JOURNAL OF VIROLOGY, Feb. 1996, p. 843–851 Vol. 70, No. 2
0022-538X/96/$04.00+0

Analysis of the T-Cell Receptor Repertoire of Human T-Cell Leukemia Virus Type 1 (HTLV-1) Tax-Specific CD8$^+$ Cytotoxic T Lymphocytes from Patients with HTLV-1-Associated Disease: Evidence for Oligoclonal Expansion

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Received 29 August 1995/Accepted 2 November 1995

Human T-cell leukemia virus type 1 (HTLV-1)-associated myelopathy/tropical spastic paraparesis (HAM/TSP) is a chronic, progressive neurological disease characterized by marked degeneration of the spinal cord and the presence of antibodies against HTLV-1. Patients with HAM/TSP, but not asymptomatic carriers, show very high precursor frequencies of HTLV-1-specific CD8$^+$ T cells in peripheral blood and cerebrospinal fluid, suggestive of a role of these T cells in the pathogenesis of the disease. In HLA-A2$^+$ HAM/TSP patients, HTLV-1-specific T cells were demonstrated to be directed predominantly against one HTLV-1 epitope, namely, Tax11-19. In the present study, we analyzed HLA-A2-restricted HTLV-1 Tax11-19-specific cytotoxic T cells from three patients with HAM/TSP. An analysis of the T-cell receptor (TCR) repertoire of these cells revealed an absence of restricted variable (V) region usage. Different combinations of TCR Vα and Vβ genes were utilized between, but also within, the individual patients for the recognition of Tax11-19. Sequence analysis of the TCR showed evidence for an oligoclonal expansion of few founder T cells in each patient. Apparent structural motifs were identified for the CDR3 regions of the TCR β chains. One T-cell clone could be detected within the same patient over a period of 3 years. We suggest that these in vivo clonally expanded T cells might play a role in the pathogenesis of HAM/TSP and provide information on HTLV-1-specific TCR which may elucidate the nature of the T cells that infiltrate the central nervous system in HAM/TSP patients.

Human T-cell leukemia virus type 1 (HTLV-1), a member of the mammalian type C oncovirus family, is the only known human RNA virus that is associated with a human malignancy, adult T-cell leukemia. In addition, the virus is also implicated in the pathogenesis of a slowly progressive neurological disease termed HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (38). Further, HTLV-1 has been suspected to play a role in autoimmune diseases such as rheumatoid arthritis and Sjögren's syndrome (46), systemic lupus (23), and multiple sclerosis (47). However, the evidence for any association with autoimmune diseases is highly controversial.

Exposure to the virus leads to a persistent infection that rarely results in disease progression. The majority of seropositive individuals (more than 95%) remain asymptomatic. What causes progression to disease is unknown but might involve genetic predisposition (44), different viral strains (18), the route of transmission, and differences in the host immune responses.

HAM/TSP is characterized by a thoracic spinal cord atrophy involving perivascular demyelination and axonal degeneration. Although the mechanism leading to clinical disease is not clearly understood, some differences between asymptomatic carriers and HAM/TSP patients were identified, suggesting the disease to be the outcome of an immunopathological mechanism. First, in contrast to asymptomatic carriers, patients with HAM/TSP show extraordinarily high levels of circulating CD8$^+$ HTLV-1-specific cytotoxic T lymphocytes (CTL) (20), which can be demonstrated directly from peripheral blood lymphocytes (PBL) by cytotoxicity assays. The precursor frequencies range from 1 in 75 to 1 in 280 among CD8$^+$ PBL (11). In affected HLA-A2-positive individuals the vast majority of these CD8$^+$ HTLV-1-specific CTL recognize one viral epitope presented by HLA-A2 (11). This immunodominant epitope is derived from the HTLV-1 Tax regulatory protein and spans amino acids 11 to 19 (22, 45).

Second, immunohistochemical analysis revealed the presence of infiltrating CD8$^+$ T lymphocytes in the spinal cord lesions that are characteristic for HAM/TSP (33, 43, 49). The numbers of infiltrating cells increased with the duration of disease (25, 43). In HAM/TSP patients of long duration, the phenotype of the inflammatory cells in spinal cord lesions was almost exclusively CD8$^+$ T cells (33). Although the specificity of these infiltrating T lymphocytes is not known, it is compelling to consider that these CD8$^+$ inflammatory cells might be HTLV-1 specific as (i) HTLV-1-specific T lymphocytes in large numbers have been detected in the cerebrospinal fluid in affected individuals (11), and (ii) the presence of HTLV-1 gene products in material from the central nervous system has been reported (26, 27).

In the present study we characterized the T-cell receptor (TCR) usage of HTLV-1 Tax-specific T cells restricted by HLA-A2 because those cells constitute the majority of virus-specific T cells in HLA-A2-positive individuals. Additionally, the immunodominant Tax epitope (Tax11-19) has been well characterized (22, 45), and the structural conformation of the Tax11-19/HLA-A2 complex has recently been resolved by crystallography (29). Our analysis demonstrates that the TCRs used within each patient display a very limited heterogeneity, indicative of an oligoclonal in vivo expansion of HTLV-1-

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TABLE 1. TCR Vα- and Vβ-family-specific primers used for the TCR analysis[a]

Primer	Sequence	Primer	Sequence
Vα1	aga gcc cag tcg gtg acc cag	Vβ1	gag aga gca aaa gga aac att ctt gaa
Vα2	gt gtt cca gag gga gcc att g	Vβ2	tcc aag gcc aca tac gag caa ggc gtc
Vα3	g gtg aac agt caa cag gga ga	Vβ3	gaa aaa gga gat att cct gag ggg tac
Vα4	t gat gct aag acc acc cag cc	Vβ4	gag gcc aca tat gag agt gga ttt gtc
Vα5	g gcc ctg aac att cag gag gg	Vβ5	a gag aga ggc aga gga aac ttc cct g
Vα6	g tca ctt tct agc ctg ctg aa	Vβ6	aaa gct caa cta gac aaa tca ggg
Vα7	a gga gcc att gtc cag ata aa	Vβ7	agt gtg cca agt cgc ttc tca cct gaa
Vα8	gga gag agt gtg gag ctg cat c	Vβ8	gtt ccg ata gat gat tca ggg atg ccc
Vα9	atc tca gtg ctt gtg ata ata	Vβ9	t ata aat gaa aca gtt cca aat cgc tt
Vα10	acc atg tac tgc aat tcc tca ac	Vβ10	att cag aaa gca gaa ata atc aat gag
Vα11	a gaa agc aag gac caa gtg tt	Vβ11	aca gag aag gga gat ctt tcc tct gag
Vα12	cag aag gta act caa gcg cag act	Vβ12	gac aaa gga gaa gtc tca gat ggc tac
Vα13	agt gcc cag gtt tgc tgt gtg	Vβ14	agg cag ggg cct cca gct gct ctt cta
Vα14	gtg atc tcc acc tgt ctt gaa t	Vβ15	ata aac aaa gga gag atc tct gat gga
Vα15	agt atg agt aga gga gag gat	Vβ16	cat gat aat ctt tat tgg tat cga cgt
Vα16	agt ggg ctg aga gct cag tca	Vβ17	cag aaa gga gat ata gct gaa ggg tac
Vα17	gtg act ata cta aca gca tgt	Vβ18	gag tca gga atg cca aag gaa cga ttt
Vα18	atg aca agg gaa gca aca aag	Vβ19	gaa acg gag atg cac aag aag cga ttc
Vα19	caa agt cct cag tct ctg att gtc	Vβ21	gta gac gat tca cag ttg cct aag gat
Vα22	caa cag cta atg cag agt cct caa	Vβ22	gaa atc tca gag aag tct gaa ata ttc
Vα23	gag gtg aca cag att cct gca gct	Vβ23	ttc ctc att tcg ttt tat gaa aag atg
		Vβ24	ttc cac tac tat gac aaa gat ttt aac
CαExt	agc gtc atg agc aga tta aac ccg	CβExt	gat ggt ggc aga cag gac ccc ttg ctg

[a] The primer specific for Vα10 also amplifies Vα27 and Vα30; primer Vα19 amplifies the single family members of Vα19, -20, -21, -24, -26, and -28; and primer Vα22 amplifies Vα22 and -25. The above-mentioned Vα families are expressed rarely and were grouped together according to homologies and to reduce the amount of material required for each TCR Vα or Vβ analysis. The Vβ13 family members were grouped with the Vβ12 family members on the basis of their high homology. Vβ20 is a pseudogene.

specific CTL. Furthermore, sequence analysis revealed common structural motifs in the CDR3 regions of HTLV-1 Tax-specific $CD8^+$ CTL. If indeed virus-specific CTL contribute to the pathology of HAM/TSP, these CTL would be expected to be present in central nervous system lesions. The knowledge of the sequences of virus-specific TCRs is a first step towards the resolution of this question.

