Long-term effect of IFN-beta 1a therapy on CCL2 (MCP-1) chemokine in patients with multiple sclerosis

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Chemokines play an important role in pathogenesis of multiple sclerosis (MS), mediating migration of leukocytes into the central nervous system. CCL2 (MCP-1) chemokine is expressed in astrocytes in MS lesions. The aim of the study was to evaluate the effect of a two-year treatment with IFN-beta 1a on serum CCL2 level in MS patients.

CCL2 concentration in sera of 18 relapsing-remitting MS (RR-MS) patients, and of 16 healthy controls was measured by ELISA. MS patients were treated with interferon-beta 1a (Avonex) in a dose of 30 μg i.m. once weekly.

Significantly lower serum CCL2 level was found in MS patients in comparison with results of the control group. CCL2 concentration increased significantly after one year of therapy with IFN-beta, and remained high after the two-year treatment.

The therapy of relapsing-remitting MS patients with interferon beta 1a is associated with a significant increase in CCL2 serum concentration.

key words: multiple sclerosis, interferon-beta, MCP-1

INTRODUCTION

Multiple sclerosis (MS) is regarded as an immune-mediated disease. The influx of activated T cells starts the autoimmune response, which leads to formation of demyelinated plaques and damage of axons [19]. Chemokines may play an important role in pathogenesis of MS, mediating migration of leukocytes into the central nervous system. They form concentration gradients, which attract leukocytes, and activate leukocyte integrins increasing adherence and extravasation [13, 20]. Chemokines can also stimulate leukocytes to release metalloproteinases, which may degrade components of the basement membrane, and facilitate the transfer of immune cells through the blood-brain barrier [32]. So far, significantly increased CSF concentration of chemokines (CXCL9 (Mig), CXCL10 (IP-10), CCL3 (MIP-1α) and CCL5 (RANTES)) was detected in patients with MS attacks [14, 17, 24, 27]. Increased concentration of CXCL10 (IP-10) and CCL5 (RANTES) in sera of MS patients has also been described [24,26]. It was found that T cells, which expressed CXCR3 receptors (for CXCL9 and CXCL10) and CCR5 receptors (for CCL3 and CCL5), were enriched in the CSF of MS patients in comparison with T cells in the peripheral blood [27].

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CCL2 chemokine (MCP-1, monocyte chemoattractant protein-1) belongs to CC subfamily of chemokines (the two N-terminal cysteines are adjacent to each other) [33]. This small protein acts as a chemoattractant for monocytes and activated lymphocytes T by binding to CCR2 receptors located on target cells [2]. Astrocytes, microglia, endothelial cells and macrophages can produce CCL2 [13]. Simpson et al. [25] described its expression in astrocytes in MS lesions. In experimental autoimmune encephalomyelitis (EAE), an animal model for MS, expression of CCL2 in astrocytes was reported, and anti-CCL2 antibodies blocked relapses of EAE [7, 21]. Surprisingly, significantly lower CSF concentrations of CCL2 chemokine (MCP-1) in active MS patients were found [3, 14, 26, 27]. Sörensen et al. [27] suggested that decreased CSF CCL2 concentration in MS patients in relapses may be explained by increased activity of Th1 cells and higher secretion of Th1 proinflammatory cytokines during MS attacks. On the other hand, CCL2 expression in astrocytes is intensified by TGF-beta 1, a Th2 cytokine with immunosuppressive properties [29]. CCL2 itself is likely to increase IL-4 production to induce the differentiation of Th0 cells into Th2 [8, 9].

In therapy of multiple sclerosis, various immunomodulatory agents are used. One of them is interferon beta 1a: a recombinant form of human IFN-beta. It reduces the exacerbation rate, the development of new lesions in MRI and EDSS between 1.0 and 4.0. Interferon beta 1a therapy: age 18–60 years, at least two exacerbations during last 24 months, multiple lesions on MRI, and may delay the progression of disability [4]. One of the possible mechanisms of action of IFN-beta in MS could be its influence on chemokines production.

The aim of our study was to evaluate the effect of a two-year treatment with IFN-beta 1a on CCL2 chemokine concentrations in sera of MS patients and to compare the results with those of a control group.

**MATERIAL AND METHODS**

We studied 18 patients with MS, according to McDonald’s et al. [15] criteria. All patients had the relapsing-remitting (RR-MS) form of the disease (clearly defined disease relapses with full recovery or with sequelae and residual deficit upon recovery). For diagnosis, we used the brain MRI, detection of oligoclonal bands and measurement of intrathecal IgG synthesis.

MS patients studied included 10 women and 8 men. The mean age was 30.1 ± 6.6 years, mean duration of the disease 4.2 ± 2.1 years and mean EDSS [10] 2.5 ± 0.8. All patients fulfilled inclusion criteria to interferon beta 1a therapy: age 18–60 years, at least two exacerbations during last 24 months, multiple lesions in MRI and EDSS between 1.0 and 4.0. Interferon beta 1a (Avonex) was given in a dose of 6 MIU (30 μg) once a week i.m. The control group consisted of 16 healthy blood donors (9 women and 7 men; age and sex matched the MS group). The patients had neither received any immunosuppressive treatment for at least 1 year nor any corticosteroids for at least 3 months before beginning the study.

Oligoclonal bands in the CSF and sera were detected by the isoelectric focusing method with silver staining [16]. Concentrations of IgG and albumin in the CSF and sera were measured by using the Behring Turbi-Time System. The IgG index was subsequently calculated [11]. All patients had oligoclonal bands in the CSF and an increased IgG index.

