Table I. Therapeutic trials in HAM/TSP patients (Quotation from [51])

A. Therapies focusing on immunomodulatory effects	
Effects	a) suppression of immune activation, particularly for activated HTLV-I-infected cells b) inhibition of transmigration of activated HTLV-I-infected cells to the spinal cord c) reduction of chronic inflammation in the spinal cord
Therapies	1) corticosteroid hormone 2) blood purification 3) pentoxifylline 4) heparin 5) high dose-intravenous gamma globulin 6) intermittent high-dose vitamin C 7) fosfomycin and erythromycin 8) fermented milk drink
B. Therapies focusing on anti-viral effects	
Effects	a) suppression of HTLV-I expression and/or replication b) inhibition of proliferation of HTLV-I-infected cells c) elimination of HTLV-I-infected cells
Therapies	1) interferon-α and -β 2) reverse transcriptase inhibitors 3) humanized anti-Tac 4) histone deacetylase enzyme inhibitor 5) prosultiamine

sphincteric disturbance [19,23,58]. The primary pathological feature of HAM/TSP is chronic myelitis characterized by chronic inflammation in the spinal cord, mainly the lower thoracic cord, with perivascular cuffing and parenchymal infiltration of mononuclear cells [28]. With the discovery of HAM/TSP, it has become evident that HTLV-I has the remarkable capacity for not only aggressive lymphoproliferation, as in adult T-cell leukaemia (ATL), but also profound chronic inflammation. However, the exact mechanisms by which HTLV-I causes these disparate clinical conditions, an aggressive lymphoproliferative malignancy on the one hand and chronic neuroinflammation on the other, are still unknown. Also, although HTLV-I infects approximately 10-20 million people worldwide [10], with large endemic areas in southern Japan and the tropical zone [23] as described above, it is still unclear why only a very small proportion of HTLV-I-infected individuals develop either of these HTLV-I-associated diseases.

Although the molecular mechanisms by which HTLV-I infection induces the chronic inflammation in the spinal cord still remain unresolved, a long-standing bystander mechanism, such as the destruction of surrounding tissues by the interaction between HTLV-I-infected $CD4^+$ T cells and HTLV-I-specific $CD8^+$ cytotoxic T cells (CTL), is probably critical in the pathogenesis of HAM/TSP [25,60]. That is, chronic inflammation, induced by soluble factors, such as inflammatory cytokines released during the encounter between transmigrated HTLV-I-infected $CD4^+$ T cells and HTLV-I-specific $CD8^+$ CTL in the spinal cord, is operative (Fig. 1). In a point of view on the virological abnormalities in HAM/TSP, it is well known that the HTLV-I proviral load in the peripheral blood is significantly higher in HAM/TSP patients than HTLV-I asymptomatic carriers, and high HTLV-I proviral load in the peripheral blood is the most important prerequisite in the development of HAM/TSP [31,47]. On the other hand, numerous immunological dysregulations mostly mediated by HTLV-I tax expression are detected in the peripheral blood of HAM/TSP patients [5,50]. Therefore, when considering the immunopathogenesis of HAM/TSP, it is plausible that the increase

of HTLV-I-infected CD4+ T cells possessing up-regulated transmigrating activity to the spinal cord plays an important role in the development of HAM/TSP because HTLV-I-infected CD4+ T cells are the first responders.

Since the discovery of HAM/TSP, more than 20 years have passed. During that period, numerous therapeutic approaches have been proposed and tested on various aspects of HAM/TSP [51]. Based on the evidence, such as the involvement of the immune-activated status in the immunopathological process in the spinal cord of HAM/TSP patients as mentioned above, immunomodulatory therapy has been the main treatment administered to HAM/TSP patients. Although these treatments have produced good results, their overall efficacy is still controversial. In addition, whether or not these treatments can be tolerated as a long-term or lifelong treatment is uncertain. Viewed within the context that HAM/TSP is an infectious disease, treatment should primarily target HTLV-I-infected cells in the peripheral blood. Therefore, the therapeutic strategy for HAM/TSP should focus on the suppression of HTLV-I expression and/or replication, the inhibition of the proliferation of HTLV-I-infected cells, and/or the elimination of HTLV-I-infected cells. Of these, the ideal treatment is, most importantly, the elimination of HTLV-I-infected cells from the peripheral blood.

In this review, the focus will be on the role of HTLV-I-infected CD4+ T cells as activated Th1 cells in the immunopathogenesis of HAM/TSP, and on the therapeutic strategies aimed at targeting the elimination of HTLV-I-infected cells in HAM/TSP patients.

The role of HTLV-I-infected CD4+ T cells as activated Th1 cells in the immunopathogenesis of HAM/TSP

Activated Th1 status in HAM/TSP patients

Helper T cells are generally divided into two distinct populations, Th1 and Th2, based on their cytokine-production profiles [44, 64]. The differentiation to the former or the latter induces cell-mediated immunity or humoral immunity, respectively. Interferon-γ (IFN-γ) or interleukin-4 (IL-4) are important cytokines for the differentiation to Th1 or Th2, respectively [44,64]. We have previously reported up-regulated mRNA expression of IFN-γ concomitant with tumour necrosis factor-α (TNF-α), granulocyte-macrophage colony stimulating factor (GM-CSF), and IL-1α in the peripheral blood mononuclear cells (PBMC) of HAM/TSP patients [71]. We also demonstrated that the spontaneous production of IFN-γ, TNF-α and GM-CSF, but not IL-4, increased simultaneously in cultured peripheral blood CD4+, but not CD8+, T cells of HAM/TSP patients [55]. These findings suggested that the Th1 rather than the Th2 cell population predominates in the peripheral blood CD4+ T cells of HAM/TSP patients. The predominance of Th1 activation in HAM/TSP patients is also supported by the findings of both increased Th1 with decreased Th2 cytokine signalling activities [53] and increased serum level of IFN-γ [16]. It has also been reported that intracellular IFN-γ+/IL-4+ cell ratio in the peripheral blood CD4+ T cells is increased in HAM/TSP patients [24].

HTLV-I-infected CD4+ T cells in the peripheral blood have characteristics of Th1 cells in HAM/TSP patients

We previously reported, in the analyses of Th1 signalling molecules, that there was a positive correlation between IL-12 receptor β2, which is a reliable marker of Th1 cells [20], and HTLV-I tax mRNA expression in the peripheral blood of HAM/TSP patients but not anti-HTLV-I-antibody positive carriers, suggesting that HTLV-I tax expression is connected with Th1 activation in HAM/TSP patients [53]. Hanon and associates showed that the HTLV-1 tax expression in short-term cultured peripheral blood is associated with rapid up-regulation of IFN-γ in HTLV-I-infected individuals [21]. More recently, Furukawa and colleagues clearly demonstrated, in a comparative study of intracellular cytokine expression levels in HAM/TSP patients and HTLV-I asymptomatic carriers with a high HTLV-I proviral load equivalent to those of HAM/TSP patients, the abundance of IFN-γ- and TNF-γ-producing cells in the HTLV-I tax-expressing cell population, but not tax-non-expressing cells, in HAM/TSP patients [15]. HTLV-I proviral load in the peripheral blood is significantly higher in HAM/TSP patients than HTLV-I asymptomatic carriers, as mentioned before. Therefore, these findings strongly suggested that HTLV-I-infected cells having the characteristics of Th1 are increased in HAM/TSP patients, compared to HTLV-I asymptomatic carriers, leading to activated Th1 status in the immunological balance between Th1 and Th2 in the peripheral blood of HAM/TSP patients.

Exaggerated transmigrating activity of peripheral blood HTLV-I-infected CD4+ T cells in HAM/TSP patients

In order to trigger the bystander mechanism, the transmigration of peripheral blood HTLV-I-infected CD4+ T cells to the spinal cord is, at first, necessary. Accordingly, we found that the transmigrating activity of peripheral blood CD4+ T cells of HAM/TSP patients was significantly higher than that of either HTLV-I-seropositive carriers or HTLV-I-seronegative controls through a reconstituted basement membrane (RBM), using Transwell cell-culture chambers [17]. Subsequently, we found that HTLV-I proviral load in transmigrated CD4+ T cells from HAM/TSP patients was significantly higher than in non-transmigrated CD4+ T cells. By contrast, no significant difference was found in HTLV-I proviral load in transmigrated and non-transmigrated CD4+ T cells from HTLV-I-seropositive carriers. These results strongly suggested that peripheral blood CD4+ T cells of HAM/TSP patients, particularly HTLV-I-infected CD4+ T cells, have an exaggerated transmigrating activity with the ability to accumulate in the tissues. Indeed, Nagai and co-workers reported that HTLV-I proviral load in cerebrospinal fluid (CSF) cells was significantly higher than that of the matched PBMC in HAM/TSP patients [48]. Interestingly, Lezin and colleagues also reported that the percentage of HTLV-I-infected cells in CSF and the CSF cell : PBMC HTLV-I proviral load ratio were always > 10% and > 1, respectively, in HAM/TSP patients but were always < 10% and < 1, in HTLV-I asymptomatic carriers in a comparative study [39]. Therefore, HTLV-I-infected CD4+ T cells of HAM/TSP patients have the potential to trigger the bystander mechanism in the spinal cord by its accumulative activity through the increased transmigrating activity. In addition, although inflammatory diseases, such as Sjögren's syndrome, arthropathy, alveolitis, uveitis, interstitial cystitis and polymyositis, occasionally occur in conjunction with HAM/TSP, the increased transmigrating activity of HTLV-I-infected CD4+ T cells to the tissues might be involved even in the trigger of these inflammatory diseases [50].

Cells having the potential to transmigrate into tissues of HAM/TSP patients have the characteristics of Th1 cells

We suggested that the immunological status of HAM/TSP patients is under systemic Th1 activation, based on an increased number of HTLV-I-infected Th1 cells. On the other hand, we also suggested that HTLV-I-infected CD4+ T cells of HAM/TSP patients have an exaggerated transmigrating activity with the ability to accumulate in the tissues. Then, how do these situations connect with the trigger of the pathological process in the spinal cords of HAM/TSP patients?

In the first step of T-cell transmigration into the tissues, selectin and its ligands, which are expressed on vascular endothelial cells (EC) and T cells, respectively, play an important role [37]. Of these, sialyl Lewis^x antigen (sLe^x) is a ligand for both E- and P-selectin [69]. Therefore, T cells expressing sLe^x have the potential to transmigrate into tissues. In addition, it was reported that Th1 cells, but not Th2 cells, can bind to E- and P-selectin, indicating that each ligand, sLe^x, for each selectin is a phenotypic markers of Th1 cells [3]. We compared the frequency of sLe^{x+} cells, together with production of cytokines, such as IFN-γ and IL-4 as Th1 and Th2 cytokine, respectively, in the peripheral blood CD4+ T cells between HAM/TSP patients and controls, including anti-HTLV-I-seropositive carriers. In addition, we also compared HTLV-I proviral load between sLe^{x+} and sLe^{x-} cell population in the peripheral blood CD4+ T cells of HAM/TSP patients [32]. We found that the frequency of sLe^{x+} cells in the peripheral blood CD4+ T cells of HAM/TSP patients was significantly higher than in controls, suggesting that the cells having the potential to transmigrate into the tissues are increased in the peripheral blood of HAM/TSP patients. When we compared the activity of IFN-γ production in the sLe^{x+} cell population in the peripheral blood CD4+ T cells between HAM/TSP patients and controls, HAM/TSP patients had significantly increased activity as compared to controls, suggesting that Th1 cells of HAM/TSP patients are obviously also under the activated status of Th1 function. Furthermore, when we compared the activity of both IFN-γ and IL-4 production between sLe^{x+} and sLe^{x-} cell populations of the peripheral blood CD4+ T cells of HAM/TSP patients, IFN-γ production was significantly higher in the former than in the latter population, but vice versa for IL-4 production. However, there was no significant difference in the production of both cytokines between sLex+ and sLe^{x-} cell populations of the peripheral blood CD4+ T cells in controls. These findings suggest that the cells having the potential to transmigrate into tissues have activated Th1 function and this cell population is increased in the peripheral blood CD4+ T cells of HAM/TSP patients. On the other hand, what is the relationship between Th1 function

and HTLV-I infection in the peripheral blood $CD4^+$ T cells of HAM/TSP patients? When we compared the HTLV-I proviral load between sLe^{x+} and sLe^{x-} cell populations of the peripheral blood $CD4^+$ T cells of HAM/TSP patients, HTLV-I proviral load in the sLe^{x+} cell population was two- to eight-fold higher than in the sLe^{x-} cell population, indicating that HTLV-I-infected $CD4^+$ T cells are concentrated in the cell population having the potential to transmigrate into the tissues. Overall, our data suggested that the cells having the potential to transmigrate into the tissues have the characteristics of Th1 cells and are constituted of HTLV-I-infected cells compared to the cells which lacked the potential to transmigrate into tissues. Therefore, these findings suggested that HTLV-I-infected sLe^{x+} cells in the peripheral blood of HAM/TSP patients cells having the potential to transmigrate into the tissues have the characteristics of Th1 cells and the increase of this cell population plays a very important role in the triggering of the development of the pathological process in the spinal cords of HAM/TSP patients.

