T Cell Receptor V$_\alpha$-V$_\beta$ Repertoire and Cytokine Gene Expression in Active Multiple Sclerosis Lesions

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Summary

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system with presumed autoimmune etiology. A recent study has suggested the presence of a restricted T cell receptor (TCR) V$_\alpha$ repertoire in MS lesions. The presence of such a restricted TCR repertoire at the site of inflammation would have important consequences for the pathogenesis and the ultimate treatment of MS. To further characterize the TCR V$_\alpha$ and V$_\beta$ repertoire in MS plaque tissue, we examined a series of 26 histologically well-characterized central nervous system (CNS) tissue specimens from six MS patients as well as samples from five normal postmortem cases and a case of subacute sclerosing panencephalitis. RNA was extracted from frozen sections and cDNAs were amplified by polymerase chain reaction using primers for TCR V$_\alpha$ (V$_\alpha$1-18) and V$_\beta$ (V$_\beta$1-19) gene families. This analysis demonstrated a broad TCR V$_\alpha$-V$_\beta$ repertoire in active lesions, while fewer TCR V genes were detected in chronic plaques and control samples. Even though a large number of TCR V$_\alpha$ and V$_\beta$ gene segments were present in the majority of active lesions, there were clear differences in the TCR repertoire between plaques from the same case, suggesting that local events influence the TCR repertoire at the level of T cell recruitment or T cell expansion. Examination of cytokine mRNAs demonstrated that IL-1 mRNA was present in the majority of acute and subacute plaques, while IL-2 and IL-4 mRNA were detected in only a few acute lesions. These data demonstrate that the TCR repertoire in MS plaques is polyclonal. However, autoreactive α/β T cells thought to be critical in the initiation of the inflammatory process probably represent a minor fraction of T cells in active MS plaques and may use a limited number of TCR V gene segments for recognition of the autoantigen.

M ultiple sclerosis (MS)[1] is a chronic inflammatory disease of central nervous system (CNS) myelin. Early MS lesions are characterized by the focal accumulation of activated T cells around small venules (1-5). Later, there is myelin degeneration associated with marked perivenular inflammation consisting of T cells, B cells, and macrophages. T cells can also be found at the leading edge of plaques and in the surrounding normal-appearing white matter (1-5). Since myelin is important for saltatory excitation along axons, demyelination leads to loss of neurologic function (1).

Clonally expanded T cell populations using a restricted set of TCR gene segments may be important in the pathogenesis of MS and human autoimmune diseases. An analysis of the TCR V$_\alpha$-V$_\beta$ repertoire in a MS brain may allow such expanded T cell populations to be studied in the target organ of the disease. A recent study has suggested that a restricted TCR V$_\alpha$ repertoire is present in MS plaque tissue (6). To further examine the TCR repertoire in MS plaques, we asked the following questions. (a) Is there a restricted TCR V$_\beta$ repertoire in MS plaques? (b) Is the TCR repertoire similar between plaques from the same case and between different cases of MS? (c) Is there a correlation between the TCR repertoire in MS plaque tissue and the stage of the inflammatory process? (d) Which cytokine genes are expressed in MS plaques, and does cytokine gene expression correlate with the degree of inflammation and demyelination? The TCR V$_\alpha$-V$_\beta$ repertoire was examined in a series of histologically well-defined plaques from six postmortem MS brains, samples from five cases without neurological disease, and one case of subacute sclerosing panencephalitis (SSPE). These samples represent stages of plaque development ranging from early, acute lesions to chronic plaques, allowing the TCR repertoire to be correlated with the stage of the inflammatory process.

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[1] *Abbreviations used in this paper:* CNS, central nervous system; MS, multiple sclerosis; SSPE, subacute sclerosing panencephalitis.

Materials and Methods

Histology. Tissue blocks (~1 cm^3) were dissected from the CNS of six clinically and neuropathologically definite cases of MS, one case of subacute sclerosing panencephalitis, and five control cases without neurological disease (Table 1). The unfixed tissue blocks were placed in O. C. T. compound (Merck, Poole, UK) on cork disks, snap-frozen in isopentane cooled on liquid nitrogen, and stored at $-70°C$ in sealed containers (7). From each block, cryostat sections (10 μm) were cut onto glass slides. Then, 20 consecutive sections were placed in a sterile Eppendorf tube for RNA extraction. Sections cut before and after the sections taken for RNA extraction were stained with hematoxylin and eosin as well as oil red O using standard methods. The extent of positive oil red O staining was assessed under light microscopy by two independent observers.

Immunohistology. Acetone-fixed (4°C, 10 min) sections on gelatin-coated slides were incubated with mouse mAbs directed against CD2, CD4, CD8 (Dako, High Wycombe, UK), IL-2R (CD25) (Becton Dickinson & Co., Mountain View, CA), Vic-Y1 (the cytoplasmic chain of HLA-DR) (a gift from Dr. W. Knapp, University of Vienna), TCR-β (BF1) (gift from Dr. Mike Brenner, Harvard Medical School, Boston, MA), and the myelin lipid galactocerebroside (7) to demonstrate myelin loss in plaques. Sections were then immunoperoxidase stained (8) using a biotinylated anti–mouse IgG antibody and an avidin-biotin complex (Vector Laboratories, Inc., Burlingame, CA), with 0.05% (wt/vol) 3,3'-diaminobenzidine tetrahydrochloride (Sigma Chemical Co., St. Louis, MO), 0.013% (wt/vol) hydrogen peroxide, and 0.04% (wt/vol) nickel (II) chloride hexahydrate (9, 10). The noncounterstained sections were dehydrated and cleared in ethanol-xylene and mounted in D. P. X. mountant (BDH). Staining with these antibodies was graded by two independent observers (table 2). Tissue from one MS patient (case 481), who had had a rapidly progressive disease course over 2 yr, was examined by hematoxylin and eosin staining but not by immunohistology. This analysis demonstrated large numbers of actively demyelinating plaques in the cerebrum and cerebellum.