MATERIALS AND METHODS

Patients. Peripheral blood mononuclear cells were obtained after informed consent from three HTLV-1-seropositive patients diagnosed with HAM/TSP at the National Institutes of Health, Bethesda, Md. HLA typing was performed in the HLA Typing Laboratory, Department of Transfusion Medicine, National Institutes of Health. Patient 1 (HLA: A1, 2; B7, 35; Cw1,7; DR1,4; DQ5,8; Dw53), a 54-year-old Hispanic male, was first diagnosed with HAM/TSP 10 years ago, patient 2 (HLA: A2, 66; B44, 53; Cw4; B1.1201, B1.1501, B1.0301, B1.0602, B3.0202, B5.0101), a 54-year-old black male, was diagnosed 9 years ago, and patient 3 (HLA: A2, 28; B18,44; Cw6,7; DR5,7; DQ2,3; Dw52), a 53-year-old Caucasian male, was diagnosed 13 years prior to this study. All three patients showed antibodies to HTLV-1 in their sera and cerebrospinal fluid and did not present lesions in the head or spine as measured by magnetic resonance imaging.

Synthetic peptides. Peptides were purchased from Synthecell (Gaithersburg, Md.). The sequences for HTLV-1 Tax11-19 and influenza virus A M1 58-66 were LLFGYPVYV and GILGFVFTL, respectively. The peptides were stored as stock solutions in phosphate-buffered saline with 50% dimethyl sulfoxide at 1 mg/ml.

Generation of HTLV-1 Tax11-19-specific HLA-A2-restricted CTL. PBL from three patients with HTLV-1-associated myelopathy (HAM/TSP) were sorted for $CD8^+$ cells, using magnetic beads coated with anti-CD8 antibodies (Dynabeads M-450 CD8; Dynal, Lake Success, N.Y.). $CD8^+$ cells were detached from the beads, using a polyclonal anti-immunoglobulin antibody according to the procedures recommended by the manufacturer (DetachaBeads; Dynal). Following one in vitro stimulation with peptide-pulsed (1 μg of Tax11-19 per ml for 1 h at 37°C), irradiated (3,000 rads) autologous PBL, the cells were set up in limiting dilution at 10 cells per well in 96-well tissue culture plates (Costar, Cambridge, Mass.). The cells received 4 U of recombinant interleukin 2 per well on day 1 and 20 U per well on day 7. The first visible T-cell lines appeared on day 10 after limiting dilution. The T-cell lines were tested for cytotoxicity and antigen specificity, and all positive clones were expanded as previously described (15).

Cytotoxicity assay. The cytolytic activity, antigen specificity, and HLA restriction of T-cell clones were assayed in a standard chromium release assay as previously described (15). Target cells were Hmy2.C1R cells (42) transfected with HLA-A2 (41) or HLA-A3 (10) and pulsed with either Tax11-19 or M1 58-66. The results of cytotoxicity assays are expressed as mean percent specific lysis of triplicate determinations (15).

RNA extraction and cDNA synthesis. T-cell lines were kept without feeder or antigen-presenting cells for at least 12 days prior to RNA preparation. RNA from 0.2×10^6 to 2×10^6 T cells was extracted by lysis with a guanidinium-isothiocyanate solution, followed by phenol-chloroform extraction and precipitation with isopropanol as described elsewhere (6). First-strand cDNA was transcribed with oligo(dT) and random hexanucleotides as primers, using a cDNA synthesis kit (Pharmacia, Piscataway, N.J.). Synthesis reactions were performed as suggested by the manufacturer. The first-strand cDNA was used for the analysis of TCR usage by PCR.

TCR analysis. An analysis of the TCR usage of T-cell lines was undertaken, using 21 TCR Vα- and 22 TCR Vβ-family-specific primers together with primers annealing in the respective constant regions of the TCR α- and β-chain genes. A summary of all TCR Vα- and Vβ-family-specific primers used in the PCRs is shown in Table 1. Briefly, PCRs were set up as batches containing cDNA, 1× PCR buffer (Boehringer, Mannheim, Germany), deoxynucleoside triphosphates (Perkin-Elmer), constant-region primer, and *Taq* polymerase (Boehringer). Fifteen microliters of the mixture was distributed to 21 (for TCR Vα analysis) or 22 (for TCR Vβ analysis) tubes each containing 5 μl of a 20 μM solution of a respective Vα or Vβ primer. First-strand cDNA representing material from 2,000 to 5,000 cells was used for each individual PCR. The applied PCR conditions (30 cycles; 1 min at 94°C, 20 s at 60°C, 1 min at 72°C, followed by 10 min at 72°C) proved stringent and excluded cross-annealing. The amplification products were separated on 1% agarose gels and visualized with ethidium bromide. The band sizes ranged from 650 to 800 bp. The identities of the TCR V regions were verified for most cases by a second independent PCR using V-region primers containing one *Eco*RI site. These restriction sites allowed cloning of the PCR products in pUC18 via *Eco*RI. Multiple colonies were sequenced in each case to confirm V-region usage and to determine the productivity of a rearrangement. Generic primers annealing in the 5′ regions of the two constant regions were used to obtain CDR3 region sequences. All colonies sequenced from an individual amplified TCR confirmed the previous V-region analyses, and all appeared homogeneous.

RESULTS

Establishment of HTLV-1 Tax11-19-specific CTL. Previous studies of the virus-specific T-cell responses in HAM/TSP patients had shown precursor frequencies of 1 in 75 to 1 in 280 among $CD8^+$ PBL (11). The vast majority of these HTLV-1-specific CTL recognized one epitope derived from the viral

regulatory protein Tax. This epitope consists of amino acids 11 to 19 (LLFGYPVYV) of the protein and is presented by HLA-A2 (22, 45). Three HAM/TSP patients were initially selected for analysis of their Tax-specific CTL repertoires on the basis of the following criteria: (i) at least a 9-year duration of disease, (ii) HLA-A2 positivity, and (iii) high precursor frequencies for HLA-A2-restricted Tax11-19-specific $CD8^+$ CTL in their PBL. A panel of HLA-A2-restricted Tax11-19-specific CTL were established from each of these patients as described in Materials and Methods. All CTL lysed only Hmy2.C1R cells that had been transfected with HLA-A2 and pulsed with Tax11-19. The same targets pulsed with the HLA-A2-restricted peptide M1 58-66 were not lysed, and Tax11-19-pulsed Hmy2.C1R cells transfected with HLA-A3 were also not lysed. Representative results are shown in Fig. 1A. Titration experiments were performed to determine the concentration of the Tax11-19 peptide required to produce half-maximal specific lysis. Two of the 11 CTL tested were able to specifically lyse target cells pulsed for 1 h with 10^{-17} M Tax11-19 (Fig. 1B). All CTL had half-maximal lysis values at $\leq 10^{-9}$ M Tax11-19 (Fig. 1B).

TCR repertoire of Tax11-19-specific CTL. The TCR repertoire was determined for a total of 8 CTL from patient 1, 5 from patient 2, and 11 from patient 3. To analyze the TCR genes expressed by these HLA-A2-restricted Tax11-19-specific CTL, two sets of TCR Vα- and Vβ-family-specific primers were used for PCR amplifications. CTL displaying more than one prominent Vβ signal were excluded from the study, as they were thought not to represent a clonal T-cell population. Figure 2 shows the representative results of a TCR Vα and Vβ analysis for CTL clone 2F2 from patient 1 (Fig. 2A), clone 10B7 from patient 2 (Fig. 2B), and clone 1A7 from patient 3 (Fig. 2C).

