

# Analysis of Cerebrospinal Fluid Cell Populations with Monoclonal Antibodies

P. ADAM<sup>a</sup>, O. SOBEK<sup>a</sup>, C.S. SCOTT<sup>b</sup>

<sup>a</sup>Laboratory of Reference for Cerebrospinal Fluid and Neuroimmunology, Homolka Hospital, 150 30 Prague, Czechia

e-mail pavel.adam@homolka.cz

fax +420 257 272 426

<sup>b</sup>Abbott Laboratories, Wiesbaden-Delkenheim, Germany

Received 19 April 2007

Revised version 16 July 2007

**ABSTRACT.** Sixty-five samples of cerebrospinal fluid (CSF) were evaluated using an automated cytoflow method with the CD-Sapphire hematology analyzer in order to investigate possible relationships between cell population patterns and diagnostic groups and better understand the biology of neurological disease. A basic panel of CD markers, including CD3/4/8/19/138/HLA-DR, was used to analyze CSF samples from clinical and laboratory confirmed cases of multiple sclerosis, neuroborreliosis, viral and bacterial neuro-infective diseases, malignant infiltrations of meninges and scavenger macrophagic reactions of the central nervous system. The principles of immune response and the contribution of cytological ‘disease-related patterns’ for these nosological entities are described. The distinct patterns of lymphocyte subpopulations in neuroborreliosis appear to be characteristic and could possibly serve as diagnostic indicators. Further verification and research will be necessary to clarify the significance and nature of CD4<sup>+</sup> CD8<sup>+</sup> positive subset in cerebrospinal fluid.

## Abbreviations

|      |                                    |      |  |
|------|------------------------------------|------|--|
| CD   | cluster of differentiation         | Mab  | monoclonal antibody(ies)               |
| CNS  | central nervous system             | MIM  | malignant infiltration of meninges     |
| CSF  | cerebrospinal fluid                | MS   | multiple sclerosis                     |
| IND  | inflammatory neurological diseases | NB   | neuroborreliosis                       |
| FL   | fluorescent channel                | NIND | non-inflammatory neurological diseases |
| FITC | fluorescein isothiocyanate         | PE   | phycoerythrin                          |

As a precondition *sine qua non* for precise cytological diagnosis of nosological entities impairing the CNS and peripheral nervous system, permanent cytological preparations using classical and specialized cytological staining procedures are made according to routine protocols. However, relatively little is known about the patterns of lymphocyte subsets, as defined immunologically with Mab, in CSF. Most published reports describing Mab only describe changes in highly specialized (*e.g.*, activation) markers and, with the notable exception of specific procedures designed to assess the possible presence of malignant leukemic or lymphomatous CNS infiltration (Hausler *et al.* 2003; Babušíková and Železníková 2004), have limited diagnostic relevance, due to impossibility to express sufficiently the composition of antigen-presenting cells in CSF and due to absence of description of basic principles of CD marker changes in complete spectrum of etiological entities.

No matter how the humoral processes of neuroinflammation are described relatively in detail-concerning immunoglobulins (Svatoňová 2006), concretely in MS and NB (Bednářová 2006), as well as inflammatory markers (Adam *et al.* 2001), the cellular characterization of neuroinflammation is also rather well understood (Cepok *et al.* 2006), nevertheless the principal changes during the inflammatory reaction are rather neglected, although some insights into changes associated with tick-borne encephalitis (Holub *et al.* 2002) and NB (Jacobsen *et al.* 2003; Kivisakk *et al.* 2003) have been documented, and again, with description of activation markers without respect to global changes of lymphocyte subsets during neuroinflammation. The possibility that lymphocyte subset changes could be diagnostically important is supported by studies of multiple sclerosis describing the presence of plasmablasts with CD138 positivity as a rare but highly specific marker in MS (Cepok *et al.* 2005), which is a substantial observation, including evidence of late B-cell differentiation (Corcione *et al.* 2004; Haubold *et al.* 2004) and the presence of  $\gamma/\delta$  cells (Murzenok *et al.* 2002), present also in peripheral blood. A CSF T<sub>H</sub>1 shift has also been suggested in CIDP (Mei *et al.* 2005), which could be of clinical relevance, while the trafficking of CD4<sup>+</sup> memory T-cells expressing gut- or skin-

specific determinants through the choroid plexus and meninges (Kivisiakk *et al.* 2003) represent only interesting observations without consequences to diagnostics or therapy.

