was not limited to IFN-γ but was also observed for TNF-α (Figure 4C) and IL-2 (Figure 4D). Expression level of the *HBZ* gene transcript was much higher than that of HBZ-Tg mice (supplemental Figure 2). IL-4 production was

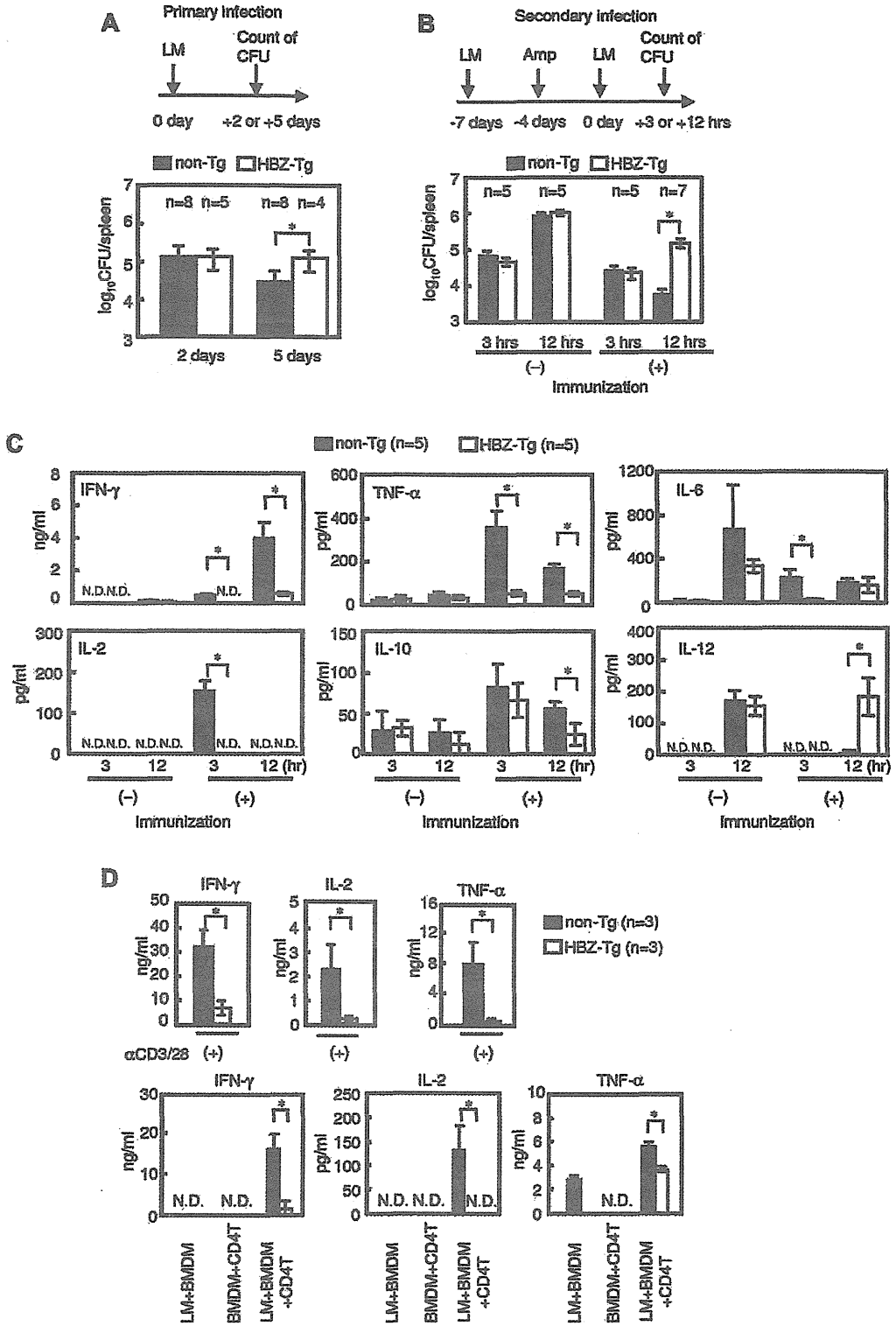

Figure 2. HBZ-Tg mice show decreased immune response to primary and secondary infection with LM. Bacterial loads of spleens from mice challenged with LM in primary (A) and secondary (B) infection are shown. (C) Concentrations of IFN-γ, TNF-α, IL-2, IL-6, and IL-12 in serum and IL-10 in homogenized spleen supernatant from the secondarily infected mice. (D) Cytokine production by CD4 T cells from secondarily infected mice. Mice were immunized as shown in panel B. CD4 T cells were stimulated ex vivo with mAbs to CD3 and CD28 or with LM-infected WT-BMDMs. Bars represent the mean ± SD of all mice per genotype. Two independent experiments have been performed; representative results are shown. *$P < .05$ by Student t test. N.D. indicates not detected.

not detected in CD4 T cells (supplemental Figure 3A). Although production of Th1 cytokines was reduced in sHBZ-expressing CD4 T cells, IL-6 and IL-10 production was not altered by sHBZ expression (supplemental Figure 3B-C). These results collectively suggest that sHBZ expression in HTLV-1–infected CD4 T cells inhibits transcription of the *IFN-γ*, *TNF-α*, and *IL-2* genes, which