Involvement of p38 MAPK signalling pathway for Th1 activation in the peripheral blood of HAM/TSP

Even if sLe^{x+} antigen is a ligand for the recruitment of Th1 cells into tissues [3, 36], HTLV-I tax protein can up-regulate the expression of this antigen on HTLV-I-infected cells [22], indicating that the characteristics of Th1 cells, such as up-regulated IFN-γ expression, are not explainable by only the expression of this ligand in HTLV-I-infected status. Indeed, leukaemia cells in patients with ATL and the related cell lines strongly express this ligand [22,57]. In addition, the pattern of chemokine receptor expression suggests that ATL cells originate from Th2 or regulatory T cells [76,77]. Although IFN-γ expression is up-regulated in HTLV-I-infected cells of HAM/TSP patients, it is still not clear which intracellular signalling induces such a status. Thus, what type of signalling molecule is involved in Th1 activation in HTLV-I-infected cells from HAM/TSP patients?

Numerous signalling molecules are involved in the regulation of IFN-γ expression [45]. Since IFN-γ (representative Th1 cytokine) expression in HAM/TSP patients is spontaneously increased and not dependent on T cell receptor (TCR) [55], we focused on p38 mitogen-activated protein kinase (p38 MAPK) because this signalling pathway functions in TCR or signal transducers and activators of transcription 4 (STAT4) independently [75,78]. We analyzed the relationship between IFN-γ expression and p38 MAPK activation in IL-2 dependent HTLV-I-infected T cell lines derived from HAM/TSP patients, compared to ATL patients [14]. Western blot analysis revealed that phosphorylated (activated) p38 MAPK was detected in only cell lines derived from HAM/TSP patients producing large amounts of IFN-γ, but not in cell lines derived from ATL patients producing little IFN-γ. To confirm whether p38 MAPK signalling was functionally activated for IFN-γ induction in cell lines derived from HAM/TSP patients, we analyzed the effect of a p38 MAPK-specific inhibitor, SB203580, on spontaneous IFN-γ production by these cell lines. SB203580 suppressed dose-dependently, up to about 50%, IFN-γ production by these cell lines, suggesting that p38 MAPK signalling is involved in IFN-γ expression in HTLV-I-infected cells of HAM/TSP patients. Indeed, this is supported by the fact that SB203580 significantly suppressed spontaneous IFN-γ production from peripheral blood $CD4^+$ T cells of HAM/TSP patients, but not control patients [14].

Thus, the p38 MAPK signalling pathway must be involved, as one of the signalling pathways toward the up-regulated IFN-γ expression in HTLV-I-infected cells, in Th1 activation in HAM/TSP patients. However, the exact mechanisms by which this signalling is activated in HTLV-I-infected cells of HAM/TSP patients are still unknown. In this regard, constitutive activation of p38 MAPK signalling was observed in IL-2- dependent HTLV-I-infected T cell lines derived from HAM/TSP patients, but not ATL patients, suggesting that the aberrant IL-2 to p38 MAPK signalling induces constitutive Th1 activation for HTLV-I-infected cells of HAM/TSP patients (unpublished data).

Therapeutic strategies for HAM/TSP

To date, numerous therapeutic approaches have been presented on various aspects of HAM/TSP [51], principally in two directions (Fig. 1 and Table I), namely immunomodulatory therapy and anti-viral therapy. Of these, immunomodulatory therapies for the suppression of chronic inflammatory status based on immune-activated status as mentioned above were mainly performed in HAM/TSP patients. This strategy is mainly directed to anti-inflammatory effects, such as (a) the suppression of immune activation, particularly for activated HTLV-I-infected cells, (b) the inhibition of the transmigration of these cells to the spinal cord, and

(c) the reduction of chronic inflammation in the spinal cord, through the down-regulation of inflammatory cytokines and/or adhesion molecules expression (Fig. 1 and Table I). The regimens exhibit the effects also for activated HTLV-I-non-infected cells, which are subsequently induced by the activation of HTLV-I-infected cells. Of these, treatment with corticosteroid hormones, such as prednisolone, is most popular [49]. However, the efficacy of this treatment is still controversial [19,33]. When considering the therapeutic strategies in HAM/TSP, the primary target is the HTLV-I-infected cells of the peripheral blood because HTLV-I-infected $CD4^+$ T cells are the first responders in the immunopathogenesis of HAM/TSP.

Interferon-α and -β

IFN-α and -β, which are type I IFNs, have a variety of biological actions including anti-viral effects as well as growth regulation and modulation of the cellular immune response [7,34,68]. Therefore, treatment with these regimens might be suitable for HAM/TSP because they can target the immunological dysregulation based on high HTLV-I proviral load in the peripheral blood.

IFN-α has proven to be effective in a multicentre, randomized, double-blind, controlled trial [29] and has been approved as a therapeutic agent for HAM/TSP by the Ministry of Health, Labour and Welfare in Japan. In a controlled trial of IFN-α treatment against HAM/TSP, in about 70% of HAM/TSP patients treated with 3.0 million international units of natural IFN-α human lymphoblastoid interferon (HLBI, Sumiferon) (Sumitomo Pharmaceutical Co., Osaka, Japan) by intramuscular injection, daily for 4 weeks, motor dysfunction, even urinary disturbances in some cases, improved, and its effectiveness continued for 4 weeks after discontinuation of therapy without serious adverse effects. Previously, we had also demonstrated similar efficacy of HLBI treatment among 17 HAM/TSP patients in an open trial [65]. In our trial, the most striking change of the immunological markers in the peripheral blood was the significant decrease of spontaneous PBL proliferation *in vitro* leading to the recovery of the response to lectin, such as phytohaemagglutinin. Although spontaneous PBL proliferation is one of the major immunological abnormalities observed *in vitro* in patients with HAM/TSP [27], the exact mechanisms are still unclear. However, this phenomenon is thought to consist of the proliferation of HTLV-I-infected $CD4^+$ T cells and the expansion of HTLV-I-specific $CD8^+$ CTL against virus-expressing cells concomitant with the involvement of aberrant signalling of both interleukin-2 (IL-2) and IL-15 [4,30]. It was reported that HTLV-I proviral load and HTLV-I tax mRNA expression correlated with the frequency of HTLV-I tax specific $CD8^+$ CTL in the peripheral blood of HAM/TSP patients [46,74]. Therefore, IFN-α treatment might induce the reduction of HTLV-I proviral load or HTLV-I tax mRNA expression in the peripheral blood of HAM/TSP patients. Indeed, Saito and co-workers recently reported that HTLV-I proviral loads in the peripheral blood were significantly decreased, concomitant with the reduction of memory T cells in $CD8^{high+}$ T cells, after IFN-α treatment [63]. In addition, $CXCR3^+$ T cells (Th1 cells) were also significantly decreased by this treatment. Thus, IFN-α treatment seems to also induce the correction of Th1/Th2 imbalance, which deviates toward Th1 in HAM/TSP [50]. Other reports also demonstrated that both the percentage of $CCR5^+$ cells (Th1 cells) in $CD4^+$ T cells and the ratio of intracellular IFN-γ^+/IL-4^+ T cells in the peripheral blood were significantly decreased by IFN-α treatment [12].

On the other hand, the efficacy of treatment with IFN-β1a, which has already been approved as a treatment for multiple sclerosis [6], was also reported for HAM/TSP [56]. This treatment induced an improvement of motor dysfunction with the reduction of both HTLV-I tax mRNA load and the frequency of HTLV-I-specific $CD8^+$ CTL in the peripheral blood. Although up-regulated expression of HTLV-I tax itself in HTLV-I-infected cells might be one of the important factors for the development of HAM/TSP [2,74], IFN-β1a treatment might be able to target this point. In addition, the reduction of spontaneous PBL proliferation was also observed, the same as it was in IFN-α treatment. However, HTLV-I proviral load in the peripheral blood remained unchanged. With regard to the change of HTLV-I proviral load, the reasons for the discrepancy between IFN-α and IFN-β1a treatment are unclear. However, although IFN-α treatment as mentioned above induced a significant reduction of HTLV-I proviral load in the total study population, HTLV-I proviral load was actually increased in about 30% of the total study population [63], suggesting that anti-viral effects of IFN are different among individuals.

High HTLV-I proviral load in the peripheral blood is the most important prerequisite in the development of HAM/TSP [31,47]. At the present time, the

increased proliferation of HTLV-I-infected cells is thought to play an important role mainly in the maintenance of high HTLV-I proviral load in the peripheral blood [5,9,72]. Neither IFN-α nor IFN-β treatment seems to target this point. However, it is certain that these regimens have anti-viral activity although its mechanism is obscure. In addition, these regimens also have the activities to correct various immunological dysregulations, such as the imbalance of Th1/Th2 status, in the peripheral blood of HAM/TSP patients. Therefore, these treatments have considerable benefits in therapeutic strategies for HAM/TSP. However, whether or not these treatments are well tolerated over long-term or lifelong treatment is uncertain.

Reverse transcriptase inhibitors

Some nucleoside analogues have been shown to block HTLV-I replication by inhibition of reverse transcriptase (RT). Zidovudine (azidothymidine, AZT) can inhibit HTLV-I replication *in vitro* although its inhibitory dose for HTLV-I is higher than for human immunodeficiency virus [42]. However, a recent clinical trial of combination therapy using zidovudine and lamivudine, the thymidine and cytosine analogue, in a randomized, double-blind, placebo-controlled study gave a pessimistic view of RT inhibitors for the regimen for HTLV-I targeting as a treatment for HAM/TSP [66]. Taylor and colleagues have conducted a controlled study on 6 months of combination therapy with these two RT inhibitors for 16 HAM/TSP patients. Comparing the clinical effects and the changes of laboratory markers in the peripheral blood including HTLV-I proviral load between each group treated by combined therapy or placebo therapy, no significant changes were seen between the two arms. This finding strongly suggests that both RT inhibitors have no activities to reduce HTLV-I proviral load, at least, *in vivo* in HAM/TSP patients. If the increased proliferation of HTLV-I-infected cells, rather than new infection through cell-to-cell spread, plays an important role mainly in the maintenance of high HTLV-I proviral load in the peripheral blood of HAM/TSP patients [5,9,72], the inefficacy of the treatment with RT inhibitors might be reasonable.

