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Accumulation of $V_\alpha 7.2$–$J_\alpha 33$ invariant T cells in human autoimmune inflammatory lesions in the nervous system

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Abstract

T cells expressing an invariant TCR α chain and NK cell markers are expected to exhibit unique functions. Whereas much attention has been paid to CD1d-restricted NKT cells, a second NKT cell population bearing an invariant AV19–AJ33 TCR has recently been identified in mice and humans. Selection and/or expansion of this population require B cells, and would involve a non-classical class I-related molecule MR1. Although their preferential distribution in the gut mucosa indicates their role in the host response at the site of pathogen entry, it remains unknown whether they play an alternative role at different sites or in immunological disorders. Using single-strand conformation polymorphism clonotype analysis, we investigated the presence of the human AV19–AJ33 T cells ($V_\alpha 7.2$–$J_\alpha 33$ T cells) in autopsy samples from multiple sclerosis (MS) patients as well as in nerve biopsy samples from chronic inflammatory demyelinating polyneuropathy (CIDP) patients. Here we report that the $V_\alpha 7.2$–$J_\alpha 33$ T cells are accumulated in some of the central nervous system lesions of MS and in the majority of the peripheral nerve samples from CIDP. We have previously revealed that CD1d-restricted, $V_\alpha 24$–$J_\alpha Q$ NKT cells are remarkably reduced in the peripheral blood from MS. However, $V_\alpha 7.2$–$J_\alpha 33$ T cells are not reduced in the peripheral blood from MS and could be detected in a large majority of the cerebrospinal fluid samples obtained during relapse of MS. The present results indicate that the $V_\alpha 7.2$–$J_\alpha 33$ T cells are involved in the autoimmune inflammatory lesions.

Introduction

Conventional T cells display a wide and diverse repertoire with regard to TCR that are made by random recombination of V, D and J segments as well as junctional deletion and insertion of nucleotides. In addition to these mainstream T cells that have undergone thymic selection for cognate peptides bound to MHC molecules, there also exist discrete 'invariant' lymphocyte populations, characterized by limited repertoire diversity due to their expression of an invariant receptor chain (1). The most well-characterized 'invariant' T cells are CD1d-rectricted NKT cells, which constitute a major component of lymphocyte populations expressing TCR and NK cell markers. The NKT cells are reactive to glycolipid presented by CD1d molecules and express an invariant TCR α chain paired with particular V_β segments [$V_\alpha 24$–$J_\alpha Q$ α chain paired with $V_\beta 11$ in humans; $V_\alpha 14$–$J_\alpha 281$ with $V_\beta 8.2$ and 7 in rodents] (2–4). Although the natural ligand for the CD1d-restricted NKT cells still remains unknown, they could produce large amounts of cytokines shortly after TCR ligation, allowing us to speculate that they play a critical role in regulation of various immune responses that would maintain the state of health or cause damage to self-tissues.

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Several lines of evidence indicate that the T cell repertoire in humans contains 'invariant' T cells distinct from CD1d-restricted NKT cells (2,5–7). It has recently been proposed that T cells expressing an invariant AV19–AJ33 TCR (the canonical $hV_\alpha 7.2$–$J_\alpha 33$ or $mV_\alpha 19$–$J_\alpha 33$ TCR rearrangement) represent a second 'invariant' T cell subset that would develop in the absence of CD1d (8–11). Independent studies by Lantz *et al.* (8,11) and Shimamura *et al.* (9,10) have shown that the $V_\alpha 19$–$J_\alpha 33$ T cells do not require CD1d for their development and expansion *in vivo*. They are present in TAP-1 knockout mice (8), but are absent in β_2-microglobulin-deficient mice (8,9), suggesting that they probably recognize a non-peptide antigen associated with a non-classical MHC class Ib molecule other than CD1d. Although surface phenotypes of the invariant T cells remain to be fully characterized, the $V_\alpha 19$–$J_\alpha 33$ T cells were enriched in the $NK1.1^+CD3^+$ population isolated from CD1d-deficient mice (9), allowing us to refer to the 'invariant' T cells as a second type of NKT cells. Very recently, Lantz *et al.* (11) have reported that the 'invariant' T cells are enriched in the gut lamina propria, and that their selection and/or expansion require B cells and commensal flora. The distribution of the cell population would indicate that the novel 'invariant' T cells are possibly involved in the host response at the site of pathogen entry. Finally, MR1, a non-classical class I-related molecule (12), has been identified as a restriction element involved in the selection of the $V_\alpha 19$–$J_\alpha 33$ T cells.

