Clinical Effect of Neutralizing Antibodies to Interferon Beta That Persist Long After Cessation of Therapy for Multiple Sclerosis

Laura F. van der Voort, MD; Francesca Gilli, PhD; Antonio Bertolotto, MD; Dirk L. Knol, PhD; Bernard M. J. Uitdehaag, MD, PhD; Chris H. Polman, MD, PhD; Joep Killestein, MD, PhD

Objectives: To confirm that neutralizing antibodies (NAb) to interferon beta can persist after therapy withdrawal and to evaluate whether persisting NAb are associated with a worse clinical disease course in multiple sclerosis (MS).

Design: Retrospective study.

Setting: Tertiary referral center in the Netherlands.

Patients: A total of 71 patients with relapsing-remitting multiple sclerosis treated with interferon beta in the past.

Main Outcome Measures: Persisting NAb after therapy withdrawal were tested using the cytopathic effect assay. Patients with and without persisting NAb were compared on several outcomes: the change in annualized relapse rate from prior to interferon beta treatment initiation to after cessation of treatment, time to sustained disability on the Kurtzke Expanded Disability Status Scale, and the use of disease-modifying treatments after cessation of treatment with interferon beta.

Results: Seventeen of 71 patients (24%) tested NAb positive after a median interval of 25 months (interquartile range, 10-51 months) after interferon beta treatment cessation. Eleven of these 17 patients (15%) were high-titer NAb positive (>150 10-fold reduction units per mL). Persisting NAb were associated with an increase in the annualized relapse rate ($P=0.04$) and a reduction in time to reach a sustained Expanded Disability Status Scale score of 6.0, ie, the need for unilateral assistance to walk 100 m ($P=0.02$). Moreover, NAb-positive patients were treated with second-line therapy significantly more often, especially mitoxantrone ($P=0.006$).

Conclusion: Anti–interferon beta NAb can persist after interferon beta treatment withdrawal and are associated with overt clinical disease activity. This is made apparent by an increase in relapse rate and faster disability progression and is supported by the observed need for more aggressive therapy after interferon beta treatment cessation. Prospective studies are warranted to confirm these results.


MULTIPLE SCLEROSIS (MS) is a chronic, immune-mediated disorder of the central nervous system and one of the leading causes of permanent disability in young adults. Interferon beta has been shown to be a safe and effective treatment for relapsing-remitting MS and is widely used as a first-line treatment. Long-term use of recombinant protein-based therapeutics such as interferon beta, insulin, growth hormone, and factor VIII can lead to an immune response directed against the drug that is mainly based on breaking B-cell tolerance. Antidrug antibodies may cross-react with their endogenous counterpart, influence the therapeutic efficacy of the drug, or be associated with allergic reactions. The clinical significance of anti–interferon beta neutralizing antibodies (NAb) continues to be a controversial issue in the MS community. Increasing evidence suggests that, during treatment, bioactivity of interferon beta is influenced by NAb, and efficacy of the treatment is decreased with persisting NAb. Some studies have shown that NAb can persist even after cessation of interferon beta treatment. The dynamics and clinical effect of persisting NAb, however, are largely unknown. In this study, our primary aim was to confirm the occurrence of persisting NAb after withdrawal of interferon beta therapy and to evaluate potential predisposing factors. Secondly, as persistent NAb after cess-
sation of interferon beta therapy will probably inhibit endogenous interferon β, we correlated persisting NAb status to measures of clinical disease activity.

METHODS

Potential participants were retrospectively identified by a medical record review of all well-monitored patients who had started interferon beta treatment from 1994 to 2006 at the MS center in Amsterdam. Consecutive patients were invited to participate when they were treated with interferon beta for at least 12 months and subsequently ceased treatment for at least 3 months. In general, patients were seen at baseline, 1, 3, 6, and 12 months, and annually thereafter. Patients had additional visits when indicated. We collected data concerning age at disease onset, sex, disease duration at start interferon beta treatment, treatment duration, and interferon beta product used. Furthermore, we evaluated the use of disease-modifying treatment after interferon beta treatment cessation. The number of relapses was assessed in the 2 years before interferon beta treatment initiation and in the period after interferon beta treatment cessation, and both were converted into annualized relapse rates. Disability status was determined at the start of interferon beta treatment for all subjects by using the Kurtzke Expanded Disability Status Scale (EDSS) and repeated at the most recent visit to the outpatient clinic. Neutralizing Ab titer levels were measured by a previously described cytopathic effect assay in the Centro Riferimento Regionale Sclerosi Multipla, Orbassano, Italy. Titers were calculated according to the Kawade formula and expressed in 10-fold reduction units per milliliter.

