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IMMUNOPATHOGENSIS OF HTLV-I ASSOCIATED NEUROLOGIC DISEASE: MOLECULAR, HISTOPATHOLOGIC, AND IMMUNOLOGIC APPROACHES

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1. ABSTRACT

Human T-cell lymphotropic virus type I (HTLV-I) infection is associated with a variety of human diseases including HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP), a chronic progressive inflammatory neurological disease. An important risk factor for the development of HAM/TSP is thought to be a high HTLV-I proviral load. Histopathological studies have demonstrated the presence of HTLV-I virus in the affected areas of spinal cords from HAM/TSP patients. Furthermore, T-cell infiltrations have been shown in spinal cord lesions. The precise mechanism for disease development is still unknown. Virus-host immune interactions are considered to play an important role in disease pathogenesis. This review focuses on current molecular, histopathological, and immunological approaches to understand the immunopathogenesis of HAM/TSP.

2. INTRODUCTION

Human T-cell lymphotropic virus type I (HTLV-I) is a member of the exogenous human retroviruses that have tropism for T-lymphocytes. HTLV-I is the causative agent for adult T-cell leukemia (ATL) and a progressive neurological disease known as HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP) (1, 2). The clinical and laboratory guidelines for the diagnosis of HAM/TSP have been formulated based on the recommendation of the World Health Organization guidelines (3, 4). 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 also shown to be associated with several other inflammatory diseases, such as alveolitis, polymyositis, arthritis, uveitis, and Sjogren syndrome. There are also less certain associations with chronic infective dermatitis, Bechet disease, pseudohypoparathyroidism, and systemic lupus erythematosus (5). Another closely related virus, human T-cell lymphotropic virus type II (HTLV-II), has also been implicated in development of neurological disorders (6-8).

It is estimated that approximately 10-20 million people worldwide are infected with HTLV-I (9-11). Endemic areas of HTLV-I are in southern Japan, Central and West Africa, the Caribbean, Central and South America, the Middle East, Melanesia (12, 13), and there are also smaller foci in the aboriginal populations of Australia, Papua New Guinea, and northern Japan. In Europe and North America, the virus is found chiefly in immigrants from these endemic areas and in some communities of intravenous drug users. Within the endemic areas, the seroprevalence varies from 1% to 20%. In contrast to the human immunodeficiency virus (HIV), the majority of infected persons remain asymptomatic and only 5% will develop HAM/TSP or ATL (1, 2). While a significant number of molecular, histopathological, and immunological studies have been reported regarding HTLV-I and HTLV-I

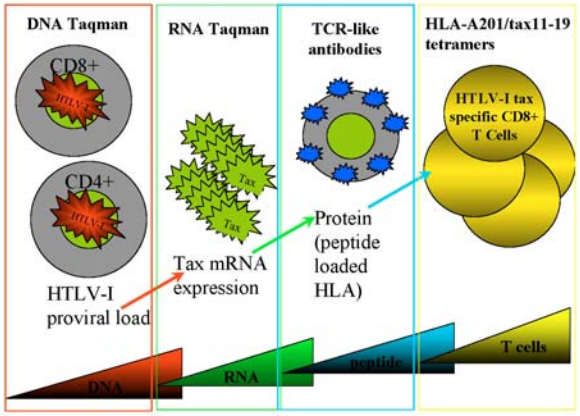

Figure 1. Schematic of induction of HTLV-I specific CD8+ T cells responses associated with immunopathogenesis of HAM/TSP. Quantitative PCR demonstrates high HTLV-I proviral loads in HAM/TSP patients that are directly proportional to increased HTLV-I mRNA expression. Elevated HTLV-I mRNA expression leads to increases of HTLV-I protein expression. This protein can be processed into immunodominant peptides such as Tax11-19 peptide. HTLV-I Tax11-19 peptide strongly binds to HLA-A*0201 molecules and stimulate a virus-specific CD8+ T cells response. This Tax11-19/HLA-A*0201 complex can be detected by TCR-like antibody and frequency of virus-specific CD8+ T cells can be determined by HLA-A*0201/Tax11-19 tetramer. These antigen-specific responses are expanded in the CSF of HAM/TSP patients and may contribute to disease progression by recognition of HTLV-I-processed antigens in the CNS associated with lysis of HTLV-I-infected inflammatory cells or HTLV-I-infected glial cells and/or through induction of proinflammatory cytokines and chemokines.

associated disorders, it is still unknown why only a small proportion of HTLV-I infected individuals develop disease.

HTLV-I is transmitted via three major routes: (i) transmission from mother to child through breast milk; (ii) transmission by sexual contact (mainly from male to female); (iii) transmission by way of infected blood through blood transfusion or contaminated needle usage. Although HTLV-I can infect a wide range of vertebrate cells *in vitro* (14), it has been thought to preferentially infect CD4+ T-cells *in vivo* (15). Therefore, CD4+ T-cells have been considered an important component contributing to the inflammatory process in HAM/TSP. Recently, however, several studies have indicated that CD8+ T-cells were also infected with HTLV-I *in vivo* (16, 17). The mechanisms through which HTLV-I causes HAM/TSP are not completely understood, although virus-host immune interactions have been suggested to play a role in the pathogenesis of the disorder.