It is of note that the novel MR1-restricted NKT cells share several characteristics with CD1d-resticted NKT cells. For example, both of the 'invariant' populations can be detected in unrelated individuals with different ethnic backgrounds and a majority of the cells resides in the $CD4^-CD8^-$ T cell population (7,8). As noted, the TCR α chain represents the canonical V_α–J_α rearrangement, whereas the β chain sequence is restricted by the use of particular V_β segments ($hV_\beta 11$ for CD1d-restricted NKT cells and $hV_\beta 2$ and 13 for MR1-restricted T cells). Both of the 'invariant' populations have a 'natural' memory phenotype ($CD44^{high}$). Furthermore, the invariant TCR sequences as well as their restriction elements (CD1d and MR1) are highly conserved across the species. These characteristics are consistent with the idea that the 'invariant' lymphocyte populations might exert an immediate response against phylogenetically conserved antigens at the frontier between innate and adaptive immunity (1). In accordance with this idea, CD1d-restricted NKT cells produce large amounts of IL-4 and IFN-γ within hours of TCR engagement (3,13–15). Through the explosive release of cytokines and chemokines, they are capable of initiating a cascade of immunological events involved in regulation of autoimmunity and vital defense against microbial agents or tumor cells (1). In contrast, very little is known about the function of the MR1-resricted T cells. Although accumulating data indicate that they may promptly respond to antigen by producing IL-4 (Shimamura *et al.,* unpublished observations), their ability to produce cytokines and chemokines needs to be systematically analyzed in the coming years.

A numerical reduction or functional alterations in CD1d-rectricted NKT cells bearing the $V_\alpha 24$–$J_\alpha Q$ invariant chain have been documented in various human autoimmune diseases (3,4). Using three different approaches, the RT-PCR single-strand conformation polymorphism (SSCP) clonotype method (16), anti-$V_\alpha 24$ and anti-$V_\beta 11$ antibodies, and glycolipid-loaded CD1d tetramers (17), we have recently revealed that the $V_\alpha 24$–$J_\alpha Q$ NKT cells are greatly reduced in number in the peripheral blood of multiple sclerosis (MS), a putative autoimmune disease mediated by $T_h 1$ autoimmune T cells (18,19). We also examined the distribution of the $V_\alpha 24$–$J_\alpha Q$ NKT cells in the central nervous system (CNS) lesions from patients with MS as well as in the peripheral nerve biopsy samples derived from chronic inflammatory demyelinating polyneuropathy (CIDP) patients (16). Although expression of non-invariant $V_\alpha 24$ rearrangement was ubiquitous in the CNS samples of MS, the $V_\alpha 24$–$J_\alpha Q$ clonotype specific for the NKT cells appeared to be missing in most of the CNS lesions. In contrast, the NKT cell clonotype could be readily detected in a large majority of the biopsy samples of CIDP, which is a chronic demyelinating disease of the peripheral nervous system (PNS) with a presumed autoimmune origin (20).

Using the same samples previously analyzed for the $V_\alpha 24$–$J_\alpha Q$ NKT cells (16), we conducted experiments to address the following questions. (i) Are the invariant $V_\alpha 7.2$–$J_\alpha 33$ T cells reduced in the peripheral blood of MS? (ii) Are they involved in the inflammatory lesions of MS and CIDP or could they be missing from the lesions? (iii) Are they present in the cerebrospinal fluid (CSF) derived from MS? Here, we report that unlike the $V_\alpha 24$–$J_\alpha Q$ NKT cells, the $V_\alpha 7.2$–$J_\alpha 33$ T cells are not reduced in the peripheral blood of MS. More strikingly, they were detected in some of the pathological samples obtained from MS and in the majority of nerve biopsy samples from CIDP, and could be detected in the CSF samples from MS. Comparison with other T cell populations indicated a selective accumulation of the $V_\alpha 7.2$–$J_\alpha 33$ T cells in the inflammatory lesions. We propose that the invariant $V_\alpha 7.2$–$J_\alpha 33$ T cells do not only play a role in protection against pathogen entry in the gut (11), but also in the regulation of autoimmune tissue inflammation.

Methods

Selection of patients with MS or CIDP

We obtained peripheral blood and CSF from patients with 'definite MS' according to the diagnostic criteria proposed by Poser *et al.* (21). The diagnosis of MS was further assisted by magnetic resonance imaging and evoked responses. All patients had remitting-relapsing MS and did not receive immunosuppressive agents at the time of investigation. Diagnosis of CIDP was based on the criteria of the American Academy of Neurology (22).

Peripheral blood mononuclear cells (PBMC), CSF, CNS and PNS samples

Heparinized blood (20 ml) was taken and PBMC were isolated by Ficoll density gradient centrifugation. CSF samples were obtained within 1 week after the onset of exacerbation. The sural nerve biopsy samples were taken for other diagnostic purposes with standard procedure (23). Samples were snap-frozen and were stored at –70°C along with frozen brain samples until analysis had been performed. The histopatho-

logical characterization of the MS plaques was performed as described previously (24).

Isolation of mRNA and synthesis of cDNA

mRNAs were isolated from 10^7 PBMC and from CSF sediment using the QuickPrep Micro mRNA purification kit (Amersham-Pharmacia Biotech, Uppsala, Sweden). The air-dried pellet was resuspended in 20 μl of RNase-Free Water and used for cDNA synthesis by the First-Strand cDNA synthesis kit (Amersham-Pharmacia Biotech, Uppsala, Sweden) using oligo-dT as primer. The mRNAs previously isolated from autopsy CNS samples and sural nerve biopsy samples (16) were also converted to cDNA by the same approach.

