

# Patterns of cerebrospinal fluid pathology correlate with disease progression in multiple sclerosis

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## Summary

Multiple sclerosis is a chronic inflammatory and demyelinating disease of the CNS with, as yet, an unknown aetiology. Temporal profile, intensity and treatment responses are highly variable in multiple sclerosis suggesting pathogenetic heterogeneity. This hypothesis has been supported by histopathological studies disclosing at least four different subtypes of acute demyelinating lesions. Although stratification of multiple sclerosis patients into these categories would be extremely helpful for clinical studies, this approach is impractical as it requires brain biopsy. In this study we investigated CSF cytology from 60 multiple sclerosis patients by flow cytometry. We identified different patterns of CSF cytology, which were independent of

immunological parameters in the peripheral blood. The most variable CSF parameter was the B cell to monocyte ratio, which remained stable during different phases of disease in selected patients. The ratio correlated with disease progression but not with disability or disease duration in a retrospective, consecutive analysis. A high ratio (predominance of B cells) was associated with more rapid disease progression, whereas a low ratio (predominance of monocytes) was found in patients with slower progression. Our study demonstrates the existence and potential clinical relevance of different CSF cytology patterns. We hypothesize that CSF cytology patterns may reflect the heterogeneity in the pathogenesis of multiple sclerosis.

**Keywords:** multiple sclerosis; cerebrospinal fluid; B cells; monocytes

**Abbreviations:** APC = allophycocyanin; B/M ratio = B cells/monocytes ratio; EDSS = Expanded Disability Status Scale; FCS = foetal calf serum; FITC = fluorescein isothiocyanate; mAb = monoclonal antibody; NIND = non-inflammatory neurological disease; NK = natural killer; PB = peripheral blood; PE = phycoerythrin; PerCP = peridinin chlorophyll protein; PP = primary progressive; RR = relapsing–remitting; SP = secondary progressive; TCR = T-cell receptor

## Introduction

Multiple sclerosis is the most common inflammatory CNS disease usually affecting young adults (Noseworthy, 1999). Although the role of genetic (Ebers and Dyment, 1998) and environmental factors (Sibley *et al.*, 1985) in the pathogenesis has been well established, the cause of multiple sclerosis is still unknown. Based on observations in experimental animal models (Wekerle *et al.*, 1994) it is widely believed that multiple sclerosis is mediated by an autoimmune response against CNS myelin antigens (Martin *et al.*, 1992). However, target antigens and the mechanisms leading to chronic inflammation and tissue destruction are largely unknown.

Differences in the clinical phenotype with respect to the temporal profile and disease intensity (Weinshenker, 1998), as well as the highly variable responses to therapies

(Noseworthy, 1999), suggest the existence of different pathogenetic mechanisms involved in the development of multiple sclerosis. Recent MRI findings (Lee *et al.*, 1999) and histopathological studies (Brosnan and Raine, 1996; Lucchinetti *et al.*, 1998) strongly support this hypothesis. In a systematic histopathological analysis of <230 acute demyelinating lesions from 83 biopsies or autopsies, four distinct patterns were defined (Lucchinetti *et al.*, 2000). These were stratified according to the quality of inflammation, the pattern of demyelination, the extent of oligodendrocyte damage and the occurrence of remyelination. The patterns were heterogeneous between patients, but homogeneous in different lesions of individual patients. However, brain biopsies are only performed in multiple sclerosis patients to

diagnose atypical disease or to exclude other CNS disorders. Therefore, it would be difficult to correlate the pattern of histopathological changes to the clinical course in a representative group of multiple sclerosis patients.

Since inflammatory parameters in the CSF correlate with the extent of CNS lesions (Farlow *et al.*, 1987; Frederiksen *et al.*, 1992), CSF abnormalities could reflect lesion pathology. In our study, we focused on CSF abnormalities in a representative group of multiple sclerosis patients in order to search for cytological heterogeneity in relation to clinical parameters. Although many studies compared cellular and protein changes in CSF between multiple sclerosis patients and control donors or patients with other inflammatory CNS diseases (Matsui *et al.*, 1990; Svenningsson *et al.*, 1993; Oreja-Guevara *et al.*, 1998), no study has systematically addressed the question of heterogeneity of CSF cytology in multiple sclerosis patients. In this study we determined the cellular and protein changes in CSF and peripheral blood (PB) of 60 patients with clinically or laboratory-supported definite multiple sclerosis by nephelometry, isoelectric focusing and flow cytometry. The results were then correlated with clinical disease status and disease progression [Expanded Disability Status Scale (EDSS)/disease duration] in a retrospective, consecutive analysis. The aim of the study was to identify distinct patterns of CSF cytology and relate them to clinical disease parameters.

