

## $\gamma\delta$ T-cell receptor repertoire in acute multiple sclerosis lesions

KAI W. WUCHERPFENNIG, JIA NEWCOMBE\*, HONG LI\*, CHRISTINE KEDDY, M. LOUISE CUZNER\*,  
AND DAVID A. HAFLER

\*Center for Neurologic Diseases, Division of Neurology, Department of Medicine, Brigham & Women's Hospital and Harvard Medical School, Boston, MA 02115; and †The Multiple Sclerosis Society Laboratory, Institute of Neurology, London, WCIN 1PJ, U.K.

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**ABSTRACT**  $\gamma\delta$  T cells are a distinct lymphocyte population that can exhibit reactivity with heat shock proteins overexpressed at inflammatory sites. As  $\gamma\delta$  T cells may be involved in the central nervous system (CNS) inflammatory process in multiple sclerosis (MS), we examined T-cell populations in MS plaque tissue by quantitative immunohistochemistry and sequence analysis of T-cell antigen receptor (TCR)  $\delta$  chains.  $\gamma\delta$  T cells that express the variable (V) gene segments  $V_{\delta 1}$ ,  $V_{\delta 2}$ , and  $V_{\delta 2}$  ( $V_{\gamma 9}$ ) were found to accumulate in acute, demyelinating MS plaques and appeared to have undergone clonal expansion, most likely because of recognition of a specific CNS ligand. Further, 60-kDa and 90-kDa heat shock proteins (hsp60 and hsp90), which may be target antigens for autoreactive  $\gamma\delta$  T cells, were found to be expressed in normal CNS tissue and overexpressed in acute MS plaques. In acute plaques, hsp60 was found in foamy macrophages, while hsp90 was detected in reactive astrocytes. These results provide evidence for a role of  $\gamma\delta$  T cells in active stages of MS.

$\gamma\delta$  T cells are characterized by the expression of rearranged T-cell antigen receptor (TCR)  $\gamma$  and  $\delta$  chains and are thought to participate in the immune surveillance of epithelial tissues (1, 2). They are also involved in inflammatory responses against mycobacteria, which are potent activators of  $\gamma\delta$  T cells expressing the human  $V_{\delta 2}$  ( $V_{\gamma 9}$ ) gene segment. A fraction of mycobacteria-reactive  $\gamma\delta$  T cells is specific for the 65-kDa heat shock protein (hsp65), which is highly conserved between bacteria and eucaryotes (3–6). As heat shock proteins are overexpressed by stressed cells,  $\gamma\delta$  T cells that crossreact with a bacterial and a self heat shock protein may accumulate at an inflammatory site and lyse stressed cells (7). Based on this model, heat shock protein-reactive  $\gamma\delta$  T cells have been postulated to participate in autoimmune processes (reviewed in ref. 6).

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system (CNS) myelin characterized by focal T-cell and macrophage infiltrates. Early pathological lesions in MS consist of lymphocyte cuffing around small venules in the CNS white matter (8). The inflammatory process leads to a loss of myelin (demyelination) and a loss of neurological function. A recent report also has demonstrated the presence of  $\gamma\delta$  T cells in chronic active MS plaques by immunohistochemical techniques.  $\gamma\delta$  T cells and oligodendrocytes expressing hsp65 were found to be colocalized in the center and at the edge of plaques (9). To further examine the potential role of  $\gamma\delta$  T cells in the pathogenesis of MS, we have examined the  $\gamma\delta$  TCR repertoire in MS plaque tissue. A limited  $\gamma\delta$  TCR repertoire in MS plaques would suggest antigen-driven clonal expansion and implicate  $\gamma\delta$  T cells in the pathogenesis of MS.

## MATERIALS AND METHODS

**Immunohistochemistry.** Tissue blocks ( $\approx 1$  cm<sup>3</sup>) were dissected from postmortem CNS specimens and snap frozen in OCT compound (Gurr). Acetone-fixed sections were incubated with monoclonal antibodies (mAbs) directed against TCR  $\beta$  chain (BF1), TCR  $\gamma$  chain ( $\alpha$ -C $\gamma$ M1) (gifts from M. Brenner), CD2 (DAKO, Carpinteria, CA), and the interleukin 2 receptor (CD25, Becton Dickinson). Selected sections were immunostained with mAbs against human 60-kDa heat shock protein (hsp60; 4B9/89, a gift from M. Sharif) and fungal 90-kDa heat shock protein (hsp90; AC88, Stressgen Biotechnologies, Sidney BC V8L3S1, Canada). Sections were then immunoperoxidase-stained by using a biotinylated anti-mouse IgG antibody and an avidin–biotin–peroxidase complex (Vector Laboratories) (10).