SSCP analysis

We conducted RT-PCR SSCP following amplification with V_α and C_α primers as described previously (16,25). Primers and probes were designed based on the previously published sequences (2,3,5,8,26). To detect the $V_\alpha 7.2$–$J_\alpha 33$ invariant chain, 1 μl of the diluted cDNA was used for each PCR reaction with $V_\alpha 7.2$-specific sense primer (GTCGGTCTAAAGGGTACAGT) and anti-sense C_α-specific primer (CAGCTGAGAGACTCTAAAT). cDNAs obtained from PBMC samples were amplified by PCR for 39 cycles, while cDNAs from autopsy/biopsy samples and CSF were amplified for 40 cycles. Quantities of 0.2 μg of sense and 0.2 μg of anti-sense primers (30 pmol) were added to 50-μl reactions containing 5 μl of 10 × ExTaqBuffer, dNTPs and 2.5 U of ExTaq DNA polymerase (Takara, Tokyo, Japan). Amplified DNAs were diluted (1:3) and heat-denatured. Aliquots of 4 μl of the diluted samples were electrophoresed in non-denaturing 4% polyacrylamide gel. DNAs were transferred to Immobilon-S (Millipore Intertech, Bedford, MA) and hybridized with biotinylated C_α-specific (AAATATCCAGAACCCTGACCCTGCCGTGTACC), $J_\alpha 33$-specific (TATCAGTTAATCTGGGGCGCTGGGACCAAGCT) or invariant $V_\alpha 7.2$–$J_\alpha 33$-specific internal probe (TGTGCTGTGAGAGATAGCAACTATCAGTTAATCTG). To detect the $V_\alpha 24$–$J_\alpha Q$ invariant chain, cDNAs were PCR amplified with $V_\alpha 24$-specific sense primer (ACACAAAGTCGAACGGAAG) and C_α-specific anti-sense primer (GATTTAGAGTCTCTCAGCTG), and then hybridized with a probe specific for the invariant $V_\alpha 24$–$J_\alpha Q$ sequence (TGTGTGGTGAGCGACAGAGGCTCAACCCTG) as previously described (16).

SSCP clonotypes were visualized by incubation with streptavidin, biotinylated alkaline phosphatase and a chemiluminescent substrate system (Phototope; New England Biolabs, Bedford, MA). cDNAs for human IL-4 and IFN-γ were amplified by RT-PCR as described previously (16).

Real-time $V_\alpha 7.2$ clonotypic RT-PCR

Quantitative RT-PCR (LightCycler; Roche Molecular Biochemicals, Mannheim, Germany) was performed with the $V_\alpha 7.2$ sense primer and with an anti-sense primer matching the CDR3α region of the $V_\alpha 7.2$–$J_\alpha 33$ T cells (TGATAGTTGCTATCTCTCAC). An aliquot of 1 μl of the cDNA was amplified by PCR for 40 cycles using quantification with a commercial kit (LightCycler DNA Master SYBR Green I; Roche Molecular Biochemicals). Quantities of 0.2 μg of sense and 0.2 μg of anti-sense primers (30 pmol) were added to 50-μl reactions containing 5 μl of 10 × ExTaqBuffer, dNTPs and 2.5 U of ExTaq DNA polymerase (Takara). All PCR reactions were controlled by β-actin expression (sense primer: AGAGATGGCCACGGCTGCTT; anti-sense primer: ATTTGCGGTGGACGATGGAG) (27). Based on the standard values of control samples, the relative expression for each sample was determined with the LightCycler software.

TCR DNA sequencing

In brief, small areas of the SSCP gel corresponding to the clonotypes of interest were cut out and DNAs were eluted as described previously (25). A second PCR was performed with the corresponding V_α-specific and C_α-specific primers using the eluted DNAs as template. The PCR products were cloned into pCR 2.1-TOPO Vector using TOPO TA cloning kit (Invitrogen, Carlsbad, CA) and recombinants were sequenced using an ABI 377 DNA sequencer (Applied Biosystems, Foster City, CA).

Results

Invariant $V_\alpha 7.2$–$J_\alpha 33$ T cells represent a dominant $V_\alpha 7.2^+$ subset in the peripheral blood of healthy subjects (HS)

First, we examined if the RT-PCR SSCP clonotype method (16,25,28,29) could be used to detect the invariant $V_\alpha 7.2$–$J_\alpha 33$ sequence in peripheral blood derived from HS. Following mRNA isolation from nine PBMC samples, the TCR α chain sequences encompassing the αCDR3 region were RT-PCR amplified with a set of $V_\alpha 7.2$ and C_α primers. The amplified cDNAs were denatured and electrophoresed on the SSCP gel, and hybridized with C_α-, $J_\alpha 33$- or invariant $V_\alpha 7.2$–$J_\alpha 33$-specific probe. Hybridization with the C_α probe has revealed several bands in the smear background on the gel, indicating that T cells using the $V_\alpha 7.2$ segment are restricted, but heterogeneous (Fig. 1, upper panel). However, hybridizing with the $J_\alpha 33$ probe revealed a solitary band in each sample at a same position (Fig. 1, middle panel), indicating that a single clonotype may dominate the TCR $V_\alpha 7.2$ and $J_\alpha 33$ rearrangements found in the peripheral blood. Given that the invariant $V_\alpha 7.2$–$J_\alpha 33$ clonotype was identified as an expanded clonotype in prior studies (7,8), we speculated that the solitary band that hybridized with the $J_\alpha 33$-specific probe might correspond to the invariant $V_\alpha 7.2$–$J_\alpha 33$ sequence. Consistent with this speculation, the invariant $V_\alpha 7.2$–$J_\alpha 33$-specific probe detected a distinct band at the same position where the $J_\alpha 33$-specific probe hybridized (Fig. 1, lower panel).

