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# Correlations between IL-4, IL-12 levels and CCL2, CCL5 levels in serum and cerebrospinal fluid of multiple sclerosis patients

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**Summary.** Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS). Both cytokines and chemokines have been implicated in the pathogenesis of MS. The aim of the study was to assess whether cytokine levels are correlated with chemokine levels during a different stage of relapsing-remitting MS (RR-MS). The study included 53 patients with RR-MS (20 subjects in stable stage and 18 patients with relapse). By ELISA method, the levels of the interleukin-4 (IL-4), interleukin-12 (IL-12), CCL2 and CCL-5 chemokines were measured both in serum and cerebrospinal fluid (CSF) of all patients. The serum IL-4 and IL-12 levels and CSF CCL5 level of patients with stable RR-MS were significantly different from the control level and the IL-12 levels were correlated with CCL5 levels in serum. During the relapse, a significant change in chemokine levels both in serum and CSF and IL-12 in CSF were noted, however no correlations were found between cytokines and chemokines.

**Keywords:** Multiple sclerosis, IL-4, IL-12, CCL2, CCL5.

## Introduction

Multiple sclerosis (MS) is an inflammatory disease of the central nervous system (CNS) presumed to be Th1 type cell mediated autoimmune disease. MS is characterized by abnormalities of the cytokine network in the peripheral blood, cerebrospinal fluid (CSF) and brain tissue. Cytokines have been identified as the major regulators of the immune system and attempts have been made to correlate cytokine levels with disease activity in MS (Laman, 1998; Bar-Or, 1999). Moreover, chemokines have been implicated in the pathogenesis of multiple sclerosis. They are the mediators of inflammation with selective chemoattractant properties. They regulate the recruitment and migration of cells to sites of inflammation. During the active stage of MS both high levels of Th1

cytokines – TNF-alfa, IFN-gamma, interleukin-1, interleukin-12 (IL-12) and CC or CXC subfamily chemokines – CCL2, CCL5, CXC10 have been described. The stable stage of the disease is linked predominantly with Th2 cytokines – interleukin-4 (IL-4), interleukin-10, TGF-beta (Laman, 1998; Bar-Or, 1999; Sørensen, 1999; Franciotta, 2001; Sindern, 2001; Mahad, 2002). Recent experimental studies suggest that the production of chemokines during autoimmune inflammation is regulated by proinflammatory cytokines. TNF-alfa, IFN-gamma and IL-12 preferentially stimulate the CCL5 production (Teran, 1999; Matejuk, 2002; Losana, 2002) and IL-4 may be involved in regulating CCL2 (Matejuk, 2002). However, their interactions in vivo have not been clearly established yet. To address this issue, we analyzed relations between cytokine and chemokine in serum and cerebrospinal fluid (CSF) of MS patients during the stable and acute stage of the disease. We focused on anti-inflammatory cytokine – IL-4, pro-inflammatory cytokine IL-12 and two CC chemokines – CCL2 and CCL5.

## Patients and methods

The patients group included 53 subjects with definite relapsing-remitting multiple sclerosis (RR-MS) according to McDonald's criteria (McDonald, 1983). Among MS patients, 20 subjects (14 F/6 M, age $31.8 \pm 5.5$ year, EDSS $2.4 \pm 0.8$, CSF cell counts $5 \pm 3\,\mathrm{cell/mm^3}$) were in the stable stage of the disease – they had no relapse within the 3 months preceding the study and 18 patients (12 F/6 M, age $31.2 \pm 5.7$ year, EDSS $4.4 \pm 1.2$, CSF cell counts $8 \pm 7\,\mathrm{cell/mm^3}$) were with relapse symptoms defined according to Schumacher criteria (Schumacher, 1974). All patients with relapse received methylprednisolon therapy (MP) at the dose of 1.0 g iv through five successive days. The control group consisted of 15 subjects (10 F/5 M, age $30.9 \pm 8.4$ year, CSF cell counts $3 \pm 2\,\mathrm{cell/mm^3}$) suffering from non inflammatory neurological diseases (tension headache or lumbar discopathy). Paired blood and CSF samples were obtained from all the patients. The samples for analysis were centrifuged and supernatants were removed and frozen at $-80^{\circ}$C until analysis. The levels of the IL-4, IL-12, CCL2 and CCL-5 chemokines were determined both in serum and CSF. We determined the total IL12 level. No subtypes of IL12 were examined. All assays were determined in duplicate by enzyme-linked immunoassay (ELISA) according to the manufacturer's instructions (Quantikine, R&D Systems, Minneapolis). The lower detection limits were for IL-12 – 0.5 pg/ml, for IL-4 – 0.13 pg/ml, for CCL2 – 5 pg/ml and for CCL5 – 8 pg/ml. If the level was below the detection limit, a concentration of 50% between 0 and the value of the detection limit was assumed for statistical calculations. The study was approved by the Local Ethics Committee and all subjects gave informed consent. Nonparametric Mann-Whitney U test and Spearman correlation test were used for statistical calculations. p was considered statistically significant when $p < 0.05$.

