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Differential Expression of NK T Cell Vα24JαQ Invariant TCR Chain in the Lesions of Multiple Sclerosis and Chronic Inflammatory Demyelinating Polyneuropathy[1]

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Human Vα24$^+$ NK T cells are a unique subset of lymphocytes expressing the Vα24JαQ invariant TCR chain. Because they can rapidly produce large amounts of regulatory cytokines, a reduction of NK T cells may lead to the development of certain autoimmune diseases. Using a single-strand conformation polymorphism method, we demonstrate that a great reduction of Vα24JαQ NK T cells in the peripheral blood is an immunological hallmark of multiple sclerosis, whereas it is not appreciable in other autoimmune/inflammatory diseases such as chronic inflammatory demyelinating polyneuropathy. The chronic inflammatory demyelinating polyneuropathy lesions were often found to be infiltrated with Vα24JαQ NK T cells, but multiple sclerosis lesions only rarely expressed the Vα24JαQ TCR. It is therefore possible that the extent of NK T cell alteration may be a critical factor which would define the clinical and pathological features of autoimmune disease. Although the mechanism underlying the NK T cell deletion remains largely unclear, a remarkable contrast between the CNS and peripheral nervous system diseases allows us to speculate a role of tissue-specific elements such as the level of CD1d expression or differences in the CD1d-bound glycolipid. *The Journal of Immunology,* **2000, 164: 4375–4381.**

Natural killer T cells are a unique lymphocyte population characterized by the expression of markers common to NK cells and the canonical Vα-Jα TCR rearrangement (1–6). Although expression of the Vα14Jα281 canonical sequence characterizes rodent NK T cells (1, 2), human NK T cells express a Vα24JαQ invariant chain which is highly homologous to the murine Vα14Jα281 sequence (3–6). Unlike conventional $\alpha\beta$ T cells, both rodent and human NK T cells are restricted by the CD1d molecule and can be triggered by a CD1d-restricted glycolipid ligand (7–9). NK T cells can produce large amounts of IL-4 and IFN-γ within hours of TCR engagement (1, 2, 10–12), and the potential to produce IL-4 suggests their regulatory role in polarizing unprimed T cells toward a Th2 population. Although a requirement of NK T cells for a Th2 immune response is not absolute (13, 14), accumulating evidence supports the role of NK T cells in the regulation of autoimmune diseases (15–20).

It has recently been reported that NK T cells may be numerically or functionally altered in certain autoimmune diseases. A decreased number of NK T cells was demonstrated in human systemic sclerosis (15), insulin-dependent diabetes mellitus (16), and spontaneous autoimmune diseases in rodents (17–19). However, it is difficult to assess the role of NK T cells in some studies because the kinetics of the NK T cell reduction was not studied and because the status of NK T cells in disease controls was not examined with the same assay. Moreover, none of the studies have addressed whether NK T cells may participate in the local regulation of autoimmune diseases.

We initiated this study to clarify whether a reduction of Vα24JαQ NK T cells in the periphery may be seen in the relapsing/remitting type of multiple sclerosis (MS).[4] To detect the presence of NK T cells, we used a novel method relying on single-strand conformation polymorphism (SSCP) of TCR nucleotide chains (21–23). This is a simple and powerful method to examine the presence of a particular TCR rearrangement in any sample that allows detection of TCR genes. In this study, we amplified the Vα24 TCR products in various samples by PCR, displayed these PCR products on a SSCP gel after denaturation, and then visualized the presence of the Vα24JαQ invariant chain of NK T cells using the specific probe. Utilizing the SSCP method, we were able to monitor the presence of the Vα24JαQ invariant chain along with the overall profile of Vα24$^+$ T cell clonotypes.

First, we obtained evidence that Vα24JαQ NK T cells are greatly reduced in the peripheral blood of MS, particularly during clinical remission. Interestingly, the NK T cell reduction was not appreciable in control autoimmune/inflammatory diseases affecting muscles or peripheral nerves, including chronic inflammatory

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[4] Abbreviations used in this paper: MS, multiple sclerosis; CIDP, chronic inflammatory demyelinating polyneuropathy; CSF, cerebrospinal fluid; HS, healthy subject; NND, non-neurological disease; OND, other neurological disease; PNS, peripheral nervous system; SSCP, single-strand conformation polymorphism.

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demyelinating polyneuropathy (CIDP) (24–27). CIDP is pathologically characterized by T cell infiltration with macrophage activation and up-regulation of MHC class II expression within the peripheral nervous system (PNS) (25), reminiscent of MS. We further explored whether Vα24JαQ NK T cells are engaged in the local pathology of MS, CIDP, or control diseases. The invariant Vα24JαQ TCR was not detected in the control CNS or PNS samples and could be seen only rarely in the CNS plaques of autopsied MS patients. In contrast, the invariant TCR was detected in 6 of 10 biopsy lesions from CIDP. Based on the collective data, we postulate that although Vα24JαQ NK T cells may not efficiently regulate MS because of their deficiency in vivo, they may play a role in the local regulation of CIDP.