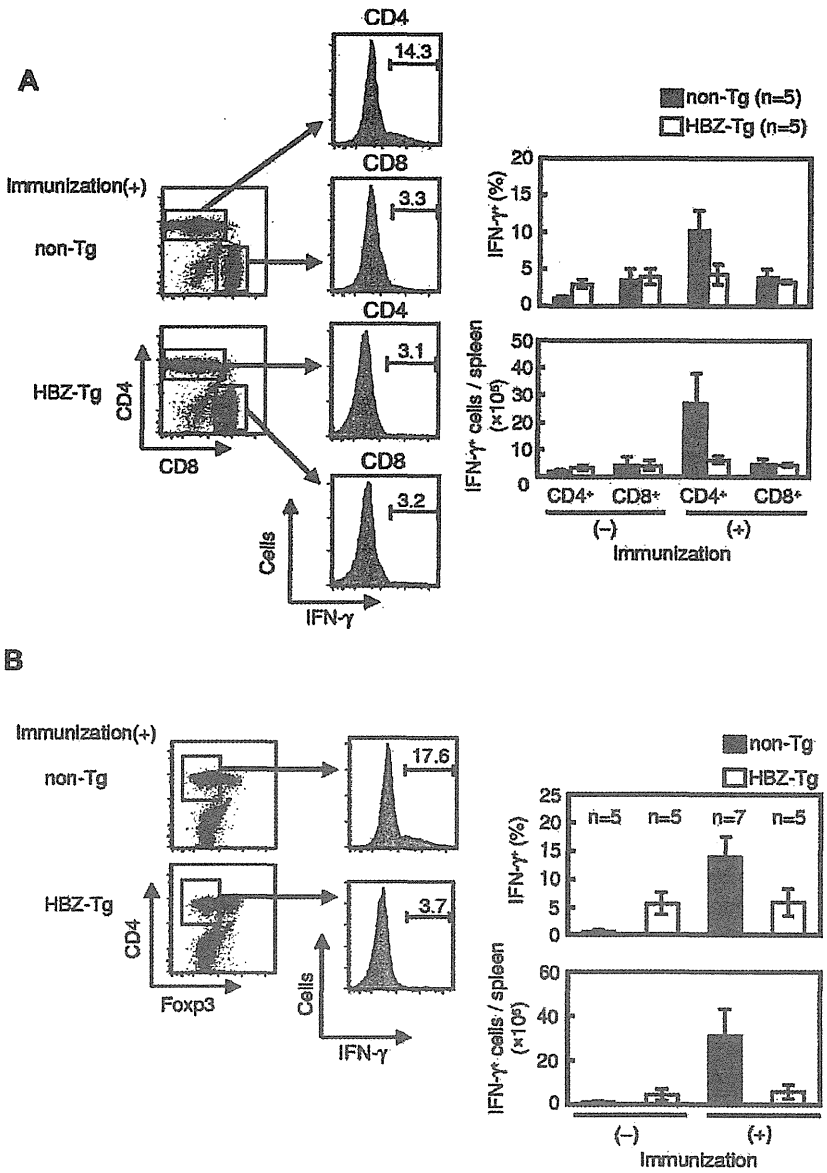

Figure 3. IFN-γ production by CD4 splenocytes from LM secondarily infected HBZ-Tg mice decreases in CD4+ Foxp3− T cells. Mice were immunized and challenged as shown at the top of Figure 2B, and their splenocytes were harvested at 12 hours after challenge and analyzed for intracellular IFN-γ production. (A) Splenocytes were gated by CD3 expression, and IFN-γ production was measured in living CD4 or CD8 T cells using FACS. (B) IFN-γ production in $CD3^+$ $CD4^+$ $Foxp3^-$ cells was determined. Bars represent the mean ± SD of all mice per genotype. Two independent experiments have been performed.

play important roles in the immune response against foreign pathogens.

sHBZ suppresses the activity of the IFN-γ promoter by inhibiting the NFAT and AP-1 signaling pathways

To further elucidate the mechanism of sHBZ-mediated IFN-γ inhibition, we performed a promoter assay using a human −670 to +64 IFN-γ promoter construct in the human T-cell line Jurkat. Previous reports have demonstrated that NFAT, AP-1, and NF-κB signaling pathways are involved in the regulation of *IFN-γ* transcription.[34] We found that PMA and ionomycin treatment enhanced IFN-γ promoter activity, and sHBZ suppressed this enhancement in a dose-dependent manner (Figure 5A). In contrast, another viral protein, Tax, enhanced the promoter activity as reported previously (Figure 5B),[35] an observation that is in line with previous findings that Tax is capable of activating the NF-κB and AP-1 signaling pathways.[36] Previous studies have demonstrated that the level of *sHBZ* transcripts in ATL patients and HTLV-1 carriers is approximately 4-fold higher than the level of *tax* transcripts.[15] The activation of the IFN-γ promoter by Tax was inhibited by sHBZ when sHBZ was expressed at levels similar to those in HTLV-1 carriers (Figure 5C), suggesting that sHBZ can have an inhibitory effect on Tax-mediated IFN-γ induction in HTLV-1 infected cells.

To identify the region of the IFN-γ promoter responsible for sHBZ-mediated suppression, we conducted further analyses using serially deleted promoter constructs. The human IFN-γ promoter (−670 to +64) contains NFAT, AP-1, STAT, ATF, and T-bet binding regions, and these transcription factors are reported to be involved in IFN-γ expression. The suppressive effect of sHBZ on the IFN-γ promoter was reduced by the deletion between dM2 and dM3 ($P < .001$; Figure 5D: a deletion, which removes 2 NFAT sites, an AP-1 site, and a STAT binding site). Because HBZ has a suppressive effect on the NFAT and AP-1 signaling pathways,[17,19] these binding sites might be associated with the suppressive effect of sHBZ. To further explore this possibility, we generated the promoter constructs with point mutation for each NFAT or AP-1 sites, and performed the promoter assay. The point mutation for

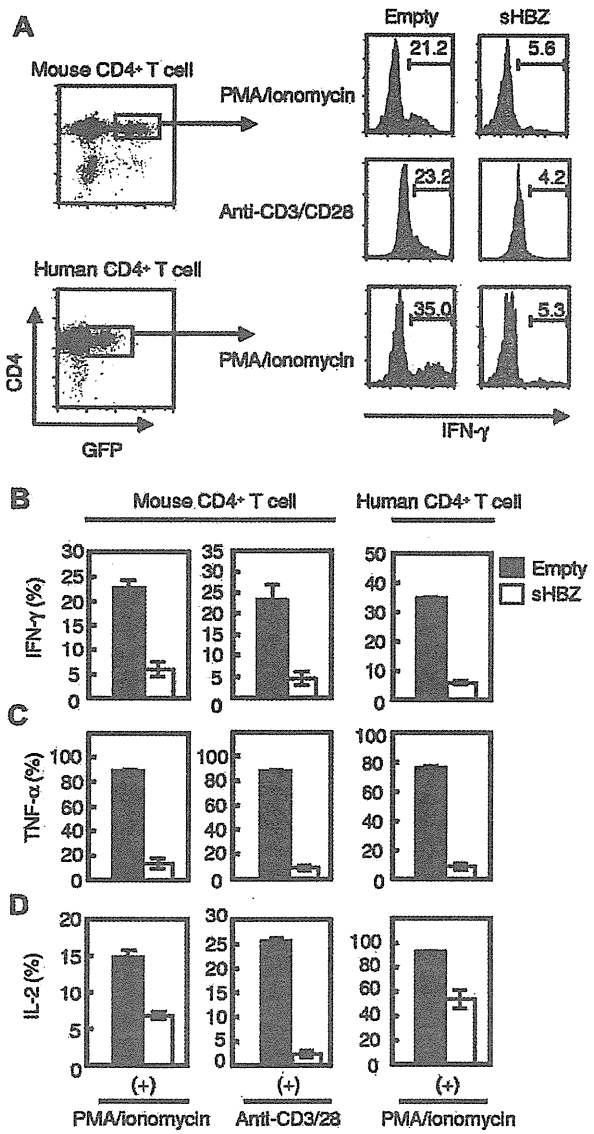

Figure 4. sHBZ directly inhibits IFN-γ production in both human and mouse CD4 T cells. Mouse and human CD4 T cells were transduced with recombinant retroviruses or lentiviruses, respectively, expressing sHBZ, and stimulated with PMA and ionomycin or antibodies to CD3 and CD28. Then, intracellular cytokines in living HBZ-expressing CD4 T cells were measured using FACS. (A) GFP$^+$ and CD4$^+$ cells were gated as shown in the left panel and evaluated for intracellular production of IFN-γ, TNF-α, or IL-2 by flow cytometry. Representative histograms of IFN-γ are shown. (B-D) Percentages of IFN-γ$^+$ (B), TNF-α$^+$ (C), or IL-2$^+$ (D) cells in mouse and human CD4 T cells. Representative data from 2 independent experiments in triplicate (mean ± SD) are shown.

−163 to −153 (P = .025) but not −279 to −269 (P = .057) NFAT binding site remarkably reduced suppressive effect of promoter activity by HBZ (Figure 5E). We next characterized effect of sHBZ on AP-1 binding sites in the IFN-γ promoter. The point mutation for −193 to −183 AP-1 binding site partially impaired the inhibitory effect (P = .042; Figure 5F). Three point mutations of all AP-1 binding sites much more reduced the HBZ-mediated suppressive effect on the promoter (P = .001; Figure 5F). These results indicate that NFAT and AP-1 binding sites are involved in the suppressive effect of HBZ on this promoter.

