Lack of restriction of T cell receptor β variable gene usage in cerebrospinal fluid lymphocytes in acute optic neuritis

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Abstract

Objectives—There have been many studies reporting restricted patterns of T cell receptor usage in established multiple sclerosis and these have led to clinical trials of immunomodulation directed at deleting clonal T cell populations. The present study aims to test the hypothesis that highly restricted T cell populations are also present in the CSF in the earliest clinical stages of acute demyelinating disease of the CNS.

Methods—T cell receptor V β (TCRBV) gene expression was studied in CSF and blood in nine patients with acute optic neuritis within 7 days of onset of symptoms, six patients with an acute relapse of multiple sclerosis, and 13 control subjects. RNA was extracted and cDNA synthesised from unstimulated CSF and blood lymphocytes, and TCRBV gene segments were amplified from the cDNA by polymerase chain reaction (PCR) using 21 family specific primers. PCR products were separated by polyacrylamide gel electrophoresis and detected via a labelled oligonucleotide probe. A semiquantitative analysis of band intensity was performed by laser densitometry.

Results—TCRBV mRNA was detected in the CSF of eight of nine patients with optic neuritis, six of six patients with multiple sclerosis, and five of 13 controls, and was closely correlated with the presence of oligoclonal IgG. Usage of a single TCRBV family was demonstrated in two of nine patients with optic neuritis and two of six patients with multiple sclerosis. The number of TCRBV families expressed in the other patients ranged between 5 and 15 (optic neuritis) and 4 and 17 (multiple sclerosis).

Conclusions—There is a relative lack of restriction of TCRBV usage by CSF lymphocytes in the very earliest stages (<7 days) of acute optic neuritis. This may imply either that multiple sclerosis is not a monoclonal disease even at onset, or that the autoimmune response has widened before the disease becomes clinically evident. This may have important consequences for the design of immune therapies in multiple sclerosis. Further studies are required to determine whether the CSF T cell repertoire at presentation has prognostic importance. Longitudinal studies are required to follow the CSF T

cell repertoire from the time of presentation and to determine whether it may have prognostic significance.

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Keywords: T cell receptor usage; cerebrospinal fluid; optic neuritis

It has been postulated that in multiple sclerosis there may be a specific population of CNS infiltrating myelin reactive T cells expressing limited types of T cell receptors.¹ Such restriction of the T cell repertoire has been demonstrated in experimental allergic encephalomyelitis,² supporting the concept that a small population of highly specific T cells is responsible for disease pathogenesis. When T cell receptor (TCR) variable (V) gene family usage was examined in encephalitogenic T cells from both rats and mice with experimental allergic encephalomyelitis, a consistent bias towards TCR V β 8.2 and V α 2 was found.³⁻⁴ These findings formed a basis for experimental treatment, whereby administration of TCR V β 8.2 specific monoclonal antibody successfully blocked experimental allergic encephalomyelitis in PL mice,³ and vaccination of Lewis rats with TCR V β 8.2 peptide both prevented experimental allergic encephalomyelitis and reduced disease severity in affected animals.⁵

Studies of T cell receptor variable β gene (TCRBV) usage in HLA-DR2 positive patients with multiple sclerosis have found biases toward the use of TCRBV5.2 and 6.1 in peripheral blood lymphocytes,⁶ and in established brain lesions.⁷ These reports suggest that TCRBV5.2 or 6.1 may encode a T cell receptor which confers reactivity against myelin basic protein (MBP), which is a major candidate for a multiple sclerosis autoantigen, and has led to clinical trials in which patients with multiple sclerosis have been immunised with TCRBV5.2 and 6.1 peptides,⁸⁻¹⁰ or with other dominant TCRBV genes (TCRBV3, 9 and 12.2).¹¹ In another study,¹² TCRBV6 was found to be overrepresented in activated CSF lymphocytes in patients with multiple sclerosis, and this has led to vaccination with TCRBV6.5 peptides.¹³ All of these clinical trials have shown promising early results.

