Effect of treatment with methylprednisolone on the serum levels of IL-12, IL-10 and CCL2 chemokine in patients with multiple sclerosis in relapse

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ABSTRACT

Objectives: Interleukin-12 (IL-12), a proinflammatory cytokine produced by Th1 cells, and interleukin-10 (IL-10), a product of Th2 cells, are involved in the pathogenetic mechanisms of multiple sclerosis (MS). CCL2 chemokine expression is induced by Th2 cytokines and is decreased in MS relapse. The mechanisms responsible for the beneficial effects of IV methylprednisolone in attacks are not clearly established and the duration of the effect of this treatment remains controversial.

Patients and methods: We measured by enzyme-like immunosorbent assay (ELISA) serum levels of IL-12, IL-10 and CCL2 before, 5 days and 1 month after the initiation of treatment with IVMP in 20 patients with MS in relapse.

Results: A significant increase of IL-10 and decrease of CCL2 serum levels was observed \((p=0.0028\) and \(0.045\) respectively) five days after the onset of steroid treatment but not after one month. Steroid treatment had no influence in serum levels of IL-12.

Conclusions: The clinical improvement of our MS patients with relapse following the treatment with methylprednisolone may be associated with an immediate but not a long-term modification of serum levels of IL-10 and CCL2. IL-12 may not be influenced by steroid treatment.

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1. Introduction

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS). Its cause remains unknown, although there is evidence of a complex and multifactorial aetiology with an underlying genetic susceptibility associated with unknown environmental factors [1]. Lymphocyte activation, extravasation and infiltration into the CNS together with activation of potentially immunocompetent glial cells are considered to lead to myelin destruction [2,3]. Proinflammatory T-Helper (Th)-1 cytokines such as Tumor Necrosis Factor, Interferon-γ and Interleukin-2 (IL-2) seem to be related to inflammation and tissue damage in MS [4–7]. In the contrary, Th-2/Th3 cytokines may contribute to suppress disease activity and progression [8–10].

Interleukin-12 (IL-12) is a heterodimeric cytokine produced by activated antigen-presenting cells (APC), such as dendritic cells (DC), monocytes/macrophages and microglia, in response to bacterial products and immune signals [11]. IL-12 is secreted as two subunits, IL-12p40 and IL-12p35 which combine to form biologically active IL-12p70. It enhances differentiation and proliferation of T cells and increases production of proinflammatory cytokines, such as INF-γ and TNF-α [12]. IL-12 may be responsible for the induction of experimental autoimmune encephalomyelitis (EAE), the animal model of autoimmune inflammatory disease of the central nervous system (CNS) [13,14]. IL-12p40 levels are increased in MS peripheral blood mononuclear cells (PBMC) and acute CNS plaques, while IL-12p35 levels are decreased [15,16]. IL-12 levels correlate with clinical measures of disease activity (EDSS) and presence of MRI lesions [17].

Interleukin-10 (IL-10) is a pleiotropic cytokine produced both by dendritic cells, B and T lymphocytes (mainly Th2) and mononu-
Table 1
Clinical and demographic features of MS patients (mean ± SD; range in parentheses)

<table>
<thead>
<tr>
<th></th>
<th>Age years</th>
<th>Disease duration years</th>
<th>Annual relapse rate</th>
<th>EDSS scores</th>
<th>EDSS scores day 30</th>
</tr>
</thead>
<tbody>
<tr>
<td>MS relapse group (n = 20)</td>
<td>33.75 ± 6.4 (25–40)</td>
<td>8.35 ± 5.69 (1–17)</td>
<td>0.55 ± 0.45 (0.1–2)</td>
<td>2.75 ± 0.93 (1.5–4.0)</td>
<td>1.67 ± 0.91 (1.0–1.5)</td>
</tr>
<tr>
<td>MS relapse group day 30 (n = 20)</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Abbreviations: MS relapse group = patients in relapse before treatment; MS day 30 = patients in relapse 1 month after initiation of therapy; EDSS = Expanded Disability Status Scale.

clear phagocytes including microglia [18–20]. IL-10 acts to decrease inflammatory cytokine production by macrophages [21]. IL-10 secretion by PBMC from MS patients is decreased prior to relapse and increased during remission [7,22,23].

Chemokines are involved in the recruitment of immune cells. They are released by activated macrophages, microglia, astrocytes, and inflammatory cells acting like mediators of inflammation with selective chemoattractant properties. They regulate recruitment and migration of cells to sites of inflammation. Depending on the number of an intervening amino acid between the first two cysteines, chemokines are subdivided into four groups: α-CXC, β-CC, Cx3C and C [24,25]. In MS activated T lymphocytes migrate from the blood to CNS through cheoattraction and recruitment performing by chemokines. Recent studies have reported expression of chemokines in the serum of MS patients mainly during active stage of the disease [26,27]. CCL2 is an attractant for monocytes and memory T cells [28]. Its production may be a result of IL-4, a Th2 suppressor cytokine [29].

