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Randomized study of antibodies to IFN-γ and TNF-α in secondary progressive multiple sclerosis

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Studies of cytokines in multiple sclerosis (MS) have shown that immune mechanisms connected with disturbance of the synthesis of cytokines probably play critical roles in the initiation and prolongation of MS. In a double-blind, placebo-controlled trial, 45 patients with active secondary progressive MS were randomized to three groups of 15 patients, each receiving a short course of antibodies to IFN-γ, to tumor necrosis factor (TNF)-α, or a placebo. After 12 months with analysis of disability (Expanded Disability Status Scale scores), accompanied by interval determinations of lymphocyte subpopulations, cytokine production levels, MRI, and evoked potentials, it was found that only patients who received antibodies to IFN-γ showed statistically significant improvement compared to the placebo group – a significant increase in the number of patients without confirmed disability progression. This was supported by MRI data (a decrease in the number of active lesions) and systemic changes in cytokine status (a decrease in IL-1β, TNF-α, and IFN-γ concentrations in supernatants of activated blood cells of these MS patients and an increase in TGF-β production). Neutralization of IFN-γ could be a new approach to treating secondary progressive MS. Long-term administration of humanized monoclonal antibodies to IFN-γ and simultaneous use of antibodies to IFN-γ together with IFN-β products are planned.
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Introduction

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS). The pathological process in brain tissue during MS is thought to be the result of chronic, mainly cell-mediated, autoimmune reactions directed against numerous myelin and possibly other endo- and exogenous antigens. Studies of cytokines in MS patients indicate that several immune mechanisms connected with the disturbance of the synthesis of these cytokines, particularly of interferon (IFN)-γ, probably play critical roles in the initiation and prolongation of the pathological process in MS.[1] For this reason, the cytokine system is the target for numerous immunomodulating approaches to treating MS, both in experimental models and in clinical trials. More than 20 years ago one of us proposed that the development of autoimmune diseases was connected with the disturbance of the synthesis of certain cytokines and that the removal of these cytokines could be therapeutic.[2] The first successful anti-cytokine therapy was performed in 1975 in patients with severe rheumatoid arthritis.[3] Later it was proposed to treat MS by anti-cytokine therapy.[4] Disease-modifying treatment of relapsing – remitting MS with IFN-β products became a great achievement in MS care.[5] Recent data on the use of IFN-β in treating secondary progressive MS are promising,[6] but the treatment of secondary progressive MS where the degenerative process might be irreversible still remains a great problem.

Cytokines are low-molecular weight soluble proteins involved in signaling between cells of the immune system. They are released by leukocytes as well as other cell types, including cells of the CNS, and modulate both local and systemic immune reactions.[7] MS is postulated to be a Th1 type cell-mediated autoimmune disease. Cytokines upregulating cellular immunity and produced at least by monocytes (interleukin-1 [IL-1], tumor necrosis factor-α [TNF-α]) and by Th1 helpers (IL-2, IFN-γ, lymphotoxin or TNF-β) are believed to contribute to propagating the pathological process and tissue injury in MS, while cytokines produced by Th2 cells (IL-4, IL-10) have been shown to suppress Th1-cells and cell-mediated immunity. Th3-cells can also participate in Th1 suppression, producing transforming growth factor-β (TGF-β). Human endogenous retroviruses (HERVs) have been detected in patients with MS,[8] schizophrenia,[9] and certain other autoimmune diseases and may play a causative role in these diseases. Activation of this retrovirus could lead to the production of significant amounts of IFN-γ, which may directly damage central and peripheral nerve cells and lead to neurological autoimmune diseases.

One possible mechanism for the action of IFN-β in MS may be through its inhibition of IFN-γ-induced immune activation.[10]

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Anti-cytokine therapy remains one of the most exciting directions in immunomodulation in MS.[11] We report results of one of the first trials of antibodies to the pro-inflammatory cytokines TNF-α and IFN-γ in secondary progressive MS.

Methods

Antibodies

Healthy female goats were immunized with recombinant IFN-γ or recombinant-TNF-α (Peprotech) until titer of the plasma reached 7×10^4 and 8×10^4 IU/ml respectively. The goats were plasmapheresed and the plasma was pooled. IgG was isolated and purified. The final concentration of the antibody was 8×10^4 u/ml and 9×10^4 IU/ml respectively (ELISA). It was aliquoted to 2 ml/vial. Each ml of preparation consisted of 48–50 mg of protein.

