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Clonal expansions of activated γ/δ T cells in recent-onset multiple sclerosis

(autoimmune disease/T-cell receptor repertoire)

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ABSTRACT **Multiple sclerosis (MS) is a chronic disease characterized by focal demyelination of the white matter of the brain and spinal cord. Central nervous system damage appears to be mediated by infiltrating T lymphocytes and macrophages, and a central role for autoreactive CD4⁺ T cells has been proposed. However, the initial immune events that lead to the chronic process of MS remain unidentified. We now present evidence that a subset of T lymphocytes bearing γ/δ T-cell antigen receptors has been activated in patients with recent-onset disease. Cells recovered from the cerebrospinal fluid of subjects with MS were cultured for short periods of time in medium supplemented with T-cell growth factors. Expansions of $V_\delta1$ and $V_\delta2$ T-cell receptor-bearing lymphocytes were found only in cell populations obtained from subjects with recent-onset disease. Similar populations were not expanded in subjects with chronic MS or other neurological diseases. Junctional region sequencing showed the expanded γ/δ T cells to be oligoclonal in nature, suggestive of specific stimulation by antigen. These results reveal a fundamental difference in the immunopathogenesis of acute vs. chronic disease and provide additional insight into the autoimmune nature of MS.**

Previous investigations into an immunological basis for multiple sclerosis (MS) have centered on the involvement of T lymphocytes and macrophages because of their detection in MS brain white matter lesions (1). $CD4^+$ α/β T-cell receptor (TCR) lymphocytes restricted in antigen reactivity by class II major histocompatibility complex molecules have been implicated due to the HLA-DR2, Dw2 association with MS (2). In addition, $CD4^+$ lymphocytes mediate experimental allergic encephalomyelitis, an animal model of central nervous system disease with similar pathology as human MS, usually elicited by immunization with myelin basic protein (MBP, ref. 3). Along these lines, several groups have described the limited expression of TCR α/β molecules by $CD4^+$ lymphocytes from chronic MS brain lesions [variable region $(V)_\alpha10$, ref. 4, and $V_\beta5.2$, J. Oskenberg, M. Panzara, A. Begovich, D. Mitchell, H. Erlich, R.S.M., R.S., M. Sherritt, J. Rothbard, C. Bernard, and L. Steinman, unpublished work], or by MS peripheral blood $CD4^+$ T cells after *in vitro* stimulation with MBP ($V_\beta12,17$, ref. 5; $V_\beta15$, ref. 6; and $V_\beta5.2$, ref. 7). Although evidence has been presented that T-cell reactivity to MBP is involved in MS, most of these studies involved patients with long-standing disease, and it is unclear at what stage T-lymphocyte reactivity to MBP might play a role. In addition, a viral etiology combined with a genetic predisposition to disease has been postulated for MS (3, 8, 9). Therefore, although MBP-reactive $CD4^+$ T cells might be speculated to contribute to the chronic pathogenesis of MS, the initiating immune events in this disease remain undefined. Recently, lymphocytes bearing γ/δ TCR, a genetically distinct group of TCRs for antigen (10), have been detected in both acute and chronic MS brain lesions (11, 12). The presence of γ/δ T lymphocytes early in bacterial infections (13, 14) has led to speculation on the role of γ/δ cells in the initial stages of inflammation. We report here that MS subjects with active recent-onset disease, but not those with long-standing disease, have an oligoclonal population of activated γ/δ T cells in their cerebrospinal fluid (CSF) that can be expanded *in vitro*.

MATERIALS AND METHODS

Subjects. CSF and peripheral blood mononuclear leukocytes (PBL) were obtained from subjects who were clinically evaluated and assigned a diagnosis (by R.S.M. and J.A.B.) before any knowledge of TCR phenotyping results. All subjects exhibited a neurological deficit that together with clinical laboratory data and/or previous history supported a diagnosis of clinically definite or probable, laboratory-supported MS (15–17), or other neurological disease.

***In Vitro* T-Lymphocyte Expansion.** Cells from CSF were pelleted by centrifugation and cultured in medium supplemented with 10% pooled A^+ normal human serum and 8% Lymphocult T-lectin free and 2% Lymphocult T-highly purified (Biotest Diagnostics, Denville, NJ) as sources of T-cell growth factors including interleukin (IL) 2 and IL-4 (referred to as IL-2–CSF). The mean number of CSF cells cultured was 2.27×10^4; the range was $0.24–7.4 \times 10^4$. Peripheral blood was also taken on the same day as CSF, and PBL were isolated by Ficoll/Hypaque gradient centrifugation. PBL (2×10^5) were cultured separately under identical conditions (referred to as IL-2–PBL). After 6–8 days of *in vitro* lymphokine-supplemented culture, the surviving blast cells were expanded by stimulation with anti-CD3 antibody (18) in the presence of 4000-R-irradiated PBL filler cells (1 R = 0.258 mc/kg) and culture medium supplemented with lymphokines, as indicated above. By 5–7 days after anti-CD3 expansion, when the cells were slowing in proliferation, aliquots of cells were harvested for analysis.