Materials and Methods

Subjects and samples

All of the MS patients fulfilled the diagnostic criteria for definite MS (28), and results of magnetic resonance imaging further assisted the diagnosis. No medication had been given to the patients for 3 mo before blood and/or cerebrospinal fluid (CSF) samples were obtained. In this study, we operationally defined "MS in remission" as those who have been clinically stable for more than 3 mo, and "MS in relapse" as those who have recently developed an apparent exacerbation. In the "relapse" patients, blood samples were obtained within 1 wk after the onset of exacerbation. Diagnosis of CIDP was based on the criteria of the American Academy of Neurology (24), and biopsy samples of sural nerves were obtained with a standard procedure (29). PBMC were isolated on a Ficoll density gradient. Autopsy samples of CNS tissues as well as the biopsy samples of sural nerves were snap-frozen and stored at −70°C until use. The histopathological characterization of the MS plaques was performed as described previously (30).

PCR and SSCP analysis

SSCP clonotype analysis (21–23) was applied to identify the Vα24JαQ TCR chain among PCR-amplified Vα24$^+$ clonotypes. In brief, mRNA was isolated from PBMC, CSF, CNS, or peripheral nerve samples with a QuickPrep Micro mRNA purification kit (Pharmacia Biotech, Uppsala, Sweden) and converted to cDNA by using a first-strand cDNA synthesis kit (Pharmacia Biotech). One microliter of the diluted cDNA reaction was then mixed with a Vα24-specific sense primer (ACACAAAGTCGAACG GAAG) and a Cα-antisense primer (GATTTAGAGTCTCTCAGCTG) (30 pmol for each). PCR was performed in 50-μl reactions containing 5 μl of 10× ExTaqBuffer (Takara, Tokyo, Japan), 4 μl of dNTPs, and 2.5 U of ExTaq DNA polymerase in a thermal cycler 480 (Takara). Although PBMC samples were amplified for 35 cycles, CSF, CNS, and PNS samples were amplified for 38 cycles (30 s at 94°C, 30 s at 60°C, and 1 min at 72°C). The PCR products were diluted in a denaturing solution (95% formamide/10 mM EDTA/0.1% bromophenol blue/0.1% xylencyanol) and incubated at 90°C for heat denaturation. The samples were then loaded onto a nondenaturing 4% polyacrylamide gel containing 10% glycerol. After electrophoresis, the DNA was transferred to Pall Biodyne nylon membranes (Pall Biosupport, Port Washington, NY) and hybridized either with a biotinylated Cα-specific internal probe (AAATATCCAGAACCCT GACCCTGCCGTGTACC) or with a biotinylated probe for the invariant Vα24JαQ sequence (TGTGTGGTGAGCGACAGAGGCTCAACCCTG). The DNA was visualized by subsequent incubation with a chemiluminescent substrate system (Phototope Star detection kit; New England Biolabs, Beverly, MA). GAPDH was used as an internal control for all PCR reactions (GAPDH primer set; Stratagene, La Jolla, CA). Under the same conditions, cDNAs for human CD1d, IL-4, and IFN-γ were amplified by PCR with a set of primers: CD1d forward, GGTTTATCGAAGCAGCTTCAC and CD1d reverse, CACTTGAATGGCCAAGTTTAC; IL-4 forward, ACTGCAAATCGACACCTATTA and IL-4 reverse, ATGGTGGCTGTA GAACTGC; and IFN-γ forward, ATGTAGCGGATAATGGAACTC and IFN-γ reverse, AACTTGACATTCATGTCTTCC.

Results

Detection of the invariant Vα24JαQ sequence in peripheral blood of healthy subjects

First, we examined PBMC samples obtained from healthy subjects (HS). After PCR amplification with a set of the Vα24 and Cα primers, the amplified products were electrophoresed on the SSCP gel and hybridized with the biotinylated Cα probe. All of these