## Material and methods

### Nomenclature

The cluster of differentiation terminology for the description of immune system antigens is used throughout the manuscript.

### Patients

Multiple sclerosis patients and controls were recruited at the Department of Neurology at the Philipps-University, Marburg. Sixty patients (mean age 37 years, range 17–69 years) with clinically definite or laboratory-supported definite multiple sclerosis according to the Poser criteria were included in the study (Poser *et al.*, 1983). Forty-seven patients had relapsing remitting (RR) multiple sclerosis, seven patients had secondary progressive (SP) multiple sclerosis and five patients had primary progressive (PP) multiple sclerosis. All patients had lumbar spinal tap and MRI of the CNS using T<sub>1</sub>-weighted sequences, with and without gadolinium, and T<sub>2</sub>-weighted sequences. At the time of the spinal tap, five out of 60 patients had immunosuppressive or immunomodulatory therapy. Intrathecal IgG synthesis or oligoclonal bands were detected in 57 out of 60 patients. An independent blinded neurologist examined all patients with regard to the EDSS score and the clinical course (Rudick *et al.*, 1996). All patients were carefully interviewed to check for the occurrence of unequivocal first symptoms compatible with multiple sclerosis (visual symptoms, motor and sensory disturbances

and ataxia) by one of the study physicians. The information about the first symptoms was compared with the medical chart to obtain reliable data about disease duration. The calculation of disease progression ( $\Delta$ EDSS) was determined by dividing the EDSS score by the number of years since onset of disease (first symptoms compatible with multiple sclerosis, as determined retrospectively; Weinshenker *et al.*, 1997). We assumed an EDSS score of 0 for all patients before developing initial symptoms. This approach was necessary to overcome the statistical difficulties with the non-linear EDSS scale. To obtain reliable results on disease progression, only patients with a disease duration of at least 3 years (mean duration 11.8 years, range 3–37 years) were included. Twenty-one of those patients had RR multiple sclerosis, six SP multiple sclerosis and four PP multiple sclerosis. Six patients had an immunomodulatory or immunosuppressive therapy during the observation period. Six patients had a second spinal tap. Five of those patients were examined during a clinical active and a silent disease phase.

The control group (mean age 42 years, range 16–79 years) consisted of patients with non-inflammatory neurological diseases (NINDs) without evidence of intrathecal immune response (normal white cell count and IgG, IgA and IgM levels as defined by the Reiber formula) (Reiber, 1995). The NIND group included 21 patients: eight with headache, four with Bell's palsy, two with pseudotumour cerebri, one with lower back pain, four patients with non-inflammatory polyneuropathy, one with hypertension-induced small vessel disease and one with benign intracranial hypertension. Multiple sclerosis patients gave written informed consent. The study was approved by the University of Marburg ethics committee.

### CSF specimens

Eight to fifteen millilitres of CSF was obtained by lumbar spinal tap from multiple sclerosis patients and NIND patients. At the same time, 2 ml peripheral blood was collected. CSF white cell count was determined in all patients. CSF and serum were examined for protein, albumin and immunoglobulin G, A and M levels by nephelometry (BN II; Behring, Marburg, Germany). The specific intrathecal production of IgG, IgA and IgM was calculated according to the Reiber formula for each patient (Reiber, 1995). CSF and serum were analysed for oligoclonal bands by isoelectric focusing and IgG immunoblot following the manufacturer's instructions (Titan Gel; Rolf Greiner Biochemica, Flacht, Germany).

### Flow cytometry on whole blood

Peripheral blood staining was performed as described (Muraro *et al.*, 2000). Briefly, fresh blood was diluted 1:1 with phosphate-buffered saline supplemented with 5% foetal calf serum (FCS), and 200  $\mu$ l of the mixture added to each well of a round-bottom 96-well plate (Nunc, Roskilde, Denmark).

