Short-lived plasma blasts are the main B cell effector subset during the course of multiple sclerosis

Sabine Cepok,1 Berit Rosche,1 Verena Grummel,1 Friederike Vogel,1 Dun Zhou,1 Joachim Sayn,2 Norbert Sommer,2 Hans-Peter Hartung1 and Bernhard Hemmer1

1Department of Neurology, Heinrich Heine-University, Duesseldorf and 2Department of Neurology, Philipps-University, Marburg, Germany

Correspondence to: Bernhard Hemmer, M.D., Neuroimmunology Group, Department of Neurology, Heinrich Heine-University, Moorenstr. 5, 40225 Duesseldorf, Germany
E-mail: bernhard.hemmer@uni-duesseldorf.de

Multiple sclerosis is a chronic inflammatory and demyelinating disorder of the CNS with an unknown aetiology. Although intrathecal immunoglobulin G (IgG) synthesis is a key feature of the disease, little is still known about the B cell response in the CNS of multiple sclerosis patients. We analysed the phenotype and kinetics of different B cell subsets in patients with multiple sclerosis, infectious disease (IND) and non-inflammatory neurological disease (NIND). B cells were detected in the CSF of multiple sclerosis and IND patients, but were largely absent in NIND patients. In the CSF, the majority of B cells had a phenotype of memory B cells and short-lived plasma blasts (PB); plasma cells were absent from the compartment. The proportion of PB was highest in multiple sclerosis patients and patients with acute CNS infection. While PB disappeared rapidly from the CSF after resolution of infection in IND patients, these cells were present at high numbers throughout the disease course in multiple sclerosis patients. CSF PB numbers in multiple sclerosis patients strongly correlated with intrathecal IgG synthesis and inflammatory parenchymal disease activity as disclosed by MRI. This study identifies short-lived plasma blasts as the main effector B cell population involved in ongoing active inflammation in multiple sclerosis patients.

Keywords: Plasma blasts, B cells, multiple sclerosis, cerebrospinal fluid

Abbreviations: EAE = experimental autoimmune encephalomyelitis; FCS = fetal calf serum; Gd = gadolinium; Ig = immunoglobulin; IgIF = intrathecally produced fraction of Ig; IND = infectious CNS disease; mAb = monoclonal antibody; NB = neuroborreliosis; NIND = non-inflammatory neurological disease; PB = plasma blasts; PBS = phosphate-buffered saline; PC = plasma cells; VM = viral meningitis


Introduction

Multiple sclerosis is a chronic inflammatory disease of the CNS leading to demyelination and neurodegeneration (Noseworthy et al., 2000; Steinman, 2001). It is widely believed that T cells play a central role in disease pathogenesis, but evidence is accumulating that B cells are also important in multiple sclerosis (Archelos et al., 2000; Cross et al., 2001; Hemmer et al., 2002). Intrathecal synthesis of immunoglobulin G (IgG) and the occurrence and persistence of oligoclonal IgG bands in the CSF are observed in the majority of multiple sclerosis patients and are, until now, the only immunological markers of diagnostic significance (Kabat et al., 1942). Intrathecal IgG antibody production, dominance of B cells and the occurrence of oligoclonal immunoglobulin M (IgM) bands in CSF are associated with a more progressive disease course (Cepok et al., 2001; Izquierdo et al., 2002; Villar et al., 2002, 2003). Severe multiple sclerosis relapses respond to plasmapheresis, suggesting that the humoral immune response contributes to CNS damage (Weinshenker et al., 1999). While the target of the B cell response is still uncertain, many studies have investigated B-cell receptor (BCR) rearrangement in CNS lesions and CSF (Owens et al., 1998, 2003; Qin et al., 1998; Baranzini et al., 1999; Colombo et al., 2000). The local B cell compartment in multiple sclerosis contains dominant clonotypes with signs of somatic
immune response in the CNS during neuroinflammation. Infectious diseases to define the kinetics of the humoral analysis of the B cell response in multiple sclerosis and acute defined. Here, we performed cross-sectional and longitudinal injection and retraction of B cell responses in the CNS has not been defined. Here, we performed cross-sectional and longitudinal analyses of the B cell response in multiple sclerosis and acute infectious diseases to define the kinetics of the humoral immune response in the CNS during neuroinflammation.