To further elucidate the involvement of the AP-1 or NFAT signaling pathway in the sHBZ-induced impairment of IFN-γ production, we used sHBZ mutants, which are unable to exert an inhibitory effect on NFAT or AP-1 signaling. We have reported that activation and central domains of HBZ interacted with NFAT.[17] We constructed deletion mutants and 7 amino-acid substitution mutants of sHBZ central domain and assessed their abilities to function in the NFAT or AP-1 signaling pathway (Figure 6A-B; supplemental Figure 4A-C). We found 2 mutants of interest: sHBZ-CDm7 and sHBZ-ΔAD. sHBZ-CDm7 contained amino acid substitutions in the central domain of sHBZ, and these mutations abrogated the inhibitory effect of sHBZ on the activity of an NFAT reporter plasmid (Figure 6A). In contrast, sHBZ-ΔAD, which contains a deletion of the activation domain of sHBZ, did not have suppressive activity on the AP-1 signaling pathway (Figure 6B). We confirmed that expression levels of the sHBZ mutants were comparable with that of WT-sHBZ (supplemental Figure 4D). Consistent with the findings of the reporter assay with the deleted promoters, sHBZ-CDm7 and sHBZ-ΔAD showed remarkable reduction in the inhibitory effect on the IFN-γ promoter (Figure 6C). Furthermore, we generated retrovirus vectors that express these sHBZ mutants, transduced them to mouse CD4 T cells, and evaluated their effect on IFN-γ production. We found that these 2 sHBZ mutants lost their inhibitory effect on IFN-γ production compared with WT-sHBZ (Figure 6D). Previous reports have shown that bZIP domain of HBZ plays a role in suppression for transcriptional activity of AP-1 family, including c-Jun and JunB.[19,37] In this study, deletion mutant of bZIP domain in sHBZ did not influence NFAT and AP-1 pathway in Jurkat cell (Figure 6A-B) and IFN-γ production in mouse CD4$^-$ T cell (supplemental Figure 5A), indicating that not bZIP domain but activation domain of HBZ is essential for suppression of AP-1 pathway in this study.

In addition, we performed a ChIP assay to explore recruitment of the transcription factors NFAT and AP-1 to the IFN-γ promoter in the presence of sHBZ. This experiment showed that sHBZ inhibited recruitment of NFATc2 and c-Jun to the IFN-γ promoter containing 2 NFAT sites and one AP-1 binding site (Figure 6E). These results suggest that sHBZ physically inhibits DNA binding of c-Jun and NFATc2 and suppresses the NFAT and/or AP-1 signaling pathways, which are critical for IFN-γ production in CD4 T cells.

Impaired production of IFN-γ in primary ATL cells

Jurkat T cells express *IFN*-γ gene transcripts after stimulation with PMA and ionomycin. sHBZ expression in Jurkat cells remarkably reduced the level of *IFN*-γ mRNA (Figure 7A). It is critical to study IFN-γ expression in naturally HTLV-1–infected T cells. Therefore, we examined IFN-γ production in PBMCs from ATL patients (supplemental Table 1). PBMCs were stimulated by PMA and ionomycin for 5 hours, and intracellular IFN-γ was stained. We found that IFN-γ production by CD4 T cells was remarkably decreased in ATL patients compared with healthy donors (Figure 7B). In addition, TNF-α and IL-2 production also was suppressed in CD4 T cells from ATL patients. These data suggest that impaired production of IFN-γ is observed not only in HBZ-Tg or ectopically transfected cells but also in primary CD4 T cells from ATL patients.

Discussion

Viruses that cause chronic infections, including hepatitis C virus, HIV, Epstein-Barr virus, and HTLV-1, have strategies to evade the host immune system and to replicate in vivo despite detectable immune responses.[38] For HTLV-1, it has been reported that p12 binds to free human major histocompatibility complex class

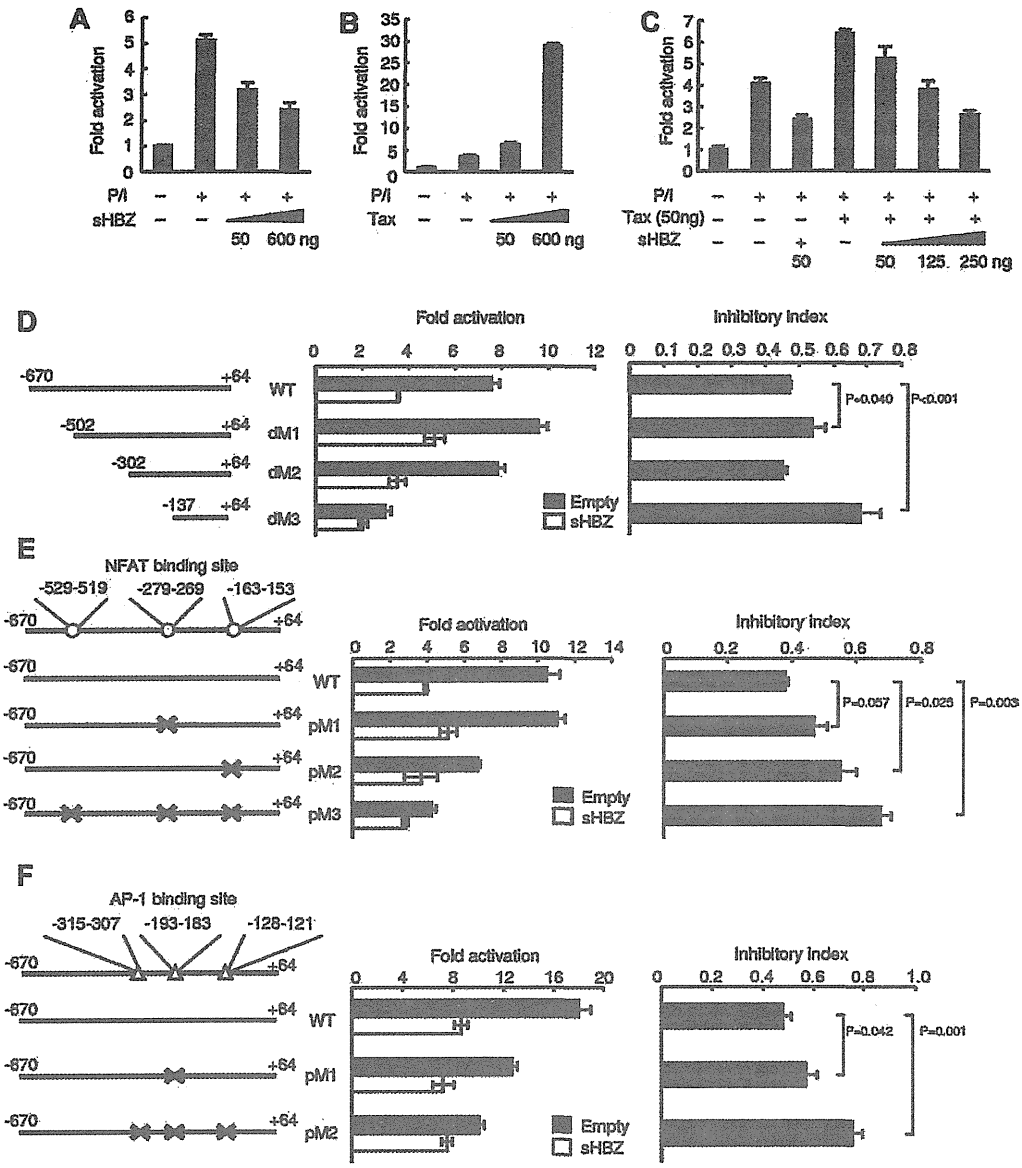

Figure 5. sHBZ suppresses IFN-γ promoter activity. Luciferase assay of the IFN-γ promoter reporter constructs (−670 to −64) cotransfected with an expression plasmid for sHBZ (A), Tax (B), or both (C) is performed in Jurkat cells, which were stimulated with PMA and ionomycin. Luciferase assays of reporter plasmids containing deletions (D) or point mutations in the NFAT (E) or AP-1 (F) consensus-binding region of IFN-γ promoter are performed. The positions of the deleted or mutated regions are indicated in the left of each graph. Consensus sequences for NFAT and AP-1 binding sites were mutated. Inhibitory index is represented as a ratio of fold activation with empty vector or HBZ expression vector. Representative data (mean ± SD) from 2 independent experiments in triplicate are shown.