RNA Extraction and cDNA Synthesis. 20 consecutive frozen sections (10 μm) from each block were placed in a sterile Eppendorf tube for RNA extraction. RNA was extracted using the method of Chomczynski and Sacchi (11). To each tube, 1 ml of cold RNAzol (Cinna Biotex Friends, Wood, TX) was added and the tube vortexed. After a 5-min incubation on ice, 100 μl of chloroform was added, and the samples were centrifuged for 15 min at 4°C. The upper phase was then transferred to a clean tube containing an equal volume of isopropanol and 10 μg of tRNA as a carrier. After a 15-min incubation on ice, the sample was again centrifuged at 4°C for 15 min, the supernatant aspirated off, and the RNA pellet washed with 1 ml of cold 70% ethanol. The air-dried pellet was resuspended in 10 μl of autoclaved distilled water and stored at $-70°C$. cDNAs were synthesized using AMV-reverse transcriptase (Bethesda Research Laboratories, Rockville, MD), oligo-dT (Sigma Chemical Co.), and 5 μl of RNA sample in 20-μl reactions as described previously (12). cDNA reactions were diluted with autoclaved distilled water to 500 μl and stored at $-70°C$. 10 μl of diluted cDNA was used for each PCR reaction, thus allowing 50 amplifications to be performed from each sample.

PCR Amplification and Southern Blotting. Samples were amplified by PCR for 30 cycles (1 min at 94°C, 2 min at 55°C, 3 min at 72°C) when using TCR V$_\alpha$, TCR V$_\beta$, TCR C$_\beta$, and actin primers, while 35 cycles were used for IL-1, IL-2, and IL-4 amplifications. 1 μg of forward and 1 μg reverse primers (30 mers) were added to 50-μl reactions containing 5 μl of 10× taq buffer, dNTPs, and 2.5 U of taq polymerase (Cetus Corp., Emeryville, CA) according to the manufacturer's suggestions. Amplified products were

Table 1. *MS and Control Cases Examined*

Patients	Brain	Age	Sex	Duration of disease	Pathology	Death-freezing time
		yr				*h*
MS	285	35	M	8 mo	Acute plaques throughout spinal cord, rare lesions in brain	28
	481	40	M	2 yr	Acute lesions in brain (no autopsy on spinal cord)	4
	194	26	M	3 yr	Acute plaques throughout spinal cord and brain	18
	214	43	M	6 yr	Acute and chronic lesions in brain and spinal cord	34
	279	36	F	10 yr	Acute plaques in spinal cord, chronic periventricular lesions	33
	302	71	F	50 yr	Chronic plaques in brain and upper spinal cord	42
Control	284	50	F			10
	289	43	M			2
	290	67	M			18
	292	42	M			21
	293	20	M			33

resolved on 1% agarose gels, denatured, neutralized, and transferred to nitrocellulose membranes (Schleicher & Schuell, Inc., Keene, NH). Southern blots were hybridized with internal oligonucleotide probes labeled with [^{32}P]ATP and T4-polynucleotide kinase. Hybridizations were done at 37°C for 18 h in 6× SSC, 5×

Denhardt's, 0.05% pyrophosphate, 100 μg/ml of denatured herring sperm DNA, 0.5% SDS, and 10^6 cpm of labeled oligonucleotide/ml of hybridization buffer. Blots were washed at a final stringency of 6× SSC at 70°C and processed for autoradiography (12).

