Neutralizing antibodies against interferon-beta

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Abstract: The development of neutralizing antibodies (NAbs) is a major problem in multiple sclerosis (MS) patients treated with interferon-beta (IFN-\(\beta\)). Whereas binding antibodies (BAbs) can be demonstrated in the vast majority of patients, only a smaller proportion of patients develop NAbs. The principle in NAb in vitro assays is the utilization of cultured cell lines that are responsive to IFN-\(\beta\). The cytopathic effect (CPE) assay measures the capacity of NAbs to neutralize IFN-\(\beta\)'s protective effect on cells challenged with virus and the MxA induction assay measures the ability of NAbs to reduce the IFN-\(\beta\)-induced expression of MxA, either at the mRNA or the protein level. A titer of \(\geq 20\) neutralizing units/ml traditionally defines NAb positivity. NAbs in high titers completely abrogate the in vivo response to IFN-\(\beta\), whereas the effect of low and intermediate titers is unpredictable. As clinically important NAbs appear only after 9–18 months IFN-\(\beta\) therapy, short-term studies of two years or less are unsuitable for evaluation of clinical NAb effects. All long-term trials of three years or more concordantly show evidence of a detrimental effect of NAbs on relapses, disease activity on MRI, or on disease progression. Persistent high titers of NAbs indicate an abrogation of the biological response and, hence, absence of therapeutic efficacy, and this observation should lead to a change of therapy. As low and medium titers are ambiguous treatment decisions in patients with low NAb titres should be guided by determination of in vivo mRNA MxA induction and clinical disease activity.

Keywords: multiple sclerosis, interferon-beta, binding antibodies, neutralizing antibodies, anti-interferon antibodies, bioactivity

Introduction

Interferon-beta (IFN-\(\beta\)) preparations are, like other protein-based biopharmaceuticals produced by recombinant gene technologies, potentially immunogenic. Antibodies against IFN-\(\beta\) develop as result of breakdown of the immune tolerance associated with presentation of the self-antigen in a repetitive way [Schellekens, 2002]. Antibodies to IFN-\(\beta\) can weaken or abrogate the cellular response to IFN-\(\beta\) and neutralize the therapeutic effect of IFN-\(\beta\); consequently these antibodies are named neutralizing antibodies (NAbs) [Sorensen et al. 2003; Ross et al. 2000].

The detrimental effects of NAbs on the clinical response to IFN-\(\beta\) in multiple sclerosis (MS) patients have been recognized even from the first pivotal study of IFN-\(\beta\)-1b [Duquette et al. 1993], and it might therefore be hard to understand the long-lasting controversies about whether NAbs do neutralize the effect of IFN-\(\beta\) in MS.

Today, consensus has been reached about the existence of NAbs and their ability to reduce the bioavailability of IFN-\(\beta\) [Fox et al. 2007; Namaka et al. 2006; Sorensen et al. 2005a]. However, it is still debated when measurements of NAbs should be performed in daily practice, how the results of NAb testing should be interpreted, and how NAb-positive patients should be managed [Fox et al. 2007; Noronha, 2007; Oger and Gibbs, 2006; Giovannoni and Goodman, 2005; Sorensen et al. 2005a].

The difference in opinion is mainly a transatlantic disagreement based on the availability of NAb testing and the experience of dealing with NAb-positive patients. Whereas measurements of NAbs and use of NAb measurement results for several years have been a part of daily clinical
practice in many European MS clinics, this has with a few exceptions not been the case in North America.

The disparity in opinions is reflected by the differences between the European Guidelines on use of anti-IFN-β antibody measurements in multiple sclerosis, produced by an European Federation of Neurological Societies Task Force [Sorensen et al. 2005a] and the American Academy of Neurology report on NAbs to IFN-β and assessment of their clinical and radiographic impact, produced by a working group under the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology [Goodin et al. 2007a].

In the working group established by the subcommittee of the American Academy of Neurology, no consensus could be reached and the two European members of the task force were unable to sign the final edition of the report and had to leave the working group and produce a letter of dissent [Sorensen and Bertolotto, 2007].