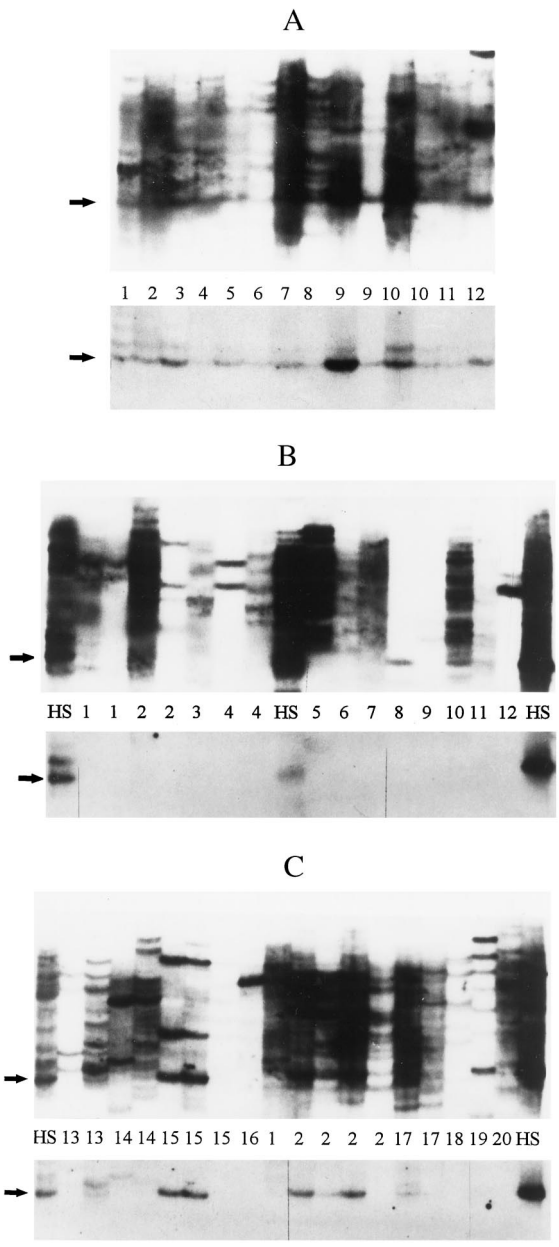

FIGURE 1. Demonstration of the Vα24$^+$ TCR rearrangement and the invariant Vα24JαQ TCR in PBMC. PBMC samples from HS (*A*), MS in remission (*B*), and MS in relapse (*C*) were examined with the SSCP technique described in *Materials and Methods*. The SSCP profiles of the total Vα24$^+$ TCR products are shown in the *upper panels*, whereas the same SSCP samples hybridized with the biotinylated probe for the Vα24JαQ TCR are demonstrated in the *lower panels*. Arrows indicate the position for the invariant TCR. Results of representative experiments are shown here. See also Table I summarizing the pooled data. *A*, Analysis of HS. Fourteen PBMC samples from 12 HS were examined in this experiment. The numerical code shown in the middle corresponds to each subject, implying that blood samples were taken from subjects 9 and 10 at two different time points. *B*, Analysis of MS in remission. Fifteen PBMC samples from 12 MS patients in remission were examined in this experiment. The numerical code shown in the middle corresponds to each patient, implying that blood samples were taken from patients 1, 2, and 4 at two different time points in remission. HS indicates a lane for a PBMC sample from HS, illustrating the position of the invariant Vα24JαQ band. *C*, Analysis of MS in relapse. Here, we examined 18 PBMC samples from 10 MS patients in relapse. The numerical code corresponds to each patient (the same codes are used in *B* and *C*). The relapse samples were obtained twice from patients 13, 14, 15, and 17, and four times from patient 2. HS indicates a PBMC sample from HS to illustrate the position of the invariant Vα24JαQ band.

Table I. *Detection frequency of the Vα24$^+$TCR and the invariant Vα24Jα Q in blood and CSF samples*
This table summarizes the results of all of the SSCP experiments for PBMC and CSF (see also Figs. 1 and 2).

Samples	Vα24$^+$ Samples/All Samples[a]	Vα24JαQ TCR$^+$ Samples/All Samples[b]
PBMC		
HS	18/18 (100%)	18/18 (100%)
MS in remission	18/18 (100%)	0/18 (0%)*
MS in relapse	26/26 (100%)	7/26 (26.9%)*†
OND[c]	20/20 (100%)	18/20 (90.0%)
CSF		
MS relapse	24/24 (100%)	11/24 (45.8%)‡
OND[d]	6/9 (66.6%)	1/9 (11.1%)