A number of precautions were applied to prevent accidental con-

Table 2. *Immunohistochemical and PCR Analysis of Plaque Tissue*

| Patients	Case	Location	Pathology	Oil red O stain	Immunohistology					PCR		
					CD2	CD4	CD8	IL2R	Ia	IL-1	IL-2	IL-4
MS	285 I-6	Spinal cord	Early, acute plaque	+ +	+ + + + +	+ + + +	+ + +	+ + +	+ + + +	+ + +	–	–
	I-7	Periventricular	White matter	–	+ + +	+ +	+ +	–	+ + +	ND		
	I-8	Corpus callosum	Early, acute plaque	+	ND	+	+ +	ND	+ +	+ + +	+ +	–
	I-9	Periventricular	White matter	–	+̃ + +	–	+ + +	+ +	+ + +	+	–	–
	194 I-16	Spinal cord	Acute plaque	+ + + + +	+ + + +	+ + +	+ + + +	+	+ + + + +	+ + +	+ + +	–
	I-17	Periventricular	Acute plaque	+ + + + +	+ + + +	+ + +	+ + +	–	+ + + + +	+ +	+ +	+
	I-18	Pons	Acute plaque	+ + + +	+ + +	+ +	+ + +	+	+ + + +	+	–	–
	I-19	Medulla	Acute plaque	+ + + +	+ + + +	+ + +	+ + +	+	+ + + +	+ + +	–	–
	I-20	Meninges		+	+ + +	+ + +	+ + +	+	+ + + +	+	–	–
	279 II-2	Spinal cord	Subacute plaque	+ + +	+ +	+ +	+ +	–	+ +	+ + +	+	+ + +
	II-3	Spinal cord	Subacute plaque	+ + +	+ +	+ +	+ +	+ / –	+ +	+ + +	–	–
	II-5	Periventricular	Mixed plaques	+	+ + +	+ +	+ + +	–	+ +	+ + +	–	–
	II-6	Corpus callosum	Chronic plaque	–	+ + +	+ +	+ + +	–	+ +	+	–	–
	302 II-8	Periventricular	Chronic plaque	–	+ +	+	+ +	+	+ +	–	–	–
	II-9	Periventricular	Chronic plaque	–	+ + +	+ +	+ + +	+	+ +	–	–	–
	II-11	Periventricular	Chronic plaque	–	+ + +	+	+ + +	+	+ +	–	–	–
	II-12	Periventricular	Chronic plaque	–	+ + +	+ +	+ + +	–	+ +	–	–	–
	214 II-14	Spinal cord	Chronic plaque	–	+	+	+	–	+ + +	–	–	–
	II-15	Cerebellum	Mixed plaques	+	+ + +	+	+ + +	–	+ + +	–	–	–
	II-17	Periventricular	White matter	–	+ + +	+	+ +	–	+ +	–	+	–
	II-18	Periventricular	White matter	–	+ +	+	+ +	–	+ +	–	–	–
Control	292 I-1	Spinal cord		–	+	+	+ +	–	+	ND		
	I-2	Periventricular		–	+ +	+	+ +	–	+ +	+	–	–
	I-3	Corpus callosum		–	+ +	+	+ +	–	+	ND		
	I-4	Periventricular		–	+	+	+	–	+	+	–	–
	293 I-11	Spinal cord		–	+	+	+	–	+ +	–	–	–
	I-12	Periventricular		–	+ +	+	+	–	+	ND		
	I-13	Pons		–	+	+	+	–	+	ND		
	I-14	Subcortical		–	+ +	+	+ +	–	+	ND		
	I-15	Corpus callosum		–	+	+	+	–	+	ND		
	289 II-1	Spinal cord		–	–	–	–	–	+	+	+	–
	II-4	Periventricular		–	+	–	+	–	+	–	–	–
	290 II-7	Periventricular		–	+	–	+	–	+	–	–	–
	II-10	Periventricular		–	+	+	+ +	–	+	+	–	–
	284 II-13	Periventricular		–	+	–	+	–	+	+	–	–
	II-16	Periventricular		–	+	–	+	–	+	+ +	+	–
SSPE												
	II-20	White matter		+ +	+ + + +	+ + +	+ +	+ +	+ + + +	ND		
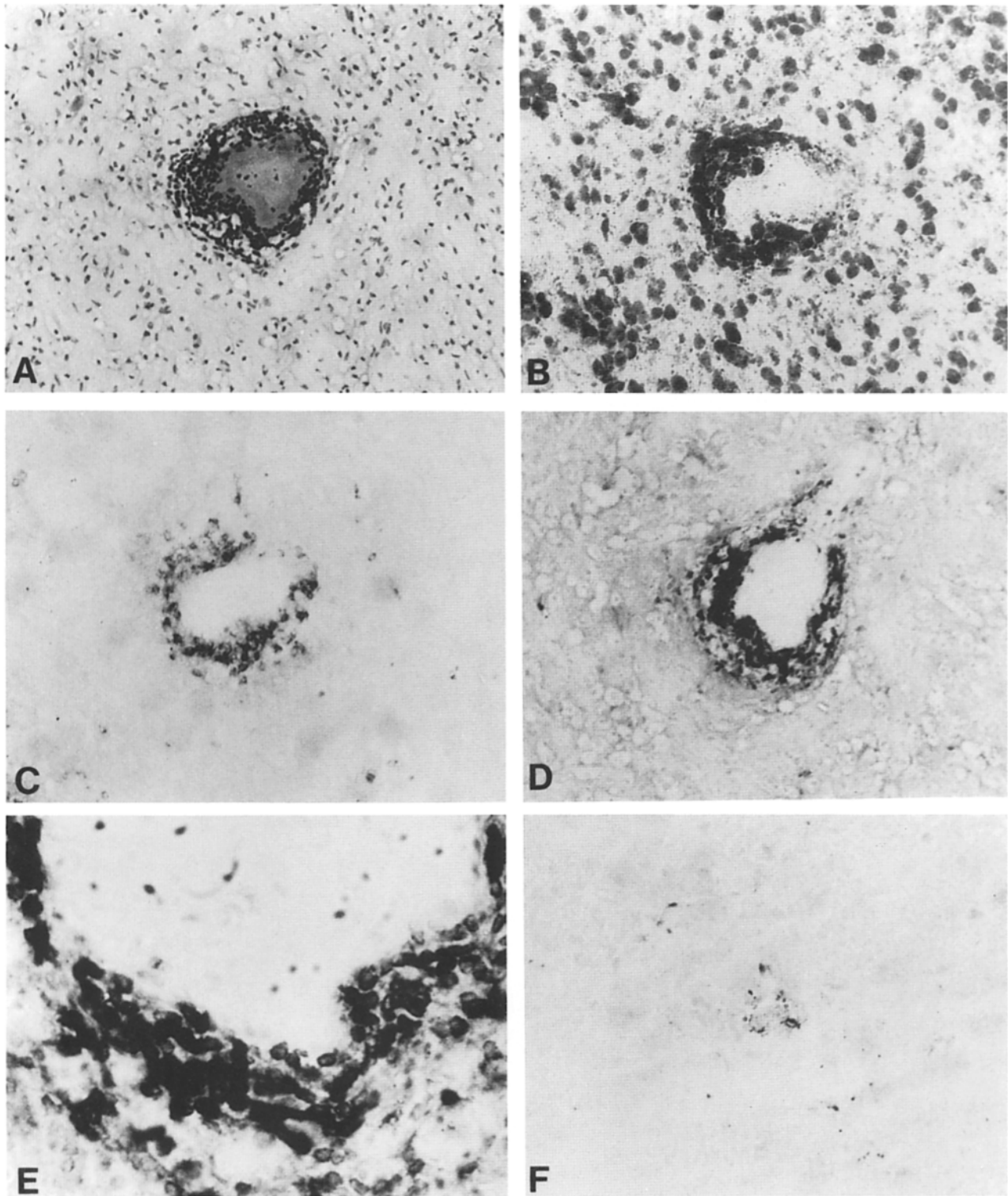
Figure 1. (*A–E*) Cryostat sections of a perivenular inflammatory cuff and the surrounding tissue in a pons demyelinating plaque (sample I-18). (*A*). Hematoxylin and eosin staining; (*B*) oil red O staining of degenerating myelin in microglia and macrophages. (*C–E*) immunoperoxidase staining of the same cuff; (*C*) anti-CD2 antibody; (*D*) anti-TCR β antibody (all ×165). (*E*) Anti-TCR β antibody (×660); (*F*) anti-TCR β immunostaining of a pons blood vessel in a normal control case (sample I-13) (×165). No hematoxylin counterstaining of nuclei in *B–F*.

