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# Sural nerve T-cell receptor Vβ gene utilization in chronic inflammatory demyelinating polyneuropathy and vasculitic neuropathy

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**Article abstract—***Objective:* To investigate the utilization of T-cell receptor (TCR) variable (V) regions in infiltrates of sural nerve biopsies of patients with chronic inflammatory demyelinating polyneuropathy (CIDP) and vasculitic neuropathy. *Background:* The presence of infiltrating T lymphocytes in sural nerve biopsies may suggest a T cell-mediated immune mechanism in the pathogenesis of CIDP and vasculitic neuropathy. *Patients and methods:* The utilization of TCR Vβ regions in sural nerves of 13 patients with CIDP and five patients with vasculitic neuropathy was determined by immunohistochemistry, reverse-transcription PCR, and nucleotide sequence analysis. These techniques were also applied in four patients with chronic idiopathic axonal polyneuropathy (CIAP) who acted as noninflammatory controls, and in five autopsy controls. *Results:* The TCR Vβ utilization of infiltrating T cells in sural nerves of patients with CIDP, vasculitic neuropathy, and noninflammatory controls is heterogeneous. A dominant TCR Vβ utilization was not found in any of the patients or controls. *Conclusion:* There is no evidence for the presence of clonally expanded T cells in sural nerves of patients with CIDP and vasculitic neuropathy.

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Evidence for a pathogenic role of T cells in chronic inflammatory demyelinating polyneuropathy (CIDP) includes an increased frequency of circulating activated peripheral T cells,[1,2] and elevated serum levels of interleukine-2 (Il-2) and soluble Il-2 receptors.[3] In both CIDP and vasculitic neuropathy, increased numbers of T cells in the sural nerve biopsy specimens may indicate a role for T cells in the disease mechanism.[4-6] It is currently not clear whether sural nerve T cells, their numbers increased or not, are disease-specific autoreactive T cells, or whether these are attracted nonspecifically to the peripheral nervous tissue.

Analysis of T-cell receptors (TCR) in affected tissue may show 1) a broad TCR repertoire without Vβ family overrepresentation (similar to that found in peripheral blood or normal lymph nodes), indicating that the T cells have been attracted nonspecifically by a proinflammatory environment; 2) a restricted TCR repertoire with Vβ family overrepresentation but without evidence of clonal expansion, indicating that there may be a superantigen-driven T cell expansion; or 3) a restricted TCR repertoire with Vβ family overrepresentation and clonal expansion, indicating that the T cells have been stimulated by specific antigens.[7] Patterns of restricted TCR Vβ gene expression have been described in studies of multiple sclerosis,[8] inflammatory myopathies,[9-16] psoriasis,[17] and Sjögren's syndrome[18]; whereas other studies of MS,[19] rheumatoid arthritis,[20] and anti-Hu paraneoplastic encephalomyelitis/sensory neuronopathy[7] have indicated a more heterogeneous TCR Vβ utilization of T cells in situ.

Additional material related to this article can be found on the *Neurology* Web site. Go to www.neurology.org and scroll down the Table of Contents for the January 9 issue to find the title link for this article.

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***Table 1*** *Clinical, laboratory, and pathologic patient data*

| Patient | Sex | Age, y | Nadir, mo | Biopsy, mo | Rankin | | CSF protein, mg/dL | MNF, density/mm$^2$ |
|---|---|---|---|---|---|---|---|---|
| | | | | | Max | Min | | |
| CIDP | | | | | | | | |
| 1 | F | 45 | 9 | 4 | 4 | † | 340 | 6911 |
| 2 | F | 49 | 5 | 5 | 5 | 4 | 350 | 2456 |
| 3 | M | 48 | 3 | 3 | 3 | 2 | 100 | 6048 |
| 4 | M | 31 | 2 | 2 | 3 | 1 | 40 | 5065 |
| 5 | F | 47 | 10 | 10 | 3 | 2 | 174 | 508 |
| 6 | M | 72 | 15 | 14 | 3 | 3 | NA | 1434 |
| 7 | M | 45 | 15 | 14 | 3 | 2 | 80 | 6922 |
| 8 | M | 59 | 25 | 19 | 2 | 2 | 72 | NA |
| 9 | F | 54 | 2 | 2 | 4 | 1 | 130 | 4663 |
| 10 | F | 27 | 21 | 19 | 3 | 3 | 40 | 4880 |
| 11 | M | 55 | 19 | 22 | 2 | 2 | 80 | 2850 |
| 12 | M | 47 | 2 | 9 | 3 | 3 | 500 | 3085 |
| 13 | M | 53 | 28 | 28 | 4 | 1 | 100 | 4378 |
| Vasculitis | | | | | | | | |
| 14 | F | 66 | | NA | NA | | NA | 1881 |
| 15 | F | 62 | | NA | 2 | | NA | NA |
| 16 | F | 58 | | 8 | 3 | | NA | 16 |
| 17 | M | 64 | | 5 | 3 | | 60 | 1610 |
| 18 | F | 78 | | 2 | 3 | | 30 | 1454 |
| CIAP | | | | | | | | |
| 19 | M | 67 | | 24 | 2 | | 44 | 3319 |
| 20 | M | 64 | | 102 | 1 | | NA | 3091 |
| 21 | M | 63 | | 52 | 2 | | 55 | NA |
| 22 | M | 53 | | 180 | 2 | | NA | 3954 |

†Deceased.