Analysis of TCR Expression by Immunofluorescence. Freshly isolated PBL or cells harvested from culture were washed and stained with either biotinylated monoclonal antibody (mAb) WT-31 (recognizing mostly TCR α/β cells

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Abbreviations: MS, multiple sclerosis; TCR, T-cell receptor; MBP, myelin basic protein; CSF, cerebrospinal fluid; PBL, peripheral blood mononuclear leukocytes; IL, interleukin; V, D, J, and C, variable, diversity, joining, and constant regions, respectively; mAb, monoclonal antibody.

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but with some cross-reactivity for a small subset of TCR γ/δ cells, ref. 19), fluoresceinated mAb TCR δ1 (recognizing all TCR γ/δ, ref. 20), or fluoresceinated mAb TCSδ-1 [recognizing V_δ1–joining region (J) $_\delta$1, ref. 21] plus biotinylated mAb BB3 (recognizing V_δ2, ref. 22). Cells were then washed and incubated with phycoerythrin–avidin. Samples were evaluated on either a FACScan (Becton Dickinson) or Profile (Coulter) flow cytometer, analyzing 5,000–25,000 gated viable lymphocytes per sample.

Analysis of TCR V_δ Sequences. PCR amplifications were done by using oligonucleotide primers specific for V_δ1 (TACTCAAGCCCAGTCATCAGTATCC) and constant region $(C)_\delta$ (CCAAGCTTGACAGCATTGTACTTCC), and the V_δ1 (GGGGTCGACAAGTTGGTGGTCATATTA) and V_δ2 (GGGGTCGACCCTCAGGTGCTCCATGAA) primers described by Krangel *et al.* (23). Amplifications were done on cDNA reverse-transcribed from ≈1–2 μg of total lymphocyte RNA with 35 cycles of 1 min at 96°C, 2 min at 55°C, and 3 min at 72°C, as described (4). PCR products of the appropriate size (450–600 bp) were excised from agarose gels, purified, ligated to a "TA" cloning vector (Invitrogen), and transformed into competent *Escherichia coli* host bacteria. Double-stranded vectors containing TCR V_δ PCR products were isolated and sequenced by using the dideoxynucleotide Sequenase enzyme (United States Biochemical).

RESULTS AND DISCUSSION

To enrich for disease-relevant T cells, which we speculated would be *in vivo* activated and, therefore, expressing receptors for T-cell growth factors (24–26), cells isolated from patients' CSF and blood were cultured in lymphokine-supplemented medium. For a subset of patients, cytofluorographic analysis of lymphokine-expanded T cells demonstrated large numbers of γ/δ lymphocytes; 5/18 PBL samples from subjects with MS contained high percentages (>10.4%, Table 1 legend) of γ/δ T cells after culture, whereas most MS PBL samples did not expand under these culture conditions. Two of four PBL cultures from non-MS subjects also contained increased percentages of γ/δ T cells. Freshly isolated PBL did not contain high γ/δ T-cell percentages (range of 1.1–10%), which in several MS subjects (M_5, W_1, W_2) either did not change or even decreased after *in vitro* culture. Both V_δ1 and V_δ2 expression predominated in γ/δ cells expanded from MS PBL, whereas the two elevated samples from non-MS subjects contained predominantly $V_\delta1^+$ cells.