**RNA Extraction, cDNA Synthesis, PCR Amplification, and Sequence Analysis.** RNA was extracted from 20 consecutive frozen sections (10  $\mu$ m) cut from each tissue block, and adjacent sections were stained with hematoxylin/eosin. cDNA synthesis and PCR amplification were performed as described (10). For sequence analysis of the TCR  $\delta$  chain, amplified products were digested with *Sal* I and *Eco*RI restriction endonucleases, cloned into phage M13 mp19, and sequenced by the dideoxy chain-termination method.

## RESULTS

**Accumulation of  $\gamma\delta$  T Cells in Acute MS Plaques.**  $\alpha\beta$  and  $\gamma\delta$  T-cell populations were examined in frozen tissue specimens from five postmortem cases with MS, one case with subacute sclerosing panencephalitis (SSPE), and five cases without neurological disease. Frozen sections were stained with hematoxylin/eosin, oil red-O (ORO), and mAbs specific for CD2 and the interleukin 2 receptor. Both the clinical data and a detailed immunohistological analysis of these cases are published in another article with the same designations for CNS samples as in this report (10). Plaques with periventricular inflammation (Fig. 1), hypercellularity, and foamy macrophages containing ORO-positive degenerating myelin throughout the lesion were considered to be actively demyelinating (cases 285 and 194), while hypocellular ORO- and galactocerebroside-negative demyelinated plaques were classified as chronic lesions. Subacute plaques had ORO-positive cells only in the borders of demyelinated lesions (Table 1).

Preliminary experiments showed that acute lesions contain relatively large amounts of TCR  $\beta$ - and  $\delta$ -chain constant region ( $C_{\beta}$  and  $C_{\delta}$ ) mRNA even though  $\gamma\delta$  T cells are a minor population among blood T cells.  $\gamma\delta$  and  $\alpha\beta$  T-cell populations in MS plaque tissue were quantitated on sections stained with mAbs specific for TCR  $\beta$  and TCR  $\gamma$  chains (Table 1).  $\gamma\delta$  T cells were found to accumulate in acute MS plaques (5.8% to

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Abbreviations: V, variable; hsp60, hsp65, and hsp90, 60-, 65-, and 90-kDa heat shock proteins; mAb, monoclonal antibody; MS, multiple sclerosis; CNS, central nervous system; TCR, T-cell antigen receptor; SSPE, subacute sclerosing panencephalitis.

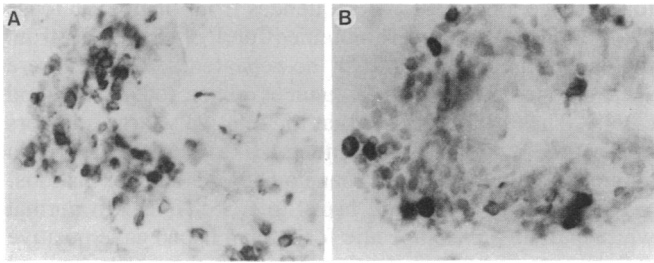


FIG. 1. Immunohistochemical analysis of  $\alpha\beta$  and  $\gamma\delta$  T-cell populations in a periventricular inflammatory cuff of an acute, demyelinating MS plaque (temporal lobe, periventricular white matter of case 194, sample I-17). Immunoperoxidase staining was with anti-TCR  $\beta$ -chain antibody (BF1) (A) and anti-TCR  $\gamma$ -chain antibody ( $\alpha$ C $\gamma$ M1) (B); there was no hematoxylin counterstaining of nuclei. ( $\times 325$ .)

25% of total T cells) and in the periventricular white matter from an early case of MS (case 285, samples I-7 and I-9), where  $\gamma\delta$ T cells represented 14.5% and 31.6% of total T cells, respectively (Table 1). In contrast,  $\gamma\delta$  T cells constituted a minor population in chronic MS plaques, normal white matter, and spleen. These data indicate that  $\gamma\delta$ T cells specifically accumulate in MS plaques during early inflammatory stages.