Nadir = time from disease onset to maximum disability; biopsy = time from disease onset to biopsy; Rankin = modified Rankin scale; max = maximal disability score; min = minimal disability score; MNF = myelinated nerve fibers; CIDP = chronic inflammatory demyelinating polyneuropathy; NA = not available; CIAP = chronic idiopathic axonal polyneuropathy.

We used a panel of TCR Vβ specific monoclonal antibodies on cryosections of sural nerve biopsy specimens from patients with CIDP or nonsystemic vasculitic neuropathy, as well as from control patients with chronic idiopathic axonal polyneuropathy (CIAP) and autopsy controls. The results were compared with PCR analysis of Vβ gene family utilization and matched with peripheral blood T lymphocyte populations. In individual cases, nucleotide sequence analysis of Vβ regions was performed.

**Patients and methods.** *Patients.* We investigated sural nerve biopsies, taken between 1990 and 1997 from 13 patients who fulfilled established criteria for CIDP,[21] and five patients with nonsystemic vasculitic neuropathy and sural nerve involvement. None of the patients had received treatment prior to the biopsy being taken, except for Patients 8 and 13, both of whom had received intravenous immunoglobulins a few months previously. For normal controls we used five sural nerves from autopsy patients without a known peripheral nerve disease. For disease controls we used four sural nerves from patients with a noninflammatory chronic idiopathic axonal polyneuropathy (CIAP). CIAP has a slowly progressive course, and during a 5-year follow-up no cause was found.[22,23] Clinical data of patients are listed in table 1. Biopsy findings were not used to assign diagnoses except in cases of vasculitis, for which well-established morphological criteria exist.[24] Eleven patients with CIDP, one patient with CIAP, all patients with vasculitis, and one normal control were consecutively included in this study. Two patients with CIDP (Patients 1 and 2), three patients with CIAP (Patients 19, 20, 21), and four autopsy controls (Patients 23 through 26) were selected because of their relatively high number of T cells in the total sural nerve area, as reported in our previous study.[4]

*Immunohistochemistry.* Immunohistochemical staining was performed on 6 μm-thick transverse acetone-fixed frozen sections of sural nerves, as well as on sections of tonsil as a positive control. As antibodies against the

variable part of the β-chain of the T cell receptor (anti-Vβ-antibodies) were not yet regularly used for immunohistochemistry, these antibodies were first tested on tonsil, muscle, and nerve with extensive infiltrates of T cells. The anti-Vβ-antibodies tested were monoclonal antibodies raised in mice and included anti-Vβ2-(IgG1), Vβ3-(IgG2a), Vβ5.1-(IgG2a), Vβ5.2-(IgG1), Vβ5.3-(IgG1), Vβ6-(IgM), Vβ8-(IgG2a), Vβ9-(IgG2a), Vβ11-(IgG2a), Vβ12-(IgG2a), Vβ13.1-(IgG2b), Vβ13.6-(IgG1), Vβ14-(IgG1), Vβ16-(IgG1), Vβ17-(IgG1), Vβ18-(IgG1), Vβ20-(IgG), Vβ21-(IgG2a), Vβ22-(IgG1), and Vβ23-(IgG1) antibodies (Beckman Coulter, Fullerton, CA). The optimal dilutions were determined for each Vβ-antibody. Insufficient or no staining in control tissues was observed with anti-Vβ5.2-, Vβ5.3-, Vβ6-, Vβ9-, Vβ11-, Vβ13.1-, Vβ18-, and Vβ20-antibodies. The anti-Vβ3-antibody specifically stained T cells in tonsil and muscle, but the background staining in nerve was unacceptably high. The anti-Vβ-antibodies which gave reliable staining and were used for further analysis were anti-Vβ2-(1:200), Vβ5.1-(1:100), Vβ8-(1:100), Vβ12-(1:200), Vβ13.6-(1:50), Vβ14-(1:25), Vβ16-(1:50), Vβ17-(1:50), Vβ21-(1:200), Vβ22-(1:50), and Vβ23-(1:50) antibodies. For further characterization of infiltrating cells, consecutive sections were incubated with the following primary antibodies diluted in phosphate-buffered saline (PBS) with 5% horse or goat serum: rabbit-anti-CD3 (Pan-T cells, 1:200; DAKO, Carpinteria, CA), mouse-anti-CD4 (helper/inducer T cells, 1:40; DAKO), mouse-anti-CD8 (cytotoxic/suppressor T cells, 1:400; DAKO), mouse-anti-CD20 (B cells, 1:200; DAKO), and mouse-anti-γδ (pan-γ/δ T cell receptor, 1:200; PharMingen, San Diego, CA). As secondary antibody, biotinylated goat anti-rabbit IgG or horse anti-mouse IgG (1:220, Vector Labs, Burlingame, CA) was used. Labeling was visualized by the avidin:biotinylated enzyme complex (ABC) method (Vector Labs). Color was developed using diaminobenzidine with cobalt and nickel intensification, and sections were counterstained with nuclear fast red. Omitting the primary antibody and incubating with some of the anti-Vβ antibodies mentioned above did not produce any staining. As CD4 can also be expressed on macrophages,[25] $CD4^+$ cells were examined but not quantified. In order to calculate the percentage of Vβ-positive cells in the entire T cell population, each section incubated with a specific Vβ-antibody (or CD8-antibody or γδ-antibody) was compared with a consecutive section incubated with the anti-CD3-antibody (figure 1).