We have investigated the biological nature of CD-marker-defined cell populations in CSF with a restricted panel of Mab reagents. To date, 65 samples of CSF have been analyzed using anti-CD3, CD4, CD8, CD19, CD138 and HLA-DR antibodies with the immunofluorescent flow cytometric capability of the Cell-Dyn Sapphire (*Abbott Laboratories*, Santa Clara, USA) hematology analyzer.

## MATERIALS AND METHODS

A total of 65 CSF samples were obtained for routine diagnostic reasons from patients with IND and NIND. These comprised 8 samples of clinical and laboratory-confirmed cases of MS, 9 cases of NB, 7 of viral and 8 of bacterial neuroinfective diseases (3 with granulocyte predominance and 5 with mononuclear cell predominance), 7 malignant infiltrations of meninges and 19 scavenger macrophagic reactions of the CNS (including 10 with subarachnoidal hemorrhage). Further 13 samples were classified according to biochemical, serological and cytological findings as normal.

The samples were initially examined using classical cytological procedures; cell count in Fuchs-Rosenthal chamber and evaluation of permanent cytological preparations (Giemsa-Romanowsky staining). Subsequent Mab analysis with the CD-Sapphire instrument was undertaken with fluorochrome-labeled monoclonal antibodies against CD3 (*Caltac*, UK), CD4, CD8, CD19, CD138 and HLA-DR (*DakoCytomation*, Denmark). The minor proportions of CSF sample events that were characterized as non-viable or degenerate were specifically excluded from the analysis.

Residual CSF sample remaining after routine laboratory analysis was used and, because of the technical limitations of low CSF cellularity, preliminary concentration by careful centrifugation was necessary prior to Mab staining and processing.

Raw data files were downloaded from the CD-Sapphire and analyzed using FCS Express software (<http://www.denovosoftware.com>), and the distributions and numbers of marker-defined populations related to the different CSF diagnostic categories were collected.

## RESULTS

There were several notable observations in these preliminary studies. The first was the clear demonstration of lymphocytes co-expressing both CD4 and CD8 antigens (Fig. 1). These CD4<sup>+</sup> CD8<sup>+</sup> lymphocytes were seen with the highest relative frequency in multiple sclerosis (7.9 %, Table I) although they were also prominent in some cases of NB. Additional observations suggest that these represent a specialized subset of CD3<sup>+</sup> T-lymphocytes and further investigation with modified Mab reagent systems is carried out to validate the relationships with other lymphocyte population disturbances, diagnostic type and disease stage. A second observation suggested that CSF samples from patients with bacterial infection, particularly in the later stages where there was a mononuclear cell predominance, had a clear imbalance between the numbers of CD3<sup>+</sup> T-cells and the combined numbers of CD4<sup>+</sup> and CD8<sup>+</sup> T-cell subsets. The possibility that these represent CD3<sup>+</sup> CD4<sup>-</sup> CD8<sup>-</sup> components is also being further investigated.

Despite having used the restricted panel of immunological Mab reagents and having been well aware of the limited number of evaluated samples, our results further suggest some basic cellular patterns as follows:

*Normal:* The typical CSF cytological finding was defined by a cell concentration of <5/mL and a predominance of lymphocytes, a few monocytes and, in samples contaminated by peripheral blood during lumbar tap, the presence of variable numbers of red cells and occasional neutrophils. CD marker analysis demonstrated a predominance of T-cells expressing the CD3 antigen.

