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IFN-β1a and IFN-β1b have different patterns of influence on cytokines

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Abstract

Multiple sclerosis is characterized by elevated levels of proinflammatory cytokines produced by Th1 cells and decreased levels of anti-inflammatory cytokines produced by Th2 cells. IFN-β treatment shifts the immune response from the Th1 to Th2 pattern, thus enhancing the production of anti-inflammatory Th2 cytokines such as IL-4, IL-10, and decreasing the production of proinflammatory Th1 cytokines such as IFN-γ. To determine which IFN-β has the stronger immunomodulatory effect we compared the levels of IL-4, IL-10, and IFN-γ of 12 relapsing-remiting MS patients treated with IFN-β1b (Betaferon®) with those of 10 patients treated with IFN-β1a (Avonex®). There were no statistically significant differences in duration of disease, number of relapses before and during treatment, and in EDSS after 2 years of treatment. After 1 year of treatment the concentration of IFN-γ was significantly lower in the Betaferon® group, and concentrations of IL-4 and IL-10 were significantly higher in the Avonex® group. It appears that IFN-β1b has a downregulatory effect on both Th1 and Th2 cytokines, while IFN-β1a causes a shift of the cytokine profile toward the Th2 phenotype. These two IFN have different influences on the pattern of cytokines in MS: IFN-β1a enhances the production of anti-inflammatory cytokines IL-4 and IL-10 and IFN-β1b decreases the production of the proinflammatory cytokine IFN-γ.

Keywords: Multiple sclerosis; Cytokines; Interferon-β

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) of suspected autoimmune origin. Its pathological hallmarks are of breakdown of the blood–brain barrier and perivenous inflammatory infiltrates in the CNS that lead to demyelination and axonal damage. The inflammatory cells produce a plethora of cytokines that appear to play a crucial role in the pathogenesis of MS [1] The disease is characterized by elevated levels of proinflammatory cytokines produced by Th1 cells such as tumor necrosis factor α (TNF-α), interleukin-2 (IL-2), and interferon-γ (IFN-γ) in the peripheral blood [2–4], CSF [5,6], and in brain lesions [7], whereas anti-inflammatory cytokines produced by Th2 cells such as interleukin-4 (IL-4) and interleukin-10 (IL-10) are downregulated [8]. Interferon-β (IFN-β), a recognized long-term treatment for MS [9,10], has downregulatory effects on Th1 cytokines and causes a shift of the cytokine profile toward the Th2 phenotype [11–13]. IFN-β suppresses the production of IFN-γ and induces IL-10, whereas the production of IL-4 is not altered [14]. Two different recombinant IFN-β, IFN-β1a (Avonex® and Rebif®) and IFN-β1b (Betaferon®), are approved for the treatment of RRMS. They differ in dosage, and in route and frequency of administration, and consequently most probably also in clinical effectiveness. The purpose of this study is to compare the immunomodulatory activity of IFN-β1a (Avonex®) and IFN-β1b (Betaferon®) by studying IL-4, IL-10, and IFN-γ levels before and during treatment.

2. Materials and methods

The study was approved by the hospital's ethics committee and was carried out according to Declaration of Helsinki protocol.

Twenty-two patients with definite [15] RRMS, aged 18–50 years, with an EDSS [16] 0–5.5, and with at least two relapses in the last 2 years were enrolled in the study from February 2000 to December 2000. None had previously

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Table 1
Clinical characteristics of patients in IFN-β1b and IFN-β1a group

	IFN-β1b group	IFN-β1a group	*P*-value
No. of subjects	12	10	
Sex (F/M)	10/2	6/4	
Mean age (years)	40.7 (S.D. ± 0.08)	32.7 (S.D. ± 5.4)	0.013
Mean EDSS	3.2 (S.D. ± 0.7)	2.1 (S.D. ± 1.1)	0.014
Mean disease duration (years)	7.7 (S.D. ± 5.3)	6.4 (S.D. ± 4.9)	n.s.
No. of relapses in the last 2 years	3.5 (S.D. ± 1.1)	3.3 (S.D. ± 1.5)	n.s.

been treated with immunomodulatory or immunosuppresive drugs. Twelve patients (10 women, 2 men) were treated with Betaferon®, 250 μg (8 MIU) injected subcutaneously every other day, and 10 patients (6 women, 4 men) with Avonex®, 30 μg (6 MIU) injected intramusculary once a week. Peripheral blood samples were obtained before treatment, and, at least 24 h after the last injection, after 1 week, 1 month, 6 and 12 months of treatment. Patients were treated for 2 years. The number of relapses and the EDSS were assessed every 12 months. The patients' characteristics are shown in Table 1.