To avoid cross-contamination, each well containing blood was left surrounded by empty wells. The plate was centrifuged at 200 *g* for 5 min, the supernatant discarded and the top of the plate briefly dried on paper towels. The plate was then placed on ice and cell pellets were resuspended adding directly the monoclonal antibody (mAb) combinations from cluster tubes and mixing for 10 s with a multi-channel pipette. The mAbs were conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP) or allophycocyanin (APC), respectively. After 30 min of incubation on ice in the dark, erythrocytes were lysed in two steps using 200  $\mu$ l of lysing solution (BD, NJ, USA) following the manufacturer's instructions. The plate was then centrifuged and the supernatants discarded as described above. After two additional washes with 200  $\mu$ l of ice-cold PBS (phosphate-buffered saline) 2% FCS, cells were transferred to 5 ml Falcon-tubes (BD) and analysed on a Flow Cytometer (FACScalibur; BD). The following mAbs were used: anti-CD4-APC (clone RPA-T4), anti-CD19-APC (clone HIB19), anti-CD45-APC (HI 30) (Pharmingen, NJ, USA); anti-CD138-PE (clone B-B4), anti-CD14-FITC (clone RMO52), anti-T-cell receptor (TCR)  $\alpha/\beta$ -PE (clone BMA 031), anti-TCR  $\gamma/\delta$ -FITC (clone Immu 510), anti-CD3-FITC (clone UCHT-1) (Immunotech, NJ, USA); anti-CD8-PerCP (clone SK1), anti-CD16-PE (clone B 73.1), anti-CD56-PE (clone NKAM 16.2), anti-CD3-PerCP (clone SK 7) (all BD). These stainings were performed in the following combinations: (i) CD3-FITC, CD56/16-PE, CD8-PerCP and CD4-APC; (ii) CD14-FITC, CD138-PE, CD3-PerCP and CD19-APC; (iii) TCR  $\gamma/\delta$ -FITC, TCR  $\alpha/\beta$ -PE, CD3-PerCP and CD45-APC; and (iv) IgG-FITC, IgG-PE, IgG-PerCP and IgG-APC (isotype control staining).

### Flow cytometry on CSF cells

Fresh CSF was immediately spun down at 200 *g* for 10 min, the supernatant removed and the pellet resuspended in an appropriate volume of phosphate-buffered saline 5% FCS, depending on the white cell count of individual samples. A minimum number of 4000 cells was required for each staining combination, and a volume of 30  $\mu$ l was used for each staining. The staining was performed as described for whole blood cells (Muraro *et al.*, 2000). No lysing step was applied to CSF cells because no red blood cells were contained in these specimens. Cells were then washed once with PBS 5% FCS and analysed as described above. Because of the small cell number obtained from control donors and some multiple sclerosis patients, staining combinations (iii) and (iv) were not performed in every patient.

Lymphocytes and monocytes were gated according to forward and sideward scatter properties. For each stained blood sample 10 000 events, and for each CSF sample at least 1000 events, were usually acquired. Subpopulations of T cells were analysed in the lymphocyte gate (R1 in Fig. 1A). The percentages of CD4<sup>+</sup>, CD8<sup>+</sup> T cells,  $\alpha/\beta$  T cells and  $\gamma/\delta$  T cells were determined in the R1 gate. The numbers

of natural killer (NK) cells and NK-like T cells were also determined in the lymphocyte gate R1 to avoid contamination by CD56<sup>+</sup> and CD16<sup>+</sup> monocytes using the combination of mAbs against CD16/CD56 and CD16/CD56/CD3, respectively. B cells, monocytes and plasma cells were determined in the lymphocyte/monocyte gate (R2 in Fig. 1A). To exclude possible cell debris or occasional erythrocytes, a sample from each individual patient was stained with an mAb for CD45 to determine the number of nucleated haematopoietic cells. The results are given as percentages of all CD45 positive cells.

