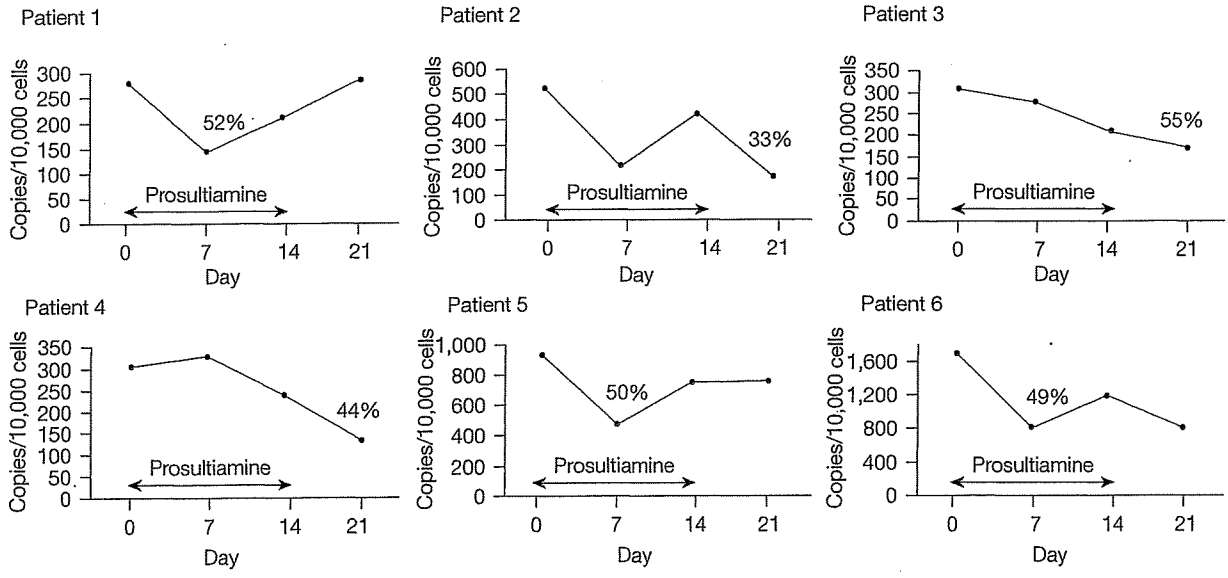
the HTLV-I proviral copy number was not statistically significant (P=0.056) at day 14 when this point was designated as the primary endpoint of this trial. However, when we analysed each result individually, as shown in Figure 5, the HTLV-I proviral copy number gradually decreased to 33%, 55%, 44% and 49% of the number at pretreatment in patients 2, 3, 4 and 6, respectively, until 7 days after treatment. Although the HTLV-I proviral copy number at day 21 was almost the same as at pretreatment in patients 1 and 5, the copy number of HTLV-I provirus transiently decreased to 52% and 50%, respectively, from the number at pretreatment at day 7. Overall, this preliminary therapeutic trial with prosultiamine showed the possibility that the clinical improvement was induced by the decrease of HTLV-I-infected cells in the peripheral blood of HAM/TSP patients.

Discussion

HAM/TSP should be treated as an infectious disease. Therefore, in the therapeutic strategy for treating HAM/TSP, we should focus on the suppression of HTLV-I expression and/or replication, the inhibition of the proliferation of HTLV-I-infected cells or the elimination of HTLV-I-infected cells. To our knowledge, this is the first report to demonstrate that the disulfide moiety in allicin or prosultiamine can induce apoptosis in HTLV-I-infected cells derived from HAM/TSP patients and that prosultiamine has the potential to be a new therapeutic tool targeting HTLV-I-infected cells in HAM/TSP. We showed that allicin can induce caspase-dependent apoptosis in HTLV-I-infected cells. However, it is not available for clinical use because of its instability. Therefore, we focused on prosultiamine as a treatment for HAM/TSP patients. In our analyses of the cytotoxic effects of prosultiamine on HTLV-I-infected T-cell lines derived from HAM/TSP patients, this compound induced effects on these cell lines that were similar to those of allicin as expected. Although there were differences in cytotoxic effects by both allicin and prosultiamine among the three HTLV-I-infected cell lines, they might have been caused by the anti-apoptotic activity that each cell line originally has. The analyses involving HCT-1 cells showed that prosultiamine could induce caspase-dependent apoptosis through the mitochondrial pathway. Our results from these analyses with regards to prosultiamine are consistent with the recent report that allicin, in HTLV-I non-infected cell lines, can induce apoptosis through the mitochondrial pathway [22]. Although the upstream of caspase-dependent apoptosis through the mitochondrial pathway induced by disulfide moiety is still obscure, the disruption of the intracellular redox system by the interaction between the Trx/Trx reductase system and the disulfide moiety, leading to the activation of apoptosis signal-regulating kinase 1 (ASK1), might be involved as one of the mechanisms because

Figure 5. Decrease of HTLV-I proviral copy numbers in the PBMCs of HAM/TSP patients as a result of a clinical trial with prosultiamine



We treated six human T-lymphotropic virus type-I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) patients with intravenous prosultiamine at a dose of 40 mg daily for 14 days. Total cellular DNA prepared from peripheral blood mononuclear cells (PBMCs) was subjected to measurement of HTLV-I proviral copies by quantitative PCR analyses. We monitored the copy numbers of HTLV-I provirus in the peripheral blood at pretreatment (day 0), during treatment (days 7 and 14) and 7 days after treatment (day 21), and they were observed to decrease to 33–55% of the numbers at pretreatment at their nadir in all HAM/TSP patients.

the reduced form of Trx is a physiological negative regulator of ASK1 activation [23–25]. Activation of ASK1 signalling, leading to stress-induced apoptosis, through the activation of p38 mitogen-activated protein kinase or c-Jun N-terminal kinase by the oxidation of Trx through a disulfide moiety was supposed. Further investigations of this mechanism are underway.

On the basis of data on the differential sensitivities of the cytotoxicity of prosultiamine between HTLV-I-infected and non-infected cell lines, we treated peripheral blood $CD4^+$ T-cells of HAM/TSP patients with prosultiamine *in vitro*. These treatments significantly induced a decrease of HTLV-I proviral copy numbers, selectively showing apoptosis of HTLV-I-infected T-cells. This evidence prompted us to administer the treatment with prosultiamine to HAM/TSP patients because this agent is very frequently prescribed as a safe treatment regimen for thiamine deficiency in Japan. We treated six HAM/TSP patients with prosultiamine. As a result, we confirmed the efficacy of prosultiamine treatment in bringing about a decrease of HTLV-I proviral copy numbers in PBMCs in this subset of HAM/TSP patients. A finding worthy of mention is that prosultiamine treatment, even when administered for a short-term, such as 14 days, induced approximately 50–70% decrease of the copy numbers of HTLV-I provirus in the PBMCs of HAM/TSP patients. However, a rebound or fluctuation of HTLV-I proviral copy numbers was observed during the treatment course in four of six patients. Although the exact reason of this phenomenon is unknown, these findings might suggest the limitations of prosultiamine treatment by the present protocol. Although the degree of clinical improvement was not as great in HAM/TSP patients who had a long-term duration of illness, it is a noteworthy result that prosultiamine treatment induced a marked improvement of motor function in a patient who had a short-term duration of illness of 2 years (patient 6).

In conclusion, we showed that compounds containing a disulfide moiety in their structures, such as allicin and prosultiamine, can induce apoptosis in HTLV-I-infected cells in HAM/TSP patients. Our results suggest that prosultiamine has the potential to be a new therapeutic tool targeting HTLV-I-infected cells by inducing apoptosis in HAM/TSP. Further investigation of long-term treatment for HAM/TSP patients with prosultiamine is needed.

Acknowledgements

This work was supported in part by a Grant-in-Aid for Research on Brain Science and the Health and Labour Sciences Research Grant on Intractable Diseases (Neuroimmunological Diseases) from the Ministry of Health, Labour and Welfare of Japan. Special thanks to Masakatsu Motomura, Akira Tsujino, Katsuya Satoh, Taku Fukuda, Youhei Tateishi, Masahiro Tokuda (First Department of Internal Medicine, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki, Japan) and Toshiro Yoshimura (Health Sciences, Nagasaki University, Nagasaki, Japan) for their cooperation in the clinical study.

Disclosure statement

The authors declare no competing interests.

References

1. Osame M, Matsumoto M, Usuku K, *et al.* 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.
2. Iwasaki Y. Pathology of chronic myelopathy associated with HTLV-I infection (HAM/TSP). *J Neurol Sci* 1990; 96:103–123.
3. Nakamura T. Immunopathogenesis of HTLV-I-associated myelopathy/tropical spastic paaparesis. *Ann Med* 2000; 32:600–607.
4. Osame M. Pathological mechanisms of human T-cell lymphotropic virus type I-associated myelopathy (HAM/TSP). *J Neurovirol* 2002; 8:359–364.
5. Nakagawa M, Nakahara K, Maruyama Y, *et al.* Therapeutic trials in 200 patients with HTLV-associated myelopathy/tropical spastic paraparesis. *J Neurovirol* 1996; 2:345–355.
6. 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.
7. Izumo S, Goto I, Itoyama Y, *et al.* Interferon-alpha is effective in HTLV-I-associated myelopathy: a multicentercentre, randomized, double-blind, controlled trial. *Neurology* 1996; 46:1016–1021.
8. Nakamura T, Nishiura Y, Fukushima N, Furuya T. The role of HTLV-I-infected $CD4^+$ T cells as activated Th1 cells in the immunopathogenesis of HTLV-I-associated myelopathy/tropical spastic paraparesis. In Broglio PV (Editor). *Neuroimmunology Research Focus*. New York: Nova Science Publishers 2007; pp. 43–84.
9. Ankri S, Mirelman D. Antimicrobial properties of allicin from garlic. *Microbes Infect* 1999; 1:125–129.
10. Hirsch K, Danilenko M, Giat J, *et al.* Effect of purified allicin, the major ingredient of freshly crushed garlic, on cancer cell proliferation. *Nutr Cancer* 2000; 38:245–254.
11. Wong WW, Macdonald S, Langler RF, Penn LZ. Novel synthetic organosulfur compounds induce apoptosis of human leukemic cells. *Anticancer Res* 2000; 20:1367–1374.
12. Lawson LD, Wang ZJ. Pre-hepatic fate of the organosulfur compounds derived from garlic (*Allium sativum*). *Planta Med* 1993; 59:A688–A689.
13. Freeman F, Kodera Y. Garlic chemistry – stability of *S*-(2-propenyl)-2-propene-1-sulfinothioate (allicin) in blood, solvents, and simulated physiological fluids. *J Agric Food Chem* 1995; 43:2332–2338.
14. Fujiwara M, Watanabe H, Matsui K. 'Allithiamine' a newly found derivative of vitamine B1. I. discovery of allithiamine. *J Biochem* 1954; 41:29–39.
15. Matsukawa T, Yurugi S. On the structure of allithiamine. *Proceedings of the Japan Academy* 1952; 28:146–149.
16. Fukushima N, Nakamura T, Nishiura Y, Ida H, Aramaki T, Eguchi K. HTLV-I production based on activation of integrin/ligand signaling in HTLV-I-infected T cell lines derived from HAM/TSP patients. *Intervirology* 2008; 51:123–129.

17. Fukushima N, Nishiura Y, Nakamura T, Yamada Y, Kohno S, Eguchi K. Involvement of p38 MAPK signaling pathway in IFN-γ and HTLV-I expression in patients with HTLV-I-associated myelopathy/tropical spastic paraparesis. *J Neuroimmunol* 2005; 159:196–202.
18. Shibatomi K, Ida H, Yamasaki S, *et al.* A novel role for interleukin-18 in human natural killer cell death: high serum levels and low natural killer cell numbers in patients with systemic autoimmune diseases. *Arthritis Rheum* 2001; 44:884–892.
19. Nakamura H, Kawakami A, Ida H, Koji T, Eguchi K. EGF activates PI3K-Akt and NF-κB via distinct pathways in salivary epithelial cells in Sjögren's syndrome. *Rheumatol Int* 2007; 28:127–136.
20. Osame M. Review of WHO Kagoshima meeting and diagnostic guidelines for HAM/TSP. In Blattner WA (Editor). *Human Retrovirology: HTLV.* New York: Raven Press 1990; pp. 191–197.
21. Matsuo H, Tsujihata M, Satoh, A, *et al.* Plasmapheresis in treatment of human T-lymphotropic virus type-I associated myelopathy. *Lancet* 1988; 332:1109–1113.
22. Miron T, Wilchek M, Sharp A, *et al.* Allicin inhibits cell growth and induces apoptosis through the mitochondrial pathway in HL60 and U937. *J Nutr Biochem* 2008; 19:524–535.
23. Saitoh M, Nishitoh H, Fujii M, *et al.* Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J* 1998; 17:2596–2606.
24. Hayakawa T, Matsuzawa A, Noguchi T, Takeda K, Ichijo H. The ASK1-MAP kinase pathways in immune and stress responses. *Microbes Infect* 2006; 8:1098–1107.
25. Masutani H, Ueda S, Yodoi J. The thioredoxin system in retroviral infection and apoptosis. *Cell Death Differ* 2005; 12:991–998.