To confirm if the clonotype hybridized with the $J_\alpha 33$-specific probe represents the invariant $V_\alpha 7.2$–$J_\alpha 33$, we eluted DNA from the sites of the gel corresponding to the band, and amplified the DNA by a second PCR with $V_\alpha 7.2$ and C_α primers. The amplified cDNAs were cloned and 10–15 clones from each individual were sequenced. Analysis of four randomly selected HS has shown that 70–90% of the clones from each subject possess the $V_\alpha 7.2$–$J_\alpha 33$ invariant sequence (GTG AGA) in the CDR3 region. Another CDR3 DNA sequence (GTG ATG combined with $J_\alpha 33$) was detected repeatedly in two of the four individuals. However, all the other sequences differed from each other and were not shared by different individuals. This analysis assured that the solitary band of our

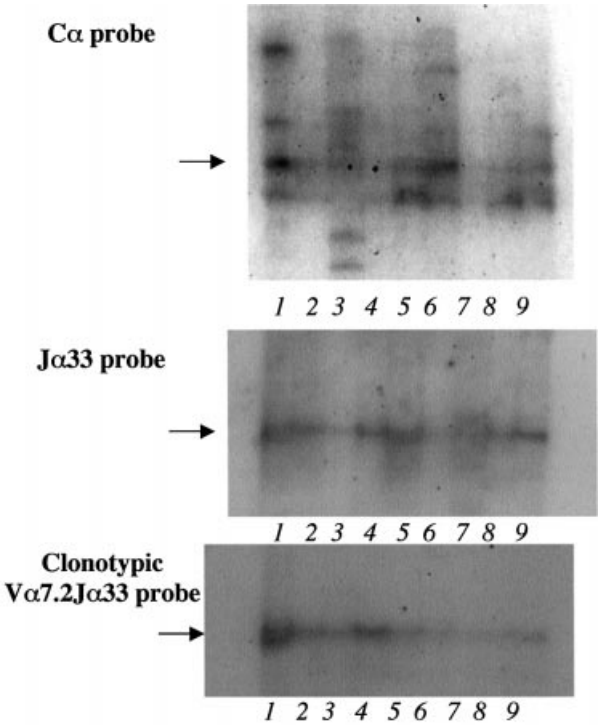

Fig. 1. Detection of the invariant $V_\alpha 7.2$–$J_\alpha 33$ TCR in the peripheral blood of HS. The $V_\alpha 7.2^+$ T cell repertoire in the peripheral blood was analyzed with the SSCP technique. The $V_\alpha 7.2^+$ TCR amplified from nine HS were hybridized with the C_α-specific probe (upper panel), the $J_\alpha 33$-specific probe (middle panel) or the invariant $V_\alpha 7.2$–$J_\alpha 33$ clonotype-specific probe (lower panel). Arrows indicate the position for the invariant $V_\alpha 7.2$–$J_\alpha 33$ clonotype. The numerical code corresponds to each subject.

interest corresponds to the clonotype expressed by the MR1-restricted T cells.

Invariant $V_\alpha 7.2$–$J_\alpha 33$ T cells do not expand in response to α-galactosylceramide (α-GalCer)

$V_\alpha 24$–$V_\alpha 11$ NKT cells are known to proliferate in response to α-GalCer, which is a prototype ligand for the NKT cells (4,17). Additionally, we examined if the invariant $V_\alpha 7.2$–$J_\alpha 33$ T cells respond to α-GalCer. In brief, PBMC from healthy individuals were stimulated with α-GalCer as previously described (17) and the α-GalCer-stimulated PBMC cultures were harvested at different time points for SSCP analysis. We observed that the invariant $V_\alpha 7.2$–$J_\alpha 33$ clonotype would gradually diminish, while the $V_\alpha 24$–$V_\alpha 11$ clonotype remarkably expanded shortly after stimulation with α-GalCer (data not shown). This result confirms that α-GalCer is not a stimulatory ligand for the invariant $V_\alpha 7.2$–$J_\alpha 33$ T cells.

Invariant $V_\alpha 7.2$–$J_\alpha 33$ T cells are not reduced in the peripheral blood of MS

In previous studies, we have demonstrated that the $V_\alpha 24$–$J_\alpha Q$ NKT cells are remarkably reduced in the peripheral blood of MS, particularly in the remission phase (16,17). In fact, SSCP analysis for the $V_\alpha 24$–$J_\alpha Q$ clonotype detected the NKT cell clonotype in all the HS (18 of 18, 100%) (16), but the clonotype was not found in any of the MS patients in remission (Table 1).