### Statistical analysis

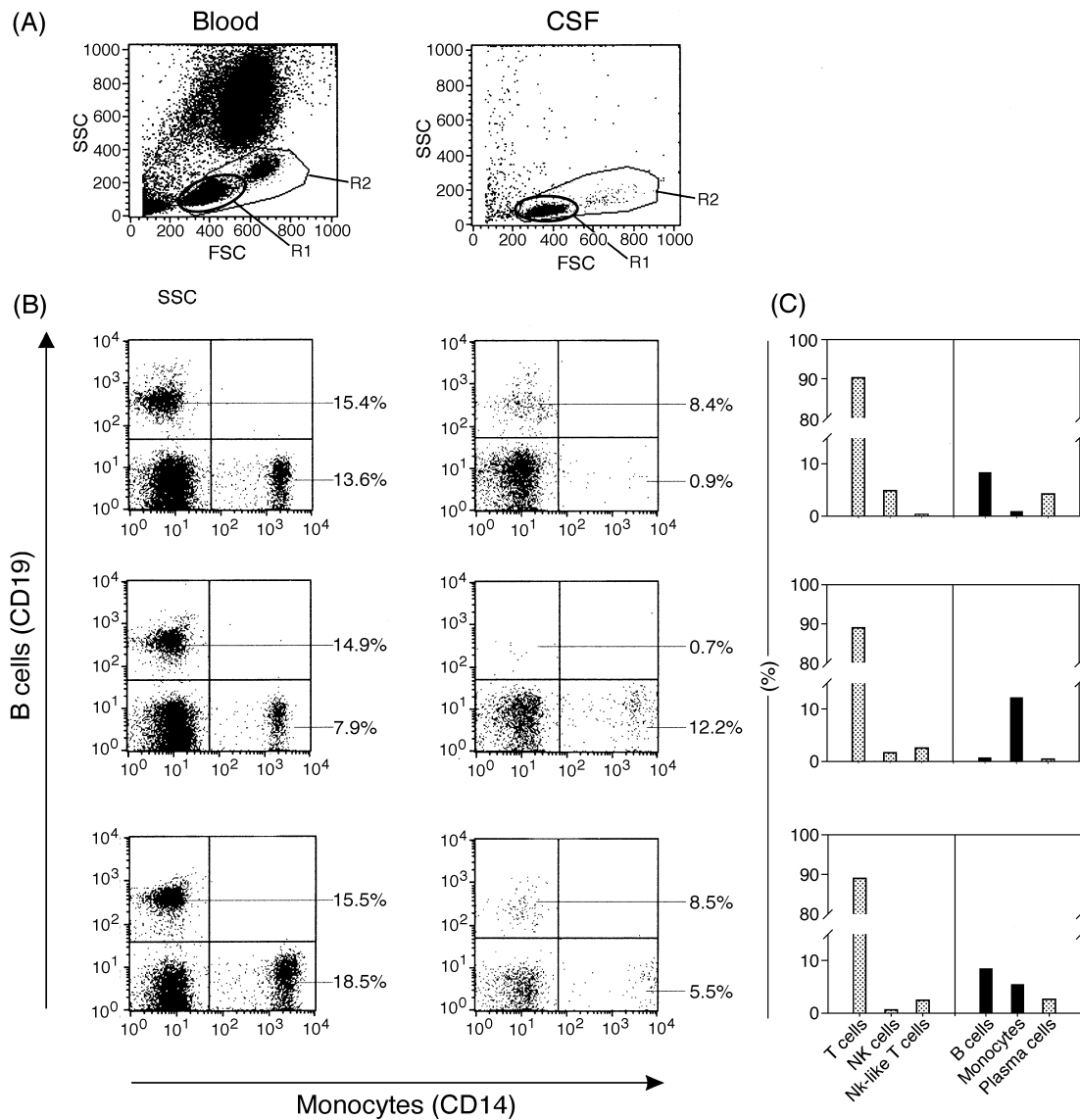
We used the paired *t*-test to compare the mean percentages of expression of each subpopulation between PB and CSF in each patient, as the differences within patients can be assumed to be normally distributed. The Mann-Whitney *U*-test was used for the comparison of the median percentages of expression between multiple sclerosis patients and the control groups. Spearman's rank correlations between CSF and clinical parameters were calculated. Disease progression ( $\Delta$ EDSS) was defined by the EDSS divided by the disease duration. Only patients with a disease duration of at least 3 years were included. To adjust for multiple testing of a total of 43 hypotheses, the significance threshold of 5% was Bonferroni-corrected. Hence, a *P* value of  $0.05/43 = 0.0012$  was considered significant.

## Results

### Comparison of CSF and blood leucocyte populations from multiple sclerosis patients and control donors

CSF and PB from 60 multiple sclerosis patients with clinically definite multiple sclerosis or laboratory-supported definite multiple sclerosis were analysed. Multiple sclerosis patients were compared with a group of 21 patients with NIND. The initial comparison included levels of protein, albumin, IgG, IgA and IgM in serum and CSF. In addition, we determined the phenotype of the cellular compartments using monoclonal antibodies specific for CD45 (all nuclear haematopoietic cells), CD3 (T cells), CD19 (B cells), CD138 (plasma cells), CD16/CD56 (NK and NK-like T cells), CD14 (monocytes), CD4 (helper T cells), CD8 (cytotoxic T cells), TCR- $\alpha/\beta$  and TCR- $\gamma/\delta$ . These mAbs enabled determination of the absolute number of each cell type and the relative percentage of all subpopulations from the pool of leucocytes in PB and CSF.