However, despite the commencement of these T cell receptor based therapies, other studies have shown the overexpression of TCRBV gene families in multiple sclerosis to follow a different pattern,¹⁴⁻¹⁶ or to be more heterogeneous.¹⁷⁻¹⁹ These conflicting data may be explained in part by differing genetic back-

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grounds, for example due to HLA polymorphisms,¹⁶ or by disease heterogeneity. Alternatively, the TCRBV repertoire may be influenced differently when T cell clones are raised against different putative multiple sclerosis autoantigens such as MBP^{6,14} or proteolipid protein (PLP).¹⁸ Another possible explanation of particular interest could be that an initially restricted immune response widens as the demyelinating process progresses,²⁰ and that the heterogeneity of TCRBV usage found in previous studies has arisen by concentrating on patients with established disease.²¹

Because the diagnosis of multiple sclerosis, requiring as it does dissemination in both time and place, is by necessity delayed²² we decided to study patients presenting very early with a syndrome consistent with acute demyelinating optic neuritis. These patients have a substantial risk of developing multiple sclerosis^{23,24} and they often seek medical attention very early. We have therefore studied the T cell (TCRBV) repertoire in the CSF of patients with optic neuritis, within 7 days of their first symptoms appearing, to test the hypothesis that there is highly restricted TCR usage by autoaggressive T cells in the earliest clinical stages of acute CNS demyelinating disease.

Methods

Nine patients (three men, six women; aged 20–38) with an acute syndrome consistent with unilateral demyelinating optic neuritis were studied within 7 days of symptoms first appearing. Brain MRI was obtained in five of nine patients at presentation. Six patients (all women; aged 26–42) undergoing an acute relapse of established multiple sclerosis were also studied. In addition, 13 control patients were studied (four men, nine women; aged 18–76; table 1).

A sample of CSF was obtained via lumbar puncture and collected into sterile tubes. One portion of the sample was concentrated and studied for the presence of oligoclonal IgG bands, using SPE II agarose gel electrophoresis (Beckman, Fullerton, CA, USA) and immunofixation with goat antiserum to IgG (Kallestad, Chaska, MN, USA), and another portion of the sample (1–5 ml) was centrifuged to obtain lymphocytes for TCRBV usage analysis. A peripheral blood sample was concurrently obtained for comparison, and lymphocytes were isolated by density gradient centrifugation. In addition, MHC class II (HLA-DR) typing was performed on patient blood samples by the NSW Red Cross Tissue Typing Laboratories.

The lymphocytes from both the CSF and peripheral blood were stored at -70°C before RNA extraction. Total RNA was extracted using UltraspecTM RNA extraction reagent (Biotecx, Houston, TX, USA) and cDNA was prepared using a standard reverse transcription technique,²⁵ with the following reaction mixture: 500 ng random hexamer primers (Promega, Madison, WI, USA), 10 mM dithiothreitol, 40 U recombinant RNasin (Promega, Madison, WI, USA), 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂,

Table 1 Presence or absence of oligoclonal IgG bands and T cell receptor (TCRBV) genes in the CSF of optic neuritis (ON), multiple sclerosis (MS), and control patients

Patient	Disease	Oligoclonal IgG bands in CSF	TCRBV in CSF
ON-1	ON	+	+
ON-2	ON	0	+
ON-3	ON	0	+
ON-4	ON	0	0
ON-5	ON	0	+
ON-6	ON	+	+
ON-7	ON	+	+
ON-8	ON	+	+
ON-9	ON	+	+
MS-1	MS	+	+
MS-2	MS	+	+
MS-3	MS	+	+
MS-4	MS	+	+
MS-5	MS	+	+
MS-6	MS	+	+
C-1	Demyelinating neuropathy	0	+
C-2	Stiff man syndrome	0*	0
C-3	Guillain-Barré syndrome	0	0
C-4	Guillain-Barré syndrome	0	0
C-5	Lymphoma and neuropathy	0	+
C-6	Cryptococcal lung infection	0	0
C-7	Meningitis	0	0
C-8	Viral encephalitis	+	+
C-9	Encephalitis	+	+
C-10	Astrocytoma	0	0
C-11	Functional visual loss	0	0
C-12	Peripheral neuropathy	0	+
C-13	Seizures	0	0

*Single band detected.

500 µM dNTPs, and 200 U M-MLV RNase H⁻ reverse transcriptase (Life Technologies/Gibco BRL, Gaithersburg, MD, USA).