I heavy chains and inhibits its expression, which results in escape of infected cells from host immune system.[39] A number of viruses evade the host immune response by perturbing the production of cytokines. It has been reported that the core protein of HCV decreases IL-2 production via suppression of mitogen-activated protein kinase.[40] The vaccinia virus double-strand RNA binding protein E3 inhibits the PKR, NF-κB, and IRF3 pathways, thus suppressing IFN-β, TNF-α, and TGF-β production.[41] The HIV-1 Tat protein perturbs signal transduction by IFN-γ.[42] However, it has not been known precisely how HTLV-1 evades the host immune system. In this study, we show that sHBZ inhibits the effector function of CD4 T cells via interaction with NFAT and AP-1, leading to a suppressive effect on the production of Th1 cytokines, such as IFN-γ. This is probably a mechanism of the cellular immune deficiency observed in HTLV-1 infection.

It is well known that NF-κB, AP-1, and NFAT are involved in T-cell receptor signaling pathways.[43] Tax is broadly recognized to play a crucial role in the pathogenesis of HTLV-1, including oncogenesis and inflammation. Previous studies showed that Tax could activate cellular signaling pathways, including NF-κB, and AP-1.[36] Thus, Tax has an enhancing effect, not a suppressive effect, on the immune response of infected cells. On the other hand, HBZ is constitutively transcribed in infected cells and suppresses cellular signaling pathways, including the CREB, AP-1, and canonical NF-κB pathways.[44] These findings suggest that HBZ, rather than Tax, is probably responsible for the immune deficiency in HTLV-1 infection and may act through the impairment of effector cytokine production. Indeed, this study shows that sHBZ suppresses the *IFN-γ* transcription through interaction with NFAT and c-Jun.

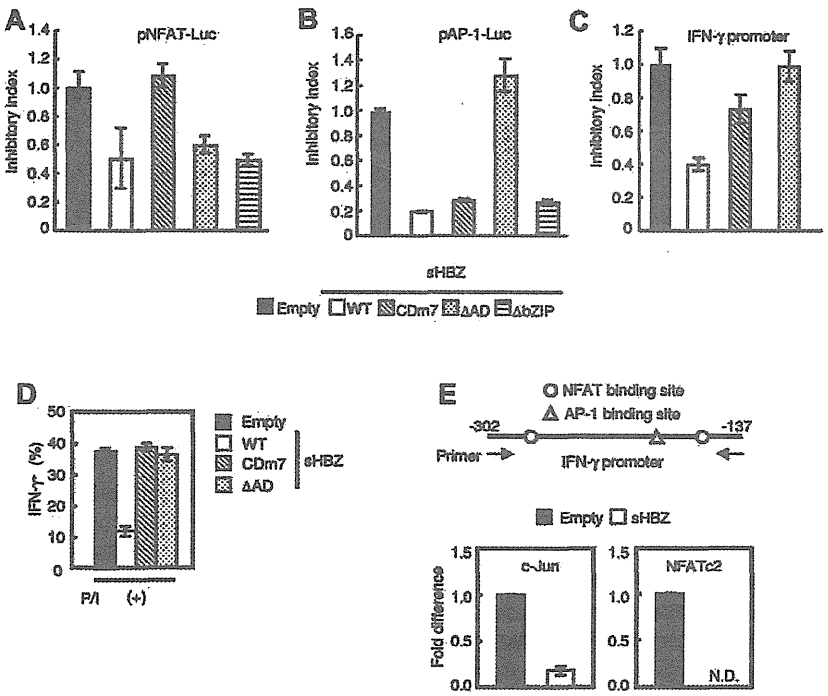

Figure 6. NFAT and AP-1 signaling pathways are responsible for HBZ-mediated inhibition of IFN-γ production. (A-C) Effects of wild-type and mutant sHBZ on (A) an NFAT-Luc reporter, (B) an AP-1-Luc reporter, and (C) the IFN-γ promoter. (D) The suppressive effect of sHBZ mutants on IFN-γ production from primary mouse CD4 T cells. Retroviruses expressing wild-type and mutated HBZ were transduced to primary mouse CD4 T cells, stimulated with PMA and ionomycin, and stained. (E) ChIP assay of the NFAT and AP-1 binding sites of IFN-γ promoter. sHBZ-expressing Jurkat cells were stimulated with PMA and ionomycin, and ChIP assay was performed using anti-NFATc2 or anti–c-Jun antibodies. The IFN-γ promoter (−302 to −137) was amplified by real-time PCR. The data from stimulated empty-transfected Jurkat cells were used as a reference. Representative data (mean ± SD) from 2 or 3 independent experiments are shown. N.D. indicates not detected.

We have recently reported that the HBZ-Tg mice used in this study harbor increased numbers of $CD4^+$ $Foxp3^+$ Tregs compared with non-Tg mice.[17] Tregs are known as negative regulators of the host immune response to pathogens[45]; hence, an increase in the number of Tregs might contribute to the suppression of effector T-cell responses against HSV-2 or LM in vivo. Tregs suppress the memory CD8 T-cell response.[46] However, we found that the production of IFN-γ was impaired in sHBZ-expressing CD4 T cells but not in CD8 T cells (Figure 3A). IFN-γ production was impaired in a CD4 T cell–intrinsic manner. In addition, the suppressive effect of Tregs on IFN-γ production by effector CD4 T cells was not observed in mice immunized with LM (supplemental Figure 6). Taken together, these data imply that the increased number of Tregs is not the main cause of the CD4 T-cell specific reduction of IFN-γ production; rather, sHBZ expression in CD4 T cells may lead directly to suppressed production of IFN-γ.

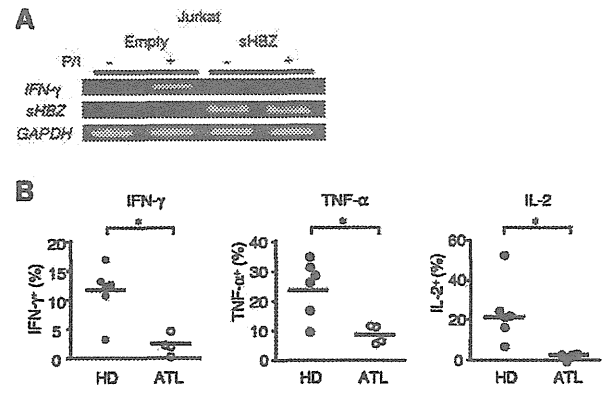

Figure 7. IFN-γ production is suppressed in sHBZ-expressing Jurkat cells and PBMCs of ATL patients. (A) sHBZ inhibits *IFN-γ* gene transcription after stimulation with PMA and ionomycin. Transcripts of the *IFN-γ* and *sHBZ* genes were analyzed by RT-PCR. (B) IFN-γ, TNF-α, and IL-2 production by CD4 T cells in PBMCs from healthy donors (HD; n = 6) and ATL patients (n = 4). PBMCs were separated from the peripheral blood and then stimulated with PMA and ionomycin for 5 hours. Thereafter, intracellular production of Th1 cytokines in living cells was measured by flow cytometry. The y-axis indicates the percentages of cytokine-producing cells in CD4 T cells. *$P < .05$ by Student *t* test.