*Quantification of T cells.* T cells were quantified by light microscopy (40× objective). Numbers of $CD3^+$, $CD8^+$, $CD20^+$, and all $V\beta^+$ T cells were measured in the total endoneurial and epineurial area. In the total sural nerve areas, the endoneurial areas, and the epineurial areas, we calculated *the percentages* of specific $V\beta^+$ cells as: ($V\beta n^+$ cells / $CD3^+$ cells) × 100%; of $CD8^+$ T cells as: ($CD8^+$ cells / $CD3^+$ cells) × 100%; of $\gamma\delta^+$ T cells as: ($\gamma\delta^+$ cells / $CD3^+$ cells) × 100%. We calculated *the percentage* of endoneurial $CD3^+$ cells as: (endoneurial $CD3^+$ cells / total $CD3^+$ cells) × 100%. To obtain reliable numbers of Vβ positive cells, the whole staining procedure was repeated, up to a maximum of four times, until approximately 100 $CD3^+$ cells in the total sural nerve area had been counted for each patient.

*Fluorescence-activated cell sorter (FACS) analysis.* Peripheral blood mononuclear cells of patients and four healthy donors were isolated from heparin blood by Ficoll gradient separation. The TCR Vβ gene products were analyzed after stepwise incubation with unlabeled anti-Vβ monoclonal antibody (1:20, Beckman Coulter, Fullerton, CA), fluorescein isothiocyanate (FITC)-labeled-goat-anti-mouse IgG (1:80, Becton-Dickinson, Franklin Lakes, NJ), mouse serum and phycoerythrin-labeled anti-CD3 (1:2, Becton-Dickinson). The cells were then subjected to two-color analysis using FACScan flow cytometer (Becton-Dickinson). All results were expressed as percentage of $CD3^+$ cells.

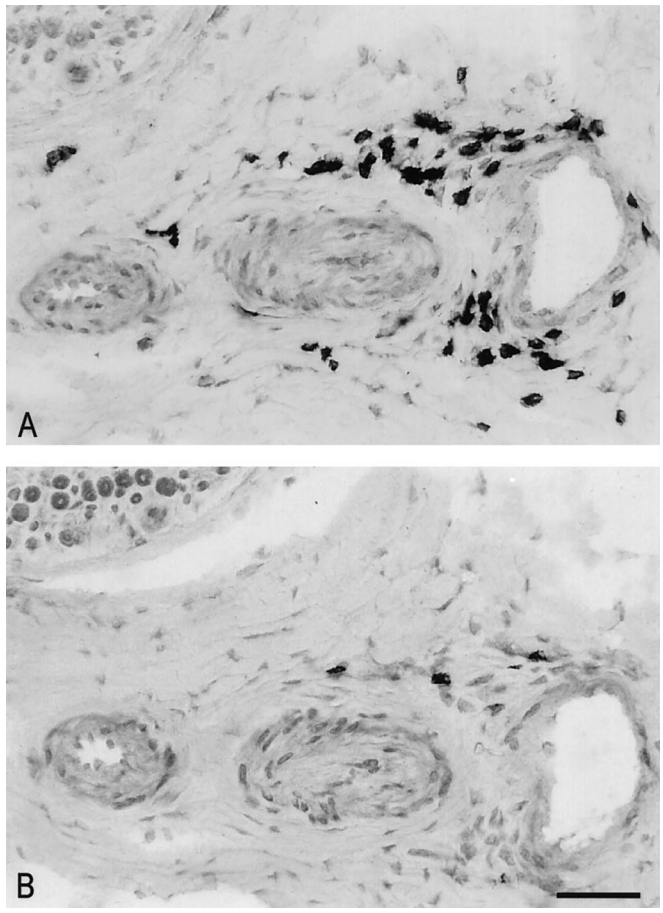


*Figure 1. Consecutive transverse sections of the sural nerve of Patient 3 with (A) anti-CD3-staining and (B) anti-Vβ17-staining. Bar = 50 μm.*