Humanized anti-Tac

It is well known that IL-2 and IL-2 receptor α (IL-2Rα) are induced by HTLV-I tax transactivation in HTLV-I-infected cells [26,67]. This dysregulation of cellular gene expression by HTLV-I tax initiates a process of T cell activation and proliferation by the autocrine or paracrine loop. Therefore, the blockade of the IL-2/IL-2Rα system might lead to decreased HTLV-I-infected cells *in vivo* through apoptosis of such cells by IL-2 deprivation. The efficacy of humanized anti-Tac antibody (daclizumab), the humanized form of monoclonal antibody against IL-2Rα which blocks the interaction of IL-2 with IL-2Rα, has been demonstrated in several immune-mediated diseases, such as renal allograft rejection, non-infectious uveitis, multiple sclerosis, pure red cell aplasia, aplastic anaemia, psoriasis, and T-cell malignancy [70].

The efficacy of daclizumab treatment for HAM/TSP patients was reported concomitant with the reduction of the number of circulating activated T cells expressing IL-2α receptor and the decrease of spontaneous PBL proliferation *ex vivo* [35]. Furthermore, HTLV-I proviral load in the peripheral blood was reduced by an average of 52% following treatment, suggesting that humanized anti-Tac can selectively remove HTLV-I-infected cells expressing IL-2Rα from the peripheral blood of HAM/TSP patients.

Histone deacetylase enzyme inhibitor

Histone deacetylase (HDAC) enzyme inhibitor has lately attracted considerable attention as a therapeutic regimen against various diseases, including malignancies and neurodegenerative diseases [1,8]. Although acetylated histones are associated with transcriptionally active chromatin and deacetylated histones with inactive chromatin, chromatin acetylation is regulated by the balance between histone acetyltransferases and histone deacetylases (HDACs) as epigenetic control under physiological conditions. Histone acetylation plays an important role also in the regulation of HTLV-I gene expression [11,40]. Therefore, inhibition of HDAC activities leads into histone hyperacetylation followed by increases in HTLV-I gene expression.

The relationship between HTLV-I proviral load and/or expression and the host immune system, such as HTLV-I-specific CTL, is at equilibrium in the peripheral blood [5]. Therefore, if HTLV-I proviral load is increased based on up-regulation of HTLV-I expression (e.g., in cells infected with latent or silent form), HTLV-specific CTL are more activated and the number of HTLV-I-infected cells might be reduced in the peri-

pheral blood. Based on the new concept of "gene activation therapy", a clinical trial of orally administered 20 mg/kg/day valproate (VPA), a HDAC inhibitor, over 3 months was performed in 16 HAM/TSP patients [38]. Although HTLV-I proviral loads in the peripheral blood were transiently increased in the early stages after administration, they significantly decreased in all patients by 2.3- to 89.3-fold (mean, 24-fold) at the end point. Although the authors did not describe the changes of clinical status in detail, they mentioned that VPA treatment induced a reduction of spasticity in all patients. Although there is one report that HDAC inhibitors decreased the activity of HTLV-I-specific CTL leading to a reduction of the efficiency of CTL surveillance against HTLV-I [43], VPA might be a potentially useful anti-HTLV-I agent. Since VPA is an anti-epileptic drug with a good safety profile as long-term therapy and is easily available, case-controlled studies of VPA treatment in HAM/TSP patients are warranted.

Prosultiamine

As another therapeutic strategy for HAM/TSP, HTLV-I-infected cells may be targeted in the peripheral blood. If HTLV-I-infected cells can be selectively removed, for example by apoptosis, from the peripheral blood, using inexpensive drugs which are well tolerated with few adverse events over long periods, this may become an ideal therapy for HAM/TSP. Very recently, we reported the efficacy of prosultiamine treatment against HAM/TSP patients [54] with the decrease of HTLV-I proviral load in the peripheral blood.

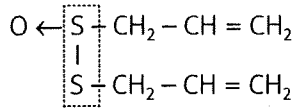
Although prosultiamine (Alinamin®), a product of Takeda Pharma Co. Inc. (Osaka, Japan), is very frequently prescribed for vitamin B1 deficiency in Japan, this compound is a homologue of allithiamine, originally synthesized by thiol type vitamin B1 and allicin (diallyl thiosulfinate) derived from garlic (*Allium sativum*) [13]. For the stability in the blood and the efficient access of vitamin B1 to the tissues, prosultiamine was developed after allyl disulfide derived from allicin was substituted to propyl disulfide in the structure of allithiamine [41] (Fig. 2). Although the mechanisms by which cytotoxic effects are induced by allicin are poorly understood, the disruption of the intracellular redox system induced by the chemical reaction of a disulfide moiety in its structure with thiol-containing intracellular molecules, such as thioredoxin, thioredoxin reductase, glutathione, etc., seems to have an important role for triggering cell death [73]. As shown in Fig. 2, prosultiamine has a disulfide moiety in its structure like allicin. Therefore, prosultiamine is expected to have the same biological activity as allicin. Indeed, we showed that prosultiamine, like allicin, has cytotoxic activity against HTLV-I-infected T cell lines derived from HAM/TSP patients by caspase-dependent apoptotic activity through the mitochondrial pathway [54]. Based on the data showing that prosultiamine *in vitro* treatment against peripheral blood $CD4^+$ T cells of HAM/TSP patients also induced a significant decrease of HTLV-I proviral copy numbers by apoptosis of HTLV-I-infected cells, we treated 6 HAM/TSP patients with intravenous prosultiamine at dosages of 40 mg daily for 14 days. As a result of this treatment, the copy numbers of HTLV-I provirus in peripheral blood decreased to about 30-50% of their pre-treatment levels, with some clinical benefits in all patients, suggesting that prosultiamine has the potential to be a new therapeutic tool targeting HTLV-I-infected cells by inducing apoptosis in HAM/TSP [54]. Although prosultiamine is safely prescribed even in long-term treatment, case-controlled studies of its treatment in HAM/TSP patients are warranted.

Conclusions

Although long-standing bystander mechanisms, such as the destruction of the surrounding tissues by the interaction between HTLV-I-infected $CD4^+$ T cells and HTLV-I-specific CTL, are probably critical in the immunopathogenesis of HAM/TSP, the entire molecular process by which the mechanisms are induced in the spinal cord are still obscure. In this review, when considering the role of HTLV-I-infected $CD4^+$ T cells in the peripheral blood as the first responders in this mechanism, I have showed the importance of the increase of these cells having the characteristics of Th1-activated status based on activation of p38 MAPK signalling with the transmigrating activity into the tissues enough to trigger chronic inflammation in the spinal cord. However, the exact mechanisms of how these abnormalities are induced in the peripheral blood of HAM/TSP patients remain unresolved.

During the past 20-year period, since the association of HTLV-I and spastic paraparesis, numerous findings have been presented on various aspects of HAM/TSP. Unfortunately, these findings have not translated into an optimal therapeutic strategy for this chronic, progressive neurological disease. Given that the patho-

A. Allicin



B. Prosultiamine

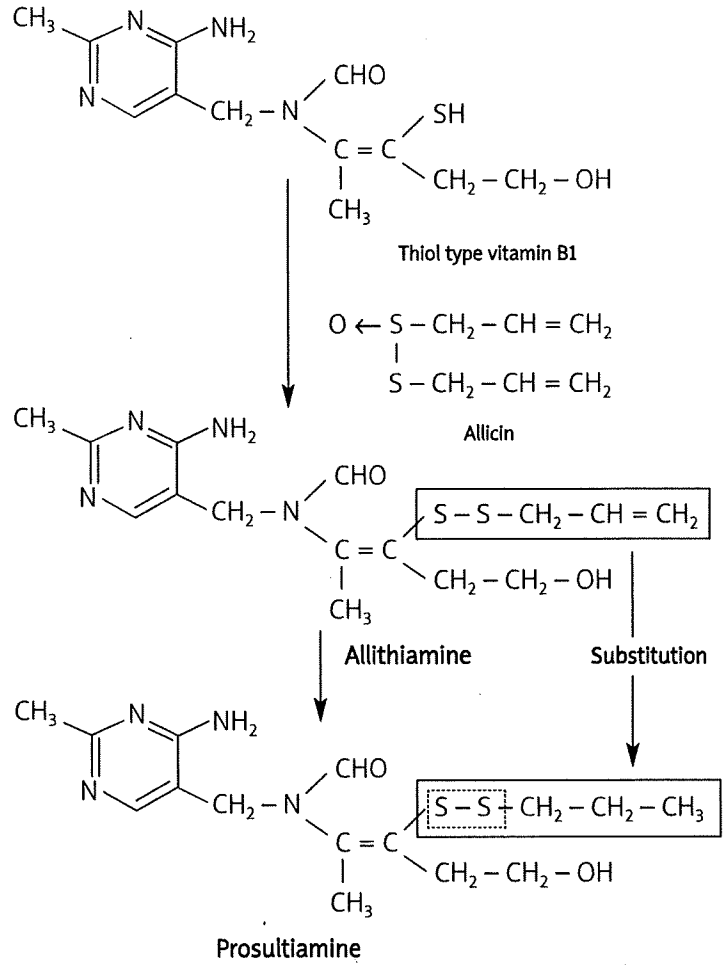


Fig. 2. Structure of allicin (A) and the generation of prosultiamine (B). Allithiamine was originally synthesized by thiol type vitamin B1 and allicin. Prosultiamine was developed after allyl disulfide derived from allicin was substituted to propyl disulfide in the structure of allithiamine as shown by ▭. Prosultiamine and allicin have a disulfide moiety in their structure as shown by ⬚. Quotation from [51].

physiology of HAM/TSP involves chronic inflammation triggered by HTLV-I infection, the treatment of HAM/TSP must be done as one of the infectious diseases because HTLV-I-infected cells are the first responders in the development of HAM/TSP. Therefore, therapeutic strategies that decrease or eliminate HTLV-I-infected cells seem appropriate for HAM/TSP. In addition, these strategies must be well tolerated, and inexpensive, even in

long-term treatment. In this regard, either VPA or prosultiamine might function as a new anti-viral agent against HTLV-I. Clinical trials for targeting the depletion of HTLV-I-infected cells provide the next important steps in assessing this new therapeutic strategy against HAM/TSP.

