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Review

HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP): a chronic progressive neurologic disease associated with immunologically mediated damage to the central nervous system

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This review examines information on clinical, pathological and immunological events in the slowly progressive neurologic disorder associated with the human T lymphotropic virus type-I (HTLV-I) termed HTLV-I associated myelopathy/tropical spastic paraparesis (HAM/TSP). The role of cellular immune responses to HTLV-I in patients with HAM/TSP and how these responses may be associated with the pathogenesis of this disorder will be discussed. While a number of immunologic responses have been shown to be abnormal in HAM/TSP patients, studies on HTLV-I specific cytotoxic T cell responses (CTL) will be specifically examined. By defining such antigen specific functional cellular host responses to HTLV-I we hope to better understand the underlying mechanisms that may be involved in the neuropathology of HTLV-I associated neurologic disease. This has led to a number of HTLV-I associated immunopathogenic models that may be operative in HAM/TSP patients. Importantly, based on these models, potential immunotherapeutic strategies for disease intervention can be devised. Moreover, such an analysis may have significant implications for our understanding of other HTLV-I associated clinical disorders and other neurological diseases in which viral etiologies have been suggested.

Keywords: HTLV-I; HTLV-I associated myelopathy; immunopathogenesis; cytotoxic T lymphocyte; neuropathology

Introduction

The Human T-cell Lymphotropic Virus Type I (HTLV-I) was the first human retrovirus isolated (Poeisz *et al*, 1980) and was shown to be the causative agent of Adult T-cell Leukemia (ATL) (Poeisz *et al*, 1980; Uchiyama *et al*, 1977; Yoshida *et al*, 1984). HTLV-I is endemic in the southern region of Japan, the Caribbean, the equatorial regions of Africa and in South America (McFarlin and Blattner, 1991; Gessain *et al*, 1985; Osame *et al*, 1986). Approximately 5 years after its discovery, epidemiological data linked HTLV-I infection with a chronic progressive disease of the central nervous system (CNS) termed HTLV-I Associated Myelopathy (HAM) in Japan (Osame *et al*, 1986) and Tropical Spastic Paraparesis (TSP) in the Caribbean (Gessain *et al*, 1985). The two syndromes were determined to be the same disease and were termed HAM/TSP (Bucher *et al*, 1990; Hollsberg and Hafler, 1993; Gessain and Gout, 1992). The adult HTLV-I seroprevalence rate in some of these endemic areas can be as high as 30%, however only 1–5% develop HAM/TSP or ATL, the remainder are asymptomatic carriers of the virus (Hollsberg and Hafler, 1993). The risk factors for infection with HTLV-I are the same as those for Human Immunodeficiency Virus (HIV), which include sexual contact, exchange of blood products and vertical transmission (mainly due to a long duration of breast feeding) (Gessain, 1996; Hollsberg and Hafler, 1993). Why one group of HTLV-I seropositive individuals develop a neurologic disease, another leukemia and the majority remain clinically well is unknown and an area of intense investigation. Possible differences between the

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diseased and asymptomatic states currently under study include analysis of virus strain (Daenke *et al*, 1990; Nishimura *et al*, 1993; Niewiesk *et al*, 1994), human histocompatibility leukocyte antigen (HLA) (Usuku *et al*, 1988), viral load (Gessain *et al*, 1989, 1990; Kira *et al*, 1991; Kubota *et al*, 1994; Richardson *et al*, 1990; Yoshida *et al*, 1989) and immune function (Hira *et al*, 1994; Itoyama *et al*, 1988; Jacobson *et al*, 1990; Kitajima *et al*, 1988; Moritmoto *et al*, 1985). The focus of this review is to describe the role that infection with HTLV-I and the immune response to this retrovirus play in the development of HAM/TSP.

Clinical disease

HAM/TSP is characterized clinically by paraparesis associated with spasticity, hyper-reflexia and Babinski signs of the lower extremities (McFarlin and Blattner, 1991; Hollsberg and Hafler, 1993; Osame and McArthur, 1990; Nakagawa *et al*, 1995). The weakness is typically symmetrical and slowly progressive (McFarlin and Blattner, 1991; Hollsberg and Hafler, 1993). Diagnosis is made on clinical criteria in association with a positive serum antibody titer to HTLV-I by ELISA assay (McFarlin and Blattner, 1991; Hollsberg and Hafler, 1993). Since the ELISA assay also screens for HTLV-II and other retroviral proteins, HTLV-I positive tests are confirmed by Western blot analysis which are designed to discriminate between HTLV types (Lipka *et al*, 1991, 1992). The incubation from time of infection to onset of disease is typically from years to decades, but can be as short as 18 weeks following blood transfusion with HTLV-I contaminated blood (Osame *et al*, 1986b; Gout *et al*, 1990). The age of onset is usually 35 to 45 years, but can be as early as 12 years of age (McFarlin and Blattner, 1991). HAM/TSP is three times more prevalent in women than men (Hollsberg and Hafler, 1993; Nakagawa *et al*, 1995). Urinary incontinence (typically from a spastic bladder) and bowel disturbances are common (Hollsberg and Hafler, 1993; Osame and McArthur, 1990; Nakagawa *et al*, 1995). Male patients sometimes complain of sexual dysfunction (Hollsberg and Hafler, 1993; Nakagawa *et al*, 1995). Sensory symptoms of the lower extremities such as paresthesias are common, however severe objective sensory signs are unusual and motor dysfunction almost always predominates compared to sensory symptoms (Hollsberg and Hafler, 1993; Nakagawa *et al*, 1995). Although the clinical presentation of HAM/TSP may be similar to the primary progressive form of multiple sclerosis, a relapsing-remitting course as is typically seen in multiple sclerosis, almost never occurs (Hollsberg and Hafler, 1993). A recent review of eight patients evaluated at NIH (Table 1) indicated that all patients progressed consistent with an ascending myelopathy (Table 1), that neurologic signs referable to the corticospinal tract predominate (weakness, spasticity, hyperactive reflexes – see Table 1), and that most patients also have abnormal neurologic signs of the upper extremities including hyper-reflexia and Hoffman's signs (Table 1). Also, some were beginning to complain of hand weakness (Table 1). Cerebrospinal fluid (CSF) analysis typically shows a mild lymphocytic pleocytosis, mild protein elevation, elevated IgG synthesis and IgG index and oligoclonal bands (Table 1) (Jacobson *et al*, 1990; Ceroni *et al*, 1988; Link *et al*, 1989). Some of the oligoclonal bands are directed to HTLV-I proteins (Kitze *et al*, 1995). MRI of the spinal cord

