Oligoclonal T cell repertoire in cerebrospinal fluid of patients with inflammatory diseases of the nervous system

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Abstract

***Objective*—To evaluate the T cell receptor β chain variable region (TCRBV) gene usage ex vivo in CSF cells and peripheral blood mononuclear cells (PBMCs) collected from patients with autoimmune and inflammatory diseases of the nervous system.**

***Methods*—A novel sensitive seminested-polymerase chain reaction coupled with heteroduplex analysis was developed.**

***Results*—Under these experimental conditions, the minimal number of cells required for the analysis of the whole T cell repertoire was established at 2.5×10^4—sufficient to evaluate most of the samples collected during diagnostic lumbar punctures. In the 21 patients examined, restrictions in TCRBV gene family usage were not seen. However, using heteroduplex analysis, oligoclonal T cell expansions were found in the CSF of 13 patients and monoclonal expansions in five patients. The T cell abnormalities found did not correlate with intrathecal IgG production or with any clinical variable considered.**

***Conclusion*—T cell clonal expansions, useful for further characterisation of pathogenetic T cells, can be found during the course of nervous system inflammations, but this abnormality is probably not disease specific.**

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Keywords: T cell receptor genes; heteroduplex analysis; cerebrospinal fluid; multiple sclerosis

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T lymphocytes play a key part in the pathogenesis of multiple sclerosis and of other autoimmune diseases of the nervous system.[1 2] Identification and characterisation of abnormalities in the T cell repertoire of patients with autoimmune diseases of the nervous system could provide a tool for the development of selective immunotherapies.[3] However, no abnormality has consistently been described in these human diseases.[4 5] So far most studies have focused on the peripheral blood compartment because of the easy access and the many T cells available. Thus far scant data are available directly from the CNS.[6–8] Considering that biopsy of lesions is not applicable in diseases that are not life threatening, the CSF is currently considered the best available source of cells infiltrating the CNS.[5] In addition, studies on multiple sclerosis and on infectious diseases of the nervous system have shown that the population of CSF lymphocytes reflects with a good approximation of the repertoire of inflammatory cells infiltrating the parenchyma.[9–12] However, the number of cells that can be collected from a diagnostic lumbar puncture is limited. Therefore investigations on immune responses in this compartment have mostly been focused on the study of humoral immunity.[11] This approach showed that the presence of intrathecal Ig synthesis with an oligoclonal pattern is a fingerprint of chronic inflammatory states of the CNS. This production reflects an individual specific intrathecal clonal expansion of B cells that, at least in multiple sclerosis, is quite stable with time.[11] On the other hand, over 70% of CSF lymphocytes belong to the T cell population and experimental studies have shown that transfer of T cells, but not of antibodies, may induce passive experimental autoimmune encephalomyelitis (EAE).[13] It may therefore be hypothesised that during CNS inflammatory states, clonally expanded T cell subpopulations in the CSF are particularly relevant for the pathogenesis. Indeed it has been reported that T cell clones isolated from the CSF of patients with multiple sclerosis show restricted T cell receptor (TCR) usage,[7 14 15] but this finding is still controversial.[16 17] These studies indicate that the CSF compartment is probably a useful source of cells for the study of the pathogenesis of autoimmune diseases of the nervous system. However CSF T cell abnormalities detected using in vitro manipulated cell populations cannot reflect the real composition of the in vivo repertoire and in vitro expanded T cell clones cannot be representative of the whole T cell population present in the CSF. In addition, studies focused on the evaluation of abnormalities within TCRBV gene families, cannot rule out the presence of clonal expansions in T cell populations bearing the same TCRBV gene. The development of novel molecular biology techniques focused on the study of gene expression, now makes possible a new approach to this analysis. Particularly, heteroduplex analysis, a simple and sensitive method used to evaluate clonal responding populations can detect cells at a frequency of 1/10 000 and in lymphocyte samples allows tracking of individual clone expansions identified by unique molecular footprints.[18–21]