Accepted for publication 30 January 2009

Disclosure statement: The authors have declared no conflicts of interest.

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1 Wirrwar A, Schramm N, Vosberg H, Muller-Gartner HW. High resolution SPECT in small animal research. Rev Neurosci 2001;12:187–93.
2 Ostendorf B, Scherer A, Wirrwar A *et al.* High-resolution multipinhole single-photon-emission computed tomography in experimental and human arthritis. Arthritis Rheum 2006;54:1096–104.
3 Ostergaard M, Peterfy C, Conaghan P *et al.* OMERACT Rheumatoid Arthritis Magnetic Resonance Imaging Studies. Core set of MRI acquisitions, joint pathology definitions, and the OMERACT RA-MRI scoring system. J Rheumatol 2003;30:1385–6.
4 Keen HI, Wakefield RJ, Grainger AJ, Hensor EM, Emery P, Conaghan PG. Can ultrasonography improve on radiographic assessment in osteoarthritis of the hands? A comparison between radiographic and ultrasonographic detected pathology. Ann Rheum Dis 2008;67:1116–20.
5 Tan AL, Grainger AJ, Tanner SF *et al.* High-resolution magnetic resonance imaging for the assessment of hand osteoarthritis. Arthritis Rheum 2005;52:2355–65.
6 Bergman AG, Willén HK, Lindstrand AL, Pettersson HT. Osteoarthritis of the knee: correlation of subchondral MR signal abnormalities with histopathologic and radiographic features. Skeletal Radiol 1994;23:445–8.
7 McQueen FM. A vital clue to deciphering bone pathology: MRI bone oedema in rheumatoid arthritis and osteoarthritis. Ann Rheum Dis 2007;66:1549–52.

Rheumatology 2009;48:854–855
doi:10.1093/rheumatology/kep072
Advance Access publication 20 May 2009

Low prevalence of ectopic germinal centre formation in patients with HTLV-I-associated Sjögren's syndrome

SIR, We have proposed that HTLV-I infection can be a possible environmental factor for SS based on high prevalence of an anti-HTLV-I antibody in primary Sjögren's syndrome (pSS) in Nagasaki [1–3], and recently confirmed that labial salivary glands (LSGs) of the HTLV-I-seropositive SS patients are less damaged in radiography [4]. Amft *et al.* [5] revealed the prominent expression of B cell-attracting chemokine 1 (BCA-1/CXCL13) on endothelial cells and lymphocytic aggregates in the ectopic germinal centre (GC) of LSGs in SS, speculating that ectopic GC is associated with the autoantibodies production as well as the salivary destruction [5].

We focused on the presence of ectopic GC formation *in situ* as well as the expression of CXCL12/CXCL13. Sixty-four pSS patients were registered and classification of pSS was determined by the revised criteria proposed by the American-European Consensus group [6]. LSGs from a control subject who complained of sicca but did not meet the pSS criteria have been obtained. The presence of anti-HTLV-I antibody was determined by ELISA (Eitest-ATL kit; Eisai, Tokyo, Japan) or particle agglutination assay (Serodia-ATL Kit; Fujirebio, Tokyo, Japan). Informed consent for the usage of samples obtained by the biopsy was obtained from all the participating patients as of the commencement of the study, and the study was conducted with the approval of the Human Ethical Committee of our institution. Immunohistochemistry was performed by the labelled streptavidin–biotin method (Histofine Staining Kit; Nichirei, Tokyo, Japan) using mouse anti-CXCL12 monoclonal antibody or goat anti-CXCL13 polyclonal antibody (R&D Systems, Minneapolis, MN, USA) [2] with microwave epitope retrieval for the detection of CXCL13. Negative control sections was treated with normal mouse IgG or normal goat serum. Mann–Whitney U-test, the chi-square test or Fisher's exact probability test was used for the statistical analysis. A P-value < 0.05 was statistically significant.

The gender and age were similar in 32 HTLV-I-seronegative (male/female 3/29; age 56.9 ± 14.9 years) and 32 HTLV-I-seropositive (male/female 4/28; age 58.5 ± 12.6 years) pSS patients, as well as in 9 HTLV-I-associated myelopathy (HAM)–pSS patients (male/female 1/8; age 61.6 ± 8.9 years). Sicca symptoms were observed in 83.3–100% of patients among the groups. Differences in anti-SS-A/SS-B antibodies (Mesacup SS-A/Ro test and SS-B/La Test; Medical & Biological Laboratories, Nagoya, Japan) and IgG concentrations at the time of biopsy were not statistically significant irrespective of HTLV-I infection. Strikingly, ectopic GC was low in HTLV-I-seropositive pSS (1/32, 3.1%) as compared with HTLV-I-seronegative pSS (6/32, 18.8%) ($P = 0.045$), and 0% in HAM–pSS.

Expression of CXCL13 was observed in 0–10% of mononuclear cells (MNCs) of HTLV-I-seronegative pSS without ectopic GC patients or HTLV-I-seropositive pSS patients (Fig. 1). In HTLV-I-seronegative pSS ($n = 6$) with ectopic GC patients, the expression of CXCL13 was found dominantly in the light zone of ectopic GC. All of the cases showed >50% of MNCs

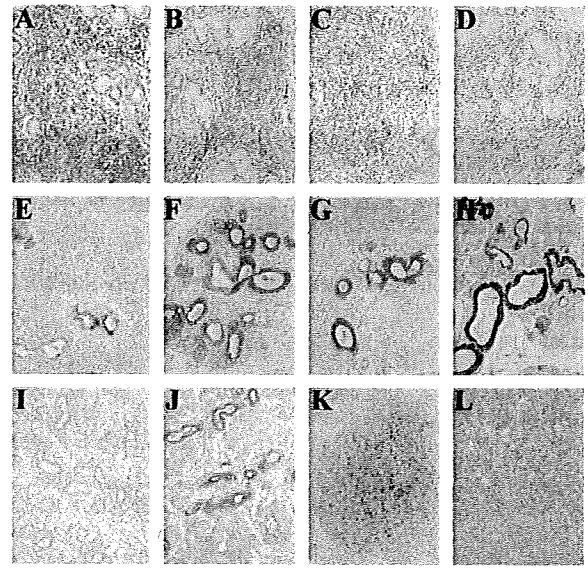

FIG. 1. Expression of CXCL13 and CXCL12 in HTLV-I-seronegative and HTLV-I-seropositive patients with SS. Immunohistochemical analysis of CXCL13 (BCA-1) in minor LSGs was demonstrated (**A–D**). After pre-treatment with microwave epitope retrieval, goat anti-CXCL13 polyclonal antibody was used to detect the expression of CXCL13. Expression of CXCL12 (SDF-1) was examined using mouse anti-CXCL12 monoclonal antibody (**E–H**). (**A** and **E**) Expression of CXCL13 and CXCL12 in minor LSG from an HTLV-I-seronegative SS patient with ectopic GC. (**B** and **F**) Expression of CXCL13 and CXCL12 in minor LSG from an HTLV-I-seronegative SS patient without GC. (**C** and **G**) Expression of CXCL13 and CXCL12 in minor LSG from an HTLV-I-seropositive SS patient without GC. These are representative of five patients with HTLV-I-seronegative SS with ectopic GC, HTLV-I-seronegative SS patients without ectopic GC and HTLV-I-seropositive SS patients without GC, respectively. (**D** and **H**) Expression of CXCL13 and CXCL12 in minor LSG from an HAM–SS patient. (**I** and **J**) Expression of CXCL13 and CXCL12 in minor LSG section from a normal subject, respectively. (**K** and **L**) Expression of CXCL13 in the light zone and CXCL12 in mantle zone of human tonsil tissue as a positive control, respectively. Haematoxylin and methyl green were used for counterstaining of CXCL13 and CXCL12, respectively (original magnification ×100).

in the light zone of ectopic GC expressing CXCL13. One pSS in HTLV-I carrier showed a relatively small size of ectopic GC whose CXCL13 expression pattern was similar. However, interestingly, the MNCs of HAM–pSS patients demonstrated no expression of CXCL13. In contrast to CXCL13, CXCL12 was commonly expressed on ductal epithelial cells of all the pSS patients irrespective of anti-HTLV-I antibody. In a normal subject, no expression of CXCL13 was observed with positive expression of CXCL12 similar with pSS.

The lymphoid aggregates of LSGs are responsible for autoantibody production that locally occurs in ectopic GC [5, 7, 8]. Radiographic destruction of the ductal structure in HTLV-I-seropositive pSS occurs to a lesser extent than in HTLV-I-seronegative pSS, which is a unique characteristic of the former [4].

The chemokines have been found to regulate ectopic GC formation of SS [5]. Xanthou *et al.* [9] also demonstrated the significance of lymphoid chemokines for lymphoid structure formation in SS, while others have demonstrated an association of CXCL13 expression and ectopic GC formation in SS [7, 8]. Barone *et al.* [8] found a B cell-dominant expression pattern, whereas the selected expression in acinar and ductal epithelial cells was observed by Salomonsson *et al.* [7], although the exact roles of these results remain unclear.

Our data suggest an important interaction of CXCL13 and ectopic GC in sialadenitis in SS. The tendency towards low levels of radiographic damage in patients with HAM–pSS suggests that salivary-specific cytotoxicity is modified by HTLV-I infection. Due to even expression of CXCL12 irrespective of HTLV-I infection, HTLV-I presumably affects the CXCL13 expression of infected $CD4^+$ T cells. Via inflammatory mediators modulated by HTLV-I tax protein, dysfunction of MNC-lineage cells due to HTLV-I infection is supposed to play an important role.

Rheumatology key message

- Low prevalence of ectopic GC is a characteristic of HTLV-I-associated SS with CXCL13 on MNCs.

Disclosure statement: The authors have declared no conflicts of interest.

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Accepted 6 March 2009

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1 Terada K, Katamine S, Eguchi K *et al.* Prevalence of serum and salivary antibodies to HTLV-1 in Sjögren's syndrome. Lancet 1994;344:1116–9.
2 Nakamura H, Eguchi K, Nakamura T *et al.* High prevalence of Sjögren's syndrome in patients with HTLV-I associated myelopathy. Ann Rheum Dis 1997; 56:167–72.
3 Nakamura H, Kawakami A, Tominaga M *et al.* Relationship between Sjögren's syndrome and human T-lymphotropic virus type I infection: follow-up study of 83 patients. J Lab Clin Med 2000;135:139–44.
4 Nakamura H, Takagi Y, Kawakami A *et al.* HTLV-I infection results in resistance toward salivary gland destruction of Sjögren's syndrome. Clin Exp Rheumatol 2008;26:653–5.
5 Amft N, Curnow SJ, Scheel-Toellner D *et al.* Ectopic expression of the B cell-attracting chemokine BCA-1 (CXCL13) on endothelial cells and within lymphoid follicles contributes to the establishment of germinal center-like structures in Sjögren's syndrome. Arthritis Rheum 2001;44:2633–41.
6 Vitali C, Bombardieri S, Jonsson R *et al.* Classification criteria for Sjögren's syndrome: a revised version of the European criteria proposed by the American-European Consensus Group. Ann Rheum Dis 2002;61:554–8.
7 Salomonsson S, Jonsson MV, Skarstein K *et al.* Cellular basis of ectopic germinal center formation and autoantibody production in the target organ of patients with Sjögren's syndrome. Arthritis Rheum 2003;48:3187–201.
8 Barone F, Bombardieri M, Rosado MM *et al.* CXCL13, CCL21, and CXCL12 expression in salivary glands of patients with Sjögren's syndrome and MALT lymphoma: association with reactive and malignant areas of lymphoid organization. J Immunol 2008;180:5130–40.
9 Xanthou G, Polihronis M, Tzioufas AG, Paikos S, Sideras P, Moutsopoulos HM. "Lymphoid" chemokine messenger RNA expression by epithelial cells in the chronic inflammatory lesion of the salivary glands of Sjögren's syndrome patients: possible participation in lymphoid structure formation. Arthritis Rheum 2001;44: 408–18.

Rheumatology 2009;48:855–857
doi:10.1093/rheumatology/kep094
Advance Access publication 27 April 2009

Monozygotic twins with distinct forms of idiopathic inflammatory myositis

Sir, A 56-year-old female (Patient A) presented with leg weakness of 4 years duration. Examination showed weakness of hip flexors and extensors, knee flexors and extensors and weakness of finger flexors confirmed with dynomometry. Manual muscle testing showed that power was 3+/5 in finger flexors, 4/5 in forearm pronators and supinators and 4/5 in hip rotation. Investigations revealed: creatine kinase (CK), 605 U/l [normal range (NR)<150]; lactate dehydrogenase (LDH), 382 U/l (NR 115–200); CRP, 1 mg/l (NR<6); RF 48 IU/l (NR<40); negative antibodies to ENAs; and negative ANA and ANCA. IgG antibodies directed to PL7, PL12, PMScl, Mi-2, Ku, Jo-1 and Ro52 were negative (Euroline Kit, ESL Biosciences, Parramatta, New South Wales, Australia). Muscle biopsy showed polyfocal, polyphasic muscle fibre necrosis, endomysial lymphocytic infiltration and rimmed vacuoles consistent with IBM (Fig. 1a and b). She was commenced on prednisolone 50 mg/day but showed gradual deterioration in power.