3. RISK FACTORS FOR DEVELOPMENT OF HAM/TSP

The prevalence of developing HAM/TSP is between 0.1% and 3% among HTLV-I infected individuals (18). A number of risk factors have been suggested to contribute to the disease development. A high proviral load has been shown to be an important risk factor, where patients with HAM/TSP have significantly higher proviral load as compared to HTLV-I infected asymptomatic carriers (19, 20). Other known risk factors including gender and host genetic factors have also been associated with disease development. (21-25).

3.1. Variants of HTLV-I in HAM/TSP

HTLV-I is classified phylogenetically into three major lineages: Melanesian, Central African, and cosmopolitan. Cosmopolitan variants are further divided into several subgroups: transcontinental (A), Japanese (B), West African (C), and North African (D). Although a variety of HTLV-I strain variants have been isolated from HTLV-I infected individuals, it is unclear whether a specific HTLV-I variant is preferentially associated with HAM/TSP. Furukawa *et al.* analyzed the HTLV-I *tax* sequence, a gene that encodes for a transactivator protein which is essential for virus expression and plays important roles in activation of cellular genes, of HAM/TSP patients and HTLV-I asymptomatic carriers. In this study, the authors demonstrated four nucleotide substitutions in the HTLV-I *tax* gene region of cosmopolitan A subgroup. In addition, this sequence was also seen more frequently in HAM/TSP patients as compared to asymptomatic carriers (26). It is unclear what role this HTLV-I *tax* variant plays in the pathogenesis of HAM/TSP. The HTLV-I Tax protein is a strong transactivator of many host genes including inflammatory cytokines, and is also a dominant epitope recognized by HTLV-I-specific CD8+ cytotoxic T lymphocytes (CTLs) (27). It is possible that this variation in HTLV-I *tax* leads to alterations of a number of host immune functions that are associated with disease progression. Further support for the hypothesis that a particular HTLV-I strain might be a risk factor for HAM/TSP was demonstrated in a study that showed different HTLV-I variants in monozygotic twins with discordant clinical outcomes (28).

3.2. HTLV-I proviral load

An important contributing factor for HAM/TSP disease development is the HTLV-I proviral load (19, 20). Recently, a real time quantitative polymerase chain reaction (PCR) assay (TaqMan) was developed that allows HTLV-I proviral DNA to be measured in peripheral blood mononuclear cells (PBMCs). This assay was used to quantify the respective HTLV-I proviral DNA loads in HAM/TSP patients and asymptomatic carriers where a 16-fold increase in HTLV-I proviral load was observed in HAM/TSP patients compared to asymptomatic carriers (19). Similar results have also been reported in patient cohorts from Jamaica (20). The prevalence of developing HAM/TSP rises sharply once the proviral load exceeds 1% of total PBMCs (19). Collectively, these observations strongly suggest that a high proviral load plays an important role in the etiology of HAM/TSP. It has been hypothesized that a high HTLV-I proviral load results in higher viral antigen presentation that may drive expansion of antigen-specific T cells and subsequently these T cells are involved in the development of HAM/TSP. Such a model is presented in Figure 1. Most individuals infected

with HTLV-I have been shown to mount strong CTL responses to viral antigens (29, 30). It is thought that this strong CTL response functions to protect against disease development by reducing the proviral load (22). However, this increase in proviral load that results in a marked increase in HTLV-I antigen-specific CTL expansion has also been suggested to contribute to a HTLV-I specific inflammatory process seen in HAM/TSP (19, 22).

3.3. Host genetic factors

Other determinants that are associated with increased risk of developing HAM/TSP are host genetic factors. Nagai *et al*. demonstrated higher HTLV-I proviral loads in asymptomatic carriers of families with HAM/TSP patients than those of unrelated asymptomatic carriers (19). It has been reported that a higher risk of developing HAM/TSP is associated with the human leukocyte antigen (HLA)-A*02$^-$, HLA-Cw*08$^-$, HLA-DR1$^+$, and tumor necrosis factor (TNF)-863A$^-$(22-25). It is reported that HLA-A*02 and Cw*08 were associated with a lower HTLV-I proviral load and a lower risk of developing HAM/TSP. Expression of HLA-B*5401 was associated with higher proviral load and an increased risk of developing HAM/TSP (22, 23). HLA class II alleles such as HLA-DRB1*0101 have also been associated with increased risk of developing HAM/TSP (22, 24). Moreover, individuals with promoter of the TNF-863A allele were predisposed to developing HAM/TSP, whereas stromal cell-derived factor 1 (SDF-1) +801A 3'untranslated region, and interleukin (IL)-15 191C alleles conferred protection (25).