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References

1. Abel T, Zukin RS. Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders. Curr Opin Pharmacol 2008; 8: 57-64.
2. Asquith B, Mosley AJ, Heaps A, Tanaka Y, Taylor GP, McLean AR, Bangham CR. Quantification of the virus-host interaction in human T lymphotropic virus I infection. Retrovirology 2005; 2: 75.
3. Austrup F, Vestweber D, Borges E, Löhning M, Bräuer R, Herz U, Renz H, Hallmann R, Scheffold A, Radbruch A, Hamann A. P- and E-selectin mediate recruitment of T-helper-1 but not T-helper-2 cells into inflamed tissues. Nature 1997; 385: 81-83.
4. Azimi N, Jacobson S, Leist T, Waldmann TA. Involvement of IL-15 in the pathogenesis of human T lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis: Implications for therapy with a monoclonal antibody directed to the IL-2/IL-15Rbeta receptor. J Immunol 1999; 163: 4064-4072.
5. Bangham CRM, Osame M. Cellular immune response to HTLV-I. Oncogene 2005; 24: 6035-6046.
6. Bermel RA, Rudick RA. Interferon-beta treatment for multiple sclerosis. Neurotherapeutics 2007; 4: 633-646.
7. Borden EC, Sen GC, Uze G, Silverman RH, Ransohoff RM, Foster GR, Stark GR Interferons at age 50: past, current and future impact on biomedicine. Nat Rev Drug Discov 2007; 6: 975-990.
8. Carey N, La Thangue NB. Histone deacetylase inhibitors: gathering pace. Curr Opin Pharmacol 2006; 6: 369-375.
9. Cavrois M, Leclercq I, Gout O, Gessain A, Wain-Hobson S, Wattel E. Persistent oligoclonal expansion of human T-cell leukemia virus type I-infected circulating cells in patients with tropical spastic paraparesis/HTLV-1 associated myelopathy. Oncogene 1998; 17: 77-82.
10. de-Thé G, Bomford R. An HTLV-I vaccine: why, how, for whom? AIDS Res Hum Retroviruses 1993; 9: 381-386.
11. Ego T, Ariumi Y, Shimotohno K. The interaction of HTLV-I tax with HDAC1 negatively regulates the viral gene expression. Oncogene 2002; 21: 7241-7246.
12. Feng J, Misu T, Fujihara K, Misawa N, Koyanagi Y, Shiga Y, Takeda A, Sato S, Takase S, Kohnosu T, Saito H, Itoyama Y. Th1/Th2 balance and HTLV-I proviral load in HAM/TSP patients treated with interferon-alpha. J Neuroimmunol 2004; 151: 189-194.
13. Fujiwara M, Watanabe H, Matsui K. "Allithiamine" a newly found derivative of vitamin B1. I. discovery of allithiamine. J Biochem 1954; 41: 29-39.
14. Fukushima N, Nishiura Y, Nakamura T, Yamada Y, Kohno S, Eguchi K. Involvement of p38 MAPK signaling pathway in IFN-gamma and HTLV-I expression in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis. J Neuroimmunol 2005; 159: 196-202.
15. Furukawa Y, Saito M, Matsumoto W, Usuku K, Tanaka Y, Izumo S, Osame M. Different cytokine production in Tax-expressing cells between patients with human T cell lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis and asymptomatic HTLV-I carriers. J Infect Dis 2003; 187: 1116-1125.
16. Furuya T, Nakamura T, Fujimoto T, Nakane S, Kambara C, Shirabe S, Hamasaki S, Motomura M, Eguchi K. Elevated levels of interleukin-12 and interferon-gamma in patients with human T lymphotropic virus type I-associated myelopathy. J Neuroimmunol 1999; 95: 185-189.
17. Furuya T, Nakamura T, Shirabe S, Nishiura Y, Tsujino A, Goto H, Nakane S, Eguchi K, Nakamura H, Nagataki S. Heightened transmigrating activity of CD4-positive T cells through reconstituted basement membrane in patients with human T-lymphotropic virus type I-associated myelopathy. P Assoc Am Physician 1997; 109: 228-236.
18. Gessain A, Barin F, Vernant JC, Gout O, Maurs L, CalenderA, de-Thé G. Antibodies to human T-lymphotropic virus type-I in patients with tropical spastic paraparesis. Lancet 1985; ii: 407-410.
19. Gessain A, Gout O. Chronic myelopathy associated with human T-lymphotropic virus type I (HTLV-I). Ann Intern Med 1992; 117: 933-946.
20. Grogan JL, Locksley RM. T helper cell differentiation: on again, off again. Curr Opin Immunol 2002; 14: 366-372.
21. Hanon E, Goon P, Taylor GP, Hasegawa H, Tanaka Y, Weber JN, Bangham CRM. High production of interferon gamma but not interleukin-2 by human T-lymphotropic virus type I-infected peripheral blood mononuclear cells. Blood 2001; 98: 721-726.
22. Hiraiwa N, Hiraiwa M, Kannagi R. Human T-cell leukemia virus-1 encoded Tax protein transactivates alpha1 -> 3 fucosyltransferase Fuc-T VII, which synthesizes sialyl Lewis^x, a selectin ligand expressed on adult T-cell leukemia cells. Biochem Biophys Res Commun 1997; 231: 183-186.

23. Höllsberg P, Hafler DA. Pathogenesis of diseases induced by human lymphotropic virus type I infection. N Engl J Med 1993; 328: 1173-1182.
24. Horiuchi I, Kawano Y, Yamasaki K, Minohara M, Furue M, Taniwaki T, Miyazaki T, Kira J. Th1 dominance in HAM/TSP and the optico-spinal form of multiple sclerosis versus Th2 dominance in mite antigen-specific IgE myelitis. J Neurol Sci 2000; 172: 17-24.
25. Ijichi S, Izumo S, Eiraku N, Machigashira K, Kubota R, Nagai M, Ikegami N, Kashio N, Umehara F, Maruyama I, Osame M. An autoaggressive process against bystander tissues in HTLV-I-infected individuals: a possible pathomechanism of HAM/TSP. Med Hypotheses 1993; 41: 542-547.
26. Inoue J, Séiki M, Taniguchi T, Tsuru S, Yoshida M. Induction of interleukin 2 receptor gene expression by p40x encoded by human T cell leukemia virus type I. EMBO J 1986; 5: 2883-2888.
27. Itoyama Y, Minato S, Kira J, Goto I, Sato H, Okochi K, Yamamoto N. Spontaneous proliferation of peripheral blood lymphocytes increased in patients with HTLV-I-associated myelopathy. Neurology 1988; 38: 1302-1307.
28. Iwasaki Y. Pathology of chronic myelopathy associated with HTLV-I infection (HAM/TSP). J Neurol Sci 1990; 96: 103-123.
29. Izumo S, Goto I, Itoyama Y, Okajima T, Watanabe S, Kuroda Y, Araki S, Mori M, Nagataki S, Matsukura S, Akamine T, Nakagawa M, Yamamoto I, Osame M. Interferon-alpha is effective in HTLV-I-associated myelopathy: a multicenter, randomized, double-blind, controlled trial. Neurology 1996; 46: 1016-1021.
30. Jacobson S. Immunopathogenesis of human T cell lymphotropic virus type I-associated neurologic disease. J Infect Dis 2002; 186 (Suppl 2): S187-192.
31. Jeffery KJ, Usuku K, Hall SE, Matsumoto W, Taylor GP, Procter J, Bunce M, Ogg GS, Welsh KI, Weber JN, Lloyd AL, Nowak MA, Nagai M, Kodama D, Izumo S, Osame M, Bangham CR. HLA alleles determine human T-lymphotropic virus-I (HTLV-I) proviral load and the risk of HTLV-I-associated myelopathy. Proc Natl Acad Sci USA 1999; 96: 3848-3853.
32. Kambara C, Nakamura T, Furuya T, Nishiura Y, Kawakami A, Ichinose K, Shirabe S, Eguchi K. Increased sialyl Lewis^x antigen-positive cells mediated by HTLV-I infection in peripheral blood CD4$^+$ T lymphocytes in patients with HTLV-I-associated myelopathy. J Neuroimmunol 2002; 125: 179-184.
33. Kira J, Fujihara K, Itoyama Y, Goto I, Hasuo K. Leukoencephalopathy in HTLV-I-associated myelopathy/tropical spastic paraparesis: MRI analysis and a 2 year follow-up study after corticosteroid therapy. J Neurol Sci 1991; 106: 41-49.
34. Kovarik P, Sauer I, Schaljo B. Molecular mechanisms of the anti-inflammatory functions of interferons. Immunobiol 2008; 212: 895-901.
35. Lehky TJ, Levin MC, Kubota R, Bamford RN, Flerlage AN, Soldan SS, Leist TP, Xavier A, White JD, Brown M, Fleisher TA, Top LE, Light S, McFarland HF, Waldmann TA, Jacobson S. Reduction in HTLV-I proviral load and spontaneous lymphoproliferation in HTLV-I-associated myelopathy/tropical spastic paraparesis patients treated with humanized anti-Tac. Ann Neurol 1998; 44: 942-947.
36. Ley K, Kansas GS. Selectins in T-cell recruitment to non-lymphoid tissues and sites of inflammation. Nature Rev Immnol 2004; 4: 325-335 .
37. Ley K, Tedder T. Leukocyte interactions with vascular endothelium. New insights into selectin-mediated attachment and rolling. J Immunol 1995; 155: 525-528.
38. Lezin A, Gillet N, Olindo S, Signaté A, Grandvaux N, Verlaeten O, Belrose G, Bittencourt MC, Hiscott J, Asquith B, Burny A, Smadja D, Césaire R, Willems L. Histone deacetylase-mediated transcriptional activation reduces proviral loads in HTLV-1-associated myelopathy/tropical spastic paraparesis. Blood 2007; 110: 3722-3728.
39. Lezin A, Olindo S, Olie`re S, Varrin-Doyer M, Marlin R, Cabre P, Smadja D, Cesaire R. Human T Lymphotropic Virus Type I (HTLV-I) Proviral Load in Cerebrospinal Fluid: A New Criterion for the Diagnosis of HTLV-I-Associated Myelopathy/Tropical Spastic Paraparesis? J Infect Dis 2005; 191: 1830-1834.
40. Lu H, Pise-Masison CA, Linton R, Park HU, Schiltz RL, Sartorelli V, Brady JN. Tax relieves transcriptional repression by promoting histone deacetylase 1 release from the human T-cell leukemia virus type 1 long terminal repeat. J Virol 2004; 78: 6735-6743.
41. Matsukawa T, Yurugi S. On the structure of allithiamine. Proceedings of the Japan Academy 1952; 28: 146-149.
42. Matsushita S, Mitsuya H, Reitz MS, Broder S. Pharmacological inhibition of in vitro infectivity of human T lymphotropic virus type 1. J Clin Invest 1987: 80: 394-400.
43. Mosley AJ, Meekings KN, McCarthy C, Shepherd D, Cerundolo V, Mazitschek R, Tanaka Y, Taylor GP, Bangham CR. Histone deacetylase inhibitors increase virus gene expression but decrease CD8$^+$ cell antiviral function in HTLV-1 infection. Blood 2006; 108: 3801-3807.
44. Mosmann TR, Coffman RL. Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. Annu Rev Immunol 1989; 7: 145-173.
45. Murphy KM, Ouyang W, Farrar JD, Yang J, Ranganath S, Asnagli H, Afkarian M, Murphy TL. Signaling and transcription in T helper development. Annu Rev Immunol 2000; 18: 451-494.
46. Nagai M, Kubota R, Greten TF, Schneck JP, Leist TP, Jacobson S. Increased activated human T cell lymphotropic virus type I (HTLV-I) tax 11-19-specific memory and effector CD8$^+$ cells in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis: correlation with HTLV-I provirus load. J Infect Dis 2001; 183: 197-205.
47. Nagai M, Usuku K, Matsumoto W, Kodama D, Takenouchi N, Moritoyo T, Hashiguchi S, Ichinose M, Bangham CRM, Izumo S, Osame M. Analysis of HTLV-I proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers: high proviral load strongly predisposes to HAM/TSP. J Neurovirol 1998; 4: 586-593.
48. Nagai M, Yamano Y, Brennan MB, Mora CA, Jacobson S. Increased HTLV-I proviral load and preferential expansion of HTLV-ITax-specific CD8$^+$ T cells in cerebrospinal fluid from patients with HAM/TSP. Ann Neurol 2001; 50: 807-812.
49. Nakagawa M, Nakahara K, Maruyama Y, Kawabata M, Higuchi I, Kubota H, Izumo S, Arimura K, Osame M. Therapeutic trials in 200 patients with HTLV-I-associated myelopathy/tropical spastic paraparesis. J Neurovirol 1996; 2: 345-355.
50. Nakamura T, Furuya T, Nishiura Y, Ichinose K, Shirabe S, Eguchi K. Importance of immune deviation toward Th1 in the early immunopathogenesis of human T-lymphotropic virus type I-associated myelopathy. Med Hypotheses 2000; 54: 777-782.

