

Human T-cell lymphotropic virus type I and neurological diseases

Masahiro Nagai and Mitsuhiro Osame

Third Department of Internal Medicine, Faculty of Medicine, Kagoshima University, Kagoshima, Japan

Human T-cell lymphotropic virus type I (HTLV-I) infection is associated with a variety of human diseases. In particular, there are two major diseases caused by HTLV-I infection. One is an aggressive neoplastic disease called adult T-cell leukemia (ATL), and another is a chronic progressive inflammatory neurological disease called HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP). It is still unknown why one virus causes these different diseases. With regard to HAM/TSP, virus-host immunological interactions are an considered to be important cause of this disease. Coexisting high HTLV-I proviral load and HTLV-I-specific T cells (CD4+ T cells and CD8+ T cells) is an important feature of HAM/TSP. Histopathological studies indicate the existence of an inflammatory reaction and HTLV-I-infected cells in the affected lesions of HAM/TSP. Therefore, the immune response to HTLV-I probably contributes to the inflammatory process of the central nervous system lesions in HAM/TSP patients. *Journal of NeuroVirology* (2003) 9, 228–235.

Keywords: HAM/TSP; HTLV-I; pathomechanism

Introduction

Human T-cell lymphotropic virus (HTLV) is a member of the exogenous human retroviruses that have a tropism for T lymphocytes. HTLV-I has been demonstrated to be the etiological agent in adult T-cell leukemia (ATL) and a progressive neurological disease called HTLV-I-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (Gessain *et al*, 1985; Osame *et al*, 1986). HTLV-II is another virus that is closely related to HTLV-I. Although a few case reports suggested the possibility of a relation between HTLV-2 and neurological diseases (Lehky *et al*, 1996; Silva *et al*, 2002), there has been no convincing evidence to support this. HTLV-I is estimated to infect approximately 10 million people worldwide. There are large endemic areas in southern Japan, the Caribbean, Central and West Africa, the Middle East, Melanesia, and equatorial regions of Africa. In Europe and North

America, the virus is found chiefly in immigrants from the endemic areas and in some communities of intravenous drug users. HTLV-I is transmitted via three major routes: (i) transmission from mother to child by breast feeding; (ii) transmission from male to female (more frequent than from female to male) by sexual contact; (iii) transmission by infected blood, either by blood transfusion or by the contaminated needles among drug abusers. Within the endemic areas, the seroprevalence varies between 1% and 20%. In contrast to the human immunodeficiency virus (HIV), the vast majority of HTLV-I-infected individuals are clinically asymptomatic; less than 5% of infected individuals develop HAM/TSP. Clinically, HAM/TSP is characterized by muscle weakness, hyperreflexia, spasticity in the lower extremities, and urinary disturbance associated with preferential damage of the thoracic spinal cord. HTLV-I has been shown to be associated not only with HAM/TSP but also with several inflammatory diseases, such as alveolitis, polymyositis, arthritis, and Sjögren syndrome (Kubota *et al*, 2000). Although the knowledge about HTLV-I and HTLV-I-associated diseases has accumulated, the following main questions still remain unsolved. (i) Why does only a small proportion of HTLV-I-infected people develop the disease, whereas the majority of HTLV-I-infected individuals are

Address correspondence to Mitsuhiro Osame, Third Department of Internal Medicine, Faculty of Medicine, Kagoshima University, 8-35-1 Sakuragaoka, Kagoshima, Japan. E-mail: osame@m2.kufm.kagoshima-u.ac.jp

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clinically asymptomatic? (ii) What factors determine the diversity of HTLV-I-associated diseases? (iii) Why is the thoracic spinal cord in HAM/TSP preferentially damaged? Many studies have tried to address these questions and so far, virus-host immunological interactions has been suggested to play a role in the pathogenesis of HAM/TSP.

In this review, the pathomechanism of HAM/TSP will be discussed based on the histopathological, immunological, and molecular points of view.