In this study, we evaluated the cell-mediated immunity of HBZ-Tg mice against HSV-2 and LM. The protective immune response to these pathogens is mediated by IFN-γ production by NK cells, CTLs, and/or Th1 cells.[47] IFN-γ up-regulates major histocompatibility complex molecules, and inducible nitric oxide synthase, activates NK cells and macrophages, and induces Th1 development,[47] thus leading to the elimination of HSV-2 and LM. Lack of IFN-γ function (because of mutation of IFN-γ or its receptor, or because of the presence of IFN-γ specific antibody) in vivo increases susceptibility to many pathogens, including lymphocytic choriomeningitis virus, *Mycobacterium tuberculosis*, and *Leishmania major*.[47] Of particular interest is the fact that protection against infection with *Cryptosporidium parvum*,[48] or *Candida albicans*,[49] which cause opportunistic infections in immune compromised hosts, depends on IFN-γ production from CD4 T cells. In addition, previous reports have shown that a lack of CD4 T-cell help during primary infection results in an incomplete memory immune response in which CTL activity and antibody production by plasma cells are impaired.[50] Our current results, therefore, indicate that the reduced production of helper cytokine caused by sHBZ expression in CD4 T cells may contribute to the immunodeficiency observed in HTLV-1–infected persons and in HBZ-Tg mice.

Previous studies reported that activation and bZIP domains of HBZ played important roles in suppressive effects on the AP-1 pathway.[19,37] However, this study showed that only activation domain was critical in T cells when stimulated by PMA and ionomycin. Deletion of bZIP domain partially impaired AP-1 activation by Tax (supplemental Figure 5B). Previous studies used 293T cells and stimulated them by expression of c-Jun or Tax to analyze suppressive function of HBZ for the AP-1 pathway.[19,37] Therefore, this difference might be because of not only cell type, but also stimulator. HTLV-1 infects CD4 T cells and IFN-γ is

produced by stimulation of T cells, indicating that activation domain of HBZ plays an important role in suppression of AP-1 signaling.

The immune deficiency observed in ATL patients is one of the major factors in their poor prognosis. The mechanisms of HTLV-1–associated oncogenesis have been extensively investigated, yet there are only a limited number of reports regarding HTLV-1-related immune deficiency. Our results contribute to the understanding of this phenomenon by identifying a new mechanism of HTLV-I–induced immunodeficiency.

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Authorship

Contribution: K.S., Y.S., J.Y., H.H., M. Mitsuyama, and M. Matsuoka conceived and designed the experiments; K.S., Y.S., and K.O. performed the experiments; K.S., Y.S., J.Y., H.H., K.O., M. Mitsuyama, and M. Matsuoka analyzed the data; A.U. and M. Mitsuyama contributed reagents/materials/analysis tools; and K.S., Y.S., J.Y., M. Mitsuyama, and M. Matsuoka wrote the paper.

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Adult T-cell leukemia: a review of epidemiological evidence

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Adult T-cell leukemia (ATL) is an aggressive T-cell malignancy caused by human T-cell leukemia virus type I (HTLV-1) infection and often occurs in HTLV-1-endemic areas, such as southwestern Japan, the Caribbean islands, Central and South America, Intertropical Africa, and Middle East. To date, many epidemiological studies have been conducted to investigate the incidence of ATL among general population or HTLV-1 carriers and to identify a variety of laboratory, molecular, and host-specific markers to be possible predictive factors for developing ATL because HTLV-1 infection alone is not sufficient to develop ATL. This literature review focuses on the epidemiology of ATL and the risk factors for the development of ATL from HTLV-1 carriers, while keeping information on the epidemiology of HTLV-1 to a minimum. The main lines of epidemiological evidence are: (1) ATL occurs mostly in adults, at least 20–30 years after the HTLV-1 infection, (2) age at onset differs across geographic areas: the average age in the Central and South America (around 40 years old) is younger than that in Japan (around 60 years old), (3) ATL occurs in those infected in childhood, but seldom occurs in those infected in adulthood, (4) male carriers have about a three- to fivefold higher risk of developing ATL than female, (5) the estimated lifetime risk of developing ATL in HTLV-1 carriers is 6–7% for men and 2–3% for women in Japan, (6) a low anti-Tax reactivity, a high soluble interleukin-2 receptor level, a high anti-HTLV-1 titer, and high levels of circulating abnormal lymphocytes and white blood cell count are accepted risk factors for the development of ATL, and (7) a higher proviral load (more than 4 copies/100 peripheral blood mononuclear cells) is an independent risk factor for progression of ATL. Nevertheless, the current epidemiological evidence is insufficient to fully understand the oncogenesis of ATL. Further well-designed epidemiological studies are needed.

Keywords: adult T-cell leukemia, ATL, epidemiology, human T-cell leukemia virus type I, HTLV-1

INTRODUCTION

Adult T-cell leukemia (ATL) was first reported as a distinct clinical entity in Japan in 1977 (Takatsuki et al., 1977; Uchiyama et al., 1977). The clustering of patients in the southwestern part of Japan propelled Japanese investigators to the interest that the disease could be virally induced. Subsequently, human T-cell leukemia virus type I (HTLV-1) was discovered as the causative virus for ATL (Poiesz et al., 1980; Yoshida et al., 1982). The discoveries of ATL and HTLV-1 ushered in the development of virology, oncology, molecular biology, epidemiology, and other fields of medicine.

The etiological association of HTLV-1 with ATL was established on the basis of the following findings: (1) all patients with ATL have antibodies against HTLV-1 (Hinuma et al., 1981; Hinuma et al., 1982), (2) geographical areas of high incidence of ATL patients correspond closely with those of high incidence of HTLV-1 carriers (The T- and B-Cell Malignancy Study Group, 1985), (3) HTLV-1 immortalizes human CD4 T cells *in vitro* (Hattori et al., 1981), and (4) monoclonal integration of HTLV-1 proviral DNA was demonstrated in ATL cells (Yamaguchi et al., 1984). Subsequently, the Japanese Lymphoma Study Group proposed the first diagnostic criteria for ATL in 1991, and the disease was classified into four clinical subtypes; acute, lymphoma, chronic, and smoldering (Shimoyama, 1991).

ATL patients have been reported mainly from HTLV-1-endemic areas. The global geographical distribution of HTLV-1 seropositive individuals has been well documented (Proietti et al., 2005). Areas with seroprevalence of more than 2% are recognized as high endemic regions (Gessain, 1996). The main endemic areas are Japan, the Caribbean islands, Central and South America, Central and South Africa, a part of the Middle East and Melanesia, and Aboriginal regions in Australia (IARC, 1996). Moreover, regional clustering of virus positivity and high incidence of ATL has been detected even within the endemic areas. The prevalence of HTLV-1 carriers in Europe, North America, China, and Korea is low (Proietti et al., 2005).

This literature review focuses on the epidemiology of ATL and the risk factors for the development of ATL from HTLV-1 carriers with asymptomatic status, while keeping information on the epidemiology of HTLV-1 to a minimum. A variety of study designs and settings, e.g., case series, nation wide surveys, and regional population-based studies using cancer registries were reported to assess incidence, prevalence, and other epidemiological

information on ATL from many countries, mostly from Japan. However, there have been few prospective cohort studies to assess reliable incidence rate of ATL. Readers should keep in mind that all epidemiological studies have individual limitations in the case accumulation and the population setting.

INCIDENCE AND PREVALENCE

JAPAN

In Japan, approximately one million individuals are carriers of HTLV-1 (Tajima, 1990; Satake et al., 2012). Both HTLV-1 and ATL have been shown to be endemic in southwest districts (Kyushu and Shikoku Islands; Tajima, 1990; Satake et al., 2012). Several epidemiological studies have been conducted to estimate annual incidence of ATL in HTLV-1 carriers or general population, but the exact annual incidence of ATL is still unclear. Most of the studies estimated the incidence of ATL just by merging the number of cases of ATL in one population to the number of people in another population such as demographic statistics, blood donors positive for HTLV-1, or an existing group of HTLV-1 carriers. Few prospective studies were conducted (**Table 1**).