4. PATHOLOGICAL ANALYSIS IN HAM/TSP

4.1. Histopathologic features in the CNS

Pathological analysis indicates that HAM/TSP affects the spinal cord, predominantly at the thoracic level (31, 32). There is degeneration of the lateral corticospinal tract as well as of the spinocerebellar or spinothalamic tract of the lateral column. These findings are consistent with neurological symptoms such as spastic paraparesis of the lower limbs (33). 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 with perivascular and parenchymal lymphocytic infiltrations with the presence of foamy macrophages, proliferation of astrocytes, and fibrillary gliosis. There is also widespread loss of myelin and axons, particularly in the corticospinal tracts of the spinal cord (2, 34, 35). Previous neuropathological studies revealed that both myelin destruction and axonal loss are histological hallmarks of actively inflamed lesions of HAM/TSP. Particularly, axonal loss is responsible for the persistent disability characterized by spastic paraparesis and urinary disturbance. Pathological study shows that beta-amyloid precursor protein, used as a marker of early axonal damage in HAM/TSP lesions, is more intensively expressed in areas of active inflammatory lesions than those of inactive-chronic lesions (36). The proximity to the areas containing inflammation, activation of macrophage/microglia, indicates that axonal damage is closely associated with inflammation in active-chronic lesions. 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 cords and brains (37). A nonrandom distribution of affected regions was suggested by autopsy studies, which showed the regions that are mainly affected are the so-called 'watershed' zones of the spinal cord in HAM/TSP patients (33). This has partly been addressed by a magnetic resonance imaging study that showed increased abnormal-intensity lesions in the white matter of the brain of HAM/TSP patients (38). These results suggest that inflammatory changes occurred simultaneously in the spinal cord and in the brain, with the distribution of inflamed vessels closely correlated with the characteristic vascular architecture of the brain and the spinal cord, which led to a slowing of blood flow.

4.2. T-cell subsets and cytokine expression in the CNS

Histopathological studies have demonstrated T-cell infiltrations in spinal cord lesions of HAM/TSP patients. The proportion of infiltrating cells shifted with the duration of the disease. HAM/TSP patients with short duration of illness, up to 5 years after onset, showed an even distribution of CD4+ T cells, CD8+ T cells, B cells, and foamy macrophages in damaged areas of the spinal cord parenchyma (34, 39). Immunohistochemistry showed that inflammatory cytokines, including TNF-alpha, IL-1beta, and interferon-gamma (IFN-gamma) were expressed on perivascular infiltrating macrophages, astrocytes, and microglia (40). HLA class I and beta2-microglobulin were expressed on endothelial cells and infiltrating mononuclear cells (41, 42). Up regulation of HLA class II expression was also found on endothelial cells, microglia, and infiltrating mononuclear cells in the affected lesions. In contrast, in patients with duration of illness from 8 to 10 years, there was a predominance of CD8+ T cells within spinal cord lesions with a concomitant down-regulation of proinflammatory cytokine expression, with the exception of IFN-gamma (34, 40). Such lesions also express increased levels of HLA class I antigens from which HTLV-I specific CD8+ CTLs were isolated (43). Although many hematogenous macrophages could be found in the active-chronic lesions, markers of monocyte/macrophage recruitment and activation were down-regulated as the duration of illness progressed (44). These studies demonstrated that immune responses in the spinal cord lesions of HAM/TSP patients gradually change concomitantly with the duration of illness. Collectively, these histopathological studies suggested that an inflammatory process in the central nervous system (CNS) is involved in the pathogenesis of HAM/TSP.

Fas/Fas ligand (FasL) interaction regulates a major pathway in apoptosis, which may be important in mediating both the antiviral effects and the inflammatory process in neurological diseases. The level of soluble Fas was found to be increased in the sera of HAM/TSP patients compared with controls (45). Furthermore, the levels of soluble FasL were higher in both the sera (45) and the cerebrospinal fluid (CSF)(46) of HAM/TSP patients during the active stages. In addition, FasL mRNA expression was