The aim of this study was to detect clonal expansions in unmanipulated CSF T cells of patients with inflammatory diseases of the nervous system using a seminested RT-polymerase chain reaction (PCR) protocol for parallel amplification of 26 different TCRBV

Table 1 Clinical and experimental data of the included patients, TCRBV gene usage, and HD abnormalities

Patient	Disease	Age	Time from disease onset	Number of CSF cells recovered	TCRBV gene families showing oligoclonal expansions	
					CSF	PB
1	RR-MS	41	4 y	4.6×10^4	2, **9***, 13.1, 19, 20, 22	9, 22
2	RR-MS	21	2 months	2.5×10^4	7	—
3	RR-MS	26	2 y	3.2×10^4	3	—
4	RR-MS	29	2 months	3.0×10^4	**5.1**	—
5	RR-MS	39	2 months	11.7×10^4	—	—
6	RR-MS	28	9 months	3.2×10^4	—	—
7	RR-MS	55	34 y	3.3×10^4	—	—
8	RR-MS	39	6 y	8.0×10^4	—	—
9	CP-MS	60	9 y	3.0×10^4	8, 14, 22	6, 13.1
10	CP-MS	68	8 y	2.5×10^4	5.2, 6, 11, 12	—
11	CP-MS	41	20 y	3.4×10^4	—	10, 24
12	Encephalitis	50	12 months	2.7×10^4	—	—
13	Encephalitis	52	1.5 y	4.0×10^4	2, 4, 5.1, 5.2, 6, 9, 10, 12	—
14	Encephalitis	40	9 months	15.0×10^4	12, **21**	—
15	Myelitis	33	10 months	6.0×10^4	5.2	—
16	Myelitis	61	1 y	2.5×10^4	2, 4, 24	—
17	Neuropathy	49	1 months	6.5×10^4	—	—
18	Neuropathy	40	7 months	3.0×10^4	**6**, 13.1, 17, **22**	—
19	Neuropathy	39	1 months	2.8×10^4	5.2	—
20	CNS vasculitis	34	4 y	2.8×10^4	9, **12**, 22	—
21	CNS vasculitis	21	4 y	3.7×10^4	—	—

RR-MS=Relapsing-remitting multiple sclerosis; CP-MS=chronic-progressive multiple sclerosis.
*The bold TCRBV gene families show monoclonal patterns.

gene families combined with the analysis of hetero/homoduplex formation of the PCR products. Noteworthy, this method allowed direct ex vivo analysis of the few T cells that can be obtained by routine diagnostic lumbar puncture.

Methods

PATIENTS

Twenty one patients admitted to the inpatient Clinic of the University of Florence Department of Neurology undergoing through diagnostic lumbar puncture yielding at least 2.5×10^4 cells, were included: mean age 41 years, range 21–68 (table 1). All patients had given written informed consent to the procedure. From these patients blood and CSF were collected at the same time.

The patients included had the following diagnoses: relapsing-remitting multiple sclerosis according to Poser *et al*,[22] eight patients (five women and three men; mean age 34 years; range 21–55; mean disease duration 6 years); chronic progressive multiple sclerosis,[22] three patients, (two men and one woman; mean age 56 years; range 41–68; mean disease duration 12 years); encephalitis, three patients (one woman and two men, mean age 47, range 40–52, mean time from onset 14 months); myelitis, two patients (two men, mean age 47, range 33–61, mean disease duration 11 months); neuropathy, three patients (one woman and two men, mean age 43, range 39–49, mean disease duration 8 months); CNS vasculitis, two patients (one woman and one man, mean age 28, range 21–34, time from onset 4 years) (table 1).

The patients included had not received corticosteroids in the previous 6 weeks or drugs active on the immune system in the previous 3 months. In addition the patients included had an erythrocyte sedimentation rate of less than 15 mm/h, blood leucocyte number/mm^3 less than 9000, were afebrile, and did not have symptoms of infection (for example, bladder infections).