Table 1. Detection frequency of the invariant $V_\alpha 7.2$–$J_\alpha 33$ TCR versus invariant $V_\alpha 24$–$J_\alpha Q$ TCR in various samples

	$V_\alpha 7.2$–$J_\alpha 33$	$V_\alpha 24$–$J_\alpha Q$
PBMC-HS	9/9 (9)	18/18 (18)
PBMC-MS	13/15 (15)	0/18 (18)
CNS-MS	7/14 (7)	1/14 (8)
CNS-control	1/6 (1)	0/6 (0)
CSF-MS	8/11 (10)	11/24 (24)
PNS-CIDP	8/10 (10)	6/10 (10)
PNS-OND	0/11 (4)	0/11 (4)

Using the SSCP clonotype method, we have previously examined the presence of the $V_\alpha 24$–$J_\alpha Q$ clonotype in various types of samples (16). Here we evaluated the presence of the $V_\alpha 7.2$–$J_\alpha 33$ clonotype in the same samples with a similar methodology. Data represent the number of samples containing the invariant TCR/the total number of samples. In the parentheses, we give the number of samples from which $V_\alpha 7.2^+$ or $V_\alpha 24^+$ TCR could be amplified.
The examined samples include PBMC from HS (PBMC-HS) or from MS in remission (PBMC-MS), CNS plaques from MS (CNS-MS), CNS samples from autopsy cases without neurological disease (CNS-control), CSF samples from MS during relapse (CSF-MS), and sural nerve biopsy samples from CIDP (PNS-CIDP) and from other neurological disease (PNS-OND).

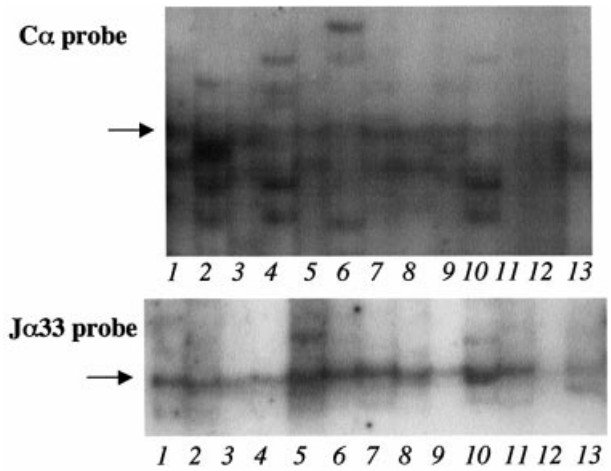

Fig. 2. Detection of the invariant $V_\alpha 7.2$–$J_\alpha 33$ TCR in the peripheral blood of MS. The $V_\alpha 7.2^+$ TCR amplified from the peripheral blood of 15 MS patients were analyzed by SSCP followed by hybridization with a C_α (upper panel)- or $J_\alpha 33$ (lower panel)-specific probe. Thirteen out of the 15 samples displayed the invariant clonotype (arrow). The presence of clonotypes distinct from the invariant $V_\alpha 7.2$–$J_\alpha 33$ clonotype indicates expansion of conventional $V_\alpha 7.2^+$ T cells. See also Table 1 summarizing the pooled data.

Given the notable similarities between CD1d-restricted NKT cells and MR1-resricted T cells, we speculated that the invariant $V_\alpha 7.2$–$J_\alpha 33$ clonotype might also be reduced in the peripheral blood of MS. However, the invariant clonotype could be readily detected by the SSCP method in 13 of 15 peripheral blood samples from MS patients in remission (Fig. 2 and Table 1). To further evaluate the frequency of the $V_\alpha 7.2$–$J_\alpha 33$ T cells in PBMC of MS, we applied a real-time RT-PCR with $V_\alpha 7.2$- and $V_\alpha 7.2$–$J_\alpha 33$ clonotype-specific primers for quantitative analysis. Using this assay, we measured relative

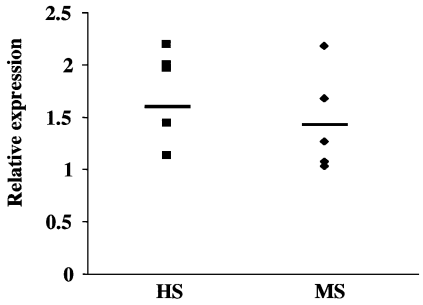

Fig. 3. Real-time RT-PCR assay for the invariant $V_\alpha 7.2$–$J_\alpha 33$ TCR. Expression of the invariant $V_\alpha 7.2$–$J_\alpha 33$ mRNA in the peripheral blood was quantitatively analyzed by applying quantitative RT-PCR (LightCycler). The PBMC from five HS and five MS were examined, and expression of the invariant $V_\alpha 7.2$–$J_\alpha 33$ TCR was quantified in comparison to that of β-actin. The values represent relative expression of the invariant TCR in each sample, where the lowest value is indicated as 1. The horizontal lines show the mean (HS 1.726 ± 0.44, MS 1.416 ± 0. 48).

expression of the invariant $V_\alpha 7.2$–$J_\alpha 33$ mRNA in the PBMC derived from five MS in remission and from five HS. As shown in Fig. 3, there was no significant difference between HS and the patients with MS. Taken together, we conclude that MR1-restricted T cells are conserved in number in the patients with MS.