Her previously well monozygotic twin (Patient B) aged 56 years presented with 6 years of myalgia and dysphagia. Examination showed isolated weakness of neck flexors (dynomometry confirmed normal power elsewhere). Manual muscle testing showed that power was 3+/5 in neck flexors and normal elsewhere. Investigations showed: CK, 230 U/l (NR<150); LDH, 236 U/l (NR 115–200); ESR, 23 mm; CRP, 3 mg/l (NR<6); RF, 49 IU/l (NR<40); negative ANA; negative antibodies to ENA; and negative IgG antibodies to PL7, PL12, PMScl, Mi-2, Ku, Jo-1 and Ro52 (Euroline Kit). Muscle biopsy showed polyfocal, polyphasic muscle fibre necrosis, patchy endomysial lymphocytic infiltration, non-necrotic fibres infiltrated by lymphocytes and absence of rimmed vacuoles consistent with PM (Fig. 1c). She responded well to prednisolone 25 mg/day and MTX. At 8 years follow-up, Patients A and B are on 7.5 mg/day and 2.5 mg/day prednisolone, respectively.

We present a case of disease disconcordant monozygotic twins, who developed idiopathic inflammatory myositis (IIM) with distinct clinical and histological features, and varied response to immunosuppressive treatment. Reports of IIM in monozygotic twins are sparse, with one report of childhood DM [1], and another of focal myositis [2] in monozygotic twin pairs.

Disease disconcordant monozygotic twins suggest that environmental factors play a critical role in determining disease phenotype. Neither twin had smoked cigarettes. Patient A was a former day-therapy coordinator, whereas Patient B had not been

Therapeutic Strategies in HTLV-I-Associated Myelopathy/Tropical Spastic Paraparesis (HAM/TSP)

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Abstract: Human T lymphotropic virus type I (HTLV-I)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is chronic progressive myelopathy characterized by bilateral pyramidal tracts involvement with sphincteric disturbances. HTLV-I infects approximately 10-20 million people worldwide. There are large endemic areas in southern Japan, the Caribbean, Central and South America, the Middle East, Melanesia, and equatorial regions of Africa. Since the primary neuropathological feature of HAM/TSP is chronic inflammation caused by HTLV-I infection in the spinal cord, various treatments focusing on immunomodulatory or anti-viral effects were performed for HAM/TSP patients until now. However, there are still many of problems, such as insufficient effects, side effects and expensive costs in long-term treatments, etc., in these treatments. Therefore, an ideal therapeutic strategy against HAM/TSP is still not established yet. Although only a small proportion of HTLV-I-infected individuals develops HAM/TSP, neurological symptoms are certainly progressive once myelopathy develops, leading to deterioration of the quality of life. Therefore, we now need the therapeutic regimens to protect the development, or be able to commence the treatments as soon as possible after the development safely and inexpensively even in long-term course or lifelong course of treatment. 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 article, we will review the therapeutic strategies against HAM/TSP up to now and will introduce our new therapeutic approach focusing on the targeting of HTLV-I-infected cells in HAM/TSP patients.

Keywords: HTLV-I, HAM/TSP, Treatment.

INTRODUCTION

Human T lymphotropic virus type I (HTLV-I) is a member of the exogeneous human retroviruses and the causative agent for both adult T cell leukemia (ATL) and HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) [1, 2]. Since the discovery of HAM/TSP, it was revealed that HTLV-I has the potentials to not only act as one of the oncoviruses but also cause a chronic inflammation by the immunologic activation. However, the exact mechanisms underlying the entirely different clinical conditions caused by HTLV-I, such as aggressive lymphoproliferative malignancy and chronic inflammation, are still unknown. HTLV-I infects approximately 10-20 million people worldwide [3]. Although there are large endemic areas in southern Japan, the Caribbean, Central and South America, the Middle East, Melanesia, and equatorial regions of Africa [4], it is still not clear why only a small proportion of HTLV-I-infected individuals develops either of these HTLV-I-associated diseases.

HAM/TSP is chronic progressive myelopathy characterized by bilateral pyramidal tracts involvement, clinically presented as spastic paraparesis, with sphincteric disturbances [5]. The primary pathological feature of HAM/TSP is chronic myelitis formed by chronic inflammation in the spinal cord, mainly the lower thoracic cord, characterized by perivascular cuffing and parenchymal infiltration of mononuclear cells [6]. Although the bystander mechanisms, such as the destruction of the surrounding tissues by the interaction between HTLV-I-infected $CD4^+$ T cells and HTLV-I-specific $CD8^+$ cytotoxic T cells (CTL),are probably critical as the cause of chronic inflammation in the spinal cord [7, 8] (Fig. (**1**)), the exact molecular mechanisms of the development of HAM/TSP still remain unresolved. However, it is well known that HTLV-I proviral load in the peripheral blood is significantly higher in HAM/TSP patients than HTLV-I asymptomatic carriers [9, 10]. On the other hand, numerous immunological dysregulations mostly mediated by HTLV-I tax expression are detected in the peripheral blood of HAM/TSP patients [11, 12]. Therefore, it is strongly supposed that the increase of HTLV-I-infected cells possessing immune-activated status, such as Th1 activation, is involved in the immunopathogenesis of HAM/TSP [13] although it is still controversial whether or not HTLV-I exists in the neuronal components of the central nervous system.

When considering the therapeutic strategies in HAM/TSP, they are divided to two ways of the direction as shown in Table **1** and Fig. (**1**) although both ways connect to each other in some part; 1) immunomodulation therapy, mainly directed to anti-inflammatory effects, 2) anti-viral therapy. Once the myelopathy developed, the neurological symptoms are certainly progressive, leading to the deterioration of

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1871-5249/09 $55.00+.00

Table 1. Therapeutic Strategies in HAM/TSP

1) The therapies focusing on immunomodulatory effects: Mainly directed to anti-inflammatory effects;
a) the suppression of immune activation, particularly for activated HTLV-I-infected cells b) the inhibition of the transmigration of activated HTLV-I-infected cells to the spinal cord c) the reduction of chronic inflammation in the spinal cord
2) The therapies focusing on anti-viral effects:
a) the suppression of HTLV-I expression and/or replication b) the inhibition of the proliferation of HTLV-I-infected cells c) the elimination of HTLV-I-infected cells

the qulity of the life. Therefore, we now need the therapeutic regimens to protect the development, or be able to commence the treatments as soon as possible after the development safely and inexpensively even in long-term or lifelong course of treatment. A number of therapeutic approaches for HAM/TSP were carried out until now as shown in Table **2** although almost trials were performed under an open, non-randomized, uncontrolled studies. Of them, immunomodulation therapies for the suppression of chronic inflammatory status based on immune-activated status as mentioned above were mainly performed for HAM/TSP patients. Indeed, as it is conceivable that the immune-activated status in the peripheral blood involved in the process of chronic inflammation of the spinal cord is one of the targets for the treatments, almost all treatments produced the good results in their own ways. However, since HTLV-I-infected $CD4^+$ T cells are the first responders in the immunopathogenesis of HAM/TSP as mentioned above, the primary target for HAM/TSP treatment is HTLV-I-infected cells themselves of the peripheral blood. Therefore, we should focus on the targeting of HTLV-I-infected cells themselves or the suppression of HTLV-I expression and/or replication in the peripheral blood.

In this review, we will refer to the therapeutic strategies, such as the therapies focusing on immunomodulatory effects or anti-viral effects, against HAM/TSP up to now. In addition, we will introduce our new therapeutic approach focusing on the targeting of HTLV-I-infected cells in HAM/TSP patients.

1. The Therapies Focusing on Immunomodulatory Effects

This strategy is mainly directed to anti-inflammatory effects as shown in Table **1** and Fig. (**1**), such as 1) the suppression of immune activation, particularly for activated HTLV-I-infected cells, 2) the inhibition of the transmigration of these cells to the spinal cord, 3) the reduction of chronic inflammation in the spinal cord, through the down-regulation of inflammatory cytokines and/or adhesion molecules expression, etc. The regimens, of course, exhibit the effects also for the activated HTLV-I-non-infected cells, which are subsequently induced by the activation of HTLV-I-infected cells.

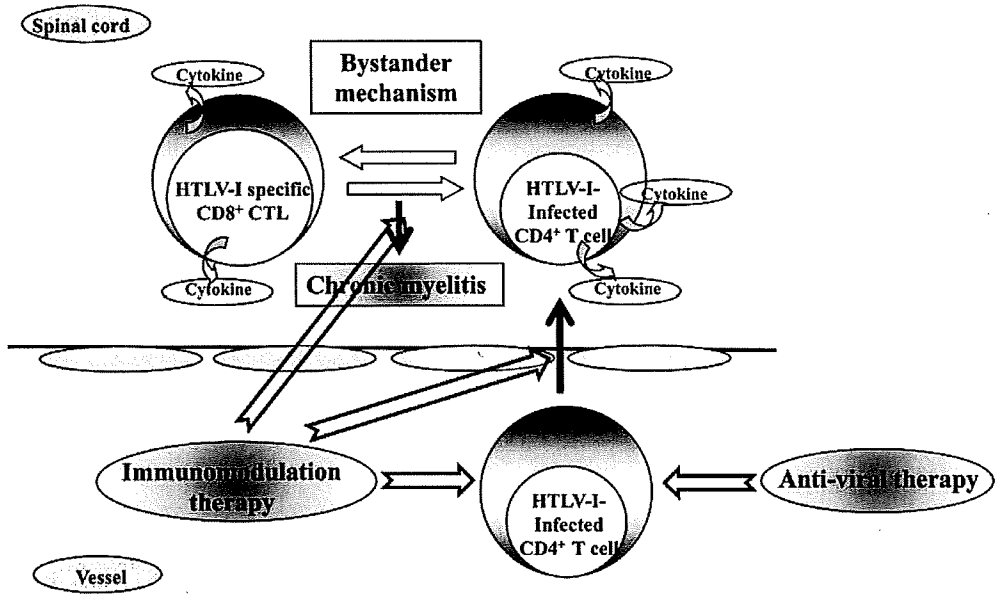

Fig. (1). The immunopathogenesis of and the therapeutic strategies in HAM/TSP. The bystander mechanism, such as the destruction of the surrounding tissues by inflammatory cytokines expression, etc., during the interaction between HTLV-I-infected $CD4^+$ T cells and HTLV-I-specific $CD8^+$ CTL, plays an important role as the cause of chronic myelitis in the spinal cord. The therapeutic strategies in HAM/TSP are divided to two ways of the direction as shown by $\Rightarrow$; 1) immunomodulation therapy, such as a) the suppression of immune activation, particularly for activated HTLV-I-infected cells, b) the inhibition of the transmigration of activated HTLV-I-infected cells to the spinal cord, c) the reduction of chronic inflammation in the spinal cord 2) anti-viral therapy, such as a) the suppression of HTLV-I expression and/or replication, b) the inhibition of the proliferation of HTLV-I-infected cells, c) the elimination of HTLV-I-infected cells.

