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# Neurotrophic factors in relapsing remitting and secondary progressive multiple sclerosis patients during interferon beta therapy

Marcella Caggiula [a,b], Anna Paola Batocchi [a], Giovanni Frisullo [a], Francesco Angelucci [a], Agata Katia Patanella [a], Cristina Sancricca [a], Viviana Nociti [a], Pietro Attilio Tonali [a], Massimiliano Mirabella [a,*]

[a] *Institute of Neurology, Catholic University, Largo Gemelli 8, 00168 Rome, Italy*
[b] *Fondazione Pro Iuventute Don Carlo Gnocchi, Rome, Italy*

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**Abstract**

Although interferon (IFN) beta is a widely used disease-modifying therapy in multiple sclerosis (MS), the mechanisms responsible for its effects are not fully understood. Some studies demonstrated that IFNbeta induces nerve growth factor (NGF) secretion by astrocytes and by brain endothelial cells. In this study, we determined the production of various neurotrophins (brain-derived neurotrophic factor, BDNF; NGF; glial cell line-derived neurotrophic factor; neurotrophin 3; neurotrophin 4) by peripheral blood mononuclear cells (PBMCs) in relapsing–remitting (RR) and secondary progressive (SP) MS patients during IFNbeta treatment. There were no main variations in neurotrophin production either among all MS patients globally considered or in the group of SPMS subjects. Instead, in the group of RRMS patients who did not present clinical exacerbation of disease up to the end of the study, we found a significant increase in BDNF production as from 6 months after starting therapy.


*Keywords:* Multiple sclerosis; Disease-modifying therapy; Interferon beta; Neurotrophins; Brain-derived neurotrophic factor; Nerve growth factor; Glial cell line-derived neurotrophic factor; Neurotrophin 3; Neurotrophin 4/5; Neuroprotection

## Introduction

Multiple sclerosis (MS) is a human chronic inflammatory disease of the central nervous system and the most common disabling neurological disease in young adulthood. Interferon (IFN) beta is a widely used disease-modifying therapy in MS. It has been shown to significantly reduce relapse rate and the appearance of new lesions on magnetic resonance imaging (MRI) [1]. Moreover, recent studies demonstrated that IFN beta therapy also reduces atrophy rate, mainly in the early phases of disease [2,3]. The mechanisms underlying IFNbeta therapeutic effect seem to be mainly related to a reduction of the influx of T cells in the central nervous system (CNS) and to a shift towards a Th2-type response [4]. Non-immune mechanisms may also be responsible for its effects. In fact, some studies demonstrated that IFNbeta is a potent inducer of nerve growth factor (NGF) production by astrocyte cultures [5]. Furthermore, NGF production by human brain microvascular endothelial cells is triggered by interaction with T lymphocytes derived from MS patients, and it was potentiated by pre-treating lymphocytes with IFN beta in vitro and by using lymphocytes derived from MS patients treated with IFN beta in vivo [6].

The increased production of neuronal trophic factors may represent an indirect action of IFNbeta on remyelination and can help account for some of the efficacy of this treatment in MS, but to date, there are no data about neurotrophin production during IFNbeta therapy.

In this study, we determined neurotrophin (brain-derived neurotrophic factor, BDNF; NGF; glial cell line-derived neurotrophic factor, GDNF; neurotrophin 3, NT3; neurotrophin 4, NT4) production by peripheral blood mononuclear cells (PBMCs) in relapsing–remitting (RR) and secondary progres-

* Corresponding author. Fax: +39 06 35501909.
*E-mail address:* mirabella@rm.unicatt.it (M. Mirabella).

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sive (SP) MS patients during the first year of treatment with IFNbeta, and we correlated neurotrophin production with clinical outcome of patients.