Risk factors for HAM/TSP

The prevalence of HAM/TSP is between 0.1% and 2% of HTLV-I-infected individuals. The lifetime risk of developing this disease among carriers is estimated to be 0.23% in Japan (Kaplan *et al*, 1990). The most important risk factor for HAM/TSP is a high HTLV-I proviral load. In HAM/TSP patients, the amount of HTLV-I proviral DNA in the peripheral blood mononuclear cells (PBMCs) was remarkably (approximately 16-fold) higher compared with that in the HTLV-I-asymptomatic carriers. The prevalence of HAM/TSP rises sharply once the proviral load exceeds 1% of PBMCs. These observations suggest that a high proviral load plays an important part in the etiology of HAM/TSP. It is still unclear what influences the HTLV-I proviral load. The HTLV-I proviral loads of HTLV-I-asymptomatic carriers in the families of HAM/TSP patients were higher than those of unrelated asymptomatic carriers (Nagai *et al*, 1998). These data suggest that genetic factors may influence the HTLV-I proviral load. Jeffery *et al* (1999, 2000) found evidence that human leukocyte antigen (HLA)-A*02 and Cw*08 were associated with a lower HTLV-I proviral load and low risk of developing HAM/TSP, whereas expression of HLA-B*5401 was associated with higher proviral load and an increased risk of developing HAM/TSP. HLA-A2 has been known as a strong binder of HTLV-I Tax peptide. As HLA class II, the existence of HLA-DRB1*0101 also increased the risk of HAM/TSP (Usuku *et al*, 1988; Jeffery *et al*, 1999). Moreover, the promoter tumor necrosis factor (TNF) ~863A allele predisposed to HAM/TSP, whereas stromal cell-derived factor 1 (SDF-1) +801A 3'untranslated region (UTR), and interleukin (IL)-15 191C alleles conferred protection (Vine *et al*, 2002). With regards to ATL, it has been reported that allele frequencies of HLA-A*26, -B*4002, -B*4006, and -B*4801 were significantly higher in ATL patients than those in HAM/TSP patients and HTLV-I-asymptomatic carriers (Yashiki *et al*, 2001). Interestingly, HTLV-I Tax peptide sequence completely lacks anchor motifs for binding to HLA-A*26, -B*4002, and -B*4006 molecules.

Focusing on the virus side as a risk factor of HAM/TSP, the existence of specific HTLV-I variants that may be related to HAM/TSP has been suspected. Furukawa *et al* (2000) analyzed the HTLV-I *tax* sequence of HAM/TSP patients and HTLV-I-

asymptomatic carriers. Their study demonstrated that there were four nucleotide substitutions in the HTLV-I *tax* gene that were associated with a higher risk of development of HAM/TSP (Furukawa *et al*, 2000). It is unclear how this HTLV-I *tax* variant is directly associated with the pathogenesis of HAM/TSP. The HTLV-I Tax protein is a strong transactivator of many host genes, including inflammatory cytokines and their receptors, and is a dominant epitope recognized by HTLV-I-specific CD8+ T cells. It is possible that a variation HTLV-I *tax* alters a number of host immune functions that are associated with disease progression.

Histopathologic features in HAM/TSP

Histopathological findings of HAM/TSP central nervous system (CNS) tissue have demonstrated that the affected site was predominantly the spinal cord, especially the thoracic region. Damage is most severe in the middle to lower thoracic regions. These findings are consistent with a patient's neurological symptoms such as spastic paraparesis of the lower limbs. There is degeneration of the lateral corticospinal tract as well as of the spinocerebellar or spinothalamic tract of the lateral column (Izumo *et al*, 1992). In parallel with the clinical findings, damage to the anterior and posterior columns is more variable and less extensive compared with the damage to the lateral column. These lesions are associated predominantly with perivascular and parenchymal T-cell (CD4+ and CD8+ T cells) infiltration and the presence of macrophages, proliferation of astrocytes, and fibrillary gliosis (Umehara *et al*, 1993). There is also widespread loss of myelin and axons. Early axon damage was also demonstrated using immunoreactivity for μ -amyloid precursor protein, which is a sensitive marker for the impairment of first axon transport (Umehara *et al*, 2000).