Table 1 Neurologic evaluation of eight HAM/TSP patients

Patient characteristics	
Age (range)	35–74
Sex	3M 5F
Duration of Disease (range)	5–15 years
Risk Factor	3 (blood) 2 (endemic) 3 (unknown)
Neurologic Exam	*Number of Patients*
Initial Symptom	4 (gait or weakness) 4 (urine dysfunction)
Progressive Disease	8
Weakness	2 (upper extremities) 8 (lower extremities)
Spasticity	4 (upper extremities) 8 (lower extremities)
Hyperactive Reflexes	3 (jaw jerk) 7 (upper extremities) 8 (lower extremities) 6 (Hoffman's sign) 8 (Babinski sign)
Abnormal Gait	8
Sensory Dysfunction	7 (mild vibratory loss of lower extremities)
Laboratory findings	*Number of Patients*
MRI Abnormalities	4 (spinal cord atrophy) 1 (white matter brain lesion)
CSF Abnormalities	white cells >2/hpf 4 (range 0–12 wbc) protein >45 mg/dl 0 IgG >4.1 mg/dl 6 (range 1.9–11.8 mg/dl) IgG Index >0.62 7 (range 0.58–1.5) Oligoclonal bands 7

may show atrophy, and MRI of the brain shows periventricular white matter lesions in as many as 50% of patients (Nakagawa *et al*, 1995; Mattson *et al*, 1987; Cruickshank *et al*, 1989; Godoy *et al*, 1995). An example of an MRI from one patient who presented with neurologic signs referable to the corticospinal tracts including paraplegia, upper extremity weakness with hyper-reflexia and dysphagia is shown in Figure 1. Consistent with the neuropathology (see next section), Figure 1A (arrow) demonstrates spinal cord atrophy. In addition, there is evidence of corticospinal tract damage as shown by degeneration of the corticospinal tract throughout the brainstem in the coronal plane (Figure 1B – arrowheads) and confirmation of this damage in the axial plane (arrowheads in Figure 1C – an example from the pons). Furthermore, there is damage at the point of origin of the corticospinal tract, as shown by the abnormal white matter signal adjacent to the central sulcus that includes the pre- and post-central gyrus, the cortical brain areas where corticospinal tract neurons originate (Figure 1D – arrowheads).

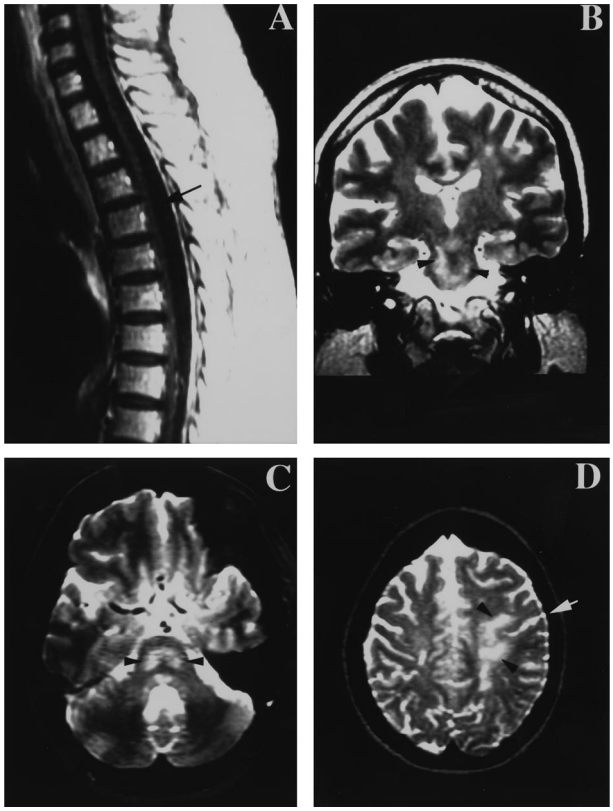

Figure 1 MRI of the spinal cord and brain of a patient with HAM/TSP in which neurologic signs and symptoms of the corticospinal tract predominate (A) T1 weighted sagittal view of the spinal cord shows thoracic cord atrophy (arrow). (B) T2 weighted coronal section of the brain demonstrates corticospinal tract damage in the brainstem (bright areas within the brainstem parenchyma outlined by arrowheads). (C) T2 weighted axial section of the brain taken from the same area as B confirms corticospinal tract damage (bright areas within the brainstem parenchyma outlined by arrowheads). (D) T2 weighted axial section of the cortex in area adjacent to the central sulcus (white arrow) shows white matter damage in the pre- and post- central gyrus (bright signal demonstrated by arrowheads) where corticospinal tract neurons originate.

Correlation of neuropathology and immune function

The neuropathology of CNS autopsy specimens from patients with HAM/TSP has been carefully investigated and provides a model for the study of retroviral associated immune mediated damage to CNS tissues. Importantly, the clinical and neuro-

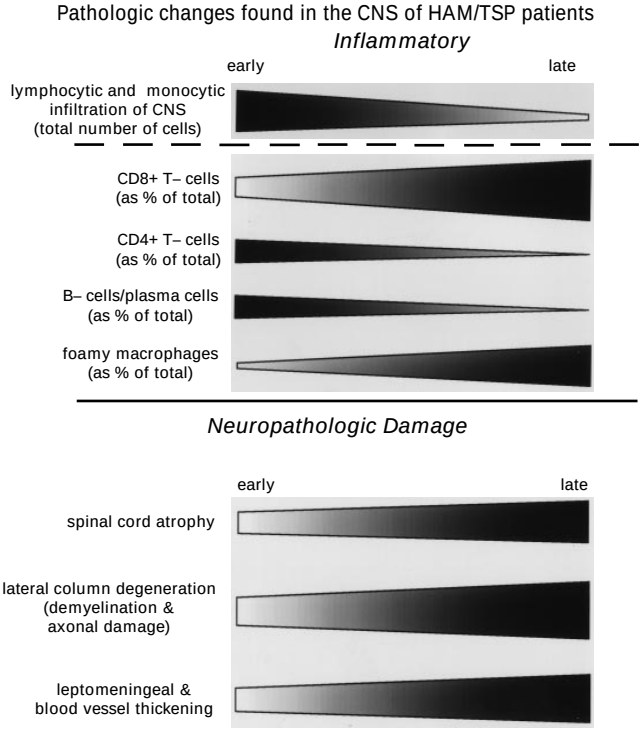

Figure 2 Summary of inflammatory infiltrates and neuropathologic damage found in central nervous system autopsy specimens of HAM/TSP patients. The top panel describes the inflammatory infiltrates. Early in disease (typical less than 2 years) there is an infiltration of lymphocytes and monocytes that decreases dramatically over the course of disease (top panel – line 1). Early in disease there are similar numbers of $CD8^+$ cells (top panel – line 2), $CD4^+$ cells (top panel – line 3) and B-cells (top panel – line 4) and a strong macrophage response (top panel – line 5). Over time, B-cells essentially disappear (top panel – line 4), $CD4^+$ cells are sparse (top panel – line 3) and $CD8^+$ cells persist and become the predominant immune cell infiltrate (top panel – line 2). Foamy macrophages also persist late in disease (top panel – line 5). The bottom line describes the neuropathologic damage. Lateral column damage, predominantly secondary to corticospinal tract damage, is present early in disease (bottom panel – line 2) and continues to progress significantly over the course of the disease (bottom panel – line 2). Spinal cord atrophy (bottom panel – line 1) along with leptomeningeal and blood vessel thickening (bottom panel – line 3) parallel the lateral column damage. There is also typically posterior column damage, but this is typically mild in relation to the changes described.

