Detection of Human T Lymphotrophic Virus Type I (HTLV-I) Proviral DNA and Analysis of T Cell Receptor V/3 CDR3 Sequences in Spinal Cord Lesions of HTLV-I-associated Myelopathy/Tropical Spastic Paraparesis

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出版情報：Journal of Experimental Medicine. 180 (3), pp.831-839, 1994-09. Rockefeller University Press
バージョン：

Detection of Human T Lymphotrophic Virus Type I (HTLV-I) Proviral DNA and Analysis of T Cell Receptor Vβ CDR3 Sequences in Spinal Cord Lesions of HTLV-I–associated Myelopathy/Tropical Spastic Paraparesis

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Summary

Identification of the localization of human T lymphotrophic virus type I (HTLV-I) proviral DNA in the central nervous system (CNS) is crucial to the understanding of the pathogenesis of HTLV-I–associated myelopathy (HAM)/tropical spastic paraparesis (TSP) pathogenesis. We have developed a sensitive detection method, called two-step polymerase chain reaction (PCR) in situ hybridization, which enabled us to detect the HTLV-I proviral DNA in paraffin-embedded spinal cord tissue sections from HAM/TSP patients. HTLV-I proviral DNA was detected only in the nucleus of lymphocytes that had infiltrated into the spinal cord. However, no proviral DNA was amplified in any neuronal cells, including neurons and glial cells. This indicates that the demyelination of the spinal cord by HTLV-I as a result of viral infection of oligodendrocytes or neuronal cells is unlikely. The T cell receptor Vβ gene sequence from lymphocytes in the spinal cord lesions taken from the same HAM/TSP autopsy cases revealed unique and restricted CDR3 motifs, CASSLXG(G) (one-letter amino acid. X is any amino acid), CASSPT(G), and CASSGRL which are similar to those described in T cells from brain lesions of multiple sclerosis (MS) and in a rat T cell clone derived from experimental allergic encephalomyelitis (EAE) lesions. The present results suggest that T cells containing restricted Vβ CDR3 motifs, which are also found in MS and EAE, become activated upon HTLV-I infection and infiltrate into the spinal cord lesions of HAM/TSP patients.

Human T lymphotrophic virus type I (HTLV-I)[1] is known to be a causative agent of adult T cell leukemia (ATL) (1, 2) and HTLV-I–associated myelopathy/tropical spastic paraparesis (HAM/TSP) (3, 4). HAM/TSP is a chronic neurological disease characterized by spastic paraparesis and urinary dysfunction. HTLV-I is endemic in the Kyushu area of Japan, the Caribbean, Africa, and the South Pacific area. This disease has some clinical aspects similar to those of multiple sclerosis (MS) for which the etiology is still unknown. Less than 1% of HTLV-1 carriers develop clinical disease. This suggests that individual differences in genetic predisposition may be related to the pathogenesis of HAM/TSP. The pathogenesis of HAM/TSP is still controversial. There are several hypotheses: (*a*) a slow viral infection of HTLV-1 which might cause neuronal degeneration; (*b*) an immune response by cytotoxic T cells against HTLV-I–infected neuronal cells or antibody-dependent cellular cytotoxicity which induces destruction of neuronal cells; (*c*) demyelination caused by cytokines secreted from HTLV-I–infected microglial cells; and (*d*) the activation of T cells reactive to a neuronal self-antigen as a result of HTLV-I infection.

The aim of this study was to clarify the pathogenesis of HAM/TSP, therefore we focused on the localization of HTLV-I proviral DNA in the spinal cord lesions of HAM/TSP

[1] *Abbreviations used in this paper:* ATL, adult T cell leukemia; CNS, central nervous system; EAE, experimental allergic encephalomyelitis; HAM/TSP, HTLV-I–associated myelopathy/tropical spastic paraparesis; HTLV-I, human T lymphotrophic virus type I; MBP, myelin basic protein.

autopsy cases. Furthermore, to elucidate whether an autoimmune mechanism is involved in the pathogenesis of HAM/TSP, we analyzed the TCR Vβ sequence of lymphocytes that infiltrated spinal cord lesions.

Materials and Methods

Patients and Control Samples. Frozen spinal cord tissues and paraffin-embedded samples were obtained within 24 h of postmortem intervals from autopsy cases with typical HAM/TSP cases (case 1, 74-yr-old female; case 2, 63-yr-old male; and case 3, 57-yr-old female), and ATL patient (39-yr-old male) and nonneurological disease controls (71-yr-old male and 88-yr-old female). PBLs were taken from healthy HTLV-I carriers (58-yr-old male and 59-yr-old female).