Three of the eight CTL clones analyzed for patient 1 (3F9, 2F2, and 1G5) expressed Vα16 and Vα18 together with Vβ17 (Table 2; Fig. 2A). The three TCR genes were subsequently cloned and sequenced for each of the CTL clones to verify the PCR analysis. All belonged to their predicted TCR V-region family. The Vα18 gene showed the same nonproductive rearrangement in each CTL line (data not shown). Each Vα16 gene was productively rearranged to Jα28; each Vβ17 gene was productively rearranged to Jβ2.1 and Cβ2 (Table 3). The nucleotide sequences for the TCR α- and β-chain genes were found to be identical for all three CTL lines (Table 3). These results clearly prove that the three CTL lines are identical progeny of the same clone. Two other CTL lines from patient 1 (1B6 and 3G12) each used Vα19 and Vα6 together with Vβ5 (Table 2). The Vβ5 genes of these two CTL lines were sequenced and verified as Vβ5.14 genes productively rearranged to Jβ2.1 and Cβ2 (Table 3) the same Jβ that had been used by the three clones described above. 1B6 and 3G12 also appear to be the offspring of a single clone. Two additional CTL lines from patient 1(2F12 and 3F2) expressed Vα12 and Vβ3 (Table 2). Sequence analysis of their Vβ3 genes revealed identical sequences (Table 3), again identifying them as the progeny of a single clone. Thus, for patient 1, seven of the eight CTL lines established represent the progeny of three founder clones.

Analysis of the five Tax11-19-specific CTL lines established from patient 2 demonstrated that two CTL lines (10C2 and 10C7) expressed Vα2 and Vα4 (Table 2). The two genes from each CTL line were cloned and sequenced. Both Vα4 genes were nonproductively rearranged and showed identical nucleotide sequences (data not shown). The Vα2 genes were both productively rearranged with Jα24 and were identical in their nucleotide sequences (Table 3). Both 10C2 and 10C7 expressed Vβ12. Therefore, the two CTL clones are derived from one founder clone. Two additional CTL lines from patient 2 expressed Vα17, Vα5, and Vβ12 (Fig. 2B). Sequence analysis showed that the genes in both lines were identical and that Vα5 was nonproductively rearranged. Vα17.2 was connected with Jα54, and Vβ12 was connected with Jβ2.7 and Cβ2 (Table 3). CTL clone 10D2 expressed Vα1.2 combined with Vβ16. The productive rearrangement of both TCR chains was verified by sequence analysis, and Vβ16 expression was additionally confirmed through fluorescence-activated cell sorter analysis using a TCR Vβ16-specific monoclonal antibody (data not shown). Thus, four of the five CTL lines from patient 2 originated from two founder CTL clones.

The TCR repertoire of 11 CTL lines was analyzed for patient 3. Three of these lines (1H2, 2A3, and 1A7) expressed Vα5, Vα8, and Vβ4 (Fig. 2C). The only productively rearranged Vα gene, Vα5, was combined with Jα6. Vβ4 was productively rearranged with Jβ1.2 and Cβ1. The nucleotide sequences were identical for all three CTL lines, indicative of clonal identity. Of the remaining eight CTL lines from patient 3 four expressed Vβ12, suggesting a preferential usage of this V-region gene in this patient. Interestingly, the TCR β-chain gene sequence for CTL 2G4 (Vβ12.3 rearranged with Jβ2.7) proved identical to the TCR β-chain gene sequence of CTL A6 (44a), a T-cell clone generated 3 years earlier from the same patient and with the same HLA restriction and antigen specificity. Also, both CTL A6 and 2G4 expressed Vα2.

Comparison of TCR sequences expressed in HLA-A2-restricted Tax11-19-specific CTL. A total of 7 unique TCR α-chain and 11 unique TCR β-chain sequences were examined for the occurrence of common motifs in their complementarity determining region 3 (CDR3), the part of a TCR gene that is created through the rearrangement of a V-region and a J-region gene and which is additionally shaped through N additions. A compilation of all TCR α- and β-chain sequences obtained is shown in Table 3.

The TCR Vα region genes were found to be rearranged with multiple Jα genes (Table 3). No significant predominance of a certain Jα was observed. Interestingly, the only two sequences derived from two patients that had used Vα2 were both rearranged with Jα24. In all TCR α-chain sequences two hydrophobic amino acids were found following directly after the last cysteine in the V region, irrespective of the V region used. Two of the seven sequences showed a subsequent arginine; three more had a serine or threonine at this position. No other apparent motifs were found within the CDR3 of TCR α-chain sequences.

Eight of the 11 unique TCR Vβ chain sequences were rearranged with Jβ2.1 or Jβ2.7. These two junctional genes show strong homologies with each other, both displaying the sequence Y(N)EQXFGPGTRLTV (X represents phenylalanine or tyrosine). Five of 11 sequences contained a proline followed by a glycine in the CDR3, generated either by the combination of N additions with a diversity region gene or solely by N addition. Two additional TCR β chains contained a proline in the CDR3 with no glycine following.

We determined the sequences of TCR Vα/Vβ heterodimers for 10 CTL clones generated from three patients (Table 4). The 10 heterodimers represented six unique TCR pairs, thus allowing the comparison of TCRs with identical HLA restriction and epitope specificities but differing in Vα/Vβ composition. Arginine created through N addition was present in the CDR3 regions of three of the six unique TCR pairs. The other three pairs showed a glutamine or glutamic acid at the same position. A proline/glycine motif was observed within the CDR3 regions of TCR β chains in four of the six TCR heterodimers. In each instance, this motif was created at least

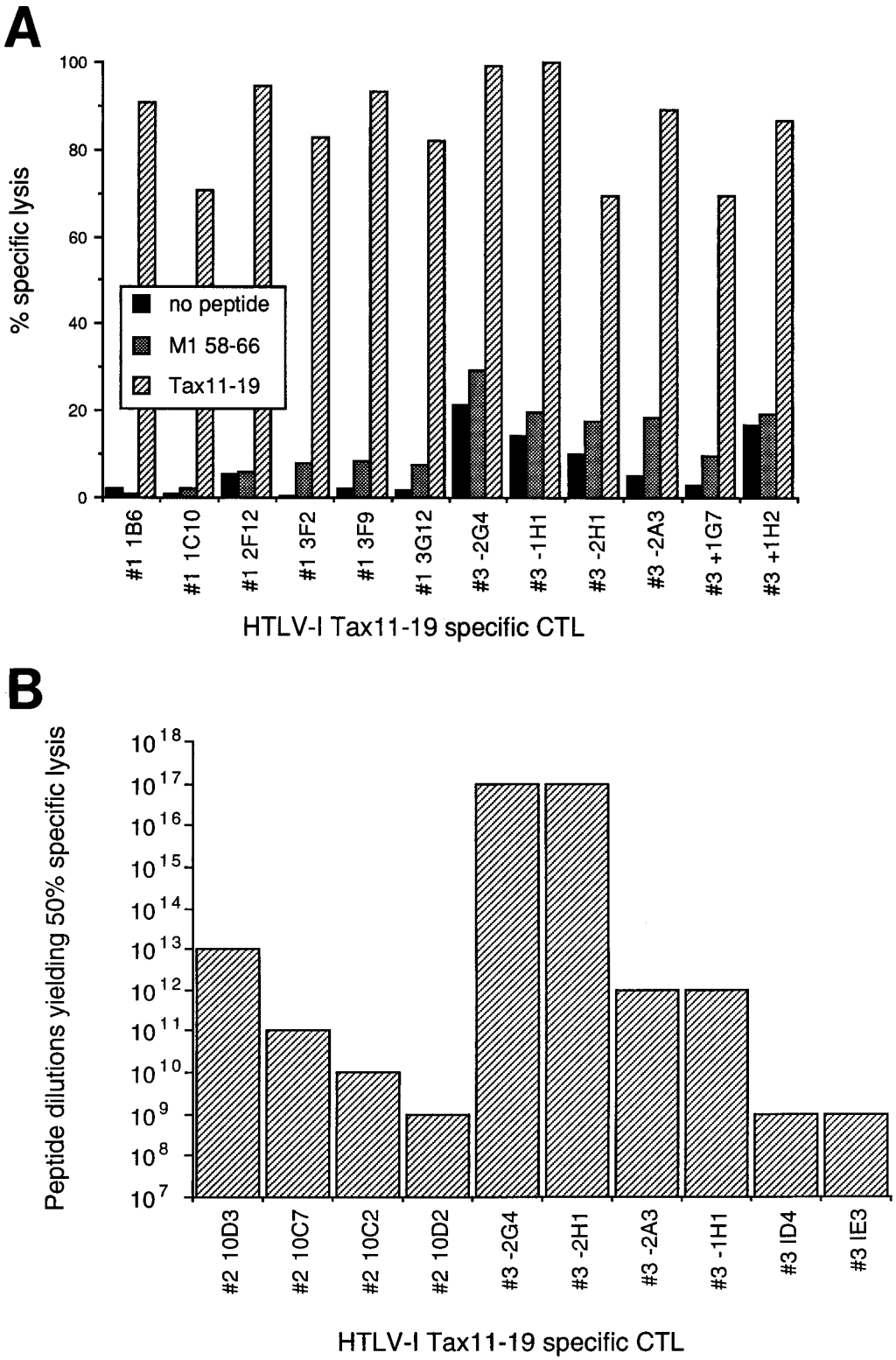

FIG. 1. (A) Antigen specificity and HLA restriction of HTLV-1 Tax11-19-specific HLA-A2-restricted CTL generated from two HAM/TSP patients. Hmy2.C1R cells transfected with HLA-A2 were pulsed with 1 μg of Tax11-19 or M1 58-66 per ml, or no peptide was added. The target cells were incubated for 1 h, and cytotoxicity assays were performed as described in Materials and Methods. Untransfected or HLA-A3-transfected Hmy2.C1R cells were not lysed by any of the T-cell lines (data not shown). (B) Determination of the concentration of Tax11-19 at which 50% specific lysis can still be obtained. Hmy2.C1R cells transfected with HLA-A2 were incubated for 1 h with 10-fold dilutions of Tax11-19 starting with 1 μg/ml (1 μM). Dilutions (molar) of Tax11-19 are indicated on the *y* axis. The corresponding CTL and patients are indicated on the *x* axis.