[a] The number of the samples from which Vα24$^+$TCR products could be amplified/the number of all of the samples examined.
[b] The number of the invariant Vα24JαQ TCR$^+$ samples/the number of all of the samples examined.
[c] The ONDs are categorized into noninflammatory CNS diseases including Parkinson disease ($n = 3$), motor neuron disease ($n = 1$), inherited ataxia ($n = 2$), and multiple system atrophy ($n = 1$); inflammatory/autoimmune non-CNS diseases including myasthenia gravis ($n = 2$), polymyositis ($n = 1$), CIDP ($n = 6$), Fisher syndrome ($n = 1$), and Guillain-Barré syndrome ($n = 1$); and noninflammatory non-CNS diseases including diabetic neuropathy ($n = 1$) and hereditary motor and sensory neuropathy ($n = 1$) (numbers in the parentheses, the total number of patients examined). See Ref. 36 for detailed information on each neurological disease. One of the two Parkinson disease patients and one inherited ataxia patient were negative for Vα24JαQ TCR.
[d] The ONDs include Guillain-Barré syndrome, Fisher syndrome, myelitis of unknown etiology, polymyositis, Parkinson disease, hereditary spastic paraplegia, cerebrovascular disease, and progressive external ophthalmoplegia.
* Significantly reduced ($p < 0.01$) compared with HS.
‡ Significantly higher ($p < 0.05$) than OND.
† Significantly higher ($p < 0.05$) than MS in remission.

samples demonstrated multiple bands on a smear background or a dense smear without appreciable bands, showing clonal heterogeneity of the Vα24$^+$ T cells (Fig. 1*A*, *upper panel*). Of note, these samples appeared to share identical SSCP clonotypes as they electrophoresed at the same positions. After removing the Cα probe, the membrane was hybridized with the biotinylated probe specific for the invariant Vα24JαQ TCR. One of the bands shared in all the samples apparently hybridized with this probe (Fig. 1*A*, *lower panel*; Table I), showing the presence for the Vα24JαQ TCR. Furthermore, by sequencing the TCR products eluted from corresponding areas of gels as reported previously (23), we confirmed that they would correspond to the invariant Vα24JαQ TCR, a marker of human NK T cells (data not shown).

Analysis of the invariant Vα24JαQ chain in MS patients in remission vs relapse

Using the PBMC samples from HS as positive control, we studied PBMC samples from MS patients in remission. The amplified Vα24$^+$ TCR products (Fig. 1*B*, *upper panel*) tended to show more limited numbers of bands and/or lesser degrees of smear density on SSCP gels as compared with HS. Unlike the samples from HS, occurrence of oligoclonal expansion was suggested in a proportion of these samples. More remarkably, none of the samples would hybridize with the probe for the invariant Vα24JαQ (Fig. 1*B*, *lower panel*; Table I). It was of note that not only samples expressing a few bands but those with dense smears (such as those from patient 5, 6, 7, or 10) did not hybridize with the Vα24JαQ probe. Although the PCR-SSCP analysis is not an absolute frequency measurement, these results indicate a great reduction of Vα24JαQ NK T cells along with a biased composition of Vα24$^+$ T cells. In parallel, we analyzed PBMC samples obtained from MS patients in relapse. Although the invariant Vα24JαQ was absent in the remission samples, 7 of the 26 relapse samples expressed the invariant TCR (Fig. 1*C*). This indicates that Vα24JαQ NK T cells could sometimes be repopulated during relapse. Of note, a long-term follow-up of one patient (patient 2) suggested the probable association between the NK T cell repopulation and clinical relapse (Table II): although the invariant Vα24JαQ chain was absent in the two samples obtained during remission, three of the four relapse samples expressed the invariant chain. In addition, repopulation of NK T cells was suggested in one of the two relapses in patient 13 (Table II). We also examined PBMC samples of other neurological diseases (ONDs, Table I), including 7 with noninflammatory CNS diseases, 11 with immune-mediated PNS or muscle diseases (including 6 with CIDP), and 2 noninflammatory PNS diseases (details described in the footnotes to Table I). Except for two samples from the noninflammatory CNS diseases (Parkinson disease and hereditary ataxia), the PBMC samples of the ONDs were all positive for the invariant TCR. The presence of the invariant TCR in the immune-mediated diseases such as CIDP and polymyositis indicates that a great reduction in NK T cells is not a general feature of immunological/inflammatory diseases.

Table II. *Temporal profiles for the appearance of Vα24JαQ TCR in the PBMC and CSF*

Patient Code[a]	Date	Clinical Condition	Vα24JαQ TCR[b] In PBMC	Vα24JαQ TCR[b] In CSF
1	September 1995	Relapse	−	
	March 1996	Remission	−[c]	+[c]
	June 1996	Remission	−	
2	August 1995	Relapse	+	
	February 1996	Remission	−	
	June 1996	Remission	−	
	November 1996	Relapse	−	
	April 1997	Relapse	+	
	June 1997	Relapse	+[c]	+[c]
13	July 1996	Relapse	−[c]	−[c]
	October 1996	Relapse	+	
17	January 1997	Relapse	+	
	June 1997	Relapse	−[c]	−[c]
20	March 1997	Relapse	−[c]	+[c]
	June 1997	Relapse	−	

[a] The patient code corresponds to that used in Fig. 1, *B* and *C*.
[b] The presence of the Vα24JαQ TCR was evaluated by the SSCP-based method as shown in the figures.
[c] The PBMC and CSF samples on the same line were obtained as a pair at the same time point.