Two-step PCR In Situ Hybridization. Human brains and spinal cords of the three cases with HAM/TSP and one case with ATL and two normal subjects were fixed with neutralized 10% formalin solution for 2 wk. The samples were embedded in paraffin and cut into 5-μm-thick sections. The sections were incubated at 65°C for 1 h and then deparaffinized with xylene. After treatment with proteinase K (10 mg/ml) for 12 min at 37°C, 100 μl of reaction mixture with 1 U of DNA Taq polymerase (Perkin Elmer-Cetus, Norwalk, CT), 10 μl of 10× reaction buffer, 200 μM each of deoxynucleotide triphosphates, and 50 nM of each pX primer (5′ primer, 5′-ATAGCAAACCGTCAAGCACAGC-3′; 3′ primer, 5′-GAGCCGATAACGCGTCCATCG-3′) was added to the slide glass which was then covered with a cover glass. The first PCR profile used began with denaturation at 94°C for 60 s, annealing at 37°C for 7 min, and extension at 72°C for 90 s for 20 cycles, followed by a second PCR profile with denaturation at 94°C for 60 s, annealing at 55°C for 2 min, and extension at 72°C for 90 s for 20 cycles on a flat-type DNA thermal cycler (model PHC-3; Techne, Ltd., Cambridge, UK). The reaction was terminated at 20°C. Internal pX probe (5′-CGGATACCCAGTCTACGT-3′) was tailed with biotin-dUTP by terminal deoxynucleotidyl transferase. In situ hybridization was done by utilizing the In Situ Hybridization and Detection System (BRL Life Technologies, Bethesda, MD). Sections were examined with a Zeiss Axiophot photomicroscope equipped with Nomarski optics. For a positive control, β globin primers (5′-ACACAACTGTGTTCACTAGC-3′ and 5′-GGAAAATAGGACCAATAGGCAG-3′; internal probe 5′-GAAGTTGGTGGTGAGGCCCT-3′) were used to show the sensitivity of this method. Immunoperoxidase staining of the consecutive sections was performed to identify T and B cells and macrophages with mAbs for T cell (MT-1, Bio-Science, Emmenbrücke, Switzerland [5]; and UCHL-1, Dakopatts, Copenhagen, Denmark), B cell (MB-2; Bio-Science, Emmenbrücke, Switzerland), macrophage (HLA-DR, Dakopatts), and microglia markers (polyclonal antibody against ferritin; Dakopatts), respectively. The colored reaction product was developed with diaminobenzidine.

PCR. Total RNA was extracted from the thoracic spinal cord tissue of autopsy cases and PBLs from healthy HTLV-I carrier using the guanidine-phenol method (6). mRNA was purified using Oligotex-dT30. First-strand cDNA was made using 2 μg mRNA by Moloney-murine leukemia virus reverse transcriptase (BRL), then the cDNA was amplified with 20 Vβ-specific oligonucleotide primers (Vβ1, 5′-AAGAGAGAGCAAAAGGAAACATTCTTGAAC-3′; Vβ2, 5′-GCTCCAAGGCCACATACGAGCAAGGCGTCG-3′; Vβ3, 5′-AAAATGAAAGAAAAAGGAGATATTCCTGAG-3′; Vβ4, 5′-CTGAGGCCACATATGAGAGTGGGATTTGTCA-3′; Vβ5, 5′-CAGAGAAACAAAGGAAACTTCCCTGGTCGA-3′; Vβ6, 5′-GGGTGCGGCAGATGACTCAGGGCTGCCCAA-3′; Vβ7, 5′-ATAAATGAAAGTGTGCCAAGTCGCTTCTCA-3′; Vβ8, 5′-AACGTTCCGATAGATGATTCAGGGATGCCC-3′; Vβ9, 5′-CATTATAAATGAAACAGTTCCAAATCGCTT-3′; Vβ10, 5′-CTTATTCAGAAAGCAGAAATAATCAATGAG-3′; Vβ11, 5′-TCCACAGAGAAGGGAGATCTTTCCTCTGAG-3′; Vβ12, 5′-GATACTGACAAAGGAGAAGTCTCAGATGGC-3′; Vβ13, 5′-ATGGCTACAATGTCTCCAGAT-3′; Vβ14, 5′-GTGACTGATAAGGGAGATGTCCTGAAGGG-3′; Vβ15, 5′-GATATAAACAAAGGAGAGATCTCTGATGGA-3′; Vβ16, 5′-CATGATAATCTTTATCGACGTGTTATGGGA-3′; Vβ17, 5′-TTTCAGAAAGGAGATATAGTGAAGGGTAC-3′; Vβ18, 5′-GATGAGTCAGAATGCCAAAGGAACGATTT-3′; Vβ19, 5′-CAAGAAACGGAGATGCACAAGAAGCGATTC-3′; Vβ20, 5′-ACCGACAGGCTGCAGGCAGGGGCCTCCAGC-3′) and Cβ primer (5′-GGCAGACAGGACCCCTTGCTGGTAGGACAC-3′) (7). PCR samples were run in a 2% agarose gel and transferred onto nylon membrane (Zeta-probe; Bio-Rad Laboratories, Richmond, CA) after denaturation with 0.4 N NaOH. Filters were fixed under UV lights, then prehybridized for 3 h at 42°C in 5× SSPE/5× Denhardt's solution/0.1% SDS containing salmon sperm DNA (100 μg/ml) and hybridized overnight at 42°C with 10^6 cpm per ml of ^{32}P-kinased internal Cβ probe (5′-TTCTGATGGCTCAAACACAGCGACCTCGGG-3′) as described (7). Actin PCR was done as previously described (8). Filters were washed in 2× SSC/0.1% SDS and 0.5× SSC/0.1% SDS each at room temperature, twice for 20 min, and then in 0.1× SSC/0.1% SDS at 42°C twice for 20 min. Kodak XAR-5 film with intensity screens were used for autoradiography at −70°C for 5 h.