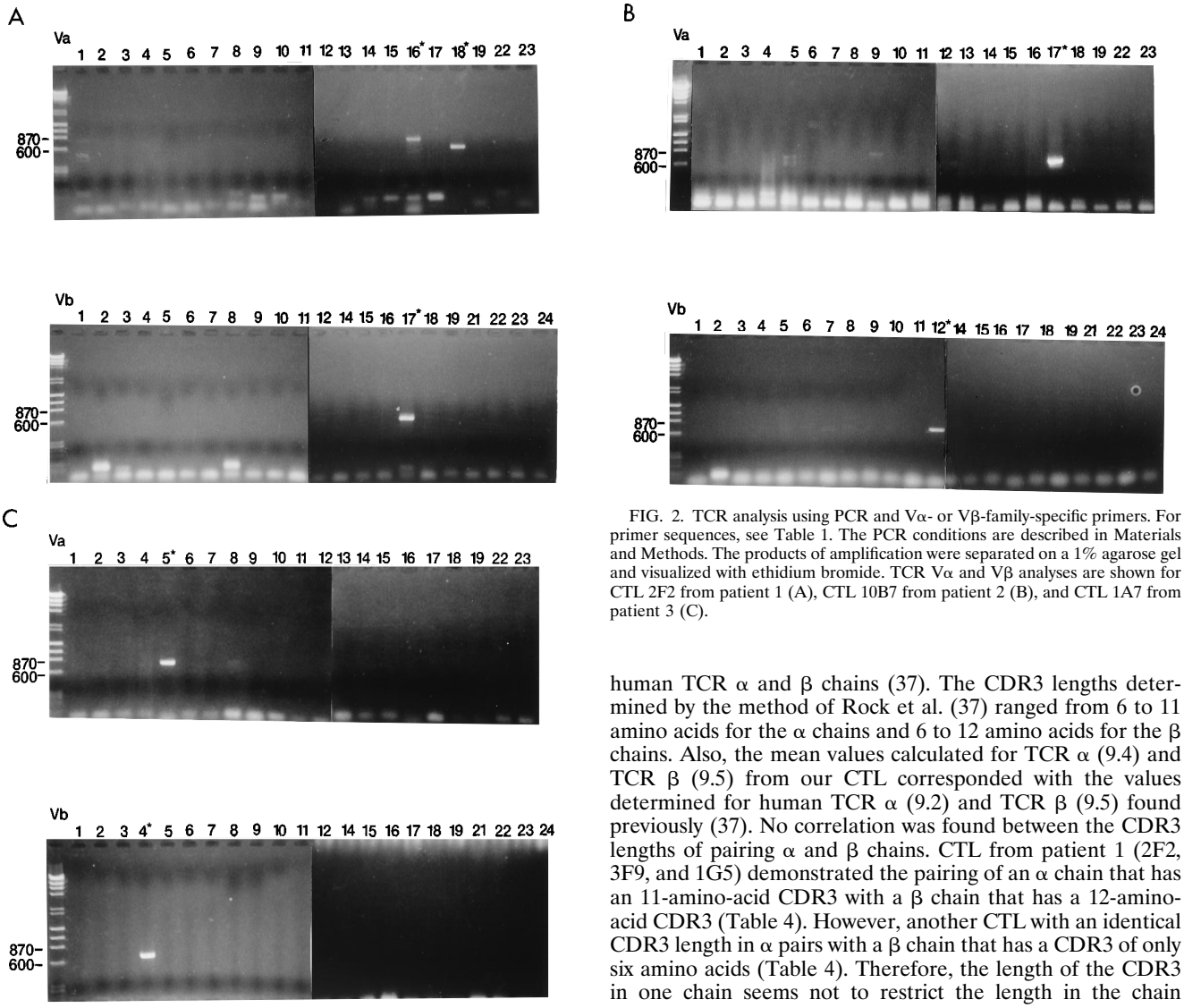

FIG. 2. TCR analysis using PCR and Vα- or Vβ-family-specific primers. For primer sequences, see Table 1. The PCR conditions are described in Materials and Methods. The products of amplification were separated on a 1% agarose gel and visualized with ethidium bromide. TCR Vα and Vβ analyses are shown for CTL 2F2 from patient 1 (A), CTL 10B7 from patient 2 (B), and CTL 1A7 from patient 3 (C).

partially through N additions, and all four TCR β chains were rearranged with Jβ2.1 or Jβ2.7. The proline/glycine motif was found in combination with Vβ12.3, Vβ16, and Vβ17. Overall, common structural motifs within the CDR3 regions were observed for the TCR β chains but not within the TCR α chains. That motifs must not predict the avidity of a TCR is demonstrated by the proline/glycine motif. CTL clones 2G4 and ID4, both generated from patient 3, express Vβ12 and contain the proline/glycine motif in their CDR3. However, 2G4 lyses target cells pulsed with peptide amounts as low as 10^{-17} M, whereas ID4 requires at least 10^{-9} M for 50% specific lysis (Fig. 1B). CTL 2G4 expresses Vα2; the Vα gene expressed by ID4 could not be determined. It is likely that the α chain of a TCR contributes to its affinity and that motifs in one CDR3 might not be sufficient for mediation of high affinity. Also, single amino acid differences might have dramatic effects and increase or diminish affinity, as was seen for single amino acid substitutions in major histocompatibility complex (MHC) genes and peptides.

The CDR3 lengths of the 7 unique TCR α chains and 11 unique TCR β chains were within the range typically found for human TCR α and β chains (37). The CDR3 lengths determined by the method of Rock et al. (37) ranged from 6 to 11 amino acids for the α chains and 6 to 12 amino acids for the β chains. Also, the mean values calculated for TCR α (9.4) and TCR β (9.5) from our CTL corresponded with the values determined for human TCR α (9.2) and TCR β (9.5) found previously (37). No correlation was found between the CDR3 lengths of pairing α and β chains. CTL from patient 1 (2F2, 3F9, and 1G5) demonstrated the pairing of an α chain that has an 11-amino-acid CDR3 with a β chain that has a 12-amino-acid CDR3 (Table 4). However, another CTL with an identical CDR3 length in α pairs with a β chain that has a CDR3 of only six amino acids (Table 4). Therefore, the length of the CDR3 in one chain seems not to restrict the length in the chain partner.

The comparison of all sequence data demonstrates that the recognition of the Tax11-19/HLA-A2 complex can be facilitated by TCR with quite diverse Vα/Vβ combinations. However, certain Vα/Vβ combinations were found to reoccur within individual patients and were shown to be the result of an oligoclonal expansion of few founder CTL.

DISCUSSION

The HTLV-1-encoded regulatory protein Tax is a major target for virus-specific CTL in HAM/TSP patients. CTL specific for Tax are almost always found in HAM/TSP patients (20, 22) but are mostly absent in asymptomatic carriers (22). Our study demonstrated the presence of oligoclonally expanded $CD8^+$ CTL that recognize Tax in several HAM/TSP patients examined. This finding is indicative of an in vivo activation of virus-specific CTL and corresponds well with the documented high precursor frequencies for HTLV-1-specific T cells in HAM/TSP patients (11, 20). The dramatic expansion of a limited number of $CD4^+$ as well as $CD8^+$ T cells following viral infections has been demonstrated in the past, most recently in patients newly infected with influenza virus (32).

