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HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP): the role of HTLV-I-infected Th1 cells in the pathogenesis, and therapeutic strategy

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Abstract

Human T lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a chronic progressive myelopathy characterized by bilateral pyramidal tract involvement with sphincteric disturbances. The primary neuropathological feature of HAM/TSP is chronic myelitis characterized by perivascular cuffing and parenchymal infiltration of lymphocytes. Although the exact cellular and molecular events underlying the induction of chronic inflammation in the spinal cord by HTLV-I are still unclear, a long-standing bystander mechanism, such as the destruction of surrounding nervous tissue by the interaction between HTLV-I-infected $CD4^+$ T cells and HTLV-I-specific cytotoxic T cells in the spinal cord, is probably critical in the immunopathogenesis of HAM/TSP. In this review, the role of HTLV-I-infected $CD4^+$ T cells as activated Th1 cells in the peripheral blood will be discussed as the first responders of this mechanism in the immunopathogenesis of HAM/TSP.

Since the discovery of HAM/TSP, various therapeutic approaches, such as immunomodulatory or anti-viral drugs, have been used for HAM/TSP patients. However, an effective therapeutic strategy against HAM/TSP is still unavailable. As HTLV-I-infected $CD4^+$ T cells are the first responders in the immunopathogenesis of HAM/TSP, the ideal treatment is the elimination of HTLV-I-infected cells from the peripheral blood. In this review, the focus will be on therapeutic strategies aimed at targeting HTLV-I-infected $CD4^+$ T cells in HAM/TSP patients.

Key words: *HAM/TSP, HTLV-I, Th1, treatment.*

Introduction

Human T lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a chronic progressive myelopathy caused by HTLV-I, a member of the exogenous human retroviruses [19,58]. In 1985, Gessain and colleagues first reported the high prevalence of anti-HTLV-I antibodies in the sera of patients with TSP in Martinique (French West Indies) [18]. Subsequently, Rodgers-Johnson and Gajdusek reported similar findings in

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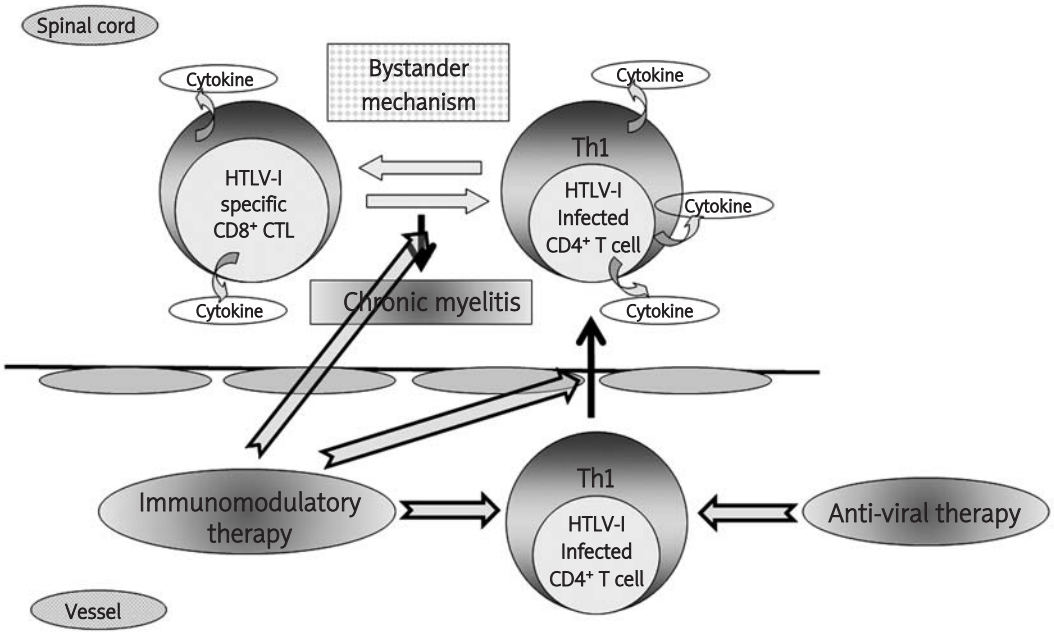


Fig. 1. Immunopathogenesis of and therapeutic strategies in HAM/TSP. The bystander mechanism, such as the destruction of surrounding tissues by inflammatory cytokine expression, during the interaction between HTLV-I-infected $CD4^+$ T cells and HTLV-I-specific $CD8^+$ CTL, is probably critical as the cause of chronic myelitis. The increase of HTLV-I-infected $CD4^+$ T cells, in the peripheral blood of HAM/TSP patients, having the characteristics of Th1-activated status with the transmigrating activity into the tissues enough to trigger this mechanism, plays an important role in the first step of the immunopathogenesis of HAM/TSP. The therapeutic strategies in HAM/TSP are divided into two directions as shown by ⟹; 1) immunomodulatory therapy, such as a) suppression of immune activation, particularly for activated HTLV-I-infected cells, b) inhibition of the transmigration of activated HTLV-I-infected cells to the spinal cord, c) reduction of chronic inflammation in the spinal cord; and 2) anti-viral therapy, such as a) suppression of HTLV-I expression and/or replication, b) inhibition of the proliferation of HTLV-I-infected cells, c) elimination of HTLV-I-infected cells. Quotation from Ref. [51].