**$\gamma\delta$  TCR Repertoire in Acute MS Plaques.** Since only small amounts of tissue from histologically characterized acute plaques were available, RNA was extracted from thin frozen sections. The TCR  $V_\gamma$  and  $V_\delta$  gene repertoire was examined by PCR amplification of  $V_\gamma 1$  to  $V_\gamma 4$  ( $V_\gamma 1$  to  $V_\gamma 11$ ) and  $V_\delta 1$  to  $V_\delta 5$  gene segments from cDNA samples (refs. 11 and 12; the Lefranc nomenclature for  $V_\gamma$  segments is given in brackets). TCR  $V_\gamma 1$  to  $V_\gamma 4$  and  $V_\delta 1$  to  $V_\delta 4$  gene segments could be amplified from blood cDNA (positive control); amplification of  $V_\gamma 2$  ( $V_\gamma 9$ ) and  $V_\delta 2$  segments gave the strongest signals, as they are used by the majority of blood T cells (data not shown).

Strong signals for TCR  $V_\gamma$  and  $V_\delta$  gene segments were only seen with active MS samples, thus confirming the histological analysis.  $V_\gamma 2$  ( $V_\gamma 9$ ) was predominant in all acute and subacute plaques examined, while  $V_\gamma 1$  ( $V_\gamma 1$ - $V_\gamma 8$ ),  $V_\gamma 3$  ( $V_\gamma 10$ ), and  $V_\gamma 4$  ( $V_\gamma 11$ ) were detected in some plaques. In active plaques (cases 285 and 194),  $V_\delta 1$  and  $V_\delta 2$  were the most prevalent  $V_\delta$  gene segments. Only  $V_\delta 1$  rearrangements were detected in a white matter sample (cases 285, sample I-9) that had large numbers of  $\gamma\delta$  T cells and was adjacent to an early acute lesion. In contrast, there was no evidence for  $\gamma\delta$ T-cell accumulation (by both immunohistochemistry and PCR) in a case of SSPE, a chronic inflammatory CNS disease following

Table 1. Quantitative immunohistology of  $\alpha\beta$  and  $\gamma\delta$  T-cell populations in MS plaque tissue

Sample	Pathology	CNS samples		Spleen samples	
		T-cell count $\gamma\delta/\alpha\beta$	$\gamma\delta$ T cells, %	T-cell count $\gamma\delta/\alpha\beta$	$\gamma\delta$ T cells, %
<b>MS cases</b>					
285 I-6	Early acute plaque	253/759	25.0	53/2344	2.2
I-7	White matter	22/130	14.5		
I-8	Early acute plaque	16/120	11.8		
I-9	White matter	118/256	31.6		
194 I-16	Acute plaque	238/849	21.9	ND	
I-17	Acute plaque	89/294	23.2		
I-18	Acute plaque	43/353	10.9		
I-19	Acute plaque	10/162	5.8		
279 II-2	Subacute plaque	20/264	7.0	44/2170	2.0
II-3	Subacute plaque	4/ 95	4.0		
II-5	Mixed plaques	5/222	2.2		
II-6	Chronic plaque	6/243	2.4		
302 II-8	Chronic plaque	22/164	11.8	44/1925	4.2
II-9	Chronic plaque	18/170	9.6		
II-11	Chronic plaque	7/180	3.7		
II-12	Chronic plaque	2/120	1.6		
214 II-14	Chronic plaque	19/ 82	18.8	ND	
II-15	Mixed plaques	80/539	12.9		
II-17	White matter	26/356	6.8		
II-18	White matter	3/ 31	8.8		
<b>Control cases</b>					
292 I-1	White matter	1/ 88	1.1	102/2084	4.7
I-2		21/184	10.2		
I-3		5/ 34	12.8		
I-4		3/ 44	6.4		
293 I-11		7/156	4.3	10/162	5.8
I-12		12/147	7.6		
I-13		0/ 27	0.0		
I-14		3/ 98	3.0		
I-15		12/131	8.4		
289 II-1		0/ 9	0.0	ND	
II-4		0/ 5	0.0		
290 II-7		0/ 31	0.0	ND	
II-10		0/ 0	0.0		
284 II-13		0/ 5	0.0	19/1709	1.1
II-16		3/ 34	8.1		

T cells present in MS plaques and control samples were immunostained with antibodies against TCR  $\beta$  chain (BF1) and TCR  $\gamma$  chain ( $\alpha$ -C $\gamma$ M1). Immunostained T cells were counted by two independent observers in the five largest periventricular cuffs of each section. ND, not detected.

measles infection (Fig. 2, sample II-20). These data indicate that the accumulation of  $\gamma\delta$  T cells is an early event during lesion development.