The current analysis of TCRs expressed by CTL specific for

TABLE 2. TCR usage of HTLV-1-specific HLA-A2-restricted CTL[a]

CTL	TCR(s)	
	TCR Vα	TCR Vβ
Patient 1		
3F9	16^b ($18^{b,c}$)	17^b
2F2	16^b (*$18^{b,c}$*)	17^b
1G5	16^b ($18^{b,c}$)	17^b
1B6	19 (6)	5^b
3G12	19 (6)	5^b
2F12	12, 11	3^b
3F2	12	3^b
1C10	12, 2	24^b
Patient 2		
10B7	17^b ($5^{b,c}$)	12^b
10D3	17^b ($5^{b,c}$)	12^b
10C7	2^b ($4^{b,c}$)	12
10C2	2^b ($4^{b,c}$)	12
10D2	1^b	16^b
Patient 3		
1H2	5^b ($8^{b,c}$)	4^b
1A7	5^b (8)	4^b
2A3	5 (8)	4^b
2H1	2	4
2G4[d]	2	12^b
ID4	NI[e]	12^b
1G7	NI	12
D3	NI	12
C7	4	NI
IE3	NI	8^b
1H1	6^b	9^b

[a] TCR usage of individual T-cell clones was determined by PCR using TCR V-region family-specific primers as described in Materials and Methods.
[b] Confirmed through sequence analysis.
[c] Nonproductively rearranged TCR Vα chains.
[d] The sequence for the TCR β-chain gene of CTL 2G4 was identical to that of the TCR β-chain gene from CTL A6, an HLA-A2-restricted HTLV-1 Tax11-19-specific cytotoxic T-cell clone that had been generated from the same patient 3 years earlier.
[e] NI, not identified.

Tax and restricted by HLA-A2 revealed a limited number of distinct founder T cells that are clonally expanded within each patient. These T cells used different TCR Vα/Vβ combinations between but also within individual subjects. TCRs with quite diverse V-region combinations and no apparent amino acid sequence similarities were found to recognize the same antigen/MHC complex (Table 4). This diversity argues for a concerted action of both TCR chains in the recognition of a single peptide/MHC complex and an involvement of all CDR regions. An overrepresentation of certain Vβ chains as reported for responses to other immunodominant viral epitopes, such as the influenza A virus matrix peptide $M1_{57–68}$ (28, 34), an epitope that is also restricted by HLA-A2, was not observed for Tax11-19. Interestingly, HLA-A2-restricted responses to $M1_{57–68}$ both in humans and in mice transgenic for HLA-A2 (30) lead to the selection of dominant Vβ genes. However, the V-region genes utilized in mice were not homologous to the V-region genes used in humans for the recognition of the same antigen/MHC complex. This was likely due to the different TCR repertoires in the two species. A preferential usage of dominant V-region genes was also observed for responses to an antigenic peptide from vesicular stomatitis virus, restricted by H-$2K^b$ (17), and to a peptide derived from Epstein-Barr virus nuclear antigen 3 restricted by HLA-B8 (1). A restricted V-region usage by autoreactive T cells, especially for the TCR β chain, has been reported for a number of autoimmune diseases (2, 24, 52). This led to a V-region disease hypothesis which tried to link the occurrence of autoimmune diseases to the usage of certain Vβ regions (14). More recent data argue in favor of a greater heterogeneity in TCR usage, even in animal systems for autoimmune diseases such as experimental allergic encephalomyelitis. This new evidence has led to a rejection of the V-region disease hypothesis (48).

One of the best studied autoreactive T-cell responses is the one to myelin basic protein in multiple sclerosis (MS) patients (2, 12, 24, 31, 36, 39, 50). MS represents a human demyelinating disease with striking similarities to HAM/TSP, and both diseases have been postulated to involve an autoimmune mechanism. Consequently, TCR genes expressed by T cells that infiltrate the spinal cord or central nervous system in HAM/TSP and MS patients have been studied and compared with TCRs expressed by myelin basic protein-specific T cells (13, 35). Both, Hara et al. (13) and Oksenberg et al. (35) found supposed CDR3 region homologies based on only one or two amino acids that recurred in a few of the sequences and at different positions. The sequences of the Tax-specific TCR detected by us did not resemble the sequences of the infiltrating T cells described by Hara et al. (13) for HAM/TSP patients. However, as no HLA haplotypes had been reported for those patients, these infiltrating T cells could have been restricted by HLA molecules other than HLA-A2. Also, HTLV-1-specific T cells recognizing epitopes other than Tax11-19 might be present in the central nervous system. Therefore, the question of whether infiltrating T cells in HAM/TSP patients represent virus-specific CTL or autoreactive T cells recognizing myelin antigens, as suggested by some, remains unanswered.

On the basis of a comparison with immunoglobulin genes, the CDR3 regions of a TCR were proposed to be of primary importance for peptide recognition (7–9), a hypothesis strengthened by the observation that the amino acids in a selected CDR3 region change in response to the substitutions made in an antigenic peptide (5, 21). We compared the sequences of the CDR3 regions of both α and β chains in order to determine the potential contribution of each to the recognition of the Tax/HLA-A2 complex. Overall, the sequences of the α chains showed no recurring motifs within their CDR3 regions. However, common structural motifs within the CDR3 regions were observed for the β chains. The TCR β chains rearranged predominantly with Jβ2.1 or Jβ2.7, and four of six distinct TCR pairs contained a proline followed by glycine at a position within the CDR3 region that is thought to be important for peptide recognition. The crystallographic structure of the Tax11-19/HLA-A2 complex has been determined (29), and the most prominently exposed amino acid within the nonamer peptide was found to be a proline. Additionally, two tyrosine residues and one phenylalanine seem to be accessible for TCR contact. These three amino acids carry aromatic side chains and might favor the selection of CDR3 residues with aliphatic side chains. The recent resolution of the crystal structure for a mouse TCR β chain suggests an involvement of all three CDR regions and a fourth hypervariable region in the recognition of antigen/MHC complexes (3). Highly heterogeneous CDR3 regions despite identical restriction and epitope specificities have also been reported for class II-restricted TCRs recognizing the tetanus toxin peptide 830–843 (4). The heterogeneity observed by us and Boitel et al. (4) argues in favor of distinct TCRs being able to "see" an antigen/MHC complex by binding to it in more than one way. In other words, the primary TCR contact residues may vary within a given peptide/TCR combination.

Interestingly, the TCR β-chain sequences of CTL clone 2G4, which had been generated from patient 3 for this study,

TABLE 3. Amino acid and nucleotide sequences of TCR α- and β-chain genes expressed by HTLV-1 Tax11-19-specific HLA-A2-restricted CTL established from three patients with HAM/TSP