Table 2. Therapeutic Trials Against HAM/TSP Patients

	References
1) The therapies focusing on immunomodulatory effects:	
a) Corticosteroid hormone	[14, 17]
b) Blood purification	[18]
c) Pentoxifylline	[21, 22, 24]
d) Heparin	[29]
e) High dose-intravenous gammaglobulin	[31]
f) Intermittent high-dose vitamin C	[32]
g) Fosfomycin and Erythromycin	[14, 33, 34]
h) Fermented milk drink	[37]
2) The therapies focusing on anti-viral effects:	
a) Interferon-α and -β	[43, 44, 49, 51]
b) Reverse transcriptase inhibitors	[57, 58, 59, 60]
c) Humanized anti-Tac	[64]
d) Histone deacetylase enzyme inhibitor	[69, 70]

a) Corticosteroid Hormone

As well known, this reagent has been shown to be a useful in various inflammatory or autoimmune diseases. Although high-doses of methylprednisolone are sometimes given intravenously, oral administration of prednisolone (PSL) is most popular treatment against HAM/TSP in Japan. Nakagawa *et al.* recommended that 1 to 2 mg/kg of PSL are given every day or every other day for 1 to 2 months and the dose of PSL, thereafter, is tapered off 5 to 10 mg every other day or is terminated at 6 to 12 months after commencement [14]. They mentioned that 107 (81.7%) of 131 HAM/TSP patients showed improvement of motor function in this therapy. On the other hand, there are some reports that corticosteroid therapy was non-efficacious or the effect of this treatment was transient [15, 16]. Therefore, the efficacy of this treatment for HAM/TSP patients is still controversial although this treatment might show the efficacy in a short-term trial. In addition, there are many adverse events , such as infection, osteoporosis, gastroduodenal ulcer, glucose intolerance, hypertension, and myopathy etc. in a long-term administration of corticosteroid. However, the fact that HTLV-I proviral load in the peripheral blood of HAM/TSP patients was significantly decreased in PSL treatment during 5 years might be noteworthy [17] although its mechanism is unclear.

b) Blood Purification

There are two methods, such as plasmapheresis and lymphocytapheresis. We previously treated 18 HAM/TSP patients with plasmapheresis using AP-05H plasma separator or IM-T350 immunoadsorbent column (Asahi Medical Co, Tokyo, Japan) [18]. In 11 of 18 HAM/TSP patients (61.1%) motor, sensory, and/or shincteric disturbance improved with plasmapheresis (4 to 6 sessions in 2 weeks). Although no adverse events were observed, the effects were transient as maintained only for 2 to 4 weeks. It is supposed that the efficacy of this treatment is based on the elimination of some humoral factors involved in the damage of nervous tissues, such as inflammatory cytokines. However, the exact mechanisms how plasmapheresis treatment induces clinical improvements in HAM/TSP patients are still unclear.

c) Pentoxifylline

Pentoxifylline (PTX) (3,7-dimethyl-1-(5-oxohexyl) xanthine), an inhibitor of phosphodiesterase, is a methylxanthine derivative used in the treatment of vascular diseases [19]. In addition to rheological effects, it is known that PTX has anti-inflammatory or immunomodulatory activity through the increase of intracellular cAMP, such as the suppression of inflammatory cytokines expression including tumor necrosis factor-α, interferon-γ and granulocyte-monocyte colony stimulating factor and the down-regulation of adhesion molecules expression [20, 21]. We previously treated 15 HAM/TSP patients by oral administration of 300 mg/day of PTX for 4 weeks [22]. In 13 of 15 patients, motor disability, especially spasticity, improved concomitant with the suppression of spontaneous peripheral blood lymphocyte (PBL) proliferation, which is one of the major immunological abnormalities observed *in vitro* in patients with HAM/TSP [23]. No adverse events were observed. The fact that the clinical efficacy was correlated with the elevation of serum Th2 cytokine levels in PTX treatment suggests that PTX has the potentials to regulate the balance of Th1/Th2 activity [24]. Thus, although the exact mechanisms with regard to the efficacy of PTX treatment is still obscure, the correction of the imbalance of Th1/Th2 activity in HAM/TSP patients [25] concomitant with the down-regulation of adhesion molecules, such as integrins expression, might be involved in the efficacy as a one of the mechanisms.

d) Heparin

The main regions in the pathological changes of HAM/TSP are the lower thoracic cord in the spinal cord as mentioned above [6]. These regions are anatomically watershed zones of the spinal cord [26], where the stagnant lymphocytes could easily transmigrate to the the tissues and evoke the immune reactions because of decreased blood flow. Indeed, the efficacies of heparin treatment based on the inhibition of lymphocytes trafficking to the tissues were also reported in another inflammatory diseases, such as multiple sclerosis and experimental autoimmune encephalomyelitis [27, 28]. Therefore, heparin was administered to HAM/TSP patients with the expectation of the clinical improvement by the inhibition of the transmigration of activated T cells to the lower thoracic cord in the spinal cord due to the improvement of the microcirculation. We treated 10 HAM/TSP patients by intravenous administration of 5000 - 10,000 units/day of heparin for 9 - 93 days [29]. In 7 patients, motor dysfunction improved substantially and the effect continued for more than a month after the discontinuation of therapy. No adverse events were observed. Most striking change of the immunological markers in the peripheral blood during treatment was the significant decrease of spontaneous PBL proliferation *in vitro*. Although the exact mechanisms of this phenomenon are unclear, it is supposed that heparin can induce not only the amelioration of the microcirculation but

also the down-regulation of immunological activations based on HTLV-I infection. Considering the efficacy of heparin treatment together with PTX treatment, they seem to originate in the inhibition of the transmigration of HTLV-I-infected cells to the spinal cord based on immunomodulatory effects with rheological effects.

e) High-Dose Intravenous Gammaglobulin

High-dose intravenous gammaglobulin (IVIG) is used in the treatment in various inflammatory or immune-mediated diseases [30]. Kuroda *et al.* reported that 10 of 14 HAM/TSP patients had presented the improvement of motor disability within 7 days of the commencement by administration of 10g/day or 400 mg/kg/day of gammaglobulin for 5 consecutive day and the effects were sustained for more than 3 weeks in some patients [31]. Although the exact mechanisms how IVIG exhibits the efficacy, even in another immune-mediated diseases, are still unclear [30], they mentioned the possibility of the suppression of perivascular inflammation from the fact that the clinical improvement was preferentially observed in patients with high anti-HTLV-I antibodies titer in cerebral spinal fluid (CSF), a high CSF IgG level and a severe white-matter lesion on brain MRI.

f) Intermittent High-Dose Vitamin C

Kataoka *et al.* reported the therapeutic efficacy of intermittent high-dose vitamine C. They treated 7 HAM/TSP patients by oral administration of 35-40 mg/kg/day of vitamin C in following manner, 3 to 5 successive days followed by a 2-day withdrawal period, for a mean period of 9.7 months [32]. All of patients presented the improvement of motor function with the decrement of serum level of immuno-suppressive acidic protein, suggesting the suppression of macrophage activation. However, the mechanisms how the down-regulation of activated macrophages are involved in the efficacy of this treatment are not unclear.

g) Fosfomycin and Erythromycin

Of 14 HAM/TSP patients treated with intravenous administration of 2 g/day of fosfomycin for 2 weeks followed by oral administration of 2 g/day of fosfomycin for 2 weeks, 11 patients showed the improvement of motor function [14]. Of 25 HAM/TSP patients treated with oral administration of 600 mg/day of erythromycin for 1 to 3 months, 12 patients showed moderate improvements in their motor function [33]. Since these regimens have not only anti-bacterial effects but also immunomodulatory functions [34, 35], the efficacy by these regimens is supposed to be based on the down-regulation of inflammatory cytokines or chemokines expression.

h) Fermented Milk Drink

Lactobacillus casei strain Shirota (LcS) is one of probiotic agents, which have immnomodulatory functions through the interactions with the gastrointestinal mucosal immune system [36]. Matsuzaki *et al.* reported that oral administration of 4 x 10^{10} viable LcS, twice a day for 4 weeks induced the improvement of motor dysfunction, particularly decrease of spasticity, and of urinary symptoms in all of 10 HAM/TSP patients treated [37]. In addition, the increase of NK cell activity, which was generally decreased in HAM/TSP patients [38, 39], in the peripheral blood was observed in LcS treatment. However, there were no significant changes of not only lymphocytes surface markers but also HTLV-I proviral load in the peripheral blood in the course of LcS treatment. Although the mechanisms how the up-regulation of NK cell activity without the decrement of HTLV-I proviral load in the peripheral blood are involved in clinical improvement is unclear, the benefit which can use safely as the supplement during a long-term might be valuable for the treatment against HAM/TSP patients.

2. The Therapies Focusing on Anti-Viral Effects

This strategy is mainly directed to anti-viral effects as shown in Table **1** and Fig. (**1**), such as 1) the suppression of HTLV-I expression and/or replication, 2) the inhibition of the proliferation of HTLV-I-infected cells, 3) the elimination of HTLV-I-infected cells.

a) Interferon-α and -β

Interferon (IFN)-α and IFN-β, which are type I IFNs, have a variety of biological actions including not only anti-viral effects but also cell growth regulation and modulation of the cellular immune response [40-42]. Therefore, treatment with these regimens might be suitable for HAM/TSP because it can target on the immunological dysregulation based on high HTLV-I proviral load in the peripheral blood of HAM/TSP.

In various treatments against HAM/TSP, only IFN-α has been proved to be effective in a multicenter, randomized, double-blind, and controlled trial [43] and has been approved as the therapeutic agent against HAM/TSP by the Ministry of Health, Labor and Welfare in Japan. In controlled tial of IFN-α treatment against HAM/TSP as mentioned above, 48 HAM/TSP patients were divided to three groups treated with 0.3 million international units (MU) of natural IFN-α (human lymphoblastoid interferon (HLBI, Sumiferon) (Sumitomo Pharmaceutical Co., Osaka, Japan), 1.0 MU, and 3.0 MU by intramuscular injection, respectively, daily for 4 weeks. In about 70 % of HAM/TSP patients treated with 3.0 MU, motor dysfunction, even urinary disturbances in some cases, improved in significant therapeutic response and its effectiveness continued for 4 weeks after completion of therapy without serious adverse effects. The therapeutic response in the 3.0-MU group was significantly higher than in the 0.3-MU group. We, previously, had also demonstrated a similar efficacy of HLBI treatment against 17 HAM/TSP patients in open trial [44]. In this trial, 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 phytohemoagglutinin. Although spontaneous PBL proliferation is one of the major immunological abnormalities observed *in vitro* in patients with HAM/TSP as mentioned above [23], the exact mechanisms of it 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 the aberrant signalings of both Interleukin-2 (IL-2) and IL-15 [45, 46]. It was reported that HTLV-I proviral load and HTLV-I tax mRNA expression correlate the frequency of HTLV-I tax specific $CD8^+$ CTL in

the peripheral blood of HAM/TSP patients [47, 48]. 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 *et al.* 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 [49]. 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 [25]. Indeed, another 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 cell in the peripheral blood were significantly decreased by IFN-α treatment [50].

On the other hand, the efficacy of IFN-β, which is another type I IFN, treatment against HAM/TSP was also reported [51]. Twelve patients with HAM/TSP were treated with escalating doses of IFN-β1a, which has already been approved as the treatment against multiple sclerosis [52], over the course of a relatively long-term, 28 weeks. This treatment induced the 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 important factors for the development of HAM/TSP [48, 53], IFN-β1a treatment might be able to target this point. In addition, the reduction of spontaneous PBL proliferation was also observed as same as it 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 of the discrepancy between IFN-α and IFN-β1a treatment are unclear. However, although IFN-α treatment as mentioned above induced the significant reduction of HTLV-I proviral load in total study population, HTLV-I proviral load was rather increased in about 30 % out of total study population [49], suggesting that anti-viral effects of IFN-α are different among each individual.

High HTLV-I proviral load in the peripheral blood is the most important prerequisite in the development of HAM/TSP [9, 10]. At 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 [12, 54, 55]. Either IFN-α or IFN-β treatment does not seem to target this point. However, it is certain that these regimens have somewhat 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 on therapeutic strategies for HAM/TSP. However, whether these treatments are tolerable as a long-term or lifelong treatment or not is uncertain.

b) Reverse Transcriptase Inhibitors

Some nucleoside analogues have been shown to block HTLV-I replication by inhibition of reverse transcriptase (RT). Zidovudine (azidothymidine, AZT), which is the thymidine analogue, can inhibit HTLV-I replication *in vitro* although its inhibitory dose for HTLV-I is higher than for human immunodeficiency virus [56]. Zidovudine treatment against 5 HAM/TSP patients during 6 months did not induce the clinical benefits at a first study [57]. However, zidovudine, with higher dose than it in a first study, treatment against 10 HAM/TSP patients for 24 weeks induced the clinical benefits in some patients [58]. However, these studies did not refer to the change of HTLV-I proviral load in each treatment. On the other hand, the clinical trial against HAM/TSP patients with lamivudine, which is the cytosine analogue, was also reported [59]. Although lamivudine treatment against 5 HAM/TSP patients during about 10 months did not induce any symptomatic improvements except one patient, who is a case with recent-onset HAM/TSP, the significant reduction of HTLV-I proviral load in the peripheral blood was observed in all 5 HAM/TSP patients. In addition, the reduction of viral load was associated with the decrease of the frequency of HTLV-I tax specific CTL in one patient who had the clinical efficacy. Thus, RT inhibitors seemed to have some clinical benefits with HTLV-I targeting in the treatment against HAM/TSP.

However, unfortunately, the result of recent clinical trial with combination therapy by zidovudine and lamivudine in a randomized, double blind, placebo controlled study made RT inhibitors pessimistic for the regimen for HTLV-I targeting as the treatment against HAM/TSP [60]. Same group, which reported the efficacy of lamivudine treatment against HAM/TSP, has conducted a controlled study of 6 months by combination therapy with these two RT inhibitors for 16 HAM/TSP patients. As far as they compared the clinical effects including motor disbility score, gait, and bladder function, etc., and the changes of laboratory markers in the peripheral blood including HTLV-I proviral load and T cell subpopulation between each group treated by combined therapy or placebo therapy, no significant changes were seen between two arms although the treatment was well tolerated with no unexpected side effects. 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. In addition, the reasons of the discrepancy of the results between two studies conducted by same group are unclear. However, 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 [12, 54, 55], the inefficacy of the treatment with RT inhibitors might be reasonable.

c) Humanized Anti-Tac

It is well known that interleukin-2 (IL-2) and IL-2 receptor α (IL-2Rα) are induced by HTLV-I tax transactivation in HTLV-I-infected cells [61, 62]. This dysregulation of cellular genes expression by HTLV-I tax initiates a process of T cell activation and proliferation by autocrine or paracrine loop. Therefore, the blockade of IL-2/IL-2Rα system might lead to the direction toward the decrease of HTLV-I-infected cells *in vivo* through apoptosis of HTLV-I-infected cells by IL-2 deprivation. The efficacies of humanized anti-Tac antibody (daclizumab), which is the humanized form of monoclonal antibody against IL-2Rα and blocks the interaction of

IL-2 with IL-2Rα, treatments were demonstrated in several immune-mediated like diseases, such as renal allograft rejection, noninfectious uveitis, multiple sclerosis, pure red cell aplasia, aplastic anemia, and psoriasis, and T-cell malignancy [63].