*Reverse-transcription PCR.* Because the panel of Vβ-antibodies used in the immunohistochemical analysis covered only approximately 39% of the entire T cell population in blood (Beckman Coulter, Fullerton, CA), a reverse-transcription PCR with 25 Vβ-family specific primers was carried out to further analyze TCR Vβ gene utilization in the sural nerve biopsy specimens and peripheral blood lymphocytes. RNA extraction and cDNA synthesis were performed using standard methods.[26,27] PCR amplification of cDNA was performed with a Cβ reverse primer (sequence: CTCCTTCCCATTCACCCACCAGCTCAGCTC) in combination with a specific forward primer for each of the following Vβ TCR: Vβ1 to Vβ4, Vβ5a (recognizes family members 5.1 and 5.4), Vβ5b (recognizes family members 5.2 and 5.3), Vβ6 to Vβ24, and Vβ26. Control PCR amplifications for actin, hypoxanthine-guanine phosphori-

bosyltranferase (HPRT), TCR Cα, and TCR Cβ were performed with each sample to confirm cDNA integrity. A negative control reaction (no cDNA) was systematically run in each experiment. PCR amplification was carried out for 35 cycles under the following conditions: denaturation (94 °C for 30 seconds), annealing (60 °C for 45 seconds), and elongation (72 °C for 60 seconds). The PCR amplification reaction was performed for a second time for each Vβ with another Cβ primer (sequence: CACAAACTCGGTAGTCTTCGTCTCTACAGGGTGTGGGT) as reverse primer. The PCR products were run on 2% agarose gels, blotted onto a nitrocellulose membrane and hybridized with a 32p end-labeled Cβ probe (sequence GAGGACCTGAAAAACGTGTTC). The blots were exposed to x-ray film for 4 to 18 hours.

*Sequence analysis.* To establish whether the expanded TCR $V\beta^+$ T cell populations were monoclonal, oligoclonal, or polyclonal, PCR products were subjected to nucleotide sequence analysis. To that end, PCR products were ligated into the pGEM-1 Vector (Promega, Madison, WI) and transfected into the *Escherichia coli* strain XL1-Blue. Clones containing inserts of the correct size were sequenced using the 373 DNA sequencer (Applied Biosystems, Weiterstadt, Germany).

*Statistical analysis.* The Mann-Whitney *U* test was used to compare percentages of the TCR Vβ utilization of each Vβ between patients with CIDP and autopsy controls, and between patients with vasculitic neuropathy and autopsy controls.

**Results.** *Immunohistochemical analysis of Vβ utilization in sural nerve.* The total number of T cells and the percentage of T cells with a specific TCR Vβ for the total sural nerve section of each patient are presented in table 2. The panel of Vβ antibodies detected between 15% and 58% of the $CD3^+$ T cell population in the sural nerve biopsy specimens. The TCR Vβ utilization in the sural nerve biopsy specimen was heterogeneous in all patients and controls analyzed. No specific Vβ family was dominant or persistently absent in any of the patient groups or controls, and differences in percentages of each Vβ between patients with CIDP or vasculitic neuropathy and autopsy controls were not significant. In particular, in Patients 1 through 4 with CIDP, who had a higher number of T cells than the noninflammatory controls, there was no overrepresentation of a specific Vβ family. Only in Patient 14, who had a vasculitic neuropathy and a highly increased number of infiltrating T cells, did the percentage of $V\beta13.6^+$ T cells appear to be increased (15%) compared with percentages of Vβ 13.6 found in peripheral blood lymphocytes (2%). In this patient, nucleotide sequence analysis on Vβ13 was performed (see below). The distribution of TCR Vβ gene families was similar in the epineurium and endoneurium in all patients and controls. The percentage of endoneurial T cells in patients with CIDP ranged from 8 to 47% (median 22); in patients with vasculitic neuropathy, from 4 to 31% (median 5%); in patients with CIAP, from 14 to 40% (median 17%); and in autopsy controls, from 14 to 62% (median 22%). The percentage of $CD8^+$ T cells in patients with CIDP ranged from 37 to 96% (median 51%); in patients with vasculitic neuropathy, from 33 to 43% (mean 38%); in patients with CIAP, from 33 to 100% (median 71%); and in autopsy controls, from 35 to 63% (median 60%). The percentage of $\gamma\delta^+$ T cells in patients with CIDP ranged from 0 to 10% (median 1%); in patients with vasculitic neuropathy, from 0 to 1% (median 1%); in patients with CIAP, from 0 to 2% (median 0%); and in autopsy controls, from 0 to 4% (median 0%).