CTL (no. of colonies sequenced)	Sequence[a]			
	V region	N or N(D)N	J region	C region
α chain				
Patient 1				
3F9 Vα16 Jα28 (8/8)	VSDSALYFCA tac ttc tgt gct	VRS gtg aga t	YSGAGSYQLTFGKGTKLSVIP ca tac tct ggg gct ggg agt tac	NIQNP
2F2 Vα16 Jα28 (6/6)	VSDSALYFCA tac ttc tgt gct	VRS gtg aga t	YSGAGSYQLTFGKGTKLSVIP ca tac tct ggg gct ggg agt tac	NIQNP
1G5 Vα16 Jα28 (4/4)	VSDSALYFCA tac ttc tgt gct	VRS gtg aga t	YSGAGSYQLTFGKGTKLSVIP ca tac tct ggg gct ggg agt tac	NIQNP
Patient 2				
10B7 Vα17.2 Jα54 (8/8)	PGDSAVYFCA tac ttc tgt gca	AME gca atg g	GAQKLVFGQGTRLTINP ag gga gcc cag	NIQNP
10D2 Vα1.2 Jα48 (13/13)	MSDAAEYFCA tac ttc tgt gct	VSD gtg agt gac c	PNFGNEKLTFGTGTRLTIIP ct aac ttt gga aat gag	KSQNP
10C7 Vα2.8 Jα24 (4/4)	LSDSATYLCV tac ctc tgt gtg	LS ctt t	TDSWGKFQFGAGTQVVVTP ca act gac agc tgg ggg	DIQNP
10C2 Vα2.8 Jα24 (1/1)	LSDSATYLCV tac ctc tgt gtg	LS ctt t	TDSWGKFQFGAGTQVVVTP ca act gac agc tgg ggg	DIQNP
Patient 3				
A6 Vα2.3 Jα24 (10/10)	PSDSATYLCA tac ctc tgt gcc	VT gtt a	TDSWGKLQFGAGTQVVVTP ca act gac agc tgg ggg	DIQNP
+1H2 Vα5 Jα6 (1/1)	PEDSATYLCA tat ctc tgt gct	VQAHS gtg cag gcc cac t	SGGSYIPTFGRGTSLIVHP ca tca gga gga agc tac	YIQNP
+1A7 Vα5 Jα6 (3/3)	PEDSATYLCA tat ctc tgt gct	VQAHS gtg cag gcc cac t	SGGSYIPTFGRGTSLIVHP ca tca gga gga agc tac	YIQNP
−1H1 Vα6.1 Jα50 (1/1)	LGDSAMYFCA tat ttc tgt gca	MREGE atg aga gag ggc ga	KTSYDKLIFGPGTSLSVIP g aaa acc tcc tacgac	NIQNP
β chain				
Patient 1				
1B6 Vβ5.14 Jβ2.1 (3/3)	LGDSALYLCASS tgc gcc agc agt	IWG att tgg ggt	YNEQFFG**P**GTRLTVL tac aat gag	EDLKN
3G12 Vβ5.14 Jβ2.1 (6/6)	LGDSALYLCASS tgc gcc agc agt	IWG att tgg ggt	YNEQFFG**P**GTRLTVL tac aat gag	EDLKN
3F9 Vβ17 Jβ2.1 (7/7)	KNPTAFYLCASS tgt gcc agt agt	IS**PG**QGAQ atc tct cca gga cag ggg gcg cag	NEQFFG**P**GTRLTVL aat gag	EDLKN
2F2 Vβ17 Jβ2.1 (4/4)	KNPTAFYLCASS tgt gcc agt agt	IS**PG**QGAQ atc tct cca gga cag ggg gcg cag	NEQFFG**P**GTRLTVL aat gag	EDLKN
1G5 Vβ17 Jβ2.1 (1/1)	KNPTAFYLCASS tgt gcc agt agt	IS**PG**QGAQ atc tct cca gga cag ggg gcg cag	NEQFFG**P**GTRLTVL aat gag	EDLKN
1C10 Vβ24.1 Dβ2.1 Jβ2.7	LGDAAMYLCATS tgt gcc acc agc	RI(TSG)E**P** aga att (act agc gg) a gag ccc	YEQYFG**P**GTRLTVT tac	EDLKN
3F2 Vβ3 Jβ2.1 (5/5)	TNQTSMYLCASS tgt gcc agc agt	LGV**P** tta ggg gtt cc	EQFFG**P**GTRLTVL t gag cag ttc	EDLKN
2F12 Vβ3 Jβ2.1 (7/7)	TNQTSMYLCASS tgt gcc agc agt	LGV**P** tta ggg gtt cc	EQFFG**P**GTRLTVL t gag cag ttc	EDLKN
Patient 2				
10D2 Vβ16.1 Jβ2.7 (9/9)	LEDSGVYFCASS tgt gccagc agc	QD**PSG**MI caa gac cct agc ggg atg at	YEQYFG**P**GTRLTVT c tac	EDLKN
10B7 Vβ12.3 Jβ2.7 (3/3)	PSQTSVYFCASS tgt gcc agc agt	Y**PG**GGF tac ccg ggg ggg ggg ttt	YEQYFG**P**GTRLTVT tac gag	EDLKN
10D3 Vβ12.3 Jβ2.7 (7/7)	PSQTSVYFCASS tgt gcc agc agt	Y**PG**GGF tac ccg ggg ggg ggg ttt	YEQYFG**P**GTRLTVT tac gag	EDLKN
Patient 3				
ID4 Vβ12.3 Jβ2.1 (4/4)	PSQTSVYFCASS tgt gcc agc agt	Y**PG**QGV tat ccg gga cag gga gta	NEQFFG**P**GTRLTVT aat gag	EDLKN
2G4 Vβ12.3 Dβ2.1 Jβ2.7	PSQTSVYFCAS tgt gcc agc	R**P**(**G**LAGG)RP agg ccg (gga cta gcg gga ggg) cga cca	EQYFG**P**GTRLTVT gag	EDLKN
A6 Vβ12.3 Dβ2.1 Jβ2.7	PSQTSVYFCAS tgt gcc agc	R**P**(**G**LAGG)RP agg ccg (gga cta gcg gga ggg) cga cca	EQYFG**P**GTRLTVT gag	EDLKN
IE3 Vβ8 Jβ2.5 (4/4)	PRDSAVYFCASS tgt gcc agc agt	FLGGG ttc ttg ggg ggt ggc	QETQYFG**P**GTRLLVL caa gag	EDLKN
1A7 Vβ4 Jβ1.2 (8/8)	PEDSSIYLCS tgc agc	ETGLEG gag aca ggt ttg gaa ggc	YTFGSGTRLTVV tacacc	EDLKN
1H2 Vβ4 Jβ1.2 (6/6)	PEDSSIYLCS tgc agc	ETGLEG gag aca ggt ttg gaa ggc	YTFGSGTRLTVV tacacc	EDLKN
2A3 Vβ4 Jβ1.2 (4/4)	PEDSSIYLCS tgc agc	ETGLEG gag aca ggt ttg gaa ggc	YTFGSGTRLTVV tacacc	EDLKN
1H1 Vβ9 Jβ1.6 (5/5)	LGDSAVYFCASS tgt gcc agc agc	KGGL aaa ggg ggg tta	NSPLHFGNGTRLTVT aat tca	EDLKN

[a] The last 10 (α chain) or 12 (β chain) amino acids of the V region followed by N additions and the J-region gene and including the first 5 amino acids of the C region are shown. The nucleotide sequence is listed below each amino acid sequence, detailing the origin of the amino acid codons and demonstrating the productive rearrangement.

TABLE 4. TCR α/β heterodimers expressed by 10 HLA-A2-restricted CTL clones specific for HTLV-1 Tax11-19 from three HAM/TSP patients

CTL	Sequence				CDR3 length[a]
	V	CDR3	J	C	
Patient 1					
2F2, 3F9, 1G5					
Vα16 Jα28	FCA	**V R**	SYSGAGSYQLTFGKGTKLSVIP	NIQNP	11
Vβ17 Jβ2.1	CASS	IS **PG**	QGAQN**EQ**FFG**P**GTRLTVL	EDLKN	12
Patient 2					
10D2					
Vα1.2 Jα48	FCA	**V** S DP	NFGNEKLTFGTGTRLTIIP	KSQNP	10
Vβ16.1 Jβ2.7	CASS	**Q** D**PSG**	MIY**EQ**YFG**P**GTRLTVT	EDLKN	11
10B7, 10D3					
Vα17.2 Jα54	FCA	**A** M E	GAQKLVFGQGTRLTINP	NIQNP	7
Vβ12.3 Jβ2.7	CASS	Y **PG**	GGFY**EQ**YFG**P**GTRLTVT	EDLKN	10
Patient 3					
A6, 2G4					
Vα2.3 Jα24	LCA	**V** T TD	SWGKLQFGAGTQVVVTP	DIQNP	8
Vβ12.3 Dβ2.1 Jβ2.7	CAS	**R** **PG**	LAGGRP**EQ**YFG**P**GTRLTVT	EDLKN	11
1H1					
Vα6.1 Jα50	FCA	**M R**	EGEKTSYDKVIFGPGTSLSVIP	NIQNP	11
Vβ9 Jβ1.6	CASS		KGGLNSPLHFGNGTRLTVT	EDLKN	9
1H2					
Vα5 Jα6	LCA	**V Q**	AHSSGGSYIPTFGRGTSLIVHP	YIQNP	11
Vβ4 Jβ1.2	CS	ET	GLEGYTFGSGTRLTVV	EDLKN	6

[a] Calculated by the method of Rock et al. (37). Briefly, length is defined as the distance between the C-terminal V-region cysteine and the first glycine encoded in the J-region triplet glycine/X/glycine minus 4 amino acids.

and of CTL clone A6, which had been established from the same patient 3 years earlier, proved identical. This implicates a selection, expansion, and maintenance of this CTL clone over a time period of at least 3 years. Such a persistence of individual T-cell clones has been observed for myelin basic protein-specific T cells in MS patients (40, 51).