CELL ISOLATION, PREPARATION OF RNA, AND cDNA CDNA SYNTHESIS

Cells were obtained from the CSF by centrifugation; peripheral blood mononuclear cells (PBMCs) were separated on Ficoll Hypaque gradients. The amount of PBMCs was adjusted to prepare total RNA from exactly the same number of CSF and peripheral blood cells. The same trained person carried out the cell counts. The variability of the cell counts was less than 5%. Total RNA was prepared from each sample (2.5×10^4-1.5×10^5 CSF cells and PBMCs) by guanidium thiocyanate-phenol-chloroform extraction.[23] The RNA preparation was used to synthesise the first strand of the TCRCB specific cDNA by the First Strand cDNA Synthesis Kit (Roche Biochemicals), according to the manifacturer's instructions.

SEMINESTED RT-PCR

Primers

Twenty six different TCRBV specific oligonucleotides and two nested TCRCB-specific oligonucleotides were used as primers for the PCR amplifications. The sequences of the primers used are given in table 2.

PCR amplifications

Simultaneous amplifications were carried out by splitting each cDNA preparation in 26 microtubes, each containing one TCRBV specific primer and a TCRCB specific primer. cDNA was combined in 50 µl PCR mixture containing 1.5 U DNA polymerase, dNTPs (Roche Diagnostics, Monza, Italy) at a final concentration of 250 µM each, and 25 pmol of each primer in the enzyme buffer. The first 30 cycle amplification was carried out in a thermal automatic cycler (9600 Perkin Elmer; Norwalk CT, USA), with a profile of 94°C for 30 seconds, 56°C for 25 seconds, and 72°C for 30 seconds, followed by a 10 minute extension. The nested amplification was carried out with the same profile using an aliquot of 1 µl from the first reaction as template. The length of the

Table 2 List of primers

TCRBV gene family	*3'-5' sequence*
1	GCACAACAGTTCCCTGACTTGCAC
2	TCATCAACCATGCAAGCTGACCT
3	GTCTCTAGAGAGAAGAAGGAGCGC
4	GCCCAAACCTAACATTCTCAACTC
5.1	ATACTTCAGTGAGACACAGAGAAA
5.2	TTCCCTAACTATAGCTCTGAGCTG
6	AGGCCTGAGGGATCCGTCTC
7	CCTGAATGCCCCAACAGCTCTC
8	ATTTACTTTAACAACAACGTTCCG
9	CCTAAATCTCCAGACAAAGCTCAC
10	CTCCAAAAACTCATCCTGTACCTT
11	TCAACAGTCTCCAGAATAAGGACG
12	AAAGGAGAAGTCTCAGAT
13.1	CAAGGAGAAGTCCCCAAT
13.2	GGTGAGGGTACAACTGCC
14	GTCTCTCGAAAAGAGAAGAGGAAT
15	AGTGTCTCTCGACAGGCACAGGCT
16	AAAGAGTCTAAACAGGATGAGTCC
17	AGATATAGCTGAAGGGTACAGCGT
18	GATGAGTCAGGAATGCCAAAGGAA
19	CAATGCCCCAAGAACGCACCCTGC
20	AGCTCTGAGGTGCCCCAGAATCTC
21	TTCTGCAGAGAGGCTCAAAGGAGT
22	CTCAGTTGAAAGGCCTGATGGATC
23	CTCAGCTCAACAGTTCAGTGACTA
24	CCAATCCAGGAGGCCGAACACTTC
BcDNA*	GGGCTGCTCCTTGAGGGGCTGCGG
BAI†	CCCACTGTGCACCTCCTTCC
NESTED‡	TTCTGATGGCTCAAACACAGCGAC

*TCRBC specific primer used for the cDNA synthesis.
†TCRBC specific primer used for the RT-PCR.
‡TCR nested C region specific primer used for the semi-nested RT-PCR.

PCR products was 180–220 bp. The products migrated within the expected molecular weight range.