Placebo

Commercial albumin from an American blood bank was used. Each ampoule of albumin consisted of 2 ml (50 mg of protein/ml).

Patients

A total of 45 patients with definite MS were enrolled in a randomized, double-blind, placebo-controlled, parallel group study. All subjects gave written informed consent to participation in the study. The study was approved by the institutional research ethics committee. The inclusion criteria were: (1) clinically definite MS according to Poser criteria[12] that followed a secondary progressive course; (2) with active disease, i.e., showing deterioration of at least one point on Kurtzke's Expanded Disability Status Scale (EDSS) within 12 months prior to enrolment; (3) age 18–50 years old; (4) EDSS score between 3.0 and 7.0 at selection; (5) negative skin test to goat protein. Exclusion criteria included: (1) substantial cognitive impairment that could have compromised the patient's ability to give informed consent; (2) moderate or severe diseases other then MS; (3) primary progressive or relapsing–remitting MS; (4) duration of secondary progression shorter than 1 year (to be sure of the progression) and longer than 5 years (to minimize the influence of irreversible degenerative changes); (5) present or planned pregnancy; (6) receiving or having received in the last 12 months any immunosuppressive drugs, interferons or copaxone; (7) use of corticosteriods in the 4 weeks before the first visit; (8) previous exposure to any product containing goat proteins; (9) inability to undergo MRI scanning or immunological investigation; and (10) positive skin test to goat protein. Forty-five patients were randomized using tables of random numbers. Patients with early active secondary progression as a rule with relapses (i.e., with steady progression between relapses) were selected as the target for the trial (Figure 1).

Treatment protocol

The treatment of the patients, the handling of any side effects and adverse events, and the evaluation of clinical parameters were performed by qualified blinded neurologists, whenever possible by the same physician. Assessing physicians were different from those treating. Patients were randomized to three groups, 15 in each, using a book of random numbers. The first group of patients received antibodies (AB) to TNF-α, the second–AB to IFN-γ; and the third–placebo. The randomization was controlled by a statistician, and the type of treatment received by each patient was kept concealed from the physician and patients. All antibodies were produced and supplied by Advanced Biotherapy Inc. (USA) in

