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

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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 im 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.

Rezumat: Scleroza multiplă (SM) este o boală cronică, autoimună, demielinizantă care afectează adulţii tineri. Beta interferonul (IFN - β) este principalul tratament imunomodulator în SM, utilizat de peste două decenii, dar cu mecanismul de acţiune insuficient înţeles. Evaluarea producţiei de citokine ale pacienţilor cu SM trataţi cu IFN - β este importantă, atât pentru clarificarea mecanismelor patologice ale SM, cât şi modul de acţiune al IFN - β. Obiective: Determinarea titrului seric de IL - 10 la pacienţii cu SM trataţi cu IFN - β1a im şi găsirea vreunei corelaţii între nivelul seric al IL - 10 şi datele demografice, handicapul neurologic, durata SM şi durata tratamentului cu IFN - β1a. Material şi metodă. Am inclus un număr de 17 pacienţi cu SM trataţi timp de cel puţin 12 luni cu IFN - β1a . Pentru detectarea cantitativă a IL – 10 a fost utilizată metoda ELISA, iar valorile au fost comparate cu cele ale 17 martori sănătoşi. Rezultate. Am găsit un titru seric al IL - 10 mai mare la pacienţii cu afectare neurologică mai redusă, durată de SM de maximum 5 ani şi la pacienţi care au avut cel puţin 3 ani de tratament cu IFN - β 1a. Nici una dintre aceste diferenţe nu are o semnificaţie statistică. Concluzii. Sunt necesare mai multe cercetări pentru a stabili rolul secreţiei de IL - 10 la pacienţii cu SM trataţi cu IFN – β1a.

INTRODUCTION

Interleukin-10 (IL-10) is an anti-inflammatory cytokine that is also known as human cytokine synthesis inhibitory factor. IL-10 is o 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

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 Encephalomyelitis (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-Mureş, 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 EIA-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

	All Patients	Healthy Subjects
	n=17	n=17
Age at MS onset (years) ± SD	31,29 ± 11,59	32,29 ± 7,48
Age at testing (years)±SD	40,70 ± 14,97	
Treatment duration (years) ± SD	4,29 ± 3,40	
MS duration (years) ± SD	10,11 ± 6,08	
Men/Women	5/12	5/12
EDSS± SD	2,73 ± 1,84	
Number of relapses within the previous year± SD	0,52 ± 0,87	
IL-10 (pg/dL) ± SD	2,90 ± 2,58	3,19 ± 2,77
SMSP/SMRR	5/12	

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.

CLINICAL ASPECTS

Table no. 2. Il-10 serum titre in different patients groups

	EDSS		MS duration (years)		Duration of treatment (years)		MS form	
	≤3	> 3	≤5	>5	≤3	>3	SMRR	SMSP
IL-(pg/dL)	3,11± 2,70	2,68±2,60	3,45±3,62	2,67±2,18	2,70±2,75	3,14±2,54	2,78±2,42	3,21±3,22

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-β 1a 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-β 1a 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).(6,7,8) 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 immunoreglatory 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 at el (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-β 1a treatment; c) age of patients; d) lack of physical activity for the majority of patients as exercise increases the IL-10 (21); e) inclusion of both RR and SPMS forms; f) inclusion of patients only in the nonactive phase of MS.