Measurements were performed with the same type of interferon beta-1a (Avonex; Biogen Idec, Cambridge, Massachusetts) once weekly (n=20), 22 µg (n=13) or 44 µg (n=5) of subcutaneous interferon beta-1b (Betaseron; Bayer Schering Pharma, Berlin, Germany) every other day (n=33). A statistical trend was found for an association between NAb positivity and the interferon beta product used (P=.05).

RESULTS

PATIENTS

About 525 patients with MS had started interferon beta therapy at the MS center in Amsterdam between 1994 and 2006. We examined the medical records of the 342 patients who had systematic clinical evaluations at least annually. Ninety-seven patients fulfilled the prespecified selection criteria. Seventy-one of these patients, 51 women and 20 men, gave written informed consent (for baseline characteristics see Table 1). The reasons for exclusion were either that patients were still receiving interferon beta treatment or that patients had been treated with interferon beta for fewer than 12 months. Twenty-six patients fulfilled the criteria but preferred not to participate because of either work responsibilities (n=6) or MS-related conditions (n=11). Nine patients declined participation in research without giving a specific reason.

Two hundred and thirty-six patients were treated with 30 µg of intramuscular interferon beta-1a (Avonex; Biogen Idec, Cambridge, Massachusetts) once weekly (n=20), 22 µg (n=13) or 44 µg (n=5) of subcutaneous interferon beta-1a (Rebif; Serono, Geneva, Switzerland) 3 times weekly, and 250 µg of subcutaneous interferon beta-1b (Betaseron; Bayer Schering Pharma, Berlin, Germany) every other day (n=33).
During follow-up (nine percent (35 of 71) of patients remained untreated intravenous mitoxantrone dihydrochloride (Mitoxantrone, Ireland) and 10 (14%) patients received courses of natalizumab (Tysabri; Elan Pharmaceuticals Inc, Dublin, Ireland). This is the first study reporting that patients with anti–interferon beta NAb that persist after cessation of therapy have a more active disease course. This was indicated by an increase in the annualized relapse rate after interferon beta treatment withdrawal and faster progression to an EDSS score of 6.0. Furthermore, persisting NAb are associated with a different treatment regimen after interferon beta treatment withdrawal; fewer patients remained untreated and more patients switched to second-line treatment, especially mitoxantrone.

Anti–interferon beta NAb develop in a significant proportion of patients with MS who are treated with interferon beta-1a (Avonex) (Table 1). The occurrence of persisting NAb was associated with a treatment switch after interferon beta treatment withdrawal; fewer patients received second-line treatment, especially mitoxantrone.

Seventeen patients (24%) were found to be NAb positive (NAb titer median, 320 TRU/mL; range, 22-5120 TRU/mL); 11 of these 17 patients were high-titer positive (range, 152-5120 TRU/mL). Patients positive for NAb were tested after cessation of interferon beta therapy with a median interval of 25 months (interquartile range, 10-51 months). No differences were found with respect to age at onset, sex, disease duration, duration of interferon beta treatment, EDSS at start of interferon beta treatment, and MS subtype (Table 1). Patients using 22 or 44 µg of subcutaneous interferon beta-1a (Rebif) 3 times weekly were more often persisting NAb titer positive (P = .05) than patients using either interferon beta-1b (Betaferon) or intramuscular interferon beta-1a (Avonex) (Table 1).