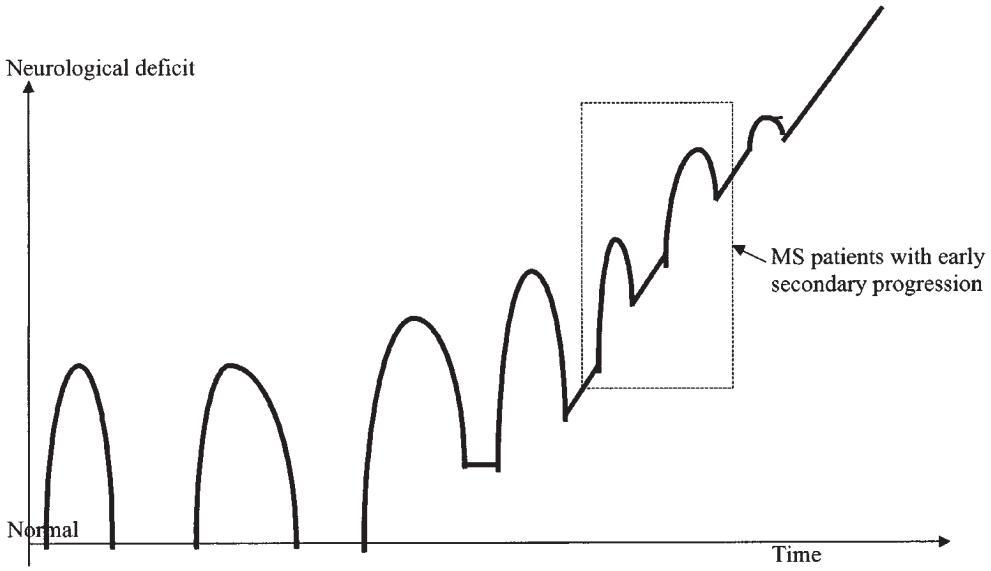

Figure 1 *Selection of patients with early secondary progressive MS*

identical-looking vials. Albumin, packaged the same way as the AB, was used as a placebo and diluted in the same manner. Purified (IgG) goat polyclonal AB were diluted in sterile, distilled water for injections and administered twice a day intramuscularly (i.m.) daily for 5 consecutive days. Syringes for injection of the ABs were prepared to be identical in appearance and administered by teams separate from the preparer and from the evaluating physician. The safety of this regimen was demonstrated in a previous trial.[8,13] Among adverse effects were a flu-like syndrome with fever and headache in one patient who received AB to TNF-α (6.7%) and mild local skin inflammatory reactions (no necrosis was registered)—in three cases who received AB to TNF-α (20%); in two—AB to IFN-γ (13.3%); and one—placebo (6.7%). No changes in liver enzymes or other hematological or biochemical characteristics were seen during the short course of AB or after it during the observation period. The side effects were never a reason for halting treatment. During the observation period, four patients dropped out because they started treatments with products of IFN-β: two from the group who received AB to TNF-α (after 8 and 10 months of follow-up) and two from the placebo group (after 7 and 8 months of follow-up). These cases cannot be analyzed as treatment failures since, independently of the treatment results, these patients at this point found money for the payment of the treatment previously shown to be effective in secondary progressive MS. All these patients were included in the analyses for the previous time points (Figure 2).

At selection, all patients underwent a medical examination, including a neurological examination. Visual and brainstem auditory evoked potentials and an MRI study were performed and blood was taken for immunological investigation. Follow-up obligatory visits were at 1, 3, 6, and 12 months. At each visit a repeat neurological examination was done and blood was taken for hematological and biochemical examination. In addition, at the 1-month visit, immunological and electrophysiological examinations were performed and at the 6-month visit—MRI and immunological examinations as well. Every patient had both MRI examinations according to universal strict protocol.[14] Five-mm axial slices were obtained, repositioning based on standardized anatomic landmarks, proton density and T2-weighted fast spin-echo sequence before contrast, axial T1-weighted spin-echo sequence after injection of gadolinium (obtained 10 min after 0.1 mmol/kg of intravenous (i.v.) gadolinium-DTPA). Scans were analyzed by the same blinded radiologists. The numbers of hyperintensive lesions at T2-weighted scans and the number of enhancing lesions at T1-weighted scans were measured. Studies of visual (VEP) and brainstem auditory evoked potentials (BAEP) were performed according to universally accepted protocol.[15,16] If the clinical status of patients worsened, they were able to visit out of order. In cases where an increased EDSS score was seen at a scheduled visit between months 3 and 12, patients were asked to come again 1 and 3 months later to confirm EDSS progression. During the observation period, MS patients visited their consulting neurologist from five to 10 times (including baseline).

The primary outcome measure was the number of patients without confirmed progression in disability as measured by an increase of at least 1.0 level on EDSS, sustained for at least 3 months, exclusive of any exacerbation. Secondary outcome measures were selected as: time without confirmed progression, number of relapses, number of patients free of relapses, number of active lesions on T1 MRI scans, changes in parameters of VEP or BAEP. During the follow-up, patients received corticosteroids if they had