tamination of reagents and samples used for PCR amplification (12). Working areas were separated for pre- and post-PCR experiments, and a set of positive displacement pipetteman was specifically designated for RNA extractions and preparation of PCR reactions. All reagents were aliquoted and systematically tested for possible contamination. Negative controls (no cDNAs) were included in experiments and MS samples run concurrently with control samples.

Results

Examination of TCR Repertoire from MS Plaques Using RNA Extracted from Frozen Sections. The TCR V_α-V_β repertoire was examined in acute, subacute, and chronic MS lesions, and control tissue that was characterized histologically for the degree of T cell infiltration and demyelination (Tables 1 and 2). Since unfixed, frozen tissue was only available in small quantities, RNA was extracted from thin frozen sections. cDNAs synthesized from these RNA samples were amplified by PCR using primers for TCR C_β and actin. Actin could be amplified from all brain samples (except I-7 and II-8) and gave an appropriately sized band on agarose gels that hybridized to an internal actin oligonucleotide probe on Southern blots. As expected, amplification of TCR C_β demonstrated that more TCR C_β mRNA was present in acute MS plaques than in chronic lesions and control samples, while the amount of actin mRNA amplified was similar (data not shown). Both actin and TCR C_β priming sites spanned an intron(s), allowing products amplified from cDNA and genomic DNA to be distinguished by size.

Histological and Immunocytochemical Analysis of MS Plaque Tissue. CNS tissue was examined histologically using oil red O, a marker for degenerating myelin, as well as hematoxylin-eosin. Plaques with perivenular inflammation, hyper-

cellularity, and oil red O–positive degenerating myelin were considered to be acute, while hypocellular galactocerebroside-negative demyelinated plaques were classified as chronic. Acute plaques were further characterized as early, acute, or subacute on the basis of oil red O and galactocerebroside staining. Both acute and chronic lesions were present in some sections, which were classified as "mixed plaques" (Table 2).

To characterize the T cell infiltrate in MS plaques and white matter samples, sections were stained with mAbs for CD2, CD4, CD8, IL-2R, and HLA-DR (Table 2). CD2$^+$ T cells were found in largest numbers around blood vessels in early acute and acute lesions, but were also present in subacute and chronic lesions (Fig. 1). Small numbers of T cells were found in control CNS samples. In general, more CD8$^+$ than CD4$^+$ T cells were present in acute and chronic plaques. Two of four samples from a patient with early disease (case 285) were found to have the greatest numbers of IL-2R-positive, activated T cells (Table 2).

Analysis of Interleukin mRNA for IL-1, IL-2, and IL-4 from Acute and Chronic Plaques. mRNAs for IL-1, IL-2, and IL-4 were examined in acute, subacute, and chronic lesions as well as white matter by PCR amplification and Southern blotting (Fig. 2). RNA extracted from a PMA ionomycin–activated T cell clone was used as a positive control for all three primer pairs. Amplification of cDNA from this activated clone resulted in a strong band for IL-1, IL-2, and IL-4, while negative controls (no cDNA) gave no signals (Fig. 2). IL-2 and IL-4 mRNA were detected in some acute and subacute lesions. In contrast, IL-1 mRNA was found in the majority of early acute, acute, and subacute plaques, but not in chronic lesions (Fig. 2). Lower levels of IL-1 mRNA were observed in some of the control samples.
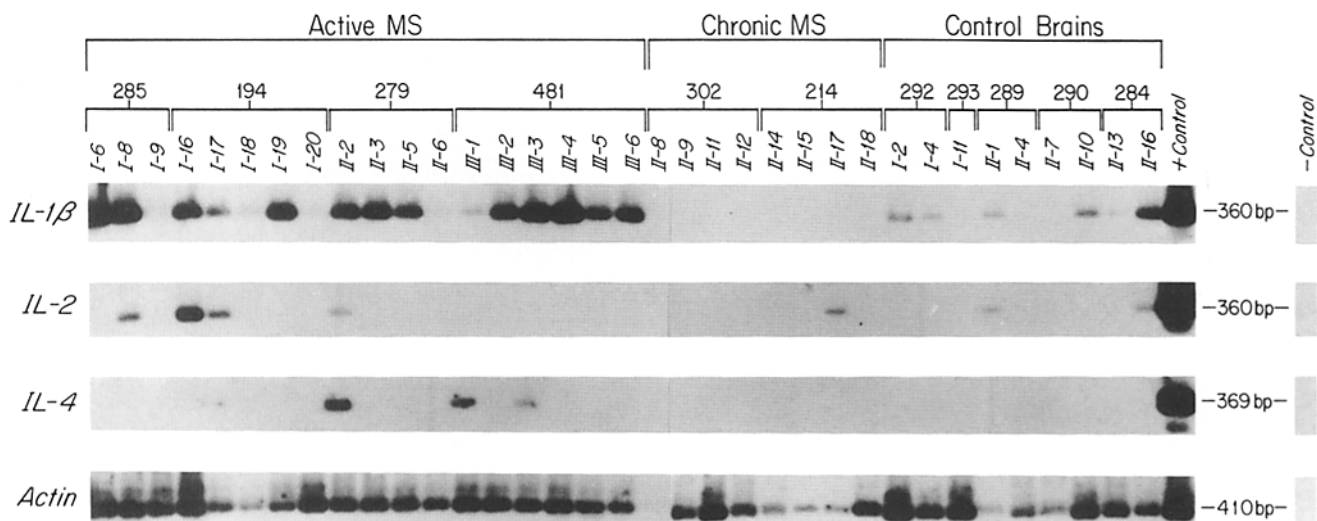