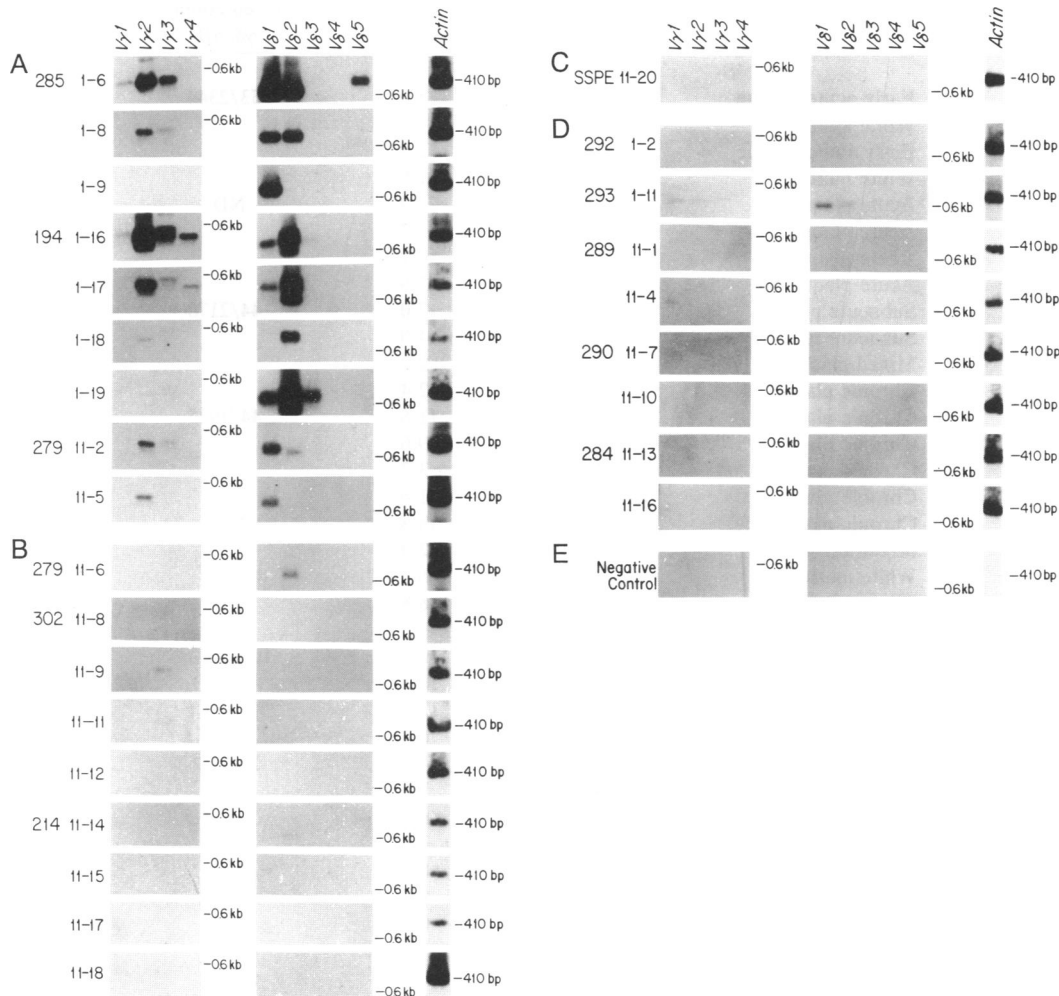
**Sequences of the TCR  $\delta$  Chains from Acute MS Plaques.** The diversity of the TCR  $\delta$  chain was examined for three acute plaques (case 285, plaque I-6; case 194, plaques I-16 and I-17) as well as one subacute lesion (case 279, plaque II-2) (Fig. 3). Amplified products were cloned into M13 and a number of recombinant clones sequenced. As a control, cDNA from blood T cells of a normal subject was used at a dilution that gave a similar level of amplification for  $V_{\delta 1}$  and  $V_{\delta 2}$  as cDNA samples from acute MS plaques; a negative control (no cDNA) was included for all steps of amplification and cloning.

The quantitative immunohistochemical analysis of  $\gamma\delta$  T cells in these samples had demonstrated 253 (case 285, plaque I-6) and 238  $\gamma\delta$  T cells (case 194, plaque I-16) around the five largest perivascular cuffs of each section, respectively. As RNA was extracted from 20 adjacent sections, more than 5060 and 4760  $\gamma\delta$  T cells, respectively, were probably present in these samples. These numbers represent a minimum estimate because only a fraction of  $\gamma\delta$  T cells was counted.

