Multiple Sclerosis: Levels of Interleukin-10-Secreting Blood Mononuclear Cells are Low in Untreated Patients but Augmented During Interferon-β-1b Treatment

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The cytokine interleukin (IL)-10 has immune response down-regulatory properties, which include suppression of the synthesis of pro-inflammatory cytokines such as interferon (IFN)-γ and of major histocompatibility complex (MHC) class II expression on monocytes. To further elucidate the involvement of IL-10 in multiple sclerosis (MS), an enzyme-linked immunospot assay was adopted to enumerate IL-10-secreting mononuclear cells (MNC) in peripheral blood. IFN-γ secreting MNC were detected in parallel. Levels of IL-10-secreting cells were lower in patients with MS compared with other neurological diseases (OND) and healthy subjects. This difference was seen only in patients with untreated MS, and not in those undergoing treatment with IFN-β-1b. No differences were observed when subgrouping the patients with MS regarding clinical phase (exacerbation, remission, secondary progression), duration of MS or disability status. Levels of IFN-γ-secreting blood MNC did not differ in patients with MS, irrespective of treatment with IFN-β-1b, compared with OND and healthy subjects. Patients with MS, but not the two groups of controls, had elevated numbers of IL-10- and IFN-γ-secreting cells upon stimulation with MBP compared with culture in the absence of antigen. The data suggest that IL-10 is decreased in MS and that treatment resulting in its up-regulation beneficially influence the disease.

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INTRODUCTION

It has become increasingly evident that cytokines play a key role in the pathogenesis of multiple sclerosis (MS). The pro-inflammatory cytokine interferon (IFN)-γ induces clinical exacerbation of MS [1]. The balance between T helper (Th)1- and Th2-type cytokines might determine whether the immune response in MS is detrimental or beneficial [2].

Interleukin (IL)-10 was originally described as a murine Th2 cytokine, inhibiting cytokine synthesis by Th1 cells [3]. The production of IL-10 is, as later studies have shown, not restricted to Th2 cells since Th0 and Th1 cells, B cells and macrophages have also been shown to express IL-10. Nevertheless, many of the effects of IL-10 are similar to, or overlap with, those of Th2 cytokines and there seems to be a close correlation between the induction of Th2-like responses and the expression of IL-10 [4].

IL-10 has been considered to have a potential therapeutic role in autoimmune diseases such as rheumatoid arthritis [5], diabetes mellitus [6] and psoriasis [7]. Administration of recombinant IL-10 to rats prevents the development of experimental autoimmune encephalomyelitis (EAE), an animal model for MS [8]. IL-10 mRNA expression within the central nervous system (CNS) correlates with clinical recovery from EAE [9, 10]. However, administration of IL-10 to rats with experimental autoimmune myasthenia gravis (EAMG), an animal model of myasthenia gravis (MG) in humans, aggravates the clinical signs of EAMG [11]. This is concordant with the profound effect of IL-10 on B-cell proliferation and antibody production, being disease promoting in EAMG and MG [12]. MS is characterized by an augmented B-cell response within the CNS, but whether the local production of excessive amounts of autoantibodies aggravates MS or has protective roles, as suggested by Rodriguez and Miller [13], remains to be clarified [14].

A number of studies have evaluated IL-10 in MS, with partly contradictory results. Elevated numbers of IL-10 mRNA-expressing mononuclear cells (MNC) have been detected in blood from patients with MS compared with MG, other neurological diseases (OND) and healthy subjects [15]. Upon culture in the presence of phytohaemagglutinin (PHA), MNC from MS patients produced higher amounts of IL-10 compared with controls [16]. CD3-induced IL-10 secretion by CD4+ T cells is increased in patients with relapsing–remitting MS compared with controls [17]. Expression of IL-10 mRNA by blood MNC is elevated during remission of MS compared with exacerbation [18]. IL-10 levels in supernatants of cultured monocytes from patients with MS and healthy subjects did not differ [19]. Decreased levels of IL-10 have, however, also been observed in MS. When the diagnosis was made, reduced IL-10 mRNA expression was observed in MS compared with healthy subjects [20]. Using enzyme-linked immunosorbant assay (ELISA), Salmaggi *et al*. [21] found lower levels of IL-10 in serum in MS patients compared with healthy subjects.