Figure 2. Examination of IL-1, IL-2, and IL-4 mRNAs in MS plaque tissue. Interleukin cDNAs were amplified by PCR for 35 cycles and Southern blots hybridized with internal oligonucleotide probes. IL-1 could be amplified from the majority of early acute, acute, and subacute plaques, while IL-2 and IL-4 were amplified from a few acute and subacute lesions. IL-1 mRNA was also found at lower levels in some of the control brains and may be derived from microglia or T cells. Controls (no cDNA) were negative after amplification with interleukin primers. IL-1, IL-2, and IL-4 could be efficiently amplified using cDNA from a PMA/ionomycin–activated T cell clone (positive control). The integrity of brain cDNAs was demonstrated using actin primers for amplification.

Table 3. *Sequences of TCR, Interleukin, and Actin Primers Used for PCR Amplification*

TCR V_α primers

$V_\alpha1$	5' CTG CTG CTC GTC CCA GTG CTC GAG GTG ATT 3'
$V_\alpha2$	5' ACA GTG ACC GAG TTT CCC AGT CCT TCT TCT 3'
$V_\alpha3$	5' GGA GTG TCT TTG GTG ATT CTA TGG CTT CAA 3'
$V_\alpha4$	5' CTC CAC AAT CAG TGG AAC TGA TTA CAT ACA 3'
$V_\alpha5$	5' AAC TAT ACA AAC TAT TCT CCA GCA TAC TT 3'
$V_\alpha6$	5' CTG AAG GTG GTC ACA GCT TCA CTG TGG CTA 3'
$V_\alpha7$	5' TGG GGA GTT TTC CTT CTT TAT GTT TCC ATG 3'
$V_\alpha8$	5' CGA GCT TTA TTT ATG TAC TTG TGG CTG CAG 3'
$V_\alpha9$	5' CCC ACC CTC ATC TCA GTG CTT GTG ATA ATA 3'
$V_\alpha10$	5' ACC CAG CTG CTG GAG CAG AGC CCT CAG TTT 3'
$V_\alpha11$	5' GCT TTG CAG AGC ACT CTG GGG GCG GTG TGG 3'
$V_\alpha12$	5' TTG AGG GCA GTC ATA GCC TCC ATC TGT GTT 3'
$V_\alpha13$	5' GCT CTG CTG GGG CTC TTG AGT GCC CAG GTT 3'
$V_\alpha14$	5' GCA TGC CCT GGC TTC CTG TGG GCA CTT GTG 3'
$V_\alpha15$	5' TGT TTC CTT GTT TAT GCT GAG CTC AGG GAA 3'
$V_\alpha16$	5' ATC TCG ATG CTT GCG ATG CTC TTC ACA TTG 3'
$V_\alpha17$	5' ATA TCT ATA AGT TCC ATT AAG GAT AAA AAT 3'
$V_\alpha18$	5' TGT CAG GCA ATG ACA AGG GAA GCA ACA AAG 3'
C_α primer	5' TTG TTG CTC CAG GCC ACA GCA CTG TTG CTC 3'
C_α probe	5' CTT GTC ACT GGA TTT AGA GTC TCT CAG CTG 3'

TCR C_β primers

TCR C_β	Forward	5' CCC GAG GTC GCT GTG TTT GAG CCA TCA GAA 3'
	Reverse	5' GGC AGA CAG GAC CCC TTG CTG GTA GGA CAC 3'
	Probe	5' TGT GCA CCT CCT TCC CAT TCA CCC ACC AGC 3'

Interleukin primers

IL-1β	Forward	5' GAC AAG CTG AGG AAG ATG CTG GTT CCC TGC 3'
	Reverse	5' TCT TTC AAC ACG CAG GAC AGG TAC AGA TTC 3'
	Probe	5' GAT ATG GAG CAA CAA GTG GTG TTC TCC ATG 3'
IL-2	Forward	5' TAC AGG ATG CAA CTC CTG TCT TGC ATT GCA 3'
	Reverse	5' GTT GCT GTC TCA TCA GCA TAT TCA CAC ATG 3'
	Probe	5' CAG TGT CTA GAA GAA GAA GAA CTC AAA CCT CTG 3'
IL-4	Forward	5' CTG CTA GCA TGT GCC GGC AAC TTT GTC CAC 3'
	Reverse	5' GAA GTT TTC CAA CGT ACT CTG GTT GGC TTC 3'
	Probe	5' GAG CAG AAG ACT CTG TGC ACC GAG TTG ACC 3'

Actin primers

Actin	Forward	5' AAC CCC AAG GCC AAC CGC GAG AAG ATG ACC 3'
	Reverse	5' GGT GAT GAC CTG GCC GTC AGG CAG CTC GTA 3'
	Probe	5' GCC CTG GAC TTC GAG CAA GAG ATG GCC ACG 3'

TCR V_α-V_β Repertoire in MS Plaque Tissue Is Polyclonal.
We then examined the TCR V_α-V_β repertoire in MS inflammatory lesions by PCR using a panel of TCR V_β primers (12). We also assembled a new panel of TCR V_α primers ($V_\alpha 1$-$V_\alpha 18$) (Table 3). All of these primers efficiently amplified $V_\alpha 1$-$V_\alpha 18$ gene segments using cDNA synthesized from PBL. TCR V_α primers were tested on a set of 11 T cell clones established from peripheral blood of a normal subject by single-cell cloning using PHA and IL-2. All clones studied were found to have a single TCR V_β rearrangement and one or two TCR V_α rearrangements (data not shown).