Methods

Patients

We recruited patients at the Departments of Neurology at the Universities of Marburg and Düsseldorf, Germany. Sixty-one multiple sclerosis patients were diagnosed according to the McDonald criteria. Patients with infectious neurological diseases (IND) had neuroborreliosis (n = 10) or viral meningitis (n = 9). Twenty-one patients with non-inflammatory neurological diseases (NNIND) and without inflammatory CSF changes (no pleocytosis, intrathecal immunoglobulin synthesis or oligoclonal bands) were included in the study. CSF samples were obtained by spinal tap during diagnostic workup with informed consent and used in the experiments. The study was approved by the ethics committees of the universities of Marburg and Düsseldorf.

Specimens

CSF (8–15 ml) was obtained by lumbar spinal tap from all patients included in the study. 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 Ig levels by nephelometry (BN II, Behring, Marburg, Germany) and for occurrence of oligoclonal bands (Titan Gel, Rolf Greiner Biochemica, Flacht, Germany). The Reiber formula was used to determine the intrathecal produced fraction of Ig (IgT) (Reiber, 1998). EDTA blood and pelleted CSF cells were used immediately for antibody staining.

Flow cytometry

Blood and CSF staining were performed as described previously (Cepok et al., 2001). Briefly, fresh blood was diluted 1:1 with phosphate-buffered saline (PBS) supplemented with 5% fetal calf serum (FCS) before 200 μl of the mixture was 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 dried briefly on paper towels. The plate was then placed on ice and cell pellets were resuspended by direct addition of monoclonal antibody (mAb) combinations. The mAbs were conjugated with fluorescein isothiocyanate (FITC), phycoerythrin (PE), peridinin chlorophyll protein (PerCP) or allophycocyanin (APC), respectively. After 30 min incubation on ice in the dark, erythrocytes were lysed in two steps using 200 μl of lysing solution (Becton Dickinson, Franklin Lakes, New Jersey, USA) according to the manufacturer’s instructions. The plate was then centrifuged and the supernatants discarded as described above. After two additional washes with 200 μl of ice-cold PBS containing 2% FCS, cells were analysed using a flow cytometer (FACScalibur; Becton Dickinson).

Fresh CSF was immediately spun down at 200g for 10 min, the supernatant removed and the pellet resuspended in an appropriate volume of PBS containing 5% FCS, depending on the white cell count of individual samples. A minimum number of 6000 cells (yielding 2500 cells for analysis) in volume of 30 μl were used for each staining. The staining was performed as described for whole blood cells. No lysing step was applied to CSF cells because no red blood cells were present in these specimens. Cells were then washed once with PBS containing 5% FCS and analysed as described above. Lymphocytes and monocytes were gated according to forward and sideward scatter properties. In control donors, one or more stainings were performed depending on the absolute CSF cell number.

To analyse the B cell subsets, the following combinations of mAbs for the cell specific markers were used: CD19 (clone HB19), CD27 (M-T271) (all Becton Dickinson); and CD138 (BB4), CD38 (LS198-4-3), HLA-DR (B8.12.2) (all Beckman Coulter (Former Immuno-techn), Krefeld, Germany). An isotype control staining was used to exclude unspecific antibody binding.

MRI

Sixty-one patients underwent cranial MRI at the time when the spinal tap was performed (mean difference: 3 days; range 0–20 days). The majority of patients were scanned on a 1.5 T MRI unit (General Electrics-Fairfield USA) according to a standardized protocol. Standard T2-weighted fast spin echo sequences [TR (repetition time) = 3250 ms, TE (echo time) = 87.8 ms] were performed routinely. T1-weighted spin echo sequences (TR = 440 ms, TE = 9 ms) were performed before gadolinium (Gd) injection and 5 min after injection in 56 patients. For standard scans, the thickness of slices was 6 mm with a 0.6 mm gap.

All MRI scans were analysed by experienced physicians who were not familiar with patient’s clinical course. We applied a semi-quantitative method involving registration of number and ranking according to lesion diameter in T1-weighted scans and calculated the resulting total Gd-enhancing lesion volume of each patient.