*Neuroborreliosis (Lyme disease):* In this frequently seen tick-borne spirochetal infection, which causes neuro-infective impairment, classical cytology reveals pleiocytosis, a predominance of activated lymphocytes and the presence of plasma cells. What is most evident in the CD analysis is the relatively high frequency of B-cells expressing CD19 and/or CD138 antigen compared to other disorders including viral neuro-infection (Fig. 2). In the course of the disease, mature CD19<sup>+</sup> B-cells appear to progressively mature to CD19<sup>-</sup> CD138<sup>+</sup> plasma cells. This distribution of lymphocyte subpopulations seems to be characteristic for NB and could possibly serve as a diagnostic criterion for distinguishing this particular infection from the CSF lymphocytoses that characterize viral diseases.

*Viral encephalitis and/or meningitis:* This is a frequent type of neuro-infective disease caused by a number of quite distinct viral agents. In classical cytology, there is a prevalence of activated lymphocytes, although in the early stages of infection there can be a relatively high prevalence of neutrophils. After immunological analysis, most lymphocytes appear to be CD3<sup>+</sup> CD4<sup>+</sup> and there is a high CD4:CD8 helper to suppressor T-cell ratio (Fig. 3, Table I).

**Table I.** Summary of cell population distribution according to diagnostic group<sup>a</sup>

| Population <sup>b</sup>                                    | Bacterial infection with<br>predominance of |                                | Viral<br>infection<br><i>n</i> = 7 | Neuroborreliosis<br><i>n</i> = 9 | Multiple<br>sclerosis<br><i>n</i> = 8 |
|--|---|--------------------------------|------------------------------------|----------------------------------|---------------------------------------|
|  | granulocyte, <i>n</i> = 3                   | MNC, <i>n</i> = 5 <sup>c</sup> |                                    |                                  |                                       |
| CD3 <sup>+</sup> T-cells, % of all cells                   | 4.8   | 50.3                           | 44.3                               | 54.7                             | 76.0                                  |
| CD3 <sup>+</sup> Ia <sup>+</sup> , % of T-cells            | <1.0  | 1.1                            | 2.0                                | 3.3                              | 6.4                                   |
| Ia <sup>+</sup> /CD19 <sup>+</sup> B-cells, % of all cells | <0.10                                       | 0.50                           | 1.4                                | 14.2                             | 3.9                                   |
| Ia <sup>+</sup> monocytes, % of all cells                  | <0.10                                       | 3.7                            | 2.9                                | 8.8                              | 2.1                                   |
| CD4 <sup>+</sup> cells, % of all cells                     | 0.60  | 7.5                            | 39.1                               | 39.6                             | 46.0                                  |
| CD8 <sup>+</sup> cells, % of all cells                     | 0.40  | 4.9                            | 6.4                                | 13.6                             | 15.6                                  |
| CD4:CD8 ratio  | 1.5   | 1.5                            | 6.1                                | 2.9                              | 2.9                                   |
| CD4 <sup>+</sup> CD8 <sup>+</sup> cells, % of all cells    | 0.14  | 0.35                           | 1.3                                | 1.7                              | 7.9                                   |

<sup>a</sup>Data obtained from merged files for all samples in each diagnostic group.

<sup>b</sup>Differentiation of monocytes from CD3<sup>-</sup> Ia<sup>+</sup>/CD19<sup>+</sup> B-cells based on combined fluorescence and optical profiles.

<sup>c</sup>Mononuclear cells (lymphocytes or monocytes).

*Bacterial purulent infections:* These are typically characterized by a predominance of neutrophils, clearly demonstrated by classical CSF cytology, although following the administration of antibiotic therapy the neutrophils are usually replaced by CD3<sup>+</sup> lymphocytes (Fig. 4).

