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Long-term favorable response to interferon beta-1b is linked to cytokine deviation toward the Th2 and Tc2 sides in Japanese patients with multiple sclerosis

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Abstract

To address the immune mechanism of the long-term beneficial effects of interferon beta (IFN-β), we measured the intracellular cytokine production patterns of IFN-γ, IL-4 and IL-13 in peripheral blood $CD4^+$ and $CD8^+$ T cells, which previously displayed alterations during the early course of IFN-β treatment, in 15 Japanese patients after long-term IFN-β administration. The patients were treated with IFN-β-1b 8×10^6 units given subcutaneously every other day for a mean period of 34.5 ± 5.5 months (range: 26–43 months). During the follow-up period, 6 patients experienced 33 relapses, while the other 9 were relapse-free. The results revealed the following cytokine alterations: (1) type 2 cytokine, such as IL-4 and IL-13, were significantly increased in producing cell percentages in both $CD4^+$ ($p = 0.0356$ and $p = 0.0007$, respectively) and $CD8^+$ ($p = 0.0231$ and $p = 0.0170$, respectively) T cells while IFN-γ, a representative type 1 cytokine, was significantly decreased in the absolute producing cell numbers ($p = 0.0125$ in $CD4^+$ T cells and $p = 0.0022$ in $CD8^+$ T cells) even after approximately 3 years of IFN-β administration; (2) the intracellular IFN-γ / IL-4 ratio tended to decrease in both $CD4^+$ and $CD8^+$ T cells ($p = 0.0535$ and $p = 0.0783$, respectively), reflecting a strong downmodulation of type 1 cytokine producing cells; and importantly (3) alterations such as the decreased intracellular IFN-γ / IL-4 ratio in $CD4^+$ T cells and increased percentage of $CD8^+$ $IL\text{-}13^+$ T cells compared with the pretreatment levels were only statistically significant in MS patients without relapse during IFN-β therapy ($p = 0.0152$ and $p = 0.0078$, respectively). Therefore, we consider that cytokine deviation toward the Th2 and Tc2 sides is linked to a long-term favorable response to IFN-β, while a higher intracellular IFN-γ / IL-4 ratio is associated with treatment failure.

Keywords: Multiple sclerosis; Interferon-β; Interferon-γ; Interleukin-4; Interleukin-13; Th1; Th2

1. Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system (CNS) that is generally considered to be mediated by myelin-autoreactive T cells. Interferon beta (IFN-β) reduces the frequency and severity of clinical relapses in relapsing–remitting MS, and the basis of these beneficial effects has been extensively studied both in vivo and in vitro.

The effects of IFN-β on the cytokine production pattern is especially important, since increasing evidence suggests that MS is largely caused by $CD4^+$ T helper 1 (Th1) cells that produce interferon gamma (IFN-γ) but not interleukin (IL)-4 [1]. Moreover, important roles of $CD8^+$ T cells have also been suggested by the selective enrichment of memory $CD8^+$ T cells in the cerebrospinal fluid (CSF) of MS patients [2] together with diffuse infiltration of clonally expanded $CD8^+$ T cells into the brain parenchyma [3,4]. However, most previous cytokine studies on the effects of IFN-β have been performed within 1 year after initiation of the therapy [5–10], although the beneficial effects of IFN-β persist for

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several years [11]. Thus, although the long-term beneficial effects of IFN-β are well established, the basis of these effects in vivo remains to be fully elucidated. We recently performed sequential measurements of multiple cytokine production patterns in both $CD4^+$ and $CD8^+$ T cells from the pretreatment period up to 48 months after initiation of the therapy, and found early increases in Th2 cytokines, such as IL-4 and IL-13, followed by late decreases in Th1 cytokines, such as IFN-γ, in both conventional MS (C-MS) and opticospinal MS (OS-MS) [12]. In the present study, we focused on the cytokines that displayed significant alterations in our previous study and evaluated the long-term effects of IFN-β on the cytokine production patterns in $CD4^+$ and $CD8^+$ T cells in the peripheral blood of MS patients several years after initiation of the drug treatment.