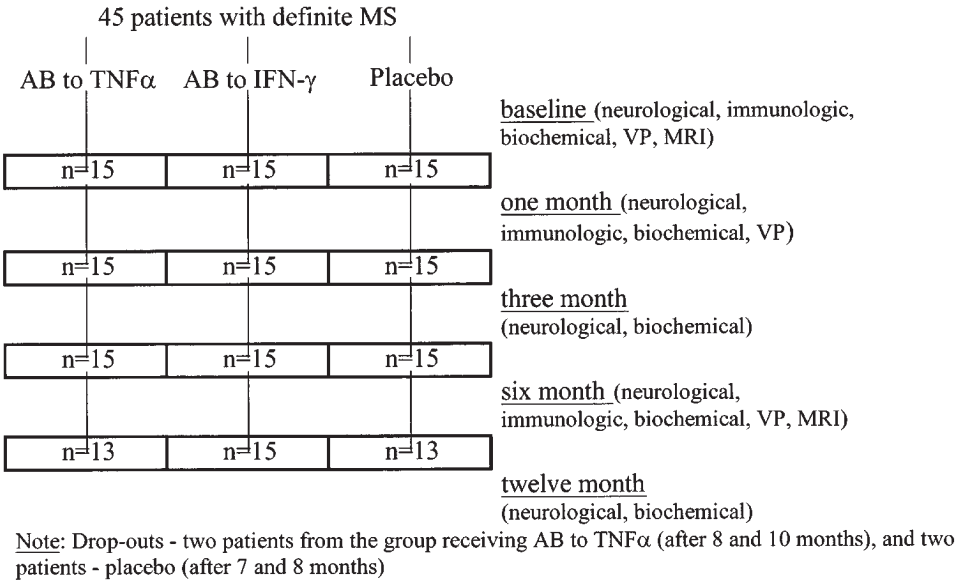

Figure 2 *Profile of the study, obligatory visits, and investigations*

relapses with severe deterioration. This treatment did not lead to exclusion from the study.

Immunological investigation
All subjects had their blood drawn between 09.00 and 11.00 h. After gentle mixing with an equal volume of Ca^{2+}/MG^{2+}-free Hank's balanced salt solution (HBSS, Sigma), blood was centrifuged with Ficoll-Paque (Pharmacia) at 400 *g* for 40 min at room temperature. Buffy coats of mononuclear cells between Ficoll and HBSS layers, carefully transferred with sterile pipettes into centrifuge tubes, were spun at 800 *g* for 10 min at 4°C three times. After cell counting of lympho/monocytes in an RPMI-1640 medium (with addition of 10% fetal calf serum (Sigma), 2 mM L-glutamine, 50 u/ml gentamicin), they were cultivated for 48 h with PHA (final concentration 5 μg/ml, Sigma). Supernatants were collected and kept at −20°C until analysis. Concentrations of cytokines were measured by a standard ELISA technique[17] with kits for IFN-γ and TGF-β (RD, USA), IL-1β, IL-2 and IL-6 (Sigma, USA) and TNF-α (Proteinoviy Kontur, Russia) according to manufacturer's guidelines. Due to technical reasons, the assay for levels of IL-1, IL-2, and IL-6 production was done for only nine patients from the AB to IFN-γ group and nine from the AB to TNF-α group, and for 10 patients from the placebo group. Study of the cell subpopulations was performed using flow cytometry (Becton-Dickenson) with mAB to CD3, CD4, CD8, CD29 and CD16 (all from Becton-Dickenson).

Statistical evaluation
Statistical evaluation was performed using SPSS and EPI INFO packages and included different methods of parametric and non-parametric statistics.

Results

Baseline characteristics of all groups did not differ significantly (Table 1). The number of patients free of progression was lower in the group who received AB to IFN-γ (Figure 3). The difference between patients who received AB to IFN-γ and placebo became significant 9 months after the short treatment course (12/15 *vs* 3/13, χ^2=6.92, *P*=0.008 with Yates correc-

Table 1 Baseline clinical-demographic characteristics of MS patients included in the study

Characteristics (number of patients)	*All* (n=*45*)	*AB to TNF-α* (n=*15*)	*AB to IFN-γ* (n=*15*)	*Placebo* (n=*15*)
Age (years): mean ± s.e. (range)	38.54 ± 1.62 (22–54)	38.33 ± 2.46 (22–51)	38.93 ± 1.95 (25–49)	38.80 ± 2.23 (24–54)
Age of onset (years): mean ± s.e. (range)	27.71 ± 1.72 (15–44)	27.93 ± 2.40 (15–44)	27.47 ± 1.69 (20–41)	27.53 ± 2.11 (16–42)
Gender (w/m)	23/22	9/6	7/8	7/8
Disease duration (years): mean ± s.e. (range)	11.31 ± 1.18 (4–30)	10.67 ± 1.84 (4–30)	11.71 ± 1.73 (4–24)	11.27 ± 1.65 (4–27)
Duration of secondary progression (years): mean ± s.e. (range)	2.65 ± 0.17 (1–5)	2.83 ± 0.37 (1–5)	2.46 ± 0.29 (1–5)	2.53 ± 0.27 (1–5)
EDSS: mean ± s.e. (range)	4.18 ± 0.19 (3.0–7.0)	3.97 ± 0.29 (3.0–6.0)	4.47 ± 0.30 (3.0–6.5)	4.13 ± 0.43 (3.0–7.0)
sFS: mean ± s.e. (range)	13.97 ± 0.67 (8–21)	14.13 ± 1.09 (8–21)	15.33 ± 0.68 (10–20)	12.80 ± 0.87 (8–18)