radiologic data in patients with HAM/TSP correlate with the neuropathological findings. Figure 2 depicts a review of the neuropathological damage and immune cell infiltration of CNS autopsy specimens from patients with HAM/TSP in relation to duration of disease (Piccardo *et al*, 1988; Moore *et al*, 1989; Iwaski, 1990; Yoshika *et al*, 1993; Akizuki, 1989a, b; Umerhara *et al*, 1993, 1994; Izumo *et al*, 1989; Wu *et al*, 1993; Hara *et al*, 1989; Kobayashi *et al*, 1989; Kishikawa *et al*, 1989). There is an extensive immune response in the spinal cord of HAM/TSP patients (Figure 2) (Piccardo *et al*, 1988; Moore *et al*, 1989; Iwaski, 1990; Yoshika *et al*, 1993; Akizuki, 1989a, b; Umerhara *et al*, 1993, 1994; Izumo *et al*, 1989; Wu *et al*, 1993; Hara *et al*, 1989; Kobayashi *et al*, 1989; Kishikawa *et al*, 1989). Leptomeninges and blood vessels are infiltrated with lymphocytes that may also penetrate the surrounding parenchyma (Piccardo *et al*, 1988; Moore *et al*, 1989; Iwaski, 1990; Yoshika *et al*, 1993; Akizuki, 1989a, b). The phenotype of infiltrating immune cells is dependent upon the length of time the patient had neurologic disease (Figure 2). In all cases, infiltrating cells include $CD8^+$ lymphocytes (Umerhara *et al*, 1993) and $CD8^+$ cells have been stained for TIA-1 (a marker for cytotoxic T lymphocytes) in active lesions (Umehara *et al*, 1994). Early in disease (less than 2 to 5 years), there are greater numbers of inflammatory cells that consist of both $CD8^+$ and $CD4^+$ T-cells, as well as B-lymphocytes (Figure 2) (Akizuki, 1989a, b; Umerhara *et al*, 1993; Izumo *et al*, 1989). In addition, foamy macrophages are present in damaged areas of spinal cord parenchyma in these patients (Figure 2) (Akizuki, 1989a, b; Izumo *et al*, 1989). In chronic HAM/TSP specimens (patients with disease greater than 5 years), inflammatory cells persist, but are less frequent, and almost exclusively are of the $CD8^+$ T cell phenotype (Figure 2) (Moore *et al*, 1989; Wu *et al*, 1993; Kishikawa *et al*, 1989). In these specimens, $CD4^+$ cells are rare, B-lymphocytes are virtually absent and foamy macrophages are reduced in number, but persist in terms of the percent of total immune cells present (Figure 2) (Moore *et al*, 1989; Wu *et al*, 1993; Hara *et al*, 1989).

Analysis of a spinal cord biopsy in our laboratory from a HAM/TSP patient with a 5 year history of rapidly progressive disease supports these observations (manuscript in preparation). The biopsy showed inflammation of the leptomeninges and parenchyma. Analysis of the mononuclear infiltrate showed $CD3^+$, $CD45R0^+$ cells (activated T-cells) which were almost entirely $CD8^+$ (Figure 3A – positive cells are stained black). $CD4^+$ cells were rare (Figure 3B – a paucity of black staining cells) and B-cells were virtually absent (Figure 3C – no positive staining). Further characterization of the immune response was studied from an autopsy of a patient with HAM/TSP for over 20 years (Wu *et al*, 1993) in which inflammatory cytokines and major histocompatibility molecules (MHC) were evaluated. In this study, $CD8^+$ cells predominated in areas of class I MHC molecule (β microglobulin and HLA-ABC) expression compared to minimal numbers of $CD4^+$ cells and class II MHC molecule (HLA-DRa) expression (which was attributed to activation, rather than CD4 interaction) (Wu *et al*, 1993). Also, interleukin 1β (IL-1β) and tumor necrosis factor alpha (TNFα) were detected by immunohistochemistry in glial cells of these spinal cord sections (Wu *et al*, 1993).

The neuropathological damage of HAM/TSP patients is characterized by extensive CNS damage

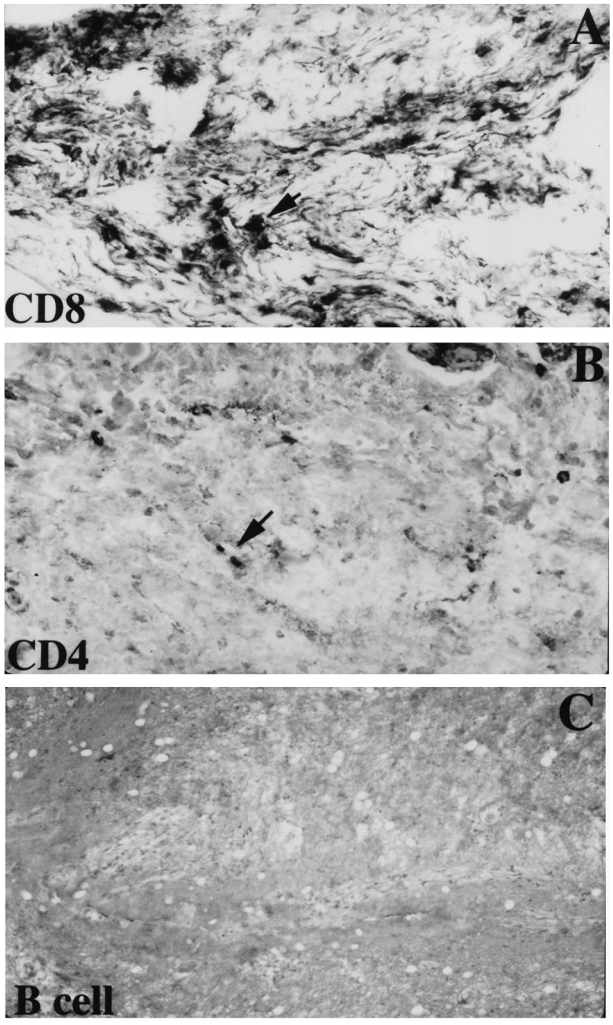

Figure 3 Immunohistochemical analysis of immune cell infiltration of the central nervous system from a spinal cord biopsy from a patient with HAM/TSP for over 5 years. (A) Staining of antigen Leu 2A (black is positive staining, the arrow shows an example of a positive cell) for $CD8^+$ T-cells demonstrates infiltration of $CD8^+$ T-cells in the specimen (methyl green counterstain) (400×). (B) Staining of antigen Leu 3A (black is positive staining, the arrow shows an example of a positive cell) for $CD4^+$ T-cells shows a sparse infiltration of $CD4^+$ T-cells (methyl green counterstain) (400×). (C) There is an absence of B-cells (staining for L26) (hematoxylin counterstain) (400×).