Most patients stopped interferon beta treatment because of a disease breakthrough (relapse and/or disability progression) while receiving therapy. Of these 41 patients, 13 (37%) tested positive for NAb after cessation of interferon beta therapy. A smaller group of patients stopped treatment because of adverse effects or because of a desire to become pregnant; 4 of these 26 patients (15%) were positive for persisting NAb. Notably, treatment decisions were made without any knowledge of NAb status during therapy, as NAb measurements were not performed in our clinic during that period. After interferon beta withdrawal, 13 patients (18%) switched to glatiramer acetate (Copaxone; Teva Pharmaceutical Industries Ltd, Petach Tikva, Israel), 13 (18%) to natalizumab (Tysabri; Elan Pharmaceuticals Inc, Dublin, Ireland) and 10 (14%) patients received courses of intravenous mitoxantrone dihydrochloride (Mitoxantrone; EBEWE Pharma GmbH, Unterach, Austria). Forty-nine percent (35 of 71) of patients remained untreated during follow-up (Table 2). Some patients who stopped receiving interferon beta treatment because of persisting NAb titer positive patients remained unchanged (P = .88). When comparing the proportion of patients with an increased relapse rate after cessation of interferon beta therapy, correcting for the use of mitoxantrone, a higher proportion was found for NAb-positive patients (P = .04; Table 3). Compared with patients without persisting NAb, the risk of an increased relapse rate after interferon beta treatment was almost 5 times higher in patients who were positive for NAb (odds ratio [OR], 4.55; 95% confidence interval [CI], 1.10-19.23). This was confirmed when analyzing with cut-off values for high titers (>150 TRU/mL; OR, 5.99; 95% CI, 1.21-29.41; P = .03). Of the 11 patients with an increased relapse rate, 5 of 11 (45%) were NAb positive. Patients positive for NAb who did not show this increase in relapse rate were more frequently treated with mitoxantrone (6 of the remaining 12 NAb-positive patients).

In both NAb status groups, approximately half of the patients reached an EDSS score of 6.0 at the time of study assessment (Table 3). Cox regression analysis, correcting for EDSS at the start of interferon beta treatment, showed that patients positive for persisting NAb after interferon beta treatment withdrawal progress faster than patients who are NAb negative (hazard ratio [HR], 2.94; 95% CI, 1.20-7.14; P = .02; Figure). For patients who are high-titer NAb positive, a statistical trend was found (HR, 2.36; 95% CI, 0.86-6.49; P = .10).

COMMENT

Overall, the mean relapse rate after interferon beta treatment cessation was significantly lower than the relapse rate of the pretreatment phase (P = .001). However, within subgroups, only NAb-negative patients had a decrease in relapse rate (P < .001), whereas the relapse rate in NAb-positive patients remained unchanged (P = .88). When comparing the proportion of patients with an increased relapse rate after cessation of interferon beta therapy, correcting for the use of mitoxantrone, a higher proportion was found for NAb-positive patients (P = .04; Table 3). Compared with patients without persisting NAb, the risk of an increased relapse rate after interferon beta treatment was almost 5 times higher in patients who were positive for NAb (odds ratio [OR], 4.55; 95% confidence interval [CI], 1.10-19.23). This was confirmed when analyzing with cut-off values for high titers (>150 TRU/mL; OR, 5.99; 95% CI, 1.21-29.41; P = .03). Of the 11 patients with an increased relapse rate, 5 of 11 (45%) were NAb positive. Patients positive for NAb who did not show this increase in relapse rate were more frequently treated with mitoxantrone (6 of the remaining 12 NAb-positive patients).

In both NAb status groups, approximately half of the patients reached an EDSS score of 6.0 at the time of study assessment (Table 3). Cox regression analysis, correcting for EDSS at the start of interferon beta treatment, showed that patients positive for persisting NAb after interferon beta treatment withdrawal progress faster than patients who are NAb negative (hazard ratio [HR], 2.94; 95% CI, 1.20-7.14; P = .02; Figure). For patients who are high-titer NAb positive, a statistical trend was found (HR, 2.36; 95% CI, 0.86-6.49; P = .10).

**PERSISTING NAb AND CLINICAL OUTCOME MEASURES**

**PERSISTING NAb AFTER CESSION OF INTERFERON BETA THERAPY**

Seventeen patients (24%) were found to be NAb positive (NAb titer median, 320 TRU/mL; range, 22-5120 TRU/mL); 11 of these 17 patients were high-titer positive (range, 152-5120 TRU/mL). Patients positive for NAb were tested after cessation of interferon beta therapy with a median interval of 25 months (interquartile range, 10-51 months). No differences were found with respect to age at onset, sex, disease duration, duration of interferon beta treatment, EDSS at start of interferon beta treatment, and MS subtype (Table 1). Patients using 22 or 44 µg of subcutaneous interferon beta-1a (Rebif) 3 times weekly were more often persisting NAb titer positive (P = .05) than patients using either interferon beta-1b (Betaferon) or intramuscular interferon beta-1a (Avonex) (Table 1).