cDNAs from MS plaque and control samples were amplified by PCR for 30 cycles using $V_\alpha 1$-$V_\alpha 18$ primers, as well as $V_\beta 1$-$V_\beta 19$ primers in combination with a C_α or a C_β primer, respectively (Fig. 3, *a* and *b*). Actin amplification was used as a positive control to demonstrate that samples contained similar amounts of cDNA. Southern blots were hybridized with internal oligonucleotide probes for C_α, C_β, and actin in order to demonstrate the specificity of amplification. The majority of samples contained functional cDNA (except I-7 and II-8). Despite variable postmortem times, most samples gave similar signals after amplification with actin primers. Actin amplification from brain samples 214 and 289 (chronic MS and control brain, respectively) gave lower signals than amplification from other samples (Fig. 2).

Using this approach, a large number of different V_α and V_β segments were detected in acute MS plaque samples. The following V_α and V_β gene segments were present in most acute and subacute specimens: $V_\alpha 2$, $V_\alpha 3$, $V_\alpha 8$, $V_\alpha 12$, $V_\alpha 14$, and $V_\alpha 17$; $V_\beta 1$, $V_\beta 2$, $V_\beta 4$, $V_\beta 5$, $V_\beta 7$, $V_\beta 8$, $V_\beta 12$, $V_\beta 14$, $V_\beta 16$, and $V_\beta 17$. A polyclonal TCR V_β gene repertoire was also found in the white matter of a case of SSPE (Fig. 3 *a*). Large numbers of CD2- and IL-2-positive T cells were detected in this white matter sample; there was also evidence of ongoing demyelination as oil red O staining revealed phagocytosed myelin in macrophages (Table 2). In chronic MS and control samples, fewer TCR V-gene segments were detected, probably due to the small number of T cells and the minute quantities of TCR mRNA present (Fig. 3, *a* and *b*).

TCR V_α-V_β Repertoire Shows Differences between Lesions from the Same Case. For each MS case examined, the TCR repertoire was analyzed for three to five plaques to determine if the same repertoire would be present among different lesions. Although most of the TCR V_α and V_β gene segments amplified were similar, clear differences between plaques obtained from the same MS case were observed (Fig. 4).

Discussion

The TCR V_α-V_β repertoire and IL-1, IL-2, and IL-4 mRNA were examined in CNS tissue from cases of MS and compared with plaque pathology. A polyclonal TCR V_α-V_β repertoire was present in acute lesions while fewer V gene segments were detected in chronic and control samples. Among different plaques from the same case, most of the TCR V_α and V_β gene segments amplified were similar, although clear differences between plaques were observed. Examination of cytokine mRNAs demonstrated that IL-1 mRNA

was present in the majority of acute and subacute plaques, while IL-2 and IL-4 mRNA were detected in only a few acute lesions. These data indicate that the TCR repertoire in MS plaques is polyclonal.

Since MS may be a T cell–mediated autoimmune disease, the analysis of the T cell repertoire involved in the inflammatory process is of importance. In particular, characterization of the TCR repertoire in the target organ could be a direct way to examine T cells important in the pathogenesis of MS. Such an analysis is unfortunately complicated by the fact that large numbers of antigen-nonspecific T cells are recruited to inflammatory lesions. For example, in experimental autoimmune encephalomyelitis (EAE) induced by injection of myelin basic protein (MBP), the frequency of MBP-specific T cells in the blood and in the brain is very low (13). However, a recent study had suggested that a restricted TCR V_α repertoire is present in MS plaques, although no pathological information was provided (6). This is a common difficulty because CNS specimens from MS patients that are adequately frozen for histological analysis can be only obtained in small quantities. For those reasons, we performed a study on the TCR V_α-V_β repertoire using samples that were histologically well characterized and extracted RNA from thin frozen sections. Our analysis demonstrates that a polyclonal TCR V_α-V_β repertoire is present in active lesions. This is not surprising since the breakdown of the blood-brain barrier in acute lesions is likely to lead to recruitment of large numbers of T cells, of which the minority is specific for the target antigen initiating the disease, as observed in EAE (13). Fewer TCR V gene segments were amplified from chronic lesions and control samples, probably due to the minute quantities of TCR mRNA present. Since antigen-specific T cells probably represent a small minority in MS plaques, we recently examined TCR V_β gene usage among peripheral blood T cells specific for an immunodominant region of MBP (residues 84–102). This analysis demonstrated that there was a preferential TCR usage in recognition of immunodominant MBP peptides (12). For these reasons, an analysis of the TCR repertoire of antigen-specific T cells cultured directly from acute MS plaques would be of great interest.

A comparison of the TCR V_α-V_β repertoire from different plaques of the same MS case as well as different cases demonstrated that a number of V_α and V_β gene segments are present in most of the active lesions. These V_α and V_β gene segments include $V_\alpha 2$, $V_\alpha 3$, $V_\alpha 8$, $V_\alpha 12$, $V_\alpha 14$, and $V_\alpha 17$; $V_\beta 1$, $V_\beta 2$, $V_\beta 4$, $V_\beta 5$, $V_\beta 7$, $V_\beta 8$, $V_\beta 12$, $V_\beta 14$, $V_\beta 16$, and $V_\beta 17$. Nevertheless, there were clear differences in the TCR repertoire between plaques from the same case, similar to what has been shown previously (6). This suggests that local events in MS plaques influence the TCR repertoire at the level of T cell recruitments or T cell expansion.