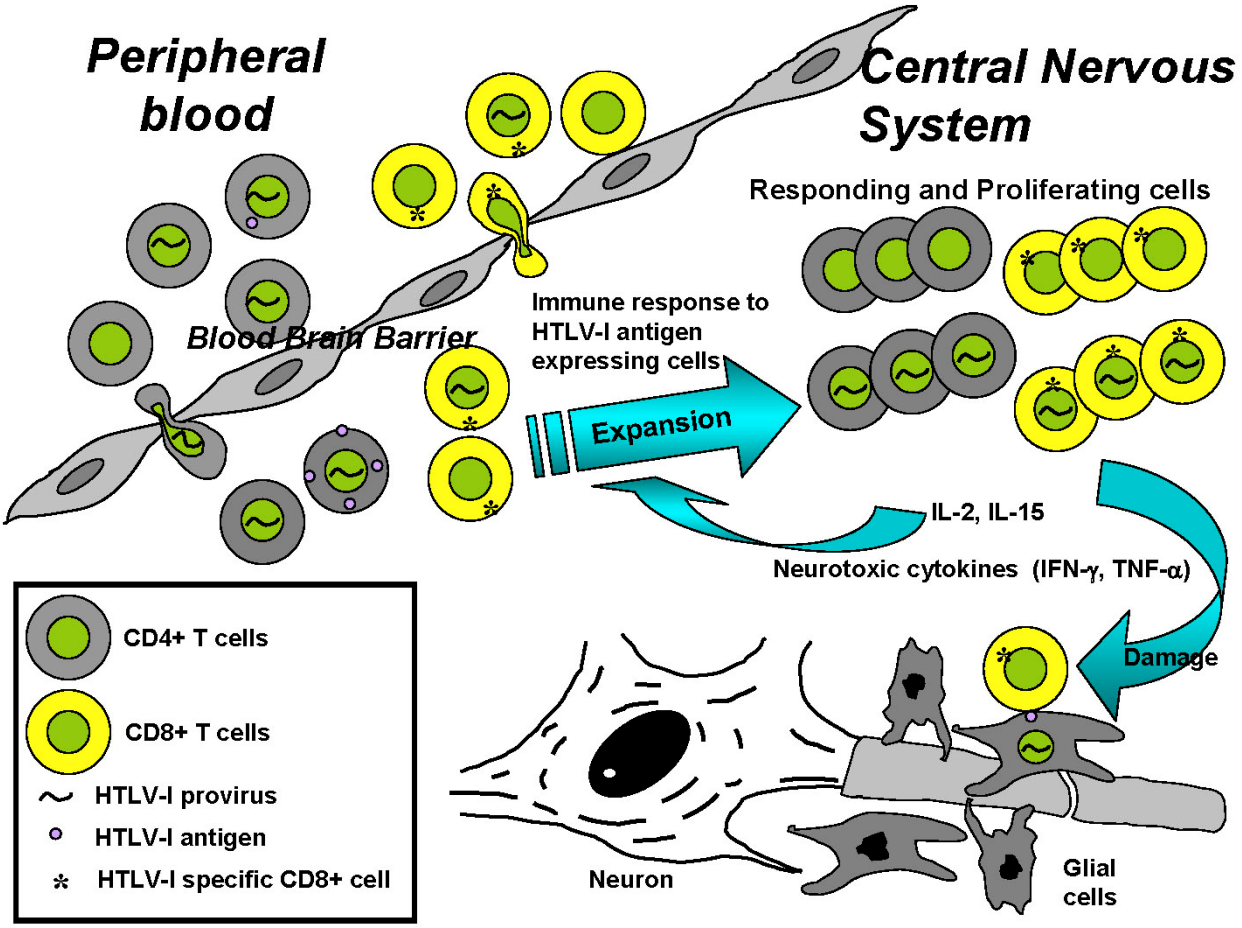

Figure 2. Model of immunopathogenesis in HAM/TSP. HTLV-I infected CD4+, CD8+, and antigen-specific T cells migrate across the blood brain barrier from the peripheral blood into the CNS. As a portion of these HTLV-I infected cells express HTLV-I antigens, antigen-specific T cells recognize these antigen-expressing cells in the CNS. By recognition, HTLV-I specific CD8+ T cells can lyse infected target cells or product proinflammatory chemokines and cytokines. Cytokines such as IL-2 and IL-15 may help bystander T cells expansion. Neurotoxic cytokines such as INF-gamma and TNF-alpha may contribute to the damage of resident glial cells and neurons in the CNS.

up-regulated in patient peripheral blood T lymphocytes (47). In the spinal cord lesions of HAM/TSP patients, Fas was shown to be preferentially expressed on infiltrating T cells (48) and FasL expression was up-regulated mainly on microglia/macrophages in active-chronic lesions. These findings suggest a pivotal role of macrophages/microglias in eliminating infiltrating T cells in the CNS of HAM/TSP. Consistent with findings that the immune response was suppressed in areas of inactive-chronic lesions, FasL expression was markedly down-regulated in lesions of HAM/TSP patients with a long duration of illness. Taken together, these data suggest that the Fas/FasL system may be an important mechanism in the modulation of immune reactions in the CNS of HAM/TSP.

4.3. CD8+ Cytotoxic T Lymphocytes in HAM/TSP Pathogenesis

To confirm the existence of CD8+ CTLs in the CNS of HAM/TSP patients, the distribution of TIA-1+ cells in spinal cord lesions was analyzed. TIA-1 (49) is a monoclonal antibody that recognizes a 15-kDa granule-associated protein contained in CTLs and NK cells. In active-chronic lesions of HAM/TSP patients, many TIA-1+ cells were distributed throughout the parenchyma and perivascular cuffs and 80% of these TIA-1+ cells also expressed CD8 (50). In contrast, TIA-1+ cells were scarcely observed in inactive-chronic lesions, even though CD8+ cells predominated in the parenchyma and perivascular cuffs. The number of TIA-1+ cells correlated with the amount of HTLV-I proviral DNA *in situ*. In active inflammatory lesions, cells undergoing apoptosis were found, most of them being identified as helper-inducer CD45RO+ T lymphocytes. Infiltrating CD8+ CTLs appeared to correlate with the presence of apoptotic CD4+ T cells in inflammatory lesions in HAM/TSP (50). Collectively, these studies support the hypothesis that HTLV-I infected CD4+ T lymphocytes enter the CNS which may drive local expansion of virus specific CD8+ CTLs. These virus-specific T cells may then either directly lyse virus infected cells and/or release a cascade of cytokines and chemokines that result in pathological changes (figure 2). It is clearly important to define which

cells might be targets of CTLs in the CNS.