**PERSISTING NAb AND DISEASE-MODIFYING TREATMENT AFTER INTERFERON BETA TREATMENT**

Most patients stopped interferon beta treatment because of a disease breakthrough (relapse and/or disability progression) while receiving therapy. Of these 41 patients, 13 (37%) tested positive for NAb after cessation of interferon beta therapy. A smaller group of patients stopped treatment because of adverse effects or because of a desire to become pregnant; 4 of these 26 patients (15%) were positive for persisting NAb. Notably, treatment decisions were made without any knowledge of NAb status during therapy, as NAb measurements were not performed in our clinic during that period. After interferon beta withdrawal, 13 patients (18%) switched to glatiramer acetate (Copaxone; Teva Pharmaceutical Industries Ltd, Petach Tikva, Israel), 13 (18%) to natalizumab (Tysabri; Elan Pharmaceuticals Inc, Dublin, Ireland) and 10 (14%) patients received courses of intravenous mitoxantrone dihydrochloride (Mitoxantrone; EBEWE Pharma GmbH, Unterach, Austria). Forty-nine percent (35 of 71) of patients remained untreated during follow-up (Table 2). Some patients who stopped receiving interferon beta treatment because of persisting NAb titer positive patients remained unchanged (P = .88). When comparing the proportion of patients with an increased relapse rate after cessation of interferon beta therapy, correcting for the use of mitoxantrone, a higher proportion was found for NAb-positive patients (P = .04; Table 3). Compared with patients without persisting NAb, the risk of an increased relapse rate after interferon beta treatment was almost 5 times higher in patients who were positive for NAb (odds ratio [OR], 4.55; 95% confidence interval [CI], 1.10-19.23). This was confirmed when analyzing with cut-off values for high titers (>150 TRU/mL; OR, 5.99; 95% CI, 1.21-29.41; P = .03). Of the 11 patients with an increased relapse rate, 5 of 11 (45%) were NAb positive. Patients positive for NAb who did not show this increase in relapse rate were more frequently treated with mitoxantrone (6 of the remaining 12 NAb-positive patients).

In both NAb status groups, approximately half of the patients reached an EDSS score of 6.0 at the time of study assessment (Table 3). Cox regression analysis, correcting for EDSS at the start of interferon beta treatment, showed that patients positive for persisting NAb after interferon beta treatment withdrawal progress faster than patients who are NAb negative (hazard ratio [HR], 2.94; 95% CI, 1.20-7.14; P = .02; Figure). For patients who are high-titer NAb positive, a statistical trend was found (HR, 2.36; 95% CI, 0.86-6.49; P = .10).

**COMMENT**

This is the first study reporting that patients with anti–interferon beta NAb that persist after cessation of therapy have a more active disease course. This was indicated by an increase in the annualized relapse rate after interferon beta treatment withdrawal and faster progression to an EDSS score of 6.0. Furthermore, persisting NAb are associated with a different treatment regimen after interferon beta treatment withdrawal; fewer patients remained untreated and more patients switched to second-line treatment, especially mitoxantrone.

Anti–interferon beta NAb develop in a significant proportion of patients with MS who are treated with inter-
neutralizing antibodies (NAb). One suggested approach to treating NAb-positive patients who are taking interferon beta is to use a strategy, as mitoxantrone, a treatment reserved for patients with aggressive inflammatory disease in our center, was prescribed significantly more often to patients who turned out to be positive for persisting NAb. However, it must be noted that most patients who discontinued interferon beta treatment because of perceived efficacy failure were not NAb positive. This suggests that treatment failure is often determined by factors other than the persistence of NAb. In our cohort, most NAb-negative patients were progressing during treatment without superimposed exacerbations and were therefore not eligible for alternative immunomodulatory treatment.

Another interesting finding of our study is that in NAb-positive patients who were treated with mitoxantrone, the immunosuppressive properties of this chemotherapeutic agent were not able to abolish persisting anti-interferon beta NAb. One suggested approach to treating NAb-positive patients who are taking interferon beta has been the induction of combination therapies with strong immunosuppressive compounds like mitoxantrone. Our data suggest that this approach, at least for the use of mitoxantrone, is not very likely to be successful. This is in line with a recent study showing that treatment failure is often determined by factors other than the persistence of NAb. In our cohort, most NAb-negative patients were progressing during treatment without superimposed exacerbations and were therefore not eligible for alternative immunomodulatory treatment.
ing NAb after interferon beta therapy withdrawal with monthly pulsed oral methylprednisolone for 6 months has no beneficial effect on NAb status or interferon beta bioactivity.26