The sequence analysis showed that the majority of TCR  $V_{\delta 1}$  and  $V_{\delta 2}$  gene segments in acute plaques are rearranged to the joining-region  $J_{\delta 1}$  gene segment (Fig. 3). Surprisingly, repeated sequences were found among different M13 clones generated from the same plaque. For example, one junctional sequence was repeated five times and a second sequence

found twice among 10  $V_{\delta 1}$  sequences from plaque I-6 (case 285). Similar results were obtained for  $V_{\delta 2}$  sequences from the same plaque. In contrast, no repeated sequences were seen among  $V_{\delta 1}$  or  $V_{\delta 2}$  sequences generated from blood cDNA. These data indicate that the relatively large numbers of repeated sequences seen among  $V_{\delta 1}$  and  $V_{\delta 2}$  sequences in active MS plaques reflect expansion of  $\gamma\delta$  T-cell populations.

**Expression of hsp60 and hsp90 in CNS Tissue.** In normal white matter, a mAb specific for human hsp60 gave positive staining of nuclei and the cytoplasm in the perikarya and processes of astrocytes, microglial cells, and oligodendrocytes (Fig. 4A). Constitutive expression of hsp90 was also seen in normal tissue, but only cell nuclei were immunoperoxidase-stained (Fig. 4B). In macroscopically normal white matter from MS cases, there was a small increase in the expression of both heat shock proteins. In actively demyelinating plaques the predominant hsp60 staining pattern was of myelin-laden foamy macrophages (Fig. 4C). In these lesions the enlarged nuclei of reactive astrocytes and the perikarya and processes of small numbers of hypertrophic astrocytes were stained with anti-hsp90 antibody (Fig. 4D). These cells were positively stained with antibodies against the astrocyte-specific antigens glial fibrillary acidic protein and glutamine synthetase. Variable numbers of lymphocytes in perivascular inflammatory cuffs were also visualized by the hsp60 and hsp90 antibodies. In chronic plaques, very weakly stained



**FIG. 2.** Southern blot analysis of the TCR  $V_{\gamma}$  and  $V_{\delta}$  repertoire in MS plaque tissue. The TCR repertoire of  $\gamma\delta$  T cells in MS plaque tissue and control samples was examined by PCR amplification of cDNAs using primers for  $V_{\gamma 1}$  to  $V_{\gamma 4}$  and  $V_{\delta 1}$  to  $V_{\delta 5}$  gene segments; actin primers were used as a positive control. (A) Acute and subacute MS plaques. (B) Chronic MS plaques. (C) SSPE sample. (D) Control samples. (E) Negative control (no cDNA). For sample I-9, no cDNA was available for analysis of TCR  $V_{\gamma}$  usage.