in association with immune cell inflammation of the spinal cord, predominantly, but not exclusively at the thoracic level (Piccardo *et al*, 1988; Moore *et al*, 1989; Iwaski, 1990; Yoshika *et al*, 1993) (Figure 2). Specifically, the spinal cord is atrophic and there is thickening and fibrosis of the leptomeninges and blood vessels which increases proportionate to the duration of disease (Figure 2) (Moore *et al*, 1989; Akizuki, 1989). There is symmetrical, widespread loss of myelin (Moore *et al*, 1989; Izumo *et al*, 1989a, b) and axonal dystrophy (Wu *et al*, 1993) of the lateral columns (Figure 2), particularly of the corticospinal tracts of the spinal cord. Damage is most severe in thoracic and lumbar regions, however corticospinal damage has been clearly described ascending to the cervical spinal cord (Akizuki, 1989a, b; Kishikawa *et al*, 1989) and brainstem (Akizuki, 1989b; Kishikawa *et al*, 1989). These findings may be secondary to Wallerian degeneration (Moore *et al*, 1989). In parallel with the clinical data, damage to the anterior and posterior columns are more variable and less extensive compared to the corticospinal tract damage (Moore *et al*, 1989; Wu *et al*, 1993). Gliosis may also be present in spinal cord white matter (Wu *et al*, 1993; Hara *et al*, 1989; Kishikawa *et al*, 1989). An autopsy performed in our lab from a patient with HAM/TSP for over 12 years showed parenchymal damage consistent with chronic HAM/TSP (unpublished observation). These findings are consistent with the patient's clinical presentation, in which corticospinal signs such as paraparesis, spasticity, hyper-reflexia and Babinski signs predominate compared to neurologic signs referable to other parts of the CNS (Hollsberg and Hafler, 1993). There was corticospinal tract atrophy at all levels of the spinal cord, with relative preservation of adjacent posterior spinocerebellar tracts and other areas on the spinal cord. An example of the specificity of corticospinal degeneration in the thoracic spinal cord is shown in Figure 4. Figure 4 shows a high power view of adjacent sections of the thoracic spinal cord of the autopsied patient stained for myelin (Figure 4B – luxol fast blue) and axons (Figure 4A – Sevier silver stain). In Figure 4B (right asterisk), there is evidence of pallor and demyelination of the corticospinal tract as shown by the reduced amount of dark staining in this area. This is in contrast to the immediately adjacent posterior spinocerebellar tract in which the dark, compact staining of myelin is normal (Figure 4B, left asterisk). In this section there is also blood vessel thickening as shown by the increased connective tissue surrounding the blood vessels (Figure 4B – arrow). In parallel, there are also decreased numbers of axons and axonal dystrophy in the corticospinal tract as shown by a decrease in the silver stain in Figure 4A (right asterisk). Specifically, surrounding the right asterisk in Figure 4A there is a decrease in the number of silver-stained black dots which are axons cut in cross section and increase in connective tissue staining between axons. This is in contrast to the adjacent posterior spinocerebellar tract (Figure 4A – left asterisk) which shows normal axonal staining: many more silver stained axons (black dots) surrounded by clear white space and a minimum amount of connective tissue staining between axons. This closely parallels the patient's neurologic exam and is typical for HAM/TSP in general, in which signs referable to the cerebellar tracts are almost always

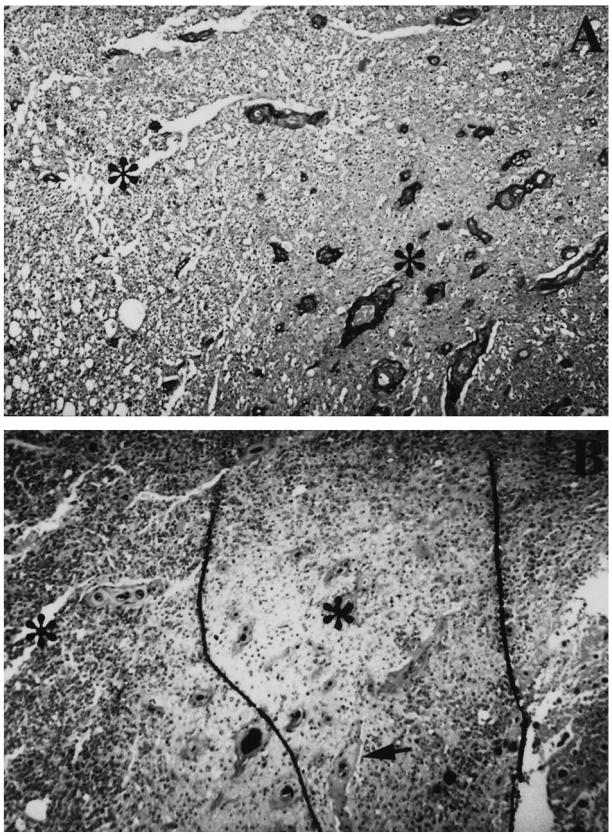

Figure 4 High power view (400×) of the thoracic spinal cord from an autopsy of a HAM/TSP patient with disease for over 12 years shows the specificity of the degeneration of the corticospinal tract. (A) Sevier silver stain which stains axons black and connective tissue light brown (grey in this black and white photograph). There are decreased numbers of black staining axons (black dots – axons cut in cross section) and increased staining of connective tissue of the corticospinal tract (right asterisk) and normal staining of axons and connective tissue of the immediately adjacent posterior spinocerebellar tract (left asterisk), which shows normal axonal staining: many more silver stained axons (black dots) surrounded by clear white space and a minimum amount of connective tissue staining between axons. (B) Luxol fast blue stain which stains myelin blue (dark, compact staining in this black and white photograph) and connective tissue pink (light grey/white staining in this black and white photograph). There is decreased staining and pallor of the corticospinal tracts (right asterisk) and normal myelin staining of the immediately adjacent posterior spinocerebellar tract (left asterisk). The dark black vertical lines are tissue fold artifacts. Both sections reveal blood vessel thickening (arrow).

absent. In addition, there was mild damage to the posterior columns (data not shown), but this was minimal compared to the corticospinal pathology. This is also consistent with the patient's neurologic exam in which motor signs referable to the corticospinal tract predominated over sensory signs referable to the posterior columns.

Many studies have attempted to localize HTLV-I in the CNS of HAM/TSP patients. HTLV-I *gag* DNA sequences were localized to the thoracic spinal cord of a HAM/TSP patient (Bhigjee *et al*, 1991). Utilizing quantitative PCR, HTLV-I-*pX* and *pol* sequences were found to be increased in the thoracic cord in areas where $CD4^+$ cells were predominate (Kubota *et al*, 1994), and this signal decreased in proportion to the length of the patient's disease (when $CD4^+$ cells decrease in number (Figure 2). In contrast, HTLV-I-*pX* and *env* (Kira *et al*, 1992) DNA sequences were localized to the spinal cord, but did not correlate with areas of lymphocyte infiltration (Kira *et al*, 1992; Ohara *et al*, 1992). These studies may not be incongruent. Early in disease, HTLV-I infected $CD4^+$ cells may carry the virus from the peripheral blood (Richardson *et al*, 1990) to the CNS and infect certain cells of the CNS. Later in disease, as the CD4 cells are cleared, localization of the virus may be found in resident CNS cells. Interestingly, HTLV-I-*pX* and *gag* RNA were localized to degenerating lateral columns of the thoracic cord in areas where lymphocyte infiltration was minimal (Kubota *et al*, 1994). In addition, *in situ* hybridization studies have more accurately localized HTLV-I RNA in CNS specimens to infiltrating $CD4^+$ T-lymphocytes in acute HAM/TSP (Moritoyo *et al*, 1996) and to astrocytes in chronic HAM/TSP (Lehky *et al*, 1995).

