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SERUM INTERLEUKIN-10 LEVELS IN MULTIPLE SCLEROSIS PATIENTS TREATED WITH IFN-B 1A

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Keywords: multiple sclerosis, interleukine-10, interferon-β treatment

Abstract: Multiple sclerosis (MS) is an autoimmune chronic, manly demyelinating disease that affects young adults. Interferon β (IFN-β) is the main immunomodulatory treatment in MS, used for more than two decades but the exact mechanism of action is not totally understood. Evaluation of cytokine production in IFN-β MS treated patients is important in clarifying both the pathological mechanisms of MS and the IFN-β mode of action. Objective. To determine the serum IL-10 titre in MS patients treated with IFN-β 1a and to establish whether there is a correlation between IL-10 and demographic data, neurological handicap, MS duration and treatment duration. Material and methods. A number of 17 MS patients treated for at least 12 months with IFN-β 1a was included. For the quantitative detection of human IL-10, the ELISA was used and the values were compared with those of 17 healthy controls. Results. We found higher IL-10 titre in patients having a reduced EDSS, MS duration of maximum 5 years and in patients who had at least 3 years of IFN-β 1a treatment. None of these differences has a significant difference. Conclusions. More research is needed to establish the role of IL-10 secretion in MS patients treated with IFN-β.

Cuvinte cheie: scleroza multiplă, interleukina-10, interferon-β


INTRODUCTION

Interleukin-10 (IL-10) is an anti-inflammatory cytokine that is also known as human cytokine synthesis inhibitory factor. IL-10 is a homodimer and other five human molecules are related to IL-10 by their α-helical protein structure: IL-19, IL-20, IL-22, IL-24 and IL-26. In humans, IL-10 is under genetic control, encoded by the IL10 gene that is located on chromosome 1 and it encodes for 5 exons. IL-10 is secreted by monocytes, type 2 T helper (Th2) lymphocytes, regulatory T cells (Treg), mastocytes and eosinophils. The expression of IL-10 is minimal in unstimulated tissues and seems to require triggering by exotoxins or endotoxins. The IL-10 secretion is regulated by many transcription factor-responsive elements like: tumour necrosis factor, endotoxins, catecholamines, vitamin D3, glucocorticoids. Its function is essential in the regulation of the immune response, having mainly a protective mechanism of regulatory T cells against autoimmune processes: it downregulates the production of proinflammatory cytokines (IL-17, IL-6), chemokines, adhesion molecules, neutrophils, T cells and reduces the expression of major histocompatibility (MHC) class II, having an immunosuppressive role. IL-10 function is mediated by its specific cell surface receptor complex that belongs to the cytokine receptor family type 2. The receptors are expressed on immune cells like: natural killers, B, T cells, monocytes, etc. The IL-10 interaction in immune cells leads to transcriptional activation of hundreds of genes, some of the being up-regulated in an important amount.(1,2,3,4)

Multiple sclerosis (MS) is an autoimmune disease of the central nervous system, characterized in the initial phase by inflammation (that can be influenced by immunomodulatory treatments) leads to demyelination, followed later by degenerative processes that are chronic and are not influenced by the treatment. MS has a genetic polymorphism, more than 130 genes were described having an impact on the susceptibility in developing MS. Several genes are situated in the MHC region. Until now, genome screening did not show a major susceptibility locus, the interactions of many genes being under

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debate. Many studies have failed to associate any IL-10 alleles with the progression of MS.(1,2)

The modern immune paradigm regarding MS, Th-17 cells play the central role in the onset of proinflammatory cascade. In Experimental Autoimmune Eencephalomyelitis (EAE), the mice experimental model of MS, IL-10 seemed to have a protective effect by producing regulatory CD4+25+T cells that in the end inhibit Th1-inducing proinflammatory cytokines.(2)

In MS immunomodulatory treatment, beta interferons (IFN-β) play a central role in the last two decades. Even though IFN-β is worldwide used, its mechanism of action is not fully understood. Many previous studies show that IFN-β exerts its anti-inflammatory action through dendrite cells and macrophages and upregulates IL-10 expression. Studies showed that IFN treatment mediates IL-10 production by stimulating antigen-specific T cells. IL-10 in turn, downregulates Th17-mediated inflammatory/autoimmune response.(4)

We determined IL-10 serum titre in MS patients treated with IFN-β 1a i.m for more than one year. Our aim was to find any correlations between the serum titre of IL-10 and the neurological handicap, age of patient, clinical form of MS, duration of MS evolution, number of MS relapses in the year prior to testing, duration of IFN-β 1a treatment.