*Multiple sclerosis:* This is the most commonly seen autoimmune disease impairing the CNS. Classical cytological preparations usually show low cell counts with a prevalence of activated lymphocytes and plasma cells which are almost certainly associated with intrathecal synthesis of immunoglobulins (especially IgG). During CD analysis, the presence of a population of cells expressing both CD4<sup>+</sup> and CD8<sup>+</sup> simultaneously can be observed in CSF samples of MS patients. Detailed immunological analysis of suspected MS CSF samples can be limited by the low cellularity of CSF samples in MS.

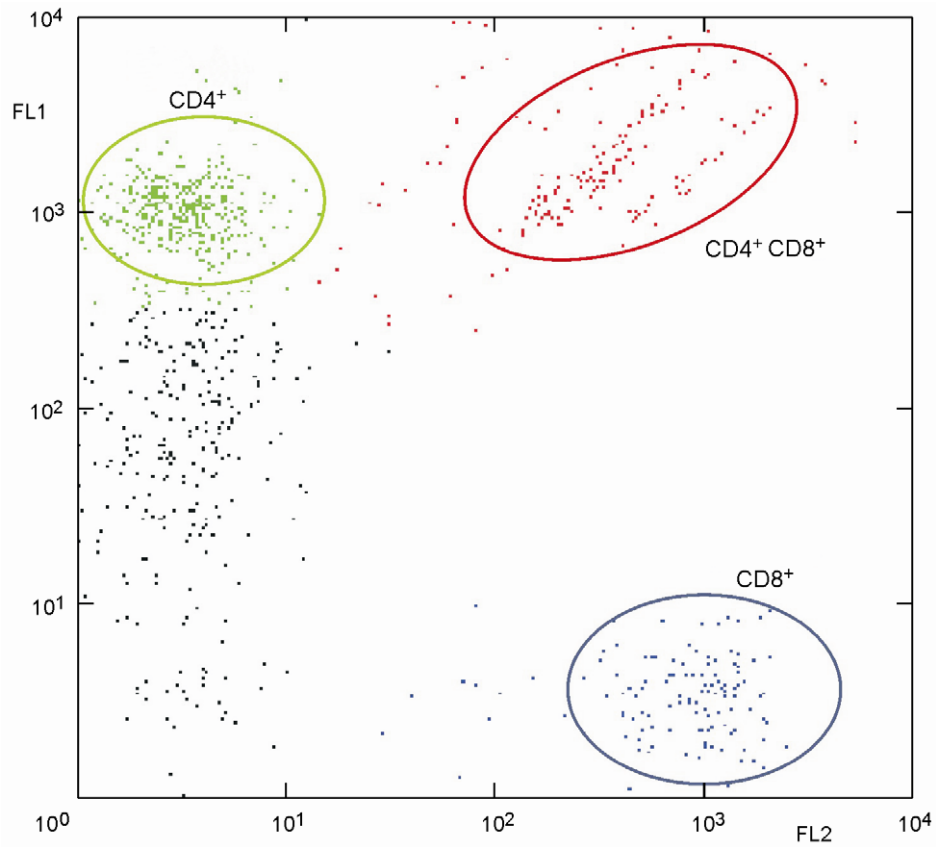
*Malignant infiltration of meninges:* Neurological infiltration and impairment of CNS often occurs in leukemia, malignant lymphoma, carcinoma and melanoma. With classical CSF cytology, the pathognomic feature is the presence of malignant cell although there can also be a predominance of activated monocytes. In leukemia and lymphomas, the use of immunological reagents can be particularly helpful in their identification.