Patients with HAM/TSP have an activated immune response that is highly specific

The neuropathology of HAM/TSP provides evidence that immune mediated mechanisms may play a significant role in the pathogenesis of the disease. A number of studies have described both cellular and humoral immune responses in patients with HAM/TSP and compared these to HTLV-I seropositive asymptomatic individuals and normal HTLV-I seronegative controls.

Immune response to HTLV-I

An immunological profile from patients with HAM/TSP is shown in Table 2. HAM/TSP patients have elevated antibody titers to HTLV-I in sera and CSF and is a diagnostic criteria for this disorder (Gessain *et al*, 1985; Osame *et al*, 1986). Patients may have hypergammaglobulinemia, oligoclonal bands in their CSF and elevated levels of lymphokines such as IL6 and soluble IL2 in sera and CSF (Hollsberg and Hafler, 1993; Jacobson *et al*, 1990; Jacobson, 1992).

Table 2 Immune response in patients with HAM/TSP

Immune response	*SERA*	*CSF*
High antibody titer to HTLV-I	X	X
Elevated levels of IgG	X	X
Elevated IgG index		X
Oligoclonal bands to HTLV-I		X
Elevated levels of cytokines	X	X
Increased activated T-cells	X	X
Spontaneous lymphoproliferation	X	
Increased HTLV-I proviral DNA load	X	
Decreased natural killer cell subsets & activity	X	
Decreased ADCC activity	X	
Elevated $\beta 2$ microglobulin		X
Oligoclonal expansion of TcR of $CD8^+$ cells	X	
Elevated neopterin levels		X
Elevated levels of complement	X	
Increased levels of VCAM-1	X	X
Increased HTLV-I specific, HLA class I -restricted CTL	X	X

Neopterin and β microglobulin levels (markers of inflammation) have been reported to be elevated in CSF of HAM/TSP patients (Nakagawa *et al*, 1995; Matsui *et al*, 1995). Also, HAM/TSP patients have increased levels of soluble vascular adhesion molecule type 1 (VCAM-1) in sera and CSF (Matsuda *et al*, 1995) and elevated levels of complement in sera (Saarloos *et al*, 1995). In addition, abnormal cellular immune responses include decreased natural killer cell activity and natural killer subsets as well as decreased antibody dependent cellular cytotoxicity (ADCC) (Morimoto *et al*, 1985; Kitajima *et al*, 1988). In the peripheral blood and CSF of HAM/TSP patients, activated T lymphocytes have been demonstrated (Jacobson *et al*, 1988, 1990; Itoyama *et al*, 1988) as shown by an increase in the number of large $CD3^+$ cells that express markers of T cell activation such as HLA-DR and IL-2 receptor molecules (Jacobson *et al*, 1988, 1990). T cell activation can also be demonstrated by the capacity of PBL of HAM/TSP patients to spontaneously proliferate *in vitro* in the absence of exogenously added antigen (Jacobson *et al*, 1988, 1990; Itoyama *et al*, 1988). This spontaneous lymphoproliferation of HAM/TSP PBL has also been described from PBL of asymptomatic HTLV-I seropositive individuals (Kramer *et al*, 1989) and in individuals infected with HTLV-II, although the magnitude of the response is higher in HAM/TSP patients (Wiktor *et al*, 1991).

HAM/TSP patients may have up to 50 times more HTLV-I proviral DNA in PBL compared to asymptomatic carriers which may be related to many of the immunologic responses discussed above (Gessain *et al*, 1989; Kira *et al*, 1991). The amount of this HTLV-I proviral load as measured by the polymerase chain reaction (PCR) can range between $2-20$ copies per 100 PBL from HAM/TSP patients compared to $0.04-8$ copies per 100 PBL in asymptomatic carriers (Kubota *et al*, 1993). How-

ever, these figures may over represent the amount of HTLV-I in PBL if the virus integrates at more than one copy per cell. This has been suggested by a study using *in situ* PCR technology which demonstrated that as little as 1 in 10 000 HAM/TSP contain HTLV-I *in situ* (Levin *et al*, 1996) and is comparable to what is seen in PBL of asymptomatic carriers. Also, semi-quantitative analysis of HTLV-I-*tax* RNA expression in PBL is similar in HAM/TSP patients and asymtomatic carriers (Furukawa *et al*, 1995). Even if these immune responses are driven by the high viral load in HAM/TSP patients, the hope is to define an HTLV-I specific immune response, that is unique to, or over represented in this disorder and determine the role that these particular responses play in disease pathogenesis. By defining such antigen specific functional cellular host responses to HTLV-I we hope to better understand the underlying mechanisms that may be involved in the neuropathology of HTLV-I associated neurologic disease.

HTLV-I specific cytotoxic T cell responses

Patients with HAM/TSP, in contrast to HTLV-I seropositive asymptomatic individuals and normal HTLV-I seronegative controls, develop a $CD8^+$ HLA class I restricted cytotoxic T-lymphocyte (CTL) response specific for immunodominant HTLV-I viral peptides that can be detected directly from peripheral blood. CTL play an important role in the normal immunologically mediated recovery from infectious disease by recognition and subsequent elimination of foreign antigens (Battisto *et al*, 1988; Askonas *et al*, 1988; Sun *et al*, 1988; Leist *et al*, 1987). CTL recognize foreign proteins as short peptide fragments in association with human histocompatibility molecules (HLA). The specificity of the CTL interaction with its peptide target is defined by a trimolecular complex involving its antigenic specific T cell receptor (TCR) bound to the peptide target which in turn is bound to an MHC (HLA) on the target cell. Each component of this trimolecular complex in relation to HTLV-I infection in patients with HAM/TSP and its potential role in the immunopathogenesis of retroviral associated chronic progressive neurologic disease will be described.

$CD8^+$ CTL recognize foreign antigens, typically as short nine amino acid peptide fragments, in the context of HLA class I molecules. $CD4^+$ CTL recognize somewhat longer peptides in association with HLA class II molecules. Both populations have been shown to be beneficial in eliminating infected cells and in recovery from viral infection (Askonas *et al*, 1988). However, virus-specific CTL can also be immunopathologic and cause disease, particularly if they destroy essential cellular structures (Sun *et al*, 1988; Leist *et al*, 1987).