The European guidelines recommended: (1) that tests for the presence of NAbs should be performed during the first 24 months of therapy (Level A), (2) that measurements should be repeated in patient with NAbs, and (3) that therapy with IFN-β should be discontinued in patients with high titers of NAbs sustained at repeated measurements with 3–6 months intervals (Level A) [Sorensen et al. 2005a].

The North American report concluded: (1) that treatment of MS patients with IFN-β is associated with the production of NAbs (Level A), (2) that it is very probable that the presence of NAbs is associated with a reduction in the radiographic and, to a lesser extent, the clinical effectiveness of IFN-β treatment (Level B), and (3) that although the finding of sustained high-titer NAbs (>100 neutralizing units (Nu)/ml) is associated with a reduction in the therapeutic effects of IFN-β on clinical and radiographic measures of MS disease activity, there is insufficient information on the utilization of NAb testing to provide specific recommendations regarding when to test, which test to use, how many tests are necessary, or which cut-off titer to apply (Level U) [Goodin et al. 2007a].

Although the conclusions seem divergent, the premises on which the conclusions rest are not very different. One reason for the different levels of recommendation is that the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology applied definitions from randomized therapeutic trials on NAb studies and, as it is not possible to randomize for NAb status, a level A recommendation is not achievable for NAb studies as such studies will never be classified as class I evidence independently of the quality and results of the study. Further, the lack of acquaintance with the use of NAbs in the management of patients treated with IFN-β may explain the rather vague North American recommendations on the use of NAb measurements. It is a fact that whereas European neurologists have ready access to NAb testing this is not the case in most places in the United States.

The present paper reviews our current knowledge of NAbs and takes into consideration new information that fills the gap of uncertainty about the clinical consequences of NAbs and the importance of incorporating NAb measurements in IFN-β-treated MS patients in daily practice.

IFN-β immunogenicity

The reason for the immunogenicity of IFN-β is not known in full detail. It is well recognized that biopharmaceuticals that are recombinant human homologs, like IFN-β, growth factors and hormones, have immunogenetic potentials, even though they may well have the same amino acid sequence as the human molecule [Schellekens, 2002]. Unlike the classic reaction to foreign proteins that produces an immune response after a single administration, antibodies against IFN-β are caused by a breakdown of the immune tolerance to self-antigens that normally exist. The self-antigen has to be presented to the immune system in a repetitive way during several months before the immune tolerance is broken [Schellekens, 2002].

There are several factors that determine whether administration of a recombinant human molecule like IFN-β to a MS patient causes development of NAbs. Some important factors are patient-linked. For example, the propensity to suffer a breakdown of the immune tolerance is genetically determined. Differences in the immune system of patients may also play a role and MS patients with an active immune system may be more prone to produce antibodies than for example cancer patients.
Currently, a promising luciferase assay uses human fibrosarcoma cells, which have been stably transfected with a luciferase reporter gene cassette. When the IFN-\(\beta\) molecule binds to its receptor it activates a transcellular signaling mechanism and causes transcription of the luciferase gene. The amount of luciferase induced can be quantified in terms of luminescent counts per second. In the presence of NAbs the response is blocked [Farrell and Giovannoni, 2007].

**Occurrence of IFN-\(\beta\) antibodies**

Antibodies binding to IFN-\(\beta\) can be detected in the blood already 3–6 months after initiation of IFN-\(\beta\) treatment and are present with different frequencies depending on the IFN-\(\beta\) preparation [Ross et al. 2006, 2000]. Low concentrations of NAbs can also be detected in vitro with sensitive assay after six months [Ross et al. 2000], whereas clinically relevant NAbs usually develop between 9 and 18 months after start of IFN-\(\beta\) therapy (Figure 2).

Patients who have been persistently NAb-negative during the first two years of IFN-\(\beta\) therapy only rarely become NAb-positive [Sorensen et al. 2005b]. NAbs develop faster with IFN-\(\beta\)-1b s.c. than with IFN-\(\beta\)-1a s.c., but after twelve months the proportions of NAb-positive patients treated with IFN-\(\beta\)-1b s.c. and IFN-\(\beta\)-1a s.c were similar [Ross et al. 2006, 2000].