Statistics

A t-test was used to compare CSF cell populations between different patient groups. Spearman’s-rank correlation was performed to analyse the relationship between antibody synthesis and CSF B cell subsets, and between B cell subsets and MRI findings. We used the paired t-test to compare the mean proportion of CSF PB within the B cell population between the first and the second time point of analysis.
Results
CNS inflammation is reflected by the presence of B cells in CSF
CSF and blood of multiple sclerosis, IND and NIND patients were analysed for the presence of B cells. The number and percentage of B cells in the CSF was determined in each specimen by flow-cytometry using monoclonal antibody for CD19. Sixty-one patients with multiple sclerosis, 10 patients with neuroborreliosis (NB), nine patients with acute viral meningitis (VM) and 21 patients with NIND were analysed for the number of B cells in their CSF and blood. B cells were rarely found in the CSF of NIND patients, but were present in almost all patients with inflammatory CNS diseases (Fig. 1A and B). The percentage of B cells in the CSF was similar in patients with multiple sclerosis and acute viral meningitis, although the absolute number of CSF cells was higher in the latter group. Patients with neuroborreliosis showed the highest number of B cells in the CSF (Fig. 1B). Notably, the range of CSF B cells in multiple sclerosis patients was variable. While B cell numbers in some multiple sclerosis patients were similar to those in NIND patients, others had levels that were only seen in patients with acute neuroborreliosis. The mean percentage (±SD) of B cells in the CSF from all analysed NIND patients was 0.67 ± 0.76 (range: 0–3.1; n = 21), and from patients with neuroborreliosis, it was 16.5 ± 10.1 (range: 1.2–31.1; n = 10). From patients with acute viral meningitis, it was 3.88 ± 4.5 (range: 0.5–13.2; n = 9) and from multiple sclerosis patients it was 4.74 ± 4.06 (range: 0.3–17.4; n = 61). In some of these patients, analysis of the number of B cells in the peripheral blood demonstrated that B cell recruitment to the CSF was independent of the number and distribution of B cells in the blood (Fig. 1A). The mean percentage (±SD) of B cells in the peripheral blood from NIND patients was 10.8 ± 4.7 (range: 3.5–18.1; n = 13), from patients with neuroborreliosis it was 10.7 ± 5.5 (range: 4.9–22.1; n = 9), from patients with acute viral meningitis it was 12.9 ± 6.7 (range: 6.4–19.8; n = 3), and from multiple sclerosis patients 12.7 ± 6.4 (range: 5.4–27.4; n = 13). There was no significant difference between the patient groups.

Fig. 1 B cells in neurological diseases. (A) The proportion of B cells in the CSF (left) and blood (right) of four patients affected by different CNS diseases was determined by flow-cytometry using mAb for CD19 (B cells, vertical axis) and CD14 (monocytes, horizontal axis). The proportion of B cells is displayed in the upper left quadrant. (B) Analysis of 21 NIND, 9 VM, 10 NB patients and 61 multiple sclerosis patients for the proportion of CSF B cells. Mean expression and standard deviation are shown by box plots. The t-test was applied for statistical analyses. A P-value of 0.05 was considered statistically significant. MS = multiple sclerosis.
Identification of different CSF B cell subsets

To further characterize CSF B cells, we performed an immunophenotyping in CSF and peripheral blood. First, we determined the percentage of memory B cells in the CSF using CD19 and CD27 antibodies. The majority of CSF B cells belonged to the memory B cell subset (CD19+CD27+), while only a minority were naïve B cells (CD19+CD27–). This distribution was observed in all patients with detectable B cell numbers in CSF, as shown for a patient with multiple sclerosis in Fig. 2A. In the peripheral blood of this and most other patients, naïve B cells were more prevalent than memory B cells (Fig. 2A).

Among CD19+CD27+ CSF cells, two distinct populations with respect to the expression of CD27 were observed; a population with high expression of CD27 (CD19+CD27++) and one with an intermediate expression (CD19+CD27+). The populations were further characterized for expression of CD138 (Syndecan-1), a molecule only present on PC, PB and some epithelial cells. High expression of CD138 was predominantly found in the CD19+CD27++ population (Fig. 2B). In contrast, CD19+CD27++ cells expressed lower levels of CD19 compared with naïve and memory B cells.

CD19+CD27++ cells were further characterized for expression of CD38, a marker upregulated on PB but not on PC (Tarte et al., 2002; Wehr et al., 2004; Odendahl et al., 2005). All CD19+CD27++ B cells expressed very high levels of CD38 (CD38++), compatible with a PB phenotype. The PB also differed from naïve and memory B cells by their increased size and density compatible with the higher activation stage (Fig. 2B).

