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Immune-cell BDNF expression in treatment-naïve relapsing-remitting multiple sclerosis patients and following one year of immunomodulation therapy

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ABSTRACT

Although neurons are the main source of neurotrophins in the healthy brain, neurotrophins can also be expressed in the immune system. We have previously shown that in relapsing-remitting multiple sclerosis (RRMS) lower immune-cell neurotrophin levels are associated with brain atrophy and cognitive impairment. The aim of the present study was to assess if immune-cell neurotrophin expression is impaired in MS as compared with the healthy controls, and to describe if these levels change in treatment-naïve RRMS patients, following one year of immunomodulation.

Fifty treatment-naïve RRMS patients were assessed at baseline and after one year of immunomodulation (beta-interferons/glatiramer acetate). The control group included 39 healthy subjects matched according to age and gender. Peripheral blood mononuclear cells (PBMCs) were isolated from heparinized blood using Ficoll-Histopaque gradient. The levels of brain-derived-neurotrophic-factor (BDNF), beta-nerve-growth-factor (beta-NGF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5) were measured in PBMC lysates with ELISA.

BDNF levels were significantly lower in MS than in the healthy controls (median 613 vs. 1657 pg/mg protein, $p < 0.001$). After one year of immunomodulation, BDNF expression did not change significantly ($p = 0.06$) on the group level. In 70% of patients there was no increase in BDNF level, and in 30% it increased. We observed no differences between treatment groups. Other neurotrophins were detected in a minority of MS samples (as opposed to the controls).

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To conclude, we have shown that immune-cell production of neurotrophins is impaired in MS patients. In our MS cohort standard immunomodulation failed to restore normal BDNF levels in PBMCs within one year of therapy.

1. Introduction

Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system (CNS), in which both, neuroinflammatory and neurodegenerative components, are responsible for endpoint disability in patients. So far it has not been established to which degree neurodegeneration can be independent of inflammation in MS. The interrelationship between the two components is complex. Although in MS inflammation drives neurodegeneration by means of oxidative stress and mitochondrial dysfunction [1], it can also exert neuroprotective effects, such as provided by immune-cell production of neurotrophic factors [2].

Neurotrophic factors are a family of polypeptides involved in neuronal development [3], survival [4] and synaptic plasticity [5]. They include neurotrophins, namely brain-derived neurotrophic factor (BDNF), nerve growth factor (NGF), neurotrophin 3 (NT-3) and neurotrophin 4/5 (NT-4/5). Neurons are the main source of neurotrophins in the healthy brain. However, when faced with MS-related damage, the additional supply of neurotrophins by peripheral blood mononuclear cells (PBMCs), which enter the brain via disrupted blood-brain barrier (BBB), could be essential in neuroprotection, especially in the periplaque area. It was previously shown, both in vivo and in vitro, that oligodendrocytes and astroglial cells serve as cellular targets for neurotrophins via trk receptors [6]. Neurotrophins, especially NT-3, were shown to regulate oligodendrocyte differentiation, which is the key process in remyelination [7]. Therefore, it may be hypothesized that in the periplaque area, where neuroinflammatory activity is the highest and so is the need for oligodendrocyte-mediated remyelination, immune cell-derived neurotrophic factors are key mediators of neuroregeneration and neuroprotection.

Following the concept of neuroprotective autoimmunity, we have previously shown that in relapsing-remitting MS (RRMS) patients neurotrophin levels are associated with general measures of brain atrophy, including brain parenchymal fraction (BPF) and corpus callosum cross-sectional area [8], and cognitive impairment [9]. We concluded that among RRMS patients, impaired immune-cell production of neurotrophins could be reflected by worse clinical outcome, as measured with brain atrophy and cognitive dysfunction parameters. Both these studies were of cross-sectional design and did not relate PBMC expression of neurotrophins in MS with the one in the healthy individuals. Therefore, one could not have assumed that all MS patients had impaired immune-cell production of neurotrophins. So far, only few studies measured neurotrophin expression within PBMCs in MS patients, mostly with regards to BDNF, while there is a number of papers assessing serum levels of neurotrophic factors. Serum BDNF levels, however, have not been considered reliable correlates of disease activity [10], mostly because the majority of serum BDNF stems from platelets, and not from immune cells [11]. As for immune cell source of neurotrophins, in one study BDNF was shown to be produced at lower levels than in PBMCs of the healthy controls [12]. In another study BDNF production by PBMCs was found to be increased during relapse phase, as compared with the remission and secondary progression [13]. On the contrary, Gielen et al. showed that BDNF expression (here assessed by mRNA levels, and not protein expression) was increased in MS as opposed to healthy controls and other neurological diseases [14].

The aim of the present study was to establish whether immune-cell production of neurotrophins is indeed impaired in treatment-naïve RRMS patients. Also, we wanted to assess if standard immunomodulatory treatment of MS could influence neurotrophin expression.

2. Methods

2.1. *Patient population*

Fifty-four patients diagnosed with relapsing-remitting multiple sclerosis according to the 2010 revised McDonald criteria [15] were screened and fifty were included in the study. The flow diagram of the study progress is presented in Fig. 1. All patients were treatment-naïve at the time of inclusion into the study. They were recruited consecutively in the Department of Neurology at the time of initiation of their immunomodulatory treatment, within 12 months of recruitment period. The study protocol was approved by the Internal Review Board at the Poznan University of Medical Sciences. All patients consented to the study in writing.

The study group consisted of 30 females and 20 males, with the mean age of 37 ± 9 years (min 18, max 64), median disease duration of 0.58 years (min 0.08, max 12.42 years) and median Expanded Disability Status Score (EDSS) of 2.0 (min 0.0, max 4.0). Clinical examination and blood sampling were performed at baseline, which was before therapy initiation, and after one year from treatment onset. All patients received standard first-line immunomodulatory drugs, namely beta-interferons (in 38 subjects) or glatiramer acetate (in 12 subjects). At follow up all patients were assessed clinically, including calculation of the Rio Score [16] and the complete set of study variables was assessed in 35 subjects.

The control group consisted of 39 healthy control subjects matched according to age and gender to MS study group. Written informed consent was obtained from control subjects, as well. Asymptomatic CNS pathology, especially radiologically isolated syndrome (RIS), was excluded in the healthy

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