Evaluation of primer specificity

The specificity of the 26 primers was confirmed by hybridising the nested PCR products, blotted on a nylon membrane, with 26 TCRBV specific DIG labelled probes. Briefly, the TCRBV specific probes were synthesised by labelling the primers themselves as follows: each of the 26 TCRBV family specific probes (50 pmol) was labelled with digoxigenin-11-ddUTP using the oligonucleotide 3'-end labelling kit (Roche Biochemicals) according to the manufacturer's instructions. Gels containing the PCR products were run and blotted onto nylon membranes (Hybond+, Amersham) using 20X SSC buffer for 16–20 hours. The transferred DNA was fixed to the membrane by UV cross linking. As with any probe, optimal hybridisation conditions were experimentally determined considering probe length and G+C content. Membranes were prehybridised for 1 hour at hybridisation temperature in 5X SSC, 0.1% N-laurylsarcosine, 0.02% SDS, and 1% blocking reagent (Roche Biochemicals), then hybridised in the same solution for 18 hours. After hybridisation, membranes were washed twice in 5X SSC, 0.1% SDS at room temperature and twice in 0.5X SSC, 0.1% SDS at a temperature previously determined. An alkali conjugated antidigoxigenin antibody was allowed to bind to the hybridised probe. The signal was detected with CSPD, chemiluminescent alkaline phosphatase substrate (Roche Biochemicals).

Each probe hybridised only the corresponding amplification product.

EVALUATION OF THE SENSITIVITY OF THE SEMINESTED PCR PROTOCOL

Freshly isolated PBMCs were collected from one healthy donor. The T cells were typed for TCRBV gene usage using a panel of 17 TCRBV-specific monoclonal antibodies (Coulter/Immunotech, Inc, Westbrook, Maine). The relative percentage of lymphocytes bearing each TCRBV gene family was determined by FACS analysis. Each sample was then serially diluted according to the percentages previously determined.

EVALUATION OF EFFICIENCY OF TCRBV SPECIFIC PRIMERS

The T cell clones were obtained as previously described.[24] The clones were typed for TCRBV gene usage by cytofluorimetry as above. T cell clones expressing TCRBV 3, TCRBV 5.1, TCRBV 16, TCRBV 20, and TCRBV 21 genes were selected. Serial dilutions of the T cell clones were obtained diluting 100, 50, 10, and 5 cells belonging to each clone in 5×10^4 T cells expressing a different TCRBV gene family. TCRCB specific cDNA prepared from each dilution was amplified according to the above seminested RT-PCR protocol.

HETERODUPLEX ANALYSIS

Clonality of T cells in each PCR product was evaluated as follows: a total of 20 µl of each PCR reaction was denatured at 95°C for 5 minutes and then maintained at 50°C for 1 hour.[18] This temperature allowed the annealing between homologous (homoduplex) or heterologous DNA strands. Heterologous DNA strands (heteroduplex) may have the same BV region but differ in the J and the N-d-N regions. The samples were kept on ice until loading. Heteroduplex and homoduplex bands were separated at room temperature on a 12% non-denaturing gel (29:1 acrylamide-bisacrylamide; gel size 16 cm×18 cm×1 mm) in TBE buffer at 150 V for 4 hours. Heteroduplex patterns were disclosed by ethidium bromide staining.

SEQUENCING OF SELECTED PCR PRODUCTS

Sequence analysis of TCRBV gene products was used to further characterise T cell clonal expansions, using the CDR3 sequence and the TCRBJ segment usage as specific markers of clonality.[25] For this purpose three amplified PCR products showing a monoclonal pattern were sequenced with an automated laser fluorescent ALF DNA Sequencer (Pharmacia LKB, Uppsala, Sweden) using a Cy5 thermo sequenase dye terminator kit. To discriminate the length of the TCR gene segments, sequences were compared with published data.[25–27]

Results

EVALUATION OF THE SEMINESTED AMPLIFICATION PROTOCOL SENSITIVITY

Sensitivity of the whole seminested PCR protocol and relative efficiency of primer pairs was evaluated using five clonal T cell populations expressing respectively TCRBV 3, TCRBV 5.1, TCRBV 16, TCRBV 20, and

Table 3 Evaluation of the sensitivity of the whole seminested PCR protocol and relative efficiency of 5 primer pairs