Although fully mature PC express at least low levels of CD19 (Odendahl et al., 2000; Wehr et al., 2004), a subpopulation of PC may completely lack CD19 expression (Harada et al., 1993; Luque et al., 1998). The occurrence of CD19−PC in the CSF of multiple sclerosis patients was suggested by a previous study (Corcione et al., 2004). To detect this population, we analysed the CD138−cell population for the expression of CD19 and CD27. Almost all CD138−cells expressed high levels of CD27, HLA DR and medium to high levels of CD19, compatible with a short-lived plasma blast phenotype (Fig. 2C). While up to 50% of all CSF B cells were PB, this subset was only observed at low numbers in the blood, with no correlation between PB levels in the CSF and blood compartment. Only a very small number of CD138−cells
in CSF and blood had a PC phenotype characterized by medium expression of CD38 and a lack of HLA-DR and CD19 expression (Fig. 2C and data not shown).

**Disease related distribution of PB in the CSF compartment**

To investigate the extent of B cell subset recruitment to the CNS, we analysed CSF from all patients described in Fig. 1. Antibodies for CD19 and CD138 were used to discriminate between B cells (CD19⁺CD138⁻), PB (CD19⁺CD138⁺) and PC (CD19⁻CD138⁺) (Fig. 3A). PB were largely absent in NIND patients, whereas they were detected in the CSF of all other groups (Fig. 3A–C). The mean percentage of CSF PB in patients with neuroborreliosis was 2.35 ± 2.05, in patients with acute viral meningitis it was 0.49 ± 0.47 and, in multiple sclerosis patients, it was 1.59 ± 1.91. In contrast, PC were low in all patient groups and did not differ between patients and NIND controls (data not shown). We also analysed the proportion of CD19⁺CD138⁺ PB within the CSF cell pool. In multiple sclerosis patients, >30% of CSF B cells were CD19⁺CD138⁺ PB, whereas in patients with acute neuroborreliosis and viral meningitis, the percentage was significantly lower (Fig. 3D).

**Different kinetics of CSF PB in IND and multiple sclerosis**

To investigate the dynamics of the humoral immune response, we performed serial CSF analyses in multiple sclerosis, neuroborreliosis and viral meningitis patients (Fig. 4A). In IND patients, CD19⁺CD138⁺ PB disappeared from the CSF within weeks after disease onset (Fig. 4A and B), although CD19⁺CD138⁺ B cells persisted for years in the CSF compartment of these patients. Only few PC were found in the CSF, and the frequency did not change during the course of disease. In multiple sclerosis, PB were detected at onset but also during the course of disease. As shown for one patient, the number of B cells and PB was stable throughout an observation period of >2.5 years (Fig. 4A). To investigate the impact of disease duration on PB numbers, we studied a group of 61 multiple sclerosis patients and 10 IND patients. In contrast to the group of IND patients, the duration of disease did not affect the number of CSF PB in multiple sclerosis patients (Fig. 4B). Twelve multiple sclerosis patients and seven IND patients were analysed serially for the proportion of PB within the CD19⁺ cell population at onset of symptoms and later during disease. Although patients differed with respect to PB numbers, the proportion within the B cell

---

**Fig. 3** CSF B cell subsets in neurological diseases. (A) Stratification of CSF and blood cells from one multiple sclerosis patient according to the expression of CD19 and CD138. The percentage of cells in each quadrant is displayed. (B) CSF cell analysis in NIND, VM and NB patients for the expression of CD19 and CD138. (C) Comparison of the proportion of PB in the CSF compartment in different neurological diseases. (D) Analysis of the proportion of PB in the B cell pool of the same patient groups. The mean percentage of PB (±SD) is displayed in the box plots. Twenty-one NIND, nine VM, 10 NB patients and 61 multiple sclerosis patients were included in the analysis. A t-test was applied for statistical analyses. A P-value of 0.05 was considered as statistically significant.
Fig. 4 Kinetics of CSF plasma blasts in patients with multiple sclerosis and IND. (A) Serial analysis of CSF B cell subsets during the course of a multiple sclerosis, an NB and a VM patient. The first analysis was performed during the initial diagnostic workup of the patients. After the initial spinal tap, the NB patient received antibiotic and the multiple sclerosis patient received steroid therapy. No therapy was performed in the VM patient. The proportion of B cells (CD19<sup>+</sup>CD138<sup>−</sup>), PB (CD19<sup>+</sup>CD138<sup>+</sup>) and PC (CD19<sup>+</sup>CD138<sup>−</sup>) cells of all CSF cells is displayed in each graph. (B) The proportion of CD19<sup>+</sup>CD138<sup>+</sup> PB in the CSF of 10 IND patients (upper graph) and 61 multiple sclerosis (lower graph) patients in relation to disease duration is shown. More than one time point is shown for the majority of IND patients. (C) The proportion of PB in the CSF B cell population at first and last follow-up examination in seven IND patients (upper graph) and in 12 multiple sclerosis patients (lower graph). The mean proportion of PB in each patient group is displayed in the graphs. A paired t-test was used to analyse difference between first and second time point. Mean interval between first and last analysis in IND was 79 days (range 7 to 315 days) and 362 days (range 6 to 933 days) in multiple sclerosis.
pool remained stable at various time points during the observation period in multiple sclerosis (Fig. 4C).