A	V $\delta$ 1	N	D $\delta$ 1	N	D $\delta$ 2	N	D $\delta$ 3	N	J $\delta$			
I-6	<u>tgctcttgggaacl</u>		<u>gaalagl</u>		<u>ctctctac</u>		<u>actgggatacg</u>		<u>acaccgataactcat</u>	J $\delta$ 1		
	tgctcttgggg	ctctgggt			ctc	tacggctccit	actggggg	gctggggg	aaactcat	J $\delta$ 1		
	tgctcttg	tgct			ctt	tggall	actggggg	g	ctcat	J $\delta$ 1		
	<u>tgctcttgggact</u>	<u>actctgtcaga</u>					<u>actgggggatac</u>	<u>gggggaga</u>	<u>cgataactcat</u>	J $\delta$ 1		
	tgctcttgggga	ccggg	tg	ggag	ctctca	tlat	actggggg	gactcct	acaccgataactcat	J $\delta$ 1		
	tgctcttgggga	ccggg	tg	ggag	ctctca	tlat	actggggg	gactcct	acaccgataactcat	J $\delta$ 1		
	tgctcttgggga	ccggg	tg	ggag	ctctca	tlat	actggggg	gactcct	acaccgataactcat	J $\delta$ 1		
	tgctcttgggga	ccggg	tg	ggag	ctctca	tlat	actggggg	gactcct	acaccgataactcat	J $\delta$ 1		
	<u>tgctcttgggga</u>	<u>ccggg</u>	<u>tg</u>	<u>ggag</u>	<u>ctctca</u>	<u>tlat</u>	<u>actggggg</u>	<u>gactcct</u>	<u>acaccgataactcat</u>	J $\delta$ 1		
	tgctcttgg	atactctaccggca				tac t	gggatac	cc	cttgggacaccgac	J $\delta$ 3		
	tgctcttgg	atactctaccggca				tac t	gggatac	cc	cttgggacaccgac	J $\delta$ 3		
	I-16	<u>tgctcttgggg</u>	<u>gactctaccggactg</u>			<u>ttc a</u>		<u>gggggata</u>	<u>t</u>	<u>acaccgataactcat</u>	J $\delta$ 1	
		tgctcttgggaa	gaggtaa			ttcac	gactaggggact	ggggatac	agttac	gataactcat	J $\delta$ 1	
		<u>tgctcttgggaa</u>	<u>gaggtaa</u>			<u>ttcac</u>	<u>gactaggggact</u>	<u>ggggatac</u>	<u>agttac</u>	<u>gataactcat</u>	J $\delta$ 1	
		tgctcttgggg	g	agt	a	cttc		actggggatac	caa	gataactcat	J $\delta$ 1	
tgctcttgggg		gccaac			toc	egt	actgggg	cactcct	acaccgataactcat	J $\delta$ 1		
tgctcttggggac		ctct			ctc		ggga	actcactc	ctttgacagcact	J $\delta$ 2		
tgggga		ggcggcaaac	gaa	catll					acaccgataactca	J $\delta$ 1*		
tggggact		gaggctctaaag			cttl	tttl	ctggggatac	tgccggat	cgataactca	J $\delta$ 1*		
Blood		tgctcttgggg		gaa	c	toc	ccactgt	actggggatac	ttcttl	acaccgataactcat	J $\delta$ 1	
		tgctcttggggac	ccc					ggga	atg	acaccgataactcat	J $\delta$ 1	
	tgctcttgggaa				toc	oca	ggga		actcat	J $\delta$ 1		
	tgctcttgggg	ctcaa			cta	t			acaccgataactcat	J $\delta$ 1		
	tgctcttggg	aa			ctt	atgagc			cgataactcat	J $\delta$ 1		
	tgctcttgg	ag			ctc	ac	ggggat	cta	acaccgataactcat	J $\delta$ 1*		
	tgctcttgggact	ga			ctac		ggggat	tcct	cgataactcat	J $\delta$ 1		
	B	V $\delta$ 2	N	D $\delta$ 1	N	D $\delta$ 2	N	D $\delta$ 3	N	J $\delta$		
		I-6	<u>tgga</u>		<u>gaalagl</u>		<u>ctctctac</u>		<u>actgggatac</u>		<u>acaccgataactcat</u>	J $\delta$ 1
			tgga	ctctg	tag	gactaga	oct		gga	gacca	cgataactcat	J $\delta$ 1
tgga			cacc					ctggggatac	caagg	cgataactcat	J $\delta$ 1	
tgga			caccgtctcaga					ggggg	gactcaagt	acaccgataactcat	J $\delta$ 1	
tgga			caccgtctcaga					ggggg	gactcaagt	acaccgataactcat	J $\delta$ 1	
tgga			caccgtctcaga					ggggg	gactcaagt	acaccgataactcat	J $\delta$ 1	