Add-on therapy with methylprednisolone administered monthly intravenously (i.v.) reduced the frequency of patients that had developed NAbs after twelve months, but did not reduce the proportion of NAb-positive patients with titers above 100 NU/ml [Pozzilli et al. 2002]. Combination of IFN-\(\beta\) with an immunosuppressive agent have shown inconsistent results [Calabresi et al. 2002; Fernandez et al. 2002].

**Disappearance of neutralizing antibodies**

In some NAb-positive patients, NAbs may subsequently disappear during continuous therapy with IFN-\(\beta\). Of the two preparations associated with high frequency of NAbs, this happens in a significantly higher proportion of patients treated with IFN-\(\beta\)-1b than in patients treated with IFN-\(\beta\)-1a s.c. [Sorensen et al. 2005b]. Approximately 50% of all NAb-positive patients treated with IFN-\(\beta\)-1b had reverted to NAb-negative status four years after they had become NAb positive [Sorensen et al. 2005b], and after reversion to the NAb-negative state patients regained the full effect of IFN-\(\beta\)-1b therapy with no negative carry-over effect from the previous NAb-positive period [Sorensen et al. 2008].

The literature furthermore shows, that reversion of NAb status largely depends on the titer [Bellomi et al. 2003]. Patients with low NAb titers (<100 NU/ml) are likely to revert to NAb negativity, whereas patients with titers above 500 NU/ml rarely become NAb negative within a time span of 2–3 years [Herndon et al. 2005; Gneiss et al. 2004b; Rice et al. 1999].

No certain explanation exists for the observed difference in the proportion of patients treated with IFN-\(\beta\)-1b and IFN-\(\beta\)-1a s.c that revert to NAb-negative status, although differences in titers may play a role. One hypothesis is that while the breakdown of immune tolerance responsible for development of NAbs seems to occur faster with injection of higher protein amounts with IFN-\(\beta\)-1b s.c., continued treatment with the same high protein load per injection might be more prone to re-establish the immune tolerance. Indeed, an increase in the dose of IFN-\(\beta\)-1b seemed to be followed by an earlier decrease in NAbs [Durelli et al. 2006].

Data from NAb-positive patients who discontinued therapy indicate that NAbs may persist for long periods after cessation of treatment [Petersen et al. 2006]. In a follow-up study of 29 IFN-\(\beta\)-treated patients, who were NAb positive at termination of therapy, only three patients reverted to a NAb-negative status during a mean follow-up time of 22 months. Of these,

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**Figure 2.** Dynamics and clinical effects of neutralizing antibodies (NAbs) by time during treatment with interferon-\(\beta\) (IFN-\(\beta\)).
Currently, a promising luciferase assay uses human fibrosarcoma cells, which have been stably transfected with a luciferase reporter gene cassette. When the IFN-β molecule binds to its receptor it activates a transcellular signaling mechanism and causes transcription of the luciferase gene. The amount of luciferase induced can be quantified in terms of luminescent counts per second. In the presence of NAbs the response is blocked [Farrell and Giovannoni, 2007].

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The literature furthermore shows, that reversion of NAb status largely depends on the titer [Bellomi et al. 2003]. Patients with low NAb titers (<100 NU/ml) are likely to revert to NAb negativity, whereas patients with titers above 500 NU/ml rarely become NAb negative within a time span of 2–3 years [Herndon et al. 2005; Gneiss et al. 2004b; Rice et al. 1999].

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Data from NAb-positive patients who discontinued therapy indicate that NAbs may persist for long periods after cessation of treatment [Petersen et al. 2006]. In a follow-up study of 29 IFN-β-treated patients, who were NAb positive at termination of therapy, only three patients reverted to a NAb-negative status during a mean follow-up time of 22 months. Of these,
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[Duquette et al. 1993]. Applying the ‘anytime positive, always positive’ method for defining NAb positivity, 11% in the placebo group, 47% in the 1.6 MIU IFN-β-1b group, and 45% in the 8 MIU IFN-β-1b group developed NABs. The annual relapse rate was significantly higher in NAB-positive patients compared with NAB-negative patients ($p < 0.001$). In fact, during the period from 13 to 36 months after start of treatment the relapse rate was 0.56 in NAB-negative patients compared to 1.08 in NAB-positive patients treated with 8 MIU, the latter not different from the relapse rate of 1.06 in placebo-treated patients. A re-analysis of the study was performed using both the ‘once positive, always positive’ definition and the ‘interval analysis’ method and although the difference in relapse rates between NAB-positive and NAB-negative patients were diminished the analysis still showed higher relapse rates in the NAB-positive compared to the NAB-negative patients [Petkau et al. 2004; Duquette et al. 1996].

