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# Increase of CD8+ T-effector memory cells in peripheral blood of patients with relapsing–remitting multiple sclerosis compared to healthy controls

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**Abstract**

CCR7 and CD45RA expression on CD4+ and CD8+ T-cells in blood (PB) of 16 patients with multiple sclerosis (MS) and 16 healthy controls and cerebrospinal fluid (CSF) of 10 patients suffering from MS were analysed by flow cytometric measurements. T-cells were divided by their distinct homing potentials and effector-functions in three groups: naïve T-cells (CCR7+, CD45RA+), central memory T-cells ($T_{CM}$) (CCR7+, CD45RA−) and effector memory T-cells ($T_{EM}$) (CCR7−, CD45RA−). There was a significant increase of CD8+ $T_{EM}$-cells in PB of MS patients compared to healthy controls, indicating systemic immune activation. Further we found a relative depletion of CD8+ $T_{EM}$-cells in CSF of MS patients compared to matching blood samples, suggesting that these cells represent the effector arm of the immune response and infiltrate the brain tissue at the sites of inflammation.


*Keywords:* Multiple sclerosis; T-cells; CCR7; CD45RA; Effector-function; Flow cytometry

## 1. Introduction

T-lymphocytes differ in their distinct homing potentials and effector-functions. They can be identified by their ability to express CCR7, a chemokine receptor that controls homing to secondary lymphoid organs, and CD45RA (Sallusto et al., 1999). Chemokines are small (8–14 kDa) structurally related molecules released by various cells. In humans more than 40 different chemokines have been identified. Interactions of chemokines with their respective cell surface receptors (seven-transmembrane G-protein coupled receptors) lead to the recruitment of specific leukocyte subpopulations to the sites of inflammation (Haegele and Sindern, 2004; Luster, 1998; Sindern, 2004; Soerensen et al., 1999). There is accumulating evidence that chemokines are important in the pathogenesis of immune-mediated inflammatory diseases of the central nervous system (CNS), especially in multiple sclerosis (MS) (Ransohoff, 1999; Trebst and Ransohoff, 2001; Zhang et al., 2000).

Naïve T-cells are able to travel to T-cells areas of secondary lymphoid organs searching for antigen presenting dendritic cells. After antigen contact they get activated and start to proliferate.

Because CCR7 is essential for lymphocyte migration to lymph nodes, the expression of this receptor might distinguish a subset of T-cells that home to lymph nodes from a subset that lost that ability. Human naïve and memory T-cells can be identified by the expression of CD45RA or CD45RO isoforms and the expression of CCR7.

Three subsets of CD4+ cell can be identified: naïve T-cells with the ability to express CCR7 and CD45 RA, T-central memory ($T_{CM}$) cells that lost the ability to express CD45RA but are still CCR7+, and T-effector memory ($T_{EM}$) cells, which are CCR7− and CD45RA− and therefore lost the homing potential for lymph nodes (Kivisakk et al., 2004; Sallusto et al., 1999). The $T_{EM}$-cells have a tissue-infiltrating potential (Kivisakk et al., 2004). This suggests that $T_{EM}$ might be the

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cell type causing inflammatory lesions in the brain of MS patients. Yet little is known about the association of these immune parameters and MS disease activity.

The aim of this study was to investigate the expression of CCR7 and CD45RA on CD4+/CD8+ T-cells in PB and CSF of MS patients. The study was divided in two parts: first an intergroup-comparison of the just mentioned parameters in PB of patients with MS and healthy volunteers, second an intragroup-comparison between PB and CSF of MS patients.

## 2. Material and methods

### 2.1. Subjects

Peripheral blood (PB) samples were analysed from 16 (11 female, 5 male) patients with clinical definite multiple sclerosis (MS) and from 16 healthy volunteers matched with the patient group for age and gender. Cerebrospinal fluid (CSF) was obtained from 10 patients with MS, who underwent diagnostic lumbar puncture in a short time interval before measuring, ranging from 1 to 24 h. Informed consent was obtained from each patient prior to the study. All patients fulfilled the McDonald criteria for MS (Mc Donald et al., 2001). Using the classification system by Lublin and Reingold (Lublin and Reingold, 1996) all patients assigned to the relapsing–remitting subtype. Most patients received diagnostic lumbar puncture within short time after relapse or even during acute relapse, though our patients have to be considered as active MS patients. None of the MS patients had received immunomodulatory therapies prior to the measurements.