Today, recombinant interferon-beta 1b (rIFN-β-1b), is an approved therapy for MS, reducing disease activity both in relapsing remitting (RR) and secondary progressive (SP) MS [22]. One of the mechanisms by which IFN-β-1b may exert its beneficial effects in MS is through an impact on cytokine production. *In vitro*, addition of IFN-β-1b to cultured monocytes from patients with MS has been shown to stimulate IL-10 secretion [19]. Anti-IL-10 antibody reduced the effect of rIFN-β-1b on IFN-γ production [23].

To further elucidate the involvement of IL-10 and, in parallel, of IFN-γ, we adopted enzyme-linked immunospot (ELISPOT) assays to enumerate the levels of IL-10 and IFN-γ secreting cells in peripheral blood from untreated and IFN-β-1b treated patients with MS and controls.

MATERIALS AND METHODS

Patients. Seventy-three patients (53 females) had clinically definite MS diagnosed according to accepted criteria [24]. Their age range was 20–72 years (mean 44 years). The duration of MS was 1–40 years (mean 13 years). Six patients were examined during exacerbation, which was defined as a sudden appearance of new symptoms and signs or the abrupt reappearance or worsening of earlier present symptoms lasting more than 24 h and occurring within 1 month before examination. Thirty-one patients were in remission. Thirty-six patients were examined during the SP phase of MS. Twenty-five patients had no or slight disability, defined as an expanded disability status scale (EDSS) score < 3, while 48 patients had moderate or severe disability defined as an EDSS score ≥ 3 [25]. The duration of MS was < 10 years in 39 patients and ≥ 10 years in 34 patients.

Twenty of the patients with MS were treated with IFN-β-1b (Betaferon; Schering AG, Berlin, Germany) at a dosage of 8 MIU units subcutaneously (sc) every other day at the time of inclusion in the study. None of the 73 patients with MS had received corticosteroids or any other immunomodulatory therapy during the 6 months prior to inclusion in the study.

In order to examine the effects of IFN-β-1b treatment on the number IL-10- and IFN-γ-secreting cells longitudinally, we followed seven patients (all females) with MS who underwent treatment with IFN-β-1b. Three patients were in remission. Four patients were examined during the SP phase of MS. Two patients had no or slight disability, defined as an EDSS score < 3, while five patients had moderate or severe disability defined as an EDSS score ≥ 3 [25]. The duration of MS was < 10 years in two patients and ≥ 10 years in five patients. We obtained blood samples before the onset of treatment, 1 week and one month after the onset of treatment.

In parallel, two control groups were examined. Thirty-two patients (24 females) had OND. Their age range was 20–77 years (mean 47 years). Ten of the OND patients had tension headache, seven had cerebrovascular diseases, five patients had vertigo, five had paraesthesia, two had epilepsy and one patient each had back-pain, sensory disturbance and polyneuropathy. Blood specimens were also obtained from 29 healthy subjects (18 females) with an age range of 23–62 years (mean 35 years).

Antigen preparation. Myelin basic protein (MBP) was prepared from bovine brain white matter [26]. The purity of MBP was checked by standard sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) [27], which showed a single band, migrating at ≈ 22 kDa. Purified protein derivative (PPD) was purchased from Statens Seruminstitut (Copenhagen, Denmark).