METHODS

Patients

This is a prospective study that included 17 consecutive patients with recurrent-remissive (RR) and secondary progressive (SP) MS patients treated with IFN-β 1a i.m (for at least 18 months) in Regional MS Centre Tîrgu-Mureș, recruited in a period of 3 months: 1-Feb.2014-1-May 2014. All patients were Caucasians, diagnosed with MS following the McDonald diagnostic criteria 2010.(2) We included adult patients with MS that had at least 30 days without any MS activity (no relapse), no steroid treatment, that signed the inform consent. The evaluation of the neurological handicap was scored using the Expanded Disability Status Scale (EDSS). Exclusion criteria for the patients: a) treated with methylprednisolone or any other corticoid previously in the last 30 days; b) any chronic disease (diabetes, other autoimmune disease, neoplasia, HIV, Lyme disease, syphilis etc.) associated with MS; c) pregnancy; d) treatment with immunosuppressive drugs. We included a number of 17 healthy controls with sex and age similar to the patients.

All subjects signed an informed consent that was approved by the local ethics committee. The study respected the Declaration of Helsinki. All MS patients had a record card containing: a) demographic data (age, sex); b) medical history; c) MS duration; d) EDSS; e) number of relapses in the year prior to serological testing.

IL-10 measurement

Blood samples were collected from 34 subjects (17 patients and 17 healthy persons) and after centrifugation at 2000 x for 15 min, serum was obtained and stored at -70°C. For the quantitative detection of human IL-10, the DRG International IL-10 ELISA kit (code DRG ELIA-4699) was used. According to the kit instructions provided, reference intervals were given only for guidance. DRG gave for guidance the result of 32 serum samples from healthy persons, ranged between 0-3.3 pg/mL with a lean value of 0.2pg/dL. As each laboratory should establish its own normal range value, the values of serum IL-10 from each patient were noted and compared with values from the healthy subjects.

Statistical methods

The data collected were placed electronically in an Excel utility. This allowed a view of the tests to be applied. Using statistical software, the data was tested for normality to see Gaussian distribution and then applied parametric or nonparametric tests properly. Parametric data are presented as mean and SD, and nonparametric data as median and range. For this we applied the Student t test, Mann Whitney, Wilcoxon. Correlation analyses were conducted using the Spearman rank correlation coefficient. P values were based on two-tailed statistical tests, with a significance level of 0.05.

RESULTS

The main clinical and demographic features of the 17 MS patients (12 women and 5 men) are summarized in table no. 1. We also included the characteristics of healthy subjects that are matched from both sex and age with our patients.

The mean age at testing of our patients was 40.7 years and they had a mean duration of disease of 10.1 years. This long period determined in 5 patients the evolution toward a SP form of MS, while the rest remained with a RR form. Our cases had few (0.5) relapses in the year prior to testing but with an important standard deviation due to a patient that had an aggressive form of MS with 3 relapses (a 26-year old female, in whom MS was diagnosed when she was 14 years-old; she was put on immunomodulatory treatment –glatiramer acetate-6 years ago; she had to stop the treatment after 4 years developing severe lipodistrophy and for the last 2 years she was put on IFN-β 1a but the disease is not well controlled with the actual EDSS in the remission phase of 3.0; the last relapse was due to a demyelinating plaque in the left cerebellum hemisphere with severe ataxia; she responded well to corticosteroid treatment; we intended to start an escalating therapy by putting her on natalizumab i.v. monthly). The majority of our patients were fully ambulatory with a mean EDSS at 2.7.

The serum level of IL-10 was more reduced in MS patients than in the healthy controls (2.9 vs 3.19).

Table no. 1. Demographic and clinical data regarding MS of the patients and healthy subjects; serum IL-10 titre in both groups

<table>
<thead>
<tr>
<th>Age at MS onset (years)± SD</th>
<th>n=17</th>
<th>Age at testing (years)±SD</th>
<th>n=17</th>
</tr>
</thead>
<tbody>
<tr>
<td>33.29 ± 11.59</td>
<td></td>
<td>40.70 ± 14.97</td>
<td></td>
</tr>
<tr>
<td>Treatment duration (years)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4.29 ± 3.40</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MS duration (years) ± SD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10.11 ± 6.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men/Women</td>
<td></td>
<td>5/12</td>
<td>5/12</td>
</tr>
<tr>
<td>EDSS± SD</td>
<td></td>
<td>2.73 ± 1.84</td>
<td></td>
</tr>
<tr>
<td>Number of relapses within</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>the previous year± SD</td>
<td></td>
<td>0.52 ± 0.87</td>
<td></td>
</tr>
<tr>
<td>IL-10 (pg/dL) ± SD</td>
<td></td>
<td>2.90 ± 2.58</td>
<td>3.19 ± 2.77</td>
</tr>
<tr>
<td>SMSP/SMRR</td>
<td></td>
<td>5/12</td>
<td></td>
</tr>
</tbody>
</table>

We searched if the serum levels of IL-10 were statistically linked with different characteristics of MS patients. In order to achieve our goal, we divided our patients in different groups according to: a) EDSS (maximum 3 when the patient is fully ambulatory without any walking restriction and more than 3 that characterizes the patient with moderate handicap); b) MS duration (maximum 5 years of evolution and more than 5 years of MS duration); c) duration of IFN-β 1a treatment (approximately short duration of maximum 5 years and more than 5 years); d) MS form (RR vs SP). The statistical data that we obtained are presented in table no. 2.
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Table no. 2. IL-10 serum titre in different patients groups