First, we compared leucocyte subsets in PB between multiple sclerosis patients and NIND patients. Although intra-individual variations were noticed for all leucocyte subsets, no significant differences were observed between multiple sclerosis and NIND patients in PB (Table 1). In contrast, highly significant differences were observed for immunoglobulin levels and leucocyte subsets in CSF between multiple



**Fig. 1** Distinct patterns of CSF pathology in patients with multiple sclerosis. **(A)** Histogram plots of all leucocytes are shown for blood (left) and CSF (right). According to forward and sideward scatter properties the region 1 (R1) was used for the analysis of T cells, NK-like T cells and NK cells; region 2 (R2) was used to determine the percentages of monocytes, B cells and plasma cells. **(B)** Analysis of three multiple sclerosis patients (upper, middle and lower row) for the expression of monocytes (CD14+) and B cells (CD19+) in peripheral blood (left) and CSF (right). The numbers represent the relative percentage of B cells (upper left quadrant) and monocytes (lower right quadrant). The relative distribution of both cell populations differed in CSF between the patients. No correlation was observed in the distribution between blood and CSF. **(C)** The distribution of the major leucocyte populations in the CSF of the three patients is shown. The subsets analysed in gate R1 are displayed on the left, the subsets analysed in R2 on the right. The percentages for B cells and monocytes are displayed in black.

sclerosis and NIND patients. As expected, the IgG levels and the absolute white cell count were increased in CSF from multiple sclerosis patients (Kabat *et al.*, 1942; Tourtellotte and Ma, 1978). We found a higher proportion of B cells and plasma cells in the CSF of multiple sclerosis patients as reported previously (Frequin *et al.*, 1993). The percentage of monocytes, NK cells and NK-like T cells was decreased in multiple sclerosis patients compared with NIND patients (Svenningsson *et al.*, 1995; Table 1).

Since CSF changes in multiple sclerosis patients could be due to alterations in the peripheral blood leucocytes composition, we compared the leucocyte distribution in both compartments. Although in general the percentages of T cells, CD4<sup>+</sup> T cells,  $\alpha/\beta$  T cells and plasma cells were increased and the percentages of  $\gamma/\delta$  T cells, B cells, monocytes, NK- and NK-like T cells were decreased in CSF, we found no correlation between the cellular composition in CSF and PB. This demonstrates that the distribution of immune cells in

**Table 1** Distribution of leucocyte subsets in peripheral blood and cerebrospinal fluid of multiple sclerosis patients and controls

CD Marker	Phenotype	Gate	PB			CSF		
			MS	NIND	P	MS	NIND	P
CD3	T cells	R1	65.9 ± 12.1	68.2 ± 8.5	0.222	91.5 ± 5.8	90.1 ± 5.4	0.177
CD3 CD4	CD4 T cells	R1	48.5 ± 8.1	46.3 ± 7.4	0.227	70.7 ± 8.1	66.9 ± 8.6	0.061
CD3 CD8	CD8 T cells	R1	19.8 ± 6.2	21.3 ± 6.2	0.135	20.0 ± 6.2	22.6 ± 6.4	0.139
TCR α/β	Alpha/beta T cells	R1	69.9 ± 7.9	69.0 ± 7.9	0.653	90.2 ± 8.7	91.5 ± 4.0	0.979
TCR γ/δ	Gamma/delta T cells	R1	2.8 ± 2.7	3.8 ± 3.4	0.115	1.4 ± 0.7	1.3 ± 0.9	0.444
CD56 CD16	NK cells	R2	12.8 ± 6.9	12.4 ± 5.1	0.816	2.6 ± 1.5	4.2 ± 2.6	0.006
CD56 CD16 CD3	NK-like T cells	R1	5.5 ± 3.6	5.0 ± 3.8	0.317	1.6 ± 1.5	3.0 ± 1.6	<0.001*
CD3	T cells	R2	57.2 ± 9.9	58.7 ± 8.4	0.172	86.2 ± 7.3	78.9 ± 9.4	<0.001*
CD14	Monocytes	R2	14.1 ± 6.6	12.6 ± 3.5	0.616	3.9 ± 4.4	16.5 ± 9.0	<0.001*
CD19	B cells	R2	11.1 ± 5.0	11.0 ± 3.8	0.954	4.6 ± 4.1	0.7 ± 0.8	<0.001*
CD138	Plasma cells	R2	0.1 ± 0.3	0.1 ± 0.1	0.553	1.3 ± 1.6	0.1 ± 0.2	<0.001*
	IgG	–	–	–	–	63.6 ± 41.4	30.3 ± 14.1	<0.001*
	Intrathecal synthesis (%)	–	–	–	–	17.2 ± 21.7	0	<0.001*
	Oligoclonal bands (%)	–	–	–	–	95	0	<0.001*

Values are mean ± standard deviation percentages. Patients with non-inflammatory neurological diseases (NIND) served as controls. CD = clusters of differentiation; MS = multiple sclerosis; P = Mann-Whitney U-test. \*Statistically significant (P ≤ 0.0012; adjusted for multiple testing).

the CNS of multiple sclerosis patients does not solely reflect the composition of PB immune cells.