51. Nakamura T, Nishiura Y, Eguchi K. Therapeutic strategies in HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). Central Nervous System Agents in Medicinal Chemistry, 2009; 9: 137-149.
52. Nakamura T. Immunopathogenesis of HTLV-I-associated myelopatrhy/tropical spastic paraparesis. Ann Med 2000; 32: 600-607.
53. Nishiura Y, Nakamura T, Fukushima N, Moriuchi R, Katamine S, Eguchi K. Increased mRNA expression of Th1-cytokine signaling molecules in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis. Tohoku J Exp Med 2004; 204: 289-298.
54. Nishiura Y, Nakamura T, Fukushima N, Nakamura H, Ida H, Aramaki T, Eguchi K. Disulfide-mediated apoptosis of HTLV-I-infected cells in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis. Antivir Ther 2009; in press.
55. Nishiura Y, Nakamura T, Ichinose K, Shirabe S, Tsujino A, Goto H, Furuya T, Nagataki S. Increased production of inflammatory cytokines in cultured CD4$^+$ cells from patients with HTLV-I-associated myelopathy. Tohoku J Exp Med 1996; 179: 227-233.
56. Oh U, Yamano Y, Mora CA, Ohayon J, Bagnato F, Butman JA, Dambrosia J, Leist TP, McFarland H, Jacobson S. Interferon-beta1a therapy in human T-lymphotropic virus type I-associated neurologic disease. Ann Neurol 2005; 57: 526-534.
57. Ohmori K, Takada A, Ohwaki I, Takahashi N, Furukawa Y, Maeda M, Kiso M, Hasegawa A, Kannagi M, Kannagi R. A distinct type of sialyl Lewis X antigen defined by a novel monoclonal antibody is selectively expressed on helper memory T cells. Blood 1993; 82: 2797-2805.
58. Osame M, Matsumoto M, Usuku K, Izumo S, Ijichi N, Amitani H, Tara M, Igata A. Chronic progressive myelopathy associated with elevated antibodies to human T-lymphotropic virus type I and adult T-cell leukemia-like cells. Ann Neurol 1987; 21: 117-122.
59. Osame M, Usuku K, Izumo S, Ijichi N, Amitani H, Igata A, Matsumoto M, Tara M. HTLV-I associated myelopathy, a new clinical entity. Lancet 1986; i: 1031-1032.
60. Osame M. Pathological mechanisms of human T-cell lymphotropic virus type I-associated myelopathy (HAM/TSP). J Neurovirol 2002; 8: 359-364.
61. Osame M. Review of WHO Kagoshima meeting and diagnostic guidelines for HAM/TSP. In: Blattner WA (ed.). Human retrovirology: HTLV. Raven Press, New York 1990; pp. 191-197.
62. Rodgers-Johnson P, Gajdusek DC, Morgan OS, Zaninovic V, Sarin PS, Graham DS. HTLV-I and HTLV-III antibodies and tropical spastic paraparesis. Lancet 1985; ii: 1247-1248.
63. Saito M, Nakagawa M, Kaseda S, Matsuzaki T, Jonosono M, Eiraku N, Kubota R, Takenouchi N, Nagai M, Furukawa Y, Usuku K, Izumo S, Osame, M. Decreased human T lymphotropic virus type I (HTLV-I) provirus load and alteration in T cell phenotype after interferon-alpha therapy for HTLV-I-associated myelopathy/tropical spastic paraparesis. J Infect Dis 2004; 189: 29-40.
64. Seder RA, Paul WE. Acquisition of lymphokine-producing phenotype by CD4$^+$ T-cells. Annu Rev Immunol 1994; 12: 635-673.
65. Shibayama K, Nakamura T, Nagasato K, Shirabe S, Tsujihata M, Nagataki S. Interferon-alpha treatment in HTLV-I-associated myelopathy: studies of clinical and immunological aspects. J Neurol Sci 1991; 106: 186-192.
66. Taylor GP, Goon P, Furukawa Y, Green H, Barfield A, Mosley A, Nose H, Babiker A, Rudge P, Usuku K, Osame M, Bangham CRM, Weber JN. Zidovudine plus lamivudine in human T-lymphotropic virus type-I-associated myelopathy: a randomized trial. Retrovirology 2006; 3: 63.
67. Tendler CL, Greenberg SL, Blattner WA, Manns A, Murphy E, Fleisher T, Hanchard B, Morgan O, Burton JD, Nelson DL, Waldmann TA. Transactivation of interleukin 2 and its receptor induces immune activation in human T lymphotropic virus type I-associated myelopathy: pathological implications and a rationale for immunotherapy. Proc Natl Acad Sci USA 1990; 87: 5218-5222.
68. Vannucchi S, Chiantore MV, Mangino G, Percario ZA, Affabris E, Fiorucci G, Romeo G. Perspectives in biomolecular therapeutic intervention in cancer: from the early to the new strategies with type I interferons. Curr Med Chem 2007; 14: 667-679.
69. Varki A. Selectin ligands. Proc Natl Acad Sci USA 1994; 91: 7390-7397.
70. Waldmann TA. Anti-Tac (daclizumab, Zenapax) in the treatment of leukemia, autoimmune diseases, and in the prevention of allograft rejection: a 25-year personal odyssey. J Clin Immunol 2007; 27: 1-18.
71. Watanabe H, Nakamura T, Nagasato K, Shirabe S, Ohishi K, Ichinose K, Nishiura Y, Chiyoda S, Tsujihata M, Nagataki S. Exaggerated messenger RNA expression of inflammatory cytokines in human T-cell lymphotropic virus type I-associated myelopathy. Arch Neurol 1995; 52: 276-280.
72. Wattel E, Vartanian JP, Pannetier C, Wain-Hobson S. Clonal expansion of human T-cell leukemia virus type I-infected cells in asymptomatic and symptomatic carriers without malignancy. J Virol 1995; 69: 2863-2868.
73. Wong WW, Macdonald S, Langler RF, Penn LZ. Novel synthetic organosulfur compounds induce apoptosis of human leukemic cells. Anticancer Res 2000; 20: 1367-1374.
74. Yamano Y, Nagai M, Brennan M, Mora CA, Soldan SS, Tomaru U, Takenouchi N, Izumo S, Osame M, Jacobson S. Correlation of human T-cell lymphotropic virus type 1 (HTLV-1) mRNA with proviral DNA load, virus-specific CD8$^+$ T cells, and disease severity in HTLV-1-associated myelopathy (HAM/TSP). Blood 2002; 99: 88-84.
75. Yang J, Zhu H, Murphy TL, Ouyang W, Murphy KM. IL-18-stimulated GADD45beta required in cytokine-induced, but not TCR-induced, IFN-gamma production. Nature Immunol 2001; 2: 157-164.
76. Yoshie O, Fujisawa R, Nakayama T, Harasawa H, Tago H, Izawa D, Hieshima K, Tatsumi Y, Matsushima K, Hasegawa H, Kanamaru A, Kamihira S, Yamada Y. Frequent expression of CCR4 in adult T-cell leukemia and human T-cell leukemia virus type 1-transformed T cells. Blood 2002; 99: 1505-1511.
77. Yoshie O. Expression of CCR4 in adult T-cell leukemia. Leuk Lymphoma 2005; 46: 185-190.
78. Zhang S, Kaplan M. The p38 mitogen-activated protein kinase is required for IL-12-induced IFN-gamma expression. J Immunol 2000; 165: 1374-1380.

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LETTER TO THE EDITORS

Efficacy of tacrolimus in Sjögren's syndrome-associated CNS disease with aquaporin-4 autoantibodies

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Sirs,

We present two patients with central nervous system (CNS) disease associated with Sjögren's syndrome (SjS) and positive for antiaquaporin-4 water channel autoantibodies (AQP4-Ab) who were treated successfully with tacrolimus. Tacrolimus is an immunosuppressant that acts as a calcineurin inhibitor and suppresses T helper 2 (Th2) cells [1]. Tacrolimus may also act as a neuroprotectant by reducing axonal and myelin damage, as shown in a mouse model of experimental autoimmune encephalomyelitis [2]. SjS is a chronic autoimmune disease characterized by lymphocytic infiltration of the exocrine glands. SjS-associated inflammation sometimes spreads into the CNS (CNS–SjS), occasionally mimics relapsing-remitting multiple sclerosis (MS), and inflammation often involves the brain, spinal cord, and optic nerve [3]. Neuromyelitis optica (NMO) is also a relapsing inflammatory disease of the CNS, characterized by severe attacks of optic nerve neuritis and longitudinally extensive transverse myelitis [4]. NMO is distinguished from MS by the presence of AQP4-Ab and differences in the distribution of inflammatory lesions and pathological findings. Combination therapy with a corticosteroid and azathioprine is the current standard treatment for preventing NMO relapse [5]; however, some patients are refractory to this therapy. Approximately 3% of patients with NMO have coexisting systemic lupus erythematosus (SLE) or SjS, and CNS–SjS patients with optic nerve neuritis or longitudinal myelitis (conditions called "NMO spectrum disorder") often present with positive findings for AQP4-Ab [4, 6, 7]. To our knowledge, this is the first reported assessment of tacrolimus in patients with CNS–SjS with AQP4-Ab. This treatment was approved by the ethical committee of our university, and the patients provided written informed consent.

A 48-year-old female (Patient 1, Fig. 1) was admitted to the hospital with rapidly progressive nausea, hiccups, dysphagia, and drowsiness. Magnetic resonance imaging (MRI) revealed T2 hyperintensities of the hypothalamus bilaterally and the dorsal medulla oblongata. After three courses of intravenous high-dose methylprednisolone (IHMP, 1 g/day for 3 days in one course), the patient recovered completely, except for mild dysphagia. One year after the first attack, she developed limb weakness. Laboratory test results revealed high levels of anti-Ro (SSA) antibodies and positive antinuclear antibody. The Schirmer test and the Saxon test revealed decreased salivary secretion (Table 1). She was diagnosed with CNS–SjS [8]. She experienced nine attacks during the entire disease course, and we started treatment with oral tacrolimus during her ninth admission. No recurrent attacks have been observed for 49 months since the start of this treatment.

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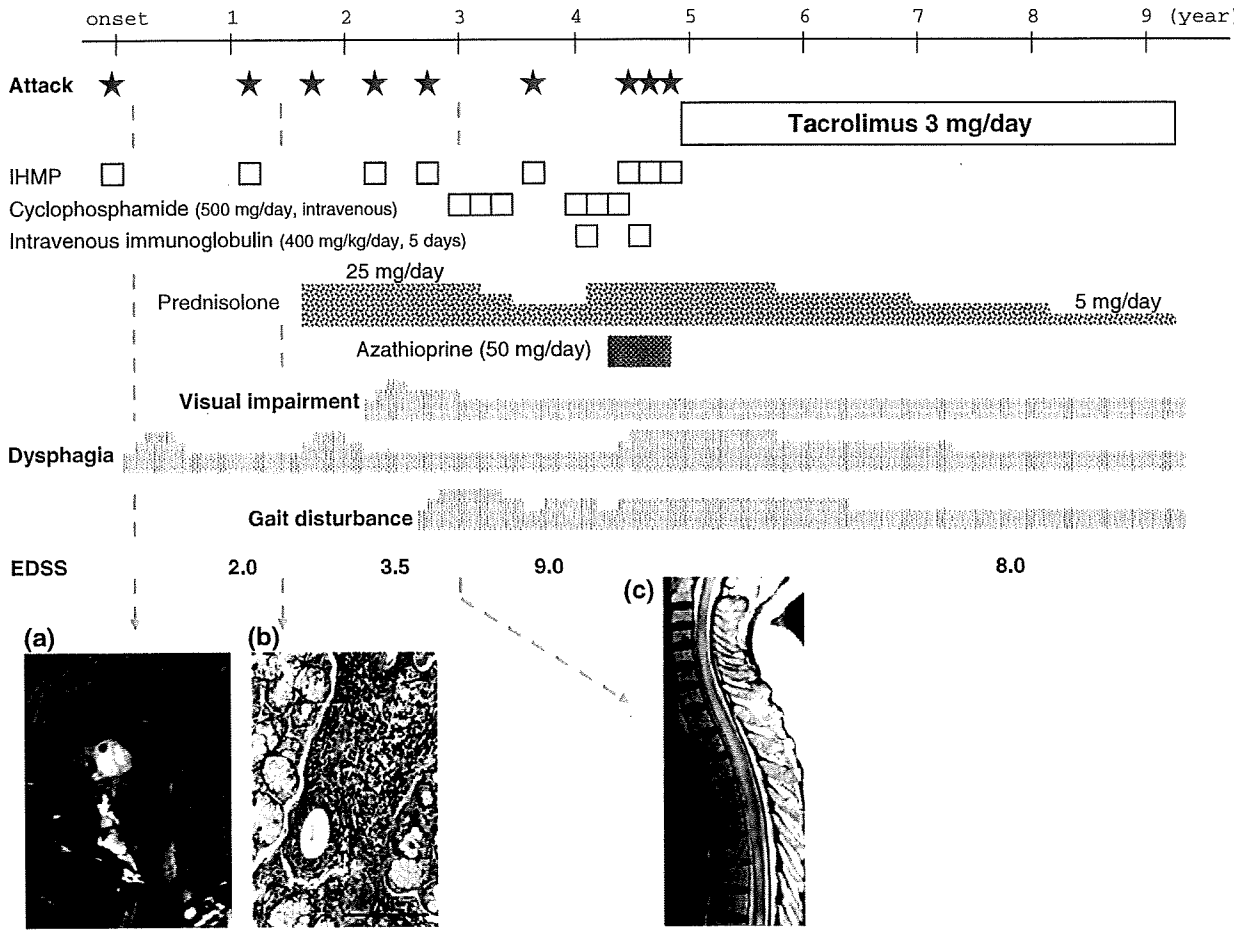