Table 1. Percentage of γ/δ T cells in CSF and PBL

Subject	Cell population	Subset staining, %				Subject	Cell population	Subset staining, %			
		α/β*	γ/δ†	V_δ1	V_δ2			α/β*	γ/δ†	V_δ1	V_δ2
MS‡											
B_5	IL-2–CSF	NA	6.9	0.5	3.5	T_1	IL-2–CSF	95	2.5	0.2	1.1
	IL-2–PBL	70	29	7.3	20		IL-2–PBL	88	8.2	4.1	3.9
	PBL	53	10	0.9	20		PBL	44	2.8	0.5	NA
B_6	IL-2–CSF	96	3.3	1.1	1.4	T_2	IL-2–CSF	87	11	11	2.3
	IL-2–PBL	91	8.4	0.0	9.2		IL-2–PBL	94	4.2	2.5	2.5
	PBL	80	6.4	0.2	7.9		PBL	45	2.1	0.9	NA
D_1	IL-2–CSF	87	12	7.1	2.9	U	IL-2–CSF	98	6.7	0.0	1.7
	IL-2–PBL	84	15	2.7	16		IL-2–PBL	84	7.4	1.3	2.9
D_3	IL-2–CSF	91	1.8	0.3	1.0		PBL	70	1.1	0.4	0.3
	IL-2–PBL	89	3.4	1.9	1.1	W_1	IL-2–CSF	50	51	21	5.0
	PBL	81	1.9	0.1	3.2		IL-2–PBL	56	5.6	0.8	4.5
E	IL-2–CSF	58	2.9	0.0	4.9		PBL	76	8.9	0.0	9.0
	IL-2–PBL	84	1.7	0.3	1.3	W_2	IL-2–CSF	60	40	35	3.5
G_2	IL-2–CSF	89	0.0	0.1	0.0		IL-2–PBL	85	5.0	2.0	2.0
	IL-2–PBL	44	44	14	20		PBL	74	4.5	1.0	4.0
	PBL	73	6.8	2.9	6.3	Y	IL-2–CSF	76	22	16	3.3
L_2	IL-2–CSF	80	1.2	0.0	0.9		IL-2–PBL	97	1.2	1.6	0.2
	IL-2–PBL	79	16	5.2	10.3		PBL	69	3.2	0.6	4.9
	PBL	71	2.4	0.6	6.8	Non-MS					
M_4	IL-2–CSF	71	24	17	6.1	F_2	IL-2–CSF	88	2.0	0.2	0.8
	IL-2–PBL	72	9.0	5.2	4.2		IL-2–PBL	80	8.2	3.7	4.6
	PBL	73	4.0	0.7	2.3		PBL	76	1.9	0.6	NA
M_5	IL-2–CSF	95	2.0	0.8	0.6	H_3	IL-2–CSF	95	2.7	2.2	0.0
	IL-2–PBL	94	2.4	0.7	1.3		IL-2–PBL	63	30	21	1.2
	PBL	56	5.5	0.9	3.4		PBL	69	6.4	1.2	6.6
P_1	IL-2–CSF	93	13	6.5	4.5	M_3	IL-2–CSF	100	1.4	0.5	1.2
	IL-2–PBL	80	17	13	2.7	M_6	IL-2–CSF	91	0.6	0.0	0.7
	PBL	72	2.5	0.8	6.9		IL-2–PBL	99	0.3	0.7	1.1
P_2	IL-2–CSF	91	5.1	1.0	3.2	N	IL-2–CSF	94	2.8	0.1	2.3
	IL-2–PBL	91	3.8	1.1	2.0		IL-2–PBL	78	19	17	0.5
	PBL	45	2.7	0.2	NA		PBL	86	2.8	1.0	2.7
R_2	IL-2–CSF	70	27	8.7	17						
	IL-2–PBL	94	5.2	1.1	3.9						
	PBL	75	1.8	1.2	0.5						

NA, data not available.

*Cells positive for TCR α/β were detected with mAb WT-31, which cross-reacts on a subpopulation of TCR γ/δ cells; therefore, cell staining may, in some cases, be >100%.

†The normal range of γ/δ T cells in human peripheral blood has been reported to be 1–8% (27). In this study we detected a mean (± SD) percentage in freshly isolated (nonmanipulated) PBL of 4.09 ± 2.1%. Values >3 SD above this mean staining (>10.4%) were considered significantly increased above normal.

‡Patient samples were evaluated in the order received, and all subjects evaluated are presented in alphabetical order.