HTLV-I Tax-specific CD8+ CTLs are hypothesized to play an important role in the development of HAM/TSP. Recently, through the development of tetramer technology, HTLV-I Tax-specific HLA-A*0201 restricted CD8+ T cells could be readily detected in HLA-A*0201 HAM/TSP patients. Tetrameric major histocompatibility antigen (MHC)-peptide complex cross-linked by streptavidin were shown to bind stably to antigen-specific T cells based on the increased avidity afforded by polyvalency (51) as well as tetramer divalent MHC class I constructs using Ig as a scaffold (52). These soluble peptide-HLA complexes that specifically bind the T cell receptor (TCR) have been useful for monitoring virus-specific T cells in laboratory and clinical settings. By using such tetramers, HTLV-I Tax 11-19-specific CD8+ cells from the PBMC of HLA-A*0201 HAM/TSP patients were found to represent an extraordinarily high proportion of the total CD8+ population (52, 53). Moreover, the frequency of these HTLV-I Tax11-9-specific CD8+ T cells was even higher in the CSF of HAM/TSP patients (54, 55). As we have shown that amount of antigen-specific cells are proportional to the amount of HTLV-I proviral DNA load and the levels of HTLV-I *tax* mRNA expression from PBMCs (56), the increased frequency of HTLV-I Tax11-19-specific CD8+ T cells in HAM/TSP CSF suggest continuous HTLV-I antigen presentation *in vivo* (figure 1). However, it is unclear what cells express HTLV-I antigens *in vivo* since no HTLV-I protein can be readily detected in PBMCs from HAM/TSP patients *ex vivo*. This may be reconciled with the observations that virus-specific T cells recognize antigens by engaging the antigen-specific TCR with peptide/HLA complexes displayed on the surface of antigen presenting cells (APCs). While tetramers have been useful for monitoring virus-specific T cells, there has been a shortage of reagents to study and visualize the ligand for the TCR: the peptide/HLA complex. Recently, by using large human antibody phage libraries, unique antibodies (Abs) with peptide-specific, HLA-restricted recognition patterns, novel human recombinant Fab antibodies (TCR-like Abs) that specifically bind to the HTLV-I Tax11-19 peptide/HLA-A*0201 complex have been isolated (57). These antibodies may prove to be crucial for studying MHC class I Ag presentation in healthy and disease in tissues as well as in peripheral blood.

4.4. Localization of HTLV-I in the CNS of HAM/TSP

HTLV-I *gag*, *pX*, and *pol* sequences have been localized to the thoracic cord in areas with increased CD4+ cells infiltration using semiquantitative PCR (39, 58). The amount of HTLV-I DNA decreased concomitantly with the number of infiltrating CD4+ cells in the spinal cord lesions of HAM/TSP patients having a long duration of illness. HTLV-I DNA has been localized to inflammatory infiltrating UCHL-1 positive cells in affected spinal cord by *in situ* PCR technique (59). HTLV-I *tax* mRNA was also detected in infiltrating CD4+ T lymphocytes in active lesions in CNS specimens from HAM/TSP patients using *in situ* hybridization (60). In addition, the HTLV-I p19 protein has been localized to spinal cord lesions (61). Collectively, these findings suggest that the main reservoir of HTLV-I may be infiltrating CD4+ T lymphocytes. Other cells may also harbor the virus. HTLV-I RNA has been shown to localize to astrocyes (62). Recent work using quantitative PCR (TaqMan) indicated that CD8+ T cells were also infected with HTLV-I *in vivo* (63). Using a sensitive flow cytometric technique, Hanon *et al*. also showed that in HTLV-I infection, a significant proportion of CD8+ T cells were infected with HTLV-I (16, 17). Interestingly, a portion of HTLV-I specific CD8+ CTLs were also infected with HTLV-I and HTLV-I protein expression in infected CD8+ T cells rendered them susceptible to cytolysis mediated by autologous HTLV-I specific CD8+ CTLs (16). These findings indicate that HTLV-I specific CTLs may serve a dual function as both target and effector cells.