Infiltration of the invariant $V_\alpha 7.2$–$J_\alpha 33$ T cells in the CIDP lesions

We could previously demonstrate an expression of the invariant $V_\alpha 24$–$J_\alpha Q$ TCR in 60% of the peripheral nerve biopsy samples from CIDP (16). A possible interpretation was that the NKT cells may be recruited to the lesions like inflammatory cells (30–32). It is very interesting to know if MR1-restricted T cells are also recruited to the CIDP lesions. Here we examined the CIDP samples for their expression of the $V_\alpha 7.2$–$J_\alpha 33$ invariant sequence. We were able to amplify $V_\alpha 7.2^+$ TCR messages from all the 10 samples. Hybridization with the C_α-specific probe (Fig. 4A) revealed a number of bands in every lesion, suggesting that not only the invariant $V_\alpha 7.2$–$J_\alpha 33$, but conventional $V_\alpha 7.2^+$, T cells may also be present in the lesions. Hybridization with $J_\alpha 33$- or invariant $V_\alpha 7.2$–$J_\alpha 33$-specific probe displayed the invariant clonotype in eight out of the 10 samples (Fig. 4A, and Tables 1 and 2). We also examined 11 nerve biopsy samples from other neurological diseases (OND) as controls. A faint single band was found in four of the 11 OND samples after hybridization with the C_α-specific probe (Fig. 4B). However, none of the samples hybridized with the $J_\alpha 33$-specific probe, excluding the presence of the invariant $V_\alpha 7.2$–$J_\alpha 33$ T cells in the control PNS lesions. These results demonstrate that not only the $V_\alpha 24$–$J_\alpha Q$ NKT cells, but also $V_\alpha 7.2$–$J_\alpha 33$ T cells, would preferentially accumulate in the inflammatory lesions of CIDP.

We classified the CIDP biopsy samples into four groups, based on the expression pattern of the $V_\alpha 7.2$–$J_\alpha 33$ and $V_\alpha 24$–$J_\alpha Q$ invariant sequences (Table 2). Eight of the 10 samples belonged to Group I ($V_\alpha 7.2$–$J_\alpha 33^+/V_\alpha 24$–$J_\alpha Q^+$) or Group II ($V_\alpha 7.2$–$J_\alpha 33^+/V_\alpha 24$–$J_\alpha Q^-$), as reflected by the frequent detection of the $V_\alpha 7.2$–$J_\alpha 33$ clonotype. We could consistently detect IL-4 mRNA in all the samples from Groups I and II. More

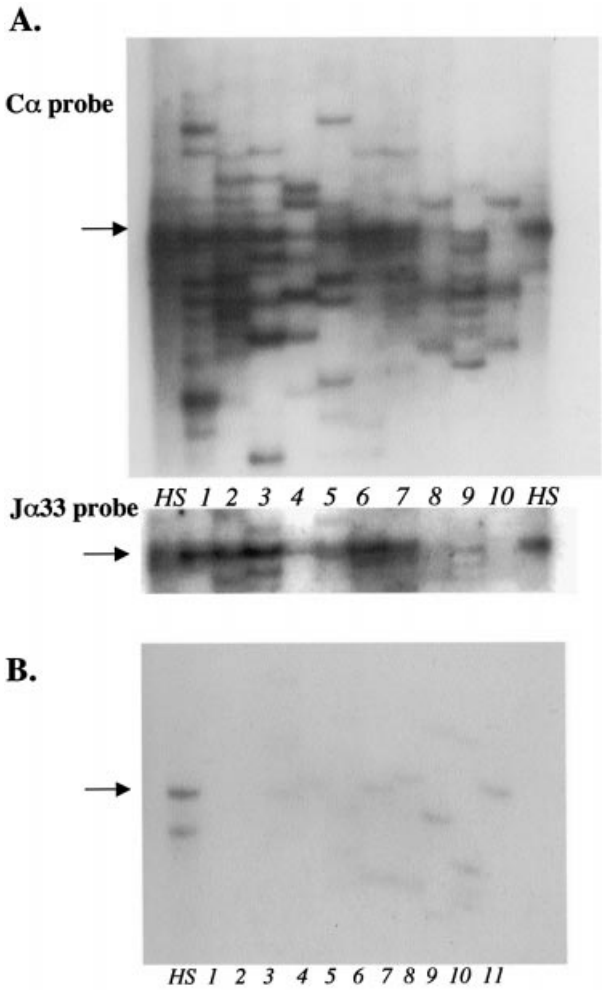

Fig. 4. Demonstration of the $V_\alpha 7.2^+$ TCR rearrangements and the invariant $V_\alpha 7.2$–$J_\alpha 33$ TCR in nerve biopsy samples. (A) Sural nerve biopsy samples from 10 CIDP patients were examined by SSCP. Amplified $V_\alpha 7.2^+$ TCR were hybridized with C_α- and $J_\alpha 33$-specific probes. HS indicates a lane for PBMC from HS, illustrating the position of the invariant $V_\alpha 7.2$–$J_\alpha 33$ clonotype (arrow). The numerical code corresponds to each biopsy sample. The same sample codes are used here and in Table 2. (B) Sural nerve biopsy samples from patients with OND were examined by SSCP. C_α-specific hybridization detected bands in four samples as shown here. However, the $J_\alpha 33$-specific probe did not detect any band (not shown). The OND include hereditary motor and sensory neuropathy, alcoholic polyneuropathy, acute demyelinating polyneuropathy, Churg–Strauss syndrome, POEMS syndrome, diabetic polyneuropathy, and Krabbe disease.

interestingly, IFN-γ was detected in four of five samples from Group I, but detected in only one of three samples from Group II. Although the number of samples was not large enough to draw any conclusion, this may indicate a possible difference between Groups I and II with regard to the cytokine milieu.