Cloning and Sequencing. After amplification of the cDNA with Vβ specific primers, a positive Vβ sample was independently run in a 2% Nusieve agarose gel and an appropriately sized fraction was excised from the gel, then cloned into pCR II vector (Invitrogen, San Diego, CA). After transformation, clones containing TCR β chain inserts were identified by colony hybridization with radiolabeled internal Cβ probe as described above. Double-stranded plasmid DNA from positive clones were prepared by alkaline method and Vβ-Dβ-Jβ-Cβ sequences were determined by the deoxy chain termination method using the Taq Dye Deoxy Terminator cycle sequencing kit (Applied Biosystems, Inc., Foster City, CA).

Results

Distribution of HTLV-1 Proviral DNA in Spinal Cord Lesions. A sensitive method we developed, two-step PCR in situ hybridization which could detect as low as two copies of target DNA per cell (Fig. 1 *f*, β globin control), was applied to detect the HTLV-I proviral DNA in the spinal cord tissue sections of three autopsy cases plus other disease controls including central nervous system (CNS) ATL. Fig. 1 shows the localization of HTLV-I proviral DNA in the spinal cord of HAM/TSP cases. HTLV-I proviral DNA was detected in the small round nucleus of lymphocyte-like cells which gathered mainly around the small blood vessels in the white matter (Fig. 1 *a*) as well as in the gray matter (Fig. 1 *b*) of the spinal cords. By morphological criteria, most of the HTLV-I–positive cells in the spinal cords appeared not to be glial cells, but rather lymphocytes. Immunohistochemistry analysis using the T cell markers, MT-1 (pan-T) and UCHL-1 (CD45RO) revealed a massive infiltration of T cells into the spinal cord (Fig. 1 *g*). We found that the distribution of the

HTLV-I proviral DNA-positive cells was almost identical to that of T lymphocytes in the serial sections when we counted the proportion of the HTLV-I proviral DNA-positive lymphocytes. Most of the infiltrated lymphocytes were positive for the T cell marker (Fig. 1 *g*) and were negative for the B cell and macrophage markers. 50–70% of the these lymphocytes were positive for HTLV-I proviral DNA (Fig. 1, *a* and *b*). Numerous HTLV-I–positive lymphocytes were also

Table 1. *Vβ CDR3 Sequences of TCR Amplified from the Infiltrated Lymphocytes in Spinal Cords of HAM/TSP and Controls, and Vβ CDR3 Sequences of PBLs from HTLV-1 Healthy Carriers*