The TCRBV repertoire was determined by the polymerase chain reaction (PCR) using 21 5' primers specific for TCRBV variable region gene families, covering TCRBV1–12, 14–19,¹⁴ 13.1, 13.2, and 20,²⁶ in conjunction with a 3' TCRBC constant region primer.²⁷ Samples from one multiple sclerosis patient and three control patients were additionally amplified with four primers specific for TCRBV21–24.²⁷ Separate reactions were prepared for each TCRBV family, based on the method of Wucherpfennig *et al.*¹⁴ The standard PCR reaction mixture (20 µl) contained 100 ng of 5' and 3' primer, 200 µM dNTPs, 10 mM Tris-HCl (pH 9 at 25°C), 50 mM KCl, 0.01% gelatin (w/v), 0.1% Triton-X 100, 2 mM MgCl₂, 0.75 U Taq DNA polymerase (Promega, Madison, WI), and 0.5 µl cDNA. Amplifications were performed for 30 cycles in a capillary thermal cycler (Corbett Research, Australia), using the following temperature profile: cycle 1: 95°C for 1 minutes, 55°C for 15 seconds, 72°C for 30 seconds; cycles 2–5: 95°C for 15 seconds, 55°C for 15 seconds, 72°C for 30 seconds, cycles 6–30: 95°C for 10 seconds, 55°C for 10 seconds, 72°C for 30 seconds. A positive control cDNA was included in each PCR run.

PCR products were size separated in 10% polyacrylamide gels and transferred to positively charged nylon membranes (Amersham, Buckinghamshire, UK) by semidry electroblotting (BioRad, Hercules, CA, USA). The membranes were hybridised with a digoxigenin (DIG) tailed (Boehringer-Mannheim Biochemica, Mannheim, Germany) internal TCRBC probe¹⁴ at 48°C, and washed twice for 15 minutes at a final stringency of 2×SSC/0.1%

SDS at 50°C. DIG chemiluminescence detection was performed according to the manufacturer's instructions (Boehringer-Mannheim Biochemica, Mannheim, Germany). The intensity of TCRBV PCR products on the resulting autoradiographs was measured by laser densitometry (Molecular Dynamics, Sunnyvale, CA, USA). This provided a semiquantitative means of determining the expression of TCRBV families. The resulting optical densities were standardised by expressing them as a percentage of the intensity of a TCRBV5 positive control PCR product, present on every membrane. A scale was created to assess the relative intensities as negative, weakly positive, or strongly positive whereby 0=no detectable signal, 1=1–100% of the control, 2=more than 100% of the control.

Results

DETECTION OF TCRBV MRNA IN CSF AND PERIPHERAL BLOOD

The expression of TCRBV gene families was detected in the CSF of all patients with an acute relapse of multiple sclerosis and in eight of nine patients with optic neuritis, compared with only five of 13 of the control group (table 1; figure). TCRBV families were detected in all paired peripheral blood samples (data not shown).

TCRBV EXPRESSION AND OLIGOCLONAL IgG

The presence of expressed TCRBV mRNA in cells found within the CSF was associated with the presence of oligoclonal IgG bands within the CSF (table 1). All optic neuritis, multiple sclerosis, and control patient CSF samples positive for oligoclonal bands were also positive for TCRBV mRNA, and more patients tested

positive for TCRBV genes than for oligoclonal IgG bands.

TCRBV REPERTOIRE IN CSF AND PERIPHERAL BLOOD

The expression of TCRBV genes in the CSF of patients with optic neuritis and those with multiple sclerosis varied considerably, both within and between patients. This heterogeneity was found in the number of TCRBV families expressed, the types of families present, and the intensity of the expression (table 2). In two patients with optic neuritis there was usage of a single TCRBV family (TCRBV12 and 17). Five to 15 TCRBV families were detected in six other patients with optic neuritis, with expression of TCRBV8 being most common (in six of nine patients), followed by TCRBV2, 7, 12, 14, 17, 18, and 19 (each occurred in five of nine patients). The family having the highest level of expression was TCRBV12. In two acute cases of multiple sclerosis there was CSF usage of a single TCRBV family (TCRBV8 and 18). Four to 17 TCRBV families were detected in the other four patients with multiple sclerosis, with expression of TCRBV8 and 18 being most common (each occurred in four of six patients). The family having the highest level of expression was TCRBV8. In comparison with both optic neuritis and multiple sclerosis, TCRBV expression was uncommon and relatively low in the control subjects, with the exception of one patient who had viral encephalitis (table 2).