**Patterns of CSF pathology observed in multiple sclerosis patients**

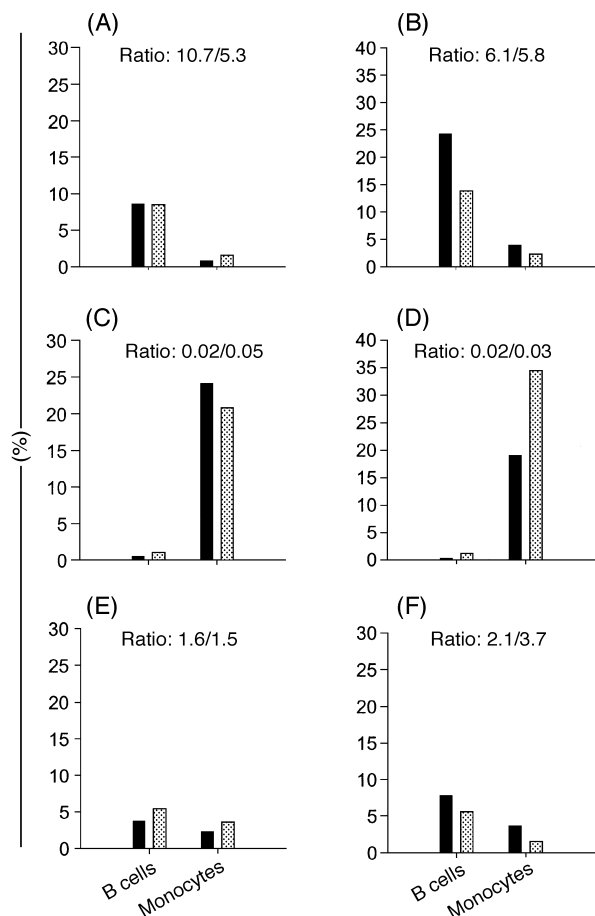
Next, we compared the CSF protein and cellular distribution between individual multiple sclerosis patients (Fig. 1). T cells represented the major CSF subpopulation in all patients. However, we observed differences in the distribution of monocytes, B cells, plasma cells, NK-like T cells, CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells and immunoglobulin levels. The most striking difference was observed for B cells and monocytes. Based on those and other cell subsets distinct patterns were stratified: a B cell dominant phenotype (high percentage of B cells, plasma cells, high CSF IgG levels, low percentage of monocytes and NK-like T cells), a monocyte dominant phenotype (high percentage of monocytes and NK-like T cells, low numbers of plasma cells, B cells and low CSF IgG levels) and an intermediate type with similar numbers of B cells and monocytes and variable IgG levels (Fig. 1B and C). These patterns did not reflect the distribution of immune cells in the PB, as shown for monocytes and B cells (Fig. 1B). A positive correlation between monocytes and NK-like T cells as well as B cells and IgG indices was observed (P = 0.002; P = 0.008). Furthermore, the ratio of CD4<sup>+</sup> and CD8<sup>+</sup> T cells varied significantly from 1.7 to 10.3 in individual multiple sclerosis patients, but so far we have not found a correlation with other cellular CSF parameters (data not shown).

**Stability of CSF cytology patterns**

Since the patterns could reflect different phases of disease, i.e. acute exacerbation or clinically silent periods, rather than heterogeneity in pathogenesis, we determined CSF patterns from selected patients at different time points (Fig. 2). In five patients the CSF was analysed during acute exacerbation and during a clinically silent period (interval 1–23 months). Although the absolute CSF white cell count varied between both analyses, the distribution of various immune cells remained stable over time. This was true for the ratio of B cells to monocytes (B/M ratio; Fig. 2) in particular, but also other cellular parameters. These findings suggest that the patterns of CSF cytology in multiple sclerosis patients do not correspond to the various disease phases, but may reflect differences in immune reactivity with a predominance of B cells in some patients and monocytes in others.