Fig. 1 Time course of relapse, therapies, symptoms, and images of Patient 1. *Stars* indicate attacks. The *thickness of each line* represents the severity of any symptom or the dose of drug. Nine attacks had occurred in spite of therapeutic approaches, and no attacks were observed after starting tacrolimus. **a** A sagittal fluid-attenuated inversion recovery (FLAIR)-weighted image at onset shows hyperintense lesions in the thalamus, the mammillary bodies, and the dorsal portion of the medulla oblongata (*arrows*). **b** A photomicrograph of a labial salivary gland biopsy at the second admission. An aggregate of lymphocytes surrounding a salivary gland. Size bar is 50 μm. **c** A sagittal T2-weighted image at the fifth admission shows hyperintense lesions throughout the spinal cord (*arrowheads*). *IHMP* Intravenous high-dose methylprednisolone (1 g/day for 3 days in one course), *EDSS* expanded disability status scale

A 50-year-old female (Patient 2) visited our hospital with acute left visual loss and depression. She was diagnosed with retrobulbar optic nerve neuritis, but she rejected steroid therapy and was not admitted to our hospital. One year later, she was sent to our hospital by ambulance due to weakness. Physical examination revealed dysphagia, dysarthria, incomplete tetraplegia, urinary retention, and depression. MRI revealed T2 hyperintensities in the corpus callosum, thalamus, midbrain, and pons. She was diagnosed with CNS–SjS based on SjS criteria (Table 1), and she was treated with IHMP. Because high doses of oral steroids may exacerbate depression, we started therapy with tacrolimus (3 mg/day) to prevent relapse. No recurrence was observed for nine months, but she could not continue the therapy due to exacerbation of depression. Two years after withdrawal, she suffered right hemiplegia, and MRI revealed a relapse lesion in the left posterior limb of the internal capsule.

The serum of both patients was positive for AQP4-Ab after the initiation of tacrolimus. Because no recurrences were observed for at least 9 months for one patient (Patient 2) and 49 months for the other (Patient 1), we concluded that tacrolimus is effective in CNS–SjS with AQP4-Ab. Considering the pathological observations that indicate loss of AQP4 with deposition of antibody and compliment in CNS lesions in NMO [9, 10], we speculate that tacrolimus may act by suppressing the humoral immunity against AQP4 through Th2 inhibition.

Table 1 Clinical features of two patients

	Patient 1	Patient 2
Age at onset (years), sex	48, female	50, female
Ethnicity	Japanese	Japanese
Dry mouth	+	+
Number of relapses[a]	9	2
MRI findings		
Optic lesion	+	+
Cerebral lesion	+	+
Pontine lesion	−	+
Medullary lesion	+	−
Longitudinal cord lesion	+	−
Autoantibodies		
ANA	+	+
SS-A	+	+
SS-B	−	+
Aquaporin-4	+	+
Diagnostic tests for SjS		
Schirmer test	+	+
Saxon test	+	+
Salivary gland biopsy	Compatible for SjS	Not done
Sialography	Not done	Compatible for SjS
CSF findings		
Cells/μl	26	37
MBP (mg/dl, <4.0)	6.2	56.3
Oligoclonal IgG bands	−	−
Diagnosis		
Criteria of SjS	Fulfilled	Fulfilled
Criteria of NMO	Fulfilled	Not fulfilled

ANA antinuclear antibody, CSF cerebrospinal fluid, IgG immunoglobulin G, *MBP* myelin basic protein, MRI magnetic resonance imaging, *NMO* neuromyelitis optica, *SjS* Sjögren's syndrome

[a] Including first attack

References

1. Xue L, Gyles SL, Barrow A, Pettipher R (2007) Inhibition of PI3 K and calcineurin suppresses chemoattractant receptor-homologous molecule expressed on Th2 cells (CRTH2)-dependent responses of Th2 lymphocytes to prostaglandin D(2). Biochem Pharmacol 73:843–853. doi:10.1016/j.bcp.2006.11.021
2. Gold BG, Armistead DM, Wang MS (2005) Non-FK506-binding protein-12 neuroimmunophilin ligands increase neurite elongation and accelerate nerve regeneration. J Neurosci Res 80:56–65. doi:10.1002/jnr.20447
3. Delalande S, de Seze J, Fauchais AL, Hachulla E, Stojkovic T, Ferriby D, Dubucquoi S, Pruvo JP, Vermersch P, Hatron PY (2004) Neurologic manifestations in primary Sjögren syndrome: a study of 82 patients. Medicine (Baltimore) 83:280–291. doi:10.1097/01.md.0000141099.53742.16
4. Wingerchuk DM, Lennon VA, Lucchinetti CF, Pittock SJ, Weinshenker BG (2007) The spectrum of neuromyelitis optica. Lancet Neurol 6:805–815. doi:10.1016/S1474-4422(07)70216-8
5. Mandler RN, Ahmed W, Dencoff JE (1998) Devic's neuromyelitis optica: a prospective study of seven patients treated with prednisone and azathioprine. Neurology 51:1219–1220
6. Jacob A, Matiello M, Wingerchuk DM, Lucchinetti CF, Pittock SJ, Weinshenker BG (2007) Neuromyelitis optica: changing concepts. J Neuroimmunol 187:126–138. doi:10.1016/j.jneuroim.2007.04.009
7. Pittock SJ, Lennon VA, Seze JD, Vermersch P, Homburger HA, Wingerchuk DM, Lucchinetti CF, Zéphir H, Moder K, Weinshenker BG (2008) Neuromyelitis optica and non organ-specific autoimmunity. Arch Neurol 65:78–83. doi:10.1001/archneurol.2007.17
8. Vitali C, Bombardieri S, Jonsson R, Moutsopoulos HM, Alexander EL, Carsons SE, Daniels TE, Fox PC, Fox RI, Kassan SS, Pillemer SR, Talal N, Weisman MH (2002) Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 61:554–558. doi:10.1136/ard.61.6.554
9. Misu T, Fujihara K, Nakamura M, Murakami K, Endo M, Konno H, Itoyama Y (2006) Loss of aquaporin-4 in active perivascular lesions in neuromyelitis optica: a case report. Tohoku J Exp Med 209:269–275. doi:10.1620/tjem.209.269
10. Roemer SF, Parisi JE, Lennon VA, Benarroch EE, Lassmann H, Bruck W, Mandler RN, Weinshenker BG, Pittock SJ, Wingerchuk DM, Lucchinetti CF (2007) Pattern-specific loss of aquaporin-4 immunoreactivity distinguishes neuromyelitis optica from multiple sclerosis. Brain 130:1194–1205. doi:10.1093/brain/awl371

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In vivo expression of the HBZ gene of HTLV-I correlates with proviral load, inflammatory markers and disease severity in HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP)

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Abstract

Background: Recently, human T-cell leukemia virus type I (HTLV-I) basic leucine zipper factor (HBZ), encoded from a minus strand mRNA was discovered and was suggested to play an important role in adult T cell leukemia (ATL) development. However, there have been no reports on the role of HBZ in patients with HTLV-I associated inflammatory diseases.

Results: We quantified the HBZ and tax mRNA expression levels in peripheral blood from 56 HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients, 10 ATL patients, 38 healthy asymptomatic carriers (HCs) and 20 normal uninfected controls, as well as human leukemic T-cell lines and HTLV-I-infected T-cell lines, and the data were correlated with clinical parameters. The spliced HBZ gene was transcribed in all HTLV-I-infected individuals examined, whereas tax mRNA was not transcribed in significant numbers of subjects in the same groups. Although the amount of HBZ mRNA expression was highest in ATL, medium in HAM/TSP, and lowest in HCs, with statistical significance, neither tax nor the HBZ mRNA expression per HTLV-I-infected cell differed significantly between each clinical group. The HTLV-I HBZ, but not tax mRNA load, positively correlated with disease severity and with neopterin concentration in the cerebrospinal fluid of HAM/TSP patients. Furthermore, HBZ mRNA expression per HTLV-I-infected cell was decreased after successful immunomodulatory treatment for HAM/TSP.

Conclusion: These findings suggest that *in vivo* expression of HBZ plays a role in HAM/TSP pathogenesis.

Background

Human T-cell lymphotropic virus type 1 (HTLV-1) is a replication-competent human retrovirus [1,2] which is associated with adult T-cell leukemia (ATL) [3,4] and with a slowly progressive neurological disorder HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [5,6]. In HTLV-1 infection, approximately 5% develop ATL [7] and another 2%-3% develop chronic inflammatory diseases involving the central nervous system (HAM/TSP), the eyes [8], the lungs [9], the joints [10], or the skeletal muscles [11]; most infected individuals, however, remain healthy in their lifetime (healthy asymptomatic carriers: HCs). Although the factors that cause these different manifestations of HTLV-1 infection are not fully understood, previous population association studies suggested that both viral and host genetic factors influence the outcome of infection [12].

Among several HTLV-1 genes, a transcriptional activator Tax encoded in the pX region is thought to play a central role in immortalization, oncogenesis and inflammation through its pleiotropic activity [13]. In HAM/TSP patients, it has been reported that several cytokines, chemokines and matrix metalloproteinases transactivated by Tax protein such as tumor necrosis factor-α (TNF-α) [14], monocyte chemoattractant protein-1 (MCP-1) [15] and matrix metalloproteinase (MMP)-9 [16] are overexpressed in the infiltrating mononuclear cells in the patients' spinal cords. In addition, a previous report from the United States suggested that the level of HTLV-1 tax mRNA expression in HTLV-1-infected cells (mRNA/DNA ratio) was significantly higher in HAM/TSP patients than HCs, and this finding correlated with the HTLV-1 proviral load, Tax-specific CD8+ T cell frequency and disease severity of the patients [17]. A report from Japan also indicated that HTLV-1 tax mRNA expression was higher in HAM/TSP than HCs, although the mRNA/DNA ratio was similar between both groups [18]. These results suggest an important role of Tax in the induction of HAM/TSP.

It has been reported that among fresh leukemic cells isolated from ATL patients, about 60% of cases do not express the tax transcript [19]. In tax transgenic mouse models, the mice develop a wide range of tumors such as neurofibrosarcomas, mesenchymal tumors, and mammary adenomas, or even skeletal abnormalities including osteolytic bone metastases [20-27]; however, no leukemias or lymphomas were identified except in three models, which used respectively the granzyme B promoter [28], Lck proximal promoter [29] and Lck distal promoter [30]. These findings suggest that Tax is required for malignant transformation but not essential for the maintenance of leukemic cells *in vivo*. Recently, a novel basic leucine zipper protein encoded by the complementary strand of the HTLV-1 genome, named HTLV-1 basic leucine zipper factor (HBZ), was characterized [31]. HBZ is expressed in all ATL cells [32], promotes proliferation of T-lymphocytes in its RNA form [32], suppresses Tax-mediated transactivation through the 5' LTR [31,33], promotes CD4+ T-lymphocyte proliferation in transgenic mice [32], and enhances infectivity and persistence in HTLV-1-inoculated rabbits [34].