A nonrandom distribution of affected regions was suggested by an autopsy study that showed that the regions mainly affected are the so-called 'watershed' zones of the spinal cord in HAM/TSP patients (Izumo *et al*, 1992). Moreover, perivascular inflammatory infiltration was seen in the brain (deep white matter and in the marginal area of the cortex and white matter) of HAM/TSP patients, and the types of infiltrating cells were similar both in the spinal cord and brains (Aye *et al*, 2000). A magnetic resonance imaging study has also showed increased abnormal-intensity lesions in the brain (white matter) of HAM/TSP patients (Kira *et al*, 1991).

The proportion of infiltrating cells were altered by the duration of the disease. HAM/TSP patients with short duration (2.5 to 4.5 years) of illness showed an even distribution of CD4+ cells, CD8+ cells, and macrophages. Proinflammatory cytokines such as IL-1 μ , TNF- μ , and interferon- μ (IFN- μ) were also detected in perivascular infiltrating cells (Umehara *et al*, 1994a). In striking contrast, patients with

long duration (8 to 10 years) of illness showed predominance of CD8+ cells over CD4+ cells, and proinflammatory cytokine expressions were down-regulated (Umehara *et al*, 1994). In addition, monocyte/macrophage recruitment and activation were also down-regulated depending on the duration of illness (Abe *et al*, 1999). These studies suggest that immune responses in the spinal cord lesions in HAM/TSP patients gradually change concomitantly with the duration of illness.

Histopathological studies suggested that the inflammatory process in the CNS is involved in the pathogenesis of HAM/TSP. Therefore, it is important to determine which cells might be targets of HTLV-I-specific T cells in the CNS. Using semiquantitative polymerase chain reaction (PCR), HTLV-I pX and pol DNA were found to be increased in the thoracic cord lesions where CD4+ cells predominated (Kubota *et al*, 1994). The amounts of HTLV-I DNA were decreased concomitant with the number of infiltrating CD4+ cells in the spinal cord lesions of HAM/TSP patients with long duration of illness. HTLV-I DNA was detected in infiltrating UCHL-1-positive cells by *in situ* PCR technique (Matsuoka *et al*, 1998), and HTLV-I tax RNA was also detected in infiltrating CD4+ T lymphocytes in active lesions in CNS specimens from HAM/TSP patients using *in situ* hybridization technique (Moritoyo *et al*, 1996). Collectively, these findings suggest that the main harbor of HTLV-I may be infiltrating CD4+ T lymphocytes. However, there are some controversial reports that showed that HTLV-I tax RNA was localized within the neural tissue (some of them were astrocytes), but not in perivascular infiltrating cells (Lehky *et al*, 1995). The reasons for the difference are not clear, but they may be related to the variations in samples or detection methods.

It is obvious that HTLV-I exists in CNS tissue in HAM/TSP patients regardless of whether resident CNS cells are infected or not. How does HTLV-I cause damage to CNS cells? It has been speculated that HTLV-I-infected T cells that have migrated may participate in a number of events leading to viral infection of resident CNS cell populations, activation of astrocytes and microglial cells, induction of proinflammatory cytokine and chemokine, recruitment of inflammatory infiltrates into the CNS, blood-brain barrier disruption, dysregulation of oligodendrocyte homeostasis, demyelination, and axonal degradation (Grant *et al*, 2002). It has been demonstrated that uptake of extracellular glutamate by astrocytes was significantly decreased after transient contact with HTLV-I-infected T cells, and recombinant HTLV-I Tax protein and TNF- μ also decreased glutamate uptake by astrocytes *in vitro* (Szymocha *et al*, 2000). These results suggested that HTLV-I-encoded protein and cytokines produced by HTLV-I-infected T cells may impair the ability of astrocytes and affect neuronal and oligodendrocytic functions and survival *in vivo*.