2.1. Measurement of cytokines

Peripheral blood mononuclear cells (PBMC) were obtained by centrifugation on Ficoll-Paque (Pharmacia, Sweden) density gradient and suspended in RPMI 1640 nutrient medium supplemented with penicillin 100 IU/l and streptomycin 100 μg/l, and fetal calf serum (conc. 5%) (Sigma, USA). The 1×10^6 cells (final culture volume 1.5 ml) were plated in 24-well culture plates (T grade, NUNC, Denmark). Polyclonal activators ionomycin (IONO; conc. 500 nM in PBMC cultures) and phorbol 12-myristate 13-acetate (PMA; conc. 3.33 ng/ml of PBMC culture) (IONO and PMA culture) were then added. The PBMC cultures were then incubated in an incubator in an atmosphere of 5% CO_2 and 95% humidity. The cell-free supernatants were collected after 40 h and stored at −70 °C before being evaluated for IFN-γ, IL-4, and IL-10 levels. The concentrations of cytokines were measured by commercial ELISA kits (Endogen-Pierce, USA).

2.2. Statistical analysis

Differences in cytokine levels between Avonex® and Betaferon® group were tested by the Student's *t*-test. A probability level of $P < 0.05$ was considered significant. Pearson's correlation coefficient was used to analyze the correlations between cytokine levels and number of relapses.

3. Results

3.1. Clinical data

Betaferon® significantly reduced the relapse rate ($P < 0.001$) and the number of required courses of steroid treatment in the first 2 years of treatment ($P < 0.01$); Avonex® only reduced the number of steroid treatments ($P = 0.02$) whereas the decrease in the relapse rate was not significant ($P = 0.24$). The EDSS increased in both groups after 2 years of treatment, but the increase was not statistically significant. The difference between the number of relapses in the 2 years before treatment and in the 2 years during the treatment was significantly higher in the Betaferon® group, but there were no significant differences in relapse rate before and during the treatment, number of steroid courses before and during the treatment, and in EDSS during the treatment, although the EDSS was significantly higher in the Betaferon® group before treatment (Table 2).

3.2. Cytokines

IFN-γ decreased significantly after 1 week of treatment with Betaferon® ($P = 0.004$) and remained so for the whole year of treatment, but before did not change significantly after treatment with Avonex®. IFN-γ was significantly higher in the Avonex® group even before as well as during the treatment (Table 3).

IL-4 decreased significantly after 1 month ($P = 0.028$) and 1 year ($P = 0.007$) of treatment with Betaferon®, but

Table 2
Clinical outcomes in IFN-β1b and IFN-β1a group

	IFN-β1b group, $n = 12$	IFN-β1a group, $n = 10$	*P*-value
Number of relapses 2 years before treatment	3.5 (S.D. ± 1.1)	3.3 (S.D. ± 1.5)	n.s.
Number of relapses 2 years during treatment	1.4 (S.D. ± 1.9)	2.6 (S.D. ± 1.6)	n.s.
Number of steroid courses 2 years before treatment	2.4 (S.D. ± 0.9)	1.8 (S.D. ± 0.8)	n.s.
Number of steroid courses 2 years during treatment	1.2 (S.D. ± 1.5)	1.2 (S.D. ± 0.8)	n.s.
EDSS before treatment	3.2 (S.D. ± 0.7)	2.1 (S.D. ± 1.2)	0.014
EDSS after 1 year of treatment	2.9 (S.D. ± 1.2)	2.2 (S.D. ± 1.4)	n.s.
EDSS after 2 years of treatment	3.6 (S.D. ± 1.7)	2.7 (S.D. ± 1.6)	n.s.
Difference in number of relapes before and during the treatment	2.1 (S.D. ± 1.0)	0.7 (S.D. ± 1.8)	0.031

Table 3
IFN-γ levels (pg/ml) before and during treatment with IFN-β1b and IFN-β1a

	IFN-β1b group, $n = 12$ (pg/ml)	IFN-β1a group, $n = 10$ (pg/ml)	P-value
Before treatment	19519 (S.D. ± 8624)	29504 (S.D. ± 12322)	0.037
After 1 week of treatment	12323 (S.D. ± 7578)	26376 (S.D. ± 9527)	0.010
After 1 month of treatment	9929 (S.D. ± 4468)	24251 (S.D. ± 13428)	0.002
After 6 months of treatment	9472 (S.D. ± 4073)	12322 (S.D. ± 5640)	0.008
After 12 months of treatment	10207 (S.D. ± 7661)	26500 (S.D. ± 13490)	0.003

Table 4
IL-4 levels (pg/ml) before and during treatment with IFN-β1b and IFN-β1a

	IFN-β1b group, $n = 12$ (pg/ml)	IFN-β1a group, $n = 10$ (pg/ml)	P-value
Before treatment	35.5 (S.D. ± 26.1)	23.2 (S.D. ± 20.1)	n.s.
After 1 week of treatment	27.3 (S.D. ± 21.4)	26.8 (S.D. ± 14.9)	n.s.
After 1 month of treatment	23.7 (S.D. ± 14.1)	28.0 (S.D. ± 17.1)	n.s.
After 6 months of treatment	29.3 (S.D. ± 19.6)	37.2 (S.D. ± 27.7)	n.s.
After 12 months of treatment	19.8 (S.D. ± 13.7)	44.4 (S.D. ± 20.7)	0.004