AB, antibodies; s.e., standard error

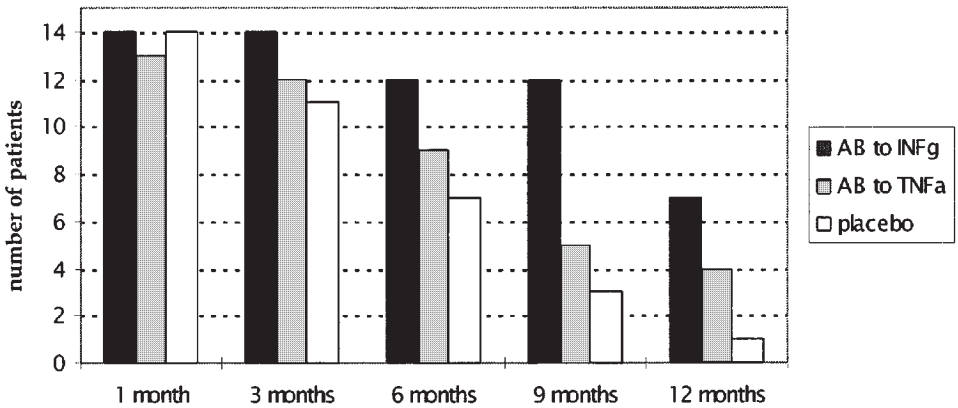

Figure 3 *Number of patients without confirmed EDSS progression for 12 months*

tion), and still remained significant 12 months later (7/15 *vs* 1/13, P=0.038 in Fisher two-sided test), while the number of such patients in the subgroup who received AB to IFN-γ decreased from the 9- to 12-month time points. No significant difference with patients who received AB to TNF-α were seen. The difference between groups was confirmed by Kaplan-Meier analyses, showing significantly less probability for these patients to progress in disability during the observation period. The time without progression in comparison to the placebo group (5.93 ± 0.94 months) was significantly longer (9.0 ± 1.06 months, P=0.042) for the AB to IFN-γ group, but not for the AB to TNF-α group (7.33 ± 1.05 months, $P > 0.05$). It is important to notice that after 3 months, the group of patients who received AB to IFN-γ were divided into two subgroups – the majority had a less active MS course and a stable or better EDSS, while there were several patients who still had active disease with an increase in EDSS score. Altogether, eight relapses during 12 months were registered in the placebo group (six had to be treated with corticosteriods), seven in the AB to TNF-α group (five were treated with corticosteroids) and five in the AB to IFN-γ group (all were treated with corticosteroids). Thus, while there was a clear decrease in these numbers for the AB to IFN-γ group, neither the number of relapses, nor the number of corticosteroid courses differed significantly between groups. The difference in the number of patients free of relapses during the 12-month period also did not reach statistical significance (for the 12-month time point, 10/15 in the AB to IFN-γ group and 6/13 in the placebo group, $P > 0.05$).

The positive trend in the whole group who received AB to IFN-γ was confirmed by MRI (Table 2). At baseline, all groups had similar MRI scans characteristics. Six months later the total number of T2-lesions was also similar, while the significant reduction in the numbers of MRI active lesions ($P < 0.05$) was seen only in the group who received AB to IFN-γ. There were no significant changes in parameters of VEP or BAEP in all groups, either in comparison to placebo or to baseline level (data not shown).

Results of the immunological studies are shown in Table 3. At baseline all patient groups had similar lymphocyte sub-population ratios and levels of cytokine production. The exceptions were an elevated CD4/CD8 ratio and percentage of $CD29^+$-cells in the AB to IFN-γ group and a percentage of $CD16^+$-cells in the AB to TNF-α group. One month later a significant decrease in levels of TNF-α, IFN-γ, and IL-1β production as well as an increase in TGF-β production was seen in the group who received AB to IFN-γ. Patients in this group also had a decrease in the CD4/CD8 ratio compared to baseline and an increased percentage of $CD16^+$-cells compared to the placebo group (insignificant changes in cell subpopulations were excluded from the table). Neither patients in the AB to TNF-α group, nor patients who received a placebo had significant changes in cytokine production. All changes in the group who received AB to IFN-γ became less prominent 6 months later.