Adult T-cell leukemia accounts for 51–59% of non-Hodgkin lymphoma (NHL) in HTLV-1 endemic areas in the Kyushu district, southwest Japan (Arisawa et al., 2000; Ohshima et al., 2002), which was extremely higher than that of nationwide data reporting that ATL accounts for 7.5% of all lymphomas (Lymphoma Study Group of Japanese Pathologists, 2000).

Annual mortality of ATL

Approximately 1,000 people die of ATL each year in Japan according to Japanese vital statistics data for 1998–2008 (Portal Site of Official Statistics of Japan, 2012; **Figure 1**). This indicates that infection with HTLV-1 was associated with approximately 1,000 deaths from ATL annually, with clustering in people aged over 50 years (Ikeda et al., 2012).

Annual incidence of ATL in nationwide studies

In the first nationwide hospital-based survey, 657 new cases of ATL were accumulated during 1986–1987, estimating the annual number of ATL in Japan to be approximately 700 cases (Tajima, 1990; Shimoyama, 1991). The new nationwide hospital-based survey was conducted recently, in which a total of 910 new cases of ATL were accumulated during 2006–2007, estimating the annual number of ATL in Japan to be approximately 1,000 cases (Yamada et al., 2011). In the new survey, two new findings were revealed in contrast to the first nationwide study. First, the age at diagnosis increased from a mean age of 52.7 years in the previous survey to 66.0 years in the new survey (**Figure 2**). Second, there were differences in the proportion of subtypes; the acute subtype accounted for the highest percentage (60.2%), followed by the lymphoma subtype (23.7%) in the previous survey, however, the percentage of the lymphoma subtype increased to 34.8%, contrary to the decrease in the acute subtype to be 46.7% in the new study. However, Takezaki et al. (1997) suggested that the annual incidence of ATL based on the nationwide hospital-based survey could be underestimated because approximately 65% of ATL cases might have been missed due to low response of the participating hospitals from endemic areas.

Annual incidence of ATL in HTLV-1 endemic areas

Results differ according to study methods and the HTLV-1 – positive rate of the study population. A series of cross-sectional survey for residents in Uwajima City (population size; 290, 464, HTLV-1-positive rate; 5.4% in men and 8.3% in women) reported that the annual incidence of ATL was estimated to be approximately 6.1 in adults aged over 30 years per 100,000 populations (Kondo et al., 1985, 1987, 1989). In another cross-sectional studies by the use of the regional cancer registry data in Nagasaki prefecture (an endemic area, the population size; 1.56 million), the age-standardized annual incidence rate of ATL (among 100,000 individuals aged 30 or older) was estimated to be 10.5 for men and 6.0 for women during 1985–1995 (Arisawa et al., 2000) and 8.7 for men and 5.5 for women during 1995–2004 (Arisawa et al., 2009). There was no significant decrease in the overall incidence rate between the two decades, however, age-specific incidence of ATL among those aged over 60 years increased significantly during 1995–2004 compared to the period of 1985–1995 (Arisawa et al., 2009).

Incidence of ATL among HTLV-1 carriers

In studies used blood donors seropositive for HTLV-1, the annual incidence of ATL was estimated to be approximately 60 per 100,000 HTLV-1 carriers over 20 years old in Japan (Tajima, 1990) or approximately 116 for men and 66 for women per 100,000 HTLV-1 carriers in Saga prefecture (an endemic area, the population size; 880,000; Tokudome et al., 1989). In a study used serological survey for residents in small cluster areas, The crude annual incidence of ATL was estimated to be 137.7 for men and 57.4 for women among 100,000 HTLV-1 carriers aged 30 years or older (Arisawa et al., 2000). Furthermore, in a study performed record linkage between the cancer registry and HTLV-1 carriers in hospital, the crude annual incidence of ATL was estimated to be 61 per 100,000 HTLV-1 carriers (Koga et al., 2010).

Lifetime risk of ATL among HTLV-1 carriers

For HTLV-1 carriers, the lifetime risk was estimated to be 4.5% for men and 2.6% for women in Saga prefecture (Tokudome et al., 1989), 6.6% for men and 2.1% for women in Nagasaki prefecture (Arisawa et al., 2000), 6.9% for men and 2.95% for women in Uwajima City (Kondo et al., 1989), and 7.29% for men and 3.78% for women in a hospital-based study (Koga et al., 2010).

In summary, in Japan, nearly 1,000 new cases of ATL are diagnosed and nearly 1,000 patients die of ATL each year over a period of 20 years. The annual incidence of ATL among HTLV-1 carriers is approximately 60 per 100,000 with the lifetime risk of 6–7% for men and 2–3% for women. The incidence was 1.35 times higher in men than in women, contrary to the higher HTLV-1-positive rate in women than in men. ATL occurs predominantly in elderly male carriers, and the mean age at diagnosis increased from the early 1950s in 1980 to the late 1960s recently. Most of Japanese epidemiological studies were population-based descriptive types using cancer registries, therefore those have limitations as follows; cases of smoldering ATL were excluded; hematological diagnoses were not performed. These limitations might have introduced an underestimation of the actual risk.

Table 1 | Epidemiological studies of ATL in literatures.

Study design	Reference	Country	Targeted population	Size of population	No. ATL cases	Incidence rate (IR)	Lifetime risk (estimated cumulative risk)
Population-based descriptive study	Kondo et al. (1989)	Japan	Inhabitants of Uwajima City (an endemic area in Japan)	Data from the Statistics Bureau in 1981	Data from a survey in 1981–1987	Annual IR:	NA
				M + F: 290,464	M: 46 F: 34	3.9 per 100,000 population 6.1 per 100,000 aged over 30 6.6 per 100,000 aged over 40	
			HTLV-1 carriers aged over 30 years	Data from HTLV-1 screening in 1981	Data from a survey in 1981–1987	Annual IR: (per 100,000 HTLV-1 carriers aged over 30 years)	(0–79 years):
				M: 4,522 F: 8,801	M: 46 F: 34	Total: 85.0 M: 145.3 F: 55.2	M: 6.9% F: 2.95%
Population-based descriptive study	Tokudome et al. (1989)	Japan	Entire residents of the Saga Prefecture (an endemic area in Japan)	Data from the Statistics Bureau in 1981	Data from a cancer registry in 1981–1983	Annual IR: (per 100,000 population aged 40–79 years)	NA
				M + F: 880,000	M: 36 F: 33	M: 4.9~12.6 (depend on age) F: 1.6~8.1 (depend on age)	
			Estimated HTLV-1 carriers in the Saga Prefecture (an endemic area in Japan)	Data calculated by multiplying HTLV-1 positivity rate among blood donors with the number of the population in Saga	Data from a cancer registry in 1981–1983	Annual IR: (per 100,000 HTLV-1 carriers aged 40–79)	(40–79 years):
				M: 14,236 F: 19,596	M: 36 F: 33	M: 115.9 F: 66.4	M: 4.5% F: 2.6%
Nationwide hospital-based survey	Tajima (1990)	Japan	Whole Japanese population	Data from the Statistics Bureau in 1986	Data from 192 hospitals 1986–1987	Annual IR: (per 100,000 adults)	NA
				Total: 120,720,000 Kyushu: 14,460,000	Total: 657	M: 4.04 (in Kyushu) F: 2.64 (in Kyushu)	
			Estimated HTLV-1 carriers in Japan	Data calculated by multiplying the HTLV-1 seropositivity rate in blood donors in an individual prefecture with the number of the population in this individual prefecture	Data from 192 hospitals 1986–1987	Annual IR (per 100,000 HTLV-1 carriers over 20 years old)	NA
				Total: 1,200,000	Total: 657	Total: 60	

(Continued)