**CSF pattern correlate with disease progression**

Next, we investigated whether clinical disease parameters of multiple sclerosis patients correlate with different CSF patterns, which were best characterized by the B/M ratio. First, we classified the patients in RR multiple sclerosis, primary-progressive multiple sclerosis and SP multiple sclerosis according to their disease course (Rudick *et al.*, 1996). We did not observe a difference between the three groups for their B/M ratio (Fig. 3A). Next, we compared the ratio with disease duration, EDSS score and disease progression. No correlation was observed between disease duration or EDSS score and B/M ratio (Fig. 3B and C) or other CSF parameters (data not shown). When we compared



**Fig. 2** Stability of CSF cytology pattern. Shown is a serial analysis of CSF cells from six different multiple sclerosis patients. Black bars represent the initial and grey bars the second analysis. The interval between the first and second analyses was 2 (A), 1 (B), 22 (C), 23 (D), 6 (E) and 2 (F) months, respectively. All but Patient F had a clinical exacerbation at one of the two time points (Patients A, B, C and E, first time point; Patient D, second time point). The percentages of B cells and monocytes, which reflect the most variable cell subsets in the CSF between individual multiple sclerosis patients, are shown. The B/M ratios at the first and second analysis are displayed in each graph. All patients had oligoclonal IgG bands in CSF and MRI abnormalities compatible with multiple sclerosis. Note that the B/M ratios are stable and differ between Patients A and C by more than two orders of magnitude.

the individual disease progression, which was defined by the EDSS score divided by the number of years since the occurrence of first neurological symptoms, we found a significant positive correlation with the B/M ratio ( $P = 0.0009$ ,  $r = 0.57$ ; Fig. 3D). When the patients were stratified according to their disease course, a similar correlation was observed for RR multiple sclerosis/SP multiple sclerosis patients ( $r = 0.59$ ) and RR multiple sclerosis patients alone ( $r = 0.63$ ). The findings suggest a higher progression rate in patients with high B cell and low monocyte numbers at least in the subgroups of patients with relapsing and secondary progressive disease course.