Number of relevant T cells	TCRBV clones				
	TCRBV 3	TCRBV 5.2	TCRBV 16	TCRBV 20	TCRBV 21
100	+	+	+	+	+
50	+	+	+	+	+
10	+	+	+	+	+
5	−	−	+	−	+

TCRBV 21. Diluting each T cell clone in other T cells bearing different TCRBV genes, the method detected the expression of the TCR gene family in as little as 5–10 cells/50 000 with no remarkable difference among the different TCRBV genes chosen (table 3). In addition, sensitivity of the method was evaluated in serial dilutions of freshly isolated normal PBMCs previously typed for the TCRBV gene repertoire by cytofluorimetry using a panel of 17 TCRBV chain specific monoclonal antibodies (table 4). The lowest number of cells that allowed amplification of TCRBV genes by a seminested PCR was determined. A complete TCRBV repertoire was consistently obtained by using as few as 2.5 (SDS 1.25)$\times 10^4$ PBMC (table 4). This amount, for the less represented T cell populations, was established as 250 (SD 12) cells.

SENSITIVITY AND REPRODUCIBILITY OF THE HETERODUPLEX ANALYSIS PROTOCOL FOR THE STUDY OF T CELL CLONALITY

To exclude the possibility that dilution determines artifactual deletion of those T cells bearing the less represented TCRBV families, heteroduplex analysis was carried out at limiting dilution of T cells using 1.25×10^4 normal PBMCs. Each of the 26 TCRBV family PCR products obtained from this number of cells were consistently polyclonal in three separate experiments (data not shown). In addition, heteroduplex analysis of the PCR products obtained from single T cell clones consistently showed the same single band of migration in two separate experiments (data not shown). These data indicate that under these experimental conditions (unselected polyclonal T cell samples), the heteroduplex analysis is sensitive enough to allow the use of as little as 1.25×10^4 PBMC, a number that for the less represented TCRBV genes bearing subpopulations may account for as little as 125 cells per TCRBV family; the heteroduplex analysis protocol used is reproducible enough to consistently allow evaluation of clonal expansions in unstimulated CSF T cells. Therefore, these data exclude the possibility of detecting artifactual mono/oligoclonal patterns of migration due to dilution.

Heteroduplex analysis results were confirmed by direct automatic sequencing carried out on three samples showing a heteroduplex analysis monoclonal pattern: the presence of the same N-d-N region sequence in each sample confirmed the validity and the sensitivity of the heteroduplex analysis protocol (data not shown).

ASSESSMENT OF T CELL CLONALITY IN CSF CELLS FROM PATIENTS WITH NERVOUS SYSTEM INFLAMMATORY DISEASES

Products of PCR obtained from CSF samples containing at least 2.5×10^4 cells and the same number of PBMC collected from 21 patients undergoing diagnostic lumbar puncture, were evaluated for the presence of clonal expansions (table 1). All patients showed a virtually complete TCRBV gene repertoire both in CSF cells and in PBMCs. However, heteroduplex analysis of TCRBV gene products obtained from different CSF samples showed discrete banding, indicating the presence of 37 clonal T cell expansions in the CNS that were not present in the periphery (fig 1). These abnormalities, although they did not segregate with any particular TCRBV gene, involved 13 out the 21 patients included (6/11 with multiple sclerosis; 2/3 with encephalitis, 2/2 with myelitis; 2/3 with inflammatory peripheral neuropathies; 1/2 with CNS vasculitis). Of note is that five out of the 37 clonal expansions showed the presence of one monoclonal TCRBV family (2/11 with multiple sclerosis; 1/3 with encephalitis; 1/3 with inflammatory peripheral neuropathies; with 1/2 CNS vasculitis). The presence of T cell oligoclonal expansions was not associated with the presence of an oligoclonal pattern of intrathecal IgG production that was seen in nine patients with CNS inflammation (data not shown). Three of the patients included showed discrete TCRBV gene banding also in PBMCs, but this banding involved TCRBV families different from those seen in the CSF. One patient showed an oligoclonal pattern of migration in PBMC T cells bearing BV9, which was detected as monoclonal in CSF.