**CSF plasma blasts are the main source for intrathecal IgG synthesis**

Next, we addressed which B cell subset is mainly involved in the intrathecal IgG synthesis in multiple sclerosis patients. We compared the percentage and number of CD19+CD138- B cells, CD19+CD138+ PB and CD19-CD138+ PC in the CSF with the amount of intrathecal IgG synthesis (determined by IgGIF). We found a strong correlation between the amount of intrathecally produced IgG and the absolute number of PB and B cells, but only a weak correlation with PC in multiple sclerosis patients (Fig. 5A). IgGIF also correlated with the percentage of CD19+CD138+ PB ($r = 0.489; P < 0.001$), CD19+CD138- B cells ($r = 0.444; P < 0.001$), but not PC ($r = -0.132; P = 0.311$). In accordance with the observed stability of the CSF PB pool, the extent of intrathecally produced IgG remained relatively stable over time in patients with high PB numbers (data not shown). In addition, intrathecal IgG synthesis correlated best with CSF PB in infectious diseases (data not shown).

**Plasma blasts in CSF correlate with parenchymal inflammation in multiple sclerosis**

The findings in multiple sclerosis and IND patients suggest that PB best reflect active CNS inflammation in these patients. To support this hypothesis, we performed cross-sectional MRI analyses in multiple sclerosis patients and compared...
B cell subsets with inflammatory MRI activity measured by Gd-enhancing lesions in T1-weighted images. CD19+CD138+ PB correlated best with the number ($r = 0.439; P < 0.001$) and volume ($r = 0.441; P < 0.001$) of Gd-enhancing lesions (Fig. 5B). While a weak correlation was also observed with CD19+CD138– memory B cells ($r = 0.285; P = 0.03$), no correlation was observed with the CD19+CD138+ PC ($P > 0.05$).

**Discussion**

Increasing evidence points towards an important role of B cells in CNS infection and autoimmunity. In experimental models of viral CNS infection, B cells and antibodies are essential to control virus replication during the chronic phase of disease (Bergmann et al., 2001; Ramakrishna et al., 2002). In EAE, administration of autoantibodies enhances disease severity (Linington et al., 1988). Less is known about the humoral immune response in human CNS inflammation.

In most inflammatory CNS diseases, including multiple sclerosis, an antibody response in the CNS is observed, which is characterized by local release of IgG and oligoclonal bands in the CSF. In addition, B cell repertoire analysis and phenotyping of CSF cells suggest that CNS B cell responses are sequestered. Dominance of B cells in the CSF, intrathecal IgG and IgM responses seem to be associated with a more progressive disease course in multiple sclerosis patients (Cepok et al., 2001; Villar et al., 2002, 2003; Izquierdo et al., 2002). Accordingly, therapies targeting antibodies and B cells show beneficial effects in multiple sclerosis (Weinshenker et al., 1999; Rastetter et al., 2004).