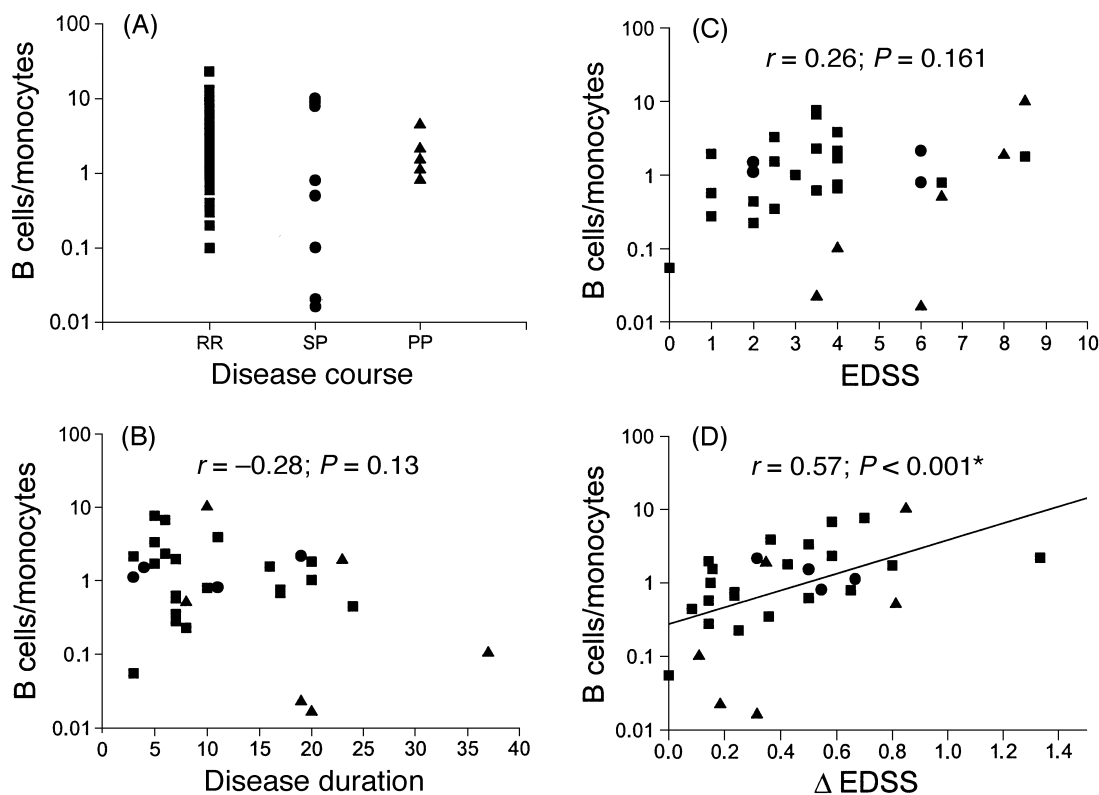
## Discussion

This study describes for the first time the existence of heterogeneity in CSF cytology in multiple sclerosis patients. We identified distinct cellular CSF patterns best defined by the ratio of B cells to monocytes. Serial analysis of CSF in clinically silent and active phases demonstrated the stability of the patterns and excluded that they reflect different disease phases. This finding is also supported by the observation that the patterns did not correlate with the EDSS score or the disease duration. In contrast, we observed a significant correlation of the B/M ratio with disease progression, although disease progression was determined retrospectively. The stability of the patterns and the correlation with disease progression suggest that the patterns may reflect different subtypes defined by distinct pathogenetic mechanisms.

Several lines of evidence support the idea of heterogeneity in multiple sclerosis pathogenesis. These include clinical, pathological and genetic findings. In particular, histopathological studies provide strong evidence for the existence of several disease subtypes. A recent study on CNS pathology in multiple sclerosis identified four patterns of acute demyelinating lesions (Lucchinetti *et al.*, 2000). Patterns I and II show macrophage and T-cell infiltration with preservation of oligodendrocytes and occurrence of remyelination. Pattern II differs from pattern I as it shows additional antibody and complement deposition. Patterns I and II have frequently been observed in acute and RR multiple sclerosis, but also in part in SP and PP multiple sclerosis. Our findings may, in fact, reflect these two types by grouping patients into those with a predominance of T cells and monocytes, and those with T cells and B cells in their CSF. In contrast, we have not yet been able to hypothesize a relationship between our patients and histopathological patterns III and IV, which are characterized by loss of oligodendrocytes and lack of remyelination.

Furthermore, we found evidence for a relationship between a strong humoral response and the severity of disease progression at least in patients with initially RR multiple sclerosis. Accordingly, a high percentage of B cells and low percentage of monocytes was found in patients with a high progression rate.

The role of antibodies in multiple sclerosis has been discussed since the discovery of intrathecal IgG synthesis and oligoclonal IgG bands in the CSF of those patients (Kabat *et al.*, 1942). In particular, studies in experimental autoimmune encephalomyelitis, a model of acute or chronic demyelinating CNS inflammation mediated by myelin-reactive T cells, have suggested a disease-influencing role of antibodies. Administration or endogenous induction of myelin-specific antibodies after initiation of autoimmune encephalomyelitis enhances severity of disease (Linington *et al.*, 1988; Litztenburger *et al.*, 1998). Induction of a T-helper type-2 response during the course of autoimmune encephalomyelitis leads to an enhanced antibody production and increased severity of disease (Genain *et al.*, 1996).



**Fig. 3** Correlation of CSF pattern with clinical parameters. The B/M ratios in CSF were correlated with (A) disease course, (B) disease duration, (C) EDSS and (D) disease progression ( $\Delta$ EDSS). Regression coefficients and  $P$  values are displayed in figures. All 60 patients were included for the correlation with disease course (A). For all other analyses only patients with a disease course of at least 3 years were selected. Those patients were classified according to their disease courses: RR = relapsing–remitting ( $n = 21$ , filled squares), SP = secondary progressive ( $n = 6$ , filled triangles) and PP = primary progressive ( $n = 4$ , filled circles). We observed a significant correlation between the B/M ratio and the individual disease progression but not with disease course, disability or disease duration. Six patients were on immunomodulatory or immunosuppressive therapy during the observation period (B/M ratios 0.05–3.5). No obvious effect of treatment as a covariant on the results was observed. \*Statistically significant after adjustment for multiple testing.

In multiple sclerosis the role of antibodies in the pathogenesis is less defined. Elevated titres of myelin-specific antibodies have been found in multiple sclerosis patients (summarized in Archelos and Hartung, 2000). B cells secreting these antibodies are found more frequently in the CSF of multiple sclerosis patients than in controls (Olsson, 1994). Myelin-specific antibodies have been detected in the CNS of multiple sclerosis patients (Wucherpfennig *et al.*, 1997; Walsh and Murray, 1998; Genain *et al.*, 1999). The co-deposition of IgG and complement factors observed in acute and active demyelinating lesions suggests a potential demyelinating role of antibodies (Storch *et al.*, 1998). Although the specificity of the cerebral B cell response is still unknown, our findings also support the role of humoral factors in the progression of multiple sclerosis. Further prospective studies are needed to clarify the implications of distinct CSF cytologies for the prognosis and therapeutic interventions in multiple sclerosis.

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