Table 1 | Continued

Study design	Reference	Country	Targeted population	Size of population	No. ATL cases	Incidence rate (IR)	Lifetime risk (estimated cumulative risk)
Population-based descriptive study	Gérard et al. (1995)	French Guiana	Whole French Guiana population	Total 115,000	Enrolled in the study in 1990–1993 Total: 18	Crude annual IR (per 100,000 entire population) Total: 3.5 Crude annual IR in an endemic region (per 100,000 population) Total: 30	NA
Cohort study (Miyazaki Cohort study)	Hisada et al. (1998a)	Japan	Residents in two HTLV-1 endemic villages in the Miyazaki Prefecture (an endemic area in Japan)	1,960 of whom 27% were HTLV-1 antibody-positive in 1984	Data in 1984–2000	NA	NA
	Okayama et al. (2004)				Total: 6		
Population-based descriptive study	Levine et al. (1999)	US	Central Brooklyn black community (an endemic area in New York)	Total: 1,184,670	Data from a survey in 1994 M: 2 F:10	NA	NA
Population-based descriptive study	Arisawa et al. (2000)	Japan	Entire residents of the Nagasaki Prefecture (an endemic area in Japan)	Data from the Statistics Bureau in 1990 M: 736,729 F: 826,230	Data from a cancer registry in 1985–1995 M: 567 F: 422	World age-standardized annual IR (cases/100,000 population): M: 10.5 F: 6.0	NA
			Residents of 4 towns on the K Islands (a cluster regions in Nagasaki)	Data from the Statistics Bureau in 1990 M: 12,820 F: 14,050	Data from a cancer registry in 1985–1995 M: 24 F: 16	Crude IR (per 100,000 person-years of residents) M: 27.4 F: 15.9	(30–79 years): M: 1.7% F: 0.7%
			HTLV-1 carriers of 4 towns on the K Islands (a cluster regions in Nagasaki)	Data from HTLV-1 screening in 1985–1996 M + F: 18,485	Data from a cancer registry in 1985–1995 M: 24 F: 16	Crude IR (per 100,000 person-years of HTLV-1 carriers) M: 137.7 F: 57.4	(30–79 years): M: 6.6% F: 2.1%

(Continued)

Table 1 | Continued

Study design	Reference	Country	Targeted population	Size of population	No. ATL cases	Incidence rate (IR)	Lifetime risk (estimated cumulative risk)
Population-based descriptive study (NAACCR)	Yamamoto and Goodman (2008)	US	General population in US	Approximately 61% of the US population	Data from cancer registry in 1997–2002	Age adjusted to the 2000 US standard population per 100,000 population	NA
					M: 248 F: 183	M: 0.05 F: 0.03	
			White population in US	NA	M: 187 F: 104	M: 0.05 F: 0.02	NA
			Black population in US	NA	M: 46 F: 69	M: 0.12 F: 0.13	NA
Population-based descriptive study	Arisawa et al. (2009)	Japan	Entire residents of the Nagasaki Prefecture (an endemic area in Japan)	Data from the Statistics Bureau in 1995	Data from a cancer registry in 1985–2004	World age-standardized annual IR (per 100,000 population)	(30–99 years):
				M: 726,894 F: 818,040	M: 1,022 F: 829	M: 8.7 F: 5.5	M: 0.88% F: 0.57%
Hospital-based and Population-based descriptive study	Koga et al. (2010)	Japan	Estimated HTLV-1 carriers in Nagasaki City (an endemic area in Japan)	Data calculated by multiplying the HTLV-1 positivity rate in the University hospital with the number of the population census in Nagasaki City	Data from a cancer registry in 1990–2005	Annual IR (per 100,000 HTLV-1 carriers)	(30–79 years):
				M: 12,755 F: 24,228	M: 188 F: 172	M: 92 F: 44	M: 7.29% F: 3.78%
Nationwide hospital-based survey	Yamada et al. (2011)	Japan	Whole Japanese population	Data from the Statistics Bureau in 2006	Data from 156 hospitals 2006–2007	Annual IR (per 100,000 population)	NA
				Total: 127,053,000 Kyushu: 13,407,000	Total: 910 Kyusyu: 544	Total: 0.91 Kyushyu: 5.11	
			Estimated HTLV-1 carriers in Japan	Data calculated by multiplying the HTLV-1 seropositivity rate in blood donors in an individual prefecture by the number of the population in this individual prefecture	Data from 156 hospitals 2006–2007	Annual IR (per 100,000 HTLV-1 carriers over 20 years old)	M:8.73%
				Total: 1,078,722	Total: 910	Total: 106	F:5.14%

NA, not available.

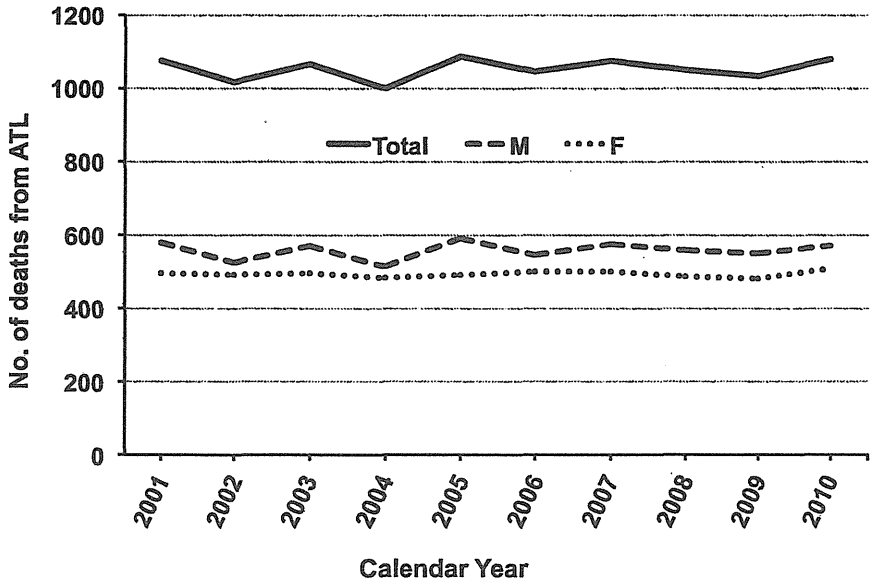

FIGURE 1 | Annual number of deaths from adult T-cell leukemia (ATL) during 2001–2010 in Japan. The raw data were obtained from vital statistics in the Portal Site of Official Statistics of Japan (e-Stat; accessed April 8, 2012). Abbreviations: M, male; F, female.

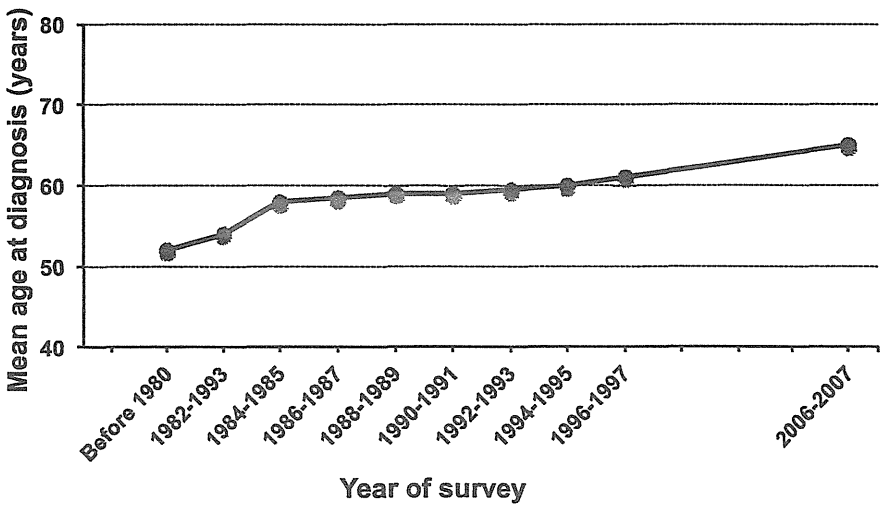

FIGURE 2 | Chronological changes in the mean age at diagnosis of adult T-cell leukemia (ATL) in Japan. The figure was modified from Figure 2 in Yamada et al. (2011).