Discussion

This is one of the first attempts to use antibodies to cytokines for the treatment of MS. In this preliminary study we have shown that patients who received antibodies to IFN-γ had a significant decrease in the production of several pro-inflammatory cytokines, and this was associated with a better course of the MS and with less activity on MRI 6 months after treatment. It is interesting that this effect was found only in patients who received antibodies to IFN-γ, but not antibodies to TNF-α. Positive clinical changes at 6 months were supported by MRI data showing a decrease in the number of active enhancing lesions. MRI has been suggested as a sensitive measure of the pathological process in MS. Serial MRI studies have shown that frequent asymptomatic disease activity occurs as evidenced by a much higher number of active lesions as revealed by MRI scans than clinical relapses.[18,19]

Table 2 Characteristics of T2 and enhancing lesions on MRI in different treatment groups at baseline and 6 months later

Characteristic	*AB to TNF-α* (n=*15*)	*AB to IFN-γ* (n=*15*)	*Placebo* (n=*15*)
Baseline			
Number of T2-lesions: mean ± s.e. (range)	16.27 ± 2.07 (7 – 30)	18.93 ± 3.02 (6 – 40)	14.73 ± 2.51 (4 – 32)
Number of enhancing lesions: mean ± s.e. (range)	2.20 ± 0.46 (0 – 6)	2.47 ± 0.56 (0 – 7)	1.93 ± 0.51 (0 – 8)
Six months later			
Number of T2-lesions: mean ± s.e. (range)	17.07 ± 2.29 (6 – 36)	19.20 ± 2.69 (5 – 40)	16.60 ± 3.04 (3 – 38)
Number of enhancing lesions: mean ± s.e. (range)	2.0 ± 0.51 (0 – 6)	1.27 ± 0.33* (0 – 4)**	2.33 ± 0.60 (0 – 9)
Patients without new enhancing lesions	8 (53.3%)	13 (86.7%)*	6 (40%)

*Difference from placebo group with $P < 0.05$; **Difference from baseline with $P < 0.05$

Table 3 Immunological parameters in different treatment groups at baseline and 6 months later (insignificant changes in subpopulation levels were excluded)

