Interleukin 17 receptor in multiple sclerosis patients treated with interferon β-1a

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Abstract

Interleukin 17 (IL-17) and its receptor IL-17R1 produced by T-helper cells named Th17 are involved in the pathology of autoimmune diseases. In contrast to the at least partially explained role of IL-17 in pathology of multiple sclerosis, the significance of IL-17R in MS is unclear. Therefore we have studied the expression of IL-17R in the stable phase of multiple sclerosis treated by interferon β-1a. The studied material consisted of 20 MS patients with relapsing-remitting form of the disease, and fulfilling the diagnostic McDonald et al. criteria.

The patients were treated subcutaneously every second day with 30 mg of interferon β-1a (Betaferon). The interleukin 17 receptor level was measured by the ELISA immunoassay test using RayBio human IL-17R ELISA kit. After three months of therapy with interferon β-1a the level of IL-17R was significantly higher than that established at the starting point. The level of IL-17R after 6 months of therapy was insignificantly higher than established in the previous study group (3 months of therapy). While it remains difficult to pinpoint the exact significance of upregulation of IL-17R in the early period of therapy, the present findings should be taken into account when considering the pharmacodynamics of interferon action in MS in view of the opinions on the crucial role of IL-17 in pathology of MS and suggestions that it may constitute a drug target in autoimmunological diseases.

Key words: interleukin 17, multiple sclerosis, interferon β-1a.

Introduction

Interleukin 17 (IL-17) and its receptor IL-17R1 produced by T-helper cells, named Th17, are involved in the pathology of autoimmune diseases. IL-17 is of importance in such processes as delayed-type hypersensitivity, contact hypersensitivity and allergic airway inflammation. According to Iwakura *et al.* [11], IL-17 promotes inflammation by inducing several proinflammatory cytokines and chemokines and recruiting neutrophils. IL-17 is increased in serum of patients with systemic lupus erythematosus (Zhao *et al.* [29]). Already in 1999, Matusevicius *et al.* [16] found elevation of IL-17 mRNA expression in blood and cerebrospinal fluid mononuclear cells in multiple sclerosis. An important observation, made by Graber *et al.* [9], was that the number of IL-17 mRNA expressing cells in blood was the highest during clinical exacerbations. MS patients in the early stage of the disease (less than 2 years) and cases with

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transverse myelitis had increased IL-17 production by stimulated peripheral blood mononuclear cells. Of similar significance are the findings of Frisullo *et al.* [4] that in patients with clinically isolated syndrome (CIS), suggesting early multiple sclerosis, the spontaneous production of IL-17 by peripheral blood mononuclear cells is increased. No overproduction of the interleukin can be detected in secondary progressive multiple sclerosis.

Very important and clarifying the significance of IL-17 in MS are studies of Tzartos *et al.* [26], who found that IL-17 production is increased in perivascular lymphocytes, in astrocytes and oligodendrocytes located in the active areas of MS lesions in the central nervous system.

The briefly presented findings seemed to document that IL-17 is involved in pathogenesis of the early phase in multiple sclerosis.

The structure and role in inflammation and autoimmunology of IL-17 receptors has been the subject of several publications [1,23,27,28] and review papers [5,6,7,24]. The IL-17 receptor superfamily comprises five receptor subunits, IL-17RA–IL-17RE. All are single transmembrane domain containing receptors. The receptor subunits contain conserved structural motifs, including extracellular fibronectin III-like domain and the cytoplasmic "SEFIR" domain. The largest member of the IL-17RA superfamily is a common signalling unit, used by multiple ligands.

IL-17RA is expressed, largely at high levels, in haematopoietic tissue, in endothelial cells and fibroblasts. IL-17RA is expressed not only in peripheral tissues but also in the central nervous tissue. Astrocytes und microglia express IL-17RA in vitro [2]. There are controversial views as to the IL-17RA-containing complexes: the prevailing opinion is that individual subunits reside in the membrane in the form of monomers.

Serum contains the soluble and transmembrane isoform of interleukin-17 receptor-like protein, but probably most of them circulate in the form of complexes with immunoglobulin (IL-17R-Ig) [25].

In contrast to the at least partially explained role of IL-17 in pathology of multiple sclerosis, the significance of IL-17R in MS is unclear. Therefore, we studied the expression of IL-17R in the stable phase of multiple sclerosis treated with interferon β-1a.