tgga			c	gaala	agg			ctggggatac	agttgggg	acaccgataactcat	J $\delta$ 1	
tgga			c	gaala	agg			ctggggatac	agttgggg	acaccgataactcat	J $\delta$ 1	
tgga			cactct					act	accgtctgtgag	cgataactcat	J $\delta$ 1	
tgga			cactct					act	accgtctgtgag	cgataactcat	J $\delta$ 1	
tgga		cactct					act	accgtctgtgag	cgataactcat	J $\delta$ 1		
I-16		tgga	caag					ctggggg	ca	acaccgataactcat	J $\delta$ 1	
		tgga	caoct					ggggg	gagggg	caaccgataactcat	J $\delta$ 1	
		tgga	ccactgg		agt	c		gggatac	ctact	acaccgataactcat	J $\delta$ 1	
	tgga	cactct					actggg	ctactgggct	acaccgataactcat	J $\delta$ 1		
	tgga	cacat					actggggatac	ct	acaccgataactcat	J $\delta$ 1		
	tgga	taocg	tag	ga			ggggatac	ct	acaccgataactcat	J $\delta$ 1		
	tgt	ggcactctgg					gggg	atg	gataactcat	J $\delta$ 1		
	tgga	ccgtgacac			cttl	gg	act	ccggg	acaccgataactcat	J $\delta$ 1		
	tgga				oct	g	gggggatac	cg	acaccgataactcat	J $\delta$ 1*		
	tgga	cc					actggggg	g	caaccgataactcat	J $\delta$ 1*		
tgga	caccg	tag	gacaaa					acaccgataactcat	J $\delta$ 1			
I-17	tgga	caccg	tag	gacaaa					acaccgataactcat	J $\delta$ 1		
	tgga	ccgtgacacg	tag	ga			ggggatac	ct	acaccgataactcat	J $\delta$ 1		
	tgga	ccgtgacacg	tag	gacaaa					acaccgataactcat	J $\delta$ 1		
	tgga	ccgtgacacg	tag	gacaaa					acaccgataactcat	J $\delta$ 1		
	tgga	ggggatg					ggggat	cccc	caaccgataactcat	J $\delta$ 1		
	tgga	cg	ata	ttc	toc	ga	gga	cc	caaccgataactcat	J $\delta$ 1		
	tgga	ccaagtg	tag	gggtaccagt			actggggg		acaccgataactcat	J $\delta$ 1		
	tgga	caccgta					gga	caa	acaccgataactcat	J $\delta$ 1		
	tgga	cagcat					actggggat	tcag	caccgataactcttt	J $\delta$ 2		
	tgga	ca			ctt	t	actgggg		ctctgggacaccg	J $\delta$ 3		
II-2	tgga	cgcta					ggggg	ccgggtggac	cgataactcat	J $\delta$ 1		
	tgga	cgcta					ggggg	ccgggtggac	cgataactcat	J $\delta$ 1		
	tgga	cgcta					ggggg	ccgggtggac	cgataactcat	J $\delta$ 1		
	tgga	cgcta					ggggg	ccgggtggac	cgataactcat	J $\delta$ 1		
	tgga	cgcta					ggggg	ccgggtggac	cgataactcat	J $\delta$ 1		
	tgga	cgcta					ggggg	ccgggtggac	cgataactcat	J $\delta$ 1		
	tgga	cgcta					ggggg	ccgggtggac	cgataactcat	J $\delta$ 1		
	tgga	cgcta					ggggg	ccgggtggac	cgataactcat	J $\delta$ 1		
	tgga	cgcta					ggggg	ccgggtggac	cgataactcat	J $\delta$ 1		
	tgga	cgcta					ggggg	ccgggtggac	cgataactcat	J $\delta$ 1		
Blood	tgga	ccc					actggggg	taagt	acaccgataactcat	J $\delta$ 1		
	tgga	tat					actggggg	ctatagggact	acaccgataactcat	J $\delta$ 1		
	tgga				oct		actggggg	ctg	acaccgataactcat	J $\delta$ 1		
	tgga	ca			oct	tggt	actggggat	t	caaccgataactcat	J $\delta$ 1		
	tgga	ccat					actggggg	ccgag	ctctgggacaccg	J $\delta$ 3		
	tgga	caccgttggccggg			tac	ctcggggggg	atcggg		caaccgataactcat	J $\delta$ 1		
	tgga	cgct					atcggggg	ccaa	acaccgataactcat	J $\delta$ 1*		
	tgga	caoct					actggggg	ctgagccc	gggacaccgacag	J $\delta$ 3*		