HTLV-I specific $CD8^+$, HLA class I restricted CTL have been demonstrated directly (without the need for *in vitro* expansion) from fresh mononuclear cells isolated from either peripheral blood lymphocytes (PBL) or CSF cells of HAM/TSP patients (Kannagi *et al*, 1994; Jacobson *et al*, 1990b; 1992b; Elovaara *et al*, 1993; Koenig *et al*, 1993; Kannagi *et al*, 1991; Parker *et al*, 1992a, b; Shida *et al*, 1987). Figure 5 shows a typical experiment comparing the CTL response from a HAM/TSP patient compared with his asymptomatic HTLV-I seropositive spouse. Cytolytic activity from HAM/TSP patients' PBL was predominantly restricted to a product of the HTLV-I-*tax* genes (p27x and p40x), although other HTLV-I antigens (HTLV-I-*env*) were also recognized (Figure 5) (Jacobson *et al*, 1990b, 1992b; Elovaara *et al*, 1993; Koenig *et al*, 1993). In contrast, there was minimal CTL activity from PBL of asymptomatic individuals (Figure 5), although recent reports have challenged this observation (Parker *et al*, 1992a, b). This may reflect the growing association of HTLV-I with other diseased states and serves to stress the importance of a detailed clinical evaluation of asymptomatic HTLV-I seropositive carriers. Moreover, it was shown that frequency of HTLV-I-*tax* specific CTL from HAM/TSP patients in both the peripheral blood and CSF was exceptionally high (one in 86 and one in 60, respectively) (Jacobson *et al*, 1992b; Elovaara *et al*, 1993). This extraordinarily high frequency of HTLV-I specific $CD8^+$ CTL should be kept in perspective. Precursor frequencies of CTL to more common viruses such as measles, mumps or influenza are typically in the range of one per 10^5 to 10^6 total lymphocytes. In contrast to the high frequency of $CD8^+$ HTLV-I specific CTL from HAM/TSP patients, it has been shown that HTLV-I seropositive, asymptomatic individuals had no or significantly lower CTL responses (Figure 5) (Jacobson *et al*, 1990b, 1992b; Elovaara *et al*, 1993; Koenig *et al*, 1993). Precursor frequency analysis also confirm these observations in which HTLV-I specific $CD8^+$ CTL were 40–100 fold less in asymptomatic carriers than from HAM/TSP patients (Elovaara *et al*, 1993). In addition, HTLV-I specific CTL from circulating PBL were not detected from two ATL patients. Collectively, these results suggest that the presence of circulating $CD8^+$ HTLV-I specific CTL is a major immunologic feature of patients with HAM/TSP and considering the predominance of $CD8^+$ T cells in the subarachnoid space and parenchyma of CNS autopsy specimens of HAM/TSP patients, would suggest that the $CD8^+$ CTL plays a significant role in immune-mediated damage to the CNS.

Peptide specificity of the CTL response

Since all HAM/TSP patients can demonstrate high levels of $CD8^+$ HTLV-I specific CTL directed towards the *tax* protein of HTLV-I (Kannagi *et al*, 1994; Jacobson *et al*, 1990b, 1992b; Elovaara *et al*, 1993; Koenig *et al*, 1993; Kannagi *et al*, 1991; Parker *et al*, 1992a, b; Shida *et al*, 1987), a more precise

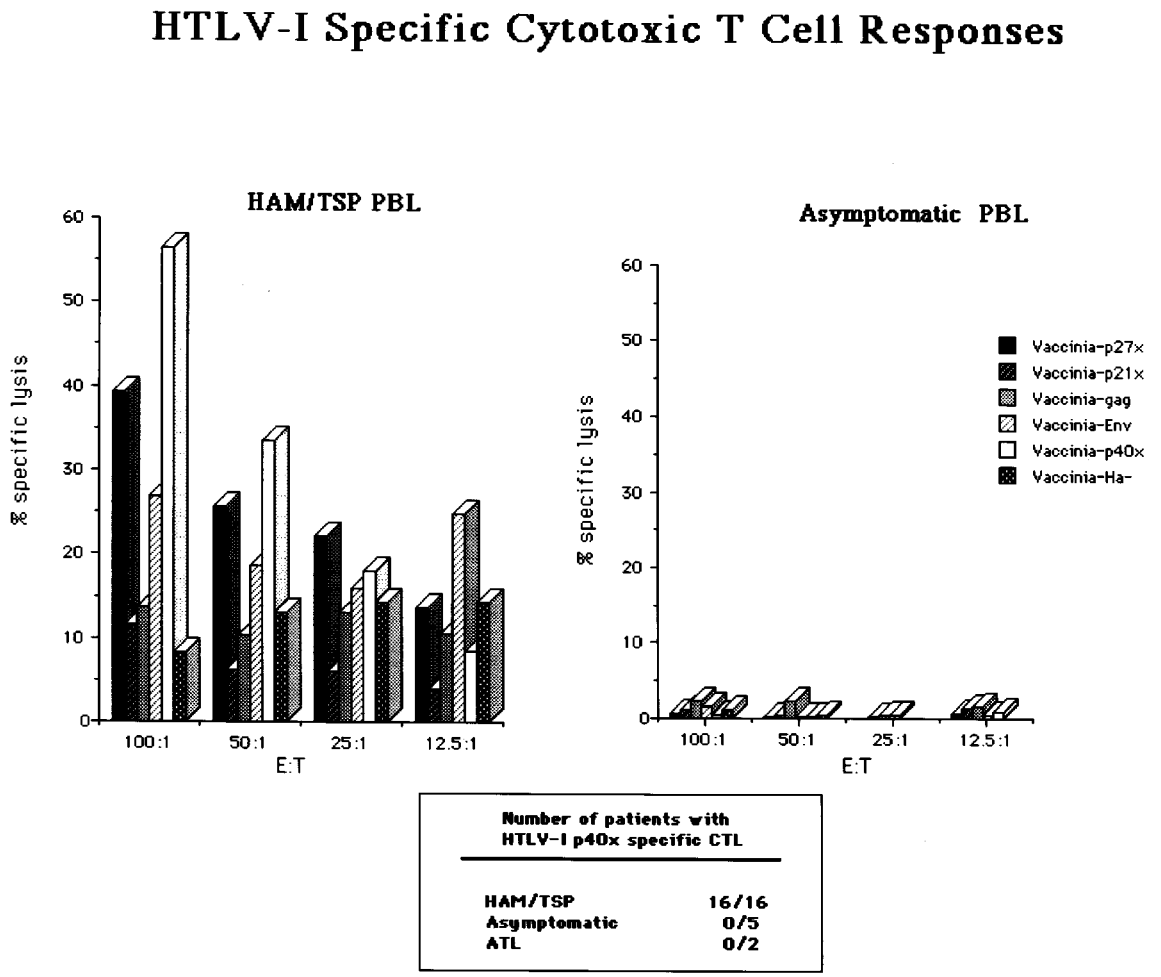

Figure 5 A typical cytotoxic T-cell (CTL) experimental profile from a patient with HAM/TSP (left) compared to his HTLV-I seropositive neurologically asymptomatic spouse (right). The percent specific lysis is represented on the Y-axis and the effector to target ratio (E : T) is represented on the X-axis. The effector cells are peripheral blood lymphocytes (PBL) isolated from the patients' blood and the target cells are autologous B-cells infected with various recombinant vaccinia-HTLV-1 peptides. The vaccinia constructs utilized are shown to the right (the vaccinia-Ha is a non-HTLV-I control for the hemaglutinin of influenza virus). The effector cells are incubated with radioactive chromium labeled target cells and the percent specific lysis calculated by measuring release of radioactivity into the supernatant. PBL from a HAM/TSP patient (left) predominantly recognized targets expressing the HTLV-I p40x and p27x constructs. There was no lysis from the HTLV-I seropositive neurologically asymptomatic individual (right) or from patients with HTLV-I associated acute T-cell leukemia or from normal controls (data not shown). The insert (bottom) shows the number of HTLV-I infected patients in which HTLV-I p40x specific CTL responses could be demonstrated from PBL.

characterization of the HTLV-I *tax* epitopes recognized by these CTL has been undertaken. Mapping of predominant CTL epitopes is important because such an analysis could lead to tailored immunotherapeutic strategies which could specifically affect CTL function and potentially alter disease progression.