Table 4 Evaluation of the TCRBV genes amplification protocol in serial dilutions of a normal PBMC sample previously typed for the TCRBV gene repertoire

Number of PBMC	TCRBV gene families																
	2	3	5.1	5.2	6	8	9	11	12	13.1	14	16	17	20	21	22	23
10^5	+ (5900)*	+ (2700)	+ (2900)	+ (400)	+ (2700)	+ (3300)	+ (2100)	+ (1200)	+ (1100)	+ (1900)	+ (1600)	+ (1800)	+ (4000)	+ (1700)	+ (2000)	+ (2600)	+ (2300)
5×10^4	+ (2950)	+ (1350)	+ (1450)	+ (200)	+ (1350)	+ (1650)	+ (1050)	+ (600)	+ (550)	+ (950)	+ (800)	+ (900)	+ (2000)	+ (850)	+ (1000)	+ (1300)	+ (1150)
2.5×10^4	+ (1475)	+ (675)	+ (725)	+ (100)	+ (675)	+ (825)	+ (525)	+ (300)	+ (275)	+ (475)	+ (400)	+ (450)	+ (1000)	+ (425)	+ (500)	+ (650)	+ (575)
2×10^4	− (1180)	+ (540)	+ (580)	+ (80)	+ (540)	− (660)	− (420)	− (240)	− (220)	+ (380)	− (320)	− (360)	+ (800)	+ (340)	+ (400)	+ (520)	+ (460)

*The absolute number of T cells expressing each TCRBV chain is reported in parentheses. It was obtained by cytofluorimetry using a panel of 17 different TCRBV chain specific monoclonal antibodies.

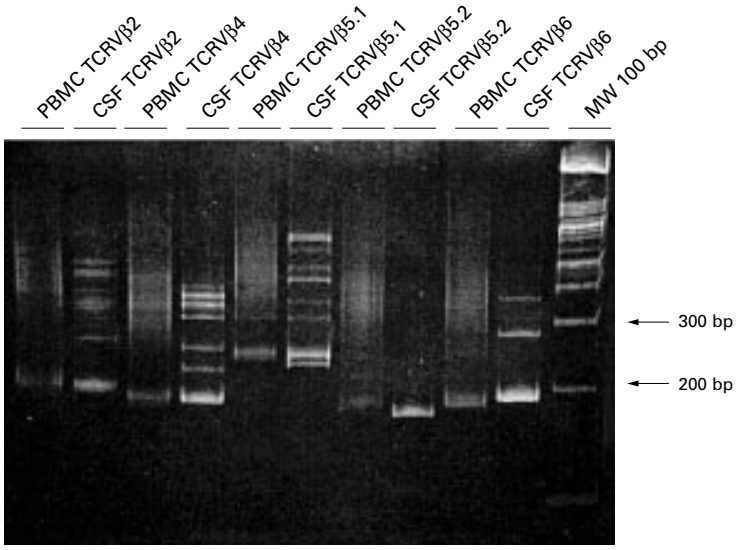

Figure 1 Heteroduplex analysis of TCRBV genes expressed by CSF cells and PBMC obtained from a patient with peripheral neuropathy. The PBMC samples analysed do not generate evidence of homo/heteroduplex bands, thus indicating a polyclonal pattern of TCRBV expression. PCR products obtained from CSF cells that express TCRBV 2, TCRBV 4, TCRBV 5.1, TCRBV 6 produce few bands, thus indicating an oligoclonal pattern of TCRBV expression. CSF cells express TCRBV 5.2 in monoclonal form, producing only one band.

Discussion

In autoimmune diseases the finding of T cell clonal expansions in target organs is considered a relevant hallmark for the presence of pathogenetic cells, but the limited number of cells that can usually be collected from biological fluids has thus far prevented ex vivo analysis of unselected T cell populations. In this study TCRBV gene usage was evaluated in unstimulated CSF cells and PBMC collected from nervous system autoimmune and inflammatory diseases. For this purpose a very sensitive method consisting of seminested RT-PCR coupled with heteroduplex analysis was applied to characterise the limited number of cells that were collected from diagnostic lumbar puncture.