4.5. T cell migration and accumulation in the CNS of HAM/TSP

Leukocyte adhesion molecules play an important role in the pathogenesis of many inflammatory diseases, including HAM/TSP. Romero *et al*. reported the enhanced adhesion and migration of HTLV-I infected lymphocytes to rat brain endothelial cells *in vitro* (64). Increased expression of adhesion molecules occurs in the spinal cord of HAM/TSP. The lesions have greater vascular cell adhesion molecule-1 (VCAM-1) expression on their endothelium compared with the spinal cord of controls (65). Expression of very late antigen-4 (VLA-4) and monocyte chemoattractant protein-1 (MCP-1) was up-regulated on perivascular infiltrating cells in active-chronic inflammatory lesions in HAM/TSP patients. These findings suggest that VLA-4/VCAM-1 interaction and MCP-1 is involved in mediating T-lymphocyte/macrophage adhesion and chemotaxis in the CNS inflammatory process. Intracellular adhesion molecule-1 (ICAM-1) and its counterpart molecule lymphocyte function-associated antigen-1 (LFA-1) are also suggested to be involved in massive infiltration of lymphocytes observed in the spinal cord (66). Additionally, it is reported that ICAM-1 surface expression was strongly upregulated in T cells carrying HTLV-1 and that the viral transactivator protein Tax was capable of inducing ICAM-1 gene expression (67, 68).

After transendothelial migration, T cells/macrophages encounter the extracellular matrix (ECM), pass through the basement membrane, and migrate into the interstitial matrix. Proteolytic disruption of ECM by matrix metalloproteinases (MMPs) is a key process for the damage of the blood brain barrier (BBB). MMPs have been reported to be involved in inflammatory disorders of the CNS. Activated HTLV-I-infected CD4+ T cells induced the production of metalloproteinases types 2, 3, and 9 and tissue inhibitors of metalloproteinases (TIMP) -1, -2, and -3 in human astrocytes by cytokines *in vitro* (69). Immunohistochemical studies revealed that collagen IV and decorin immunoreactivity on the basement membrane of CNS parenchymal vessels was partially disrupted in areas where inflammatory mononuclear cells infiltrated in active-chronic lesions of HAM/TSP (70). In these lesions, MMP-2 was detected mainly on the surface of foamy macrophages and lymphocytes, whereas MMP-9 expression was positive in the intravascular and perivascular mononuclear cells but not on foamy macrophages. In contrast, inactive-chronic

lesions of the spinal cords of HAM/TSP contained much smaller numbers of MMP-2 positive or MMP-9 positive mononuclear cells than active-chronic lesions. MMP-9 and TIMP-3 levels in the CSF of HAM/TSP patients were higher than those of HTLV-I carriers without neurological symptoms (71). Production levels of MMP-2 and MMP-9 in both sera and CSF were higher in HAM/TSP patients than those in other noninflammatory neurological disease controls. Using zymography, proMMP-9 was more frequently detected in the CSF of HAM/TSP patients than those in the control (70, 72). Taken together, these data indicate that MMP-2 and MMP-9 may play important roles in blood brain barrier breakdown and tissue remodeling in the CNS of HAM/TSP. *In vitro* experiments also showed a significant increase in the transmigrating activity of CD4+ T cells through reconstituted basement membrane in HAM/TSP patients in comparison to that of HTLV-I asymptomatic carriers (73). 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 patients, and that some CD44 v6 variant-positive cells were infected with HTLV-I as detected by *in situ* PCR (74). 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.

5. IMMUUNE RESPONSE IN HAM/TSP

HAM/TSP patients have a number of immunologic abnormalities including high HTLV-I proviral load, increased spontaneous lymphoproliferation, elevated anti-HTLV-I antibody titers in both sera and CSF, and increased cytokine production (19-21, 75-79). Abnormalities in cellular immune responses have also been described in HAM/TSP patients (52, 78-91). In comparison to HTLV-I infected asymptomatic carriers, these abnormalities are more often observed in patients with HAM/TSP, which suggests that immune dysregulation may be associated with pathogenesis of HTLV-I associated neuorlogic disease.

5.1. Cytokine expression in the peripheral blood and CSF

Dysregulation of immune responses such as elevated cytokine expression and production have been demonstrated in the peripheral blood and CSF of HAM/TSP patients. Increased levels of the cytokines IFN-gamma, TNF-alpha, and IL-6 have been reported in the sera and CSF (75). In peripheral blood lymphocytes (PBL) from HAM/TSP patients, mRNA for IL-1beta, IL-2, TNF-alpha, and IFN-gamma are up-regulated (76). ELISPOT assays have shown significant elevation of IL-2 and IFN-gamma in PBMCs isolated from HAM/TSP patients compared with both asymptomatic carriers and seronegative normal donors (77). Proinflammatory cytokines induced by HTLV-I Tax, a transactivator of cellular genes, have been suggested to play a role in HAM/TSP pathogenesis. It has been demonstrated that uptake of extracellular glutamate by astrocytes was significantly decreased after transient contact with HTLV-I infected T cells, recombinant HTLV-I Tax protein, and TNF-alpha. Therefore, it has been hypothesized that HTLV-I Tax and cytokines produced by HTLV-I infected T cells may alter the ability of astrocytes to manage steady-state level of glutamate, which in turn may result in impaired neuronal and oligodendrocytic functions and survival (92).