Nine patients with HAM/TSP were treated with administration of five doses (1 mg/kg) of humanized anti-Tac antibody at weeks 0, 2, 6, 10, 14 [64]. This treatment induced mild improvement of motor disability score in only 3 HAM/TSP patients without serious adverse effects. On the other hand, immunological studies, as expected, revealed a selective down-regulation in the number of circulating activated T cells expressing IL-2Rα receptor and a decrease of spontaneous PBL proliferation *ex vivo*. Furthermore, as most striking finding, HTLV-I proviral load in the peripheral blood was reduced an average of 52 % after this treatment. These findings suggest that humanized anti-Tac treatment have the potential to selectively remove HTLV-I-infected cells expressing IL-2Rα from the peripheral blood of HAM/TSP patients.

d) Histone Deacetylase Enzyme Inhibitor

Histone deacetylase enzyme (HDAC) inhibitor has lately attracted considerable attention as the therapeutic regimens against various diseases such as malignancies, and neurodegenerative diseases etc. [65, 66]. Although acetylated histones are associated with trascriptionally 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 [67, 68]. Therefore, inhibition of HDACs activities leads into histone hyperacetylation followed by increases in HTLV-I gene expression.

As mentioned above, 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 [12]. 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 number of HTLV-I-infected cells might be reduced in the peripheral blood. That is, the tilting of host-pathogen balance might lead to the elimination of HTLV-I-infected cells from HAM/TSP patients. Based on this new concept such as "gene activation therapy", very recently, clinical trial by oral administration of 20 mg/kg/day valproate (VPA), which is one of HDAC inhibitors, during 3 months was performed in 16 HAM/TSP patients [69]. Although HTLV-I proviral loads in the peripheral blood were transiently increased in early stages after administration as expected, they significantly decreased in all patients by 2.3- to 89.3-fold (mean; 24-fold) at the end point. Although authors did not describe the changes of clinical status in detail, they mentioned that VPA treatment induced the reduction of spasticity in all patients. This result is very intriguing because there are no reports such a significant drop of HTLV-I proviral load in the treatments against HAM/TSP until now.

However, there is one report that HDAC inhibitors including VPA, sodium butyrate, and trichostatin A, unexpectedly, decrease the activity of HTLV-I specific CTL against the increased HTLV-I expression in HTLV-I-infected cell *ex vivo* [70]. This finding suggests that HDAC inhibitors reduce the efficiency of CTL surveillance of HTLV-I and is contrary to the concept leading to the treatment with HDAC inhibitors as mentioned above. Indeed, VPA induced apoptosis in not only $CD4^+$ but also $CD8^+$ cells at relatively high frequency in short-term *in vitro* treatment [69]. Then, authors recommend caution in the use of HDAC inhibitors in non-malignant cases of HTLV-I infection such as HAM/TSP.

Overall, anyway, VPA is the anti-epileptic drug which has the good safety profiles as long-term therapy and is easily available. Since this drug might be expected as one of new anti-HTLV-I agents, we now need to perform the case controlled study by VPA treatment against HAM/TSP patients.

3. New Therapeutic Approach Focusing on Anti-Viral Effect

As another therapeutic strategy for the elimination of HTLV-I-infected cells, we can focus on the targeting of HTLV-I-infected cells themselves from the peripheral blood. If HTLV-I-infected cells is selectively removed, for example-by apoptosis, from the peripheral blood, its strategy must become one of the ideal therapeutic tools against HAM/TSP patients. With this regard, the strategy requires the regimen, which is well tolerated, additionally inexpensive, even in long-term treatment.

Allicin (diallyl thiosulfinate), which is one of natural organosulfur compounds derived from garlic (*Allium sativum*), has diverse biological activities, such as anticarcinogenic activity, antibacterial activity, antifungal activity, etc. [71, 72]. Although the exact mechanisms how cytotoxic effects described above are induced by organosulfur compounds, such as allicin, are still obscure, a disulfide moiety in their structures seems to have an important role for the trigger of cell death [73]. The disruption of the intracellular redox system induced by the chemical reaction of a disulfide moiety with thiol-containing intracellular molecules, such as thioredoxin (Trx), Trx reductase, and glutathione (GSH), etc., might be involved in cytotoxic effects [71]. However, allicin is very unstable compound as reported that this compound rapidly disappears after the injection into the blood [74, 75]. Therefore, it is difficult to use this compound in the therapeutic trial against HAM/TSP patients. Prosultiamine (*N*-[(4-amino-2-methyl-5-pyrimidinyl) methyl]- *N*-[4-hydoxy-1-methyl-2-(propyldithio)-1-butenyl]-formamide) (Alinamin®), which is the product of Takeda Pharma. Co. Inc., Osaka, Japan, is a homologue of allithiamine originally synthesized by thiol type vitamin B1 and allicin (Fig. (**2**)) [76]. 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 (Fig. (**2**)) [77]. Thus, Prosultiamine has a disulfide moiety in its structure as same as allicin (Fig. (**2**)). Therefore, it is expected that prosultiamine has the same activity as allicin. Importantly, Prosultiamine is pharmacologically stable and is very frequently

available as the regimen of vitamin B1 deficiency with the safety in Japan. Therefore, this drug has the potential to be able to immediately conduct the clinical trial against HAM/TSP patients. We showed the structure of allicin and the generation of prosultiamine (Fig. (**2**)).

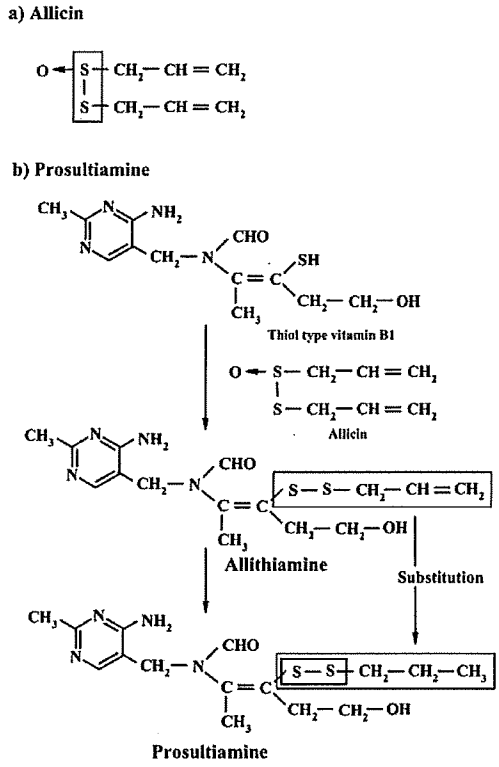

Fig. (2). The structure of allicin **(a)** and the generation of prosultiamine **(b)**. Allithiamine was originally synthesized by thiol type vitamin B1 and allicin. Prosultiamine was developed after allyl disulfide derived from allicin was substituted to propyl disulfide in the structure of allithiamine as shown by ▭. Either prosultiamine or allicin have a disulfide moiety in its structure as shown by ⬚.

a) Prosultiamine has the Cytotoxic Activity Against HTLV-I-Infected T Cell Lines Derived from HAM/TSP Patients as Same as Allicin

It is revealed, by MTS assay ((3-[4,5-dimethylthiazol-2-yl-5]-[3-carboxymethoxyphenyl]-2-[4-sulfophenyl]-2H tetrazolium) nonradioactive cell proliferation assay), that cell viability of two HTLV-I-infected T cell lines derived from HAM/TSP patients (HCT-1 and HCT-4) decreased by allicin treatment in dose-dependent manner (Fig. (**3a**)). Prosultiamine (kindly provided by Takeda Pharma Co. Inc., Osaka, Japan) treatment against these cell lines also caused the similar effect to allicin treatment as expected (Fig. (**3b**)). As shown in Fig. (**3ab**), HTLV-I-infected T cell lines were more sensitive for the treatment with each compound than HTLV-I-non-infected T cell line, Jurkat cell line. These results suggest that the cytotoxic effect against HTLV-I-infected T cell lines is based on the disulfide moiety as the common structure in these compounds (Fig. (**2**)).

b) The Cytotoxic Effect of Prosultiamine is Based on Caspase-Dependent Apoptosis

As shown in Fig. (**4a**), prosultiamine treatment against HCT-1 induced the loss of mitochondrial membrane potential with the appearance of annexin V-positive cells, suggesting that the cytotoxicity by prosultiamine was based on the cell death induced by apoptosis through the mitochondrial pathway. Indeed, as shown in Fig. (**4b**), the loss of mitochondrial membrane potential was recovered in z-VAD-fmk, which is pan-caspase inhibitor, -pretreated HCT-1. In addition, immunoblot analysis revealed that the treatment with prosultiamine resulted in the proteolytic cleavage of caspase 3, which is the effector molecule in the final process of apoptosis (Fig. (**4c**)). Overall, these results suggested that prosultiamine can induce caspase-dependent apoptosis through the mitochondrial pathway for HTLV-I-infected T cells.

c) Involvement of Activation of Apoptosis Signal-Regulating Kinase (ASK) 1 Signaling in the Induction of Caspase-Dependent Apoptosis in Prosultiamine-Treated HTLV-I-Infected Cells

As mentioned above, Trx plays an important role in the cellular reducing system, interacting with GSH [78]. Although Trx is ubiqutaously expressed in many cell types of mammalians [79], human Trx is a homologue of adult T cell leukemia-derived factor (ADF), which was originally defined as an IL-2 receptor a-chain inducer produced by HTLV-I-transformed T cells [80, 81]. Trx is a small protein (12 kDa) with two redox-active cysteine residues in an active center (-Cys-Gly-Pro-Cys-) and operates together with NADPH and Trx reductase as a protein disulfide-reducing system [79, 82]. Apoptosis signal-regulating kinase (ASK) 1 is a mitogen-activated protein (MAP) kinase kinase kinase, which is located in the upstream of p38 MAP kinase (p38 MAPK) and c-Jun N-terminal kinase (JNK) leading to cytokine- and stress-induced apoptosis [83, 84]. Trx is a redox-sensitive physiological inhibitor of ASK1 activity [85]. Reduced form of Trx binds to ASK1 and inhibits kinase activity of ASK1. However, upon the change to oxidized form of Trx such as formation of disulfides through the oxidation of cysteine residues, ASK1 dissociates from Trx and a free ASK1 is autophosphorylated in Threonine residue, subsequently activates the cascade of apoptosis signaling [86]. Therefore, prosultiamine might have the potentials to oxidize Trx in the interaction between Trx/Trx reductase system and a disulfide moiety and induce ASK1 activation leading to the induction of apoptosis of HTLV-I-infected cells. We showed the hypothetical pathway in allicin or prosultiamine-induced apoptosis of HTLV-I-infected cells (Fig. (**5**)).

As shown in Fig. (**6**), immunoblot analysis revealed that ASK1 is activated within a short period after prosultiamine treatment in HCT-1. It is reported that activation of p38 MAPK, which is located in the downstream of ASK1 signaling, connects to the mitochondrial apoptotic pathway through caspase 8 [87]. Therefore, as mentioned above, the fact that the loss of mitochondrial membrane potential was

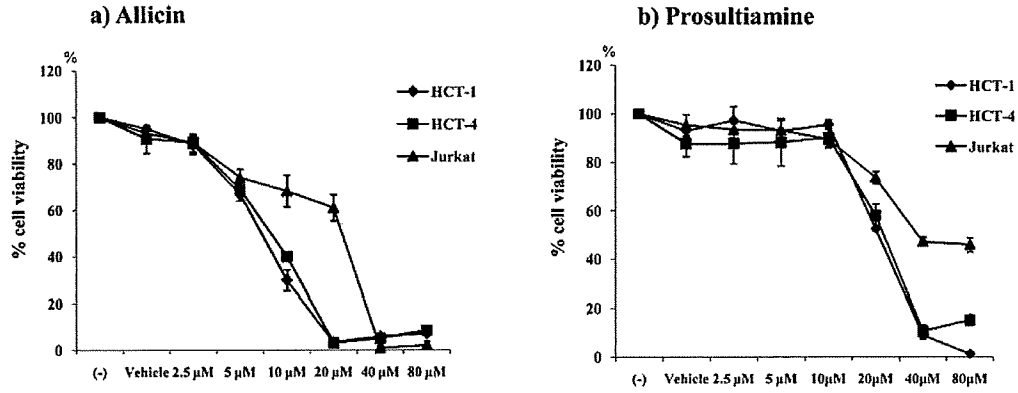

Fig. (3). The cytotoxic effect of allicin or prosultiamine against HTLV-I-infected T cell lines derived from HAM/TSP patients (HCT-1 and HCT-4) or non-infected T cell line (Jurkat). **a)** Cell viability of all cell lines decreased by the treatment with allicin for 24 hr in dose-dependent manner. **b)** Prosultiamine treatment against these cell lines also caused the similar effect to allicin treatment. Both HCT-1 and HCT-4 were more sensitive for the treatment with each compound than Jurkat cell line.
HCT-1; —◆—, HCT-4; —■—, Jurkat; —▲—. For cell viability assay, MTS assay was performed. Cell viability was determined as follows: after each OD titer at wavelength of 490 nm in triplicate cultures in the presence of allicin, or prosultiamine or vehicle / mean of OD titer at wavelength of 490 nm in triplicate cultures under medium alone was calculated, its mean ± SD was presented as the cell viability.

