Human T cell lymphotropic virus (HTLV) type I is an exogenous human retrovirus that infects 10–20 million people worldwide [1, 2]. Endemic areas of HTLV-I exist in southern Japan, the Caribbean, Central and South America, the Middle East, Melanesia, and equatorial regions of Africa [3, 4]. While the majority of infected persons remain healthy lifelong asymptomatic carriers, some 0.25%–3% develop a slowly progressive neurologic disorder termed HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP) [5, 6]. HTLV-I is also the etiologic agent in adult T cell leukemia [7] and the virus has been associated in a subset of patients with other inflammatory diseases [8–11]. How HTLV-I causes these disorders is not completely understood, although virus-host immunologic interactions have been suggested to play a role.

Neuropathology of HAM/TSP

Pathologic descriptions of HAM/TSP autopsy material indicate that the disease primarily affects the spinal cord at the thoracic level [12, 13]. Loss of myelin and axons in the lateral, anterior, and posterior columns is associated with perivascular and parenchymal lymphocytic infiltration, foamy macrophages, proliferation of astrocytes, and fibrillary gliosis [14–16]. A symmetrical loss of myelin and axonal dystrophy of the lateral columns within the corticospinal tracts is common; damage is most severe in the thoracic and lumbar regions [17].

Of importance, the neuropathology of HAM/TSP appears to change gradually during the progression of the disease. Initially, up to 5 years after onset, the leptomeninges and blood vessels are infiltrated with lymphocytes that penetrate the surrounding parenchyma. Numerous inflammatory cells including both CD8 and CD4 T cells (in equal numbers), B cells, and foamy macrophages are present in damaged areas of the spinal cord parenchyma [16, 18]. HLA class I and β2-microglobulin are expressed on endothelial cells and infiltrating mononuclear cells [17–19]. HLA class II expression also occurs in the endothelial cells, microglia, and infiltrating mononuclear cells of affected lesions. Later in the disease, immunohistochemical analysis of the affected spinal cord lesions shows the predominance of CD8 T cells [18]. Such lesions also express HLA class I antigens and have HTLV-I–specific CD8 cytotoxic T lymphocytes (CTL) [20]. CD8 CTL, thought to represent functionally cytotoxic cells, are observed frequently in active chronic lesions and occasionally in inactive chronic lesions in HAM/TSP patients [21]. The amount of proviral DNA in HAM/TSP patients correlates with the number of CTL (as defined by the monoclonal antibody TIA-1). Inflammatory cells and HTLV-I proviral DNA decrease with duration of the disease.

Since infiltrating CD8 cells are thought to play an important role in the development of HAM/TSP (and will be discussed further), an effort has been made to localize HTLV-I in the central nervous system (CNS) of HAM/TSP patients and to determine which cells might serve as targets for inflammatory CD8 cells. HTLV-I gag, pX, and pol sequences have been localized to the thoracic cord areas [15, 16] and are greater in areas of increased CD4 infiltration. HTLV-I pX and env sequences have been localized to affected spinal cord [22, 23] and HTLV-I RNA has been localized to astrocytes [24].

Increased expression of inflammatory cytokines [21] and adhesion molecules [25] occurs in the spinal cord of HAM/TSP patients with a short duration of disease. As the disease becomes chronic (>5 years), the number of inflammatory cells
decreases substantially. The expression of inflammatory cytokines is also reduced over the duration of the disease and, with the exception of interferon (IFN)-γ, becomes undetectable. Collectively, these findings support the view that inflammatory T lymphocytes (and CD8 cells in particular) may play a critical role in the immunopathogenesis of HAM/TSP.

**Immune Response to HTLV-I**

A number of immunologic parameters have been described in HAM/TSP patients including high HTLV-I proviral load, increased spontaneous lymphoproliferation, high antibody titers to HTLV-I both in sera and cerebrospinal fluid (CSF), and increased cytokine production (reviewed in [26]). Because these immune abnormalities are more often observed in patients with HAM/TSP than in HTLV-I–infected asymptomatic carriers, they suggest that immune dysregulation may be associated with pathogenesis of HTLV-I–associated neurologic disease. Increased levels of the cytokines IFN-γ, tumor necrosis factor (TNF)-α, and interleukin (IL)-6 have been reported in the sera and CSF [27], and mRNA for IL-1α, IL-2, TNF-α, and IFN-γ is up-regulated in HAM/TSP peripheral blood lymphocytes (PBL) [28]. An ELISPOT assay has shown significant elevation of IL-2, IFN-γ, and IL-4 in peripheral blood mononuclear cells (PBMC) isolated from HAM/TSP patients compared with both asymptomatic carriers and seronegative normal donors [26].