*Comparison of Vβ utilization in sural nerve biopsy specimens and peripheral blood.* The percentages of TCR Vβ utilization of T cells in sural nerve biopsy specimen of patients with CIDP, CIAP, and vasculitic neuropathy were compared with TCR Vβ utilization in peripheral blood to find evidence for expansion of T cells in nerves. The TCR Vβ utilization in the sural nerve biopsies compared with the peripheral blood lymphocytes showed only a mild increase of TCR Vβ8 in Patient 8 (11% versus 4%) and Patient 12 (9 versus 3%) with CIDP; of Vβ13.6 in Patient 13 with CIDP (10 versus 1%) and Patient 14 with vasculitis (15 versus <1%); and of Vβ17 in Patient 12 with CIDP (11 versus 5%).

*PCR analysis of Vβ utilization in sural nerve and peripheral blood.* With the Vβ primers and the PCR protocol used in our study, we were able to detect expression of all TCR Vβ gene families in peripheral blood of patients and healthy donors, except for Vβ20 in Patient 15 and Vβ23 in Patient 21. The results of the PCR analysis of the sural nerves and peripheral blood lymphocytes for patients and controls are shown in figure 2. The Vβ gene utilization was heterogeneous in all patients and controls. We found a wider array of Vβ families expressed in the patients with CIDP and vasculitic neuropathy than in the controls noninflammatory disease. Compared with the immunohistochemical analysis, the results were conflicting: some Vβ genes were not used according to the PCR analysis, whereas T cells with the same TCR Vβ were found in the sural nerve sections and vice versa (see table 2 and figure 2).

*Nucleotide sequence analysis.* In the biopsy of Patient 14 with a vasculitic neuropathy, the percentage of $V\beta13.6^+$ T cells appeared to be increased. To determine whether this increase was the result of a monoclonal expansion of T cells, RNA was extracted from the biopsy and used for first strand cDNA synthesis and PCR. The Vβ13 PCR fragments were cloned and subjected to nucleotide sequence analysis. Among 13 randomly sequenced inserts, four Vβ13 regions, as evaluated by the CDR3 region, were unique: three Vβ13 regions were found twice and one Vβ13 region was found three times. We conclude that the increased Vβ13.6 frequency in the biopsy of this patient could not be attributed to a monoclonal expansion of T cells. Similarly, we analyzed collections of TCR Vβ4 in Patient 10 and TCR Vβ9 in Patient 12, based on the observation that a strong band was found in the PCR analysis compared with the other Vβ bands. Again no evidence for monoclonality was found, as a maximum of two recurrent CDR3 regions were found in 16 and nine inserts. Also, in a normal control (Patient 27), we found a recurrent Vβ4 CDR3 region twice among seven inserts.

**Discussion.** We have characterized TCR Vβ gene expression in infiltrating T cells in sural nerve biopsy specimens of patients with CIDP, vasculitic neuropathy, CIAP, and normal controls. TCR Vβ gene expression was determined by immunohistochemistry using a panel of monoclonal antibodies specific for different TCR Vβ gene products. These results were compared with PCR analysis of the TCR

***Table 2*** *Number of CD3+ cells and percentages of T-cell receptor Vβ utilization in total sural nerve sections (immunohistochemistry)*