Detection of IL-10 and IFN-γ secreting mononuclear cells by ELISPOT assays. Peripheral blood was obtained by venous puncture between 08.00 and 12.00 a.m. Sampling from the IFN-β-1b treated patients took place 36–40 h after the last injection of IFN-β-1b. ELISPOT assay as described by Czerkinsky *et al*. [28] was adopted to detect and enumerate cells secreting IL-10 as well as IFN-γ [29]. MNC were separated by density gradient centrifugation on Lymphoprep (Nycomed, Oslo, Norway) within 2 h of blood sampling. The cells from the interphase were collected, washed three times with Dulbecco's modified Eagle's medium (DMEM; Gibco, Paisley, UK) supplemented with antibiotics, 10% fetal calf serum (Gibco), 1% minimal essential medium (Gibco) and 1% L-glutamine (Gibco), and adjusted to a concentration of 1×10^6 cells/ml. Cell viability as measured by Trypan blue exclusion always exceeded 95%. Microtitre plates with nitrocellulose bottoms (Multiscreen-HA plates; Millipore, Mulsheim, France) were coated overnight at 4 °C with 100 μg/well a monoclonal antihuman IL-10 (9-D7) or IFN-γ (1-D1K) antibody (Mabtech, Stockholm, Sweden) diluted in filtered phosphate-buffered saline (PBS, pH 7.4) to a concentration of 10 μg/ml. After removal of coating solutions by washing, 200-μl aliquots containing 2×10^5 MNC were applied to individual wells. To enumerate MBP- or PPD-reactive IL-10- or IFN-γ-secreting MNC, MBP or PPD were added in 10-μl aliquots to a final concentration of 10 μg/ml. This antigen concentration was found in preliminary experiments to give high numbers of spots in cases supposed to be positive. As a negative control, complete medium was also added to wells in the absence of MNC. Plates were incubated at 37 °C for 48 h in humidified air containing 5% CO_2, then emptied and washed. A 100-μl aliquot of a monoclonal biotinylated antihuman IL-10 (12G8) or IFN-γ (7-B6–1) antibody (Mabtech) diluted in filtered PBS to a concentration of 1 μg/ml was added overnight at 4 °C. Wells were washed and 100 μl of streptavidin-alkaline phosphatase (Mabtech) diluted 1 : 1000 was added for 1.5 h at room temperature. After incubation, plates were emptied, washed and stained with BCIP-NBT (Gibco) diluted in Tris buffer. Spots were seen after 5–15 min and the reaction was allowed to proceed for another 5 min before the wells were rinsed, emptied and dried over night. Blue immunospots, each considered to represent a cell secreting IL-10 or IFN-γ, were counted under a dissection microscope. Determinations were made in duplicate. The mean values of the

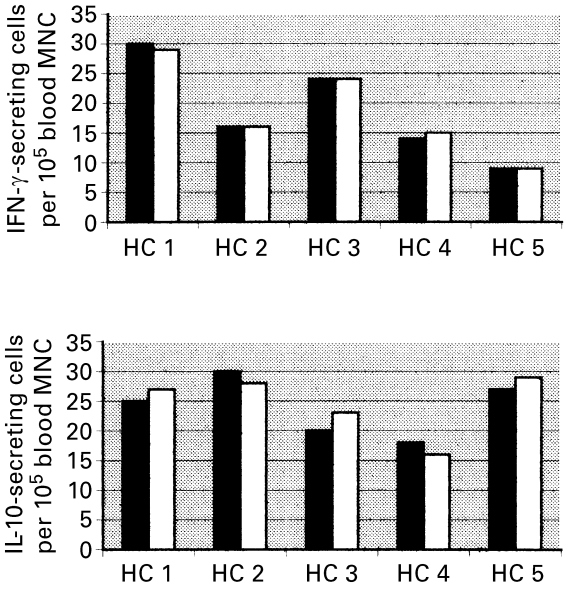

Fig. 1. Numbers of IFN-γ- and IL-10-secreting cells per 10^5 blood MNC from five healthy subjects (HC) on two consecutive days. Black columns refer to day 1 and white columns refer to day 2.

duplicates were calculated. Results were presented as numbers of IL-10- or IFN-γ-secreting cells per 10^5 blood MNC. The mean values of MBP- and PPD-reactive IL-10- or IFN-γ-secreting cells were also presented in the same way. Results with differences in spot numbers of duplicates exceeding 10% were excluded from the study.

In order to examine interplate differences, we incubated cells from three patients with MS in two different plates at the same time point and examined the numbers of IL-10- and IFN-γ-secreting cells. No difference was observed between the two plates (data not shown). Furthermore, we examined five healthy subjects on two consecutive days to test the day-to-day variability of our assay. There was less than 10% intertest variability of the number of IL-10- and IFN-γ-secreting cells from the same individual between two days (Fig. 1).

Statistical analysis. The nonparametric Kruskal–Wallis ANOVA and Mann–Whitney *U*-test were used for group comparison. Wilcoxon signed rank test was used when comparing paired samples from the same individual.