5.2. NK cell activity in patients with HAM/TSP

NK cells are believed to protect against viral invasions at an early stage of an infection before the adaptive immune response is fully activated (93). Therefore, it is conceivable that impaired NK cell activity would result in diminished control of viral infection and increase in viral replication of HTLV-I in HAM/TSP patients. NK cell activity has been reported to be significantly lower in HAM/TSP than in controls (80). In addition, NK cells from HAM/TSP patients have lower cytotoxic activity and lower antibody-dependent cell-mediated cytotoxicity than those from controls (81).

Human NK cell receptors are expressed by NK cells and also some T cells, primarily CD8+ CTLs (94). Inhibitory NK cell receptors (iNKRs) can down-regulate antigen-mediated T-cell effector functions, including cytotoxic activity and cytokine release (95, 96). It is reported that CD8+ T cells that express the NK cell inhibitory receptor were significantly decreased in HAM/TSP patients but not in asymptomatic HTLV-1 carriers (82). These receptors are suggested to play a role in regulating CD8+ T cell-mediated antiviral immune responses; therefore, a decrease in their expression may result in higher risk of developing inflammatory diseases such as HAM/TSP.

5.3. CD4+ T cell response to the virus

Although HTLV-I can infect a wide range of vertebrate cells *in vitro* (14), CD4+ T cells are the main subset of cells infected with HTLV-I *in vivo* (15). HAM/TSP patients have significantly higher frequencies of HTLV-I Env and Tax antigen specific CD4+ T cells as compared to HTLV-I infected asymptomatic carriers (87). It has been reported that CD4+ T cells in HAM/TSP patients have a more Th1-like phenotype as characterized by up-regulated secretion of proinflammatory cytokines such as IFN-gamma and TNF-alpha and down-regulated levels of Th2 cytokines such as IL4 (83-87). As suggested in the model presented in Figure 2, HTLV-I infected CD4+ T cells have increased adhesion activity to endothelial cells and transmigrating activity through basement membrane that allows migration of infected CD4+ T cells into the CNS (84). Entry into the CNS by HTLV-I CD4+ T cells that have increased production of TNF-alpha, a neurotoxic cytokine, may be responsible for the initiation inflammatory processes seen in HAM/TSP.

5.4. CD8+ T cell response to the virus

One of the most striking features of the cellular immune response in HAM/TSP patients is the highly increased numbers of HTLV-I specific HLA class I

restricted CD8+ CTLs in the PBL and CSF (29, 97). Although HTLV-I CD8+ CTLs have been detected in PBMC of some asymptomatic carriers (98), the magnitude and frequency of these responses are higher in patients with neurologic disease (99). CD8+ CTLs recognize viral and other foreign antigens, usually as small 9-aa peptides, in the context of HLA class I alleles. Although HTLV-I Env, Pol, Rof and Tof (100) could be target proteins of HTLV-I specific CTLs, HTLV-I specific CD8+ CTL activity in PBL from HAM/TSP patients is typically restricted to p27x and p40x products of the HTLV-I *tax* gene (99). In particular, the HTLV-I Tax11-9 peptide (LLFGYPVYV) has been defined as an immunodominant epitope presented in the context of HLA-A*0201 and can be recognized by CD8+ CTLs from HAM/TSP patients (101, 102). Tax11-19 conforms to a known HLA-A*0201 binding motif and has one of the highest affinities known for any peptide-HLA complex (103). Recently, HTLV-I Tax peptide loaded HLA-A*0201 dimers and tetramers were developed and used to demonstrate HTLV-I Tax-specific HLA-A*0201 restricted CD8+ T cells (52, 104). HTLV-I Tax11-19-specific CD8+ cells from the PBMC of HLA-A*0201 HAM/TSP patients were found to represent an extraordinarily high proportion of the total CD8+ population (52, 53). In patients with both HTLV-1 Tax 11-19 and cytomegalovirus peptide-specific CD8+ T cells, only HTLV-I Tax 11-19 specific CD8+ cells are found to be elevated in the CSF as detected with tetramers (54, 55). These studies suggest that the HTLV-I specific cells either are specifically expanded in the CSF or are recruited into the CNS from the periphery as depicted in Figure 2. Preferential expansion in the CSF may be associated with the recognition of HTLV-I infected cells in this compartment or in the CNS and thus may contribute to the neuropathology associated with HAM/TSP (54). Recently, it has been reported that HTLV-I *tax* mRNA expression levels in PBMCs correlated with the amount of HTLV-I Tax11-19-specific CD8+ T cells (56). These data suggest that HTLV-I specific CD8+ T cells may be continuously driven by HTLV-I antigen expression *in vivo* as shown in Figure 1.

HAM/TSP patients have high proviral loads despite vigorous virus-specific CD8+ T cell responses; however, it is unknown whether these T cells are efficient in eliminating the virus *in vivo*. Sequencing analysis revealed that epitope mutations were remarkably increased in a patient when the frequency and the degeneracy of the HTLV-1 specific CD8+ T cells were at the lowest. It was shown that the frequency and the degeneracy correlated with proviral load in the longitudinal study (88).