Migration and accumulation of HTLV-I-infected cells into the CNS

Histopathological studies demonstrated the existence of HTLV-I-infected cells in the CNS of HAM/TSP. How do the HTLV-I-infected cells migrate into the CNS, especially into the thoracic cord selectively? HTLV-I proviral load in cerebrospinal fluid (CSF) from HAM/TSP patients were significantly higher than that of the matched PBMCs (Nagai *et al*, 2001). In addition, HTLV-I-infected lymphocytes shared the same HTLV-I integration site in cellular DNA in both the CSF and peripheral blood from HAM/TSP patients (Cavrois *et al*, 2000). Experimental observations in the CSF are thought to better reflect events in the CNS than can be achieved by analysis of peripheral blood (Mor and Cohen, 1992). Collectively, these studies indicated that HTLV-I-infected cells in peripheral blood migrated to the affected CNS lesions and accumulated in the lesions in HAM/TSP patients (Figure 1). It has been considered that adhesion molecule expressions were necessary in order for the cells to migrate to targeted tissues. The spinal cord lesions in HAM/TSP patients have greater vascular cell adhesion molecule-1 (VCAM-1) expression on endothelium compared with those of controls (Umehara *et al*, 1996). Expression of very late antigen-4 (VLA-4) and monocyte chemoattractant protein-1 (MCP-1) was up-regulated on perivascular infiltrating cells in inflammatory lesions in HAM/TSP patients. Moreover, matrix metalloproteinase (MMP)-2 and MMP-9, which were important mediators for degradation of vascular basement membrane in the transmigration of lymphocytes to the tissues, are also expressed on infiltrating mononuclear cells with disruption of endothelium (Umehara *et al*, 1998). It has also been reported that the transmigrating activity through reconstituted basement membrane *in vitro* of CD4+ T cells from HAM/TSP patients was significantly increased compared to that of HTLV-I-asymptomatic carriers (Furuya *et al*, 1997). However, it is still unknown by which mechanism HTLV-I-infected cells selectively migrate to the affected lesions. One possibility was suggested by the observation that splicing variants of CD44 (v6 variants) were highly expressed in PBMCs (especially CD4+ cells) from HAM/TSP, and that some CD44 v6 variant-positive cells were infected with HTLV-I as detected by *in situ* PCR (Matsuoka *et al*, 2000). CD44 is a multifunctional cell adhesion molecule known as a lymphocyte homing receptor. In spinal cord lesions of HAM/TSP autopsy samples, CD44 v6 variants and CD4 double-positive cells were detected.

Abnormal T cells responses in HAM/TSP

The histopathology of HAM/TSP indicates that immune mechanism may play a significant role in the