Appearance of NK T cells in CSF

To know whether NK T cells may participate in the local regulation for the immunopathology of MS, we next examined the presence of the invariant Vα24JαQ TCR in CSF samples obtained from MS patients in relapse (Fig. 2). Although the Vα24$^+$ SSCP profiles were variable, the majority of these samples were characterized by a limited number (1~10) of bands without background smear. The invariant Vα24JαQ chain was detected in 11 of the 24 samples (Table I) and this TCR chain was usually a predominant one among the detectable clonotypes. We were able to directly compare five blood-CSF pairs from MS (marked with asterisk in Table II). The pairs from patients 1 and 20 revealed the presence of the invariant TCR in CSF but not in blood, whereas the other three pairs were both positive for the invariant TCR (patient 2) or both negative (patient 13 and 17). The invariant TCR was seen only in one of nine CSF samples from ONDs (Table I). These results indicate that NK T cells would sometimes (but not consistently) appear in the CSF of some MS patients, possibly owing to local clonal expansion.

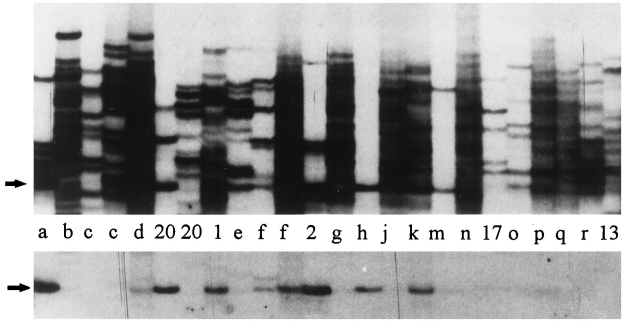

FIGURE 2. Demonstration of the Vα24$^+$ TCR rearrangement and the invariant Vα24JαQ TCR in CSF of MS. CSF samples were collected from MS patients in relapse and processed for SSCP analysis. The *upper* and *lower panels* show the total Vα24$^+$ SSCP profiles and the invariant Vα24JαQ band, respectively. The arrows indicate the position for the invariant Vα24JαQ band. Each numerical or small letter code corresponds to each patient. The numerical codes (1, 2, 13, 17, and 20) indicate the MS patients from whom PBMC samples could be also obtained at the same time or at different times. The same numerical codes are used in Fig. 1, *B* and *C*, here, and Table II. The small letter codes (a–r) indicate the patients in whom only CSF samples could be examined.

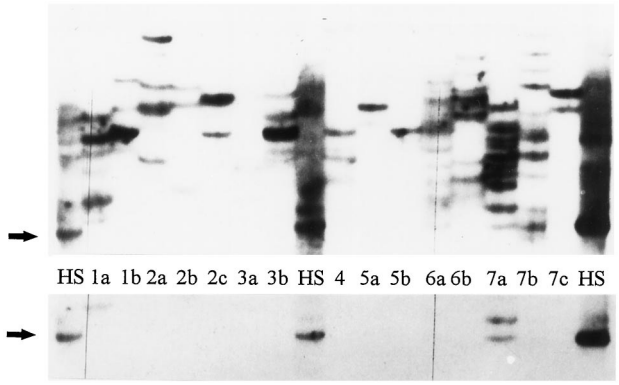

FIGURE 3. Demonstration of the Vα24$^+$ TCR rearrangement and the invariant Vα24JαQ TCR in CNS lesions of MS patients. Here, we show the SSCP profiles of autopsy tissues from which Va24 TCR messages could be PCR amplified. Each lesion is represented by a combination of patient code (number) and alphabet (except for lesion 4). Except for spinal cord lesion 5b, all of the samples were derived from brains. The samples include acute lesions (1b, 2a, 2b, 3a, 3b, 4, 6a, and 7c), chronic lesions (1a and 7b), chronic active lesions (6b), and subacute lesions (5a and 7a). Note that a subacute lesion 7a expresses two bands hybridizing with the Vα24JαQ probe. One of these is located at the position for the invariant Vα24JαQ. The arrows indicate the position for the invariant Vα24JαQ band.