Relapsing–remitting multiple sclerosis (RRMS) is characterized by periods of neurological dysfunction (relapses) followed by periods of clinical remission that may last for months or even years. High-dose intravenous methylprednisolone (IVMP) given over a period of 3–5 days has shortened the recovery period after MS relapses [30–32]. The aim of our study was to estimate serum levels of some immunological molecules resulting from Th1 such as IL-12 and Th2 activity such as IL-10 and CCL2 in MS patients with relapse before and after steroid therapy in order to assess the mechanism of action of steroids in MS and the effects of these drugs on the immune system.

2. Patients and methods

2.1. Patients

20 patients with relapsing–remitting MS in acute relapse (7 men and 13 women) were treated with IVMP (1 gr/day/5 days) and oral tapering. Serum levels of IL-12, IL-10 and CCL2 were measured before, 5 days and 1 month after the initiation of treatment. All MS patients had definite MS according to the criteria of McDonald et al. [33]. Relapse was defined as the occurrence of one or more symptoms of neurological dysfunction with a duration of at least 24 h combined with an increase of at least 1.0 point on the Expanded Disability Status Scale (EDSS) [34]. MS patients were scored by the EDSS [35] in relapse and 1 month after the initiation of therapy. The annual relapse rate and duration of the disease have also been measured. The clinical and demographic features of our patients are presented in Table 1.

Ethical consent has been obtained by all patients for studying IL-12, IL-10, and CCL2 serum levels. Venipuncture was performed just before steroid treatment, 5 days after the initiation of treatment and one month after. Serum samples were stored at −70 °C until assayed. No patient with MS had received any immunomodulatory treatment for at least 3 months prior to study. Patients had not suffered from any concomitant disease before or during the study.

Fig. 1. Scatter-plot of serum IL-12, IL-10 and CCL2 levels in the studied groups. Abbreviations: MS = patients in relapse before treatment; MS day 5 = patients in relapse 5 days after initiation of steroid therapy; MS day 30, patients in relapse 1 month after initiation of steroid therapy.
2.2. Sample collection, IL-12, IL-10 and CCL2 assay

Not fasting blood samples were obtained from a peripheral vein and centrifuged within one hour.

Serum levels of circulating IL-12 were measured by immunnoassay using a commercially available enzyme-like immunosorbent assay (ELISA) kit (Quantikine, R and D systems). The Quantikine IL-12 immunoassay was developed with a capture antibody that recognizes only the IL-12p70 heterodimer and not the individual subunits (p40 and p35) of the dimer, thus eliminating the potential for interference by these subunits. Undiluted serum samples were added in duplicate to microtiter wells and assayed according to routine procedures. Optical densities were determined by means of an automated ELISA processing system (DSX four plate, Dynex technologies Inc., Chantilly, Va, USA). Values were calculated by comparison with a standard curve that was generated with IL-12 standards. The limit of detection was 5 pg/ml.

Serum levels of circulating IL-10 and CCL2 were measured by immunnoassay using a commercially available enzyme-like immunosorbent kit (Quantikine, R and D systems). Undiluted serum samples were added in duplicate to microtiter wells and assayed according to routine procedures. Optical densities were determined by means of an automated ELISA processing system (DSX four plate, Dynex technologies Inc., Chantilly, Va, USA). Values were calculated by comparison with a standard curve that was generated with IL-10 and CCL2 standards. The limit of detection was 3.9 pg/ml for IL-10 and 5 pg/ml for CCL2.

3. Statistical analysis

Results were expressed as mean ± SD. The Mann–Whitney U test was used to compare titres of IL-10, IL-12 and CCL2 between the examined study groups. All comparisons were two-sided, with a p-value of less than 0.05 used to indicate statistical significance. The statistical software used for this analysis was Statistica 6.0.

4. Results

All patients manifested clinical improvement one month after the onset of the treatment (the p = 0.009). 14 out of these showed a decrease of at least 1 full point in the EDSS and 6 a decrease of 0.5 point.

4.1. IL-12 levels

IL-12 serum levels were not different in patients with MS in relapse before and after 5 days of the onset of MP therapy (the p = 0.65). IL-12 serum levels were not also different after 1 month of therapy (the p = 0.94) (Fig. 1, graph 1 or Table 2).

4.2. IL-10, CCL2 levels

Five days after steroid treatment, a significant increase of IL-10 and decrease of CCL2 serum levels was observed (p = 0.0028 and 0.045 respectively), whereas after 1 month, the levels of these molecules were not different compared with the levels before treatment (Fig. 1, graph 2 or Table 2).

5. Discussion

The mechanisms responsible for the beneficial effects of IVMP in attacks are not clearly established and the duration of the effect of this treatment remains controversial. Glucocorticoids (GCS) increased the secretion of Th2 type cytokines, such as interleukin-4, interleukin-10, interleukin-13, and transforming growth factor-β, while it suppressed the secretion of Th1-type cytokines, such as interferon-γ, Tumor Necrosis Factor-α, and IL-12 [36–38]. However, other studies showed that MP treatment may inhibit the IL-4 signaling pathway and did not modify the baseline low percentages of IL-4 producing cells [39,40].