Vβ	N-D-N	Jβ	Cβ	Number/ total*
HAM/TSP Case 1				
Vβ 5 LCASS	LSGGAFD	EQYFGPGTRLTVT(Jβ2.7)	EDLKN	2/5
Vβ 5 FCA	IGVR	EAFFGQGTRLTVV(Jβ1.1)	EDLNK	1/5
Vβ 5 LCASS	LAPSGRET	QYFGPGTRLTVL(Jβ2.5)	EDLKN	1/5
Vβ 5 LCASS	PGPGWGRW	YTFGSGTRLTVV(Jβ1.2)	EDLNK	1/5
Vβ 6 LCASS	LMGGG	GYTFGSGTRLTVV(Jβ1.2)	EDLNK	2/4
Vβ 6 LCASS	PTGDY	GYTFGSGTRLTVV(Jβ1.2)	EDLNK	1/4
Vβ 7 LCASS	QDPAAA	YNEQFFGPGTRLTVL(Jβ2.1)	EDLKN	1/3
Vβ 7 LCASS	QGTSY	YNEQFFGPGTRLTVL(Jβ2.1)	EDLKN	2/3
Vβ 8 LCASS	LMGGG	GYTFGSGTRLTVV(Jβ1.2)	EDLNK	2/5
Vβ 8 LCASS	LTGGSD	GYTFGSGTRLTVV(Jβ1.2)	EDLNK	2/5
Vβ 12 FCA	IGVR	EAFFGQGTRLTVV(Jβ1.1)	EDLNK	1/3
Vβ 12 FCASS	SGVSTDT	QYFGPGTRLTVL(Jβ2.3)	EDLKN	2/3
Vβ 13 LCASS	LSGGAFD	EQYFGPGTRLTVT(Jβ2.7)	EDLKN	3/5
Vβ 13 FCASS	RNPDSS	YNEQFFGPGTRLTVL(Jβ2.1)	EDLKN	2/5
Vβ 14 FCAA	GRPVM	GYTFGSGTRLTVV(Jβ1.2)	EDLNK	2/2
HAM/TSP Case 2				
Vβ 6 LCASS	PTGG	EQFFGPGTRLTVL(Jβ2.1)	EDLKN	3/5
Vβ 6 LCASS	PTSPDGG	EQYFGPGTRLTVL(Jβ2.7)	EDLKN	1/5
Vβ 8 LCASS	PEGN	EQFFGPGTRLTVL(Jβ2.1)	EDLKN	1/3
Vβ 8 FCASS	RHPDSS	YNEQFFGPGTRLTVL(Jβ2.1)	EDLKN	1/3
Vβ 8 LCASS	WTGGGDT	QYFGPGTRLTVL(Jβ2.3)	EDLKN	1/3
Vβ 17 LCASS	GRLFS	NQPQHFGDGTRLSIL(Jβ1.5)	EDLNK	5/5
Vβ 19 LCASS	PTRDYT	YNEQFFGPGTRLTVL(Jβ2.1)	EDLKN	2/4
Vβ 19 LCASS	QDGAAY	GYTFGSGTRLTVV(Jβ1.2)	EDLNK	1/4
Control 1				
Vβ 7 FCASS	YRTGVKN	TEAFFGQGTRLTVV(Jβ1.1)	EDLNK	2/2
Vβ 8 FCASS	FSRRQ	NSPLHFGNGTRLTVT(Jβ1.6)	EDLNK	2/2
Vβ 12 FCASS	EDLRGY	GYTFGSGTRLTVV(Jβ1.2)	EDLNK	1/1
Control 2				
Vβ 12 FCASS	PQGQLWQ	ETQYFGPGTRLLVL(Jβ2.5)	EDLKN	5/5
Carrier PBL				
Vβ 2 FYICS	PVPLRGGDY	GYTFGSGTRLTVV(Jβ1.2)	EDLNK	3/3
Vβ 6 LCASS	KQGE	ETQYFGPGTRLLVL(Jβ2.5)	EDLKN	4/4
Vβ 7 FCASS	RNADT	QYFGPGTRLTVL(Jβ2.3)	EDLKN	2/3
Vβ 12 FCASS	YVGRGGY	TEAFFGQGTRLTVV(Jβ1.1)	EDLNK	1/3
Vβ 12 FCAIS	ESDSG	NQPQHFGDGTRLSIL(Jβ1.5)	EDLNK	2/3
Vβ 17 LCASS	NSGTSRQD	TQYFGPGTRLTVL(Jβ2.3)	EDLNK	3/4

* The number of indicated CDR3 sequences derived from bacterial colonies containing PCR-amplified DNA per total number of colonies sequenced. These sequence data are available from EMBL/GenBank/DDBJ under accession numbers D31825–D31832 and D32014–D32038.