The question arises whether persistent virus-specific CTL, which are present in large numbers in the periphery and the cerebrospinal fluid of patients with neurological complications (19), are beneficial to the patient or contribute to the pathogenesis of HAM/TSP. This question reflects the two alternative models that currently attempt to explain the pathology of HAM/TSP (16). In the first model, activated virus-specific CTL cross the blood-brain barrier and either lyse HTLV-1-infected glial cells directly or cause central nervous system damage through a release of cytokines that kill glial cells in a bystander fashion. In the second model, HTLV-1 infection leads to the activation of autoreactive T cells that subsequently contribute to demyelination, although, to date, no autoreactive CTL have been found in the cerebrospinal fluid in HAM/TSP patients. The two models imply contrasting therapies that will attempt either to augment or to inhibit the immune response in this disease. A characterization of the antigen specificity and TCR usage of infiltrating T cells should help determine whether they represent virus-specific or autoreactive T cells.

The knowledge of the TCR sequences of the virus-specific CTL that are clonally expanded in HAM/TSP patients provides a potentially useful tool for the characterization of infiltrating T cells by PCR or in situ hybridization. If few virus-specific founder T cells are involved in the pathogenesis of HAM/TSP, they might be targeted through an induction of apoptosis or anergy using altered peptide ligands or soluble antigen/MHC complexes.

ACKNOWLEDGMENTS

We thank Petra Utz for help in the design of the TCR V-region-specific PCR primers and Tanya Lehky for help in the collection and evaluation of the patient data. We also thank Henry McFarland for critical reading of the manuscript and Netta Hamberry for manuscript preparation.

REFERENCES

1. **Argaet, V. P., C. W. Schmidt, S. R. Burrows, S. L. Silins, M. G. Kurilla, D. L. Doolan, A. Suhrbier, D. J. Moss, E. Kieff, T. B. Sculley, and I. S. Misko.** 1994. Dominant selection of an invariant T cell antigen receptor in response to persistent infection by Epstein-Barr virus. J. Exp. Med. **180:** 2335–2340.
2. **Ben-Nun, A., R. S. Liblau, L. Cohen, D. Lehmann, E. Tournier-Lasserve, A. Rosenzweig, Z. Jingwu, J. C. M. Raus, and M. A. Bach.** 1991. Restricted T-cell receptor V beta gene usage by myelin basic protein-specific T-cell clones in multiple sclerosis: predominant genes vary in individuals. Proc. Natl. Acad. Sci. USA **88:**2466–2470.
3. **Bentley, G. A., G. Boulot, K. Karjalainen, and R. A. Mariuzza.** 1995. Crystal structure of the beta chain of a T cell antigen receptor. Science **267:**1984–1987.
4. **Boitel, B., U. Blank, D. Mege, G. Corradin, J. Sidney, A. Sette, and O. Acuto.** 1995. Strong similarities in antigen fine specificity among DRB1*1302-restricted tetanus toxin tt830-843-specific TCRs in spite of highly heterogeneous CDR3. J. Immunol. **154:**3245–3255.
5. **Chien, Y.-H., and M. M. Davis.** 1993. How ab T-cell receptors 'see' peptide/MHC complexes. Immunol. Today **14:**597–601.
6. **Chirgwin, J. M., A. E. Przybala, R. J. MacDonald, and W. J. Rutter.** 1979. Isolation of biologically active ribonucleic acid from sources enriched in ribonucleases. Biochemistry **18:**5294–5301.
7. **Chothia, C., D. R. Boswell, and A. M. Lesk.** 1988. The outline structure of the