Variables	*AB to TNFα* (n=*15*)	*AB to IFN-γ* (n=*15*)	*Placebo* (n=*15*)
Baseline			
$CD3^+$ cells (%): mean ± s.e. (range)	70.27 ± 2.25 (55–85)	67.80 ± 1.23 (59–76)	68.27 ± 1.24 (60–78)
$CD4^+$ cells (%): mean ± s.e. (range)	40.40 ± 1.31 (33–49)	41.80 ± 1.0 (36–48)	38.60 ± 0.95 (32–45)
$CD8^+$ cells (%): mean ± s.e. (range)	26.80 ± 1.47 (20–38)	24.0 ± 1.10 (18–32)	24.67 ± 1.58 (15–38)
CD4/CD8 ratio: mean ± s.e. (range)	1.57 ± 0.06 (1.1–2.1)	1.81 ± 0.11 (1.1–2.7)*	1.62 ± 0.10 (0.9–2.1)
$CD29^+$ cells (%): mean ± s.e. (range)	7.27 ± 0.66 (4–12)	7.07 ± 0.42 (4–10)*	8.53 ± 0.79 (5–16)
$CD16^+$ cells (%): mean ± s.e. (range)	8.40 ± 0.86 (3–14)	8.40 ± 0.89 (4–17)	7.73 ± 0.54 (5–12)
TNF-α (pg/ml): mean ± s.e. (range)	1109.4 ± 106.7 (357–1812)	1244.6 ± 139.6 (345–2490)	1105.3 ± 256.1 (182–3800)
TGF-β (pg/ml): mean ± s.e. (range)	488.33 ± 53.98 (207–879)	402.47 ± 51.39 (120–750)	456.47 ± 64.63 (120–939)
IFN-γ (pg/ml): mean ± s.e. (range)	674.53 ± 145.5 (112–2351)	710.8 ± 127.37 (72–1497)	642.6 ± 229.26 (90–3514)
IL1-β (ng/ml): mean ± s.e. (range)	1.80 ± 0.04 (1.63–2.0)	1.75 ± 0.04 (1.55–1.90)	1.81 ± 0.04 (1.67–2.11)
IL2 (ng/ml): mean ± s.e. (range)	21.13 ± 0.57 (18.49–23.64)	21.68 ± 0.71 (19.7–26.7)	21.37 ± 0.47 (19.34–24.12)
IL6 (ng/ml): mean ± s.e. (range)	14.45 ± 1.03 (9.82–19.0)	14.87 ± 0.89 (10.3–19.1)	15.48 ± 0.54 (12.3–18.11)
One month			
CD4/CD8 ratio: mean ± s.e. (range)	1.66 ± 0.09 (0.9–2.0)	1.62 ± 0.05 (1.3–2.0)**	1.64 ± 0.12 (1.1–2.9)
$CD16^+$ cells (%): mean ± s.e. (range)	7.73 ± 0.61 (5–14)	9.6 ± 1.06 (5–19)	6.87 ± 0.38 (5–10)
TNF-α (pg/ml): mean ± s.e. (range)	1206.3 ± 137.8 (216–2160)	875.6 ± 93.1* (209–1670)**	1285.5 ± 143.0 (364–2000)
TGF-β (pg/ml): mean ± s.e. (range)	506.2 ± 77.89 (99–1180)	574.13 ± 71.4* (137–1015)**	407.47 ± 86.2 (112–1230)
IFN-γ (pg/ml): mean ± s.e. (range)	772.6 ± 123.95 (150–1992)	499.3 ± 98.98* (58–1250)**	816.2 ± 206.52 (84–2766)
IL1-β (ng/ml): mean ± s.e. (range)	1.8 ± 0.03 (1.64–1.98)	1.65 ± 0.03* (1.51–1.84)**	1.84 ± 0.04 (1.66–2.06)
IL2 (ng/ml): mean ± s.e. (range)	21.59 ± 0.58 (18.0–24.1)	22.09 ± 0.67 (20.31–26.8)	21.22 ± 0.56 (18.45–24.0)
IL6 (ng/ml): mean ± s.e. (range)	14.29 ± 0.92 (9.91–18.0)	15.81 ± 0.91 (10.1–18.9)	14.97 ± 0.6 (12.3–18.3)
Six months			
$CD16^+$ cells (%): mean ± s.e. (range)	8.53 ± 0.74 (5–16)	9.67 ± 0.70 (6–14)*	7.47 ± 0.35 (5–10)
TNF-α (pg/ml): mean ± s.e. (range)	1161.3 ± 82.39 (568–1870)	1147.7 ± 161.2 (457–2810)	1322.4 ± 120.3 (780–2270)
TGF-β (pg/ml): mean ± s.e. (range)	541.13 ± 76.69 (125–1366)	476.88 ± 65.97 (59–952)	528.53 ± 91.51 (156–1148)
IFN-γ (pg/ml): mean ± s.e. (range)	757.33 ± 101.2 (54–1560)	605.67 ± 88.7 (120–1860)	851.3 ± 121.46 (334–2060)
IL1-β (ng/ml): mean ± s.e. (range)	1.77 ± 0.04 (1.49–1.92)	1.75 ± 0.04 (1.6–1.92)	1.82 ± 0.03 (1.68–1.89)
IL2 (ng/ml): mean ± s.e. (range)	21.5 ± 0.46 (18.93–24.2)	21.79 ± 0.60 (19.5–24.3)	21.75 ± 0.50 (18.99–23.89)
IL6 (ng/ml): mean ± s.e. (range)	14.40 ± 0.70 (11.59–17.4)	15.0 ± 0.99 (10.89–19.8)	14.96 ± 0.47 (13.11–17.83)

*Difference from placebo group with $P<0.05$; **Difference from baseline with $P<0.05$

In our pilot study, the primary and two secondary outcome measures reached significant difference with placebo even with a small total number of patients. These positive clinical results of a short course of AB to IFN-γ may be related to the patient selection criteria, whereby only active early progressive patients were included. It means that within the group of secondary progressive MS patients, a subgroup of patients sensitive to anti-cytokine and other immunomodulative treatment are still present. The high percentage of such patients among secondary progressive cases might be one of the reasons for positive results in the IFN-β-1b trial in Europe.[5] The clear definition of this subgroup, close to relapsing – remitting or remitting – relapsing MS,[20] is not easy and additional clinical and MRI markers could be useful. In our study the short duration of the progressive stage, the presence of relapses during progression, and the numerous active lesions on T1-scans appeared to be suitable for such purpose, but a large further study is necessary to confirm this.