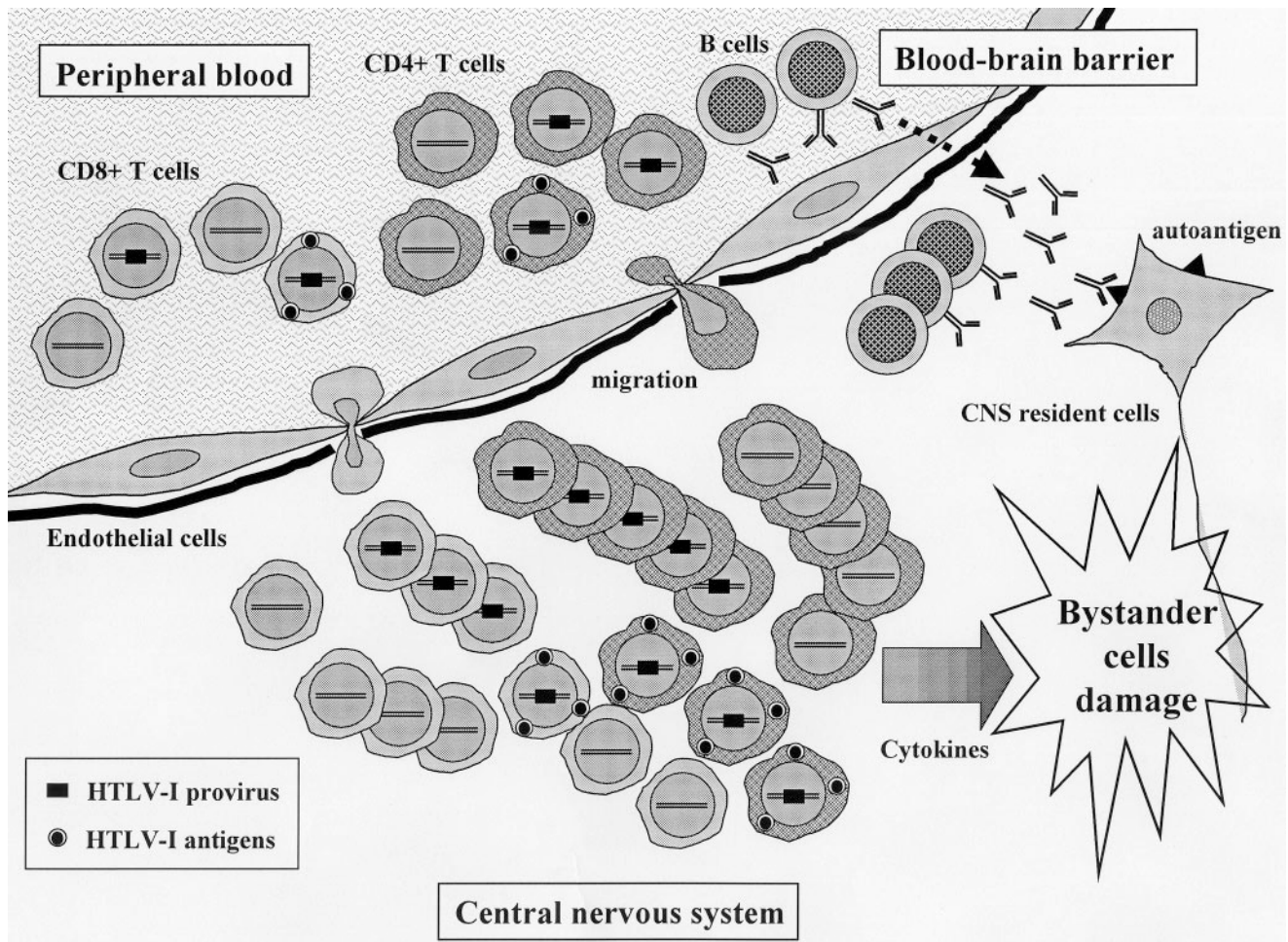


Figure 1 Postulated immunopathogenesis in HAM/TSP. Activated T cells that may be HTLV-I-infected T cells (CD4+ and CD8+ T cells) and antigen-specific T cells (CD4+ and CD8+ T cells) migrate across the blood-brain barrier (BBB) from peripheral blood to the central nervous system (CNS) and accumulate in the lesions. A portion of these HTLV-I-infected cells in the CNS express HTLV-I antigens. T-cell immune responses recognize these HTLV-I antigen-expressing cells. HTLV-I-specific T cells (CD4+ and CD8+ T cells) can result in the lysis of infected target cells or the release of a cascade of chemokines and proinflammatory cytokines. These molecules may also be produced by inflammatory HTLV-I-infected CD4+ and CD8+ T cells. Cytokines may help bystander T cells to expand and proinflammatory cytokines such as IFN- μ and TNF- μ may damage resident CNS cells such as glia and neurons. Autoimmune mechanism is also postulated. Immunoglobulin specific to HTLV-I Tax, which may pass through the BBB or be produced by B cells in CNS, cross-react with autoantigens in resident CNS cells (heterogeneous nuclear ribonuclear protein-A1 [hnRNP-A1] is a candidate antigen), and the antibody would then induce damage to the neurons.

pathogenesis of HAM/TSP. Many studies have demonstrated that both cellular and humoral immune responses are increased in HAM/TSP patients compared to that in HTLV-I-asymptomatic carriers and HTLV-I-seronegative controls. In particular, abnormality of T-cell (both CD4+ and CD8+ T cells) function has been highlighted.