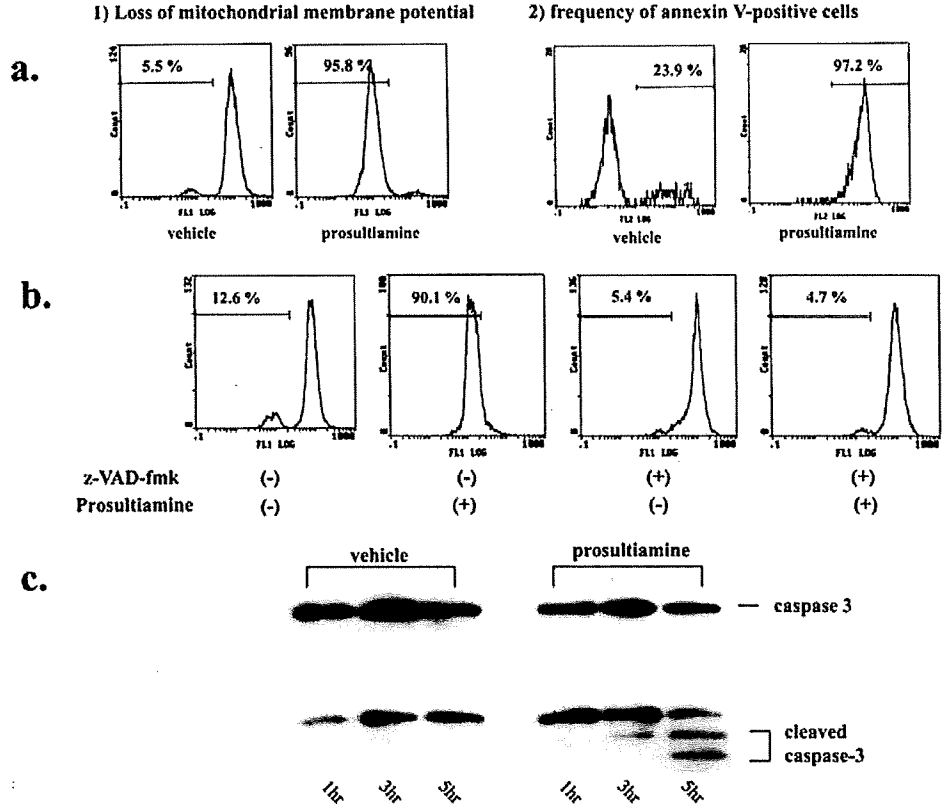

Fig. (4). The cytotoxic effect of prosultiamine is based on caspase-dependent apoptosis. **a)** The loss of mitochondrial membrane potential and the frequency of annexin V-positive cells in prosultiamine-treated HCT-1. After HCT-1 was cultured for 24 hr in the presence of 40 μM prosultiamine or vehicle, these cells was analyzed by staining the cells with the potential sensitive fluorescent dye $DiOC_6(3)$ or FITC-conjugated annexin V to evaluate $\Delta\Psi m$ or the frequency of apoptotic cells, respectively. Either the loss of $\Delta\Psi m$ or the frequency of apoptotic cells was measured by flow cytometry. **b)** The recovery of the loss of mitochondrial membrane potential in prosultiamine treatment against HCT-1pretreated with z-VAD-fmk. Before the treatment with 40 μM prosultiamine for 24 hr, HCT-1 was pretreated by 200 μM z-VAD-fmk for 1 hr. **c)** Immunoblot analysis of 40 μM prosultiamine-treated HCT-1 for the proteolytic cleavage of caspase 3. After HCT-1 was cultured for 1, 3, or 5 hr in the presence of 40 μM prosultiamine, the cells were collected and lysed for immunoblot analysis. The treatment of HCT-1 with prosultiamine resulted in the proteolytic cleavage of caspase 3.

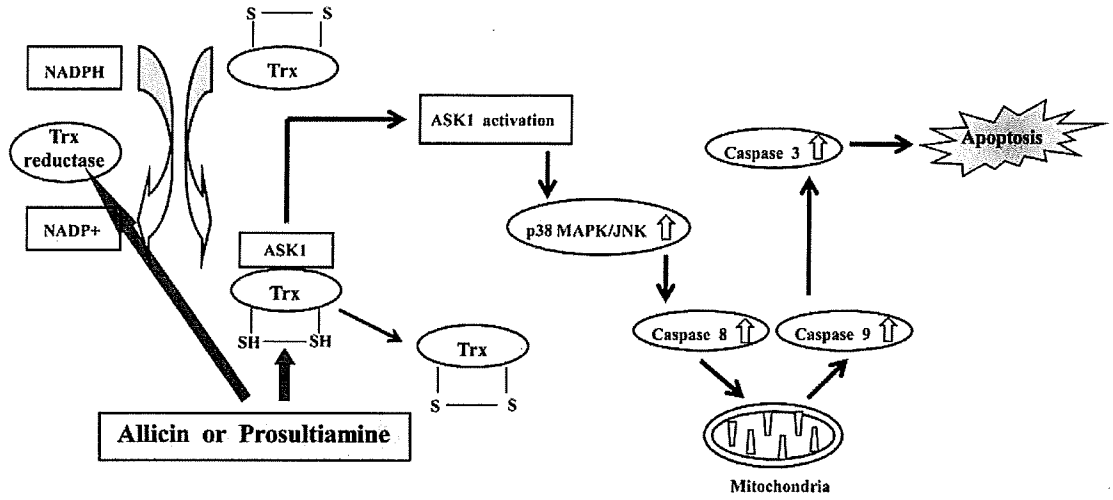

Fig. (5). Hypothetical pathway in allicin or prosultiamine-induced apoptosis of HTLV-I-infected cells. Trx has two redox-active cysteine residues in an active center (-Cys-Gly-Pro-Cys-) and operates together with NADPH and Trx reductase as a protein disulfide-reducing system. Trx is a redox-sensitive physiological inhibitor of ASK1 activity. Reduced form of Trx binds to ASK1 and inhibits kinase activity of ASK1. Upon the change to oxidized form of Trx such as formation of disulfides through the oxidation of cysteine residues, ASK1 dissociates from Trx and a free ASK1 is autophosphorylated in Threonine residue, subsequently activates the cascade of apoptosis signaling through p38 MAPK and JNK activation. Both allicin and prosultiamine might have the potentials to oxidize Trx in the interaction between Trx/Trx reductase system and a disulfide moiety in their structures and induce ASK1 activation leading to the induction of apoptosis of HTLV-I-infected cells through mitochondrial pathway. Trx; Thioredoxin, ASK1; Apoptosis signal-regulating kinase1, p38 MAPK/JNK; p38 mitogen-activated protein kinase/c-Jun N-terminal kinase.

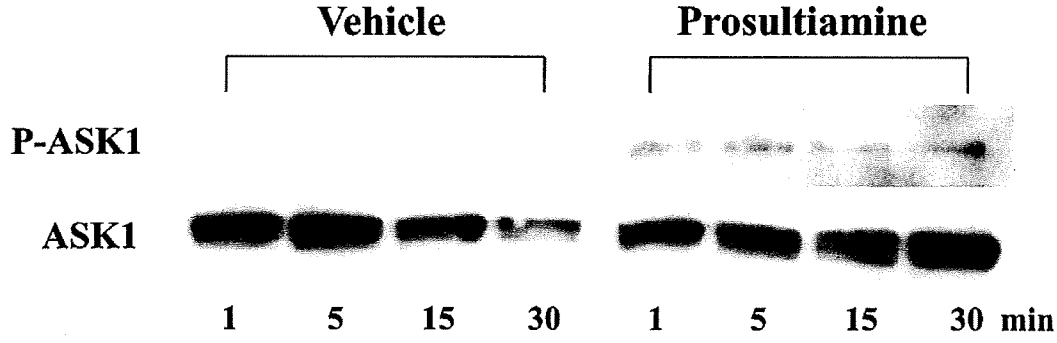

Fig. (6). Immunoblot analysis of 40 μM prosultiamine-treated HCT-1 for ASK1 activation. After HCT-1 was cultured for 1, 5, 15, or 30 min in the presence of 40 μM prosultiamine, the cells were collected and lysed for immunoblot analysis. The treatment of HCT-1 with prosultiamine resulted in phosphorylation of ASK1 P-ASK1; phosphorylated ASK1.

recovered in z-VAD-fmk-pretreated HCT-1 suggests that the activation of ASK1 signaling is involved in apoptosis of HTLV-I-infected cells by the treatment with prosultiamine.

d) The Decrease of Numbers of HTLV-I Provirus in the Peripheral Blood CD4$^+$ T Cells of HAM/TSP Patients by the in vitro Treatment with Prosultiamine and Clinical Trial with Prosultiamine Against HAM/TSP Patients

In next, we studied whether prosultiamine *in vitro* treatment can selectively target HTLV-I-infected cells of peripheral blood CD4$^+$ T cells of HAM/TSP patients or not. After the peripheral blood CD4$^+$ T cells of HAM/TSP patients were cultured in the presence of 5 μM prosultiamine or vehicle for 48 hr, total cellular DNA samples prepared from the viable cells were subjected to the measurement of HTLV-I proviral copies by quantitative PCR analysis. As shown in Fig. (**7a**), prosultiamine *in vitro* treatment against the peripheral blood CD4$^+$ T cells of 7 HAM/TSP patients induced the decrease of numbers of HTLV-I proviral copies, ranged from 29.9 - 80.2% (mean; 60.7%), compared with vehicle treatment. As far as we evaluated the cell viability by MTS assay, prosultiamine treatment did not affect the viability of total peripheral blood CD4$^+$ T cells. These data suggest that prosultiamine *in vitro* treatment can selectively induce apoptosis of HTLV-I-infected cells of the peripheral blood CD4$^+$ T cells of HAM/TSP patients.

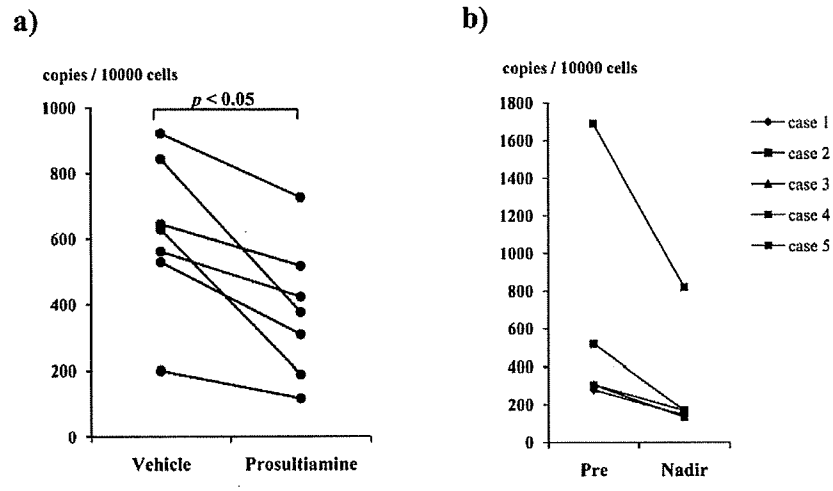

Fig. (7). The effect of *in vitro* and *in vivo* treatment with prosultiamine for the reduction of HTLV-I-infected cells. **a)** The decrease of numbers of HTLV-I provirus in peripheral blood $CD4^+$ T cells of HAM/TSP patients by the *in vitro* treatment with prosultiamine. The peripheral blood $CD4^+$ T cells of HAM/TSP patients were cultured in the presence of 5 μM prosultiamine or vehicle for 48 hr. After the dead cells induced by prosultiamine *in vitro* treatment were removed using annexin V microbead kit, total cellular DNA samples prepared from viable cells were subjected to the measurement of HTLV-I proviral copies by quantitative PCR analysis. Prosultiamine treatment induced the decrease of HTLV-I proviral copies, ranged from 29.9 - 80.2% (mean; 60.7%) ($p < 0.05$), compared with vehicle treatment. **b)** The decrease of numbers of HTLV-I provirus in the peripheral blood mononuclear cells of HAM/TSP patients by the *in vivo* treatment with prosultiamine. We treated 5 HAM/TSP patients with intravenous administration of prosultiamine at the dosages of 40 mg daily for 14 days. Total cellular DNA samples prepared from the peripheral blood mononuclear cells were subjected to the measurement of HTLV-I proviral copies by quantitative PCR analysis. As far as we monitored the copy numbers of HTLV-I provirus in the peripheral blood before treatment (day 0) (Pre), during treatment (day 7 and 14), and 7 days after treatment (day 21), copy numbers of HTLV-I provirus decreased to 33-55 % of them at day 0 at nadir in all HAM/TSP patients.