The phenomenon of spontaneous lymphoproliferation, defined as the ability of PBMCs to proliferate *ex vivo* in the absence of exogenous antigens or stimulants such as IL-2, has been well described in PBLs from HAM/TSP patients, from HTLV-I asymptomatic carriers, and from HTLV-II infected persons (78). However, the magnitude of this spontaneous lymphoproliferation is more pronounced in HAM/TSP patients than in asymptomatic HTLV-I carriers. The spontaneous lymphoproliferation of HTLV-I infected PBL is thought to consist of the proliferation of HTLV-I infected CD4+ cells and the expansion of CD8+ cells based on the demonstration of an increase in virus expressing cells concomitant with an increase in the percentage of CD8+CD28+ lymphocytes. Experimentally, CD8+ T cells, including HTLV-I specific CD8+ T cells, have been shown to be expanded during spontaneous lymphoproliferation (79).

The high frequency of HTLV-I specific CD8+ CTL in HAM/TSP patients correlates with the production of several cytokines. By intracellular cytokine staining coupled with flow cytometry, IFN-gamma, TNF-alpha, and IL-2 were all significantly elevated in the HTLV-I specific CD8+ cells of HAM/TSP patients compared with asymptomatic carriers and HTLV-I seronegative healthy controls (89). In addition, HLA-A*0201 restricted HTLV-I Tax11-19-specific CD8+ CTL lines derived from a HAM/TSP patient released IFN-gamma, and IL-2 with higher magnitude upon stimulation with Tax11-19 peptide (52).

It has been suggested that cytokine expression may be associated with an interaction of the TCR/Ag/HLA trimolecular complex. The molecular characterization of this trimolecular complex has led to major advances in the understanding of how the immune response recognizes antigen and has resulted in technologies that use these MHC-peptide complexes to directly visualize antigen-specific T cells (52). HTLV-I Tax11-19-specific CD8+ cells have been shown to possess cytolytic activity directed towards cells expressing HTLV-I Tax peptide via a perforin-dependent mechanism. Increased production of a of MMP-9, chemoattractants (macrophage inflammatory proteins 1alpha and 1beta), and proinflammatory cytokines (TNF-alpha and IFN-gamma) as a result of CD8+ T cell receptor mediated activation by HTLV-I antigens can contribute to damage of CNS tissues (52, 89-91).

5.5. Model of the autoimmune mechanism

Some studies indicate that an autoimmune mechanism may also be involved in the pathogenesis of HAM/TSP (105-108). A unique T-cell receptor CDR3 motif, which has been demonstrated in brain lesions of MS and in the animal model experimental autoimmune encephalomyelitis, was also detected in infiltrating lymphocytes in the spinal cord of HAM/TSP patients (109). HTLV-I infected CD4+ T cell clone established from PBMCs of a HAM/TSP patient proliferated to crude spinal cord protein extracts but not to lymph nodes of HTLV-I seronegative autopsy materials (110). Furthermore, Levin *et al* have provided new evidence in support of a HAM/TSP autoimmune hypothesis. Serum immunoglobulin 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 (105). IgG from brain, CSF, and serum of the HAM/TSP patients showed immunoreactivity with heterogeneous nuclear ribonuclear protein-A1 (hnRNP-A1) as the autoantigen. This antibody specifically stained human Betz cells, and infusion of autoantibodies in brain

sections inhibited neuronal firing (106-108). These data suggest that molecular mimicry between HTLV-I and autoantigens in CNS might play a role in the pathogenesis of HAM/TSP.

6. CONCLUSION

This large body of information suggests that virus-host immune interactions play a pivotal role in HAM/TSP. Although several risk factors for HAM/TSP have been identified, high HTLV-I proviral load is thought to be a critical in the development of the disease. High HTLV-I proviral loads in both CD4+ and CD8+ T cell populations drive increased HTLV-I mRNA levels that result in increased HTLV-I protein expression. Processing of HTLV-I proteins and presentation of HTLV-I specific peptides leads to activation and expansion of antigen-specific T cell responses as depicted in Figure 1. The hypothesis that HTLV-I specific CD8+ CTLs play a role in the development of HAM/TSP is supported by localization of these CTL in the CNS. Evidence for this comes from several studies. HTLV-I specific CD8+ cells could recognize productively infected cells and respond either by direct lysis of the infected cell or through the release of proinflammatory cytokines and chemokines. These molecules can act to recruit and expand additional inflammatory cells, and have been shown to be toxic to CNS tissue. Several studies suggest that HTLV-I infected lymphocytes may preferentially migrate into the CSF from peripheral blood or that HTLV-I infected lymphocytes may selectively expand in this compartment. The process is illustrated in Figure 2. It is reported that HTLV-I genomic sequences, RNA, and protein have been localized to spinal cord lesions. Therefore, all requirements for CTL recognition, including viral antigen and HLA class I expression, are present in the HAM/TSP lesion, lending support to the argument that CD8+ CTL may be immunopathogenic in this disease. Intensive studies regarding the interaction between HTLV-I specific CD8+ T cells and HTLV-I infected cells will clarify the pathogenesis of HAM/TSP.

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