| | | Vβ* | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Patient | CD3 | 2 | 5.1 | 8 | 12 | 13.6 | 14 | 16 | 17 | 21 | 22 | 23 | Total |
| CIDP | | | | | | | | | | | | | |
| 1 | 519 | 7 | 3 | 5 | 3 | 1 | 3 | 1 | 5 | 5 | 6 | 1 | 40 |
| 2 | 440 | 12 | 3 | 3 | 1 | <1 | 1 | 1 | 3 | 2 | 3 | <1 | 29 |
| 3 | 387 | 11 | 3 | 5 | 1 | 2 | 1 | 1 | 4 | 1 | 3 | 1 | 34 |
| 4 | 101 | 11 | 3 | 3 | 2 | 1 | 1 | 1 | 5 | 4 | 4 | 2 | 37 |
| 5 | 40 | 12 | 3 | 7 | 2 | 2 | 0 | 2 | 4 | 2 | 5 | 0 | 39 |
| 6 | 25 | 6 | 0 | 0 | 0 | 2 | 0 | 0 | 5 | 0 | 0 | 2 | 15 |
| 7 | 25 | 7 | 1 | 0 | 7 | 3 | 0 | 4 | 4 | 1 | 0 | 1 | 28 |
| 8 | 25 | 6 | 3 | 11 | 1 | 1 | 0 | 1 | 1 | 0 | 3 | 0 | 28 |
| 9 | 24 | 6 | 5 | 2 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0 | 17 |
| 10 | 22 | 11 | 3 | 2 | 7 | 5 | 5 | 0 | 3 | 0 | 5 | 5 | 46 |
| 11 | 20 | 7 | 0 | 5 | 3 | 3 | 3 | 1 | 7 | 4 | 3 | 0 | 36 |
| 12 | 20 | 7 | 4 | 9 | 3 | 7 | 1 | 4 | 11 | 1 | 7 | 2 | 58 |
| 13 | 17 | 10 | 8 | 6 | 6 | 10 | 1 | 2 | 2 | 1 | 3 | 2 | 49 |
| Median | | 7 | 3 | 5 | 2 | 2 | 1 | 1 | 4 | 1 | 3 | 1 | 36 |
| Vasculitis | | | | | | | | | | | | | |
| 14 | 937 | 16 | 2 | 3 | <1 | 15 | 1 | <1 | 5 | 2 | 3 | 0 | 47 |
| 15 | 629 | 8 | 7 | 4 | <1 | 1 | <1 | <1 | 4 | 1 | 5 | <1 | 30 |
| 16 | 569 | 10 | 4 | 7 | 1 | 5 | 2 | 1 | 4 | 3 | 7 | 1 | 44 |
| 17 | 372 | 10 | 4 | 3 | 3 | 6 | 1 | <1 | 2 | 1 | 5 | <1 | 36 |
| 18 | 327 | 7 | 5 | 2 | 3 | 2 | 1 | <1 | 3 | 5 | 4 | 0 | 33 |
| Median | | 10 | 4 | 3 | 1 | 5 | 1 | <1 | 4 | 2 | 5 | <1 | 36 |
| CIAP | | | | | | | | | | | | | |
| 19 | 64 | 5 | 2 | 2 | 1 | 2 | 1 | 0 | 1 | 3 | 0 | 1 | 16 |
| 20 | 57 | 5 | 2 | 2 | 1 | 1 | 0 | 0 | 1 | 0 | 2 | 1 | 16 |
| 21 | 41 | 3 | 5 | 2 | 1 | 2 | 1 | 2 | 1 | 2 | 3 | 1 | 24 |
| 22 | 19 | 10 | 7 | 3 | 2 | 1 | 0 | 0 | 3 | 1 | 3 | 1 | 31 |
| Median | | 5 | 4 | 2 | 1 | 1 | <1 | 0 | 1 | 2 | 3 | 1 | 20 |
| Normal | | | | | | | | | | | | | |
| 23 | 41 | 12 | 1 | 3 | 2 | 1 | 1 | 1 | 4 | 3 | 3 | 1 | 32 |
| 24 | 40 | 5 | 1 | 8 | 0 | 1 | 0 | 1 | 6 | 1 | 0 | 1 | 25 |
| 25 | 36 | 3 | 6 | 4 | 4 | 2 | 0 | 0 | 3 | 0 | 1 | 0 | 23 |
| 26 | 20 | 8 | 4 | 5 | 4 | 1 | 4 | 0 | 4 | 1 | 3 | 1 | 35 |
| 27 | 8 | 0 | 0 | 7 | 0 | 7 | 0 | 9 | 0 | 0 | 0 | 0 | 23 |
| Median | | 5 | 1 | 5 | 2 | 1 | 0 | 1 | 4 | 1 | 1 | 1 | 25 |

* Values are percentages.

CD3 = number of CD3 T cells in one sural nerve section; Total = total percentage of T cells detected by all available Vβ antibodies; CIDP = chronic inflammatory demyelinating polyneuropathy; CIAP = chronic idiopathic axonal polyneuropathy.

Vβ of T cells in these biopsies, and with flow cytometric and PCR analysis of TCR Vβ utilization in peripheral blood lymphocytes. The TCR Vβ utilization was heterogeneous in all patient groups, and no single Vβ gene family was consistently overrepresented. Only the utilization of the TCR Vβ13.6 in one patient with vasculitic neuropathy was mildly increased, but with nucleotide sequence analysis no evidence of monoclonal expansion of these T cells was found.