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

REFERENCES

1. Almeras L, Meresse B, de Seze J, et al. Interleukin-10 promoter polymorphism in multiple sclerosis: association with disease progression. Eur Cytokine Network 2002;13:200-206.
2. Asadullah K, Sterry W, Volk HD. Interleukin-10 therapy-review of a new approach. Pharmacol Rev 2003;55:241-269.
3. Azarpira N, Haghighi AB, Pourjafar M, et al. nterleukin 10 gene polymorphism in Iranian Patients with multiple sclerosis. Acta Neurol Taiwan 2010;19:107-111.
4. Zhang L, Yuan S, Cheng G, et al. Type I IFN promotes IL-10 production from T cells to suppress Th17 cells and Th17-associated autoimmune inflammation. PLOS ONE, 2011;6:e28432.
5. Polman CH, Reingold S, Banwell B, et al. Diagnostic criteria for multiple sclerosis: 2010 Revisions to the McDonald criteria. Ann Neurol 2011;69:292-302.
6. Ye J, Huang X, Hsueh EC, et al. Human regulatory T cells induce T-lymphocyte senescence. Blood 2012;120:2021-2024.
7. Tsuji-Takayama K, Suzuki M, Yamamoto M, et al. The production of IL-10 by human regulatory T cells is enhanced by IL-2 through a STAT5-responsive intronic enhancer in the IL-10 locus. J Immunol 2008;181:3897-3903
8. Piccio L, Cross AH. Immunology of multiple sclerosis. In: Giesser BS (Ed). Premier on multiple sclerosis. Oxford University Press 2011:47-59.
9. Huang WX, Huang P, Link H, et al. Cytokine analysis in

multiple sclerosis by competitive RT-PCR: a decreased expression of IL-10 and an increased expression of TNF-alfa in chronic progression. Mult Scler 1999;5:342-348.

10. Navikas V, Link J, Palasik W, et al. Increased mRNA expression of IL-10 in monomuclear cells in multiple sclerosis and optic neuritis. Scand J Immunol 1995;41:171-178.

11. Rudick RA, Ransohoff RM, Peppler R, et al. Interferon beta induces interleukin-10 expression: relevance to multiple sclerosis. Ann Neurol 1996;40:618-627.

12. Kvarnstrom M, ydrefors J, Ekerfelt C, et al Longitudinal interferon beta effects in multiple sclerosis: differential regulation of IL-10 and IL-17A, while no sustained effects on IFN-γ, IL-4 or IL-13. J Neurol Sci 2013;325:79-85.

13. Carrieri P, Ladogana P, Di Spigna GD, et al. Interleukin-10 and interleukin-12 modulation in patients with relapsing remitting MS on therapy with interferon-beta 1a: differences in responders and nonresponders. Immunopharmacol Immunotoxicol 2008;30:915-923.

14. Krakauer M, Sorensen P, Khademi M, et al. Increased IL-10 mRNA and IL-23 mRNA expression in multiple sclerosis: interferon-beta treatment increases IL-mRNA expression while reducing IL-23 mRNA expression. Mult Scler 2008;14:622-630.

15. Michel l, Degauque N, Garcia A, et al. Loss if IL-10 secretion by regulatory B lymphocytes in multiple sclerosis patients. J Trans Med 2011; 9 (Suppl 2): P25.

16. Sega S, Wraber B, Mesec A, et al. IFN-β 1a and IFN-β 1b have different patterns of influence on cytokines. Clin Neurol Neurosurg 2004;106:255-258.

17. Ozenci V, Kouwenhoven M, Huang Y, et al. Multiple sclerosis is associated with an imbalance between tumour necrosis factor-alpha and IL-10 screening blood cells that is corrected by interferon-beta treatment. Clin Exp Immunol 2000;120:147-153.

18. Salmaggi A, Dufour A, Eoli M, et al. Low serum interleukin-10 levels in multiple sclerosis: further evidence for decreased systemic immunosuppression? J Neurol 1996; 243:13-17.

19. Trenova AG, Manova M, Kostadinova I, et al. Clinical and laboratory study of proinflammatory and anti-inflammatory cytokines in women with multiple sclerosis. Folia Medica 2011;33:29-33.

20. Hulshof S, Montaigne L, De Groot C, et al. Cellular localization and expression patterns of interleukin-10, interleukin-4, and their receptors in multiple sclerosis lesions. Glia 2002;38:24-35.

21. Mogadasi M, Moeini A, Edalatmanesh M. Effect of resistance training on cytokines in female patients with multiple sclerosis. Ann Biol Res 2013;4:169-173.