A heterogeneous pattern of TCRBV usage was found in peripheral blood lymphocytes from optic neuritis, multiple sclerosis, and control patients, with no single gene family constituting more than 20% of the total

Analysis of TCRBV ($V\beta$) gene family usage in CSF lymphocytes from multiple sclerosis patient MS-2 by PCR, followed by oligonucleotide hybridisation and autoradiography. Band intensities were measured by densitometry and compared relative to the intensity of a TCRBV positive control PCR product (C).

Table 2 Detection of TCRBV (Vβ) gene families 1–20* in the CSF of control, multiple sclerosis (MS), and optic neuritis (ON) patients, and scored according to PCR band hybridisation intensity (0 = not detectable, 1 = weakly positive, 2 = strongly positive)

	Controls													MS													ON												
	1	2	3	4	5	6	7	8	9	10	11	12	13	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9								
Vβ 1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1								
2	1	0	0	0	0	0	0	2	0	0	0	0	0	0	0	2	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1								
3	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1								
4	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	1	0	0	1	0	0	0	1	0	0	0	0	0	0	0	1								
5	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1								
6	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1								
7	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1								
8	0	0	0	0	0	0	0	2	0	0	0	1	0	0	2	1	0	0	0	0	0	0	1	1	0	0	0	0	0	0	1								
9	0	0	0	0	0	0	0	2	1	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1								
10	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								
11	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								
12	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0								
13.1	0	—	0	0	0	0	0	0	0	0	—	0	0	0	—	0	0	0	0	0	0	0	0	0	—	0	0	0	0	0	0	1							
13.2	0	—	0	0	0	0	0	2	0	0	—	0	0	0	—	0	0	0	0	0	0	0	0	—	0	0	0	0	0	0	0	1							
14	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1							
15	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							
16	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							
17	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							
18	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							
19	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							
20	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0							
HLA-DR	7,17	9,16	n/a	13,17	7,13	n/a	n/a	17,17	15,15	9,16	n/a	1,10	11,17	13,15	15,15	4,14	4,13	11,15	11,14	15,15	7,11	1,13	n/a	n/a	1,11	15,17	15,17	17,17											

*TCRBV families 21–24 were also analysed in MS-6 (Vβ 22, 24 present), C-5 (Vβ 23, 24), C-13 (none present). All TCRBV 21–24 positive signals were scored as 1.

†TCRBV families 13.1–13.2 were not determined in some subjects, as PCR primers were not then available.

n/a = not available.

TCRBV repertoire, except in one patient with optic neuritis who had a TCRBV12 usage of >50% (data not shown).

CORRELATION OF TCRBV USAGE WITH MHC CLASS II

When CSF TCRBV usage was examined by MHC class II status it was found that TCRBV8 was present in five of six DR15 positive and in four of seven DR15 negative optic neuritis and multiple sclerosis patients (not significant). Correlation of the CSF TCRBV repertoire with other HLA-DR phenotypes showed no significant associations in any of the patient groups studied (data not shown).

MAGNETIC RESONANCE IMAGING DATA

Of the five patients with optic neuritis, three had multiple white lesions typical of multiple sclerosis and two had normal brain MRI. Two of three patients with optic neuritis with an abnormal MRI and one of two patients with optic neuritis with normal MRI at presentation developed definite multiple sclerosis within 24 months.

Discussion

The detection of TCRBV gene segments in the CSF of almost all patients with optic neuritis and patients with multiple sclerosis in this study confirms the presence of T cells within the CNS, to an extent not seen in the control subjects. The correlation between the presence of TCRBV gene expression and oligoclonal IgG bands within the CSF suggests a link between the presence of T cells and IgG production by CSF B cells. The present study has shown that the CSF TCRBV repertoire could be characterised directly by means of PCR without using T cell cloning or stimulation techniques to expand CSF cell numbers, which have been used in previous studies of CSF TCR usage.^{12 28–30} It has been reported that these may introduce the risk of an artefact bias in the TCR repertoire.³¹ The methods used are simple and rapid in detecting very low levels of gene expression and could be adapted as a clinical tool.