Analysis of CNS and PNS lesions

We next examined MS plaques (11 acute, 6 subacute, and 8 chronic plaques) from 10 autopsied cases, 6 CNS samples from 3 OND patients, and 6 from 6 non-neurological disease (NND) (Table III). Vα24 TCR messages were detected in 15 of the 25 MS plaques, showing no particular preference for acute, subacute, or chronic plaques. The invariant Vα24JαQ TCR was found only in 1 subacute lesion of the 15 Vα24$^+$TCR-expressing plaques (Fig. 3). Three of the six OND samples expressed Vα24$^+$ TCR, but the invariant Vα24JαQ chain could not be detected in these samples. The NND samples did not express any Vα24$^+$ TCR chain. Furthermore, the Vα24JαQ-expressing plaque and four randomly chosen MS plaques were examined for the presence of IFN-γ and IL-4 mRNA. The Vα24JαQ-expressing plaque did not express IFN-γ mRNA, whereas the other plaques were all positive for IFN-γ (Table IV, MS plaques). IL-4 was not detected in any of the CNS lesions.

We further examined the biopsy samples of CIDP for comparison. In contrast to the MS lesions where the invariant TCR was only rarely detected, 6 of the 10 CIDP samples definitively expressed the invariant Vα24JαQ (Fig. 4; Table IV, CIDP biopsy samples). Although IL-4 mRNA was not detected in the MS lesions, this cytokine message was detected in all of the CIDP lesions (Fig. 5; Table IV, CIPD biopsy samples). By contrast, expression of IFN-γ mRNA appeared to be less consistent in CIDP than MS. Of note, none of the OND nerve samples expressed the invariant Vα24JαQ, and IL-4 was detected less frequently in the OND as compared with CIDP (Table IV, OND biopsy samples). These results indicate that MS, CIDP, and the OND lesions are different in the cytokine milieu as well as in the local frequency of NK T cells. However, because the origins of cytokines were not determined in this study, it remains to be established whether the local IL-4 expression in the PNS lesions may reflect the local presence of Vα24JαQ NK T cells.

Finally, we examined CD1d expression in the CNS and PNS samples with PCR. Examination revealed that 18 of 25 MS lesions (72.0%) and 6 of 7 NND tissues (85.7%) expressed CD1d mRNA, regardless of the pathological classification. In contrast, CD1d mRNA was found less frequently in the PNS samples (detected in 2 of 9 CIDP lesions (22.2%) and 1 of 4 other PNS diseases (25.0%)). It is possible that the turnover of CD1d molecule in the CNS may be more rapid than in the PNS.

Table III. *Detection frequency of the Vα24$^+$ TCR and the invariant Vα24JαQ in the CNS and PNS lesions*
This table summarizes the results of all of the SSCP experiments for autopsy samples of the CNS and biopsy samples of PNS.

Samples	Vα24$^+$ Samples/All Samples[a]	Vα24JαQ TCR$^+$ Samples/All Samples[b]
CNS		
MS	15/25 (60.0%)	1/25 (4.0%)
OND[c]	3/6 (50.0%)	0/6 (0%)
NND[d]	0/6 (0%)	0/6 (0%)
PNS		
CIDP	10/10 (100%)	6/10 (60.0%)
OND[e]	4/11 (36.4%)	0/11 (0%)

[a] The number of the samples from which Vα24$^+$TCR products could be amplified/the number of all of the samples examined.
[b] The number of the invariant Vα24JαQ-TCR$^+$ samples/the number of all of the samples examined.
[c] The OND samples include two from patients with Alzheimer disease and four from those with striatonigral degeneration.
[d] Autopsy cases without neurological disease.
[e] The OND samples include the sural nerve of hereditary motor and sensory neuropathy (n = 1), nerves without remarkable pathology (n = 3), Krabbe disease (n = 1), alcoholic polyneuropathy (n = 2), diabetic polyneuropathy (n = 1), Churg-Strauss syndrome (n = 1), acute demyelinating polyneuropathy (n = 1), and POEMS syndrome (n = 1) (numbers in parentheses show the total number of the patients examined). See Ref. 36 for information on each neurological disease.

Discussion

MS is thought to be an autoimmune disease mediated by Th1 T cells specific for CNS autoantigens (31, 32). Since numerical or functional defects of NK T cells were reported in systemic as well as organ-specific autoimmune diseases (15–19), it was of particular interest to investigate the status of NK T cells in MS. In this study, we used the SSCP method (21–23) to identify and monitor the presence of the invariant Vα24JαQ TCR. The previous studies (15, 16) evaluated the frequency of the Vα24JαQ NK T cells by

Table IV. *Presence of the Vα24JαQ TCR message and cytokine expression*

	Subacute Plaques			Acute Plaques	
	1	2	3	4	5
MS plaques[a]					
Vα24JαQ	+	−	−	−	−
IL-4 mRNA	−	−	−	−	−
IFN-γ mRNA	−	+	+	+	+

	Sample Code									
	1	2	3	4	5	6	7	8	9	10
CIDP biopsy samples[b]										
Vα24JαQ	+	+	+	+	−	+	+	−	−	−
IL-4 mRNA	+	+	+	+	+	+	+	+	+	+
IFN-γ mRNA	−	+	−	+	+	+	−	+	−	+