patients with TSP in Jamaica and Colombia [62]. Thereafter, similar findings were documented from other countries of the tropical zone, and the contribution of HTLV-I infection to the development of TSP was confirmed [19,23]. Although these areas, which are highly endemic for HTLV-I, are located in the tropical zone, such as the Caribbean, equatorial Africa, the Seychelles, and Central and South America, Japan, which is located in the temperate zone, particularly southern Japan, is also an area which is highly endemic for HTLV-I. In 1986, Osame and co-workers reported the association between HTLV-I infection and spastic paraparesis and proposed that spastic paraparesis associated with elevated antibodies to HTLV-I should be named "HTLV-I-associated myelopathy (HAM)" and considered a new clinical entity [59]. Based on the accumulated evidence, a WHO scientific group on HTLV-I infections and its associated diseases, in a meeting held in Kagoshima, Japan on 1988, concluded that HTLV-I-seropositive TSP and HAM were identical diseases, and proposed the name "HAM/TSP" [61].

The principal clinical manifestation of HAM/TSP is spastic paraplegia or paraparesis, characterized by a slowly progressive course of prominent upper motor neuron involvement and mild sensory deficit with

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.

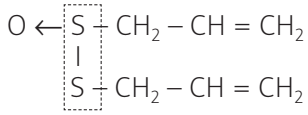
Although prosultiamine (Alinamin®), a product of Takeda Pharma Co. Inc. (Osaka, Japan), is very frequently prescribed for vitamin B1 deficiency in Japan, this compound is a homologue of allithiamine, originally synthesized by thiol type vitamin B1 and allicin (diallyl thiosulfinate) derived from garlic (*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

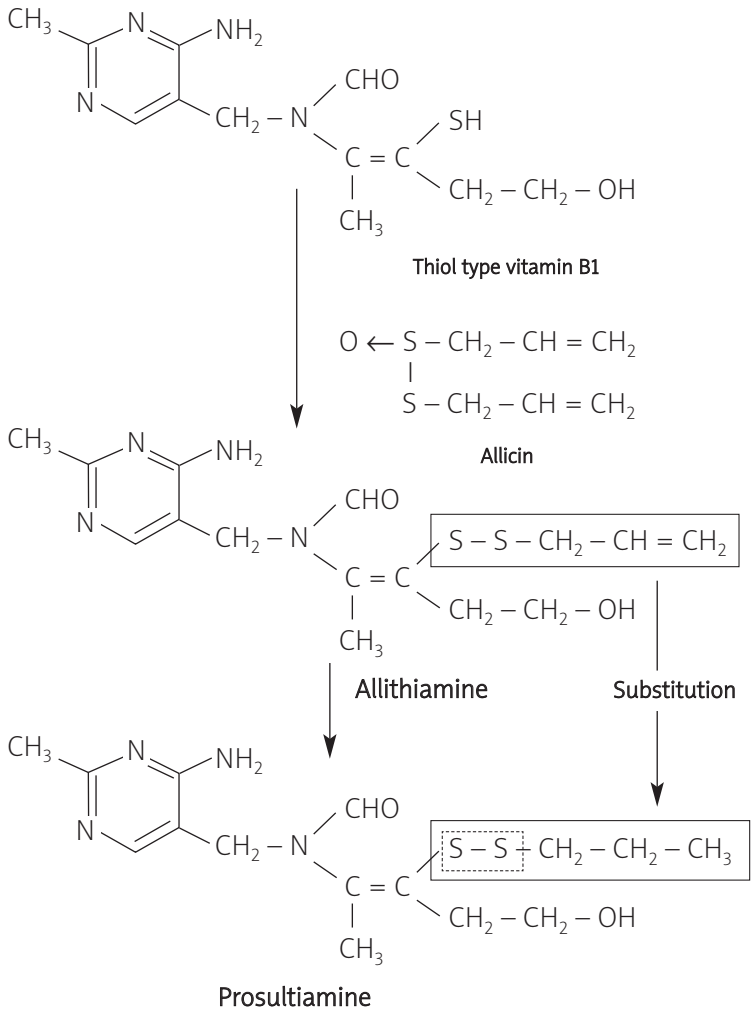


Fig. 2. Structure of allicin (A) and the generation of prosultiamine (B). Allithiamine was originally synthesized by thiol type vitamin B1 and allicin. Prosultiamine was developed after allyl disulfide derived from allicin was substituted to propyl disulfide in the structure of allithiamine as shown 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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