$CD8^+$ HLA class I restricted CTL are known to recognize short peptide fragments (usually nine amino acids) endogenously processed within virus infected cells and conformationally bound to an HLA class I molecule (Zinkernagel and Doherty, 1974; Townsend *et al*, 1986; Townsend and Bodmer, 1989). Different HLA class I molecules bind different peptides and unique binding motifs have been identified for a number of HLA class I alleles (Madden *et al*, 1993). It has been shown that HTLV-I specific CTL which recognize HTLV-I- *tax* protein are specific for a nine amino acid peptide, LLFGYPVYV (*tax* 11–19), in association with the Class I, HLA-A2 allele (Parker *et al*, 1992a, b; Davis and Bjorkman, 1988; Chothia *et al*, 1988; Marrack and Kappler, 1987). Figure 6 depicts the binding of HTLV-I-*tax* 11–19 to an HLA A2 molecule in which the HLA and T-cell receptor contact residues are shown. The *tax* 11–19 peptide has an extraordinarily high affinity for the HLA A2 molecule and can sensitize an HLA-A2 target for lysis by HTLV *tax* 11–19 specific CTL at a concentration as low as 10^{-16} M (0.1 femtomoles). Biochemical binding

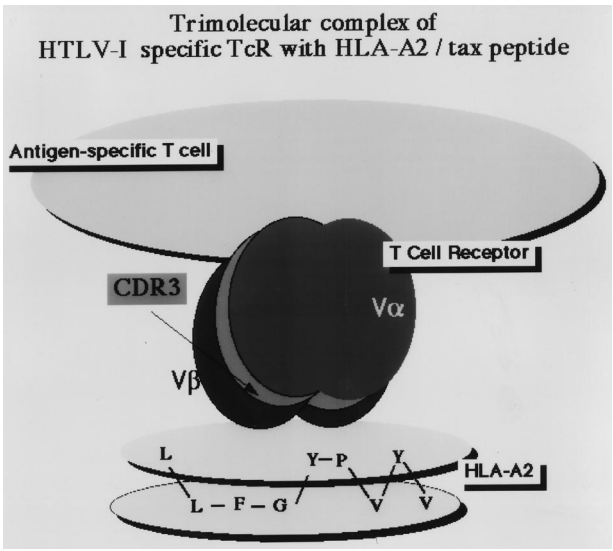

Figure 6 Trimolecular complex of HTLV-I specific TcR with HLA-A2/*tax* specific peptide. Through its antigen specific T cell receptor (TCR) consisting of a heterodimer of one α and one β chain, an HTLV-I *tax* 11–19 peptide specific T cell recognizes a 9 amino acid peptide bound to an HLA A2 molecule. Amino acids in the first (L=leucine), fifth (Y=tyrosine), sixth (P=proline), and eighth (Y=tyrosine) position of HTLV-I *tax* 11–19 are thought to make contact with the TCR while amino acids in the second (L=leucine), third (F=phenylalanine), fourth (G=glycine), seventh (V=valine) and ninth (V=valine) are bound in a pocket of the HLA A2 molecule. Highly variable complimentary determining regions (CDR3) have been defined within the TCR which are thought to bind directly to the antigenic peptide/HLA complex.

studies have demonstrated that the HTLV-I- *tax* 11–19 peptide has one of the highest affinities known for any peptide-HLA complex with a half life of 6400 min in association with HLA-A2 (Parker *et al*, 1992a, b). Precursor frequency analysis of HTLV-I-*tax* 11–19 specific CTL from PBL of HLA-A2 positive HAM/TSP patients (at a level of one in 300 $CD8^+$ cells) is also consistent with the immunodominance of this peptide (Elovaara *et al*, 1993).

T cell receptor repertoire in patients with HAM/TSP

The specificity of the CTL interaction with its peptide target is defined by a trimolecular interaction involving the MHC on the target cell bound with an antigenic peptide which in turn is recognized and bound by an antigenic specific T cell receptor (TCR). TCRs consist of a heterodimer of one α and one β chain (Davis and Bjorkman, 1988; Chothia *et al*, 1988; Marrack and Kappler, 1987). In order to recognize the enormous spectrum of peptide-MHC combinations, TCR heterogeneity is generated by the somatic rearrangement of non-contiguous genes called V, D and J genes (Davis and Bjorkman, 1988; Chothia *et al*, 1988; Marrack and Kappler, 1987), found in both the α and β TCR chains. Highly variable complimentary determining regions (CDR3) have been defined within each chain of the TCR which are thought to bind directly to the antigenic peptide/MHC complex (Davis and Bjorkman, 1988; Chothia *et al*, 1988; Marrack and Kappler, 1987). The salient features of this trimolecular complex is shown in Figure 6 with specific reference to HTLV-I specific CTL recognition of HTLV-I-*tax* 11–19 in association with HLA-A2 (a class I MHC). Since the vast majority of virus-specific CTL from HLA-A2-positive HAM/TSP patients recognize HTLV-I-*tax* 11–19, it is important to define if these CTL are dominated by a single, limited, or heterogeneous set of TCRs. With the molecular resolution of the crystal structure of the HTLV-I *tax* 11–19/HLA-A2 complex (Madden *et al*, 1993) it is possible to predict potential amino acid contact points where HTLV-I *tax* 11–19 peptide may bind to the TCR (Figure 6). Proline appeared the most prominently exposed amino acid with two tyrosine residues and one phenylalanine accessible for TCR contact (Madden *et al*, 1993). Recent studies have begun to evaluate the TCR repertoire of the highly specific CTL response in HAM/TSP patients.