Changes in cytokine production may foreshadow changes in MS course. This was shown both for relapsing – remitting and secondary progressive MS.[21–23] The concentration of IFN-γ and TNF-α in the supernatants of activated cells and the levels of these cytokine messenger RNA in cells were proposed as markers of active inflammation and demyelination.[20] In our study the decrease in IFN-γ and TNF-α production 1 month after the short course of AB to IFN-γ were accompanied by an increase in TGF-β and a decrease in IL-1β production. IL-1 is the main cytokine triggering the process of immune activation. High production of this cytokine in active MS has also been found.[24] TGF-β is an anti-inflammatory cytokine. Its production increased during the regression of exacerbations of MS.[25,26] Changes in all these cytokine levels reflect the systemic change in immunoregulation. The patients with active secondary progressive MS may have mostly unfavorable changes in cytokine status[27] and may positively react even to a short course of AB to IFN-γ.

The future of anti-cytokine therapy in MS has been widely discussed. It is generally known that administration of IFN-γ provokes MS attacks.[28] This has been attributed to a direct stimulation of lymphocyte proliferation, which leads to inflammation and demyelination. On endothelial cells, for example, IFN-γ can increase lymphocyte transmigration through the blood–brain barrier and MHC II-expression at the

sites of active demyelination in chronic active MS plaques.[29] All the new strategies for treating MS include reducing IFN-γ activity in different ways, the most accepted one by administration of IFN-β. The strategy of IFN-γ neutralization is less popular in part because of the theoretical risk of reactivation of potential virus infections. But it can be argued that neutralization may only remove hyperproduced IFN-γ, not stopping its production in the cells. Furthermore, IFN-γ removal still leaves other antiviral interferons. In some patients with active MS, this strategy may be reasonable, as was proposed for the blocking of TNF-α. However, we did not find any changes in disease course in patients given antibodies to TNF-α. In preliminary testing we also found that patients given a combination of antibodies to IFN-γ and to TNF-α did not do better than anti-IFN-γ alone. Lack of improvement from anti-TNF-α therapy agrees with the results of several trials of TNF-α modulating agents,[30,31] though such an approach was effective in treating the MS animal model of experimental allergic encephalitis (EAE).[32] At the same time, we did not see a dramatic increase in disease activity after use of anti-TNF-α antibodies as in the Lenercept MS Study,[28] nor any severe deterioration in MRI as in Van Oosten *et al*.[27] The majority of our patients continued to progress at the rate they had before. This could be explained by: (a) low activity of antibodies used or (b) specific selection of patients. TNF-α is one of the most pleiotropic cytokines and acts in a complex way. Its real role in MS is still unknown. A very severe EAE was induced in mice lacking TNF following immunization with myelin oligodendrocyte glycoprotein (MOG).[33] This is not surprising given the importance of this cytokine in apoptosis of inflammatory cells and their migration to the site of inflammation in the brain. TNF may inhibit IL-12 production, triggering production of IFN-γ.[34]

The limitations of the current results should be noted. We used a very short course of polyclonal antibodies. The course was well tolerated, but further use of these antibodies was limited due to the risk of allergic reactions and serum sickness. The true magnitude of clinical and MRI changes could not be determined given the short duration of treatment and relatively short follow-up. Levels of cytokine production varied greatly in each group of patients. Finally, there was a subgroup of patients who did not respond to the antibodies to IFN-γ and still maintained a very active course of the disease.

This study has demonstrated that even a short course of IFN-γ antibodies may bring changes in the cytokine profile associated with positive changes in MS course and MRI findings. Future trials with chimeric, humanized, or all-human monoclonal antibodies are needed. They could determine more accurately the magnitude and duration of the clinical and MRI effect of antibodies to IFN-γ on MS. The next step may be to test the use of IFN-β combined with anti-IFN-γ, both of which act to block the action of IFN-γ, for a possible stronger, more lasting effect.

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