Table 2. Classification of disease status

Subject	Onset†	CSF indices* WBC/ mm³	OCB	IgG syn. rate, mg/day	MRI‡
			Recent-onset MS		
D_1	1 mo	12	4 $IgG^{k/l}$	34	Mf-WM/Gd^+
M_4	1 mo	8	None	NA	Mf-WM+SC/Gd^+
P_1	2 mo	16	None	50	Mf-WM/Gd^+
R_2	4 mo	56	2 $IgG^{k/l}$	73	Single WM
T_2	1 mo	7	3 $IgG^{k/l}$	64	Mf-WM+SC/Gd^+
W_1	6 mo	3	4 $IgG^{k/l}$	47	Mf-WM/Gd^+
W_2	6 mo	46	None	48	Mf-WM
Y	5 mo	93	1 IgG^k	49	Mf-SC/Gd^+
			Subacute and/or chronic MS		
B_5§	7 yr	0	None	7	Normal
B_6	4 yr	4	None	5	Multi-WM/Gd^-
D_3	>6 mo	4	3-IgG^k	20	Single-WM/Gd^+
E	16 yr	3	4-$IgG^{k/l}$	32	Mf-WM/Gd^+
G_2	7 yr	0	2-$IgG^{k/l}$	12	Normal
L_2	11 yr	0	None	NA	Normal¶
M_5	8 mo	7	3-$IgG^{k/l}$	14	Normal
P_2	2 yr	10	Mult-$IgG^{k/l}$	46	Mf-WM, ST
T_1	4 yr	2	None	10	Mf-WM/Gd^-
U	4 yr	4	4-$IgG^{k/l}$	13	Mf-WM
			Non-MS‖		
F_2		0	None	5	Normal (CPS)
H_3		1	None	4	Normal (Dystonia)
M_3		122	4-$IgG^{k/l}$	18	Mf-WM/Gd^+ (ADEM)
M_6		0	None	6	Normal (PVN)
N		0	None	7	Normal (B12-def.)

NA, data not available.

*A CSF white blood cell (WBC) count of 0–4 cells per mm³ is considered the normal range for healthy persons. A CSF-specific presence of $IgG^{k/l}$ oligoclonal bands (OCB) are typical (but not specific) for MS, and an IgG synthesis (syn) rate of 0–9 mg per day is the normal range for healthy persons.

†Duration of MS symptoms at time of evaluation is indicated.

‡Magnetic resonance imaging (MRI) with gadolinium (Gd^+) was done, indicating enhancement of active single or multifocal (Mf) white matter (WM), brainstem (ST), or spinal cord (SC) inflammatory lesions.

§This subject, B_5, while having been diagnosed 7 yr previously as having MS, does not meet all criteria for clinically definite MS and is therefore categorized as "possible MS."

¶This subject, L_2, had an abnormal MRI 1 yr before this study.

‖Non-MS subjects included in this study were diagnosed with chronic pain syndrome (CPS), idiopathic dystonia, acute disseminated encephalomyelitis (ADEM), postvaccinal neuropathy (PVN), and one myelopathy secondary to vitamin B_{12} deficiency.

Only a small percentage of cells from the CSF of non-MS subjects expressed γ/δ after culture (range, 0.6–2.8%). However, cultured CSF samples from 8 of 18 subjects with MS contained >10.4% γ/δ T cells (Table 1). Two subjects demonstrated increased γ/δ percentages in both CSF and blood. Staining for TCR V_δ indicated that $V_\delta1^+$ T cells predominated in nearly all MS CSF cultures showing a γ/δ expansion; only one MS patient's CSF contained predominantly $V_\delta2^+$ cells. The striking expansion of $V_\delta1^+$ cells in only a subgroup of patients indicated that only a subset of γ/δ cells was present or had the ability to expand during culture. Experiments were also done in which CSF cells were stimulated *in vitro* with anti-CD3, instead of initially culturing in medium containing IL-2 plus IL-4. None of the anti-CD3-stimulated cultures resulted in high percentages of γ/δ cells, and in every case $CD4^+$ cells predominated. These results suggested to us that the growth factor-responsive γ/δ cells

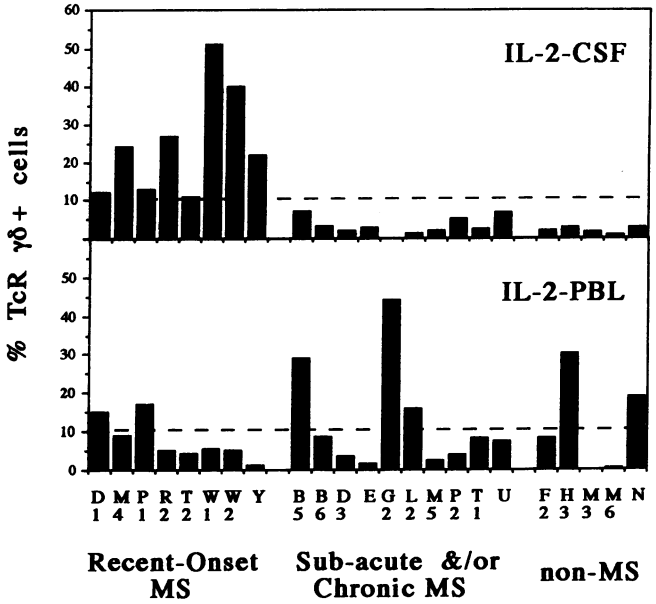

FIG. 1. Correlation of lymphokine-mediated expansion of γ/δ cells with subject disease status. Percent staining for TCR γ/δ from Table 1 was grouped according to subject disease classification from Table 2. A TCR γ/δ percentage >10.4% (see Table 1 legend) was considered significantly elevated and is indicated by dashed line.