The CD4+ T-cell response to HTLV-I is important in the immunopathogenesis of HAM/TSP. CD4+ T cells are the main subset of cells infected with HTLV-I *in vivo* (Richardson *et al*, 1990) and HTLV-I-infected CD4+ T cells spontaneously secrete proinflammatory cytokines, such as IFN- μ and TNF- μ (Hanon *et al*, 2001). HTLV-I-infected CD4+ T cells have increased adhesion activity to endothelial cells and transmigration activity through basement mem-

brane (Nakamura *et al*, 2000). These findings suggest that increased transmigration activity and production of neurotoxic cytokines by HTLV-I-infected CD4+ T cells might be involved in the early induction of the inflammatory process in the CNS of HAM/TSP (Figure 1). HTLV-I-specific CD4+ T cells are also important components of this inflammatory process. It has been recently reported that frequencies of HTLV-I Env- and Tax-specific CD4+ T cells in HAM/TSP patients were significantly higher than that in HTLV-I-asymptomatic carriers, and that the Env- and Tax-specific CD4+ T cells produced IFN- μ but not IL-4 (Goon *et al*, 2002). Thus, HTLV-I-specific CD4+ T cells as well as HTLV-I-infected CD4+ T cells in HAM/TSP patients deviated toward the Th1 phenotype.

HTLV-I-specific HLA class I-restricted CD8+ cytotoxic T lymphocytes (CTLs) have been demonstrated in the PBMCs and CSF of HAM/TSP patients (Jacobson *et al*, 1990). CTL activity was predominantly restricted to products of the HTLV-I pX gene. In particular, the HTLV-I Tax11–19 peptide (LLFGYPVYV) was defined as an immunodominant epitope by HLA-A2-restricted CD8+ CTLs (Koenig *et al*, 1993; Parker *et al*, 1994). Recently, HTLV-I Tax peptide-loaded HLA-A2(*0201) dimers and tetramers were developed and used to demonstrate HTLV-I Tax-specific HLA-A2-restricted CD8+ T cells (Greten *et al*, 1998; Bieganowska *et al*, 1999). HTLV-I Tax11–19-specific CD8+ T cells from the PBMCs of HLA-A2 HAM/TSP patients were found to represent a high proportion of the total CD8+ population (Nagai *et al*, 2001). In addition, these HTLV-I Tax11–19-specific CD8+ T cells accumulated in CSF (Nagai *et al*, 2001). How are these high proportion of CD8+ T cells maintained? It has been recently reported that HTLV-I *tax* mRNA expression levels in PBMCs correlated with the amount of HTLV-I-specific CD8+ T cells (Yamano *et al*, 2001). These data suggested that HTLV-I-specific CD8+ T cells may be continuously driven by HTLV-I antigens *in vivo*. It has also been demonstrated that IL-15 plays a major role in the maintenance of HTLV-I-specific CD8+ T cells in HAM/TSP, and IL-15 mRNA expression was upregulated in the PBMCs from HAM/TSP patients (Azimi *et al*, 2001). Experimentally, CD8+ T cells (including HTLV-I-specific CD8+ T cells) have dominantly expanded in spontaneous lymphoproliferation, which was defined as spontaneous lymphoproliferation of PBMCs in the absence of exogenous antigens or stimulants *in vitro*. This has become an immunological hallmark of HAM/TSP (Sakai *et al*, 2001). Moreover, both CD4+ and CD8+ T cells from HAM/TSP patients were greatly stimulated by contact with autologous dendritic cells pulsed with inactivated HTLV-I antigens as well as HTLV-I-infected dendritic cells (Makino *et al*, 1999).

To confirm the existence of CD8+ CTLs in the CNS inflammatory lesions of HAM/TSP patients, the distribution of TIA-1+ cells in the spinal cord lesions was analyzed. A monoclonal antibody, designated TIA-1, recognizes a 15-kDa granule-associated protein contained in CTLs and NK cells. Many TIA-1+ cells were distributed throughout the parenchyma and perivascular cuffs in active-chronic lesions of HAM/TSP patients, and 80% of TIA-1+ cells expressed CD8 (Umehara *et al*, 1994b). This study indicates that CD8+ CTLs, probably including HTLV-I-specific CTLs, exist in the spinal cord lesions. HTLV-I-specific CD8+ CTLs kill HTLV-I antigens expressing target cells directly by a perforin-dependent mechanism as well as by the production of a large amount of MMP-9, chemoattractants (macrophage inflammatory proteins 1 μ and

1 μ), and proinflammatory cytokines (TNF- μ and IFN- μ), which can damage CNS tissue (Biddison *et al*, 1997; Kubota *et al*, 1998). Therefore, it is reasonable to suggest that HTLV-I-specific CD8+ T cells contribute to the inflammatory process in CNS lesions of HAM/TSP, regardless of which cells (HTLV-I-infected CD4+ T cells or CNS resident glial cells) are target cells of the CD8+ T cells (Figure 1).