## DISCUSSION

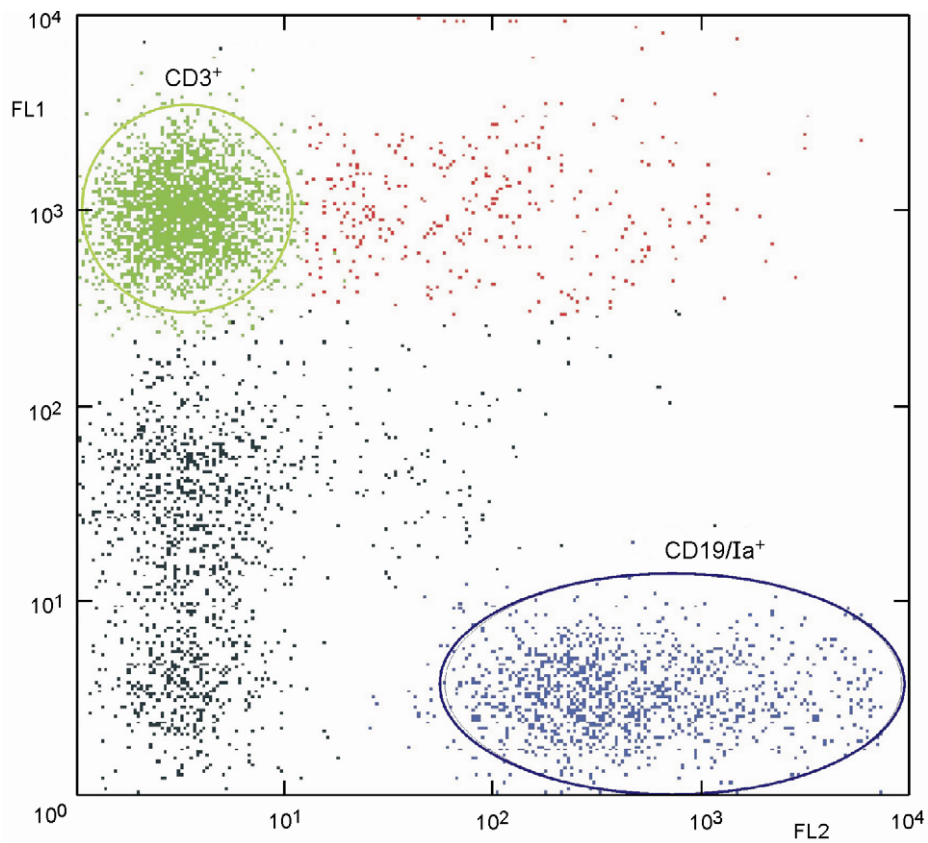
Although there are some clear limitations of this study which stem from the small groups investigated, restricted sample volume and panel of antibodies, as well as the low cellularity of CSF, still it is evident that flow cytometric analysis of CSF can significantly enhance the interpretation of morphological observations and offers a more practical alternative to classical immunocytology (Lodin 2003) and cytochemistry (Táborský *et al.* 2003).

We cannot predict the areas of further development in the field of CSF research but our initial results are highly promising. To fully understand its potential, however, it will be necessary to further verify these findings with larger patient groups and clarify the possibly important observations regarding CD4/CD8 co-expressing cells in MS and NB, and the apparent increased presence of CD3<sup>+</sup> CD4<sup>-</sup> CD8<sup>-</sup> cells in bacterial infection. Surprisingly, the co-expression of CD4<sup>+</sup> CD8<sup>+</sup> has been rarely described as a significant population in peripheral blood, having no relationship to clinical diagnostics (Sala *et al.* 1993).