The TCR of HLA-A2-restricted HTLV-I *tax* 11–19-specific CTL were analysed by PCR using two sets of TCR Vα and Vβ-family specific primers, followed by sequence analysis of the CDR3 regions (Utz *et al*, 1995). TCR chain sequences revealed heterogeneity of the CDR3 regions, and varying Vα and Jα gene usage. However, at least one T cell clone from each of three patients utilized Vα2 rearranged with Jα24. Their sequences were identical except one amino acid within the CDR3 region. TCR β chain sequences were preferentially rearranged with Jβ2.1 and 2.7, two Jβ genes with strong homologies. Half of the TCR β chain sequences contained proline followed by glycine in a position within CDR3 region thought to be important in peptide recognition as predicted by the crystallographic structure of the *Tax* 11–19/HLA A2 complex. These experiments are ongoing, however some preliminary observations in relation to the immune response in other diseases can be made. Highly heterogeneous CDR3 regions despite identical HLA restriction and peptide specificity have been reported for TCR's recognizing infectious agents such as tetanus toxin. Interestingly, a restricted TCR Vβ-region usage has been reported for a number of autoimmune diseases (Ben-Nun *et al*, 1991; Kotzin *et al*, 1991; Zamvil *et al*, 1988), including multiple sclerosis (MS), a demyelinating disease of unknown origin, with clinical similarities to HAM/TSP. Both diseases have been postulated to involve an autoimmune mechanism. Furthermore, one of the HLA-A2-restricted *Tax* 11–19-specific CTL clones could be traced in one patient over a period of 3 years. The persistence of individual T cell clones has been observed for myelin basic protein-specific T cells in MS patients (Salvetti *et al*, 1993; Wucherpfennig *et al*, 1994). However, the

question whether T cells that infiltrate the spinal cords of HAM/TSP patients represent virus-specific CTL and/or autoreactive T cells recognizing myelin antigens is still not answered. The information obtained from the analysis of TCR usage by HTLV-I *tax*-specific CTL from HAM/TSP patients could be used to devise therapeutic strategies that target a specific set of immunopathogenic TCR's. Such therapies have been initially developed for use in the murine demyelinating disease experimental allergic encephalitis (EAE) and more recently have been extended to patients with MS (Wraith *et al*, 1989; Sakai *et al*, 1989; Howell *et al*, 1989; Vanderbark *et al*, 1989, 1992; Offner *et al*, 1990; Chou *et al*, 1994; Bourdette *et al*, 1994).

Immunopathogenesis of HTLV-I associated neurologic disease

It has been over a decade since infection with HTLV-I was clearly associated with the development of HAM/TSP, and a definitive immunopathogenic mechanism describing how infection with a retrovirus results in a chronic progressive neurologic diseas remains elusive. However, a number of hypothetical models have emerged based on an HTLV-I induced immune response that either directly recognizes viral antigens or cross-reactive self peptides on the surface of a target cell in the CNS leading to damaged tissue, or indirectly causes CNS damage by developing an immune response to these peptides resulting in cytokine production which in turn leads to damaged tissue (bystander mechanism).

Models on the pathogenesis of HTLV-I associated neurologic disease reflect two major experimental observations. First, a pathological hallmark of HAM/TSP is inflammatory T cells in affected spinal cord areas (Piccardo *et al*, 1988; Moore *et al*, 1989; Iwaski, 1990; Yoshika *et al*, 1993; Akizuki, 1989a, b; Umerhara *et al*, 1993, 1994; Izumo *et al*, 1989; Wu *et al*, 1993; Hara *et al*, 1989; Kobayashi *et al*, 1989; Kishikawa *et al*, 1989) are $CD8^+$ and dominate the inflammatory response as the disease progresses (Umerhara *et al*, 1993, 1994). Second, an extraordinarily high frequency of $CD8^+$ HTLV-I specific CTL restricted to immunodominant epitopes of HTLV-I gene products has been demonstrated in both PBL and CSF of HAM/TSP patients (Jacobson *et al*, 1990b, 1992b; Elovaara *et al*, 1993; Koenig *et al*, 1993). It is compelling to ask if these $CD8^+$ T cells present in HAM/TSP lesions are HTLV-I specific CTL and what is the target for these cells? In addition to the data already presented, the most direct demonstration of HTLV-I specific CTL in the CNS of HAM/TSP patients is from a spinal cord biopsy from a HAM/TSP patient who's MRI of the spinal cord showed several enhancing lesions (manuscript submitted). Inflammatory T cells were present in this spinal cord biopsy and were almost entirely $CD8^+$. This material was cultured *in vitro* without antigenic stimulation in an attempt to expand these cells. $CD8^+$ T cell lines were generated and demonstrated to be cytotoxic, HTLV-I specific and HLA class I restricted. This patient was not HLA A2 and the HTLV-I peptide specificity of these $CD8^+$ HTLV-I specific CTL lines derived from her spinal cord biopsy were not determined. Although these results are based on CTL lines derived after short term culture, it argues strongly for the presence of functionally active HTLV-I specific CTL in the pathogenesis of HTLV-I associated neurologic disease.

There are a number of potential target cells in the CNS for the HTLV-I specific CTL and other components of the HTLV-I induced immune response. These include infiltrating immune cells as well as resident CNS cells such as endothelial cells, oligodendrocytes, astrocytes, microglia or neurons. Infection and subsequent expression of HTLV-I gene product(s) with a concomitant induction of HLA molecules could make these cells targets for cytotoxic $CD8^+$ cells that may be present at lesion sites. In support of this argument, class I expression was demonstrated in CNS autopsy specimens from HAM/TSP patients where $CD8^+$ cells predominate (Wu *et al*, 1993). Also, HTLV-I DNA sequences in the CNS of HAM/TSP patients have been reported by solution phase PCR (Bhigjee *et al*, 1991; Kubota *et al*, 1994). Unfortunately, these studies cannot ascertain which cells are infected with HTLV-I. Recently, a number of reports have shown that HTLV-I is present *in situ* in spinal cord material from HAM/TSP autopsy cases (Kubota *et al*, 1994; Lehky *et al*, 1995). In one, HTLV-I DNA was localized to inflammatory $CD4^+$ cells as demonstrated by a novel *in situ* hybridization PCR technique. In contrast, by more conventional *in situ* hybridization procedures, HTLV-I RNA did not appear to be inflammatory infiltrates but was localized to areas where lymphocyte infiltrate was minimal (38) and in one study HTLV-I RNA was localized to astrocytes (Lehky *et al*, 1995).

The demonstration of both HTLV-I infected $CD4^+$ cells and HTLV-I infected neuroglial cells in the CNS may not be mutually exclusive events. Early in disease, when $CD4^+$ cells are numerous in HAM/TSP lesions (Figure 2) (Piccardo *et al*, 1988; Moore *et al*, 1989; Iwaski, 1990; Yoshika *et al*, 1993; Akizuki, 1989a, b; Umerhara *et al*, 1993, 1994; Izumo *et al*, 1989; Wu *et al*, 1993; Hara *et al*, 1989; Kobayashi *et al*, 1989; Kishikawa *et al*, 1989), an HTLV-I infected $CD4^+$ cell from the peripheral blood may act as an antigen presenting cell stimulating an HTLV-I specific CTL. This same infected $CD4^+$ cell in periphery may traffic through the CNS, where it may trigger the CTL response locally, and upon the secretion of toxic levels of cytokines, indirectly damage CNS tissue. Alternatively, HTLV-I infected

$CD4^+$ cells may infect resident CNS cells such as astrocytes and neurons. Viral peptides may be expressed in the setting of appropriate HLA conditions, and lead to CNS damage by inflammatory HTLV-I specific T-cells. Finally, a purely autoimmune phenomena such as molecular mimicry may occur, in which the HTLV-I induced CTL response may recognize cross-reactive self-antigens in the CNS with similar structure and binding motifs as viral peptides, resulting in an immune response targeted against CNS tissue leading to CNS damage. Future studies are required to clarify which mechanism predominates the immunopathogenesis of HTLV-I associated neurologic disease so that specific therapies for this and similar chronic progressive neurologic diseases, such as multiple sclerosis, can be designed.

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