## Results

### *IL-4*

IL-4 levels in serum were above the detection limit in 12 patients with relapse (66.7%), in 18 (90%) patients with clinical stable RR-MS and in all patients in the control group. In CSF the levels above detection limit were in 16 (88.9%), in 19 (95%) and in 3 (20%), respectively. Both in serum and CSF there were no significant differences between tested MS groups, however both MS groups

had in serum and CSF significantly different levels than control group ($p < 0.05$).

### *IL-12*

Serum IL-12 levels were above the detection limit in all MS patients and in 13 (72.2%) in the control group. CSF concentrations were detectable in all MS patients during relapse, in 14 (70%) with stable stage and in 12 (66.7%) in the control group. Both in serum and CSF of MS patients the IL12 levels were significantly higher than in control group, except for CSF level during the stable stage, which was similar to the control level. During relapse, the CSF IL-12 level was significantly higher than in stable stage ($p < 0.001$). In serum the increase of the chemokine was noted as well, but the difference was insignificant.

### *CCL2*

Serum and CSF concentrations were detectable in all patients and mean levels were higher in CSF than in serum. There were no significant differences between RR-MS and control group. During relapse, the CCL2 levels both in serum and CSF were significantly lower than in RR-MS and the CSF level were lower than in control as well ($p < 0.05$).

### *CCL5*

The level of CCL5 in serum was detectable for all patients. CCL5 levels in CSF were above the detection limit in 14 patients with relapse (77.8%), in 11 (55%) patients with RR-MS and none in the control group. In RR-MS the serum level was similar to the control group but CSF levels were not detectable using method chosen in this study. High values of CCL5 were noted both in CSF and serum of patients with relapse and these were significantly higher than levels of RR-MS and serum control level ($p < 0.001$). All results are summarized in the Table 1.

**Table 1.** The cytokines and chemokines concentrations in serum and CSF samples from patients with MS and control

| Parameter | | Relapse | RR-MS | Control |
|---|---|---|---|---|
| IL-4 | Serum | $13.6 \pm 18.7^{*}$ (BD–74.9) | $6.2 \pm 9.1^{*}$ (BD–40.5) | $45.2 \pm 22.1$ (11.2–86.5) |
| | CSF | $5.6 \pm 4.7^{*}$ (BD–14.4) | $8.6 \pm 4.7^{*}$ (BD–17) | $0.1 \pm 0.3$ (BD–1.2) |
| IL-12 | Serum | $20.1 \pm 13.7^{*}$ (3.8–53.1) | $14.1 \pm 8.5^{*}$ (2.8–31) | $5.4 \pm 4.2$ (BD–14.6) |
| | CSF | $15.6 \pm 10.4^{*,\#}$ (2–49.1) | $4.9 \pm 5.0$ (1–16) | $1.4 \pm 1.0$ (BD–3.8) |
| CCL 2 | Serum | $229.0 \pm 75.8^{\#}$ (122.3–354.9) | $307.9 \pm 78.7$ (192–438) | $277.3 \pm 100.9$ (121.3–438.0) |
| | CSF | $354.5 \pm 138.1^{*,\#}$ (178.4–591.0) | $574.4 \pm 159.2$ (361.8–941.7) | $526.8 \pm 158.1$ (321.8–782.0) |
| CCL 5 | Serum | $328.2 \pm 171.1^{*,\#}$ (129.8–745.2) | $153.4 \pm 54.8$ (64.2–281.7) | $122.8 \pm 36.8$ (67.4–208.6) |
| | CSF | $29.8 \pm 22.8^{\#}$ (BD–79) | $7.2 \pm 7.1$ (BD–21.6) | BD |

The data are expressed in pg/ml as means ± standard deviation (range). * Statistically significant vs control ($p < 0.05$); # Statistically significant vs RR-MS ($p < 0.05$); *BD* below detection limit

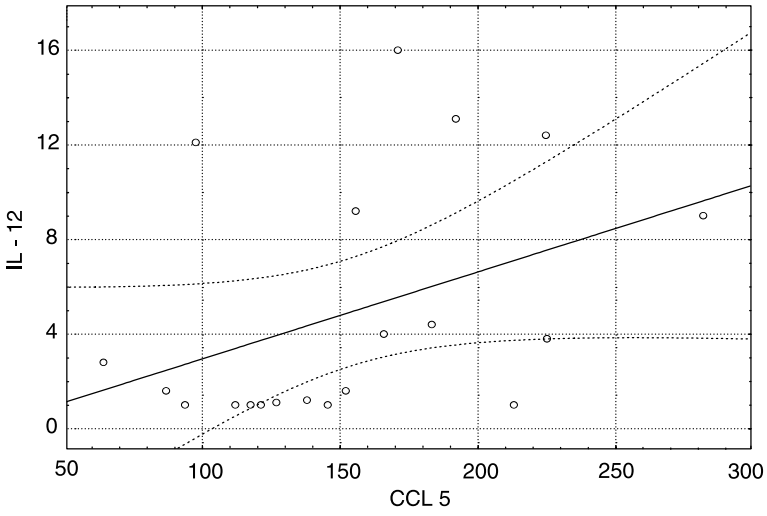


**Fig. 1.** Scatterplot of CCL5 concentrations plotted against the corresponding interleukin-12 levels. CCL5 correlated significantly with the interleukin-12 in serum of RR-MS patients ($r = 0.55$, $p = 0.01$). The linear regression line and the 5% lower and 95% upper confidence curves are shown. The chemokine levels in pg/ml

### *Correlations*

We found a significant correlation between the levels of IL-12 and CCL5 ($p = 0.01$) in serum of patients with stable RR-MS (Fig. 1). No other significant correlations were found.

