Multiple Sclerosis and Related Disorders 18 (2017) 29–32

Contents lists available at ScienceDirect

Multiple Sclerosis and Related Disorders

journal homepage: www.elsevier.com/locate/msard

Lymphocytes in the treatment with interferon beta-1 b

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ARTICLE INFO

Keywords:
Multiple sclerosis
Lymphocytes
Interferon beta – 1 b
Parameters
Treatment
Characters

ABSTRACT

Background: Multiple sclerosis (MS) is a chronic inflammatory demyelinating and neurodegenerative disease affecting the central nervous system. One of the basic medications for the treatment of a clinically isolated syndrome (CIS) or relapsing-remitting MS is interferon beta (INFβ). Although the exact mechanism of its effects is unknown, the medication has an anti-inflammatory and immunomodulatory effect. The goal of this study was to determine the characters which are affected in patients treated with INFβ.

Methods: A total of 97 patients (25 males and 72 females) were included into the study. Patients were treated by INFβ 1-b (subcutaneous injection, 250 μg, each other day). Clinical evaluations were performed by an attending neurologist. Peripheral blood samples were obtained just prior to treatment and 5 years after INFβ 1-b. Statistical analysis and processing of the obtained data were performed by using a comprehensive statistical software package MATLAB®.

Results: A significant decrease of the observed parameters after 5 years' of treatment (significant at the 1% significance level) was found in the absolute and relative CD69 count, absolute cytotoxic/suppressor T lymphocyte count, absolute total leukocyte count, absolute natural killer cells count. A significant decrease of the observed parameters after 5 years' of treatment (significant at the 5% significance level) was found in the absolute lymphocyte count, relative cytotoxic/suppressor T lymphocyte count, relative CD3 + CD69 + count and absolute CD8 + CD38 + count.

Conclusion: The treatment with interferon beta reduces clinical exacerbations in multiple sclerosis (MS) through several known immunomodulatory mechanisms. However, the exact mechanism of effect of this medication is not known. This study presents some parameters that were affected by the long-term INFβ treatment.

1. Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating and neurodegenerative disease affecting the central nervous system. Genetic predispositions and environmental factors are involved in the pathogenesis of this disease (Steinman, 2001). Although MS is an incurable disease, it is one of the autoimmune immunopathological diseases, whose pathology is quite well explored. It has its own experimental animal model, the so-called experimental autoimmune encephalomyelitis (EAE). In this model, the autoimmune immunopathological reactivity plays the main role. The release of autoantigens in the central nervous system occurs in an individual affected in this way. After processing and binding to the HLA (Human Leukocyte Antigen), these autoantigens are presented to autoreactive T lymphocytes. Antigen-presenting cells identify harmful patterns, produce pluripotent proinflammatory cytokines and give necessary costimulatory signals to T lymphocytes. The blood-brain barrier becomes permeable for lymphocytes and mononuclear phagocytes which migrate to the central nervous system (CNS). The main targets of harmful inflammation are immunodominant epitopes of the myelin basic protein (Krejsek et al., 2002). Although until recently, T lymphocytes were considered to be an absolutely crucial part of MS pathogenesis. Thus, the contribution of humoral immunity to the harmful inflammatory reaction cannot be ignored.

One of the basic medications for the treatment of a clinically isolated syndrome (CIS) or relapsing-remitting MS is interferon beta (INFβ). Although the exact mechanism of its effects is unknown, the medication has an anti-inflammatory and immunomodulatory effect. It dampens the activity of Th1 subset of T lymphocytes by induction of IL-10 production. It decreases the production of proinflammatory cytokine

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http://dx.doi.org/10.1016/j.msard.2017.08.020
Received 30 June 2017; Received in revised form 18 August 2017; Accepted 31 August 2017

IL-17. It leads to the reduction of antigen presentation and T lymphocytes proliferation. It decreases the permeability of the blood-brain barrier by blocking the adhesive interactions, dampening the effect of matrix metalloproteinases and leukocytes migration (Anderton and Liblau, 2008; Chabot et al., 1997; Chen et al., 2009; Markowitz, 2007). Nevertheless, the INFβ treatment leads specific and marked decrease of CD27+ memory B cells. In vivo IFNβ therapy specifically and highly induced apoptosis in memory B cells, in accordance with a strong increase of the apoptotic markers Annexin-V and active caspase-3, via a mechanism requiring the FAS-receptor/TACI (transmembrane activator and CAML interactor) signaling (Rizzo et al., 2016).

In this study individual populations of lymphocytes in MS patients (CIS and relapsing-remitting MS) were examined. The goal was to determine the characters which are affected in patients treated with INFβ.

2. Material and methods

2.1. Study population

All subjects (aged from 19 to 69, mean age 42 +− 11) of Caucasian origin fulfilled the McDonald criteria or revised McDonald criteria for RR MS (McDonald et al., 2001; Polman et al., 2005; Polman et al., 2011). All subjects were recruited in the Department of Neurology, Faculty of Medicine and University Hospital Hradec Králové. Between 2004 and 2016, a total of 97 patients (25 males and 72 females) were included into the study. Patients were treated by INFβ 1-b (subcutaneous injection, 250 μg, each other day).

All case participants were recruited during their hospital visit where all relevant MS information (Expanded Disability Status Scale - EDSS, disease duration, MS treatment history) were obtained. Clinical evaluations were performed by an attending neurologist. Peripheral blood samples were obtained just prior to treatment and 5 years after INFβ 1-b.

All participants gave written informed consent. The study protocol was approved by the Ethical Committee of the University Hospital Hradec Králové, reference number 201706S18P.