In the pivotal phase III placebo-controlled trial of IFN-β-1a i.m. 30 μg weekly for up to two years, there was no significant difference in relapse rate and progression between NAB-positive and NAB-negative patients [Jacobs et al. 2000; Rudick et al. 1998].

The PRISMS study compared IFN-β-1a 22 μg, 44 μg or placebo three times weekly for two years in 560 patients [PRISMS Study Group, 1998]. NAB-positive patients were defined using the ‘anytime positive, always positive’ method. No significant differences in the relapse rate or progression were seen over the two years study duration between NAB-positive and NAB-negative patients. However, in the extension phase of this study, PRISMS-4, NABs caused a clear reduction in the efficacy on relapses in the third and the fourth year [PRISMS Study Group, 2001]. A re-analysis employing both the ‘anytime positive, always positive’ method and the ‘interval analysis’ method confirmed the negative effects of NAB development [Francis et al. 2005].

The European secondary progressive MS study comprised 718 patients treated for three years with either IFN-β-1b 8 MIU s.c. every other day or placebo. Using the ‘once positive, always positive’ method NAB positive patients had a significant 45% increase in relapse rates ($p = 0.009$), but only a marginal significant effect ($p = 0.07$) of increase when the ‘interval analysis’ method was applied. Higher titers seemed to reduce the treatment effect more [Polman et al. 2003b].

The North American Placebo Control Randomized Study of IFN-β-1b in secondary progressive MS patients for three years showed a significant higher relapse rate in NAB-positive patients [Panitch et al. 2004].

The SPECTRIMS study of IFN-β-1a s.c. was the only study in secondary progressive MS that did not show a statistically significant impact of NABs on the relapse rate [SPECTRIMS, 2001]. A study of comparing IFN-β-1a i.m. 30 μg vs 60 μg once weekly for four years showed that NAB-positive patients had significantly higher relapse rate compared to NAB-negative patients ($p = 0.04$) [Kappos et al. 2005]. The INCOMIN study, an open randomized study that compared IFN-β-1b 8 MIU s.c. every other day with IFN-β-1a 30 μg i.m. weekly did not include a comparison between NAB-positive and NAB-negative patients in the IFN-β-1b arm.
An unselected comparative trial comprising a large sample of all Danish patients treated with an IFN-\(\beta\) preparation comprised 541 patients with relapsing remitting MS. Testing of NAbs was performed blindly without routinely reporting the results of NAb tests to the treating physicians. Patients were followed for up to 60 months and the effect of NAbs was assessed using the ‘interval analysis’ method. In NAb-positive periods the annual relapse rate increased more than 50% compared with NAb-negative periods, and the time to the first relapse and the proportion of relapse-free patients were significantly lower in NAb-negative patients [Sorensen et al. 2003].

In a retrospective trial 262 patients had been treated with an IFN-\(\beta\) preparation for more than three years. During the first two years of treatment, the relapse rate appeared to be unaffected by the subsequent NAb status. However, the relapse rates in the NAb-positive patients were significantly greater than in the NAb-negative patients during years three \((p<0.010)\) and four \((p<0.027)\) [Boz et al. 2007].

In a study based on rates of NAb-positive patients in tests submitted to laboratories in Australia, Europe and North America it was concluded that NAb were not an important factor in worsening of disease. In Australia where testing was mandatory for all patients, 37% of all tests were positive, whereas rates were lower in Europe (27.6%) and North America (21.3%) where testing was discretionary. Without having access to all relevant clinical data the authors assumed that all discretionary testing was prompted by treatment failure, which is a scientifically inadmissible conclusion [Goodin et al. 2007b].