HTLV-I has been thought to preferentially infect CD4+ T cells *in vivo* (Richardson *et al*, 1990). However, recent studies indicated that CD8+ T cells were also infected with HTLV-I *in vivo* (Hanon *et al*, 2000; Nagai *et al*, 2001). Interestingly, a portion of HTLV-I-specific CD8+ CTLs was also infected with HTLV-I, and HTLV-I protein expression in naturally infected CD8+ T cells rendered them susceptible to cytolysis mediated by autologous HTLV-I-specific CD8+ CTLs (Hanon *et al*, 2000). These findings indicate that HTLV-I-specific CTLs become target cells of HTLV-I-specific T cells as well as effector cells against HTLV-I-infected cells. It is therefore important to clarify whether HTLV-I-infected CD8+ T cells exist in the affected CNS lesions of HAM/TSP patients.

The autoimmune mechanism has been considered as the pathomechanism behind inflammatory neurological diseases, such as multiple sclerosis (MS) and Guillain-Barré syndrome. However, it is still controversial whether it is also involved in the pathogenesis of HAM/TSP. Some studies indicate such a possibility. A unique T-cell receptor CDR3 motif, which has been demonstrated in brain lesions of MS and experimental autoimmune encephalomyelitis, was also detected in infiltrating lymphocytes in the spinal cord of HAM/TSP patients (Hara *et al*, 1994). HTLV-I-infected CD4+ T-cell clone established from PBMCs of a HAM/TSP patient showed proliferating response to crude protein extracted from HTLV-I-seronegative spinal cord autopsy samples but not from lymph nodes (Nagai *et al*, 1996). HTLV-I Tax-specific CD8+ CTL clones recognize and lyse selfpeptide(s)-pulsed target cells *in vitro* (Hausmann *et al*, 1999). Levin *et al* (1998) provide a new evidence for the autoimmune hypothesis. Serum immunoglobulin (IgG) from HAM/TSP patients reacted to neurons in HTLV-I-uninfected human CNS but not to cells in the peripheral nervous system or other organs. This reactivity was abrogated by pretreatment with recombinant HTLV-I Tax protein (Levin *et al*, 1998). It has been recently demonstrated that this antibody reacted to heterogeneous nuclear ribonuclear protein-A1 (hnRNP-A1) as the autoantigen. This antibody specifically stained human Betz cell, and infusion of autoantibodies in brain sections inhibited neuronal firing (Levin *et al*, 2002). These data suggest that molecular mimicry between HTLV-I and autoantigens in CNS might be involved in the pathogenesis of HAM/TSP (Figure 1).

Conclusion

Several risk factors for HAM/TSP have been clarified. High HTLV-I proviral loads are an important risk factor in the development of this disorder. The recent demonstration of disease-specific HTLV-I gene (*tax*) sequences also served to highlight the importance of the virus in HAM/TSP. Accumulation of knowledge about risk factors for HAM/TSP should enable us to predict the risk of progression to HAM/TSP from HTLV-I-asymptomatic carriers. Histopathological studies indicate that an inflammatory T-cell

process is closely associated with HAM/TSP. The abnormal immune responses to HTLV-I in patients with HAM/TSP has already been demonstrated. Therefore, HAM/TSP pathogenesis may pivot around virus-specific immune responses. CD8+ T cells were also found to be HTLV-I reservoirs in addition to CD4+ T cells. Both HTLV-I-specific CD4+ T cells and HTLV-I-specific CD8+ T cells secrete proinflammatory cytokines that might be damaging to CNS tissue. Intensive studies regarding HTLV-I-infected cells and HTLV-I-specific T cells will clarify the pathogenesis of HAM/TSP.

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