RESULTS

Levels of blood MNC spontaneously secreting IL-10 and IFN-γ

IL-10-secreting cells were detected in peripheral blood from almost all patients with MS and OND, as well as in the healthy subjects (Fig. 2). The median values of IL-10-secreting cells were 10 per 10^5 blood MNC in MS, 23 per 10^5 in OND and 19 per 10^5 MNC in the healthy subjects. Corresponding frequencies were ≈ 1 per 10 000, 1 per 4500 and 1 per 5500 cells, respectively. Levels of IL-10-secreting blood MNC were lower in the patients with MS compared with OND and healthy subjects ($P < 0.01$ for both comparisons; Table 1).

IFN-γ-secreting cells were also detected in peripheral blood from almost all patients with MS and OND, as well as in the healthy subjects (Fig. 2). The median values of IFN-γ-secreting cells were 6 per 10^5 blood MNC in MS, 7 per 10^5 in OND and 7 per 10^5 MNC in the healthy subjects. The corresponding frequencies were ≈ 1 per 16 500, 1 per 14 000 and 1 per 14 000 cells, respectively. There were no significant differences for numbers of IFN-γ-secreting cells between the three groups (Table 1).

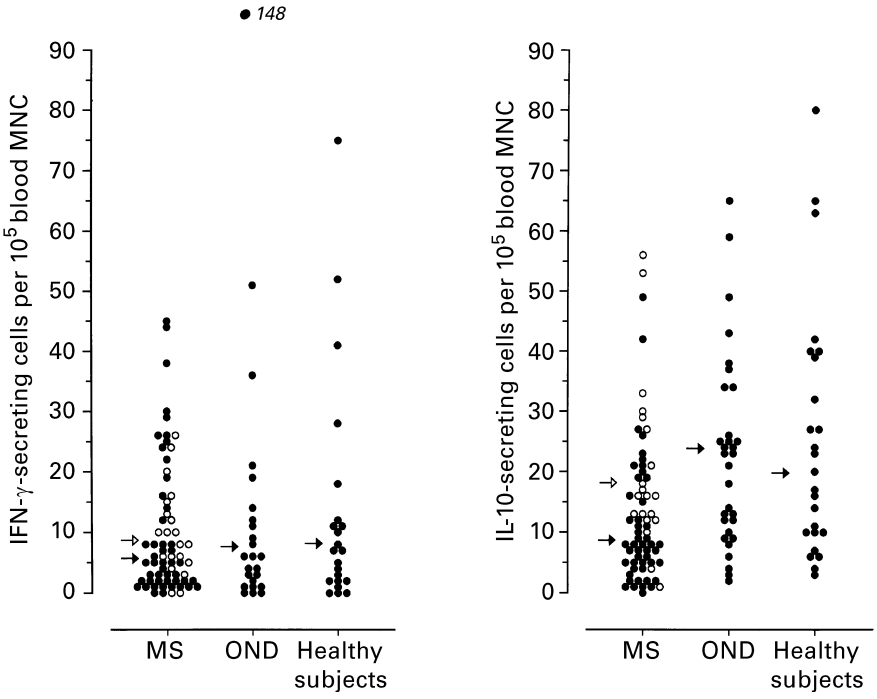

Fig. 2. Numbers of IFN-γ- and IL-10-secreting cells per 10^5 blood MNC from patients with multiple sclerosis (MS), other neurological diseases (OND) and healthy subjects. Arrows refer to median values. Open circles and open arrows refer to patients with MS treated with IFN-β-1b.

Table 1. Numbers of IFN-γ and IL-10 cells secreting cells per 10^5 blood mononuclear cells from patients with MS, OND and HC detected after culture without antigen (No antigen) and in the presence of antigen (MBP or PPD)