In contrast to our results in inflammatory neuropathy, previous studies in inflammatory myopathies were indicative for restriction of the utilization of the TCR Vβ when investigated with immunohistochemical methods,[9,12,15] or PCR analysis.[11,13,14,16] Collectively, a heterogeneous utilization of the TCR Vβ

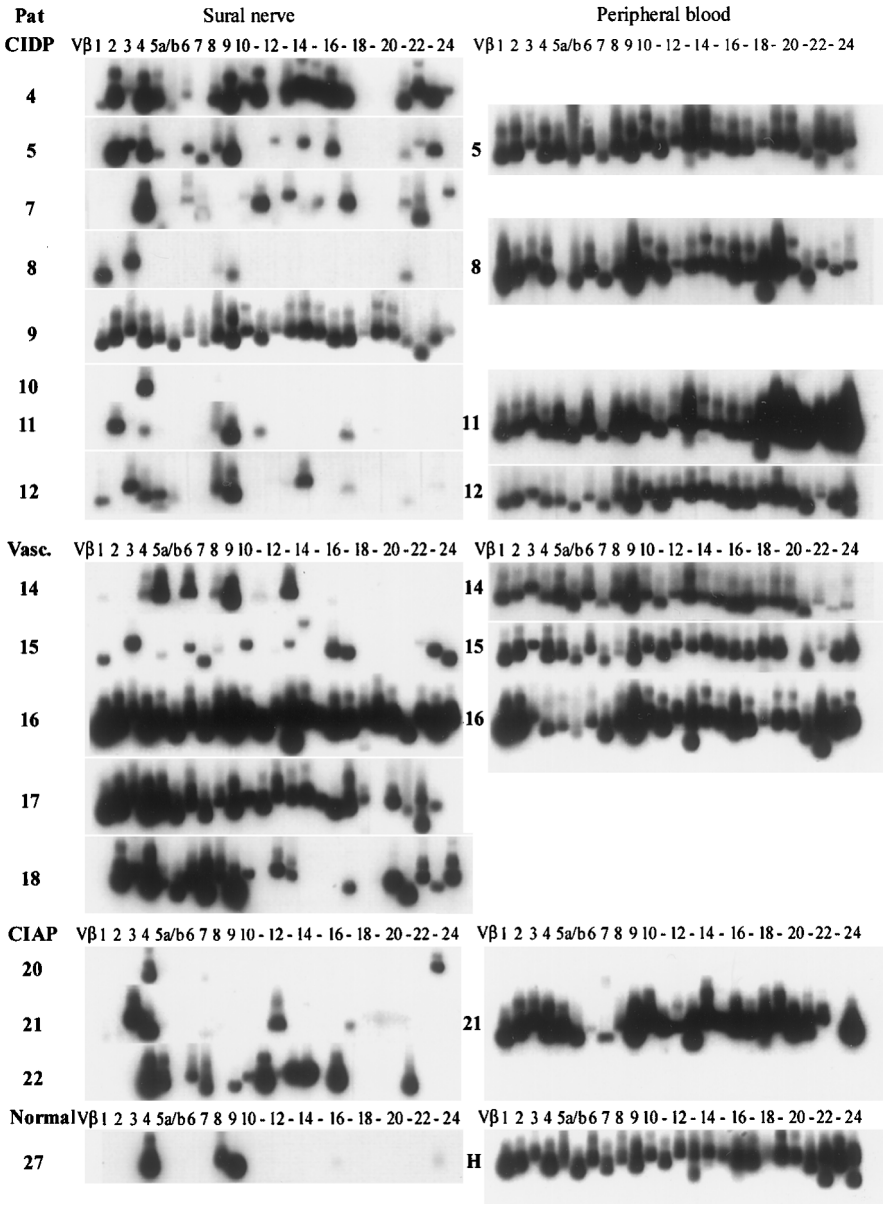


*Figure 2. Vβ T-cell receptor utilization in sural nerve and peripheral blood of patients and controls (PCR). Pat = patient; CIDP = chronic inflammatory demyelinating polyneuropathy; Vasc. = vasculitic neuropathy; CIAP = chronic idiopathic axonal polyneuropathy; Normal = autopsy control; H = healthy control.*

was found. In multiple sclerosis, the TCR Vβ repertoire in brain lesions was restricted in one immunohistochemical study,[8] and heterogeneous with PCR analysis in another study.[19] The TCR Vβ repertoire of affected tissue has never been investigated using the combination of immunohistochemical and PCR analysis with all available antibodies and primers. Both the immunohistochemical and PCR approaches have advantages and limitations. With anti-TCR antibodies it is possible to quantify and localize TCR Vβ positive T cells, but the panel of antibodies that performed well in immunohistochemical analysis of sural nerve biopsy specimens comprised only approximately 39% of the Vβ genes used in peripheral T cell populations. The PCR primers comprise the entire T cell population, and the tissue specimen analyzed is larger than the sural nerve sections used in immunohistochemical analysis, but quantification and localization of T cells is not possible. With PCR analysis, not all TCR Vβ genes were positive, which could be interpreted as restriction of TCR Vβ utilization. However, in noninflammatory and normal controls, even fewer TCR Vβ genes were positive, which is not likely to be due to clonal expansion of disease-related T cells. Moreover, the results of the PCR analysis do not correspond to the immunohistochemical analysis. It is unlikely that these conflicting results can be explained by false positivity in the immunohistochemical analysis due to cross-reactions of the anti-Vβ antibodies, as the percentages of the Vβ utilization are similar to the percentages obtained from flow cytometric analysis of peripheral blood lymphocytes. An explanation could be a methodological failure of the PCR method, but the consistent detection of the entire TCR repertoire in the peripheral blood lymphocyte samples confirmed the integrity of the TCR primer sequences used in this assay. It is still possible that the results of PCR analysis on T cells are not reliable due to a factor concerning the characteristics of the tissue specimen or its preparation.