The mean age of the MS patients was 34.69 years (S.D. 13.37; range 21.32 to 48.06 years). The controls were in average 33.06 years old (S.D. 8.744; range 24.316 to 41.804 years).

### 2.2. PB and CSF analysis

CSF was immediately centrifuged at 200 g for 20 min to obtain cells. CSF supernatants and serum were snap frozen within 20 min after sampling and maintained at −80 °C. CSF cell count was determined in a Fuchs-Rosenthal chamber, for the group of MS patients the mean CSF cell count was 8.5±3.4 cell/μl. For diagnostic analysis the CSF and serum albumin and IgG levels were measured by automated immunoprecipitation nephelometry (Array Protein System, Beckman Instruments). The CSF/albumin ratio (Qalb) was used as a measure for the function of the blood–brain barrier (BBB). Respectively with 4.88±1.34 the BBB can be considered as mainly intact.

### 2.3. Lymphocyte separation and flow cytometric analysis

From each patient 2 ml heparinized venous blood and if available 7–10 ml CSF was obtained in the morning and analysed right afterwards. Monoclonal antibodies (mAbs) were titred in blood samples of 50 μl and incubated 30 min at room temperature in the dark. Erythrocytes were lysed using FACS-lysing solution (Becton Dickinson). Samples were centrifuged at 100 g for 10 min and washed twice in ice-cold phosphate-buffered saline (PBS), containing 1% bovine serum albumin and 0.05% sodium azide. The resuspended leucocytes were fixed in 1% paraformaldehyde-PBS and kept at 4 °C until flow cytometric analysis. Figs. 1–5.

CSF was centrifuged immediately at 200 g for 20 min. The supernatant was removed, snap frozen and stored. To the pellet a defined amount of mAbs was titred and incubated 30 min at room temperature excluding light. Stained cells were washed twice in PBS, resuspended and fixed with 1% paraformaldehyde-PBS and stored at 4 °C until analysis.

For CD4/CD8 detection cytochrome™-conjugated CD4/CD8 mouse anti-human $IgG_1\kappa$ (BD Bioscience, clone RPA-T4/HIT8a), for CD45RA detection fluorescein isothiocyanate (FITC)-conjugated mouse anti-human $IgG_{2b},\kappa$ (BD Bioscience, clone HI100) and for marking CCR7 phycoerythrin (PE)-conjugated rat anti-human CCR7 (rat $IgG_{2b},\kappa$) (BD Bioscience, clone 3D12) were used. As isotype controls mouse IgM,κ (BD Bioscience, clone G155–228), cytochrome™-conjugated mouse $IgG_1,\kappa$ (BD Bioscience, clone MOPC-21) and fluorescein isothiocyanate (FITC)-conjugated mouse $IgG_{2b},\kappa$ (BD Bioscience, clone 27–35) were used. Analyses were performed on FACS Calibur flow cytometer (Becton Dickinson) using BD CellQuest software for analysis. Lymphocytes were identified on the basis of forward and 90° light scatter signals and gated approximately. For each stained blood sample 10,000 events were analysed for each antibody combination, in CSF at least 2000 events were acquired. Subpopulations of T-cells were analysed in the lymphocyte gate. The proportions of lymphocytes with positive staining for each antibody were calculated by setting four quadrants. The results of the T-cell subpopulations are given as percentages of the gated lymphocytes and as percentages of total measured events. In our study we analysed the results of the gated lymphocyte subpopulations.

### 2.4. Statistical analysis

As statistic program SPSS 11.0 for Microsoft Windows TM was used. Differences between two groups were analysed by *t*-test for independent samples, as the differences within patients can be assumed to be normally distributed. Differences between PB and CSF of MS patients were analysed by paired *t*-test. The Spearman correlation coefficient was used for assessment of the relation between variables. *P*-value of $p<0.05$ was considered significant.

## 3. Results

### 3.1. Intergroup-comparison (PB-MS patients versus PB-controls) CCR7 and CD45RA on PB-T-cells

PB samples of 16 MS patients with clinically definite MS or laboratory-supported definite MS were analysed. This