Table 3. The Profiles of Patients and the Clinical Efficacy of Prosultiamine Treatment

			The Changes of Motor Disability Score [a)]	
Case	**Age / Gender**	**Duration of Illness (Years)**	**Before**	**After**
1	58 / female	24	4	4
2	51 / male	13	8	8
3	52 / male	46	4	4
4	73 / female	13	4	4
5	53 / female	2	4	2

[a)] Motor disability score was rated from 0 to 10 according to the scale of reference [18].

These evidences prompted us to the treatment with prosultiamine against HAM/TSP patients because this agent is frequently and easily available as the regimen of vitamin B1 deficiency with the safty in Japan. We treated 5 HAM/TSP patients with intravenous administration of prosultiamine at the dosages of 40 mg daily for 14 days. We showed the profiles of the patients in Table **3**. As shown in Table **3**, although motor disability grade was not changed among 4 of 5 HAM/TSP patients (case 1-4), some clinical improvements, such as the reduction of spasticity etc., were individually observed. On the other hand, case 5, who had the short duration of illness, showed marked improvement of motor function such as the change of motor disability grade (Table **3**). No adverse events were observed. As far as we monitored the copy numbers of HTLV-I provirus in the peripheral blood mononuclear cells before treatment (day 0), during treatment (day 7 and 14), and 7 days after treatment (day 21), copy numbers of HTLV-I provirus decreased to 33-55 % of them at day 0 at nadir in all HAM/TSP patients (Fig. (**7b**)).

Overall, our results indicated that prosultiamine treatment against HAM/TSP patients have the potential to be able to induce the clinical improvement based on the decrease of HTLV-I-infected cells by apoptosis in the peripheral blood, suggesting that prosultiamine can work as the new anti-viral agent against HTLV-I.

CONCLUSION

Since the discovery of HAM/TSP, over 20 years have passed. During that period, numerous findings have been presented in the research field of HAM/TSP. Unfortunately, these findings have not translated into an optimal therapeutic strategy against HAM/TSP. Although the pathphysiology of HAM/TSP is, in a word, a chronic inflammatory status triggered by HTLV-I infection, we should treat HAM/TSP as one of the infectious diseases because HTLV-I-infected cells are the first responders in the development of HAM/TSP. Therefore, the therapeutic strategy that manages to decrease or eliminate HTLV-I-infected cells seems to be critical in considering the ideal treatment for HAM/TSP. With this regard, either VPA or prosultiamine might function as a new anti-viral agent against HTLV-I. However, the trial for the targeting of HTLV-I-infected cells toward the depletion of HTLV-I, as a new therapeutic strategy against HAM/TSP, has just opened now.

ACKNOWLEDGEMENTS

This work was supported in part by the Grant-in-Aid for Research on Brain Science and the Health and Labour Sciences Research Grant on Intractable Diseases (Neuroimmunological Diseases) from the Ministry of Health, Labour and Welfare of Japan.

ABBREVIATIONS

HTLV-I	=	Human T lymphotropic virus type I
HAM/TSP	=	HTLV-I-associated myelopathy/tropical spastic paraparesis
CTL	=	Cytotoxic T cells
PSL	=	Predonisolone
PTX	=	Pentoxifylline
IFN-α and -β	=	Interferon-α and -β
RT inhibitor	=	Reverse transcriptase inhibitor
IL-2	=	Interleukin-2
HDAC inhibitor	=	Histone deacetylase enzyme inhibitor
VPA	=	Valproate
Trx	=	Thioredoxin
Trx reductase	=	Thioredoxin reductase
GSH	=	Glutathione
MTS assay	=	(3-[4,5-Dimethylthiazol-2-yl-5]-[3-carboxymethoxyphenyl]-2- [4-sulfophenyl]-2H tetrazolium) nonradioactive cell proliferation assay
ASK1	=	Apoptosis signal-regulating kinase 1

REFERENCES

[1] Yoshida, M. Multiple viral strategies of HTLV-1 for dysregulation of cell growth control. *Annu. Rev. Immunol.*, **2001**, *19*, 475-496.

[2] 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**, *1*, 1031-1032.

[3] de Thé, G.; Bomford, R. An HTLV-I vaccine: why, how, for whom? *AIDS Res. Hum. Retroviruses* , **1993**, *9*, 381-386.

[4] Hollsberg, P.; Hafler D.A. Pathogenesis of diseases induced by human T-lymphotropic virus type I infection. *N. Engl. J. Med.*, **1993**, *328*, 1173-1182.

[5] 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.

[6] Iwasaki, Y. Pathology of chronic myelopathy associated with HTLV-I infection (HAM/TSP). *J. Neurol. Sci.*, **1990**, *96*, 103-123.

[7] 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.

[8] Osame, M. Pathological mechanisms of human T-cell lymphotropic virus type I-associated myelopathy (HAM/TSP). *J. Neurovirol.*, **2002**, *8*, 359-364.

[9] Jeffery, K.J.; Usuku, K.; Hall, S.E.; Matsumoto, W.; Taylor, G.P.; Procter, J.; Bunce, M.; Ogg G.S.; Welsh, K.I.; Weber, J.N.; Lloyd, A.L.; Nowak, M.A.; Nagai, M.; Kodama, D.; Izumo, S.; Osame, M.; Bangham, C.R. 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.

[10] Nagai, M.; Usuku, K.; Matsumoto, W.; Kodama, D.; Takenouchi, N.; Moritoyo, T.; Hashiguchi, S.; Ichinose, M.; Bangham, C.RM.; 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.

[11] Nakamura, T. Immunopathogenesis of HTLV-I-associated myelopatrhy/tropical spastic paraparesis. *Ann. Med.*, **2000**, *32*, 600-607.

[12] Bangham, C.R.M.; Osame, M. Cellular immune response to HTLV-I. *Oncogene*, **2005**, *24*, 6035-6046.

[13] Nakamura, T.; Nishiura, Y.; Fukushima, N.; Furuya, T. In *Neuroimmunology Research Focus*, Broglio P.V.; Ed.; Nova Science Publishers: New York, **2007**, pp. 43-84.

[14] 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.

[15] 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.

[16] Gesaain, A.; Gout, O. Chronic myelopathy associated with human T-lymphotropic virus type I (HTLV-I). *Ann. Intern. Med.*, **1992**, *117*, 933946.

[17] Matsuzaki, T.; Nakagawa, M.; Nagai, M.; Usuku, K.; Higuchi, I.; Arimura, K.; Kubota, H.; Izumo, S.; Akiba, S.; Osame, M. HTLV-I proviral load correlates with progression of motor disability in HAM/TSP: analysis of 239 HAM/TSP patients including 64 patients followed up for 10 years. *J. Neurovirol.*, **2001**, *7*, 228-234.

[18] Matsuo, H.; Nakamura, T.; Tsujihata, M.; Kinoshita, I.; Satoh, A.; Tomita, I.; Shirabe, S.; Shibayama, K.; Nagataki, S. Plasmapheresis in treatment of human T-lymphotropic virus type-I associated myelopathy. *Lancet*, **1988**, *ii*, 1109-1113.

[19] Ward, A.; Clissold, S.P. Pentoxifylline: a review of its pharmacodynamic and pharmacokinetic properties, and its therapeutic efficacy. *Drug*, **1987**, *34*, 50-97.

[20] Panettieri, Jr., R.A.; Lazaar, A.L.; Puré, E.; Albelda, S.M. Activation of cAMP-dependent pathways in human airway smooth muscle cells inhibits TNF-α-induced ICAM-1 and VCAM-1 expression and lymphocyte adhesion. *J. Immunol.*, **1995**, *154*, 2358-2365.

[21] Tsujino, A.; Nakamura, T.; Nishiura, Y.; Shirabe, S.; Furuya, T.; Goto, H.; Kawakami, A.; Eguchi, K.; Nagataki, S. Pentoxifylline down-regulates adhesion molecule expression and inflammatory cytokine production in cultured peripheral blood mononuclear cells from patients with HTLV-I-associated myelopathy. *J. Neuroimmunol.*, **1997**, *73*, 191-196.

[22] Shirabe, S.; Nakamura, T.; Tsujino, A.; Nishiura, Y.; Furuya, T.; Goto, H.; Suenaga, A.; Nakane, S.; Yoshimura, T.; Nagataki, S. Successful application of pentoxifylline in the treatment of HTLV-I associated myelopathy. *J. Neurol. Sci.*, **1997**, *151*, 97-101.

[23] 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.

[24] Fujimoto, T.; Nakamura, T.; Furuya, T.; Nakane, S.; Shirabe, S.; Kambara, C.; Hamasaki, S.; Yoshimura, T.; Eguchi, K. Relationship between the clinical efficacy of pentoxifylline treatment and elevation of serum T helper type 2 cytokine levels in patients with human T-lymphotropic virus type I-associated myelopathy. *Intern. Med.*, **1999**, *38*, 717-721.

[25] 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.

[26] Izumo, S.; Usuku, K.; Osame, M.; Machigashira, K.; Johnosono, M.; Nakagawa, M. In *HTLV-I and the nervous system*; Roman, G.C.; Vernant, J-C.; Osame, M.; Eds.; Alan R Liss: New York, **1989**, pp. 261-267.

[27] Weiner, H.L.; Hafler, D.A. Immunotherapy of multiple sclerosis. *Ann. Neurol.*, **1988**, *23*, 211-222.

[28] Willenborg, D.; Parish, C. Inhibition of allergic encephalomyelitis by sulphated polysaccharides (abstracvt). *J. Neuroimmunol.*, **1987**, *16*, 182183.

[29] Nagasato, K.; Nakamura, T.; Ichinose, K.; Nishiura, Y.; Ohishi, K.; Shibayama, K.; Watanabe, H.; Tsujihata, M.; Nagataki, S. Heparin treatment in patients with human T-lymphotropic virus type I (HTLV-I)-associated myelopathy: a preliminary study. *J. Neurol. Sci.*, **1993**, *115*, 161-168.

[30] Nimmerjahn, F.; Ravetch, J.V. Anti-inflammatory actions of intravenous immunoglobulin. *Annu. Rev. Immunol.*, **2008**, *26*, 513-533.

[31] Kuroda, Y.; Takashima, H.; Ikeda, A.; Endo, C.; Neshige, R.; Kakigi, R.; Shibasaki, H. Treatment of HTLV-I-associated myelopathy with high-dose intravenous gammaglobulin. *J. Neurol.*, **1991**, *238*, 309-314.

[32] Kataoka, A.; Imai, H.; Inayoshi, S.; Tsuda, T. Intermittent high-dose vitamin C therapy in patients with HTLV-I associated myelopathy. *J. Neurol. Neurosurg. Psychiatr.*, **1993**, *56*, 1213-1216.

[33] Sugimoto, M.; Matsumoto, M.; Imamura, F.; Nakashima, H.; Uyama, E.; Araki, S. Effects of erythromycin on lymphocyte function in patients with HTLV-I-associated myelopathy. *Jpn. J. Inflammation*, **1990**, *10*, 387-391.

[34] Yamano, Y.; Machigashira, K.; Ijichi, S.; Usuku, K.; Kawabata, M.; Arimura, K.; Osame, M. Alteration of cytokine levels by fosfomycin and prednisolone in spontaneous proliferation of cultured lymphocytes from patients with HTLV-I-associated myelopathy (HAM/TSP). *J. Neurol. Sci.*, **1997**, *151*, 163-167.

[35] Amsden, G.W. Anti-inflammatory effects of macrolides--an underappreciated benefit in the treatment of community-acquired respiratory tract infections and chronic inflammatory pulmonary conditions? *J. Antimicrob. Chemother.*, **2005**, *55*,10-21.

[36] Alvarez-Olmos, M.I.; Oberhelman, R.A. Probiotic agents and infectious diseases: a modern perspective on a traditional therapy. *Clin. Infect. Dis.*, **2001**, *32*, 1567-1576.

[37] Matsuzaki, T.; Saito, M.; Usuku, K.; Nose, H.; Izumo, S.; Arimura, K.; Osame, M. A prospective uncontrolled trial of fermented milk drink containing viable *Lactobacillus casei* strain Shirota in the treatment of HTLV-I associated myelopathy/tropical spastic paraparesis. *J. Neurol. Sci.*, **2005**, *237*, 75-81.

[38] Fujihara, K.; Itoyama, Y.; Yu, F.; Kubo, C.; Goto, I. Cellular immune surveillance against HTLV-I infected T lymphocytes in HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). *J. Neurol. Sci.*, **1991**, *105*, 99-107.

[39] Yu, F.; Itoyama, Y.; Fujihara, K.; Goto, I. Natural killer (NK) cells in HTLV-I-associated myelopathy/tropical spastic paraparesis-decrease in NK cell subset populations and activity in HTLV-I seropositive individuals. *J. Neuroimmunol.*, **1991**, *33*, 121-128.

[40] Vannucchi, S.; Chiantore, M.V.; Mangino, G.; Percario, Z.A.; 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.