	Sample Code										
	1	2	3	4	5	6	7	8	9	10	11
OND biopsy samples[c]											
Vα24JαQ	−	−	−	−	−	−	−	−	−	−	−
IL-4 mRNA	+	+	+	+	−	−	−	−	−	−	−
IFN-γ mRNA	−	−	+	+	−	+	+	−	+	−	+

[a] The CNS and PNS samples were examined for the local expression of the mRNA for the invariant Vα24JαQ TCR, IL-4, or IFN-γ. SSCP profiles of all of the CIDP samples and four of the OND samples are demonstrated in Fig. 3.

[b] The sural nerves were obtained from patients with CIDP 1~7 months after onset. The patients include seven males and three females, ages between 20 and 67 years old. The presence of the Vα24JαQ invariant TCR chain did not correlate with sex, age, or timing of biopsy (data not shown).

[c] The OND samples include the sural nerve of hereditary motor and sensory neuropathy (sample 1), nerves without remarkable pathology (samples 2, 5, and 8), Krabbe disease (sample 3), alcoholic polyneuropathy (samples 4 and 6), diabetic polyneuropathy (sample 7), Churg-Strauss syndrome (sample 9), acute demyelinating polyneuropathy (sample 10), and POEMS syndrome (sample 11).

isolating $CD4^-CD8^-$ T cells by flow cytometry and cloning $V\alpha24^+$ TCR. Because it is a laborious procedure, only a limited number of patient PBMC samples (four systemic sclerosis and nine insulin-dependent diabetes mellitus) were examined. In contrast, the SSCP method enables us to monitor the Vα24JαQ TCR along with the overall Vα24 repertoire and to handle a large number of samples from a variety of sources, including a minute volume of frozen sample. In this study, we examined PBMC, CSF, and inflammatory tissues obtained from MS and control diseases including CIDP. As shown in Fig. 1*A*, the Vα24JαQ probe always detected the invariant TCR as a dominant band. Although one or two additional bands were faintly detected in some samples, there was no difficulty in identifying the major band for the invariant TCR in a given sample by setting a positive control of HS PBMC. We conceive that the additional bands most probably represent the invariant Vα24JαQ TCR with an alternative conformation (21, 22) because they would migrate to the same positions (Fig. 1*A*). A possibility for the Vα24JαQ probe degeneracy was not a major concern, because of the limited number of bands.

The first remarkable observation was that all of the PBMC samples of MS in remission and three-quarters of the samples in relapse did not express the Vα24JαQ rearrangement (Table I). In contrast, all of the samples from HS and 90% of the OND samples

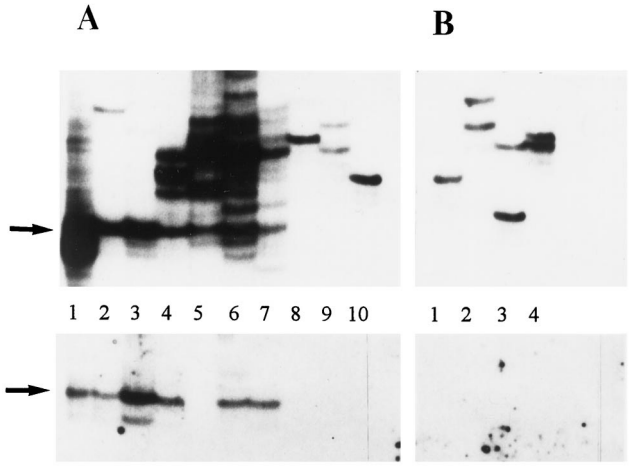

FIGURE 4. Demonstration of the $V\alpha24^+$ TCR rearrangement and the invariant Vα24JαQ TCR in biopsied peripheral nerves. In this experiment, 10 sural nerve samples from CIDP patients (*A*) and 4 from ONDs (*B*) were examined on the same SSCP gel. The same patient codes are used in this figure and Table IV. The arrows indicate the position for the invariant Vα24JαQ band.

