Flow cytometric analysis of T cell subsets in paired samples of cerebrospinal fluid and peripheral blood from patients with neurological and psychiatric disorders

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ABSTRACT

Recent studies suggest inflammatory mechanisms involved in the pathogenesis of major psychiatric disorders (MPD). T cells play a major role during inflammation, but little is known about T cell subpopulations in the cerebrospinal fluid (CSF). We investigated the frequency of cells positive for the surface markers CD4, CD8, CD25, CD45, CD69, and CD127 in 45 paired cerebrospinal fluid (CSF) and peripheral blood (PB) samples by multiparameter flow cytometry from patients with MPD of the schizophrenic and affective spectrum with normal CSF cell counts and compared them with those from patients with non-inflammatory (NIND), chronic inflammatory (CIND) neurological disorders, and meningitis (MEN). In MEN patients, $CD4^+$ cell frequency in PB, but not in CSF, was significantly increased as compared to CIND and NIND. No difference between patient groups was observed for $CD8^+$. $CD4^+CD45RO^+$ double positive cells in PB were significantly lower in CIND than in MEN or NIND. The frequency of $CD4^+CD25^+$ cells in PB was significantly higher in MEN than in MPD or CIND. For CSF, the percentage of $CD4^+CD127^{dim}$ cells was significantly lower in MEN than in MPD. $CD4^+CD127^{dim}$ in PB and CSF showed overlapping characteristic clusters between MPD and CIND and MEN patients. Overall, the hypothesis of low degree inflammation in a subgroup of MPD is supported. The analysis of lymphocyte subsets in PB and CSF constitutes a novel promising tool to understand underlying pathomechanisms in psychiatric and neurological disorders on an individual case level.

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1. Introduction

There is increasing evidence that inflammatory mechanisms contribute to the pathogenesis of both the affective and the schizophrenic spectrum of MPD (Bechter, 2007; Knight et al., 2007; Leonard and Myint, 2006; Miller and Manji, 2006; Mueller and Schwarz, 2007; Rothermundt et al., 2001). Genetic factors overlap between disorders of the affective and schizophrenic spectrum (Berrettini, 2003) and regulation of several of these genes is involved in inflammatory mechanisms (Hanson and Gottesman, 2005; Knight et al., 1992). The specific nature of inflammatory abnormalities remains to be elucidated, though providing yet a basis for therapy (Dickerson et al., 2003a; Knight et al., 2007; Mueller et al., 2006). Hypotheses to explain the immune process in MPD include the macrophage theory of depression (Smith, 1991), involvement of serotonin and kynurenine pathways with a link to neurodegeneration (Myint and Kim, 2003), the role of cytokines (Leonard and Song, 2007; Licinio and Wong, 1999; Miller and Manji, 2006), the Th1/Th2 imbalance and secondary neurotransmitter abnormalities (Mueller and Schwarz, 2007), as well as the mild encephalitis hypothesis considering that low level inflammatory mechanisms possibly are triggered by a range of pathogens (Bechter, 2007). Plausibility for the causal role of pathogens emerges from experiments in animals and from investigations in patients diagnosed with psychiatric disorders (Amminger et al., 2007; Bechter and Bogerts, 2004; Dickerson et al., 2003b, 2004; Fellerhoff et al., 2007; Leweke et al., 2004; Torrey et al., 2006). However, characterization of the underlying mechanisms and differential diagnosis of suspected immune inflammatory CNS pathology in the individual patient remain difficult and are still speculative in most cases (Bechter, 2007). In patients with acute or chronic CNS inflammatory neurological diseases, typical findings are abnormalities of CSF protein levels and cell count. However, routine analysis of T cell subsets in the CSF is not yet available. In this report, we compare for the first time CSF lymphocyte subsets including $CD4^+CD25^+CD127^{dim}$ T regulatory cells (T_{reg}) (Shevach et al., 2006; Zwar et al., 2006) in patients with psychiatric

disorders (MPD) versus patients with neurological disorders such as meningitis (MEN), chronic inflammatory (CIND), or non-inflammatory (NIND) diseases, investigated in paired samples of CSF and peripheral blood.

2. Material and methods

2.1. Patients

A total of 17 patients with psychiatric disorders (12 diagnosed as schizophrenic, five as affective spectrum disorder) and 28 patients with neurological diseases were included during their hospitalization between July 2006 and April 2007 at the Department of Psychiatry and the Department of Neurology, Ulm University. The sex and age distribution is specified in Table 1, the detailed diagnoses of the neurological patients are summarized in Table 2. Psychiatric diagnosis was obtained by clinical consensus based on lifetime data and extended procedures (brain images, EEG, ECG, blood, and CSF immunochemistry) and standardized by OPCRIT checklist. Included in the study were schizophrenic or affective spectrum disorders (ICD-10, F20-F25, and F30-F33). Excluded from the psychiatric patient group were cases diagnosed with meningitis, encephalitis, or multiple sclerosis. Neurological patients underwent standard diagnostic procedures. Lumbar puncture was done with a 22G atraumatic Sprotte needle between 3rd und 4th dorsal processes of the lumbar vertebrae in a supine position. The volume of the drained CSF was a minimum of 6 mL and a maximum of 11 mL, depending on the patient's condition. The first 2 mL fraction was used for routine analysis. Written informed consent was obtained from all patients. The study was approved by the local Ethic Committee of the Ulm University (approval No. 43/2001). Due to ethical reasons, we did not perform LP in healthy individuals and therefore considered the group of NIND patients undergoing LP for exclusion of an inflammatory process or for other clinical reasons as controls.