[41] Kovarik, P.; Sauer, I.; Schaljo, B. Molecular mechanisms of the anti-inflammatory functions of interferons. *Immunobiology*, **2008**, *212*, 895-901.

[42] Borden, E.C.; Sen, G.C.; Uze, G.; Silverman, R.H.; Ransohoff, R.M.; Foster, G.R.; Stark, G.R. Interferons at age 50: past, current and future impact on biomedicine. *Nat. Rev. Drug Discov.*, **2007**, *6*, 975-990.

[43] 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.

[44] 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.

[45] Azimi, N.; Jacobson, S.; Leist, T.; Waldmann, T.A. 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-15Rβ receptor. *J. Immunol.*, **1999**, *163*, 4064-4072.

[46] Jacobson, S. Immunopathogenesis of human T cell lymphotropic virus type I-associated neurologic disease. *J. Infect. Dis.*, **2002**, *186*(Suppl 2), S187-192.

[47] Nagai. M.; Kubota, R.; Greten, T.F.; Schneck, J.P.; Leist, T.P.; 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.

[48] Yamano, Y.; Nagai, M.; Brennan, M.; Mora, C.A.; Soldan, S.S.; 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.

[49] 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-α therapy for HTLV-I-associated myelopathy/tropical spastic paraparesis. *J. Infect. Dis.*, **2004**, *189*, 29-40.

[50] 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-α. *J. Neuroimmunol.*, **2004**, *151*, 189-194.

[51] Oh, U.; Yamano, Y.; Mora, C.A.; Ohayon, J.; Bagnato, F.; Butman, J.A.; Dambrosia, J.; Leist, T.P.; McFarland, H.; Jacobson, S. Interferon-β1a therapy in human T-lymphotropic virus type I-associated neurologic disease. *Ann. Neurol.*, **2005**, *57*, 526-534.

[52] Bermel, R.A.; Rudick, R.A. Interferon-beta treatment for multiple sclerosis. *Neurotherapeutics*, **2007**, *4*, 633-646.

[53] Asquith, B.; Mosley, A.J.; Heaps, A.; Tanaka, Y.; Taylor, G.P.; McLean, A.R.; Bangham, C.R. Quantification of the virus-host interaction in human T lymphotropic virus I infection. *Retrovirology*, **2005**, *2*, 75.

[54] Wattel, E.; Vartanian, J.P.; 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.

[55] 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.

[56] Matsushita, S.; Mitsuya, H.; Reitz, M.S.; Broder, S. Pharmacological inhibition of *in vitro* infectivity of human T lymphotropic virus type 1. *J. Clin. Invest.*, **1987**, *80*, 394-400.

[57] Gout, O.; Gessain, A.; Iba-Zizen, M.; Kouzan, S.; Bolgert, F.; de The, G.; Lyon-Caen, O. The effect of zidovudine on chronic myelopathy associated with HTLV-1. *J. Neurol.*, **1991**, *238*, 108-109.

[58] Sheremata, W.A.; Benedict, B.S.; Squilacote, D.C.; Sazant, A.; DeFreitas, E. High-dose zidovudine induction in HTLV-I-associated myelopathy: safety and possible efficacy. *Neurology*, **1993**, *43*, 2125-2129.

[59] Taylor, G.P.; Hall, S.E.; Navarrete, S.; Michie C.A.; Davis, R.; Witkover, A.D.; Rossor, M.; Nowak, M.A.; Rudge, P.; Matutes, E.; Bangham, C.R.M.; Weber, J.N. Effect of lamivudine on human T-cell leukemia virus type 1 (HTLV-1) DNA copy number, T-cell phenotype, and anti-tax cytotoxic T-cell frequency in patients with

HTLV-1-associated myelopathy. *J. Virol.*, **1999**, *73*, 10289-10295.

[60] Taylor, G.P.; Goon, P.; Furukawa, Y.; Green, H.; Barfield, A.; Mosley, A.; Nose, H.; Babiker, A.; Rudge, P.; Usuku, K.; Osame, M.; Bangham, C.R.M.; Weber, J.N. Zidovudine plus lamivudine in human T-lymphotropic virus type-I-associated myelopathy: a randomized trial. *Retrovirology*, **2006**, *3*, 63.

[61] Inoue, J.; Seiki, 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.

[62] Tendler, C.L.; Greenberg, S.L.; Blattner, W.A.; Manns, A.; Murphy, E.; Fleisher, T.; Hanchard, B.; Morgan, O.; Burton, J.D.; Nelson, D.L.; Waldmann, T.A. 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.

[63] Waldmann, T.A. 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.

[64] Lehky, T.J.; Levin, M.C.; Kubota, R.; Bamford, R.N.; Flerlage, A.N.; Soldan, S.S.; Leist, T.P.; Xavier, A.; White, J.D.; Brown, M.; Fleisher, T.A.; Top, L.E.; Light, S.; McFarland, H.F.; Waldmann, T.A.; 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.

[65] Carey, N.; La Thangue, N.B. Histone deacetylase inhibitors: gathering pace. *Curr. Opin. Pharmacol.*, **2006**, *6*, 369-375.

[66] Abel, T.; Zukin, R.S. Epigenetic targets of HDAC inhibition in neurodegenerative and psychiatric disorders. *Curr. Opin. Pharmacol.*, **2008**, *8*, 57-64.

[67] 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.

[68] Lu, H.; Pise-Masison, C.A.; Linton, R.; Park, H.U.; Schiltz, R.L.; Sartorelli, V.; Brady, J.N. 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.

[69] Lezin, A.; Gillet, N.; Olindo, S.; Signaté, A.; Grandvaux, N.; Verlaeten, O.; Belrose, G.; Bittencourt, M.C.; 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.

[70] Mosley, A.J.; Meekings, K.N.; McCarthy, C.; Shepherd, D.; Cerundolo, V.; Mazitschek, R,; Tanaka, Y.; Taylor, G.P.; Bangham, C.R. Histone deacetylase inhibitors increase virus gene expression but decrease $CD8^+$ cell antiviral function in HTLV-1 infection. *Blood*, **2006**, *108*, 3801-3807.

[71] Ankri, S.; Mirelman, D. Antimicrobial properties of allicin from garlic. *Microbes. Infect.*, **1999**, *2*, 125-129.

[72] Hirsch, K.; Danilenko, M.; Giat, J.; Miron, T.; Rabinkov, A.; Wilchek, M.; Mirelman. D.; Levy, J.; Sharoni, Y. Effect of purified allicin, the major ingredient of freshly crushed garlic, on cancer cell proliferation. *Nutr. Cancer*, **2000**, *38*, 245-254.

[73] Wong, W.W.; Macdonald, S.; Langler, R.F.; Penn, L.Z. Novel synthetic organosulfur compounds induce apoptosis of human leukemic cells. *Anticancer Res.*, **2000**, *20*, 1367-1374.

[74] Lawson, L.D.; Wang, Z.J. Pre-hepatic fate of the organosulfur compounds derived from garlic (*Allium sativum*). *Planta Med.*, **1993**, *59*(Suppl.), 688-689.

[75] Freeman, F.; Kodera, Y. Garlic chemistry – stability of *S*-(2-propenyl) 2-propene-1-sulfinothioate (allicin) in blood, solvents, and simulated physiological fluids. *J. Agric. Food Chem.*, **1995**, *43*, 2332-2338.

[76] Fujiwara, M.; Watanabe, H.; Matsui, K. "Allithiamine" a newly found derivative of vitamin B1. I. discovery of allithiamine. *J. Biochem.*, **1954**, *41*, 29-39.

[77] Matsukawa, T.; Yurugi, S. On the structure of allithiamine. *Proc. Jpn. Acad.*, **1952**, *28*, 146-149.

[78] Matsutani, H.; Ueda, S.; Yodoi, J. The thioredoxin system in retroviral infection and apoptosis. *Cell Death Differ.*, **2005**, *12*, 991-998.

[79] Lillig, C.H.; Holmgren, A. Thioredoxin and related molecules-from biology to health and disease. *Antioxid. Redox Signal.*, **2007**, *9*, 25-47.

[80] Teshigawara, K.; Maeda, M.; Nishino, K.; Nikaido, T.; Uchiyama, T.: Tsudo, M.; Wano, Y.; Yodoi, J. Adult T cell leukemia cells produce a lymphokine that augments interleukin 2 receptor expression. *J. Mol. Cell. Immunol.*, **1985**, *2*, 17-26.

[81] Tagaya, Y.; Maeda, Y.; Mitsui, A.; Kondo, N.; Matsui, H.; Hamuro, J.; Brown, N.; Arai, K.; Yokota, T.; Wakasugi, H.; Yodoi, J. ATL-derived factor (ADF), an IL-2 receptor/Tac inducer homologous to thioredoxin; possible involvement of dithiol-reduction in the IL-2 receptor induction. *EMBO J.*, **1989**, *8*, 757-764.

[82] Nakamura, H.; Nakamura, K.; Yodoi, J. Redox regulation of cellular activation. *Annu. Rev. Immunol.*, **1997**, *15*, 351-369.

[83] Ichijo, H.; Nishida, E.; Irie, K.; Ten Dijke, P.; Saitoh, M.; Moriguchi, T.; Takagi, M.; Matsumoto, K.; Miyazono, K.; Gotoh, Y. Induction of apoptosis by ASK1, a mammalian MAPKKK that activates SAPK/JNK and p38 signaling pathways. *Science*, **1997**, *275*, 90-94.

[84] Hayakawa, T.; Matsuzawa, A.; Noguchi, T.; Takeda, K.; Ichijo, H. The ASK1-MAP kinase pathways in immune and stress responses. *Microbes Infect.*, **2006**, *8*, 1098-1107.

[85] Saitoh, M.; Nishitoh, H.; Fujii, M.; Takeda, K.; Tobiume, K.; Sawada, Y.; Kawabata, M.; Miyazono, K.; Ichijo, H. Mammalian thioredoxin is a direct inhibitor of apoptosis signal-regulating kinase (ASK) 1. *EMBO J.*, **1998**, *17*, 2596-2606.

[86] Tobiume, K.; Saitoh, M.; Ichijo, H. Activation of apoptosis signal-regulating kinase 1 by the stress-induced activating phosphorylation of pre-formed oligomer. *J. Cell. Physiol.*, **2002**, *191*, 95-104.

[87] Blattacharyya, A.; Pathak, S.; Basak, C.; Law, S.; Kundu, M.; Basu, J. Execution of macrophage apoptosis by *mycobacterium avium* through apoptosis signal-regulating kinase 1/p38 mitogen-activated protein kinase signaling and caspase 8 activation. *J. Biol. Chem.*, **2003**, *278*, 26517-26525.

Received: October 29, 2008 **Revised: February 20, 2009** **Accepted: February 23, 2009**

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Neuropathologica

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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Folia Neuropathol 2009; 47 (2): 182-194

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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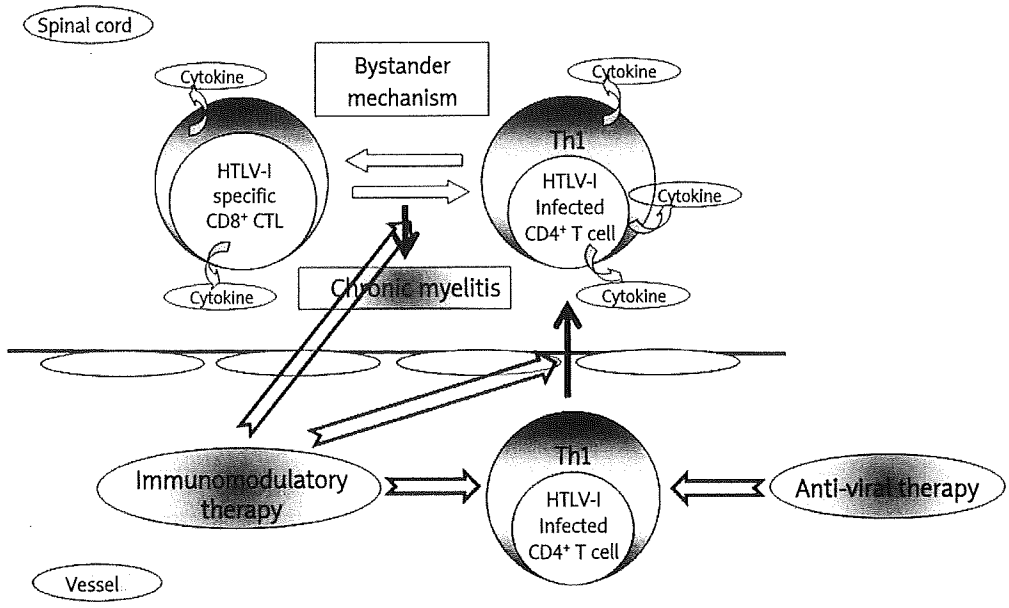

Fig. 1. Immunopathogenesis of and therapeutic strategies in HAM/TSP. The bystander mechanism, such as the destruction of surrounding tissues by inflammatory cytokine expression, during the interaction between HTLV-I-infected $CD4^+$ T cells and HTLV-I-specific $CD8^+$ CTL, is probably critical as the 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