To address the role of the humoral immune system in neuroinflammation, we investigated B cell responses in different neuroinflammatory diseases in a cross-sectional and longitudinal study. We demonstrate that neuroinflammatory diseases are characterized by accumulation of B cells in the CSF. The main proportion of these B cells express a memory phenotype (CD19+CD27+CD138–). Memory B cells remain in the CNS compartment for years after infection, suggesting that their presence is independent of antigen persistence. We also observed a population of CD19+CD27++CD138+ cells. These cells express high levels of CD38 and HLA-DR molecules, which identify them as short-lived PB (phenotype: CD19+CD27++CD138–CD38++HLA-DR++) (Odendahl et al., 2005). In contrast, PC, which express medium levels of CD38 and no HLA-DR were largely absent from the CSF (phenotype: CD19+/CD27++CD138+CD38+HLA-DR–). This finding contrasts a recent study, which described increased numbers of CD19+CD138+ PC in the CSF of patients with inflammatory CNS diseases (Corcione et al., 2004). In our study, predominantly short-lived PB were found in the CSF of patients with acute neuroinfection or multiple sclerosis. Since PB rapidly appear in the CSF following infection but disappear after clearance of the pathogen, their presence seems to be strictly dependent on the driving antigens. In multiple sclerosis, the PB subset was present throughout the disease course. Remarkably, the percentage of PB among all CSF B cells was higher in multiple sclerosis patients than all other diseases. Moreover, plasma blast numbers in CSF correlate strongly with the intrathecal IgG production and the CNS inflammation determined by MRI. These findings identify PB as the main effector B cell subset in multiple sclerosis. The high proportion of PB in the CSF of multiple sclerosis patients throughout the disease course implies—in analogy to the findings in infectious CNS diseases—ongoing local exposure of disease-relevant antigens to the immune system.

In the peripheral lymphoid system, B cells differentiate upon antigen exposure to memory B cells and highly proliferating, short-lived antibody secreting PB. After systemic infection or vaccination, PB appear in the blood, secreting large mounts of specific antibodies (Jego et al., 1999; Odendahl et al., 2005). Plasma blasts migrate from the lymphoid organs to the bone marrow and become fully mature, non-dividing and long-lived antibody-secreting plasma cells (Manz et al., 1997). The survival of PC is supported by specific survival niches such as bone marrow or inflamed tissues. Long-lived PC (Slifka et al., 1995) can survive in bone marrow for several months to years (Cassese et al., 2001; Manz and Radbruch, 2002). They continuously release antibodies providing protective humoral immunity but are also a possible source for autoimmunity (Arce et al., 2002).

Different scenarios can be envisioned how B cell responses are generated and maintained in the CNS. Upon antigen release from the CNS (e.g. during infection), antigen specific B cell responses are mounted in the draining lymph nodes (Knopf et al., 1998; Ramakrishna et al., 2002; Tschen et al., 2002). Pre-activated antigen–specific B cells, similar to T cells, can cross the blood–brain barrier and accumulate at sites of antigen exposure. While PC are not seen in the CSF, PB are highly present during active infection. Once the antigen is removed from the compartment, PB disappear in neuroinfectious diseases. This rapid disappearance may be a result of local death or redistribution of these cells to the periphery. The kinetics in acute neuroinfection is contrasted by the persistence of PB in the CSF of multiple sclerosis patients. This finding is in line with an ongoing activation of the humoral immune response in multiple sclerosis driven by persistence of disease-associated antigens in the CNS. While these antigens would also find their way to the periphery and may drive the response in the lymph nodes, they may also support continuous maturation of PB from memory B cells in the CNS. This process may take place in meningeal germinal centre-like structures which were recently described in EAE animals and some chronic-progressive multiple sclerosis patients (Magliozzi et al., 2004). Given the stability of oligoclonal bands in multiple sclerosis patients who had undergone bone marrow transplantation, it is tempting to speculate that persistent memory B cells in the CNS are the source for the continuous local development of short-lived PB in the brain (Saiz et al., 2001). However, little overlap of B cell clonotypes in the CD138+ and CD19+ subset was observed in a recent study.
Plasma blasts in multiple sclerosis (Ritchie et al., 2001). Although this study did not differentiate between CD19+CD138+ and CD19+CD138- cells, the results would rather argue against continuous maturation of memory B cells to PB in the brain. Alternatively, PB primed in the lymph nodes by antigens released from the CNS may continuously be recruited to the brain throughout the disease course.

While the site of terminal B cell activation and maturation remains controversial, our data provide strong evidence for a key role of PB in CNS inflammation during the course of multiple sclerosis. Because the target antigen(s) of the B cell response remain obscure, clarifying the specificity of CSF PB (i.e. whether they target autoantigens or proteins from infectious agents) will be essential to develop B cell directed therapies in multiple sclerosis.

Acknowledgements

We wish to thank all our patients for their continuous support and Michael Happel and Annette Hehenkamp for excellent technical support. B.H. was a Heisenberg Fellow of the Deutsche Forschungsgemeinschaft (DFG, He 2382/3–1) and S.C. was supported by a stipend of the Langheinrich-Stiftung. The research work was funded by the DFG (projects 2382/4–1 and 5–1) and the German Multiple Sclerosis Society.

References