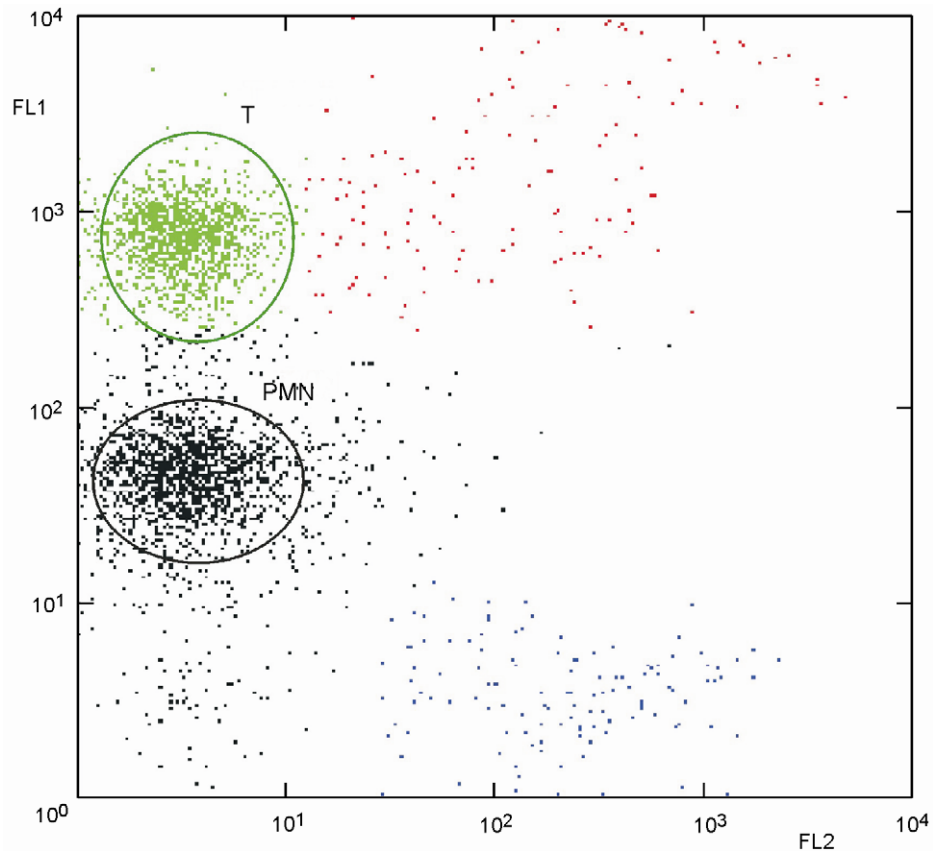
The aim of our study is to establish laboratory disease-related patterns that have relevance to the clinical diagnosis, etiological entity or disease stage. As has been demonstrated in NB, the representation of lymphocyte subpopulations seems to be characteristic, and it could possibly serve as a significant diagnostic tool in distinguishing from the immune responses of viral diseases.



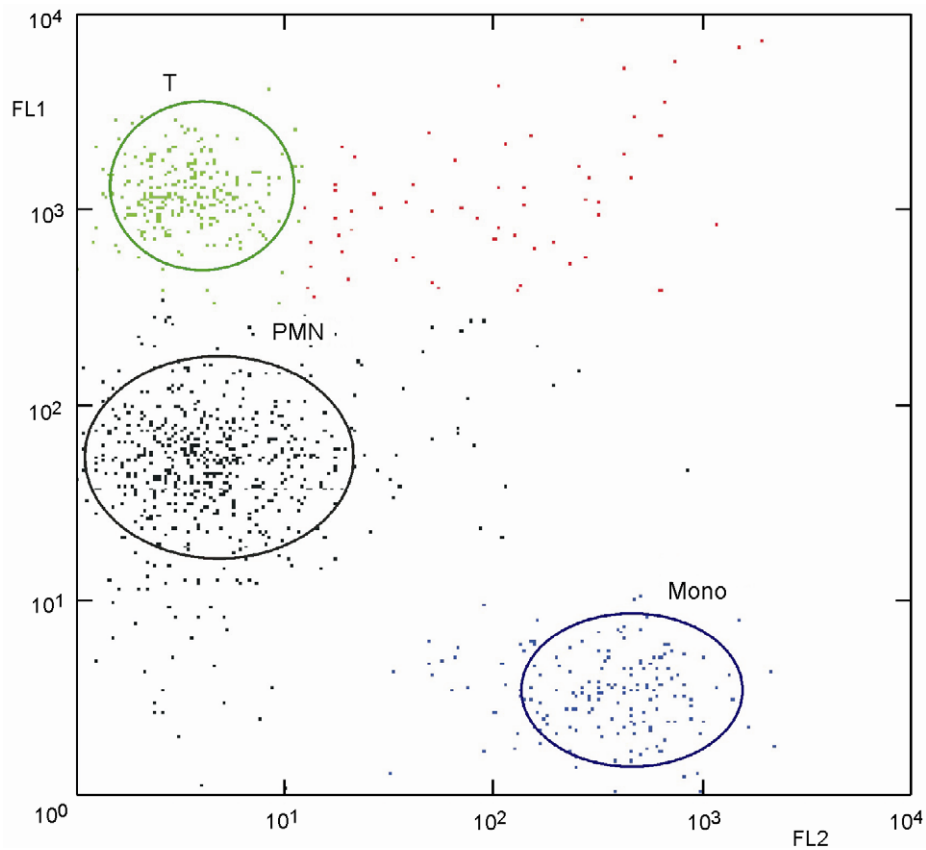
**Fig. 1.** Distinct population of  $CD4^+ CD8^+$  cells; MS, merged files ( $n = 8$ ); FL1: CD4 (FITC), FL2: CD8 (PE).