did not represent the major CSF $CD3^+$ population but were selected on the basis of other properties, presumably their activation status. Furthermore, in CSF samples from MS subjects where γ/δ cells did not expand, TCR α/β lymphocytes proliferated extensively, indicating the presence of a different activated cell population (Table 1). All CSF samples cultured *in vitro*, irrespective of subject disease status and whether the expanded T population expressed α/β or γ/δ TCR, expanded >100-fold to ≈ 1.5–2.5×10^6 cells (data not shown).

The presence of activated, lymphokine-expandable γ/δ T lymphocytes in MS subjects acquired greater significance after the disease status of the study subjects was revealed. All eight of the CSF populations generating high percentages of γ/δ T cells were obtained from MS subjects with active recent-onset disease (Table 2 and Fig. 1). These subjects all displayed clinically definite MS of an active inflammatory nature, characterized by gadolinium-enhancing white matter and/or spinal cord lesions, elevated CSF indices, and recent onset of clinical symptoms (mean, 3.3 mo; range, 1–6 mo, Table 2). Those MS subjects whose CSF γ/δ T cells did not expand after *in vitro* culture were found to have either stable subacute disease (subjects B_5, G_2, L_2, T_1) with normal magnetic resonance images and relatively low CSF white cell counts, and/or chronic MS of multiple years duration, including six subjects whose disease was active by magnetic resonance imaging and/or clinical parameters (B_6, D_3, E, M_5, P_2, and U; Table 2). Lymphokine-cultured CSF from patients with other neurological diseases (non-MS group) also did not contain activated γ/δ T cells. One subject in this group (M_3) had an acute disseminated encephalomyelitis characterized by an extremely high CSF mononuclear white cell count and a gadolinium-enhancing magnetic resonance imaging indicative of an active inflammatory process.

In contrast to CSF, the observed expansion of γ/δ T cells in lymphokine-cultured PBL populations appeared unrelated to disease status. Two patients had recent-onset MS, three had chronic MS, and two were in the non-MS group (Fig. 1). Significantly, chronic MS subject G_2's PBL contained activated $V_\delta1^+$ and $V_\delta2^+$ T cells while having no detectable CSF γ/δ cells (Table 1 and Fig. 1). Comparison of results with CSF

	Sample	Sequences Identical /Analyzed	V_{delta}	N	D_{delta1}	N	D_{delta2}	N	D_{delta3}	N	J_{delta1}
$V_\delta 1$	Germline		CTTGGGGAACT		GAAATAGT		CCTTCCTAC		ACTGGGGGATACG		ACACCGATAAACTC
	D_1 IL-2-CSF	9/11	CTTG.....TCAAAAAAAGGGGTTGGCCTTCCTAC					CT	ACTGGGGGATACG	G	...CCGATAAACTC
	D_1 IL-2-PBL	10/12	CTTGGGG....		GCCGGGGCG		...TCCTA.	AACGG	.CTGGGG.....	AAAGT	ACACCGATAAACTC
	R_2 IL-2-CSF	4/13	CTTGGGGAACT				GGCGCGGTGGGCCCTCC				ACACCGATAAACTC
		2/13	CTTGGGGA...		CTTTTATCTTGGGTACGGGGTCCCGT				ACTGGGGGA....	CTCCTCGGT	ACACCGATAAACTC
	R_2 IL-2-PBL	2/11	CTTGGGGAACT				ACACC		....GGGGAT...	CCACCC	ACACCGATAAACTC
		2/11	CTTGGGGAACT				AGATGTGT				ACACCGATAAACTC
	W_2 IL-2-CSF	12/12	CTTGGGG....		TTCTTTA		.....CTAC	G	.CTGGGGGATAC.	AG	....CGATAAACTC
	W_2 IL-2-PBL	4/11	CTTGGGGA...		CTGGCGAAGAGGACGAAGCGGGT				ACTGGGGGATACG	AGCTTTT	GACAGCACAACTC (J_{delta2})
		3/11	CTTGGG.....		AACCTGAAGTGCTC				.....GGGATAC.	TATAAACA	....CGATAAACTC
$V_\delta 2$	Germline		TGTGACACC		GAAATAGT		CCTTCCTAC		ACTGGGGGATACG		ACACCGATAAACTC
	D_1 IL-2-CSF	3/8	TGTGAC...				CCGATCCCTT		ACTGGGGGATACG	CGG	.....TAAACTC
		2/8	TGTGACACC				GTAGGT		ACTGGGGGA....	CTGA	ACACCGGTAAACTC
	D_1 IL-2-PBL	4/10	TGTGACAC.				GGTAGC		ACTGGGGGA....	CCTCATCT	ACACCGATAAACTC
	R_2 IL-2-CSF	8/10	TGTGACACC				G		..TGGGGGATA..	AAGGGGGGT	ACACCGATAAACTC
	R_2 IL-2-PBL	0/9 were repeated sequences.									