Abnormalities in cellular immune responses of HAM/TSP patients have also been identified. Natural killer cells tend to be diminished in both number and activity in HAM/TSP [29]. In particular, the phenomenon of spontaneous lymphoproliferation, defined as the ability of PBMC to proliferate ex vivo in the absence of antigenic stimulation or IL-2, has been well described in HAM/TSP PBL, in HTLV-I asymptomatic carriers, and in HTLV-II–infected persons [30]. However, the magnitude of this spontaneous lymphoproliferation is typically higher in HAM/TSP PBL. 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 [31]. Spontaneous lymphoproliferation from the PBMC of HAM/TSP patients involves both IL-2 and IL-15 [32].

**Role of HTLV-I Cellular Immune Responses in HAM/TSP: Current Status**

**Virus-specific CTL** One of the most striking features of the cellular immune response in HTLV-I patients is the highly increased numbers of CD8 HTLV-I–specific CTL in the PBL and CSF of persons with HAM/TSP [33–37]. CD8 CTL recognize viral and other foreign antigens, usually as small 9-aa peptides, in the context of HLA class I alleles. HTLV-I–specific CD8 CTL activity in HAM/TSP PBL is typically restricted to the p27x and p40x products of the HTLV-I tax gene [34]. However, CD8 CTL responses to other HTLV-I antigens, particularly the Env proteins, can occur at a lower frequency. Although HTLV-I CD8 CTL are seen in PBMC of asymptomatic carriers [38], the magnitude and frequency of these responses are higher in patients with neurologic disease (as high as 1 in 75 CD8 cells) [34]. As will be discussed, the elevated precursor frequency of HTLV-I–specific CTL in HAM/TSP PBMC is even higher with the use of newer tetramer technology. The high precursor frequency of HTLV-I–specific CTL in HAM/TSP in comparison with that of HTLV-I–infected asymptomatic carriers and in persons with other HTLV-I–associated diseases suggests that these CTL may be disease-specific and immunopathogenic in HAM/TSP.

Class I–restricted CTL recognize relatively short peptide fragments that are endogenously processed and bound to an HLA class I molecule. It was first demonstrated at the clonal level and then by precursor frequency analysis that PBMC of HAM/TSP patients preferentially recognize a 9-aa peptide derived from the HTLV-I Tax protein (Tax11-19, LLFGYPVVY). They are restricted to the HLA-A201 haplotype [39]. Tax11-19 conforms to a known HLA-A201 binding motif and has one of the highest affinities known for any peptide-HLA complex [40]. 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-γ, TNF-α, 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 [35]. In addition, HLA-A201–restricted HTLV-I Tax11-19–specific CD8 CTL lines derived from a HAM/TSP patient released IFN-γ, IL-4, and IL-2 with higher magnitude upon stimulation with Tax11-19. It has been suggested that cytokine expression may be associated with an interaction of the TCR/Ag/HLA trimolecular complex [41].

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. This was accomplished by the use of molecularly engineered tetrameric HLA peptide complexes (tetramers) that are biotinylated recombinant HLA molecules folded around a specific peptide and then cross-linked with streptavidin or HLA-peptide complexes by use of immunoglobulin as a molecular scaffold to produce a divalent peptide-MHC-IgG reagent [41]. By use of such methodologies, HTLV-I Tax11-19–specific, HLA-A201–restricted CD8 lymphocytes were visualized directly from the peripheral blood of HAM/TSP patients (figure 1) and found to be present in about 5% of the CD8 cells [41, 42] (but in up to 30% of CD8 lymphocytes of some persons; figure 1). Of importance, these cells...
were significantly elevated in patients with neurologic disease relative to asymptomatic carriers [42].

Most recently, tetramer analysis of lymphocytes isolated from the CSF of HAM/TSP patients showed even higher frequencies of HTLV-I Tax11-19–specific, HLA-A201–restricted CD8 lymphocytes compared to PBMC [42, 43] (figure 1). Tetramers to cytomegalovirus peptide-specific CD8 cells were not increased in the CSF. This suggests that HTLV-I–specific cells either are specifically expanded in the CSF or are recruited into the CNS from the periphery. Preferential expansion in the CSF may be associated with the recognition of HTLV-I–infected cells in this compartment or in CNS and thus may contribute to the neuropathology associated with HAM/TSP [42].

HTLV-I proviral and mRNA loads. As noted, HTLV-I–specific immune abnormalities are significantly elevated in HTLV-I–associated neurologic disease, specifically with regard to antigen-specific CD8 T cell responses, and the CD8 cells are thought to play a role in disease pathogenesis [26]. What drives such responses? There is considerable evidence that HTLV-I proviral loads in PBMC of HAM/TSP patients are significantly higher than in HTLV-I asymptomatic carriers [44]. Analysis of HTLV-I proviral DNA in PBMC of more than 400 subjects infected with HTLV-I [43] showed that in HAM/TSP patients, the HTLV-I proviral load was about 16-fold higher than in carriers.