Diagnosis			No antigen	MBP	PPD
MS	IFN-γ	Range	0–45	0–102	1–238
		Mean (SD)	10 (11)	18 (20)	57 (55)
		Median	6	13	46
		No. exam.	69	54	53
	P-values	MS versus OND	NS	NS	NS
		MS versus HC	NS	NS	NS
	IL-10	Range	0–56	1–128	1–271
		Mean (SD)	14 (13)	21 (22)	70 (67)
		Median	10	15	53
		No. exam	69	62	51
	P-values	MS versus OND	< 0.01	NS	NS
		MS versus HC	< 0.01	NS	NS
OND	IFN-γ	Range	0–148	1–149	2–280
		Mean (SD)	16 (30)	19 (31)	65 (58)
		Median	7	7	58
		No. exam	32	24	22
	IL-10	Range	2–65	3–66	3–280
		Mean (SD)	23 (16)	26 (17)	71(64)
		Median	23	24	53
		No. exam	31	27	22
HC	IFN-γ	Range	0–75	0–78	2–250
		Mean (SD)	14 (19)	15 (19)	66 (69)
		Median	7	7	47
		No. exam	24	19	16
	IL-10	Range	3–80	1–89	6–230
		Mean (SD)	25 (20)	25 (21)	63 (62)
		Median	19	17	48
		No. exam	25	24	21

The patients with MS had significantly lower levels of IL-10-secreting blood MNC compared with the patients with OND and HC. No such differences were detected for IFN-γ secreting cells, or for MBP or PPD reactive IL-10 or IFN-γ-secreting cells. NS = not significant.

After subgrouping the patients with MS by treatment, untreated patients with MS were found to have lower numbers of IL-10-secreting blood MNC compared with patients undergoing treatment with IFN-β-1b ($P < 0.01$) (Table 2). The median values were 8 per 10^5 blood MNC in the untreated and 17 per 10^5 MNC in treated patients. Corresponding frequencies were ≈ 1 per 12 500 and 1 per 6000 cells, respectively. The numbers of IL-10-secreting cells were lower in the untreated patients with MS compared with the patients with OND ($P < 0.0001$) and the healthy subjects ($P < 0.001$). In contrast, the numbers of IL-10-secreting blood MNC in the IFN-β-1b-treated MS patients did not differ from the numbers in the control patients with OND or the healthy subjects (Fig. 2).

Untreated and IFN-β-1b-treated patients with MS had similar numbers of blood MNC secreting IFN-γ. The median values were 5 per 10^5 blood MNC in untreated and 9 per 10^5 MNC in the

Table 2. Numbers of IFN-γ and IL-10 secreting cells per 10^5 blood mononuclear cells from patients with MS subgrouped with regard to treatment with IFN-β-1b

Diagnosis		No antigen	MBP	PPD
Untreated MS				
IFN-γ	Range	0–45	0–102	1–238
	Mean (SD)	10 (12)	19 (22)	57 (58)
	Median	5	13	46
	No. exam.	49	36	36
IL-10	Range	0–49	1–73	1–271
	Mean (SD)	11 (10)	16 (15)	57 (64)
	Median	8	9	39
	No. exam	49	42	34
IFN-β-1b -reated MS				
IFN-γ	Range	0–24	0–40	7–165
	Mean (SD)	11 (7)	16 (11)	57 (47)
	Median	9	11	47
	No. exam	20	18	15
IL-10	Range	1–56	0–62	5–237
	Mean (SD)	21 (14)	31 (18)	93 (66)
	Median	17	31	88
	No. exam	20	20	17
p-values	IFN-γ	NS	NS	NS
	IL-10	< 0.01	< 0.01	NS

P-values refer to differences between the groups of untreated versus treated MS.

treated patients, corresponding to frequencies of ≈ 1 per 20 000 and 1 per 11 000 cells, respectively. The numbers did not differ from those registered in the two control groups (Table 2).

After subgrouping the untreated MS patients by clinical variables, there were no significant differences in number of IL-10-secreting cells when comparing RR with SP course, high (EDSS ≥ 3) with low (EDSS < 3) disability, or short with long MS duration (< 10 years versus ≥ 10 years) of the disease (Table 3). Nor were any differences observed for numbers of IFN-γ-secreting blood MNC among these subgroups of patients with MS (Table 3).

We examined seven patients with MS longitudinally during IFN-β-1b treatment. Patients with MS had lower numbers of IL-10-secreting blood MNC before the onset of IFN-β-1b treatment compared with after 1 week ($P < 0.05$) and 1 month ($P < 0.01$) of treatment (Fig. 3). In contrast to IL-10 there was no difference in numbers of IFN-γ-secreting blood MNC during IFN-1b treatment (Table 4).