## Discussion

In the MS patients the changes in the levels of cytokines as well as chemokines were observed, particularly during the relapse. However, no correlations between the molecules examined during the relapse were found in vivo. Only in serum of the patients with the stable stage the IL-12 levels were correlated with the CCL5 levels.

The levels of both chemokines tested in serum and CSF of the patients with stable RR-MS were not significantly different from the values found in the control group. Marked changes were observed during the relapse when the CCL2 levels were lower while the CCL5 levels significantly higher than the levels in RR-MS and control groups. Our observations are consistent with the findings of other authors who also stress the changes in chemokine levels during the relapse both in CSF (Sørensen, 1999; Sindern, 2001; Franciotta, 2001; Mahad, 2002) and serum (Sindern, 2001; Franciotta, 2001). Alterations in the levels of chemokines, which occur only when new symptoms of the CNS lesions appear, suggest that the release of chemokines may be stimulated by factors that appear at the time of the acute stage of the disease. One of the key factors during a relapse is a dysregulation of the balance between pro- and anti-inflammatory cytokines (Laman, 1998). Upregulation of pro-inflammatory cytokines like IL-12 and downregulation of anti-inflammatory cytokines such

as IL-4 has been observed in MS patients during relapses (Fassbender, 1998; Drulovic, 1997). In our study the IL-4 level in CSF during the stable stage as well as during the relapse was significantly higher than that in the control group. However, no differences between the stable and relapse patients were observed. In contrast, the level of IL-12 in stable RR-MS was similar to the control level while its significant increase was observed during the relapse. IL-4 is one of the main anti-inflammatory cytokines and IL-12 is a pro-inflammatory cytokine. The levels found in our patients during the stable stage are consistent with the conception of balance between Th1 and Th2. On the other hand, increased levels of both these cytokines during the relapse may suggest general stimulation of the immune system, particularly Th1 cytokines. Recently, Saruhan demonstrated a significantly higher level of IL-12 in CSF of the RR-MS patients; however, the examined group was not uniform and both the stable patients and those with relapse were assessed (Saruhan-Direskeneli, 2003). In our study the clinical condition of the patients in all groups was similar and a significantly higher level of IL-12 was found during the relapse; moreover, similarly to Drulovic, the number of patients with the IL-12 level above the detection limit was higher in this group (Drulovic, 1997). The serum level of IL-4 in the stable RR-MS patients was lower and the IL-12 level was higher than that in the control group. However, during the relapse no significant changes in IL-4 and IL-12 levels were observed. This is likely to be associated with low percentage of Th1 and Th2 cells that was observed in serum of the clinically active MS patients (Franciotta, 2003).

IL-12 is an immunoregulatory cytokine regulating cell-mediated immune response by inducing the differentiation of uncommitted CD4 Th cells towards Th1 phenotype. IL-12 is produced by monocyte/macrophages and dendritic cells in response to infection (Trembleau, 1995). CCL5 is produced by T lymphocyte, endothelial cells and eosinophiles and is attractant for monocytes/macrophages and T cells (Zlotnik, 2000). In the MS patients the serum levels of both cytokines are found to be higher that the CSF levels; moreover, they are significantly increased during the relapse and are connected with the active form of the disease (Nicoletti, 1996; Fassbender, 1998). Therefore the correlation between IL-12 and CCL5 is likely to suggest that the activity of cell-mediated immune response is maintained (mainly monocytes and macrophages) also during the clinical stabilization. Our study demonstrated for the first time the correlation between IL-12 and CCL5 in vivo. This correlation, however, cannot be confirmed during the relapse or between other cytokines examined. Despite marked relations between cytokines and chemokines demonstrated in the experimental studies, our study did not reveal any significant correlations. It may be related to the nature of the cytokines themselves. They act paracrinely or autocrinely, have very short half-lives in body fluids, and effects are dose- and tissue-dependent. Moreover, the systemic immune response in MS can be non-dramatic, may develop with time in a very variable way and therefore, the measurements of circulating cytokines in body fluids are hard to interpret (Laman, 1998; Ozenci, 2002). On the other hand, our cross-sectional study included only clinical-immunological parameters. In the imaging examinations (MRI, spectroscopy), the MS activity is much higher than its

clinical manifestation (Miller, 2003). The evaluation of MS activity on the basis of MRI findings is likely reveal more explicit correlations between the studied parameters. In our study, MRI was performed due to diagnostic indications only in some patients and was not simultaneous with determinations of chemokine and cytokine levels and therefore its results were not included in the paper. Further studies which will additionally evaluate the relations between levels of chemokines and cytokines and MRI data are required.

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