**Fig. 2.** Neuroborreliosis; merged files ( $n = 9$ ); FL1: CD3 (FITC), FL2: CD19/HLA-DR (PE); distinct population of B cells (and monocytes) at FL2 axis.



**Fig. 3.** Viral meningo-encephalitis; merged files ( $n = 7$ ); FL1: CD3 (FITC), FL2: CD19/HLA-DR (PE); T cells (T) and polymorphonuclears (PMN) are gated, very low number of B cells (CD19) detected.



**Fig. 4.** Bacterial meningitis, late phase; FL1: CD3 (FITC), FL2: CD19/HLA-DR (PE); polymorphonuclears (PMN), T cells (T) and monocytes (Mono).

In comparison with the previously used methods to identify the increased number of B lymphocytes in NB which were based on visual microscopic evaluation of stained cytological preparations (Schädlich *et al.* 1980), the discussed method of flow cytometry offers more precise, non-examiner-dependent results. In MS, we confirm the presence of CD138-positive plasmablasts in CSF in comparison with observations by Cepok *et al.* (2005).

It is proposed that classical cytological methods supplemented by flow cytometry will provide a means for obtaining qualitatively new analytical information.

## REFERENCES

- ADAM P., TÁBORSKÝ L., SOBEK O., HILDEBRAND T., KELBICH P., PRŮCHA M., HYÁNEK J.: Cerebrospinal fluid. *Adv.Clin.Chem.* **36**, 1–62 (2001).
- BABUŠÍKOVÁ O., ŽELEZNIČKOVÁ T.: The value of multiparameter flow cytometry of cerebrospinal fluid involved by leukemia/lymphoma cells. *Neoplasma* **51**, 345–351 (2004).
- BEDNÁŘOVÁ J.: Cerebrospinal-fluid profile in neuroborreliosis and its diagnostic significance. *Folia Microbiol.* **51**, 599–603 (2006).
- CEPOK S., ROSCHE B., GRUMMEL V., VOGEL F., ZHOU D., SAYN J., SOMMER N., HARTUNG B.: Short-lived plasma blasts are the main B cell effector subset during the course of multiple sclerosis. *Brain* **128**, 1667–1676 (2005).
- CEPOK S., VON GELDERN G., GRUMMEL V., HOCHGESAND S., CELIK H., HARTUNG H., HEMMER B.: Accumulation of class switched IgD<sup>-</sup> IgM<sup>-</sup> memory B cells in the cerebrospinal fluid during neuroinflammation. *J.Neuroimmunol.* **180**, 33–39 (2006).
- CORCIONE A., CASAZZA S., FERRETTI E., GIUNTI D., ZAPIA E., PISTORIO A., GAMBINI C., MANCARDI G.L., UCCELLI A., PISTOIA V.: Recapitulation of B cell differentiation in the central nervous system of patients with multiple sclerosis. *Proc.Nat.Acad.Sci. USA* **101**, 11064–11069 (2004).
- HAUBOLD K., OWENS G.P., KAUR P., RITCHIE A.M., GILDEN D.H., BENNER J.L.: B-Lymphocyte and plasma cell clonal expansion in monosymptomatic optic neuritis cerebrospinal fluid. *Ann.Neurol.* **56**, 97–107 (2004).