2.2. Sample collection and sample processing

Blood samples were collected from the antecubital fossa vein. Relative numbers of cluster of differentiation CD3+, CD4+, CD8+, CD19+, CD3-/CD16+56+, CD3+CD69+, CD3+CD25+, CD4+/CD45RA+, CD4+/CD45RO+, CD8+/CD38+, CD19+/CD5+, CD40 and CD40L lymphocytes were analysed by two-colour flow cytometry. For surface staining, 100 μl of blood was added to tubes containing 10 μl of fluorochrome-labelled monoclonal antibody, including fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (clone UCHT1), anti-CD4 (clone 13B8.2), anti-CD45RA (clone ALB11), anti-CD8 (clone B9.11) and anti-CD19 (clone J3-119) and phycoerythrin (PE)-conjugated anti-CD25 (clone B1.49.9), anti-CD69 (clone TP1.55.3), anti-CD4 (13B8.2), anti-CD45RO (clone UCHL1), anti-CD38 (clone LS198-4-3), anti-CD5 (clone BL1a), anti-CD40 (clone MAB89) and anti-CD40L (clone TRAP-1), all of which were supplied by Beckman Coulter (Miami, FL, USA). Class-matched isotype immunoglobulin conjugated to FITC and PE were used as negative control monoclonal antibodies and were added simultaneously to separate tubes for all samples to detect nonspecific binding.

Subsequently, 100 μl of heparinized peripheral blood was mixed with the cocktail monoclonal antibody solution and incubated for 15 min at room temperature. After the incubation, lysing solution (OptiLyse C, Beckman Coulter) was added, and the mixture was incubated for another 10 min. Flow cytometric analysis was performed using the Cytomics FC 500 cytometer (Beckman Coulter) equipped with a 15-mW air-cooled 488-nm argon laser and a 625-nm neon diode laser. The data were analysed using the CXP Analysis Software (Beckman Coulter). Data on at least 10,000 events were acquired for each staining and stored in list mode.

2.3. Statistical analysis

Statistical analysis and processing of the obtained data were performed by using a comprehensive statistical software package MATLAB® (MathWorks, Inc., USA). A Kolmogorov-Smirnov (K-S) test for the assessment of normality and paired parametric Student's T-test to determine the different reliability was used. The critical level of significance was considered to be 5% ($p < 0.05$). The obtained data are presented as an arithmetic mean and standard error of arithmetic mean.

3. Results

During the observation, multiple peripheral blood parameters were examined in absolute and relative values. These were lymphocytes, CD4+ T lymphocytes, CD8+ T lymphocytes, CD 19 (B lymphocytes), natural killer cells (CD3-/CD16+56+), CD3+/CD69+ cells, CD5 cells, CD25 cells, CD3+/CD25+ cells, CD5+/CD19+ cells, CD4+/CD45RO+ cells, CD8+CD38+ cells, CD4+/CD45RA+ cells, CD 69 protein, CD 40 protein, CD 40 L protein in absolute and relative values and also the absolute leukocyte (white blood cells) count.

Table 1 below presents the statistical significance of the change of the level of measured values in the treatment. The data with ab represent an absolute value and the data without ab represent a relative value. The absolute values are calculated from the blood count, the relative values are then the percentage of lymphocytes.

4. Discussion

The immunopathogenesis of MS is a complex process involving T lymphocytes and B lymphocytes. In the inflammatory reaction, Th1 T lymphocytes, Th17 T lymphocytes and activated macrophages have the dominant effect. Th1 subset of T lymphocytes is responsible for the development of the cytotoxic reaction. Th1 T lymphocytes produce proinflammatory cytokines interferon γ (INFγ), Tumour Necrosis Factor β (TNFβ) and interleukin 2 (IL-2). Th17 T lymphocytes most likely have the crucial role in the immunopathogenesis of MS. This subset produces interleukins IL-17, IL-21, IL-22 and IL-26. Regulatory T (Treg) lymphocytes act in a regulatory way against the activities of subset Th17. Dysfunction of Treg T lymphocytes plays a significant role in the pathogenesis of MS (Costantino et al., 2008).

B lymphocytes are responsible primarily for specific, antibody-mediated immune response. Autoreactive B lymphocytes cross the blood-brain barrier. After stimulation by an autoantigen they go through the process of maturation and clonal expansion in the CNS. This population of B lymphocytes and plasma cells can be detected in MS lesions, cerebrospinal fluid, even in peripheral blood (Hauser, 2015). The examination of cerebrospinal fluid is a very important examination helping to determine the definitive MS diagnosis (Polman et al., 2011). In patients with MS, the isoelectric focusing of a sample of cerebrospinal fluid can help to find the so-called oligoclonal bands which are considered as evidence of oligoclonal expansion of B lymphocytes present directly in the CNS. The role of B lymphocytes in the pathogenesis of MS is very diverse. They produce antibodies; they are very effective antigen-presenting cells (APC), and they create cytokines (antiinflammatory IL-10 and proinflammatory TNFα a IL-6).

The goal in this study was to determine which immunological parameters from peripheral blood are affected by the long-term INFβ 1-b treatment. A significant decrease of the parameter CD69 was found. CD69, one of the earliest specific antigens acquired during the lymphoid activation, acts as a signal-transducing receptor involved in cellular activation events, including proliferation and induction of specific genes. CD69 belongs to a family of receptors that modulate the immune response and whose genes are clustered in the natural killer (NK) gene complex (Llera et al., 2001). The recent discovery of a ligand for CD69