A second explanation might be that the total number of T cells in the nerve biopsy specimens affected the number of positive Vβ genes detected in the PCR analysis, as positivity was highest in the vasculitis group and lowest in the noninflammatory controls. However, this cannot be the only explanation, as the correlation between number of T cells in the biopsies and PCR Vβ gene positivity within the groups of patients with vasculitis, CIDP or CIAP was not consistent. For example, in the patient (Patient 14) with most T cells per sural nerve section and a heterogeneous utilization of Vβ receptor in the immunohistochemical analysis, a majority of the PCR Vβ genes were negative. Similar results were obtained in a previous study on the TCR Vβ repertoire in MS plaques: the Vβ repertoire in normal controls seemed more restricted compared with acute MS lesions. The authors of this previous study concluded that this might be due to fewer T cells or minute quantities of TCR mRNA in the controls; but in their study, the number of T cells did not fully correlate with the number of positive TCR.[19] Moreover, in another study on the TCR Vβ of cerebrospinal fluid T cells, PCR analysis of the TCR Vβ repertoire showed negative results in some patients, whereas in other patients with the same number of T cells the results were positive.[28] It might be that the amount of mRNA from nerve tissue is so minute that suboptimal amplification of the different rearranged Vβ genes produces a negative result. A final explanation might be that TCR mRNA in the nerve tissue has already disappeared, whereas the TCR protein was still detectable. From these results one may conclude that restricted positive results of PCR analysis of the TCR Vβ regions requires careful interpretation.

The results of our study may reflect nonspecific recruitment of T cells from peripheral blood T lymphocytes to the peripheral nervous system during the course of the inflammatory response. The presence of nonspecific T cells in the peripheral nerve tissue is in accordance with the finding that T cells are also found in patients with noninflammatory neuropathies and normal controls.[4] Activated nonspecific T cells may help B cells to produce antibodies against peripheral nerve components, thereby inducing activation of either complement or antibody-dependent cytolytic cells.[29,30] In CIDP, however, there is not much evidence for a B cell or antibody mediated process.[31-33] Another function of nonspecific T cells may be to increase the blood–nerve barrier permeability and recruit macrophages, as has been hypothesized in experimental model of inflammatory neuropathy in Lewis rats.[34,35] Local accumulation of high numbers of non-neural T cells induced severe axonal degeneration and conduction failure, while lower numbers induced conduction block and mild demyelination. Neural damage could be induced by soluble proinflammatory mediators released by T cells and macrophages which could damage susceptible Schwann cells first, and then axons. However, it is also possible that T cells present at the time the biopsy was taken have a suppressive function in CIDP. In vasculitic neuropathy, it is possible that after damage of the blood vessels by immune complex deposition or cytotoxic T cells,[6,24,36,37] T cells are attracted nonspecifically into the nervous tissue.

Conversely, it should be noted that the lack of detectable shifts in the TCR Vβ repertoire between blood and nerve compartments does not necessarily exclude the causative involvement of T cells in peripheral nerve.[38] At least five reasons why antigen-driven selection of certain TCR are difficult to unveil, can be given: 1) the majority of infiltrating T cells in nerve may not recognize antigen, thus masking the antigen-specific population; 2) larger sized antigens might comprise multiple epitopes that are recognized by different TCR, thus leading to the presence of a multiclonal T cell population; 3) even a given epitope might activate T cells characterized by different receptors depending on the antigen presenting molecule(s)[38,39]; 4) at the time the biopsy was taken, antigen-specific T cells may already have disappeared; and 5) the major pathology in CIDP is within proximal portions of the peripheral nervous system, and the findings in the sural nerve at ankle level may not reflect the active process more proximally.

The heterogeneous Vβ gene utilization makes it impossible to design a single broadly effective immunotherapy that targets the Vβ gene product. Further analysis of the TCR by investigating the Vα chain will have limited results because not many antibodies to the Vα chain are available, the Vα chain is homogeneous in combination with the Vβ chain in clonal expansion, and recognition of superantigen is independent of the Vα chain.

Investigation of Vβ TCR utilization by immunohistochemical analysis, reverse-transcription PCR, and nucleotide sequence analysis in sural nerve, and flow cytometric analysis in blood did not provide evidence for predominant TCR Vβ utilization in CIDP or vasculitic neuropathy.

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