EAST ASIA (EXCLUDING JAPAN)

Although there were several reports of blood donor screening for HTLV-1, no epidemiological study of ATL has been published from East Asian countries other than Japan because of the lower prevalence of HTLV-1 (less than 0.1%). Nevertheless, several case series of ATL were available. The first case of ATL was reported in Taiwan in 1985 (Chen et al., 1985), in Korea in 1987 (Lee et al., 1987), and in China in 1995 (Zhuo et al., 1995). In Hong Kong, since the first case of ATL was reported in 1994 (Liang, 1994), all patients with T-cell lymphoma have been routinely screened for HTLV-1 antibody. In a registration study of lymphoma between 1993 and 2002 in Hong Kong, six cases of ATL were diagnosed among 5,911 lymphomas, in which ATL contributed to 0.1% of all cases of lymphoma and 1.3% of T-cell lymphoma (Au and Lo, 2005). Recently, 17 cases of ATL were reported from Taiwan (Lee et al., 2010), of those approximately 40% of the patients co-infected with HBV and HCV, which may be a characteristic of the Taiwanese ATL.

MIDDLE EAST

The prevalence of HTLV-1 infection among healthy subjects is reported to be very low, less than 0.1%, in Lebanon, Saudi Arabia, Egypt, and Kuwait (Proietti et al., 2005). However, there are some areas with a very high rate of HTLV-1 infection.

Northeast province of Iran (Mashhad, Sabzevar, and Neyshabour) and Urmia are known to be an endemic area for HTLV-1, where the prevalence of HTLV-1 infection was reported to be 0.34–0.77% in blood donors (Abbaszadegan et al., 2003; Khameneh et al., 2008), 1.7–12% in cross-sectional studies (Meytes et al., 1990; Safai et al., 1996; Hedayati-Moghaddam et al., 2011; Azarpazhooh et al., 2012), and 2–3% in community-based population (Rafatpanah et al., 2011).

Romania is also suggested to be an endemic area for HTLV-1 because antibodies to HTLV-1 were found in 0.64% of blood donors (Paun et al., 1994), which was an extremely higher seroprevalence rate than in Europe and the USA. In Israel, HTLV-1 seropositive were discovered only in 0.0018% out of 276,000 blood donations, but a very high rate of infection (over 20%) has been identified among a segregated community of Jews originated from the city of Mashhad in Iran (Miller et al., 1998).

Although, there are several clinical studies for ATL patients in the Middle East (Kchour et al., 2007, 2009), epidemiological studies regarding incidence and prevalence of ATL were not available in literature from the Middle East. There were several case reports of ATL, most of which were Mashhad origins or Romanian origins (Sidi et al., 1990; Veelken et al., 1996; Shtalrid et al., 2005; Bitar et al., 2009).

UNITED STATES

HTLV-1 and ATL are extremely rare in North America. Several ATL cases have been reported sporadically (Catovsky et al., 1982). Most of the cases were migrants from endemic areas. A population-based survey reported that the annual incidence in African Americans in central Brooklyn (population size; 1,184,670) was estimated to be approximately 3.2 per 100,000 person-years (Levine et al., 1999). An interesting finding in their study was that the male-to-female ratio of 1:3 was different from the male dominance reported in Japan. Recent cancer registry systems for hematological malignancies allow a precise evaluation of epidemiological features of ATL in the USA. In a recent report from the North American Association of Central Cancer Registries (NAACCR; Yamamoto and Goodman, 2008), a total of 431 cases (248 men and 183 women) of ATL (ICD-O-3 code; 9,827) were registered between 1997–2002, showing that the age adjusted incidence rate was 0.05 for men and 0.03 for women per 100,000 population. The study also reported a racial difference in the incidence rate, showing that African Americans had the highest rates of ATL (0.12 for men and 0.13 for women per 100,000 population). A possible explanation for this observation might be the higher number of migrants from endemic areas of the Caribbean and parts of Sub-Saharan Africa rather than a racial difference in susceptibility.

THE CARIBBEAN

In the early 1980s, eight patients were diagnosed with ATL in the USA, and all of them were Blacks from the Caribbean (Blattner et al., 1982). Since then, Central/South America and the Caribbean are known as areas of high prevalence of HTLV-1. Although there is no concrete epidemiological report regarding the incidence or prevalence of ATL from Central and South America, several case series have been published. A regional registration study of Jamaica reported a total of 126 cases of ATL (acute 46.8%, lymphoma 27%, chronic 20.6%, and smoldering 5.6%) between January 1985 and July 1995 (Hanchard, 1996). The mean age was 43 years old (17–85 years old), which is similar to that reported in Brazil (43 years; Pombo de Oliveira et al., 1995) but younger than that in Japan (50–60 years; Yamaguchi et al., 1987). There is definite evidence that the age at diagnosis in Central/South America and the Caribbean is younger than that in Japan. This difference in the age at diagnosis might be due to different environmental backgrounds.

CENTRAL AND SOUTH AMERICA

In Central and South America, HTLV-I has been shown to be endemic mainly in populations of African ancestry and in some populations of Japanese origin.

Brazil has the highest HTLV-1 seroprevalence rate in healthy subjects (approximately 1%), especially in Rio de Janeiro and Salvador (1.8%) on the northeast coast of the country where the population is largely of African descent. ATL accounts for approximately 30% of patients with T-cell malignancies in Brazil (Pombo de Oliveira et al., 1995; Farias de Carvalho et al., 1997). A Brazilian ATLL Study Group identified 195 cases of ATL in the national registry of T-cell malignancies between 1994 and 1998 (Pombo de Oliveira et al., 1999), but no epidemiological indicators were available. In Argentina, HTLV-1 infection is known to be highly prevalent among Native Americans living in the Andes, and ATL accounts for approximately 14.7% of patients with lymphoid malignancies (Marin et al., 2002).

Chile is a non-tropical country but small case series of ATL patients have been reported frequently (Cabrera et al., 1994, 1999, 2003). The characteristics of Chilean ATL were reported that the most of patients were of Caucasian origin, and age at diagnosis (50 years old) was younger than Japanese patients but older than those from other Latin American countries. According to the recent pathological study in Chile, ATL accounts for 0.5% of patients with of NHL (Cabrera et al., 2012).

French Guiana (population 115,000), an overseas French administrative district located on the northeast coast of the South American continent between Brazil and Surinam, is also known to be an area of high endemicity for HTLV-I (Plancoulaine et al., 1998; Talarmin et al., 1999, Pouliquen et al., 2004). Although the population consists of various ethnic groups, a high seroprevalence of HTLV-I (8%) and a high incidence of cases of ATL were found among the Noirs-Marrons, an isolated population descended from Surinam slaves (Gérard et al., 1995; Tuppin et al., 1995; Plancoulaine et al., 1998). An epidemiological study was performed in French Guiana to determine the prevalence and incidence of ATL (Gérard et al., 1995). Only 18 patients with ATL (8 acute forms, 8 lymphoma types, and 2 smoldering cases) were enrolled during 1990–1993 and the annual crude incidence rate was estimated to be around 3.5 per 100,000 populations. However, in a small remote ethnic group of African origin (around 6200 inhabitants), the annual crude incidence rate was the highest to be around 30 per 100,000 populations.