**Measurement of CCL2**

The CCL2 concentrations in the sera were detected by ELISA (Quantikine human MCP-1/CCL2 Colorimetric Sandwich ELISA, R&D Systems, Minneapolis, MN, USA). Briefly, 96 well pre-coated microtiter plates with murine monoclonal antibodies against CCL2 (MCP-1) were incubated with studied samples, control serum at standard room temperature for 2 hours. Serum samples were diluted in a ratio of 1:1. After incubation and washing (3 ×), 200 μl of anti-CCL2 polyclonal antibody conjugated with horseradish peroxidase was added and incubated for next 2 hours. After incubation and subsequent washing (3 ×), 200 μl of substrate solution (tetramethyl-benzidine) was added and incubated for 30 minutes at room temperature. After the reaction was stopped, optical density was determined using a plate reader (MR 250, Dynatech) set to 540 and 450 nm. The readings at 540 nm were subtracted from the readings at 450 nm for corrections according to the manufacturer’s instructions. All samples were tested in duplicates. Quantitative results were obtained in relation to standard curve for recombinant human CCL2 (MCP-1).

The sensitivity of the method (the minimum detectable dose) was < 5 pg/ml.

**Sample collection**

The blood samples from MS patients were taken before treatment after 1 and 2 years of therapy with IFN-beta 1a. All serum samples were collected and then frozen in aliquots at –80°C for CCL2 measurement. Small volumes of samples were used for determination of IgG and albumin concentrations as well as for oligoclonal bands detection.

**Statistical methods**

The statview statistical program was used. The Mann-Whitney U test was applied to determine the significance of differences between subject groups.
The study was performed with the understanding and consent of each patient and with the approval of the Local Ethics Committee.

RESULTS

Concentration of CCL2 in the sera of MS patients was 106.6 ± 23.2 pg/ml and was significantly lower (p < 0.05) than that of the control group with 150.6 ± 46.1 pg/ml.

After one year of the therapy with interferon beta 1a, the mean concentration of CCL2 in serum increased to 236.7 ± 32.1 pg/ml, and after two years it remained high (mean value, 225.5 ± 21.1 pg/ml). The differences between serum concentrations of CCL2 chemokine before and after treatment for both one and two years of the therapy were statistically significant (p < 0.001).

DISCUSSION

Interferon beta 1a as an immunomodulator is used in treatment of MS. Mechanisms of action of interferon beta 1a have not been yet fully explained. IFN-beta inhibits the proliferation of T cells, decreases antigen presentation [1], and reduces the production of proinflammatory cytokines [22, 30]. On the other hand, IFN-beta increases the secretion of anti-inflammatory cytokines: IL-4, IL-10 and TGF-beta 1 [12, 18, 23].

The influence of IFN-beta on chemokines production was also investigated. Zang et al. [31] reported that IFN-beta 1a inhibited mRNA expression for CCL5 (RANTES) and CCL3 (MIP-1alpha) and their receptor CCR5 in T cells derived from MS patients. Iarlori et al. [4] reported decreased serum concentrations of CCL5 and reduced CCL5 expression in peripheral blood adherent mononuclear cells of MS patients treated with IFN-beta 1b.

CCL2 expression and production by peripheral blood mononuclear cells (PBMC) from RR-MS patients were described by Iarlori et al. [5]. The study revealed lower CCL2 production by PBMC in relapsing than in stable MS patients. It was also found that IFN-beta 1b, in vitro, increases expression and production of CCL2 by PBMC. It is possible that IFN-beta may also affect production of CCL2 chemokine in vivo.

Sörensen et al. [28] as well as Franciotta et al. [3] studied CCL2 concentration in CSF of MS patients in relapses treated with methylprednisolone. After corticosteroid therapy CSF concentration of CCL2 increased significantly or tended to increase in comparison with CSF CCL2 concentration before therapy.

We measured CCL2 chemokine concentrations in sera of MS patients treated with IFN-beta 1a. So far no studies have been published on CCL2 serum concentration in MS patients after a long-term therapy with interferon beta 1a. Our data show that after one year of therapy, serum concentrations of CCL2 chemokine increased significantly and remained high after two years of the therapy. This finding is suggested to be due to an up-regulation of Th2 cytokines during IFN-beta therapy. Observed increase in CCL2 serum concentration in MS patients treated with IFN-beta 1a as well as results presented by Iarlori et al. [4, 5] indicate that IFN-beta may exert a complex influence on chemokines production in MS. It is possible that different changes in CCL2 (MCP-1) and CCL5 (RANTES) serum concentrations in MS patients treated with IFN-beta may reflect various roles of these chemokines in the course of multiple sclerosis.

Our study has also showed lower concentration of CCL2 in sera of MS patients before interferon treatment in comparison with concentration of CCL2 in the sera of healthy controls. Sörensen et al. [28] described similar results, comparing CCL2 concentration in CSF of RR-MS patients and control group. Sindern et al. [26] found significantly lower values of CCL2 in both CSF and sera of RR-MS patients with Gd-enhancing lesions on MRI in comparison with RR-MS patients without enhancing lesions. These data reflect probable connection of low concentration of CCL2 in serum and CSF of MS patients with down-regulation of Th2 cytokines in active MS.

In conclusion, our results suggest that the therapy of relapsing-remitting MS patients with interferon beta 1a is associated with a significant increase in CCL2 chemokine serum concentration.

REFERENCES