2. Materials and methods

2.1. Patients

Fifteen Japanese patients (8 women and 7 men; mean age ± SD: 42.13 ± 12.44 years) with relapsing–remitting MS, diagnosed according to the revised diagnostic criteria for MS [13] and treated with IFN-β-1b (Betaferon®; Shering) 8×10^6 units given subcutaneously every other day for at least 2 years (mean period ± SD: 34.5 ± 5.5 months; range: 26–43 months), were included in this study. At the time of their enrollment, none of the patients were experiencing an acute attack or had been under immunosuppressive treatment for at least the previous 3 months. The patients were clinically classified into two subtypes: C-MS (13 patients) and OS-MS (2 patients), as described previously [14]. Briefly, patients who had both optic nerve and spinal cord involvement without any clinical evidence of disease in either the cerebrum or the cerebellum were considered to have OS-MS. Patients with minor brainstem signs, such as transient double vision or gaze nystagmus, were included in this subtype. All other patients showing disseminated involvement of the CNS were considered to have C-MS. The demographic features of the patients are shown in Table 1.

2.2. Intracellular cytokine analysis by flow cytometry

The intracellular cytokine patterns were studied by flow cytometry, as described previously [12]. IFN-γ was examined as a Th1 cytokine, while IL-4 and IL-13 were investigated as Th2 cytokines. Peripheral blood mononuclear cells were collected from the patients before treatment and after 2–3 years of IFN-β-1b therapy, and treated with 25 ng/ml phorbol 12-myristate 13-acetate (Sigma, St. Louis, MO), 1 μg/ml of ionomycin (Sigma) and 10 μg/ml brefeldin A (Sigma) for 4 h. The monoclonal antibodies used were: PC5-conjugated anti-CD4 (13B8.2; Becton Dickinson, San Jose, CA), PC5-conjugated anti-CD8 (B9.11; Becton Dickinson), FITC-conjugated anti-IFN-γ (25723.11; Becton Dickinson), PE-conjugated anti-IL-4 (3010.211; Becton Dickinson) and PE-conjugated anti-IL-13 (JES10-5A2; PharMingen, San Diego, CA). The percentages of cytokine-positive $CD4^+$ and $CD8^+$ cells were determined as the % cytokine-positive $CD4^+$ population/total $CD4^+$ population and the % cytokine-positive $CD8^+$ population/total $CD8^+$ population, respectively. Pretreatment measures of cytokine production were done twice at distinct time (average 3.1 days apart) in all MS patients and 9 of 15 in post-treatment measures and means were used when measured twice. According to the results of a preliminary study using 15 subjects, interassay variabilities were as follows; 14.2% in $CD4^+IL\text{-}4^+IFN\text{-}\gamma^-$ cell percentage, 11.6% in $CD4^+IL\text{-}4^-IFN\text{-}\gamma^+$ cell percentage, 29.0% in $CD4^+IL\text{-}13^+$ cell percentage, 13.8% in $CD8^+IL\text{-}4^+IFN\text{-}\gamma^-$ cell percentage, 28.2% in $CD8^+IL\text{-}4^-IFN\text{-}\gamma^+$ cell percentage, 42.1% in $CD8^+IL\text{-}13^+$ cell percentage.

2.3. Statistical analysis

Statistical analyses comparing age at baseline, disease duration at baseline and EDSS scores were performed by the Mann–Whitney *U*-test, while those for the gender ratio were carried out by the Fisher's exact probability test. Statistical analyses comparing the cell percentage and ratio of intracellular cytokine-producing $CD4^+$ and $CD8^+$ T cells between responders and non-responders were performed by the Mann–Whitney *U*-test, and those comparing the cell

Table 1
Demographic features of the 15 MS patients before and during IFN-β-1b treatment

	Total	Non-relapsed	Relapsed
Number of patients	15	9	6
Gender (male : female)	8 : 7	6 : 3	2 : 4
Age at baseline (mean ± S.D.)[a]	42.13 ± 12.44	40.33 ± 10.92	44.83 ± 15.09
Disease duration at baseline (mean ± S.D.)[a]	5.42 ± 3.64	4.30 ± 2.92	7.11 ± 4.22
EDSS at baseline (mean ± S.D.)	4.03 ± 2.18	3.94 ± 2.48	4.17 ± 1.86
EDSS after the observation period (mean ± S.D.)	4.27 ± 2.27	4.22 ± 2.59	4.33 ± 1.72
Relapse rate during the 2 years before IFN-β-1b (mean S.D.)	3.00 ± 2.24	2.56 ± 1.88	3.67 ± 2.73
Relapse rate during 1 year of IFN-β-1b (mean ± S.D.)	0.73 ± 1.38	0.00 ± 0.00	1.83 ± 1.72
Relapse rate during 1–2 years of IFN-β-1b (mean ± S.D.)	1.07 ± 1.67	0.00 ± 0.00	2.67 ± 1.63
Relapse rate during 2–3 years of IFN-β-1b (mean ± S.D.)	0.40 ± 0.63	0.00 ± 0.00	1.00 ± 0.63

[a] Years.