FIG. 2. TCR $V_\delta 1$ and $V_\delta 2$ sequences from lymphokine-expanded MS T cells that were found to be repeated. Sequences not shown were in frame and only found once. Germ-line sequences are indicated at top. Deletions from germ line are indicated by underlined dots, and additions to germ-line sequences are nonunderlined nucleotides.

and PBL samples indicated that the presence in CSF of activated γ/δ T cells was not simply a consequence of the influx of activated peripheral blood cells.

We hypothesized that the expandable γ/δ cells had been selectively activated *in vivo*, and, therefore, would be limited in heterogeneity—i.e., oligoclonal. These cells should, therefore, express a limited number of TCR junctional regions. To test this hypothesis, cDNA clones encoding the expanded V_δ were sequenced. Samples from three active recent-onset MS patients were analyzed, and all demonstrated remarkable oligoclonality (Fig. 2). For patient W_2, all of the $V_\delta 1$ cDNA clones analyzed from CSF were identical in sequence. For patients D_1 and R_2 CSF, dominant clones expressing $V_\delta 1$ or $V_\delta 2$, respectively, were identified. Both $V_\delta 1$-restricted populations used diversity $(D)_\delta 2$, $D_\delta 3$, and $J_\delta 1$ genes with extensive N-region nucleotide additions. The restricted $V_\delta 2$ population used $D_\delta 3$ and $J_\delta 1$ genes. Surprisingly, the IL-2-cultured PBL population from recent-onset subject D_1, which demonstrated only 2.7% $V_\delta 1^+$ cells after culture, also contained a dominant $V_\delta 1$ clonotype distinct from that found expanded from the same patient's CSF. $V_\delta 2^+$ populations from this subject's CSF and PBL were also oligoclonal but less marked compared with the $V_\delta 1^+$ results. $V_\delta 1^+$ populations from R_2 CSF and W_2 PBL contained dominant clones but did not use $D_\delta 2$ and had a greater diversity of TCR sequences. In addition, one repeated $V_\delta 1$ sequence used the $J_\delta 2$ gene (W_2, Fig. 2), which was not seen in the other oligoclonal sequences obtained. Both $V_\delta 1$ and $V_\delta 2$ PBL populations from subject R_2 were heterogeneous, showing diversity in D and J region usage and junctional variations, as would be expected of polyclonal populations.

The complexity and differences in TCR V_δ junctional sequences in the PBL populations, in particular, indicated that PCR artifacts and contamination were not responsible for the observed oligoclonality of certain CSF populations. We found only one example of a CSF TCR V_δ sequence present in the same patient's PBL, a singular PBL sequence that corresponded to the dominant CSF clone (D_1, Fig. 2), emphasizing the distinct repertoire of cells found in the CSF vs. peripheral circulation. Because a TCR repertoire analysis of the original CSF samples was not possible (due to the extremely low cell numbers), we cannot rule out that the oligoclonality developed during the *in vitro* expansion period. However, the correlation of γ/δ expansion with disease status, the similarity in TCR gene usage by expanded CSF γ/δ cells among different patients, along with the diversity found in some PBL populations suggest that the oligoclonal γ/δ expansions truly reflect TCR over-represented in the starting populations. Such a restricted TCR usage is indicative of a population of cells selected *in vivo*, presumably by response to antigen. The significance of finding activated CSF γ/δ cells bearing $V_\delta 1$ or $V_\delta 2$–$D_\delta 2$–$D_\delta 3$–$J_\delta 1$ TCR in the present studies is underscored by the detection by Wuchterpfenning *et al.* (12) of the same TCR gene usage ($V_\delta 1$ or $V_\delta 2$ rearranged to $D_\delta 2$–$D_\delta 3$–$J_\delta 1$) in acute lesions. That association, along with our data showing the presence of these cells only in recent-onset MS, emphasizes their potential relevance to disease.