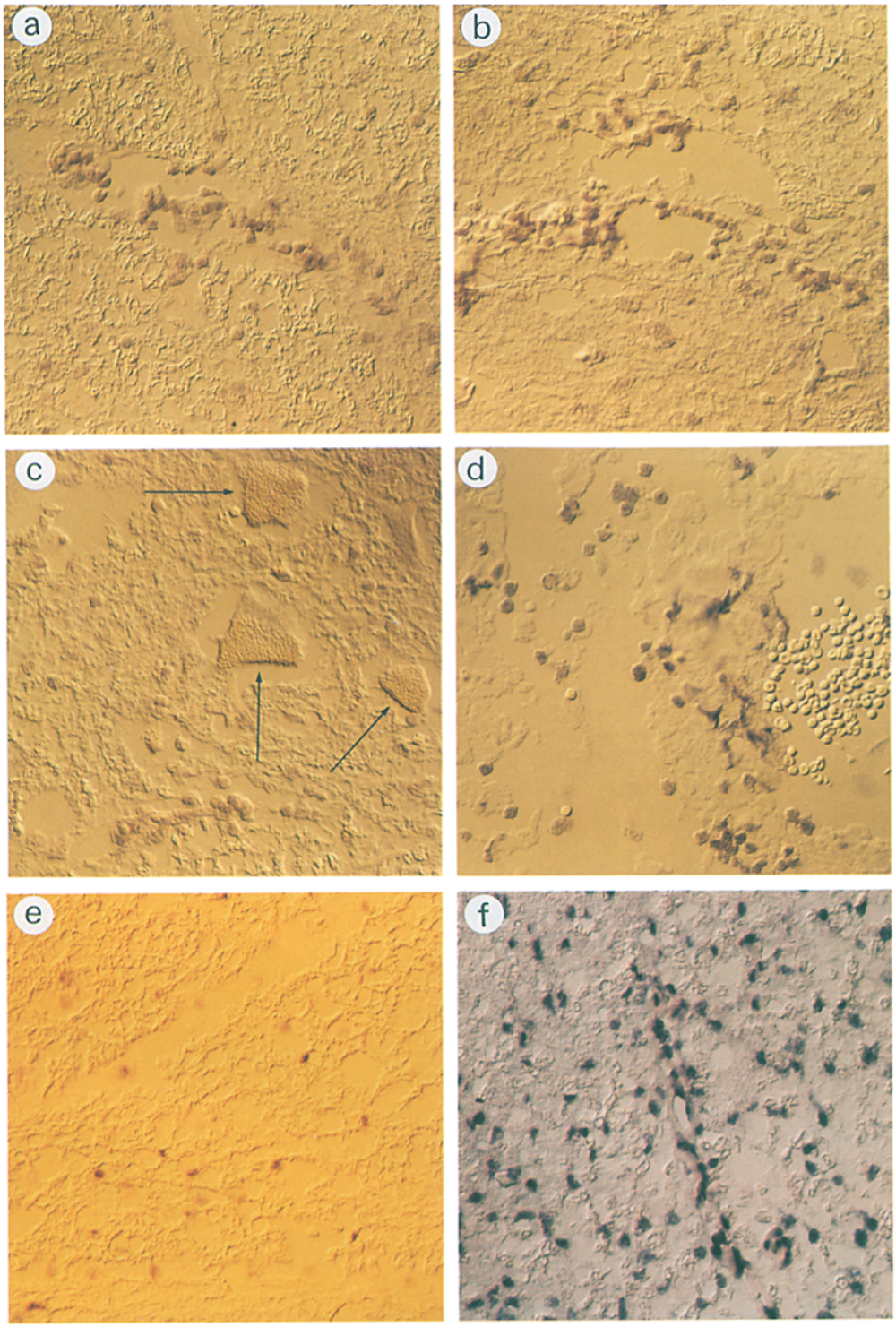
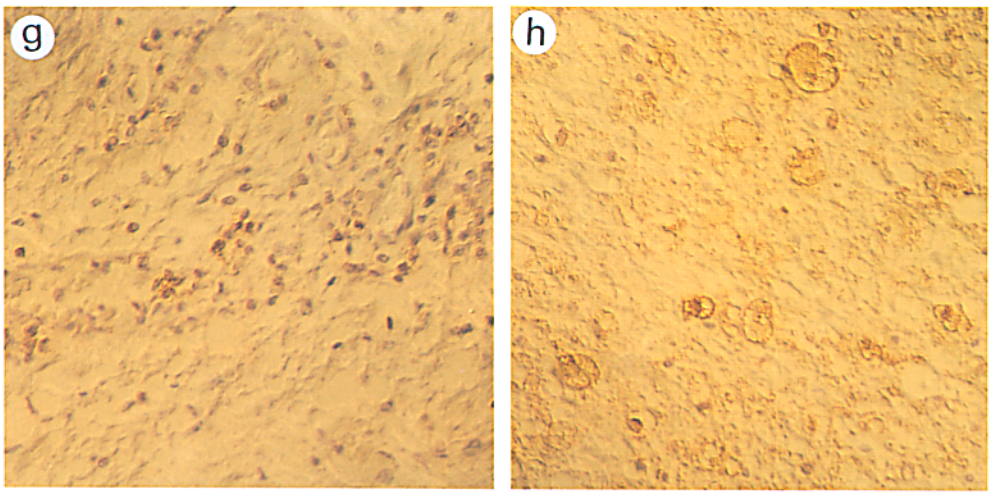


Figure 1. Distribution of HTLV-I proviral DNA in the spinal cord lesions of three HAM/TSP autopsy cases. HTLV-I was detected in the lymphocytes around the blood vessels in (*a*) white matter (HAM/TSP case 2) and (*b*) gray matter of the thoracic cord (HAM/TSP case 1), however, (*c*) not in the motor neurons (HAM/TSP case 1) (*arrows*). (*d*) HTLV-I–positive lymphocytes were also found in the thickened leptomeninges of the spinal cord (HAM/TSP case 3). (*e*) All of the infiltrated lymphocytes in the medulla oblongata ATL autopsy case were positive for HTLV-I proviral DNA. (*f*) All nuclei of cells in the spinal cord tissue were positive for control β globin. (*g*) Immunostaining with T cell marker. All of the infiltrated lymphocytes in the spinal cord of HAM/TSP case 1 were positive for T cell marker, MT-1 (pan-T) (5). They were also positive for UCHL-1 (CD45RO), and negative for B cell marker (MB-2) and macrophage marker (HLA-DR). (*h*) Activated microglia were positive for ferritin and negative for HTLV-I (HAM/TSP case 2).