Table 5
IL-10 levels (pg/ml) before and during treatment with IFN-β1b and IFN-β1a

	IFN-β1b group, $n = 12$ (pg/ml)	IFN-β1a group, $n = 10$ (pg/ml)	P-value
Before treatment	233 (S.D. ± 168)	150 (S.D. ± 64)	n.s.
After 1 week of treatment	145 (S.D. ± 109)	138 (S.D. ± 79)	n.s.
After 1 month of treatment	106 (S.D. ± 53)	113 (S.D. ± 57)	n.s.
After 6 months of treatment	128 (S.D. ± 79)	176 (S.D. ± 121)	n.s.
After 12 months of treatment	121 (S.D. ± 80)	261 (S.D. ± 129)	0.007

increased significantly after 1 year ($P = 0.008$) of Avonex®. IL-4 was significantly lower in the Betaferon® group than in the Avonex® group after 1 year of treatment (Table 4).

IL-10 decreased significantly after the first week of treatment with Betaferon® ($P = 0.036$) and for the whole year of treatment. On the contrary IL-10 significantly increased after 1 year of treatment with Avonex® ($P = 0.046$). After 1 year of treatment IL-10 was significantly higher in the Avonex® group (Table 5).

There was no correlation between the number of relapses and IL-4, IL-10, and IFN-γ levels before or during the treatment.

4. Discussion

The current study was performed to explore the effects of IFN-β1a (Avonex®) and IFN-β1b (Betaferon®) on cytokine profile. We expected that both drugs would have the same pattern of influence on Th1 and Th2 cytokines but of different magnitude. Clinical studies [9,10,17,18] and in vitro studies had shown that the effect of treatment is most probably dose and frequency of administration dependent. Higher and more frequent doses are more efficacious than lower, less frequent ones [19]. We expected that Betaferon® which is administered in higher dose and more frequently, and diminishes the relapse rate more than Avonex®, would decrease IFN-γ and increase IL-10, and probably IL-4 as well, more than the latter. However, we found different patterns of influence: Avonex® was more potent in enhancing the production of anti-inflammatory cytokines IL-4 and IL-10, and Betaferon® in decreasing the production of the proinflammatory cytokine IFN-γ. Our data indicate that Betaferon® does not affect the Th1/Th2 balance but has a downregulatory effect on both Th1 and Th2 cytokines; IFN-γ, IL-4, and IL-10 all decreased significantly after the first month of treatment and remained so during the first year of treatment. Avonex® caused a shift of the cytokine profile toward the Th2 phenotype. It had no effect on IFN-γ but significantly increased IL-4 and IL-10 after 1 year of treatment. This different pattern could be explained on the basis of differences in the molecules of IFN-β, or in routes, frequency and dosage of administration.

Our results are in contrast to Franciotta et al. [20] who found that the number of IFN-γ- and IL-4-producing cells had decreased after the treatment with Avonex®. In another study, Avonex® added to in vitro activated blood cells, induced the production of IL-4 and IL-10 [11], but Betaferon® inhibited IFN-γ secretion and increased IL-10 secretion, whereas IL-4 secretion was not affected [12]. These conflicting data could have resulted from different techniques in measurement of cytokines.

The effect of Betaferon® on cytokines production became significant after the first month of treatment, but that of Avonex® was delayed and became significant only after 1 year of treatment. This is not surprising because it has al-

ready been shown that the treatment effect can be delayed for a year or more [10].

We found no correlation between the number of relapses and the levels of IFN-γ, IL-4, and IL-10. Several studies of the relationship between cytokine abnormalities and the clinical course of the disease, have been disappointing. IFN-γ treatment worsened MS [21]. IL-10 production was shown to be low during exacerbations and subclinical activity as demonstrated on gadolinium-enhanced MRI [22], whereas it increased during the resolution of exacerbations and active MRI lesions [23]. Low IL-10 production was shown to be associated with higher disability and MRI lesion load in SP MS [24]. Kraus et al. [25] found no correlation between MRI parameters for disease activity and serum levels of IL-4, IL-10, and IFN-γ. Such limited correlations are not surprising because cytokines act mainly locally, where they are produced and may quickly bind to receptors and thus have a very short half-life [8].

The reason for the higher IFN-γ levels in the Avonex® group before treatment is not known. Both groups differ significantly only in sex ratio, age, and EDSS. We are not aware of any data on the effect of sex or age on IFN-γ levels. A positive correlation has been reported between disability in MS and intracellular IFN-γ [26], but the patients treated with Avonex® were actually less disabled than the other ones.

In summary, this study shows that Betaferon® and Avonex® differ in their effects on cytokine profiles. This may explain some of the differences in the clinical results obtained with these two therapeutic agents. The former may be more potent in decreasing the production of the proinflammatory cytokine IFN-γ, while the latter may be better at enhancing the production of anti-inflammatory cytokines IL-4 and IL-10.

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