- HAUSLER M., SELLHAUS B., SCHWEIZER K., RAMAEKERS V.T., OPLADEN T., KLEINES M.: Flow cytometric cerebrospinal fluid analysis in children. *Pathol.Res.Pract.* **199**, 667–675 (2003).
- HOLUB M., KLUČKOVÁ Z., BERAN O., ASTER V., LOBOVSKÁ A.: Lymphocyte subset numbers in cerebrospinal fluid: comparison of tick-borne encephalitis and neuroborreliosis. *Acta Neurol.Scand.* **106**, 302–308 (2002).
- JACOBSEN M., ZHOU D., CEPOK S., NESSLER S., HAPPEL M., STEI S., WILSKE B., SOMMER N., HEMMER B.: Clonal accumulation of activated CD8<sup>+</sup> T cells in the central nervous system during the early phase of neuroborreliosis. *J.Infect.Dis.* **187**, 963–973 (2003).
- KIVISAKK P., MAHAD D.J., CALLAHAN M.K., TREBST C., TUCKY B., WEI T., WU L., BAEKKEVOLD E.S., LASSMANN H., STAUGAITIS S.M., CAMPBELL J.J., RANSOHOFF R.M.: Human cerebrospinal fluid central memory CD4<sup>+</sup> cells: evidence for trafficking through choroid plexus and meninges via P-selectin. *Proc.Nat.Acad.Sci.USA* **100**, 8389–8394 (2003).
- LODIN Z.: Inflammatory and autoimmune diseases of the nervous system; possibilities of laboratory diagnostic methods in cerebrospinal fluid. *Folia Microbiol.* **48**, 839–847 (2003).
- MEI F.J., ISHIZU T., MURAI H., OSOEGAWA M., MINOHARA M., ZHANG K.N., KIRA J.: T<sub>H</sub>1 shift in CIDP versus T<sub>H</sub>1 shift in vasculitic neuropathy in CSF. *J.Neurol.Sci.* **228**, 75–85 (2005).
- MURZENOK P.P., MATUSEVICIUS D., FREEDMAN M.S.:  $\gamma/\delta$ T Cells in multiple sclerosis: chemokine and chemokine receptor expression. *Clin.Immunol.* **103**, 309–316 (2002).
- SALA P., TONUTI E., FERUGLIO C., FLORIAN F., COLOMBATTI A.: Persistent expansions of CD4<sup>+</sup> CD8<sup>+</sup> peripheral blood T cells. *Blood* **82**, 1548–1552 (1993).
- SCHÄDLICH H.J., NEKIC M., FELGENHAUER K.: The detection of activated cerebrospinal fluid B lymphocytes by peroxidase conjugated antibodies. *J.Neurol.* **224**, 77–87 (1980).
- SVATOŇOVÁ J.: Clinical evaluation of the biological role of IgM in cerebrospinal fluid in inflammatory and other diseases of the nervous system. *Folia Microbiol.* **51**, 485–491 (2006).
- TÁBORSKÝ L., ADAM P., SOBEK O., DOSTÁL M., DVOŘÁKOVÁ J., DUBSKÁ L.: Levels of apolipoprotein A-II in cerebrospinal fluid in patients with neuroborreliosis are associated with lipophagocytosis. *Folia Microbiol.* **48**, 849–855 (2003).