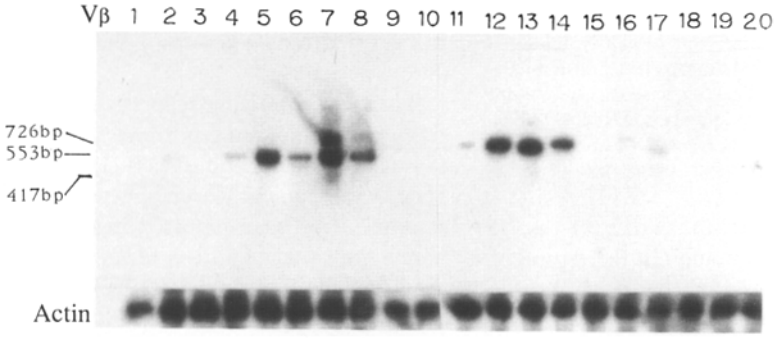
found in the thickened leptomeninges of the spinal cord (Fig. 1 *d*). These findings contrasted the fact that most T lymphocytes that penetrated into the CNS of ATL patients contained the HTLV-I proviral DNA in the nucleus (Fig. 1 *e*). On the other hand, no HTLV-I proviral DNA was detected in neuronal cells including motor neurons, astrocytes, oligodendrocytes, and microglia of the spinal cord in any of the HAM/TSP cases (Fig. 1, *c* and *h*). Likewise, nothing was found in the sections of the non-HTLV-I carriers.

Analysis of the TCR Vβ CDR3 Sequence of the Infiltrated Lymphocytes. To investigate the possibility of an autoimmune mechanism in the pathogenesis of HAM/TSP, we analyzed TCR Vβ usage of lymphocytes that infiltrated the sites of inflammation in the spinal cords from the same two HAM/TSP



autopsy cases. Rearranged Vβ 5, 6, 7, 8, 12, 13, and 14 genes in case 1 and Vβ 6, 8, 17, and 19 genes in case 2 were each identified by PCR (Fig. 2). Weak signals were observed in Vβ 4, 11, in case 1 and Vβ 5, 10, 12, 13, and 14 in case 2; these Vβ-expressed lymphocytes were a minor population, because the signal intensity was quantitatively less as compared with the actin control bands. We also sequenced these Vβ transcripts to study Vβ-Dβ-Jβ rearrangements (Table 1). Several clones from each Vβ family were picked up and sequenced. Although the CDR3 amino acid sequences were

Case 1



Case 2

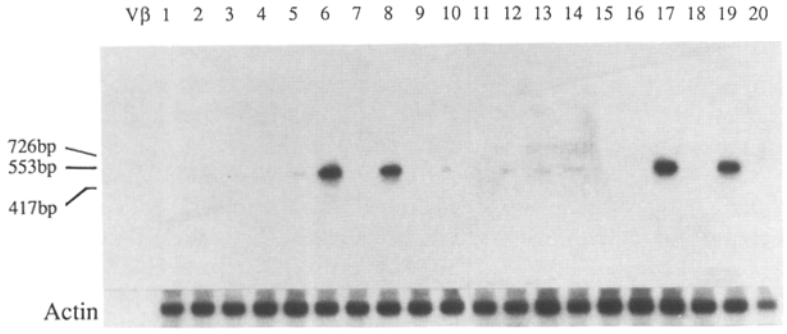


Figure 2. Southern blot analysis of TCR Vβ repertoire. Samples were taken from thoracic spinal cord of two patients with HAM/TSP case 1 and case 2. Vβ-Cβ amplified products from spinal cord cDNA were analyzed by Southern blot hybridization using Cβ probe. Actin was amplified in all Vβ samples as a positive control for successful amplification of cDNA.

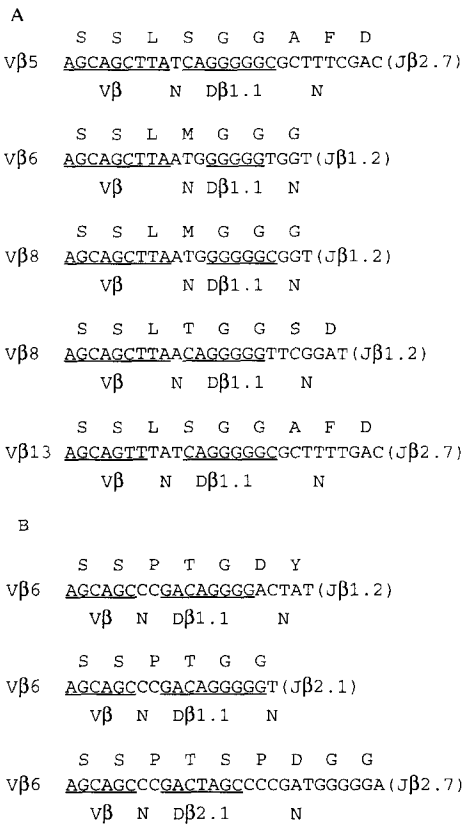


Figure 3. Vβ-Dβ-Jβ junctional nucleotide sequences of lymphocytes infiltrated in the spinal cord lesions of HAM/TSP patients. Genomic sequences of each Vβ and Dβ are underlined. These sequence data are available from EMBL/GenBank/DDBJ under accession numbers D31825–D31832.