2.2. Cell isolation

A total of 18 mL of peripheral blood (PB) were collected into vacutainer tubes containing EDTA (BD Bioscience, Heidelberg, Germany). PB samples were subjected to density gradient separation within 4 h from venipuncture. Briefly, EDTA blood was diluted 1:2 with phosphate buffered saline without calcium/magnesium (PBS, Invitrogen, Heidelberg, Germany), carefully overlaid on 15 mL Ficoll (Biochrom, Berlin, Germany) in 50 mL tubes (BD-Falcon, Heidelberg, Germany) and centrifuged for 20 min 1200g. The layer containing peripheral blood mononuclear cells (PBMC) was washed twice with 50 mL PBS. CSF samples were centrifuged at 800g for 10 min.

2.3. Immunotyping by flow cytometry

CSF and PB derived cells were stained within 2 h after obtaining the sample. In rare cases native CSF was kept up to 12 h at room temperature (RT) prior to the staining procedure. Staining of the cells was performed using fluorescence labeled monoclonal antibodies: 5 μL of mouse anti-human CD25*FITC (M-A 251), 3 μL of mouse anti-human CD127*PE (hIL-7R-M21), 5 μL of mouse anti-human CD4*PerCP (SK3), 5 μL of mouse anti-human CD69*PE-Cy7 (FN50), 5 μL of mouse anti-human CD45RO*APC (UCHL1) (all from Becton-Dickinson, Heidelberg, Germany), and 5 μL of mouse anti-human CD8*APC-AlexaFluor750 (3B5) (Caltag-Invitrogen, Heidelberg, Germany). The corresponding isotypes used were IgG*FITC, IgG*PE, IgG*PerCP, IgG*PE-Cy7, IgG*APC, and IgG*APC-AlexaFluor750 obtained from Becton-Dickinson, Heidelberg, Germany and Caltag-Invitrogen, Heidelberg, Germany. Staining was performed with 200,000–400,000 PB and 400–100,000 CSF derived cells in 1.5 mL tubes at a final volume of 100 μL PBS supplemented with 1% bovine serum albumin for 30 min at 4 °C in the dark. Cells for CSF isotype controls were limited to approximately 30% of the total cell amount due to limited CSF cell count. After incubation, cells were washed once in 1.5 mL PBS supplemented with 1% bovine serum albumin. Stained cell samples were analyzed within 4 h using a FACSAria™ flow cytometer and FACS Diva 4.0.2 software (Becton-Dickinson, Heidelberg, Germany). For PB 100,000 events and for CSF at least 500 events were collected for analysis. For each sample the appropriate isotype control was analyzed to define unspecific fluorescence.

Gating strategies for PB and CSF cells were as follows: after carefully fitting FSC/SSC lymphocyte gates independently for both CSF and PB to exclude debris and dead cells (Fig. 1A and B), PB, and CSF derived cells were characterized for CD4 versus CD8 expression (Fig. 1C and D). CD4$^+$ and CD8$^+$ cells were further characterized for the differentiation marker CD45RO$^+$ (Fig. 1E–H). Similarly, the co-expression of activation markers CD69 and CD127 was determined on CD4$^+$ T cells (Fig. 1I and J). Eventually, the subset of CD4$^+$CD25$^+$ cells was measured that comprises the regulatory T cells T$_{reg}$ (Fig. 1K and L). Cells positive for three markers (e.g. CD4$^+$CD25$^+$CD69$^+$) were gated as presented in Fig. 2.

2.4. CSF immunochemistry

CSF cell counts were visually determined in a Fuchs–Rosenthal chamber within 30 min after lumbar puncture (Fuchs and Rosenthal, 1904) excluding samples with a blood contamination above 100 RBC/μL. The concentrations of albumin and IgG, IgA, and IgM in CSF and in serum were measured using a Behring nephelometer analyser (BNA, Dade Behring, Marburg, Germany). For albumin and IgG a polyclonal antibody and for IgA and IgM a latex-enhanced antibody was used. Reproducibility was according to the standard (Reiber, 1995; Reiber et al., 2001). Detection of oligoclonal IgG bands was performed by isoelectric focusing on agarose gel and subsequent immunoblotting using IgG specific antibody staining (Keir et al., 1990). Humoral immune responses within the CNS can reliably be detected or excluded using this most sensitive test.

Table 1
Demographic characterization of patients included in the four groups of diseases

	Psychiatric patients (MPD)	Non-inflammatory (NIND)	Neurological patients meningitis (MEN)	Chronic inflammatory (CIND)
Number of patients (n)	17	16	7	5
Female (n)	6	7	4	4
Male (n)	11	9	3	1
Mean age (years)	39.5	54.6	44.8	30.5
Age range (years)	20–67	14–85	18–69	14–52