PERIPHERAL BLOOD TH17 SUBSETS IN PATIENTS WITH MULTIPLE SCLEROSIS

I.V. Kadryavtsev¹, A.M. Petrov², A.G. Ilves², K.K. Mineev², M.K. Serebriakova¹, I.D. Stolyarov²

¹ Institute of Experimental Medicine, Saint Petersburg, Russia; ² N.P. Bechtereva Institute of the Human Brain RAS, Saint Petersburg, Russia

Abstract

Using multicolor flow cytometry four main Th17 subsets were identified within total CCR6-expressing Th cells in peripheral blood from patients with multiply sclerosis (MS, n = 26) and healthy control subjects (HC, n = 44). MS showed significantly higher frequencies of CCR6+DP and “classical” Th17 subsets, while the level of Th17.1 was significantly decreased if compared to HC. Correlation analysis revealed a significant relationship between the decrease of CCR6+DN Th17 subset and EDSS worsening (r = −0.456, p = 0.022). The area under the ROC curve (AUC) for the percentage of “classical” Th17 within CCR6+ Th as a predictor of MS was 0.948 (0.882 to 1.014, p < 0.001) and for Th17.1 cells — 0.937 (0.863 to 1.011, p < 0.001). The optimal cutoff value for percentage of “classical” Th17 for predicting MS was 31.55% within CCR6+ Th cells with 88.0% sensitivity and 95.45% specificity, while for Th17.1 — 38.66% within CCR6+ Th cells with 88.0% sensitivity and 95.45% specificity. Our data indicate that the relative numbers of “classical” Th17 and Th17.1 within CCR6+ Th cells were effective in discrimination between MS and HC groups.

Keywords: multiply sclerosis; flow cytometry; “classical” Th17, Th17.1; CCR4 and CXCR3.

Introduction. Multiple sclerosis (MS) is a demyelinating autoimmune disease of the central nervous system and the underlying mechanisms of MS are not fully understood yet [3]. A leading part in MS pathogenesis was assigned to type 1 T-helper subset (Th1) able to secrete IFNg, but, recently, more studies have been aimed at examining type 17 Th cells (Th17) considered as one of the most promising targets for MS therapy [2]. Currently, the existence in humans of four distinct Th17-polarized CCR6+ Th subsets with differential expression of CCR4 and CXCR3, including “classical” CCR4+CXCR3+ Th17 cells, CCR4+CXCR3+ Th17.1 cells and two newly characterized CCR6+DN and CCR6+DP populations, was shown [5].

Material and methods. MS was diagnosed based on the McDonald criteria. All patients with relapsing-remitting form of MS (n = 26) underwent a neurological examination and were evaluated by the Expanded Disability Status Scale of Kurtzke (EDSS) that varied from 1.5 to 3.5 (median 2.5). None of the patients had received immunomodulatory or immunosuppressive drugs for at least 48 month before. The healthy control (HC) consisted of 44 healthy donors matched with patients by sex and age.

The combination of monoclonal antibodies, approach design and “gating strategy” allowed to identify main Th subsets were described previously [1]. The percentages of Th17 subsets within total CCR6-expressing Th were given in Med (Q25; Q75). The Mann–Whitney U test was used to compare MS and HC. The Spearman rank correlation was used to evaluate the relationship between the relative numbers of Th17 subsets and EDSS score. A receiver operating characteristic (ROC) analysis of the continuous parameters related to the discrimination between the groups was performed.

Results. Comparison of relative numbers of CCR6-expressing, but CXCR5-negative Th
between MS patients and HC, indicated significantly lower frequencies of these cell in MS (15.78% (14.63; 22.23) vs. 24.80% (20.06; 30.48), respectively, \( p = 0.002 \)). Then, we analyzed the Th17 population in MS versus HC, based on CCR4 and CXCR3 chemokine receptors co-expression profile. This approach allowed us to specify the phenotype of “classical” Th17 cells, Th17.1 cells as well as CCR6+DN and CCR6+DP Th17 subsets (Figure).

Scatter plots \( a, b, c \) and \( d \) showing the relative numbers of CCR6+DP Th17 (CXCR5–CXCR3–CCR6+CCR4+), “classical” Th17 (CXCR5–CXCR3–CCR6+CCR4+), Th17.1 (CXCR5–CXCR3–CCR6+CCR4+) and CCR6+DN Th17 (CXCR5–CXCR3–CCR6+CCR4+), respectively, in the peripheral blood samples for MS (\( n = 26 \), black circus) and HC (\( n = 44 \), white circus). Numbers represent the percentage of the indicated Th subset among total CCR6+ Th population. Each dot represents individual subjects, and horizontal bars represent the group medians and quartile ranges (Med (\( Q_{25} \); \( Q_{75} \)). Statistical analysis was performed with the Mann-Whitney \( U \)-test.

The comparison of these different Th17 subsets between MS and HC indicated significantly higher frequencies of CCR6+DP and “classical” Th17 subsets, while the level of Th17.1 was significantly decreased in MS. These observations indicate that MS patients have the abnormal distribution of circulating Th17 cell subsets. Next, the statistical analysis revealed a significant relationship between the decrease of CCR6+DN Th17 subset and EDSS worsening (\( r = −0.456 \), \( p = 0.022 \)). Finally, the area under the ROC curve (AUC) for the percentage of “classical” Th17 within CCR6+ Th as a predictor of MS was 0.948 (0.882 to 1.014, \( p < 0.001 \)) and for Th17.1 cells — 0.937 (0.863 to 1.011, \( p < 0.001 \)). The optimal cutoff value for percentage of “classical” Th17 for predicting MS was 31.55% within CCR6+ Th cells with 88.0% sensitivity and 95.45% specificity, while for Th17.1 — 38.66% within CCR6+ Th cells with 88.0% sensitivity and 95.45% specificity.

**Conclusion.** We found the imbalance between different Th17 subsets within CCR6+ Th from MS patients peripheral blood. The level of CCR4+CXCR3+ “classical” Th17 cells was evaluated while the relative number of CCR4+CXCR3+ Th17.1 was decrease in MS if compared with HC. Furthermore, our data indicate that the relative numbers of “classical” Th17 and Th17.1 within CCR6+ Th cells were effective in discrimination between MS and HC groups and these observations could let to uncover new cell markers to be used for assessing MS progression, predicting its severity and course.

**References**