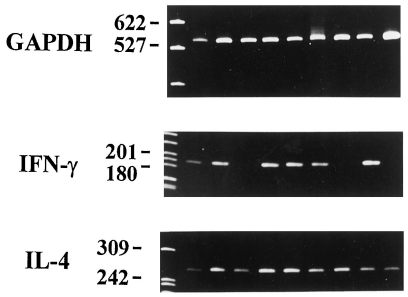

FIGURE 5. Expression of IFN-γ and IL-4 in biopsied peripheral nerves from CIDP. GAPDH, IFN-γ, and IL-4 messages were PCR amplified from nine biopsy samples from CIDP. A representative experiment of three is shown. GAPDH and IL-4 were detected in all of the samples, whereas expression of IFN-γ was seen in six of nine samples.

expressed the invariant TCR. The striking difference between MS and control groups implies that Vα24JαQ NK T cells are greatly reduced in MS. It was possible that the reduction of Vα24JαQ NK T cells in the periphery might be due to recruitment of a large number of the cells into the site of pathology. However, the detection frequency of NK T cells in the CSF and CNS samples (Tables 1 and 3) was lower than the frequency of the PBMC samples of MS in which NK T cells are greatly reduced. Furthermore, examination of PBMC-CSF pairs (Table II) showed that the absence of Vα24JαQ TCR in the PBMC is not necessarily associated with its presence in the CSF. These results do not support the idea that peripheral reduction of NK T cells may be secondary to massive recruitment of the cells into the CNS.

Studies of mice (33, 34) indicate a role of IL-12 and TCR ligand in causing the apoptotic deletion of NK T cells in vivo. However, the reduction of NK T cells in MS was more pronounced in remission than relapse, excluding a correlation of IL-12 elevation and NK T cell reduction. Although some cytokines could be involved, it is likely that an additional molecule differentially expressed in MS and CIDP may play a key role in the selective reduction of NK T cells in MS. We would speculate that the differentially displayed molecule may be a TCR ligand (possibly glycolipid/CD1d) that could specifically delete NK T cells.

On the other hand, we also demonstrated that the greatly reduced Vα24JαQ NK T cells could appear again in the course of MS. A most convincing evidence was obtained after a 2-year follow-up of patient 2 (Table II), revealing that NK T cells could probably repopulate during clinical exacerbation. It is currently recognized that NK T cells are relatively resistant to glucocorticoid-induced apoptosis as compared with conventional T cells (35). It was, therefore, arguable that production of endogenous glucocorticoids during clinical exacerbation may trigger the expansion of NK T cells by altering a balance between conventional T cells and NK T cells. However, this is unlikely since the repopulation of Vα24JαQ NK T cells was accompanied by the reappearance of other Vα24$^+$ clones (see the relapse samples from patient 2 in Fig. 1*C*). It is reported in mice that once deleted, NK T cells in liver and spleen could rapidly repopulate via regeneration of the NK T cells in bone marrow (33). If this observation can be extrapolated to humans, the NK T cell repopulation seen in MS may be explained by disruption of death signals and/or induction of survival (growth) factors that might occur in association with clinical relapse.

Most notably, we demonstrate the presence of Vα24JαQ NK T cells in the autoimmune inflammatory lesions of CIDP. To our knowledge, this is the first definitive proof for the local engagement of Vα24JαQ NK T cells. In contrast to CIDP, the invariant TCR was only rarely detected in MS lesions (Fig. 3 and Table III). This intriguing contrast can probably be based on the difference in the total number of NK T cells in vivo. However, differences in the local environment for the survival of NK T cells may also underlie the immunopathological differences between MS and CIDP.

At this moment, the role of the locally infiltrated NK T cells in CIDP or MS is not clear. Because both murine and human NK T cells have the potential to produce a large amount of IFN-γ and IL-4 (1, 2, 10–12), it is possible that the Th1 cell-mediated CNS or PNS inflammation may be up- or down-regulated by either IFN-γ or IL-4 locally produced by NK T cells. To obtain some insight, we evaluated expression of the cytokine messages in the CNS and PNS samples (Table IV). The results revealed significant differences in the cytokine milieu among the CNS and PNS diseases. To know whether the NK T cell infiltration may account for the differential expression of the cytokines, we need to perform cytokine analysis on a single-cell basis in the future.

In summary, we demonstrate that a great reduction of Vα24JαQ NK T cells in the peripheral blood is an immunological hallmark of MS, whereas it is not appreciable in CIDP. The CIDP lesions were found to be often infiltrated with Vα24JαQ NK T cells, but MS lesions only rarely expressed the Vα24JαQ TCR. It is therefore possible that the extent of NK T cell alteration may be a critical factor which would define the clinical and pathological features of the autoimmune diseases. Finally, although the mechanism underlying the NK T cell deletion remains largely unclear, a remarkable contrast between the CNS and PNS diseases allows us to speculate a role of tissue-specific elements such as the level of CD1d expression or differences in the CD1d-bound glycolipid. Namely, it is possible that interaction of NK T cells with the CNS APC strongly expressing CD1d or recognition of CD1d-associated ligand restricted to the CNS by NK T cells may lead to deletion of NK T cells via TCR engagement. This postulate could be verified in the future by analyzing the alteration of NK T cells in a wider range of tissue-specific autoimmune diseases.

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