Material and methods

The studied material consisted of 20 MS patients with relapsing-remitting form of the disease, and fulfilling the diagnostic criteria of McDonald *et al.* The MS cohort included 14 females and 6 males, of the mean age 35.9 ± 10.2 years. The duration of MS was 4.7 ± 3.0 years, and on the EDSS scale the process averaged 3.0 ± 1.18. All patients had at least one relapse in the last 12 months and half of them manifested two relapses in the last 24 months.

The patients were treated subcutaneously every second day with 30 mg of interferon β-1a (Betaferon).

Blood samples for the immunological test were taken before treatment, and after 3 and 6 months of interferon therapy. The investigations were approved by the Regional Ethics Committee in the Medical University in Poznań.

The interleukin 17 receptor level was measured in duplicate by the ELISA immunoassay test, using Ray-Bio human IL-17R ELISA kit (Georgia, USA). The assay employs a 96-well plate coated with an antibody specific for human Il-17R. Standard and study samples were pipetted into the wells and IL-17R present in a sample was bound to the wells by the immobilized antibody. All the steps were performed according to the user's manual, prepared by Ray Biotect, Inc. After washing off any unbound biotinylated antibody, HRP-conjugated streptavidin was added, the plate was washed again, then a TMB (3,3`-5.5`-tetramethylbenzidine) substrate solution was added and colour developed of intensity correlated with the amount of bound IL-17R. After use of a stop solution, intensity of the colour was measured at 450 nm in a spectrophotometer (Bender Med Systems). The results represented mean absorbance of duplicate standard, controls and samples following subtraction of the average zero standard optical density, using a standard curve.

The level of IL-17R was compared between the studied groups by Tukey (ANOVA) test. The statistical analysis was performed using the Statistica 8.0 Pl system.

Results

After 3 months of therapy with interferon β-1a the level of IL-17R was significantly higher than that established at the starting point. The level of IL-17R after 6 months of therapy was insignificantly higher

Grażyna Michałowska-Wender, Justyna Biernacka-Łukanty, Zofia Lasik, Łukasz Jernas, Mieczysław Wender

Table I. IL-17R serum level (pg/ml) in MS patients treated with interferon β-1a

Before treatment	3 months of therapy	6 months of therapy
8.3 ± 6.2	14.7 ± 8.3*	11.5 ± 10.1

*$p < 0.05$

than that established in the previous study group (3 months of therapy).

The results are presented in Table I.

Discussion

Multiple sclerosis is generally considered to be a chronic autoimmune disease [12,14]. Among immuno-modifying therapies of MS, treatment with interferon β (IFN-β) still represents the principal approach. The detailed mechanism of IFN-β action as an immuno-modulating agent in MS is not known, but the action is only extrathecal, i.e. it does not take place in the central nervous system. According to Dhib-Jalbut and Marks [3], the main modes of action involve inhibition of T-cell activation and proliferation, probably by increase of apoptosis, as well as slowed down white cell migration through the blood-brain barrier and, last but not least, cytokine modulation. IFN-β suppression of Th17 cell differentiation process, which seems to be connected with expression of IL-17 and IL-17 receptors, represents one of the most probable mechanisms of suppression of the autoimmune response in MS [20-22]. The effect of therapy with IFN-β on cytokine pattern was studied by Losy and Michałowska-Wender [18], who found a significant increase of IGF-β after 6 months of treatment. The authors could not confirm the suggestion of Karp *et al.* [15] that the basic mechanism of IFN-β action in MS is suppression of IL-12 production.

The effect of interferon β therapy on cytokine and chemokine levels is not generalized but selective and so Losy *et al.* [19] could not find any effect of treatment on MIP-1 and MCP-1 levels. In our recent studies we have established the impact of therapy with IFN-β on serum levels of IL-17 receptor, an important player in immunology of MS. While it remains difficult to pinpoint the exact significance of upregulation of IL-17R in the early period of therapy, the present findings should be taken into account when considering the pharmacodynamics of interferon action in MS in view of the opinions on the crucial role of IL-17 in the pathology of MS [8,17] and suggestions that it may constitute a drug target in autoimmunological diseases [10].

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