The mechanisms through which persisting NAb exert their effect on MS disease activity are unknown. However, anti–interferon beta antibodies probably have an effect on endogenous interferon pathways that may result in a more proinflammatory modification of the immune system and, subsequently, to an increase in MS disease activity. Alternatively, the tendency to develop and sustain anti–interferon beta NAb might be a reflection of a more active immune system. In our study, patients with persisting NAb were treated somewhat earlier and the pretreatment relapse rate was somewhat higher. This could support the hypothesis that these patients have more active disease owing to a previously more active immune system. However, although this alternative explanation cannot be fully excluded, it must be emphasized that there were no significant differences between NAb-positive and -negative patients for any of the clinical features at baseline, including disability status.

Obviously, the retrospective nature and the small sample of our study do not allow for definite conclusions to be drawn, and causality cannot be proven. On the other hand, for the purpose of this analysis, the retrospective nature also provides some strength because it guarantees that all clinical assessments and treatment decisions were not influenced by knowledge of NAb status. Altogether, our findings suggest that NAb that persist after treatment discontinuation may negatively influence the subsequent course of the disease and may require more aggressive treatment. Systematic long-term follow-up of patients exposed to NAb titers that persist after termination of interferon beta therapy is lacking and, given the possible effect on the MS disease course, conclusive studies on persisting anti–interferon beta NAb are warranted.

Accepted for Publication: April 24, 2009.
Published Online: February 8, 2010 (doi:10.1001/archneurol.2010.21).
Correspondence: Laura F. van der Voort, Department of Neurology, VU Medical Center, de Boelelaan 1117, PO Box 7057, 1007 MB Amsterdam (laura.vandervoort@vumc.nl).
Author Contributions: Study concept and design: van der Voort, Knol, Polman, and Killestein. Acquisition of data: van der Voort, Gilli, and Polman. Analysis and interpretation of data: van der Voort, Gilli, Bertolotto, Knol, Uitdehaag, Polman, and Killestein. Drafting of the manuscript: van der Voort, Gilli, Knol, and Polman. Critical revision of the manuscript for important intellectual content: van der Voort, Gilli, Bertolotto, Knol, Uitdehaag, Polman, and Killestein. Statistical analysis: van der Voort, Knol, and Uitdehaag. Obtained funding: Bertolotto and Killestein. Administrative, technical, and material support: van der Voort and Gilli. Study supervision: Gilli, Knol, Polman, and Killestein.
Financial Disclosure: Drs van der Voort, Killestein, and Uitdehaag report being involved in clinical trials of companies that market drugs for multiple sclerosis (Schering AG, Biogen Idec, Serono, and Teva) and working with companies that have development programs for future drugs for multiple sclerosis. Dr Polman reports having received consulting fees from Actelion, Biogen Idec, Bayer Schering, Teva, Merck Serono, Novartis, GlaxoSmithKline, Union Chimique Belge, Roche, and Antisense Therapeutics; lecture fees from Biogen Idec, Schering AG, Novartis, and Teva; and grant support from Biogen Idec, Bayer Schering, GlaxoSmithKline, Novartis, UCB, Merck-Serono, and Teva. Dr Gilli reports being reimbursed by Schering, Merck Serono, Sanofi-Aventis, and Biogen Dompè for attending several conferences, and for receiving fees for speaking by Biogen Dompè and Biogen Idec. Dr Bertolotto reports being reimbursed by Schering, Merck Serono, Sanofi-Aventis, and Biogen Dompè for attending several conferences, receiving fees for speaking by Biogen Dompè and Biogen, and receiving funds for research and for members of the staff by Merck Serono, Schering, Sanofi-Aventis, and Biogen Dompè. Dr Knol reports no conflicts of interest.

Funding/Support: This study was supported in part by Neutralizing Antibodies in Multiple Sclerosis, a specific targeted research project on neutralizing antibodies to interferon beta in multiple sclerosis, established by the European Commission under its 6th Framework Programme (contract No. 018926).

Role of the Sponsor: The Neutralizing Antibodies in Multiple Sclerosis group played no role in study design, collection of data, analysis, interpretation, writing of the article, or the decision to submit.

Additional Contributions: We would like to thank all participating patients for their cooperation. Furthermore, we thank Marzia Caldano, MPH, of ‘Centro di Riferimento Regionale Sclerosi Multipla e Neurobiologia Clinica’ in Orbassano for performing the neutralizing antibody assays of this study.

REFERENCES