Accumulation of the invariant $V_\alpha 7.2$–$J_\alpha 33$ T cells in the autopsy CNS lesions of MS

Next we asked if the $V_\alpha 7.2$–$J_\alpha 33$ T cells may infiltrate into the autopsy CNS lesions of MS. Using the SSCP clonotype method, we analyzed 14 CNS lesions obtained from five

Table 2. Classification of the CIDP lesions based on the expression pattern of the invariant TCR

	V_α7.2–J_α33	V_α24–J_αQ	IFN-γ	IL-4
Group I				
CIDP-1	+	+	+	+
CIDP-3	+	+	+	+
CIDP-7	+	+	+	+
CIDP-9	+	+	+	+
CIDP-5	+	+	–	+
Group II				
CIDP-2	+	–	–	+
CIDP-4	+	–	–	+
CIDP-6	+	–	+	+
Group III				
CIDP-8	–	+	–	+
Group IV				
CIDP-10	–	–	+	–

The sural nerve lesions derived from CIDP were classified into four groups based on the expression pattern of the invariant TCR of the MR1-restricted T cells and of CD1d-restricted NKT cells. Here we define those expressing both of the invariant chains as Group I and those missing both as Group IV. Lesions expressing V_α7.2–J_α33 only or V_α24–J_αQ alone were classified as Groups II and III respectively. Expression of IFN-γ and IL-4 was detected by RT-PCR.

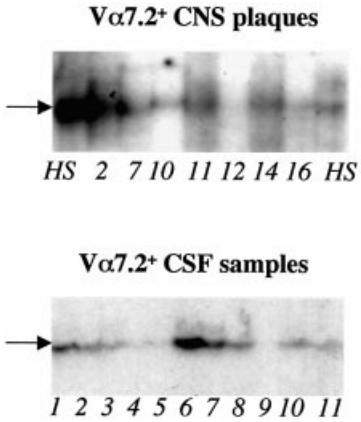

Fig. 5. Demonstration of the invariant V_α7.2–J_α33 TCR in autopsy CNS samples and CSF from MS. Fourteen CNS autopsy samples and 11 CSF samples from MS were analyzed in these experiments. The V_α7.2$^+$ samples were hybridized with a C_α-specific probe and re-hybridized with the invariant V_α7.2–J_α33-specific probe. HS indicates a lane for PBMC from a HS, illustrating the position of the invariant V_α7.2–J_α33 clonotype (arrow).

Table 3. Detection frequency of V_α19$^+$ TCR clonotypes in various samples

PBMC-HS	9/9
PBMC-MS	15/15
CNS-MS	0/14
CNS-control	0/6
PNS-CIDP	0/10
PNS-OND	1/11

Data represent the number of samples containing the V_α19 TCR/the total number of samples. The same samples were used for V_α24–J_αQ and V_α7.2–J_α33 clonotypes (Table 1).

autopsied cases with MS, including seven acute plaques, six subacute plaques and one chronic plaque. V_α7.2$^+$ TCR could be amplified by RT-PCR from seven out of the 14 lesions, although they were detected only in one of the six control CNS samples (Table 1). We found that all the V_α7.2$^+$ plaques (four acute plaques, two subacute plaques and a single chronic plaque) expressed the message for the invariant V_α7.2–J_α33 sequence (Fig. 5, upper panel and Table 1). In contrast, as reported previously (16), whereas Vα24$^+$ TCR could be amplified from eight of the 14 MS autopsy lesions, only a single subacute plaque expressed the V_α24–J_αQ clonotype, i. e. the invasion of V_α24–J_αQ NKT cells was mainly restricted to the CIDP lesions, whereas the V_α7.2–J_α33 T cells were also found in some CNS lesions from MS. To verify the postulate that the V_α7.2–J_α33 T cells are involved in the pathology of MS, we also analyzed CSF samples obtained at an acute stage of MS. We were able to detect the V_α7.2–J_α33 invariant sequence in 73% of the samples examined (Fig. 5, lower panel and Table 1), supporting that the invariant V_α7.2–J_α33 T cells are a component of the CNS infiltrates in MS.

In parallel to V_α7.2$^+$ TCR and V_α24$^+$ TCR, we examined the V_α19$^+$ TCR repertoire with the SSCP method. With the C_α-specific probe, we could detect a number of V_α19$^+$ clonotypes in all the PBMC samples obtained from HS and MS (Table 3). However, V_α19$^+$ TCR was not detected in any of the CNS samples from MS or PNS samples from CIDP. This result indicates that the presence of a particular clonotype in the MS lesions does not simply reflect that this population is present in the peripheral circulation. In other words, it implies that the presence of the V_α7.2–J_α33 clonotype may indicate the 'lesiophilic' nature of the MR1-restricted T cells.