IL-10 is a cytokine secreted by Th2 cells. Thus, it is possible that inducing IL-10 may have therapeutic effects in the treatment of MS patients. In one recent study the authors suggested that one anti-inflammatory mechanism of GCS action may be through inhibition of the release of pro-inflammatory cytokines IL-1 alpha and beta, IL-2, INF-gamma and TNF-α, and up-regulation of the anti-inflammatory cytokine IL-10 [41]. In our patients serum IL-10 levels were increased 5 days after and were recovered to levels prior treatment one month after the initiation of IVMP therapy. To our knowledge this is the first study in which the possible modification of serum IL-10 levels after MP therapy in RRMS patients in relapse has been assessed. In the contrary, serum IL-12 levels were not changed after steroid therapy. In one recent study levels of IL-12 increase in MS relapses and decreases after MP therapy [38].

Cytokine changes during interferon-beta therapy or glatiramer acetate (GA) in patients with multiple sclerosis have recently been studied. Different interferons had different patterns of influence on cytokines [42]. IFN-β treatment increased cellular IL-10 and the ratios of cellular IL-10/IL-12p40 and IL-10/IL-12p70 in stimulated peripheral blood mononuclear cells (PBMC) of patients with RRMS [43]. IL-12 and IL-10 are affected differentially by treatment of MS with GA. The authors established a significant decrease of IL-12 after 3 and 6 months of GA therapy and some insignificant differences in the level of IL-10 [44]. IL-10 has been suggested as a potential indicator of positive response to interferon-beta treatment in MS patients [45].

The findings described in one recent study suggest that the addition of prednisone in combination therapy with INF-β or IFN-β plus azathioprine is associated with a significant decrease in serum levels of IL-12 p40 [46]. These results do not agree with the findings of another study suggesting that the addition of prednisone antagonizes the effect of INF-beta-1a on the up-regulation of IL-10 [47]. The cause of the contradictory results may be the problems that the several authors have to resolve when try to measure circulating cytokines in neurological diseases. Circulating cytokines have a short half-life, act mainly in an autocrine or paracrine fashion, may reach high concentrations at sites of release, but much lower concentrations after dilution in blood, or they may bind to molecules that do not permit their detection by immunological methods.

A decrease of CCL2 in both CSF and in serum during a relapse was recently found [48–50]. The decrease in the CCL2 level may precede the emergence of clinical symptoms and is linked to the increase in the inflammatory factors in the CSF during a relapse [50]. It can result from the Th2 cell activity, because CCL2 expression is induced by Th2 cytokines, such as interleukin-4 and the immunomodulatory cytokine transforming growth factor β [51,52].

Most recent studies showed that after MP therapy the levels of CCL2 in serum or/and CSF were increased and this increase was
long-lasting (after 4 and 8 weeks) [53–55]. Following treatment with methylprednisolone, the CCL2 level rises in parallel with normalization of some inflammation markers [48]. In our study after the MP therapy, the levels of the CCL2 decreased, but this decrease did not last for a long time. One month after the onset of the treatment the levels of CCL2 were not different compared with the levels before.

Most studies (including those that have measured the levels of cytokines and chemokines before and after steroid therapy in MS patients in relapse, have not used a not-treatment MS group [38,53–55]. A comparison with cytokine levels of not-treated patients in relapse would be useful to know how the levels evolve in this group of patients. Such a group is very difficult and maybe not ethical to be, since most patients with a relapse should start treatment with steroids as soon as possible. Treatment with steroids is recommended as type-A drug in case of relapse for patients with MS [56,57].

We think that the increase of the Th2 cytokine IL-10 during the ongoing inflammation (just 5 days after treatment) suggests the influence of the treatment. The decrease of IL-10 during remission (after one month) comes to strengthen this statement, since IL-10 in remission is increased compared to IL-10 in relapse [7,22,23]. Therefore, if the change of IL-10 or CCL2 levels was the result of a normal evolution of the disease, it would present a continuous increasing or decreasing course. Nevertheless we cannot completely exclude some influence of normal evolution during the relapse in cytokine and chemokine profile.

The clinical improvement of our MS patients with relapse following the treatment with methylprednisolone (as it results from the significant decrease of EDSS score after one month of the initiation of treatment compared to score before treatment) was not associated with a long-term modification of serum levels of IL-10 and CCL2. However, the important change of their serum levels 5 days after the onset of MP therapy may suggest an immediate effect of treatment in the immunological disturbances that preceeds clinical remission. The insignificant change of IL-12 observed in the course of MP therapy in our patients seems to indicate that this cytokine may not be connected with the immunomodulatory effect of MP in MS. Further studies are needed to establish the role of steroids and other immunomodulatory treatment modalities in the immune reactions of MS.

References