Levels of MBP-reactive IL-10- and IFN-γ-secreting blood MNC

To elucidate levels of myelin antigen-reactive IL-10- and IFN-γ-secreting cells, blood MNC from patients with MS and controls were cultivated in the presence and absence of MBP. In patients with MS, stimulation with MBP resulted in increased numbers of blood MNC secreting both IL-10 and IFN-γ compared with

Table 3. Numbers of IFN-γ- and IL-10-secreting cells per 10^5 blood MNC from patients with untreated MS upon subgrouping regarding clinical variables

MS patients groups		IFN-γ	IL-10
All untreated patients	Range	0–45	0–49
	Mean (SD)	10 (12)	11 (10)
	Median	5	8
	No. exam.	49	49
MS exacerbation	Range	5–25	4–16
	Mean (SD)	12 (5)	10 (5)
	Median	9	7
	No. exam.	6	6
MS remission	Range	1–45	1–56
	Mean (SD)	14 (15)	12 (11)
	Median	7	10
	No. exam.	21	22
SP	Range	0–38	0–49
	Mean (SD)	8 (11)	12 (13)
	Median	3	5
	No. exam.	22	21
EDSS < 3	Range	1–45	1–47
	Mean (SD)	14 (15)	13 (12)
	Median	6	10
	No. exam.	20	18
EDSS ≥ 3	Range	0–38	0–49
	Mean (SD)	7 (9)	11 (12)
	Median	4	6
	No. exam.	29	31

SP, secondary progressive phase of MS. There were no differences between patients examined during exacerbation versus remission versus SP, or between patients with EDSS < 3 versus EDSS ≥ 3.

cultures without any antigen present ($P < 0.0001$ for both comparisons; Fig. 4). Increased numbers of IL-10- and IFN-γ-secreting cells were observed in cultures with MBP compared with cultures without antigen present both in MS patients receiving IFN-β-1b treatment (IL-10: $P < 0.0001$; IFN-γ: $P < 0.01$) and MS patients without any immunomodulatory treatment (IL-10: $P < 0.001$; IFN-γ: $P < 0.001$). MS patients treated with IFN-β-1b had, however, higher numbers of IL-10-secreting cells after MBP stimulation compared with untreated patients ($P < 0.01$; Table 2). After stimulation with MBP, numbers of IFN-γ-secreting cells did not differ between untreated and IFN-β-1b-treated patients with MS.

After subgrouping the untreated MS patients regarding clinical variables, there were no differences for numbers of MBP-reactive IL-10- and IFN-γ-secreting cells when comparing RR with SP course, high (EDSS ≥ 3) with low (EDSS < 3) disability, or short with long MS duration (< 10 years versus ≥ 10 years) of the disease (data not shown).

In the control groups, sporadic individuals had MBP-reactive blood MNC secreting IL-10 or IFN-γ. In contrast to the findings in MS, the levels of MBP-reactive IL-10- or IFN-γ-secreting cells in the control groups did not differ compared with levels registered upon culture in the absence of antigen.

PPD was used as a positive control antigen in all MBP-related experiments. Levels of PPD-reactive IL-10, as well as PPD-reactive IFN-γ, were also higher compared with levels observed after culture in the absence of this recall antigen. There were no statistical differences for PPD responses between the groups (Table 1).

DISCUSSION

Using ELISPOT assays, we found that patients with MS had lower numbers of IL-10-secreting blood MNC compared with patients with OND or healthy subjects. The low numbers were confined to patients with untreated MS, while no differences were encountered between patients with MS treated with IFN-β-1b and the two control groups. Furthermore, longitudinal examination of a group of patients with MS receiving IFN-β-1b treatment showed us that numbers of IL-10-secreting cells are increased during treatment. In contrast to IL-10, the patients with