In summary, our data demonstrate that the TCR repertoire in MS inflammatory lesions is polyclonal. As antigen-specific T cells that may be critical for the initiation of an inflammatory lesion are probably in the minority, autoreactive T cells involved in the disease process may nevertheless have a limited TCR V gene repertoire.
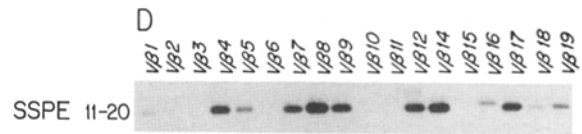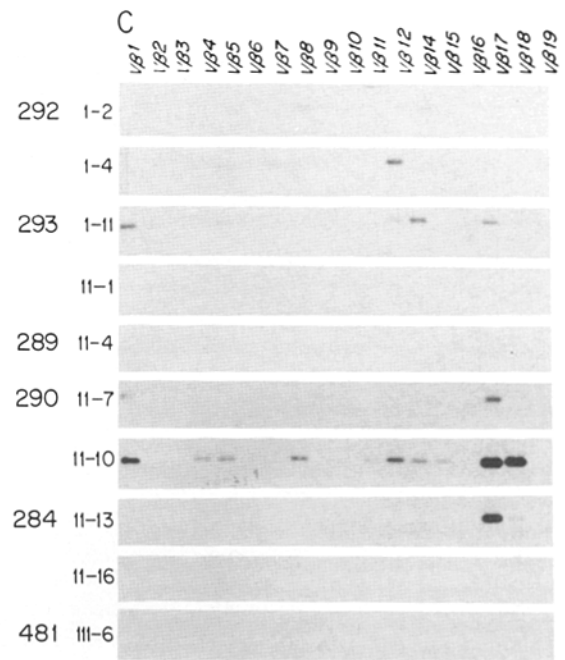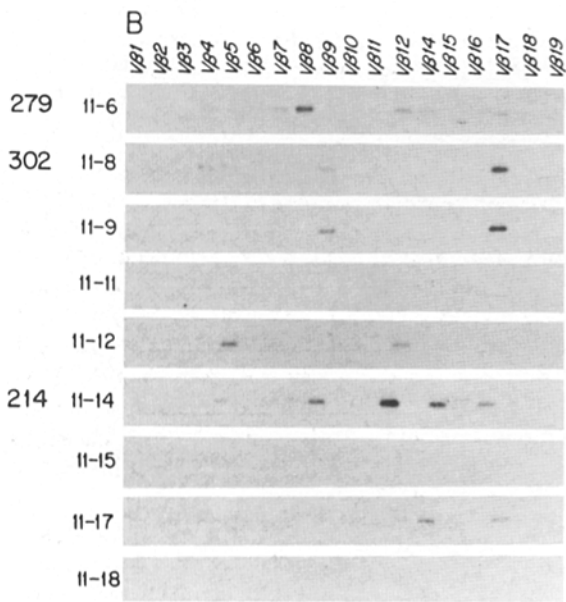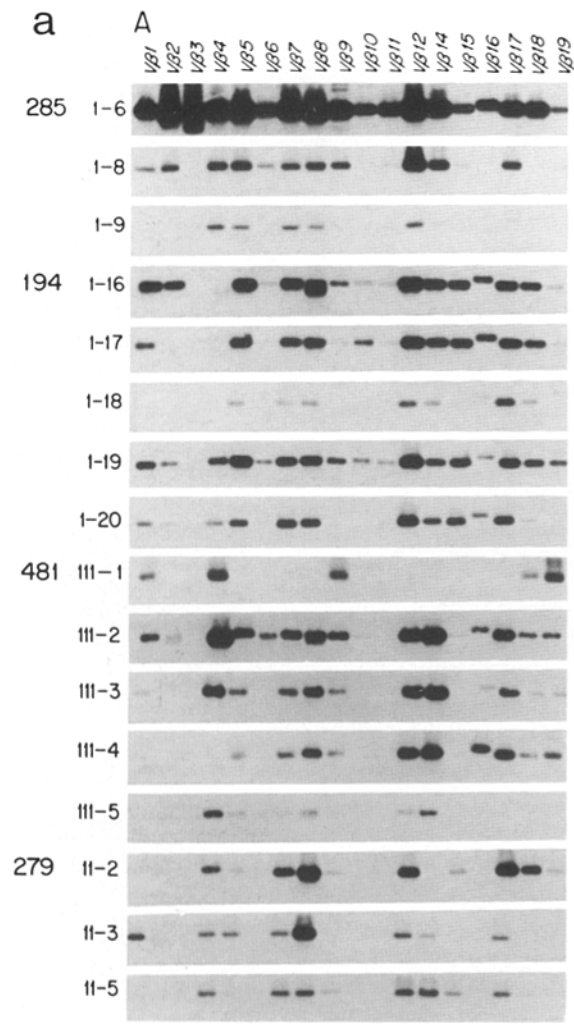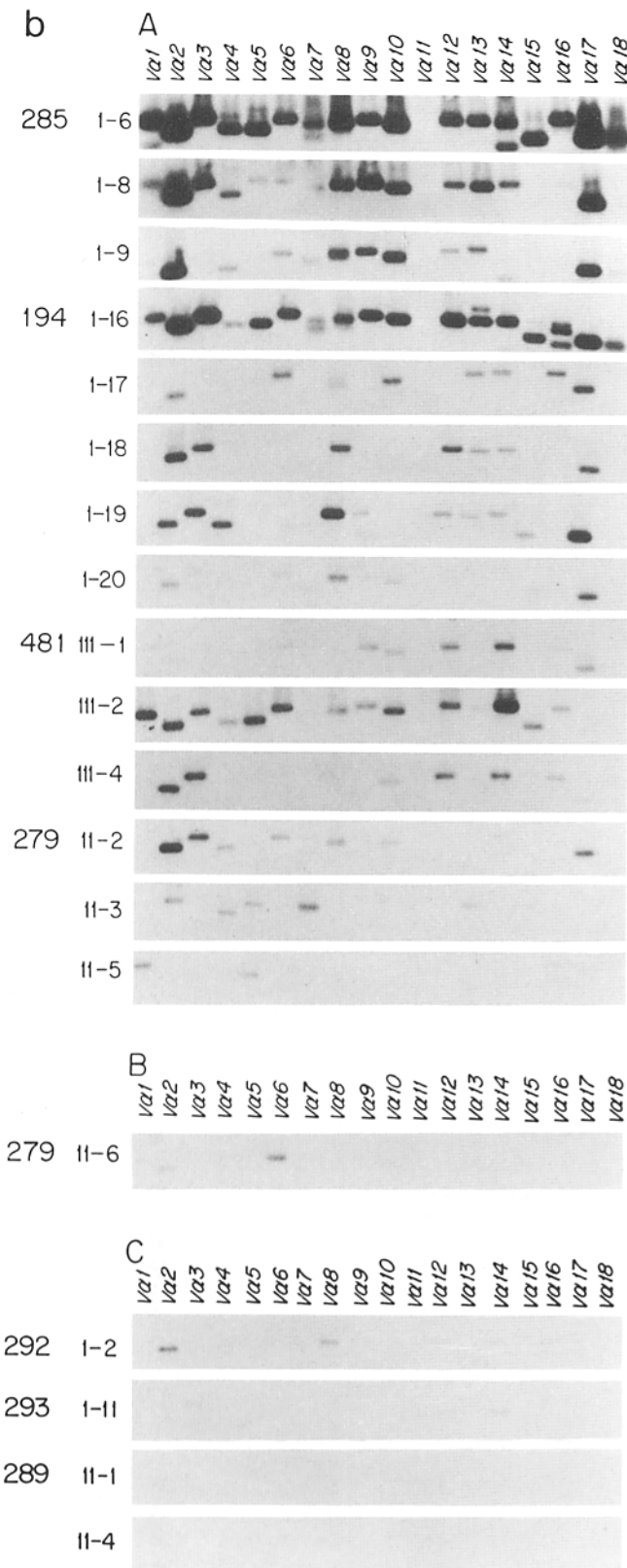
Figure 3. PCR analysis of TCR V_α and V_β repertoire in MS plaque tissue. cDNA synthesized from MS plaque RNA was amplified using TCR V_β (V_β1-19) (*a*) and TCR V_α (V_α1-18) primers (*b*). Southern blots are shown for active MS plaques (*A*) and chronic MS lesions (*B*), control