Discussion

It has been demonstrated repeatedly that CD4$^-$CD8$^-$ T cells in the human peripheral blood contain clonally expanded T cell populations (5–8). For example, CDR3 spectratyping analysis for the peripheral CD4$^-$CD8$^-$ T cells in healthy individuals has previously indicated clonal expansion of T cells bearing invariant V_α7.2–J_α33, V_α4–J_α29 or V_α19–J_α48 in addition to those expressing the V_α24–J_αQ NKT cell clonotype (7). After knowing that biopsy samples of CIDP are often infiltrated with the V_α24–J_αQ NKT cells (16), we attempted to analyze the expression of the V_α7.2–J_α33, V_α4–J_α29 and V_α19–J_α48 invariant sequences in the lesions of MS and CIDP. However, the invariant V_α4–J_α29 and V_α19–J_α48 sequences were not detected in any of the samples (data not shown). Accordingly, we focused our efforts on analysis of the V_α7.2–J_α33 T cells. In order to shed light on the difference and similarity between the V_α7.2–J_α33 and V_α24–J_αQ NKT cells, we examined the autopsy and biopsy samples as well as PBMC and CSF samples previously analyzed for the expression of the V_α24–J_αQ TCR (16).

Here, we documented that the autopsy CNS lesions from MS as well as the biopsy PNS lesions from CIDP are infiltrated with the invariant V_α7.2–J_α33 T cells. We also showed that the invariant V_α7.2–J_α33 T cells are present in a large majority of the CSF samples obtained at relapse phases of MS. In contrast, V_α19$^+$ TCR could not be detected in any of the affected tissues from MS or CIDP after PCR amplification,

although they can be detected in all the PBMC samples. Although the function of the invariant $V_\alpha 7.2$–$J_\alpha 33$ T cells remains elusive, the present results demonstrated for the first time to our knowledge that the novel invariant T cells (8–11) are present in autoimmune inflammation affecting the nervous system.

In a very recent report (11), Lantz *et al.* showed that the MR1-restricted invariant T cells are preferentially located in the gut mucosa and therefore proposed to name the population as mucosal-associated invariant T cells (MAIT). In support of the special role of this cell population in the mucosa, the number of the cells in the gut mucosa was greatly reduced in germ-free mice. This observation indicated the role of commensal flora for selection of the invariant T cells. Although an interpretation for this could be that the MR1-resricted T cells would recognize the exogenous antigen bound to MR1, analysis of the T–T hybridomas showed that they could recognize MR1 directly in the absence of bound ligand (11). If this is indeed the case, the MAIT cells could be autoreactive to MR1. To accommodate the autoreactivity with the requirement for commensal flora, it could be speculated that MR1 expression may require some ligand derived from or induced by commensal flora. An alternative possibility is that microbial products may facilitate translocation of MR1 to the cell membrane.

Provided that the invariant $V_\alpha 7.2$–$J_\alpha 33$ T cells are generated or expanded in the mucosa, how would they accumulate into the inflammatory lesions of MS and CIDP? Although this remains a conundrum, we would speculate that inflammation-associated signals such as chemokines play a role in the initial step. In fact, CD1d-restricted NKT cells would behave like inflammatory cells and rapidly accumulate into certain granuloma lesions (30), and they could be detected in non-autoimmune inflammatory lesions (31,32). Given a number of similarities between $V_\alpha 7.2$–$J_\alpha 33$ and $V_\alpha 24$–$J_\alpha Q$ NKT cells, we would postulate that the $V_\alpha 7.2$–$J_\alpha 33$ T cells also might be preferentially recruited to the inflammatory sites. To support this idea, non-autoimmune inflammatory lesions are reported to express the invariant $V_\alpha 7.2$–$J_\alpha 33$ TCR (32).

The second critical step may be the interaction of the invariant T cells with B cells expressing MR1 in the site of lesions, given that the vast majority of the inflammatory lesions are infiltrated with B cells. We could expect the $V_\alpha 7.2$–$J_\alpha 33$ T cells to regulate local immune responses by producing cytokines. If IL-4 is the major cytokine produced by the novel invariant T cells, their interaction with B cells may lead to augmentation of antibody production. Even though the encephalitogenic $T_h 1$ autoreactive T cells are down-regulated by IL-4, the direct interaction between B cells and the $V_\alpha 7.2$–$J_\alpha 33$ T cells may substantially augment the tissue damage or alter the type of lesions. However, if suppressive cytokines such as transforming growth factor-β are the major products of the $V_\alpha 7.2$–$J_\alpha 33$ T cells *in vivo*, they may down-regulate the B cells as well as inflammatory cells in the vicinity. Although we can only speculate about how they would deal with auto-immunity, it is possible that they would play an active role in the regulation of autoimmune inflammation. To verify this hypothesis, we need to systematically analyze the functions of the $V_\alpha 7.2$–$J_\alpha 33$ T cells with regard to ligand recognition and cytokine production. It is also important to know if the presence or absence of the invariant T cells may correlate with the type of pathology. The present data indicate that it is indeed a rewarding attempt.

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Abbreviations

α-GalCer	α-galactosylceramide
CIDP	chronic inflammatory demyelinating polyneuropathy
CNS	central nervous system
CSF	cerebrospinal fluid
HS	healthy subject
MAIT	mucosal-associated invariant T cell
MS	multiple sclerosis
OND	other neurological disease
PBMC	peripheral blood mononuclear cell
PNS	peripheral nervous system
SSCP	single-strand conformation polymorphism

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