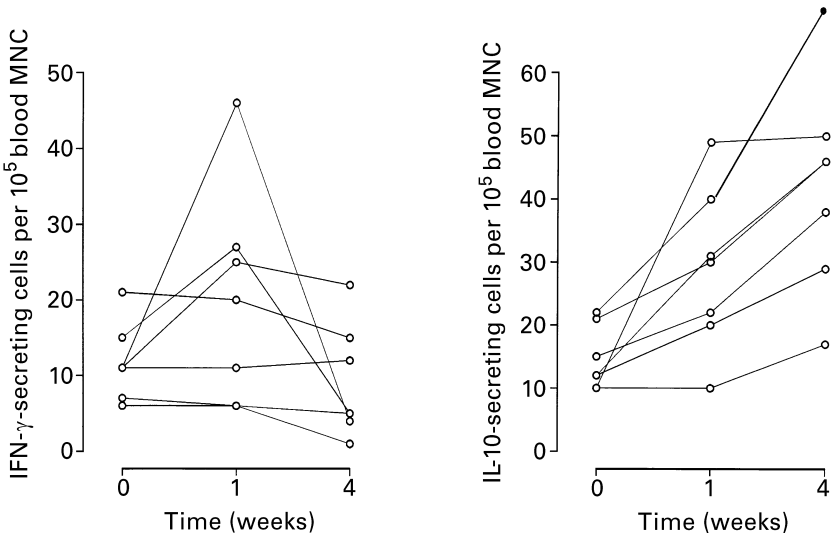

Fig. 3. Numbers of IFN-γ- and IL-10-secreting cells per 10^5 blood MNC from patients with MS, before, after 1 week and after 4 weeks of IFN-β-1b treatment.

Table 4. Numbers of IFN-γ- and IL-10-secreting cells per 10^5 blood MNC from patients with multiple sclerosis during IFN-β-1b treatment

MS patients		IFN-γ	IL-10
Before the onset	Range	6–21	10–22
of treatment	Mean (SD)	11 (5)	15 (5)
(A)	Median	11	12
	No. exam.	7	7
1 week after	Range	1–46	10–49
treatment	Mean (SD)	19 (15)	29 (13)
(B)	Median	20	30
	No. exam.	7	7
4 weeks after	Range	4–22	17–130
treatment	Mean (SD)	10 (7)	51 (37)
(C)	Median	6	46
	No. exam.	7	7
P-values			
A versus B versus C		NS	< 0.0001
A versus B		NS	< 0.05
A versus C		NS	< 0.01

The number of IL-10-secreting blood MNC is increased during IFN-β-1b treatment while there were no differences in numbers of IFN-γ-secreting blood MNC

MS had similar numbers of IFN-γ-secreting blood MNC compared with levels in patients with OND and healthy subjects. These results regarding IFN-γ-secreting blood MNC are concordant with previous observations obtained with ELISPOT assays [30, 31]. Numbers of IFN-γ-secreting cells were also found to be similar irrespective of treatment with IFN-β-1b.

To understand the role of cytokines in normal and pathological states, the source and frequency of cells secreting these factors should be established [32], since it probably represents one of the best ways currently available to detect and quantitate cytokine involvement. ELISA is usually used to determine serum cytokine levels in body fluids. Since cytokines are seldom found in unbound states [33], have a short half-life [34] and are rapidly taken up and utilized [35], results obtained by ELISA are frequently negative and/or difficult to interpret. Cytokine mRNA levels can be examined by Northern blot, semi-quantitative reverse transcription polymerase chain reaction (RT–PCR) or *in situ* hybridization, yet mRNA levels do not necessarily reflect rates of protein secretion. Immunostaining generally requires *in vitro* stimulation and cannot discriminate passively absorbed cytokines.

ELISPOT assays can circumvent many of these problems. They permit the *ex vivo* identification of cells actively secreting cytokines [36]. ELISPOT assays can detect a single cell out of a million, each cell releasing less than 100 molecules of a certain cytokine per second [37].

IL-10 has immune response down-regulatory effects that could be beneficial for MS. IL-10 promotes the development of an anti-inflammatory cytokine pattern by inhibiting the IFN-γ production of T cells and natural killer (NK) cells, particularly via the suppression of IL-12 synthesis in accessory cells [38]. IL-10 down-regulates MHC class II expression on monocytes and inhibits the synthesis of many Th1-cell-related cytokines (IFN-γ, TNF-α, TNF-β, IL-1, IL-2, IL-6) and T-cell proliferation [39]. IL-10 also suppresses cytotoxic functions of activated macrophages by inhibiting nitric oxide production [40].