In this study, we investigated whether HTLV-1 HBZ mRNA expression is associated with clinical and laboratory markers reported in HAM/TSP patients, including HTLV-1 proviral load, neopterin concentration in cerebrospinal fluid (CSF), and motor disability score. In addition, to confirm the previous observations [17,18], we have also investigated the tax mRNA expression in ATL patients, HAM/TSP patients, and HCs by using the same technology but in a larger number of subjects.

Methods

Patients and cells

Human leukemic T-cell lines (Jurkat, MOLT-4, and CEM) and HTLV-1-infected T-cell lines (C5/MJ, SLB1, HUT102, MT-1, MT-2, and MT-4) were cultured in RPMI 1640 medium supplemented with 10% FCS. The diagnosis of HAM/TSP was done in accordance with World Health Organization criteria [35]. The diagnosis of ATL was made on the basis of clinical features, hematological characteristics, serum antibodies against HTLV-1 antigens, and detection of the HTLV-1 viral genome inserted into leukemia cells by Southern blot hybridization. All the PBMC samples used in this study were collected prior to treatment by a Histopaque-1077 (Sigma) density gradient centrifugation, washed and stored in liquid nitrogen until use. This research was approved by the institutional review boards of the authors' institutions, and informed consent was obtained from all individuals.

Quantification of HTLV-I proviral load, tax and HBZ mRNA expression, anti-HTLV-I antibody titers and neopterin concentration in cerebrospinal fluid

RNA was extracted from PBMCs using RNeasy Mini Kit with on-column DNase digestion (QIAGEN, Tokyo, Japan) according to the manufacturer's instructions. Complementary DNA (cDNA) was synthesized using TaqMan Gold RT-PCR Kit (Applied Biosystems, Tokyo, Japan). For cDNA synthesis from extracted mRNA, 2 μg total RNA, 10 μl 10×TaqMan RT buffer, 22 μl $MgCl_2$ (25 mM), 20 μl dNTPs mixture (at a final concentration of 500 μM each), 5 μl random hexamers (50 μM), 2 μl RNase inhibitor (20 U/μl), and 2.5 μl (50 U/μl) Moloney murine leukemia virus reverse transcriptase were added to a total volume of 100 μl. Samples were incubated at 25°C for 10 minutes and 48°C for 30 minutes, and reactions were stopped by heating to 95°C for 5 minutes. Genomic DNA was extracted from the frozen PBMCs by QIAamp blood kit

(QIAGEN, Tokyo, Japan). We, then, carried out a real time quantitative PCR using ABI Prism 7900 HT Fast Real-Time PCR System (Applied Biosystems) to examine the HTLV-1 proviral load [36] and tax mRNA expression [17] in PBMCs or HTLV-1 infected cell lines as reported previously. The amount of the HTLV-1 proviral load was calculated using β-actin as an internal control through the following formula: copy number of HTLV-1 tax per cell = [(copy number of tax)/(copy number of β-actin/2)]. The sequences of primers for HTLV-1 provirus were as follows: 5'-CAA ACC GTC AAG CAC AGC TT-3' and 5'-TCT CCA AAC ACG TAG ACT GGG T-3', and the probe was 5'-TTC CCA GGG TTT GGA CAG AGT CTT CT-3'. HBZ mRNA expression levels were also quantified by real time quantitative PCR using the same method for tax mRNA [17]. Namely, serially diluted cDNA from HTLV-1 infected MT-2 cells was used for generating standard curves for the value of HTLV-1 tax or HBZ mRNA and hypoxanthine ribosyl transferase (HPRT) mRNA, and the relative HTLV-1 tax or HBZ mRNA load was calculated by the following formula: HTLV-1 tax mRNA load = value of tax/value of HPRT. HTLV-1 HBZ mRNA load = value of HBZ/value of HPRT. We used aliquots of the same standard MT-2 cDNA preparation for all assays and the correlation values of standard curves were always more than 99%. The sequences of primers for tax mRNA detection were as follows: 5'-ATC CCG TGG AGA CTC CTC AA-3' and 5'-ATC CCG TGG AGA CTC CTC AA-3', and the probe was 5'-TCC AAC ACC ATG GCC CAC TTC CC-3'. The sequences of primers for HBZ mRNA detection were as follows: 5'-AGA ACG CGA CTC AAC CGG-3' and 5'-TGA CAC AGG CAA GCA TCG A-3', and the probe was 5'-TGG ATG GCG GCC TCA GGG CT-3'. As the probes for tax and HBZ mRNA surrounded the splice junction site of each mRNA, we detected HBZ splicing isoform, which is the most abundant HBZ transcript and contributed significantly to HBZ protein synthesis [37-39], but not unspliced form in this study. We used the HPRT primers and probe set (Applied Biosystems) for internal calibration. The tax and HBZ probes were labeled with fluorescent 6-carboxyfluorescein (FAM) (reporter) at the 5' end and fluorescent 6-carboxy tetramethyl rhodamine (TAMRA) (quencher) at the 3' end. All assays were performed in triplicate. The sensitivity of our real-time RT-PCR assay was determined using MT-2 cells diluted serially with PBMCs from a healthy uninfected donor. The HTLV-1 mRNA signal (both tax and HBZ) could be detected in a dose-dependent manner with a sensitivity limit as low as one MT-2 cell in 10^6 PBMCs. Neopterin levels were evaluated by HPLC with fluorometric detection methods as described previously [40]. Serum HTLV-1 antibody titers were determined by a particle agglutination method (Serodia-HTLV-1®, Fujirebio, Japan).

Clinical evaluation

Motor dysfunction seen in HAM/TSP patients was evaluated by clinical neurologists according to the Osame Motor Disability Score (OMDS) [41], which grades motor dysfunction from zero (normal walking and running) to 13 (complete bedridden) as follows: 1 = normal gait but runs slow; 2 = abnormal gait; 3 = abnormal gait and unable to run; 4 = need support while using stairs; 5 = need one hand support in walking; 6 = need two hands support in walking; 7 = need two hands support in walking but is limited to 10 m; 8 = need two hands support in walking but is limited to 5 m; 9 = unable to walk but able to crawl on hands and knees; 10 = crawls with hands; 11 = unable to crawl but can turn sideways in bed; 12 = unable to turn sideways but can move the toes. We have used OMDS throughout our previous studies [41-43] because this is a neurological measure of disability weighted toward ambulation and was specifically developed to evaluate motor dysfunction seen in HAM/TSP patients. It is therefore more suitable for evaluating HAM/TSP motor symptoms than the widely used EDSS [44]. The laboratory data were examined by an investigator who was not involved in the patients' clinical care, and the neurologists who made the clinical evaluation did not have access to the laboratory data.

Statistical analysis

The Mann-Whitney U test was used to compare data between two groups. Correlations between variables were examined by Spearman rank correlation analysis. Values of $p < 0.05$ were considered statistically significant.

Results

HTLV-I tax and HBZ mRNA load in HAM/TSP, ATL and HCs

A total of 56 HAM/TSP patients, 10 ATL patients and 38 HCs completed the evaluation. Twenty normal uninfected healthy controls (NCs) were used as negative controls. The HTLV-1 proviral load in this study represents the copy number of HTLV-1 tax per cell (for HTLV-1 infected cell lines) or PBMC (for HAM/TSP, ATL and HCs) (Table 1). Therefore, the HTLV-1 proviral load represents the population of infected cells in PBMCs when one cell harbors one provirus. However, since recent data by Kamihira et al. indicated that 43 out of 321 ATL specimens (17.8%) showed two or more bands by Southern blot analysis after *EcoRI* digestion [45], we reviewed the Southern blot data of our 10 ATL patients. As a result, two distinct bands of over 9 kb were observed in *EcoRI* digestion in samples from two ATL patients, indicating at least the biclonal integration of HTLV-1 proviral DNA. The incidence of multibands in our cases (two out of ten: 20%) was comparable with the data by Kamihira et al. (17.8%). The

Table 1: HTLV-I mRNA load, proviral load and mRNA/DNA ratio in HTLV-I – infected individuals and T-cell lines.

Cell line	HBZ mRNA[a]	tax mRNA[b]	Proviral load[c]	HBZ mRNA/DNA[d]	tax mRNA/DNA[e]
C5/MJ	13.3	0.062	8.1	1.64	0.0076
HUT102	1.2	26.35	19.3	0.063	1.37
MT1	25.2	0.011	7.1	3.56	0.0015
MT2	7.8	1.24	16.2	0.48	0.077
MT4	2.4	1.71	12.6	0.19	0.135
SLB1	25.8	87.4	115.5	0.22	0.756
HAM/TSP*	0.74 (0.023–33.50)	0 (0–0.041)	0.051 (0.0008–0.41)	19.10 (0.81–273.45)	0 (0–0.32)
HCs*	0.15 (0.0013–6.42)	0 (0–0.000078)	0.0089 (0.0001–0.10)	16.67 (0.21–7358.91)	0 (0–0.11)
ATL*	31.43 (5.93–225.64)	0.000018 (0–0.59)	1.14 (0.25–2.88)	24.04 (13.77–135.83)	0 (0–0.29)

*The results represent the median and range (n = 56 for HAM/TSP, n = 38 for HCs and n = 10 for ATL)
[a]HTLV-I HBZ mRNA load = value of HBZ/value of HPRT
[b]HTLV-I tax mRNA load = value of tax/value of HPRT
[c]Proviral load: HTLV-I tax copy number per cell
[d]HBZ mRNA/DNA ratio = HTLV-I HBZ mRNA load/Proviral load
[e]tax mRNA/DNA ratio = HTLV-I tax mRNA load/Proviral load

number of HTLV-1 proviral load in MT-2 cells measured by our quantitative PCR method (16.2 copies/cell) was also comparable with the previous report (12.6 copies/cell) [46].

The HTLV-1 proviral load was significantly greater in HAM/TSP patients (median 0.051, range 0.0008–0.41) than HCs (median 0.0089, range 0.0001–0.10) (P = 0.000011, Mann Whitney U test, Table 1). The HTLV-1 HBZ mRNA level was highest in ATL, medium in HAM/TSP, and lowest in HCs with statistical significance (Table 1 and Figure 1A). It is noteworthy that we could detect HTLV-1 HBZ gene transcripts in all infected individuals tested. Interestingly, there were three cases with extremely high data of HBZ mRNA in HCs (Figure 1C). Since recent report by Shimizu et al. indicated that HTLV-1-specific T-cell responsiveness widely differed among HTLV-1 carriers [47], these extremely high data of HBZ mRNA might be explained by immunological diversity observed in HCs. In contrast, although the HTLV-1 tax mRNA levels in ATL patients was significantly higher than HCs (p = 0.014, Mann-Whitney U test), the HTLV-1 tax mRNA levels between HCs-HAM/TSP and HAM/TSP-ATL did not reach statistical difference (Figure 1B). We could not detect any HTLV-1 tax and HBZ mRNA expression in any of the 20 NCs and 3 uninfected human leukemic T-cell lines (Jurkat, MOLT-4, and CEM) tested (data not shown).