identical in Vβ6 and Vβ8 or in Vβ5 and Vβ13 for case 1, the nucleotide sequences of the 5′ Vβ and CDR3 region of each Vβ gene were different from one another (Fig. 3). One of the conserved motifs found in the spinal cord of HAM/TSP case 1 contained the sequence CASSLXG(G) (X is any amino acid) in the CDR3 region from Vβ 5, 6, 8, and 13 transcripts. The number of colonies that showed the identical CDR3 sequence per total colonies sequenced in each Vβ family is listed in Tables 1 and 2. The CDR3 LCASSLXG is similar to the CDR3 sequence found in the brain lesions of MS (9) and in a T cell clone recognizing myelin basic protein (MBP) peptide 87-106 from a MS patient (10) and in a rat T cell clone recognizing MBP peptides 85-99 and 87-99 (11) (Table 2). Other motifs found in the spinal cord of HAM/TSP cases have the sequence Vβ6, Vβ19CASSPT(G), Vβ7, Vβ9CASSQD(G), and Vβ17CASSGRL. The CDR3 sequence CASSPT(G) also has been seen in brain lesions of MS (9) and CASSQD(G) was described in (PL/J × SJL)F_1 T cell clone specific for rat MBP (12). CASSGRL was observed in a rat T cell clone recognizing MBP peptides 85-99 and 87-99 (10). None of these CDR3 sequences was seen in the Vβ sequence from the spinal cord tissues of disease control samples (total of 10 colonies sequenced) or peripheral blood T cells of healthy HTLV-I carriers (total of 20 colonies sequenced).

Discussion

Postmortem study of HAM/TSP cases revealed that there was demyelination and marked lymphocyte infiltration around small vessels in the spinal cord, especially at the thoracic level (13). However, viral antigens have not been clearly identified in CNS tissue of HAM/TSP patients by immunohistochemistry (14), mainly because the copy number of HTLV-I DNA is too low to detect. A sensitive method, as mentioned in Materials and Methods, a two-step PCR in situ hybridization (Morita, M., H. Hachisuka, and Y. Sasai, manuscript submitted for publication) has made it possible for us to detect the proviral DNA in paraffin-embedded spinal cord sections. This method can detect as low as two copies of target DNA per cell. The present results show that HTLV-I proviral DNA was present only in the nuclei of infiltrated T lymphocytes that gathered around small vessels in the spinal cord, but was absent in neuronal cells including neurons, glial cells, and microglial cells. Therefore, a slow viral infection of HTLV-I in neurons, an immunological response by cytotoxic T cells or antibodies against infected neuronal cells in the spinal cord tissue of HAM/TSP patients, is very unlikely. Although high frequency of cytotoxic $CD8^+$ T cell specific for HTLV-I pX was observed in HAM/TSP patients (15), it is still hard to reason that this is the pathogenesis of HAM/TSP because the existence of cytotoxic T cells for PX was also observed in healthy carriers of whom <1% developed the clinical disease, and because we could not find the HTLV-I proviral DNA in neuronal cells taken from the spinal cord of HAM/TSP autopsy cases.

There are increasing reports that autoimmune-like conditions such as Sjögren syndrome (16), Hashimoto disease (17), polymyositis (18), and uveitis (19) are often complicated in HAM/TSP patients. Several phenomena of increased immune response have been reported in HAM/TSP patients. They have a high titer of anti-HTLV-I antibody in the serum and cerebro-spinal fluid, whereas ATL patients have a rather low titer. The number of activated, helper/inducer T cells is also increased in the peripheral circulation of HAM/TSP patients (20). It is known that these lymphocytes start to proliferate spontaneously during in vitro culture (21). These findings led us to analyze TCR Vβ sequence from the infiltrated lymphocytes of HAM/TSP, because residues in CDR3 associated with a particular Vβ are critical for the whole affinity of a TCR for its ligand (22). Unique CDR3 sequences were found in MBP-reactive T cell clones derived from EAE animals (10, 12) and MS patients (11) as well as in the infiltrated lymphocytes from the brain lesions of MS patients (9). Wilson et al. (23) pointed out that an antigen recognition structure, which is composed of the particular sequence of the CDR3 junctional region in combination with a certain β chain variable region, may play a crucial role in the pathogenesis of EAE and MS.

TCR Vβ-Dβ-Jβ CDR3 sequences of lymphocytes that infiltrated into the spinal cords of HAM/TSP patients were highly conserved. These CDR3 motifs, CASSLXG(G), CASSPT(G), CASSQD(G), and CASSGRL, were similar to those found in the brain lesions of MS patients and in the T cell

Table 2. *Comparison of Vβ CDR3 Amino Acid Sequences among HAM/TSP, MS, and MBP-reactive T Cell Clones*