CNS samples (*C*), and white matter of a case with SSPE (*D*). Amplification of actin cDNA was used as a positive control. This analysis demonstrates that a polyclonal TCR repertoire is present in acute lesions. Control experiments (no cDNA) gave no amplification of TCR gene segments.
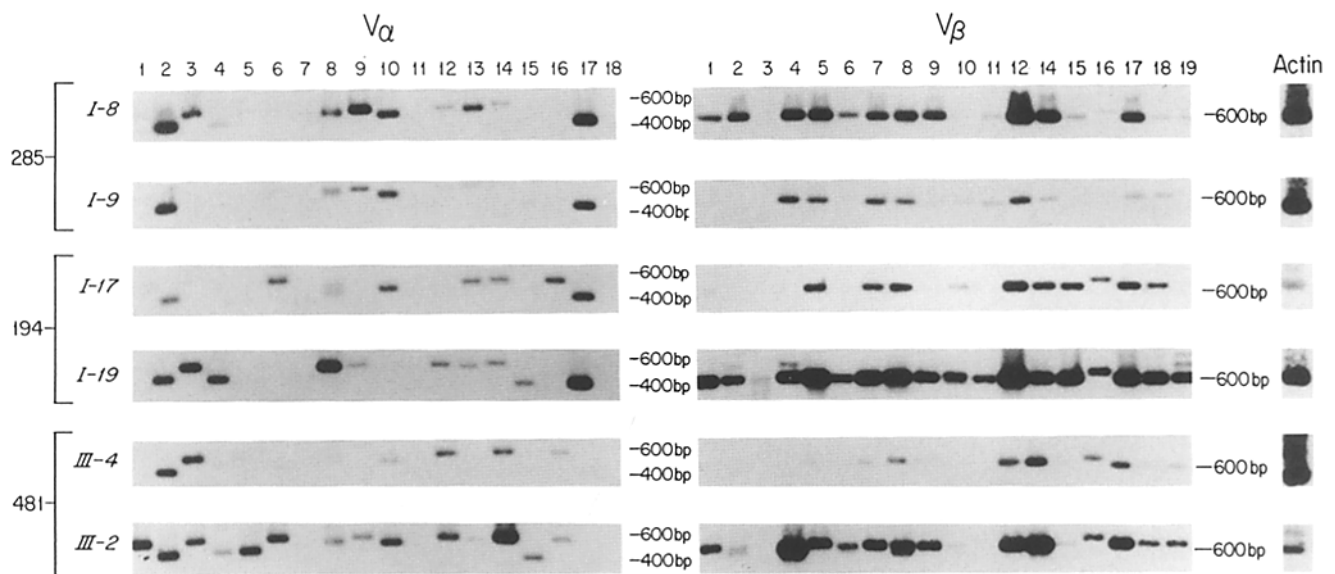
Figure 4. Plaque-to-plaque diversity of the TCR V_α and V_β repertoire. A PCR analysis of TCR V_α (V_α1-18), TCR V_β (V_β1-19) gene segments, and of actin cDNA is shown for CNS samples from three MS cases to allow comparison of the TCR repertoire between plaques from each case. Clear differences in the TCR V_α and V_β repertoire is seen between plaques, indicating that local events in the CNS influence the TCR repertoire.

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