In support of our findings, other investigators have detected γ/δ T cells in MS brain lesions (11, 12). Whereas we found a clear distinction between the presence of these cells in recent-onset versus chronic disease, those studies differed in their correlation with stage of lesion development. Selmaj *et al.* (11) found mostly mAb δTCS1-negative $\gamma\delta$ cells (non-$V_\delta 1$–$J_\delta 1^+$, ref. 28) in chronic silent lesions (extensively demyelinated areas), whereas Wuchterpfennig *et al.* (12) detected a $V_\delta 1$–$J_\delta 1^+$ presence in acute lesions (early active areas preceding extensive demyelination). Both studies detected the presence of heat shock antigens colocalized to the areas of γ/δ cell infiltration. A specific response against heat shock antigen is an attractive hypothesis to explain the $\gamma\delta$ T-cell activation, but the oligoclonal $V_\delta 1^+$ populations found here differ from the $V_\delta 2$-dominated immune response elicited by mycobacterial antigens (29–33). Also, the observed TCR

usage does not suggest a γ/δ-specific superantigen response, where V-region family-specific usage with diverse D and J regions would more likely predominate (34). Alternatively, because γ/δ T cells can recognize viral antigens (35, 36), they might be involved in the immune response against a virus, several of which have been implicated in MS (8, 9).

A role for γ/δ T cells in human autoimmune diseases such as rheumatoid arthritis (37), diabetes (38), polymyositis (39), and sarcoidosis (40, 41) has been previously proposed. All of these diseases, including MS, are characterized by involvement of α/β $CD4^+$ T cells of undefined antigen specificity, especially in the chronic phase of disease. It is interesting that γ/δ cells are prominent in the T-cell response to certain organisms, especially early after infection (13, 14, 30). The important role of γ/δ cells in autoimmune disease may also occur early, as this report suggests for MS. Elucidation of the antigen reactivity and role for γ/δ lymphocytes may be crucial to our understanding of the pathogenesis of this disease.