A preliminary evaluation of the methodology showed that the protocol was sensitive enough to allow the analysis of single TCRBV gene family usage from as little as 5–10 clonal T cells (table 3). No relevant difference was found in the amplification protocol using the 26 different TCRBV specific oligonucleotides, demonstrating that the efficiency of the various primer pairs was comparable (table 4). In addition, it was previously shown that an identical pattern of banding was consistently obtained by repeating the heteroduplex analysis on the same PCR products, indicating that these techniques can identify fingerprints of clonal expansions.[20]

However, when unselected PBMC populations are evaluated, contemporary analysis of 26 TCRBV gene families is needed. For the less represented TCRBV gene family bearing subpopulations this splitting contributed to limit the sensitivity to a total of 2.5 (SD 1.25)$\times 10^4$ mononuclear cells (table 4). This finding was consistent with that carried out on T cell clones of which the TCRBV usage was known. Indeed, in unselected PBMC samples, the less represented T cell subpopulations accounted for about 1% of the lymphocytes (250 (SD 10) cells). Therefore the genomic material used for each PCR, divided by 26 for separated amplification of each BV gene family, was equivalent to that of 10 cells. The sensitivity of the protocol used in this study was higher than the limit determined by the number of cells collected from diagnostic lumbar puncture.

Finally, the possibility that the limited number of cells could fall beyond the heteroduplex protocol sensitivity limit was investigated using PCR products obtained from half of the cell number that allowed the amplification of all the TCRBV gene families: 1.25 (SD 0.65)$\times 10^4$. Under these experimental conditions, the less represented TCRBV gene families expressing subpopulations may account of as little as 125 (SD 5) cells. Heteroduplex analysis showed a polyclonal pattern indicating that at this cell dilution, the T cell repertoire is not affected by artefacts and therefore the analysis is reliable.

Once the sensitivity limits of this method were determined, 21 patients with nervous system inflammatory diseases, whose lumbar puncture yielded at least 2.5×10^4 mononuclear cells, were included in the study. The TCRBV gene expression was analysed in matched CSF cells and PBMC samples collected from the same people at the same time. Virtually all the investigated TCRBV genes were amplified in all patients in both compartments. However, parallel heteroduplex analysis in CSF cells and in PBMCs detected the presence of oligoclonal patterns in CSF indicating the presence of CNS clonal T cell expansions in 13 patients and monoclonal expansions in five patients. Sequence analysis of three selected PCR products showing monoclonal expansion confirmed that the method can consistently detect T cell clonal expansions and validates the biological relevance of heteroduplex analysis carried out in a limited number of cells. The mono/oligoclonal pattern was detected in any TCRBV family, indicating that T cells probably relevant for the pathogenesis of these diseases are not biased for TCRBV gene usage. In addition, the clonal abnormalities were not associated with the presence of intrathecal IgG production or with any clinical variable taken into consideration.

These data indicate that T cell clonal expansions are present during the course of nervous system inflammation and that evaluation of these abnormalities is feasible in the diagnostic lumbar puncture collecting at least 2.5×10^4 cells that represent most of the samples obtained during routine clinical activity. However, T cell clonal expansions seem nonspecifically associated with different intrathecal inflammatory conditions and therefore this marker may probably not be useful for diagnostic purposes. In addition, some degree of variability was seen within each disease suggesting that T cell clonal expansions may be associated with specific stages of nervous system inflammatory diseases as well as the

genetic background of the patient. However, analysis of T cell clonal expansions in CSF cells should be useful, at least, for further characterisations of cells probably relevant for inflammatory disease pathogenesis. Indeed, the overrepresented gene products could therefore be used as markers for identifying potentially pathogenetic cell populations and for further in vitro characterisation. This procedure, narrowing the cell populations potentially relevant for specific antigen recognition, may increase the chances of successful identification of pathogenetic T cells.

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