<table>
<thead>
<tr>
<th>EDSS</th>
<th>MS duration (years)</th>
<th>Duration of treatment (years)</th>
<th>MS form</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤3</td>
<td>&gt; 3</td>
<td>≤5</td>
<td>&gt;5</td>
</tr>
<tr>
<td>IL-(pg/dL) 3.1±2.70</td>
<td>2.68±2.60</td>
<td>3.45±3.62</td>
<td>2.67±2.18</td>
</tr>
<tr>
<td>2.70±2.75</td>
<td>3.14±2.54</td>
<td>2.78±2.42</td>
<td>3.21±3.22</td>
</tr>
</tbody>
</table>

In patients that had a mild neurological handicap (EDSS ≤3) the serum level of IL-10 was higher but without statistical significance compared with MS patients more severe affected characterized by a higher EDSS. Also, the disease duration influenced the secretion of IL-10 being higher in the early stages of MS evolution but without any statistical significance. A longer IFN-β1a treatment was followed by an increased serum IL-10 but not in a statistical manner between the two groups.

DISCUSSIONS

Evaluation of cytokine production in IFN-β1a MS treated patients is important in clarifying both the pathological mechanisms of MS and the IFN-β1a mode of action. IL-10 is the hallmark of the Th-2 cells subset that has an anti-inflammatory effect in many autoimmune disease, including MS.(1,2,3)

In the majority of EAE models, IL-10 had a role in preventing the demyelinating disease, whereas its role in ongoing EAE is still under debate.(2)

Later data showed that IL-10 have a role in immune tolerance rather than in immunity per se. An important amount of IL-10 is secreted by Treg. The induction of this cytokine determines the activation of STAT5 that engages the transcription factor Foxp3 (very important in the transfer of immune tolerance, mainly self-tolerance). Although IL-10 is considered the most important anti-inflammatory cytokine having the role of inhibiting proinflammatory cytokine production, some studies found contradictory data. Lower serum levels of IL-10 were found in MS patients (9), while other investigators (10) have found elevated numbers of IL-10 mRNA-expressing blood mononuclear cells.

In MS treatment, IFN-β has an important clinical role: decreases relapse rate and severity, progression of handicap, development of new demyelinating T2 MRI lesions. The complete mechanism of action if not completely understood. Numerous studies tried to demonstrate the immunoregulatory effect of IFN-β by determining the serum titre of different inflammation biomarkers. IFN-β treatment has increased serum levels of IL-10, mainly through IL-17/IL-10-associated pathways.(11,12) Carrieri et al (13) examined the effects of IFN on IL-10 and IL-12 secretion in RRMS patients and found that in patients that responded to IFN treatment (without disease activity for one year under IFN) the IL-10 production was significantly increased. Krakauer et al (14) found an increased expression of IL-10 mRNA after IFN treatment of RRMS patients. IFN-β 1a and IFN-β 1 b have different patterns of influence on cytokines: the first enhances the production of IL-10 and IL-4 (anti-inflammatory effect) while IFN-β 1 b decreases IFN-γ that is a pro-inflammatory cytokine. IFN-β 1a produces a shift of the cytokine profile toward the Th2 phenotype.(16) IL-10 is useful to determine the role of regulatory B lymphocytes in MS: B cells of MS patients present a significant decreased secretion of IL-10, suggesting a defect in the B cells regulatory property.(15) The group from Stockholm found that untreated MS patients had lower levels of IL-10-secreting blood mononuclear cells than healthy controls but in IFN-β treated patients the difference was not found.(17) On the contrary with these findings, Salmaggi et al (18) looked to the serum IL-10 levels in MS patients who were either in an active or in a stable clinical condition. When the values were compared with healthy subjects, no significant difference was found, data consistent with our study. Still we found a decreased IL-10 titre in MS patients compared with healthy controls, demonstrating that an autoinflammatory disease such as MS needs a shift toward the proinflammatory environment with primary or secondary decrease of the anti-inflammatory cytokines as IL-10.

Another debate is the moment of MS (recurrence or remission) when IL-10 is secreted in higher quantities. It is assumed that during MS relapse, elevated IL-10 levels result from the stimulation of the regulatory mechanisms that suppress autoimmune processes.(13) Other authors like Trenova et al (19) found an increased secretion of mediators with opposite effects (IFN-γ, IL-10) during remission, demonstrating persisting regulatory dysbalance in the Th1/Th2 system.

In a study in postmortem human brain from MS patients, Hulshof et al (20) found specific patterns of protein localization and protein expression for IL-10 in MS lesions at different stages of development, demonstrating that IL-10 participate to constitute chronic MS lesions.

The discrepancy between our results and previous data are related to: a) the MS population tested (different duration of MS); b) different duration of IFN-β1a treatment; c) age of patients; d) lack of physical activity for the majority of patients with disease progression. Eur Cytokine Network 2002;13:200-206.

More research is needed to establish if decrease of IL-10 secretion in MS patients treated with IFN-β1a is a cause of and/or a consequence of the complex autoimmune mechanisms that trigger the demyelinating cascade in MS.

REFERENCES