Our data suggest that TCR usage by CSF lymphocytes in patients with acute demyelinating optic neuritis is not highly restricted at the time of clinical presentation. Some TCRBV families, notably TCRBV8 and TCRBV18, were expressed more often in the optic neuritis and multiple sclerosis patients. However, although an increased usage of TCRBV8⁷ and TCRBV18^{28 32} has been found in the CNS in previous studies of multiple sclerosis, neither of these TCRBV gene families have been used in the TCR clinical trials.^{8–11 13} In addition, TCRBV8 and TCRBV12 had a high level of expression in the multiple sclerosis and optic neuritis patients, respectively. A TCRBV12 bias has been found previously in the CNS of patients with multiple sclerosis.^{33 34} However, despite these observations, overall comparison of CSF TCRBV usage between optic neuritis and multiple sclerosis patient groups showed no significant differences in restriction patterns; usage of a single receptor was demon-

strated in two patients with optic neuritis and in two patients with multiple sclerosis, and multiple receptors were detected in all other patients. Also, in this study, the multiple sclerosis associated MHC class II phenotype (DR15) was not associated with a greater usage of any of the TCRBV gene families in the optic neuritis and multiple sclerosis patients, and there was no bias towards usage of either TCRBV 5.2 or 6.1. Thus, the data do not show highly restricted TCRBV usage to be occurring in early optic neuritis or acute relapsing multiple sclerosis; nor was a single TCRBV family commonly expressed in all patients.

What are the possible explanations for this relative lack of restriction of the CSF T cell repertoire in early optic neuritis? The first possibility is that optic neuritis/multiple sclerosis is never a truly monoclonal disease, and instead the earliest stages of the disease process are characterised by the simultaneous infiltration and activation of several different T cell families in the CNS. Secondly, if optic neuritis and multiple sclerosis are indeed initially monoclonal diseases in which there is a highly specific immune response generated against a single antigenic epitope, then by the time symptoms have appeared the pathological process has already become established and the T cell repertoire has widened to involve other TCR genes and likely other antigenic determinants—"epitope spreading".³⁵ This concept of the TCR repertoire diversifying after an initial T cell response in the early disease phase has been previously postulated from findings in experimental allergic encephalomyelitis,²⁰ and has been raised for consideration in human studies.²¹ Epitope spreading—that is, autoantigenic epitopes becoming secondarily immunogenic after an initial T cell response—has been shown in a mouse model of demyelination induced by Theiler's virus,³⁵ and this may contribute to a lack of clonality in established autoimmune disease.

The approaches used in the present study do not establish the antigenic specificities of CSF T cell clones, but nevertheless certain inferences can be made. Even if optic neuritis/multiple sclerosis are initially monoclonal, much of the inflammation and neuronal loss which occur are associated with a polyclonal autoimmune process and, hence, immunomodulatory strategies directed at clonal T cell populations may be unlikely to succeed. Some evidence in support of this possibility comes from MRI studies which have demonstrated that patients presenting with clinically isolated optic neuritis or other acute demyelinating syndromes often have multiple silent lesions present in the brain^{23 36 37} and spinal cord.³⁸ Furthermore, there is a relation between the number of MRI lesions at presentation and the subsequent risk of progression to multiple sclerosis.^{37 39} This leads us to speculate, therefore, that very early widening of the immune response, perhaps occurring even before symptoms appear, may be associated with risk of progression to multiple sclerosis. A longitudinal study to examine the persistence of specific TCRBV gene segments in the CSF

of patients with optic neuritis would allow correlation with the risk of developing multiple sclerosis and subsequently with the clinical course and quantitative MRI abnormalities. Analysis of CSF TCRBV usage before clinical presentation is an important question not easily addressed, although one possible approach would be to study asymptomatic first degree relatives of patients with multiple sclerosis, particularly those with abnormal MRI.

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