FIG. 3. Sequence analysis of TCR  $\delta$  chains in MS plaque tissue. Amplified DNA from MS plaques I-6 (case 285, early-acute MS plaque), I-16 and I-17 (case 194, acute plaques), II-2 (case 279, subacute plaque), and blood cDNA were cloned into M13, and the junctional regions were sequenced by the dideoxy method. Repeated sequences found in a plaque are boxed. (A) V $\delta$ 1 rearrangements. (B) V $\delta$ 2 rearrangements. In the one case where two separate plaques could be examined from the same brain (case 194, I-16 and I-17), two identical sequences were found (underlined). This suggests the same expanded  $\gamma$ - $\delta$  population can be found in different plaques of the same brain. In contrast, no identical sequences were observed between plaques from different brains. \*, Out of frame sequence.

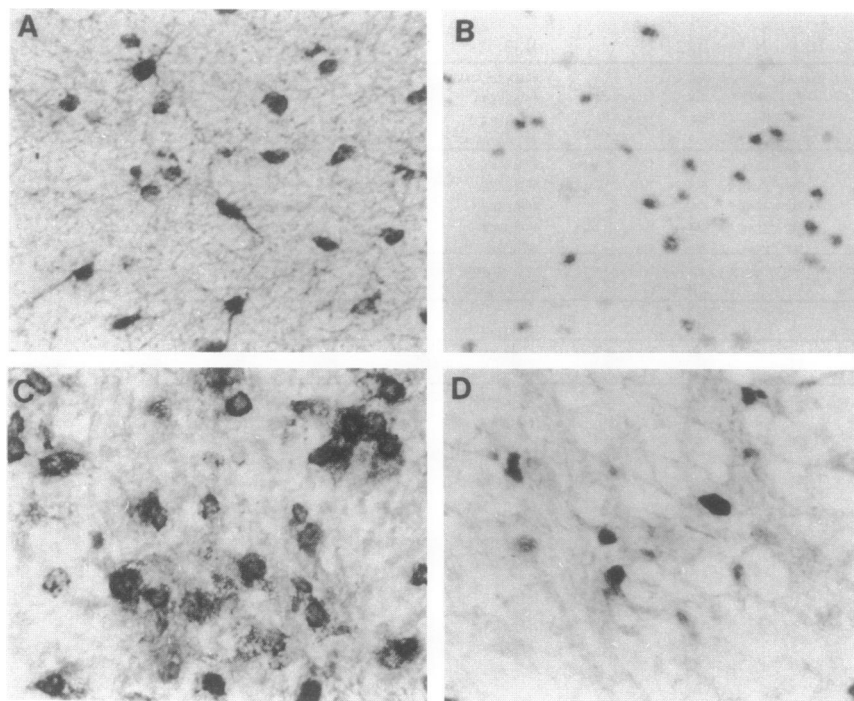


FIG. 4. Heat shock proteins in acute MS plaques. (A and B) Immunoperoxidase-stained cryostat sections of normal control white matter from case 293, adjacent to sample I-14, showing immunostaining with anti-human hsp60 antibody of nuclei and cytoplasm in the perikarya and processes of microglia and astrocytes and in oligodendrocyte perikarya (A) and immunostaining with anti-hsp90 antibody of glial nuclei but not microglia (B). (C and D) Immunoperoxidase-stained actively demyelinating plaque from MS case 194 (sample I-16), showing predominant staining with the anti-hsp60 antibody in the nuclei and cytoplasm of myelin-laden macrophages (C) and staining with anti-hsp90 antibody of the enlarged nuclei of reactive astrocytes (D). There was no hematoxylin counterstaining of nuclei. ( $\times 430$ .)

dense networks of astrocyte cell processes were seen with both antibodies (data not shown).

## DISCUSSION

An accumulation of  $\gamma\delta$  T cells that predominantly use the  $V_{\delta 1}$  and  $V_{\delta 2}$  gene segments was observed in acute MS lesions.  $\gamma\delta$  T cells appear to have undergone clonal expansion in active MS plaques, as repeated TCR  $\delta$  chain sequences were found in all plaque samples examined. This observation was not due to small cell numbers present in plaques, as a minimum of 5000  $\gamma\delta$  T cells were presented in sections from two acute plaques. Therefore,  $\gamma\delta$  T cells appear to undergo clonal expansion following recognition of a specific CNS ligand.

A recent model proposes that  $\gamma\delta$  T cells reactive with highly conserved heat shock proteins are involved in autoimmune reactions (6). According to this model, overexpression of heat shock proteins at an inflammatory site leads to recruitment of  $\gamma\delta$  T cells cytotoxic for stressed cells. In such a scenario, autoreactive  $\alpha\beta$  T cells reactive with a myelin autoantigen may initiate the inflammatory process, leading to an increased expression of specific heat shock proteins and recruitment of  $\gamma\delta$  T cells that induce demyelination. This model is supported by a recent report demonstrating that  $\gamma\delta$  T cells colocalize with hsp65-reactive oligodendrocytes in chronic-active MS lesions. Both the presence of  $\gamma\delta$  T cells and hsp65 expression were specific for MS plaques and not detected in the CNS of patients with SSPE or tropical spastic paraparesis (9).

In summary, we demonstrate that  $\gamma\delta$  T cells bearing TCR  $V_{\delta 1}$  and  $V_{\delta 2}$  gene segments accumulate in acute MS plaques where they appear to undergo clonal expansion, presumably because of recognition of a specific ligand. hsp60 and hsp90,

which are increased in expression in acute plaques, may be target antigens for such autoreactive  $\gamma\delta$  T cells. Therefore,  $\gamma\delta$  T cells may play an important role in the pathogenesis of MS.

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