Comparison of HTLV-I tax and HBZ mRNA load with HTLV-I proviral load

To test whether higher HBZ mRNA levels reflect higher proviral load, we adjusted the tax or HBZ mRNA load (i.e. value of tax or HBZ/value of HPRT) by the HTLV-1 proviral load (i.e. HTLV-1 tax copy number per cell). As a result, neither tax nor the HBZ mRNA/DNA ratio differed significantly between each clinical group (i.e. HAM/TSP-HCs, HAM/TSP-ATL and HCs-ATL) (figure 1C, D). Interestingly, although both HTLV-1 proviral load and tax mRNA/DNA ratio were higher in HTLV-1-infected cell lines (C5/MJ, SLB1, HUT102, MT-1, MT-2, and MT-4) than PBMCs, HBZ mRNA/DNA ratio was even higher in PBMCs than HTLV-1-infected cell lines (Table 1). Consistent with the previous observations that HBZ suppresses Tax mediated transactivation through the 5' LTR [31,33,48], HBZ mRNA load tended to be higher in cell lines with lower tax mRNA load, and indeed HBZ mRNA/DNA ratio was inversely correlated with tax mRNA/DNA ratio in 6 HTLV-1-infected cell lines (Spearman's rank correlation coefficient r = -0.943, P = 0.035) (Table 1 and data not shown), although such correlation was not observed between HBZ and tax mRNA/DNA ratio in PBMCs from HAM/TSP patients, ATL patients, HCs and all groups combined (data not shown). As shown in Figure 2, the HTLV-1 HBZ mRNA load was significantly correlated with HTLV-1 proviral load in HAM/TSP patients (P = 0.0005, r = 0.470 by Spearman rank correlation analysis), HCs (P = 0.0013, r = 0.528) and all groups combined (P < 0.000001, r = 0.686), but not in ATL patients (P = 0.300, r = 0.345). The tax mRNA load was correlated with the HTLV-1 proviral load in HCs (P = 0.045, r = 0.444), ATL patients (P = 0.045, r = 0.673), and all groups combined (P < 0.01, r = 0.365), but not in HAM/TSP patients (P = 0.411, r = 0.210).

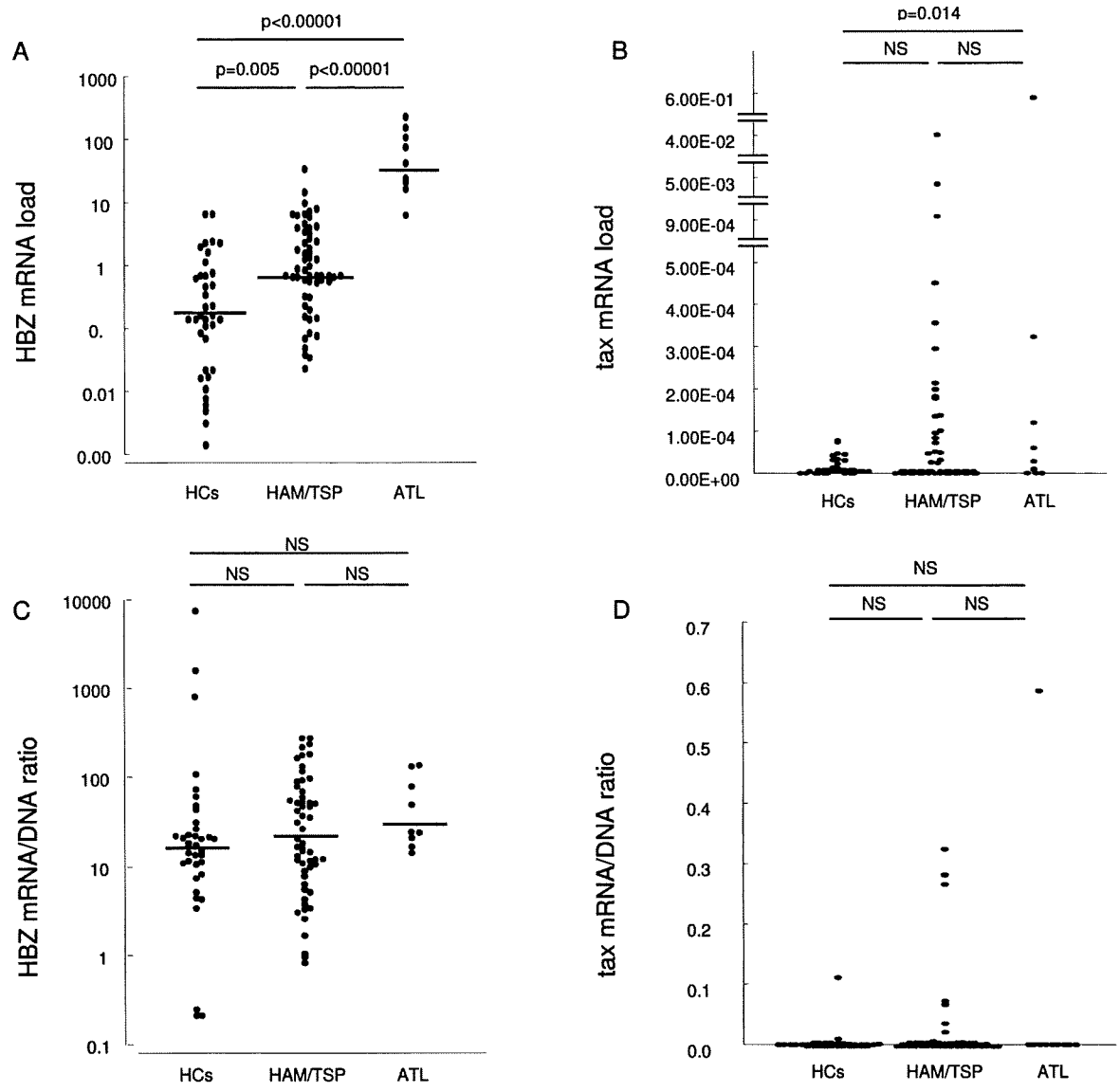

Figure 1

HTLV-I tax and HBZ mRNA load in patients with HAM/TSP, ATL and asymptomatic HTLV-I carriers. A. HTLV-I HBZ mRNA load was highest in ATL, medium in HAM/TSP, and lowest in HCs. B. The HTLV-I tax mRNA load between HCs and HAM/TSP, HAM/TSP and ATL did not reach statistical significance, although the HTLV-I tax mRNA load in ATL patients was significantly higher than HCs ($p = 0.014$, Mann Whitney U test). C and D. To normalize the HTLV-I tax or HBZ mRNA expression level per provirus, the mRNA/DNA ratio was calculated by dividing the HTLV-I tax or HBZ mRNA load by the HTLV-I proviral load. Neither the HBZ (C) nor the tax (D) mRNA/DNA ratio differed significantly between each clinical group (HAM/TSP – HCs, HAM/TSP – ATL, HCs – ATL). The zero value of tax gene transcripts was observed in 60.7% of HAM/TSP patients (34 out of 56), 71.1% of HCs (27 out of 38) and 30.0% of ATL patients (3 out of 10). The medians are represented by horizontal lines and the statistical differences between them were calculated with a Mann Whitney U test.

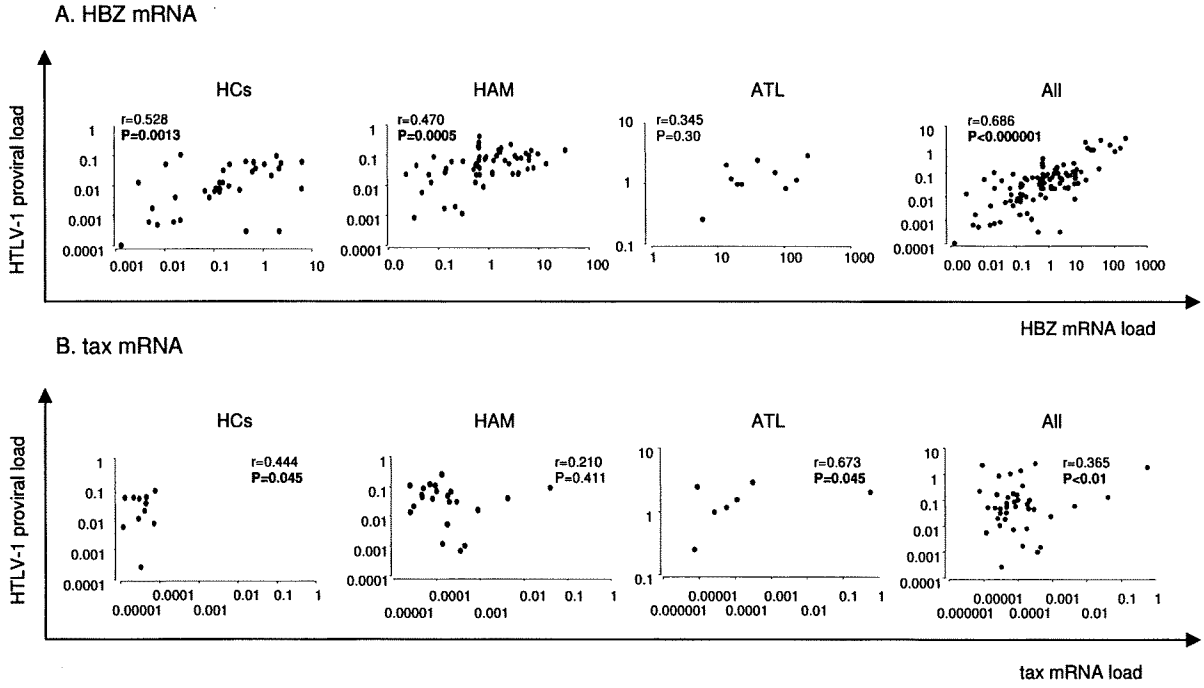

Figure 2
Correlation between HTLV-I proviral load and HTLV-I mRNA load in HTLV-I infected individuals. A. The HTLV-I HBZ mRNA load was significantly correlated with HTLV-I proviral load in HAM/TSP patients alone (P = 0.0005, r = 0.470 by Spearman rank correlation analysis), HCs alone (P = 0.0013, r = 0.528) and all groups combined (P < 0.000001, r = 0.686) but not in ATL patients (P = 0.300, r = 0.345). B. The tax mRNA load correlated with the HTLV-I proviral load in HCs (P = 0.045, r = 0.444), ATL patients (P = 0.045, r = 0.673) and both group combined (P < 0.01, r = 0.365) but not in HAM/TSP patients (P = 0.411, r = 0.210). The zero value of tax gene transcripts did not appear in the figures. Correlations were examined by Spearman rank correlation analysis.

Comparison of HBZ mRNA load with tax mRNA load among HTLV-I infected individuals in different clinical status

To investigate the mutual expression status of HBZ and tax mRNA in different clinical status, we calculated the ratio of HBZ mRNA/tax mRNA in 22 HAM/TSP patients, 11 HCs and 7 ATL patients, who express both tax and HBZ mRNA in PBMCs. HTLV-1 tax mRNA was not expressed in 60.7% (34 out of 56) of HAM/TSP patients, 71.1% (27 out of 38) of HCs and 30.0% (3 out of 10) of ATL patients, whereas HTLV-1 HBZ mRNA was expressed in all the infected individuals tested. As shown in figure 3, HBZ mRNA/tax mRNA ratio in PBMCs was significantly increased in ATL patients than HAM/TSP patients and HCs (P = 0.013 and 0.0051, Mann-Whitney U test, respectively), indicating very high HBZ transcript levels relative to tax, especially in ATL patients.

Correlation of HTLV-I HBZ mRNA load with CSF neopterin concentration and disease severity in HAM/TSP patients

To investigate the relationship between HTLV-1 mRNA load and various laboratory markers, HTLV-1 proviral load, CSF neopterin concentration and anti-HTLV-1 antibody titers were quantified and compared with motor dysfunction of HAM/TSP patients. Since neopterin is a low molecular weight pteridine compound released from macrophages upon stimulation with γ-interferon secreted by activated T cells, the measurement of neopterin concentrations in body fluids like blood serum, CSF or urine provides information about cellular immune activation in humans under the control of type 1 T helper cells [49]. As shown in table 2, we showed that the CSF neopterin level, which was positively correlated with proviral load, was also positively correlated with the HBZ mRNA load in HAM/TSP patients (Spearman's rank correlation coefficient P = 0.0052, r = 0.437). However, such a correlation was not observed between neopterin and HTLV-1 tax mRNA load (P = 0.544, r = 0.228). Motor dysfunction evaluated by OMDS significantly correlated with HTLV-1 HBZ mRNA load (P = 0.023, r = 0.328), but again not with HTLV-1 tax mRNA load (P = 0.401, r = 0.241).