The present finding of higher levels of IL-10-secreting blood MNC in patients treated with IFN-β-1b, i.e. a compound

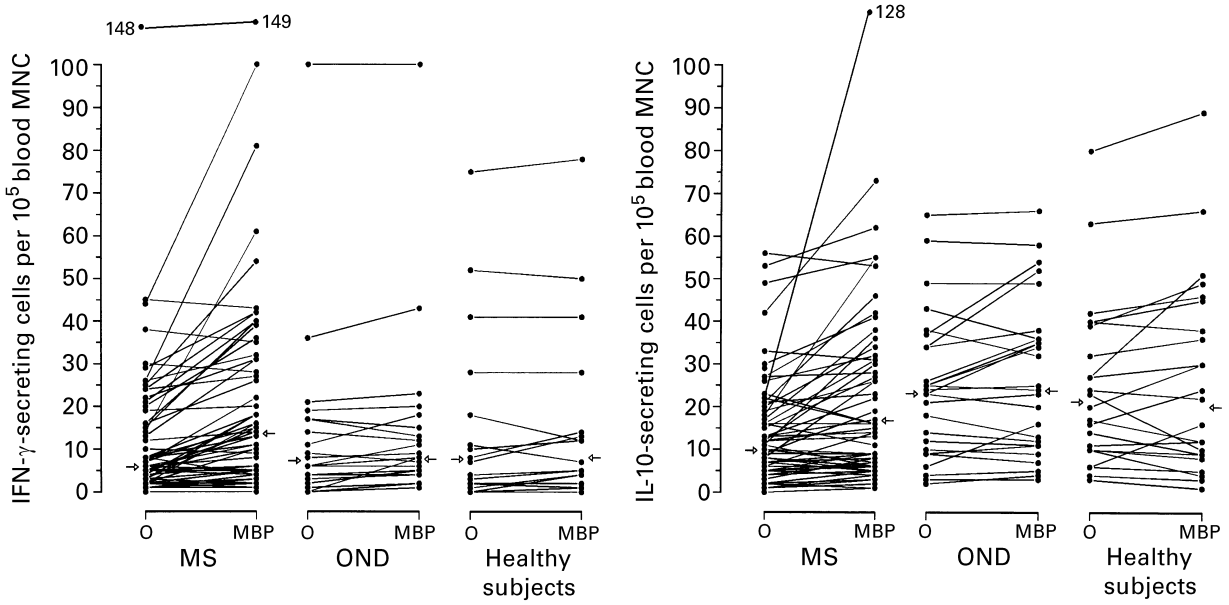

Fig. 4. Numbers of IFN-γ- and IL-10-secreting cells per 10^5 blood MNC from patients with MS, OND and healthy subjects after culture in the absence of antigen (O) and in the presence of MBP. Arrows refer to median values.

that is known to have a beneficial effect on the course of MS, compared with patients with untreated MS, is in line with a recent report that culturing blood MNC in the presence of IFN-β-1b *in vitro* increases the numbers of cells that spontaneously secrete IL-10 [41]. Also IFN-β-1a induces the accumulation of IL-10 mRNA and protein secretion *in vitro* by cultured peripheral blood MNC from healthy subjects and patients with MS [42]. Furthermore, using ELISA a relationship has been reported between increased cerebrospinal fluid IL-10 levels and clinical benefits of IFN-β-1a, suggesting that the induction of IL-10 is a mechanism underlying the effects of IFN-β-1a in MS [43].

In conclusion, lower levels of IL-10-secreting blood MNC were observed in untreated patients with MS compared with patients with OND or healthy subjects. Treatment with IFN-β-1b seemed to normalize the decreased levels of IL-10-secreting cells. The numbers of IL-10-secreting blood MNC in the IFN-β-1b-treated MS patients did not differ from the numbers in the two control groups. Such normalization could be one effector mechanism behind the effects of IFN-β-1b, but could also reflect a normalization of the immune deviations present in MS. Based on the presently demonstrated lower numbers of IL-10-secreting cells in MS and the known immune-response down regulatory effects of IL-10, further examination of IL-10 function in MS is warranted to evaluate its potential value in treatment of the disease.

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