T-cell αβ receptor. EMBO J. **7:**3745–3755.
8. **Claverie, J. M., A. Prochnicka-Chalufour, and L. Bougueleret.** 1989. Implications of a Fab-like structure for the T-cell receptor. Immunol. Today **10:** 10–14.
9. **Davis, M. M., and P. J. Bjorkman.** 1988. T-cell antigen receptor genes and T-cell recognition. Nature (London) **334:**395–402.
10. **DiBrino, M., K. C. Parker, J. Shiloach, M. Knierman, J. Lukzo, R. V. Turner, W. E. Biddison, and J. E. Coligan.** 1993. Endogenous peptides bound to HLA-A3 possess a specific combination of anchor residues that permit identification of potential antigenic peptides. Proc. Natl. Acad. Sci. USA **90:**1508–1512.
11. **Elovaara, I., S. Koenig, A. Y. Brewah, R. M. Woods, T. Lehky, and S. Jacobson.** 1993. High human T-cell lymphotropic virus type I (HTLV-I)-specific precursor cytotoxic T lymphocyte frequencies in patients with HTLV-I-associated neurological disease. J. Exp. Med. **177:**1567–1573.
12. **Giegerich, G., M. Pette, E. Meinl, J. T. Epplen, H. Wekerle, and A. Hinkkanen.** 1992. Diversity of T cell receptor α and β chain genes expressed by human T cells specific for similar myelin basic protein peptide/major histocompatibility complexes. Eur. J. Immunol. **22:**753–758.
13. **Hara, H., M. Morita, T. Iwaki, T. Hatae, Y. Itoyana, T. Kitamoto, S. Akizuki, I. Goto, and T. Watanabe.** 1994. Detection of human T lymphotropic virus type I (HTLV-I) proviral DNA and analysis of T cell receptor Vb CDR3 sequences in spinal cord lesions of HTLV-I-associated myelopathy/tropical spastic paraparesis. J. Exp. Med. **180:**831–839.
14. **Heber-Katz, E., and H. Acha-Orbea.** 1989. The V-region disease hypothesis: evidence from autoimmune encephalomyelitis. Immunol. Today **10:**164–169.
15. **Hogan, K., N. Shimojo, S. Walk, V. Engelhard, L. Maloy, J. E. Coligan, and W. E. Biddison.** 1988. Mutations in the alpha two helix of HLA-A2 affect presentation but do not inhibit binding of influenza virus matrix peptide. J. Exp. Med. **168:**725–736.
16. **Höllsberg, P., and D. A. Hafler.** 1995. What is the pathogenesis of human T-cell lymphotropic virus type I-associated myelopathy/tropical spastic paraparesis? Ann. Neurol. **37:**143–145.
17. **Imarai, M., E. C. Goyarts, G. M. van Bleek, and S. G. Nathenson.** 1995. Diversity of T cell receptors specific for the VSV antigenic peptide (N52-59) bound by the H-2K^b class I molecule. Cell. Immunol. **160:**33–42.
18. **Jacobson, S., A. Gupta, D. Mattson, E. Mingioli, and D. E. McFarlin.** 1990. Immunological studies in tropical spastic paraparesis. Ann. Neurol. **27:**149.
19. **Jacobson, S., D. McFarlin, S. Robinson, R. Voskuhl, R. Martin, A. Brewah, A. J. Newell, and S. Koenig.** 1992. Demonstration of HTLV-I specific cytotoxic T lymphocytes in the cerebrospinal fluid of patients with HTLV-I associated neurologic disease. Ann. Neurol. **32:**651–657.
20. **Jacobson, S., H. Shida, D. E. McFarlin, A. S. Fauci, and S. Koenig.** 1990. Circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. Nature (London) **348:** 245–248.
21. **Jorgensen, J. L., U. Esser, B. Fazekas de St. Groth, P. A. Reay, and M. M. Davis.** 1992. Mapping T-cell receptor-peptide contacts by variant peptide immunization of single-chain transgenics. Nature (London) **355:**224–230.
22. **Koenig, S., R. Woods, A. Y. Brewah, A. Newell, G. Jones, E. Boone, J. Adelsberger, M. Baseler, S. M. Robinson, and S. Jacobson.** 1993. Characterization of major histocompatibility complex (MHC) class I-restricted cytotoxic T cell (CTL) responses to Tax in HTLV-I infected patients with neurological disease. J. Immunol. **156:**3874–3883.
23. **Koike, T., M. Kagami, and K. Takabayashi.** 1985. Antibodies to human T cell leukemia virus are absent in patients with systemic lupus erythematosus. Arthritis Rheum. **28:**481.
24. **Kotzin, B. L., S. Karuturi, Y. K. Chou, J. Lafferty, J. M. Forrester, M. Better, G. E. Nedwin, H. Offner, and A. A. Vandenbark.** 1991. Preferential T-cell receptor b-chain variable gene use in myelin basic protein-reactive T-cell clones from patients with multiple sclerosis. Proc. Natl. Acad. Sci. USA **88:**161–165.
25. **Kubota, R., F. Umehara, S. J. Izumo, S. Ijichi, K. Matsumura, S. Yashiki, T. Fujiyoshi, S. Sonoda, and M. Osame.** 1994. HTLV-I proviral DNA correlates with infiltrating CD4+ lymphocytes in the spinal cord from patients with HTLV-I associated myelopathy. J. Neuroimmunol. **53:**23–29.
26. **Kuroda, Y., M. Matsui, M. Kikuchi, K. Kirohara, C. Endo, M. Yukitake, Y. Matsuda, O. Tokunaga, A. Komine-Sakaki, and R. Kawaguchi.** 1994. In situ demonstration of the HTLV-I genome in the spinal cord of a patient with HTLV-I associated myelopathy. Neurology **44:**2295–2299.
27. **Lehky, T. J., C. H. Fox, S. Koenig, M. C. Levin, N. Flerlage, S. Izumo, E. Sato, C. S. Raine, M. Osame, and S. Jacobson.** 1995. Detection of human T lymphotropic virus type I (HTLV-I) tax RNA in the central nervous system of HTLV-I associated myelopathy/tropical spastic paraparesis patients by in situ hybridization. Ann. Neurol. **37:**246–254.
28. **Lehner, P. J., E. C. Y. Wang, P. A. H. Moss, S. Williams, K. Platt, S. M. Friedman, J. I. Bell, and L. K. Borysiewicz.** 1995. Human HLA-A0201-restricted cytotoxic t lymphocyte recognition of influenza A is dominant by T cells bearing the Vb17 gene segment. J. Exp. Med. **181:**79–91.
29. **Madden, D. R., D. N. Garboczi, and D. C. Wiley.** 1993. The antigenic identity of peptide/MHC complexes: a comparison of the conformations of five viral peptides presented by HLA-A2. Cell **75:**693–708.
30. **Man, S., J. P. Ridge, and V. H. Engelhard.** 1994. Diversity and dominance among TCR recognizing HLA-A2.1+ influenza matrix peptide in human MHC class I transgenic mice. J. Immunol. **153:**4458–4467.
31. **Martin, R., U. Utz, J. E. Coligan, J. R. Richert, M. Flerlage, E. Robinson, R. Stone, W. E. Biddison, D. E. McFarlin, and H. F. McFarland.** 1992. Diversity in fine specificity and T cell receptor usage of the human CD4+ cytotoxic T cell response specific for the immunodominant myelin basic protein peptide 87-106. J. Immunol. **148:**1359–1366.
32. **Masuko, K., T. Kato, Y. Ikeda, M. Okubo, Y. Mizushima, K. Nishioka, and K. Yamamoto.** 1994. Dynamic changes of accumulated T cell clonotypes during antigenic stimulation in vivo and in vitro. Int. Immunol. **6:**1959–1966.
33. **Moore, G. R. W., U. Traugott, L. C. Schienberg, and C. S. Raine.** 1989. Tropical spastic paraparesis: a model of virus-induced cytotoxic T cell mediated demyelination? Ann. Neurol. **26:**523–530.
34. **Moss, P. A., R. J. Moots, W. M. Rosenberg, J. S. Rowland, H. C. Bodmer, A. J. McMichael, and J. I. Bell.** 1991. Extensive conservation of alpha and beta chains of the human T-cell antigen receptor recognizing HLA-A2 and influenza A matrix peptide. Proc. Natl. Acad. Sci. USA **88:**8987.
35. **Oksenberg, J. R., M. A. Panzara, A. B. Begovich, D. Mitchell, H. A. Erlich, R. S. Murray, R. Shimonkevitz, M. Sherritt, J. Rothbard, C. C. A. Bernard, and L. Steinman.** 1993. Selection for T-cell receptor V beta-D beta-J beta gene rearrangements with specificity for a myelin basic protein peptide in brain lesions of multiple sclerosis. Nature (London) **362:**68–70.
36. **Oksenberg, J. R., S. Stuart, A. B. Begovich, R. B. Bell, H. A. Erlich, L. Steinman, and C. C. A. Bernard.** 1990. Limited heterogeneity of rearranged T-cell receptor V alpha transcripts in brains of multiple sclerosis patients. Nature (London) **345:**344–346. (Erratum, **353:**94, 1991.)
37. **Rock, E. P., P. R. Sibbald, M. M. Davis, and Y.-H. Chien.** 1994. CDR3 length in antigen-specific immune receptors. J. Exp. Med. **179:**323–328.
38. **Roman, G. C., and M. Osame.** 1988. Identity of HTLV-I-associated tropical spastic paraparesis and HTLV-I-associated myelopathy. Lancet **i:**651.
39. **Rotteveel, F. T., I. Kokkelink, H. K. van Walbeek, C. H. Polman, J. J. van Dongen, and C. L. Lucas.** 1987. Analysis of T cell receptor-gene rearrangement in T cells from the cerebrospinal fluid of patients with multiple sclerosis. J. Neuroimmunol. **5:**243–249.
40. **Salvetti, M., G. Ristori, M. D'Amato, C. Buttinelli, M. Falcone, C. Fieschi, H. Wekerle, and C. Pozzilli.** 1993. Predominant and stable T cell responses to regions of myelin basic protein can be detected in individual patients with multiple sclerosis. Eur. J. Immunol. **23:**1232–1239.
41. **Shimojo, N., B. Anderson, D. Mattson, R. Turner, J. E. Coligan, and W. E. Biddison.** 1990. The kinetics of peptide binding to HLA-A2 and the conformation of the peptide-A2 complex can be determined by amino acid side chains on the floor of the peptide binding groove. Int. Immunol. **2:**193.
42. **Storkus, W. J., D. N. Howell, R. D. Salter, J. R. Dawson, and P. Cresswell.** 1987. NK susceptibility varies inversely with target cell class I HLA antigen expression. J. Immunol. **138:**1657–1659.
43. **Umehara, F., S. Izumo, M. Nakaga, A. T. Ronquillo, K. Takahashi, K. Matsumuro, E. Sato, and M. Osame.** 1993. Immunocytochemical analysis of the cellular infiltrates in the spinal cord lesions in HTLV-I associated myelopathy. J. Neuropathol. Exp. Neurol. **52:**424–430.
44. **Usuku K., S. Sonoda, M. Osame, S. Yashiki, K. Takahashi, M. Matsumoto, T. Sawada, T. Kimiyoshi, M. Tara, and A. Igata.** 1988. Immune responsiveness against HTLV-I in HTLV-I-associated myelopathy: comparison with adult T-cell leukemia/lymphoma. Ann. Neurol. **23**(Suppl.)**:**143.
44a. **Utz, U.** Unpublished data.
45. **Utz, U., S. Koenig, J. E. Coligan, and W. E. Biddison.** 1992. Presentation of three different viral peptides, HTLV-I Tax, HCMV gB, and influenza virus M1 is determined by common structural features of the HLA-A2.1 molecule. J. Immunol. **149:**214–221.
46. **Vernant, J. C., G. Buisson, J. Magdelaine, J. de Thore, A. Jovanelle, D. Neisson-Vernant, and N. Monplaisir.** 1988. T lymphocyte alveolitis, tropical spastic paraparesis and Sjögren's syndrome. Lancet **i:**177.
47. **Waksman, B. H.** 1989. Multiple sclerosis: relationship to a retrovirus? Nature (London) **337:**599.
48. **Wilson, D. B., L. Steinman, and D. P. Gold.** 1993. The V-region disease hypothesis: new evidence suggests it is probably wrong. Immunol. Today **14:**376–380.
49. **Wu, E., D. W. Dickson, S. Jacobson, and C. S. Raine.** 1993. Neuroaxonal dystrophy in HTLV-I associated myelopathy/tropical spastic paraparesis: neuropathologic and neuroimmunologic correlations. Acta Neuropathol. **86:** 224–235.
50. **Wucherpfennig, K. W., K. Ota, N. Endo, J. G. Seidman, A. Rosenzweig, H. L. Weiner, and D. A. Hafler.** 1990. Shared human T cell receptor V beta usage to immunodominant regions of myelin basic protein. Science **248:**1016–1019.
51. **Wucherpfennig, K. W., J. Zhang, C. Witek, M. Matsui, Y. Modabber, K. Ota, and D. A. Hafler.** 1994. Clonal expansion and persistence of human T cells specific for an immunodominant myelin basic protein peptide. J. Immunol. **152:**5581–5592.
52. **Zamvil, S. S., D. J. Mitchell, N. E. Lee, A. C. Moore, M. K. Walder, K. Sakai, J. B. Rothbard, H. O. McDevitt, L. Steinman, and H. Acha-Orbea.** 1988. Predominant expression of a T cell receptor V beta gene subfamily in autoimmune encephalomyelitis. J. Exp. Med. **167:**1586–1596. (Erratum, **168:**455.)