Although HTLV-I can infect a wide range of human and nonhuman cells in vitro, HTLV-I has been thought to preferentially infect CD4 T cells in vivo [46]. Recently, this view was challenged. By using quantitative polymerase chain reaction (PCR) methods, CD8 T cells were shown to be infected with HTLV-I in vivo [47]. In addition, a high proportion of CD8 T cells were also infected with HTLV-I when assessed by a sensitive flow cytometric technique [48]. Moreover, HTLV-I protein expression in these naturally infected CD8 T cells rendered them susceptible to cytolysis mediated by autologous HTLV-I–specific CD8 CTL [49]. HTLV-I–infected CD8 T cells may therefore have a role as a significant virus reservoir in vivo and together with HTLV-I–infected CD4 cells may drive the high HTLV-I–specific immune response observed in patients with HAM/TSP.

Analysis of HTLV-I proviral loads from lymphocytes of the CSF of HAM/TSP patients also demonstrated high levels of HTLV-I tax DNA [42]. Of importance, these levels were elevated compared with PBMC proviral loads. In HLA A201 HAM/TSP patients, the increased HTLV-I proviral DNA loads in CSF were proportional to the frequency of HTLV-I Tax11-
19-specific CD8 T cells as defined by tetramer staining (figure 1). These observations support the hypothesis that the HTLV-I proviral load may drive the increased HTLV-I-specific immune responses that have been suggested to be immunopathogenic in HAM/TSP [26].

Paradoxically, even though a high proviral DNA load is characteristic of HAM/TSP patients, the expression of HTLV-I in PBMC appears to be low [50]. These observations have led a number of investigators to consider that HTLV-I may be latent in peripheral blood. By using a newly established real-time quantitative reverse transcriptase PCR technique [37], HTLV-I mRNA load in PBMC of HAM/TSP patients was significantly higher than in HTLV-I asymptomatic carriers [51]. This is consistent with a study that detected HTLV-I Tax protein in PBMC and CSF cells of HAM/TSP patients [52] by use of laser scanning cytometry. HTLV-I Tax protein expression in CSF cells was higher than in PBMC and was more frequent in HAM/TSP patients with shorter duration of illness. This low amount of HTLV-I mRNA and Tax protein-expressing PBMC compared with the high HTLV-I proviral DNA load suggests that the majority of HTLV-I-infected cells are latent in peripheral blood, although this amount seems sufficient to continuously activate the immune system in vivo.

Immunopathogenesis Model of HAM/TSP

The large body of evidence summarized above strongly suggests that virus-host immunologic interactions play a pivotal role in HAM/TSP. As shown in figure 2, 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 and presentation of HTLV-I-specific peptides leads to activation and expansion of antigen-specific T cell responses. The hypothesis that HTLV-I-specific CD8 CTL play a role in the development of HAM/TSP is supported by localization of these CTL in the CNS. Inflammatory CD8 cells have been found in the spinal cord lesions of HAM/TSP patients [53] and tend to increase with disease progression. Activated T cells have been reported in HAM/TSP patient CSF, usually of the CD8+, CD11a+, CD45 RO−, CD28− phenotype [54].

The precursor frequency of HTLV-I-specific CTL from CSF lymphocytes is extraordinarily high [34]. HTLV-I-specific CD8 cells could recognize HTLV-I antigen-expressing target cells in the CNS and induce large amounts of proinflammatory cytokines and chemokines that can induce HLA expression in neuronal cells and damage CNS tissue [55]. As the HTLV-I proviral load in CSF of HAM/TSP patients was more than two times higher than in PBMC [42], this suggests 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. In addition, HTLV-I genomic sequences, RNA, and the HTLV-I p19 protein [56] 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. This understanding will allow for directed immunotherapeutic strategies for the treatment of this chronic progressive neurologic disease like that currently being evaluated in multiple sclerosis [57]. These experimental therapeutic strategies include inhibition of T cell activation, altered peptide ligand therapies, and transmigration through the blood-brain barrier.

References


Figure 2. Schematic of induction of HTLV-specific CD8 T cell responses associated with immunopathogenesis of HTLV-associated myelopathy/tropical spastic paraparesis (HAM/TSP) and current technologies that show the responses. Quantitative polymerase chain reaction (DNA Taqman) shows high HTLV-I proviral loads in patients with HAM/TSP that is directly proportional to increased mRNA levels for HTLV-I tax. Elevated mRNA levels (RNA Taqman) for HTLV-I tax lead to increased expression of HTLV-I protein that can be processed into immunodominant peptides. HTLV-I peptides (e.g., Tax11-19) strongly bind to HLA A201 molecules and can stimulate a virus-specific CD8 T cell response (detected by HLA A201/Tax11-19-specific tetramers). These antigen-specific responses are expanded in the cerebrospinal fluid of HAM/TSP patients and may contribute to disease progression by recognition of HTLV-I-processed antigens in the central nervous system associated with lysis of HTLV-I-infected inflammatory cells or HTLV-I-infected glial cells and/or through induction of proinflammatory cytokines and chemokines.
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