HAM/TSP				
Case 1	Vβ	N-D-N	Jβ	Number/total*
Vβ 5	CASS	LSGGAFD	EQYFGPGTRLTVT(Jβ2.7)	2/5
Vβ 6	CASS	LMGGG	GYTFGSGTRLTVV(Jβ1.2)	2/4
Vβ 8	CASS	LMGGG	GYTFGSGTRLTVV(Jβ1.2)	2/5
Vβ 8	CASS	LTGGSD	GYTFGSGTRLTVV(Jβ1.2)	2/5
Vβ 13	CASS	LSGGAFD	EQYFGPGTRLTVT(Jβ2.7)	3/5
MS brain lesions‡				
Vβ5.2	CASS	LRGAN	Jβ 2.6	
Vβ5.2	CASS	LGGSE	Jβ 2.5	
T cell clone for MBP pep 87-106 from MS patient§				
Vβ5.2	CASS	LRGAL	Jβ 2.4	
Lewis rat EAE T cell clone for MBP peptides 85-99 and 87-98‖				
Vβ8.2	CASS	LGGE	Jβ 2.5	
Vβ 6	CASS	LRG	Jβ 1.6	
HAM/TSP				
Case 1	Vβ	N-D-N	Jβ	Number/total*
Vβ 6	CASS	PTGDY	GYTFGSGTRLTVV(Jβ1.2)	1/4
Case 2				
Vβ 6	CASS	PTGG	EQFFGPGTRLTVL(Jβ2.1)	3/5
Vβ 6	CASS	PTSPDGG	EQYFGPGTRLTVL(Jβ2.7)	1/5
Vβ 17	CASS	GRLFS	NQPQHFGDGTRLSIL(Jβ1.5)	5/5
Vβ 19	CASS	PTRDYT	YNEQFFGPGTRLTVL(Jβ2.1)	2/4
MS brain lesions‡				
Vβ 5	CASS	PT	GANVLTFGAGSRLTVL(Jβ2.6)	
Lewis rat EAE T cell clone for MBP peptides 85-99 and 87-98‖				
Vβ 4	CASS	GRLGE	YAEQ . . . . . (Jβ2.1)	
HAM/TSP				
Case 1	Vβ	N-D-N	Jβ	Number/total*
Vβ 7	CASS	QDPAAA	YNEQFFGPGTRLTVL(Jβ2.1)	1/3
Case 2				
Vβ 19	CASS	QDGAAY	GYTFGSGTRLTVV(Jβ1.2)	1/4
(PL/J × SJL)F_1 T cell clone specific for rat MBP¶				
Vβ 4	CASS	QDGWGNQ	Jβ2.5	

* The number of indicated CDR3 sequences derived from bacterial colonies containing PCR-amplified DNA per total number of colonies sequenced.
‡ Oksenberg et al. (9).
§ Martin et al. (10).
‖ Gold et al. (11).
¶ Acha-Orbea et al. (12).

clones with specificity for MBP as shown in Table 2. One of such unique motifs, CASSLXGG, was also identical to a motif found in SJL/J mice T cell clones which recognizes MBP peptides 89-101 (24). Although TTA codon for the Leu residue of CASSLXGG motif is mostly encoded in germline Vβ genes (25–27), these TTA nucleotides are frequently deleted for other nucleotides are randomly added, so that this Leu residue is not always conserved in the TCR Vβ-Dβ junction of the T cell clones previously reported (9, 28). There is a possibility that GlyGly residues seen in the CASSLXGG

motif are derived from germline D segments (27). However, we could not find this CASSLXG(G) motif in any of the CDR3 sequences obtained from both the control spinal cords and PBLs of healthy carriers. Therefore, it appears that this unique motif, which is conserved in the TCR CDR3 region of MBP-reactive T cell clones from MS and EAE patients (9, 10, 11, 23), is also present in the TCR CDR3 of HAM/TSP infiltrated T cells. The fact that T cells bearing such a specific TCR CDR3 sequence are found in the spinal cord lesions of HAM/TSP patients as well as in the brain lesions of MS patients raises some thought-provoking questions on the etiology of these diseases.

The TCR CDR3 motifs found in HAM/TSP patients, which are highly conserved even though they have different Vβ families, may arise because of a genetically based, structurally determined preference for this particular VDJ recombination. Another possible explanation for the genesis of these conserved motifs may be that certain specific antigen–MHC complexes positively select for these unique CDR3 motifs. If the latter is true, self-antigens such as MBP, proteolipid protein, and heat shock protein, which are frequently released and exposed to immune system during CNS inflammation, are primary candidates to be TCR ligands.

Recently, Lunardi-Iskander, et al. (29) reported that HTLV-I infection was associated with abnormal proliferation and differentiation of T cell progenitors in vitro. Therefore, one hypothesis to explain the pathogenesis of HAM/TSP is that T cells reactive to self-antigens may exist in a state of anergy in the periphery. These self-reactive T cells become activated and propagate in some genetically predisposed individuals upon HTLV-I infection. Identification of the antigen(s) that is recognized by T cell clones bearing these unique TCR Vβ CDR3 sequences from HAM/TSP patients is now under investigation.

We thank Perry Allan Seto for reading the manuscript.

This work was supported in part by a Grant in Aid for Cancer Research (05152093) from the Ministry of Education, Science and Culture of Japan.

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Received for publication 30 August 1993 and in revised form 11 April 1994.

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