## Materials and methods

### *Patients*

We serially studied 39 consecutive patients with definite MS (28 females and 11 males) [7]; 25 subjects presented a relapsing–remitting course of disease (19 females and 6 males) and 14 a secondary progressive one (9 females and 5 males). Their mean age was 37.7 ± 12.3 years (31.2 ± 10 as to RRMS patients and 49.4 ± 5 as to SP ones), and mean disease duration was 9.4 ± 9.1 years (5.8 ± 7 for RRMS patients and 15.9 ± 9 for SP ones). None of our patients had ever been treated with any immunomodulatory drugs except corticosteroids. RRMS patients were treated with IFNbeta-1a, 10 with 30 μg given once weekly intramuscularly, 10 with 22 μg given three times a week subcutaneously and 5 with 44 μg given three times a week subcutaneously. All SPMS subjects underwent IFNbeta-1b therapy 8 MIU administered subcutaneously every other day. Clinical data and blood samples were obtained at baseline and after 3, 6 and 12 months of treatment. In patients treated with intravenous methilprednisolone (20 mg/kg/die for 5 days) for relapses, blood was not drawn until 30 days after the corticosteroid treatment, in order to avoid corticosteroid-induced variations in neurotrophin production [8]. The Expanded Disability Status Scale (EDSS) was used to score degree of disability. At the end of the follow-up, patients were divided into two groups based on the occurrence of clinical relapses or progression in disability (increase of EDSS score >1). The study was approved by the Ethical Committee of our Institution.

### *Separation of PBMCs and cell culture*

PBMCs were isolated from venous blood by density gradient centrifugation (2500 rpm, 30 min) over a Ficoll–Hypaque density gradient (Pharmacia, Uppsala, Sweden). PBMCs were then harvested by pipetting cells from the Ficoll/serum interface and washed twice. Cells were cultured for 24 h in 24-well plates at a density of $5 \times 10^6$/ml in RPMI 1640 (EuroClone, West York, United Kingdom) containing 2 nM L-glutamine and 5% fetal calf serum. At the end of incubation, cell-free supernatants were harvested and stored at −80°C until assayed.

### *Measurement of neurotrophin production by PBMCs*

Spontaneous neurotrophin (BDNF, NGF, GDNF, NT3, NT4) production was measured by enzyme-linked immunoabsorbent assay (ELISA) using commercial kits (R&D Systems, Minneapolis, USA) and following manufacturer's instructions. All assays were performed on F-bottom 96-well plates (Nunc, Wiesbaden, Germany). Tertiary antibodies were conjugated to horseradish peroxidase. Wells were developed with tetramethylbenzidine and measured at 450/570 nm.

Neurotrophin concentrations were determined from the regression line for a standard curve generated by using highly purified recombinant human neurotrophin at various concentrations, performed contemporaneously with each assay. The standard curve also served as an internal control over the sensitivity and range of each assay. All samples were assayed in duplicate and quality control pools at low, normal and high concentrations were present in each assay. Data were expressed as pg/ml.

There was no cross-reactivity or interference between related neurotrophins (BDNF, NGF, GDNF, NT3, NT4).

### *Magnetic resonance imaging (MRI)*

All patients underwent MRI before and at the end of the follow-up period and at relapse time. MRI data were acquired on high resolution 1.5 T system with 5-mm slice thickness. Scanning sessions included proton density (echo time [TE] 20/repetition time [TR] 2500), T2-weighted (TE 80/TR 2500) and T1-weighted (TE 17/TR 600) images. The T1-weighted images were acquired before and 10 min after IV injection of gadolinium-diethylenetriaminepentaacetic acid (0.1mmol/kg).

### *Statistical analysis*

Variations of neurotrophic factor (BDNF, NGF, GDNF, NT3, NT4) production by PBMCs throughout interferon treatment were assessed by two-way analysis of variance (ANOVA) with clinical outcome (occurrence or non-occurrence of relapses for RRMS patients; progression or non-progression in disability for SP ones) and time points (baseline, 3, 6 and 12 months after starting therapy) as independent variables. When significant differences were obtained, post hoc comparisons were performed using Fisher's PLSD test.

A $P$ value $< 0.05$ was considered significant.

## Results

### *Neurotrophin production by unstimulated PBMCs in MS patients under IFNbeta treatment*

During IFNbeta treatment, there were no main variations in NGF, GDNF, NT3, NT4 production among all 39 MS patients. A trend towards an increase of BDNF production was observed after 1 year of treatment versus baseline values (months 0–12: $P = 0.09$).

Levels of BDNF, NGF, GDNF, NT3 and NT4 in the supernatants of unstimulated PBMCs were comparable between RRMS and SPMS patients at baseline.

### *Neurotrophin production by unstimulated PBMCs in RR patients under IFNbeta treatment*

During the study, there were no main variations of NGF, GDNF, NT3, NT4 production among all 25 RRMS patients, while a trend towards an increase of BDNF production was found after 1 year of treatment (months 0–12: $P = 0.1$) (Fig. 1).