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1. Traugott, U., Reinherz, E. L. & Raine, C. S. (1982) *Science* **219**, 308–310.
2. Terasaki, P. I., Park, M. S., Opelz, G. & Ting, A. (1976) *Science* **193**, 1245–1247.
3. Martin, R., MacFarland, H. F. & McFarlin, D. E. (1992) *Annu. Rev. Immunol.* **10**, 153–187.
4. Oksenberg, J. R., Stuart, S., Begovich, A. B., Bell, R. B., Erlich, H. A., Steinman, L. & Bernard, C. C. A. (1990) *Nature (London)* **345**, 344–345.
5. Wuchterpfenning, K. W., Ota, K., Endo, N., Seidman, J. G., Rosenzweig, A., Weiner, H. L. & Hafler, D. A. (1990) *Science* **248**, 1016–1019.
6. Ben-Nun, A., Liblau, R. S., Cohen, L., Lehmann, D., Tounier-Lasserve, E., Rosenzweig, A., Jingwu, Z., Raus, J. C. M. & Bach, M.-A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 2466–2470.
7. Kotzin, B. L., Karuturi, S., Chou, Y. K., Lafferty, J., Forrester, J. M., Better, M., Nedwin, G. E., Offner, H. & Vandenbark, A. A. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 9161–9165.
8. Waksman, B. H. (1985) *Nature (London)* **318**, 104–105.
9. McFarlin, D. E. & Lachman, P. J. (1989) *Nature (London)* **341**, 693–694.
10. Raulet, D. H. (1989) *Annu. Rev. Immunol.* **7**, 175–207.
11. Selmaj, K., Brosnan, C. F. & Raine, C. S. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 6452–6456.
12. Wuchterpfennig, K. W., Newcombe, J., Li, H., Keddy, C., Cuzner, M. L. & Hafler, D. A. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 4588–4592.
13. Janis, E. M., Kaufmann, S. H. E., Schwartz, R. H. & Pardoll, D. M. (1989) *Science* **244**, 713–716.
14. Ohga, S., Yoshikai, Y., Takeda, Y., Hiromatsu, K. & Nomoto, K. (1990) *Eur. J. Immunol.* **20**, 533–538.
15. Poser, C. M., Paty, D. W., Scheinberg, L., McDonald, W. I., Davis, F. A., Ebers, G. C., Johnson, K. P., Sibley, W. A., Silberberg, D. H. & Tourtellotte, W. W. (1983) *Ann. Neurol.* **13**, 227–231.
16. Swanson, J. W. (1989) *Mayo Clin. Proc.* **64**, 557–586.
17. Goodkin, D. E., Doolittle, D. H., Hauser, S. S., Ransohoff, R. M., Roses, A. D. & Rudick, R. A. (1991) *Arch. Neurol.* **48**, 805–807.
18. van Wauwe, F. P., de Mey, J. R. & Goossens, J. G. (1980) *J. Immunol.* **124**, 2708–2713.
19. van de Griend, R. J., Borst, J., Tax, W. J. M. & Bolhuis, R. L. H. (1988) *J. Immunol.* **140**, 1107–1110.
20. Borst, J., van Dongen, J. J. M., Bolhuis, R. L. H., Peters, P. J., Hafler, D. A., de Vries, E. & van de Griend, R. J. (1988) *J. Exp. Med.* **167**, 1625–1644.
21. Wu, Y.-J., Tian, W.-T., Snider, R. M., Ritterhaus, C., Rogers, P., LaManna, L. & Ip, S. H. (1988) *J. Immunol.* **141**, 1476–1479.
22. Ciccone, E., Ferrini, S., Bottino, C., Viale, D., Prigione, G., Panteleo, G., Tambussi, B., Moretta, A. & Moretta, L. (1988) *J. Exp. Med.* **168**, 1–11.
23. Krangel, M. S., Yssel, H., Brockelhurst, C. & Spits, H. (1990) *J. Exp. Med.* **172**, 847–859.
24. Bellamy, A. S., Calder, V. L., Feldman, M. & Davison, A. N. (1985) *Clin. Exp. Immunol.* **61**, 248–256.
25. Stamenkovic, I., Stegano, M., Wright, E. A., Kane, S. M., Amento, E. P., Colvin, R. B., Duquesnoy, R. J. & Kurnick, J. T. (1988) *Proc. Natl. Acad. Sci. USA* **85**, 1179–1183.
26. Howell, M., Diveley, J. P., Lundeen, K. A., Esty, A., Winters, S. T., Carlo, D. J. & Brostoff, S. W. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 10921–10925.
27. Lanier, L. L., Ruitenberg, J., Bolhuis, R. L. H., Borst, J., Phillips, J. H. & Testi, R. (1988) *Eur. J. Immunol.* **18**, 1985–1992.
28. Selmaj, K., Brosnan, C. F. & Raine, C. S. (1991) *Neurology* **42**, 795–800.
29. Born, W. K., O'Brien, R. L. & Modlin, R. L. (1991) *FASEB J.* **5**, 2699–2705.
30. Modlin, R. L., Pirmez, C., Hofman, F. M., Torigian, V., Uyemura, K., Rea, T. H., Bloom, B. R. & Brenner, M. B. (1989) *Nature (London)* **339**, 544–548.
31. Fisch, P., Malkovsky, M., Kovats, S., Strum, E., Braakman, E., Klein, B. S., Voss, S. D., Morrissey, L. W., Demars, R., Welch, W. J., Bolhuis, R. L. H. & Sondel, P. M. (1990) *Science* **250**, 1269–1273.
32. DiLibero, C., Casorati, G., Giachino, C., Carbonara, C., Migone, N., Matzinger, P. & Lanzavecchia, A. (1991) *J. Exp. Med.* **173**, 1311–1322.
33. Kabelitz, D., Bender, A., Prospero, T., Wesselborg, S., Janssen, O. & Pechold, K. (1991) *J. Exp. Med.* **173**, 1331–1338.
34. Choi, Y., Herman, A., DiGusto, D., Wade, T., Marrack, P. & Kappler, J. (1990) *Nature (London)* **346**, 471–473.
35. DePaoli, P., Gennari, D., Martelli, P., Cavarzerani, V., Comoretto, R. & Santini, G. (1990) *J. Infect. Dis.* **161**, 1013–1016.
36. Johnson, R. M., Lancki, D. W., Sperling, A. I., Dick, R. F., Spear, P. G., Fitch, F. W. & Bluestone, J. A. (1992) *J. Immunol.* **148**, 983–988.
37. Holoshitz, J., Koning, F., Coligan, J. E., De Bruyn, J. & Stober, S. (1989) *Nature (London)* **339**, 226–229.
38. Lang, F. P., Schatz, D. A., Pollock, B. H., Riley, W. J., Maclaren, N. K., Dumont-Driscoll, M. & Barrett, D. J. (1991) *J. Autoimmunity* **4**, 925–933.
39. Hohlfeld, R. J., Engel, A. G., Ii, K. & Harper, M. C. (1991) *N. Engl. J. Med.* **324**, 877–881.
40. Balbi, B., Moller, D. R., Kirby, M., Holroyd, K. J. & Crystal, R. G. (1990) *J. Clin. Invest.* **85**, 1353–1361.
41. Forrester, J. M., Newman, L. S., Wang, Y., King, T. E. & Kotzin, B. L. (1992) *J. Clin. Invest.*, in press.