**Effect of NAbs on disease progression**

None of the pivotal trials in relapsing–remitting MS showed an effect of NAbs on disease progression and neither did any of the trials in secondary progressive MS [Polman et al. 2003c; SPECTRIMS, 2001; PRISMS Study Group, 1998; Rudick et al. 1998; Duquette et al. 1996; Jacobs et al. 1996]. However, all the trials were underpowered to show an effect of NAbs because IFN-\(\beta\) by itself had no or only marginal effect on disease progression. Also the Danish study showed only a trend toward more progression in NAb-positive patients [Sorensen et al. 2003].

The IFN-\(\beta\)-1a i.m. 30\(\mu\)g vs 60\(\mu\)g dose comparison study with a duration of four years showed the negative effect of NAbs on disease progression. Despite that only 1.8% of patients receiving 30\(\mu\)g and 4.8% of patients receiving 60\(\mu\)g IFN-\(\beta\)-1a became NAb positive, NAb positive patients had a higher rate of mean change in expanded disability status scale (EDSS) from baseline to month 48 compared with NAb-negative patients \((p=0.01)\) [Kappos et al. 2005].

In a study comparing IFN-\(\beta\)-1a i.m. 30\(\mu\)g weekly and IFN-\(\beta\)-1a s.c. 44\(\mu\)g three times weekly for up to five years significantly more NAb-positive patients compared with NAb-negative patients had disability progression [Minagara and Murray, 2008].

*The presence of NAbs was associated with a higher risk of developing disability during the subsequent five years in a long-term follow-up study of 68 patients receiving IFN-\(\beta\) [Tomassini et al. 2006], and in a study of 78 patients followed for three years a higher proportion of 13 persistently NAb-positive patients worsened one or more points on EDSS compared with NAb-negative patients \((p=0.013)\) [Malacchi et al. 2004].*

**Effect of NAbs on disease activity measured on magnetic resonance imaging (MRI)**

All trials have shown an effect of NAbs on disease activity, measured on MRI as gadolinium positive lesions or new T2-lesions, or MRI disease severity measured as T2-lesion load (Table 1).

In the pivotal trial of IFN-\(\beta\)-1b s.c. the mean number of new and enlarging T2-lesions was significantly larger in NAb-positive compared with NAb-negative patients [Paty and Li, 1993].

*The pivotal phase III placebo-controlled trial of IFN-\(\beta\)-1a i.m. reported a strong trend towards more gadolinium-enhancing lesion in NAb-positive patients \((p=0.062)\) [Rudick et al. 1998; Jacobs et al. 1996].*

A negative effect of NAbs could be observed in some MRI endpoints in the two-year PRISMS study [PRISMS Study Group, 1998] and NAbs caused a clear reduction in efficacy in MRI endpoints in the third and the fourth year [PRISMS Study Group, 2001].

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A negative effect of NABs could be observed in some MRI endpoints in the two-year PRISMS study [PRISMS Study Group, 1998], and NABs caused a clear reduction in efficacy in MRI endpoints in the third and the fourth year [PRISMS Study Group, 2001].

In the European secondary progressive MS study, NAB-positive patients showed a higher...
Although 48 weeks was far too short to provide an estimate of the negative effects of NAbs, the EVIDENCE trial comparing IFN-β-1a 44 μg s.c. with IFN-β-1a 30 μg i.m. showed higher MRI activity in NAb-positive compared to NAb-negative patients [Panitch et al. 2002].

The comparative study of IFN-β-1a i.m. 30 μg vs 60 μg once weekly for four years showed that disease activity measured on MRI either as gadolinium-positive lesions or new T2-lesions was negatively affected by NAbs [Kappos et al. 2005].

A three-year open follow-up study of 30 patients treated with IFN-β-1b i.m. for relapsing–remitting MS showed that NAb-positive patients had significantly more gadolinium-positive lesions and a higher T2-lesion load compared to NAb-negative patients [Frank et al. 2004].

Implications for clinical practice

Today, evidence of the detrimental effects of NAbs has accumulated and it is generally acknowledged that NAbs reduce the biological and clinical efficacy of IFN-β. Patients with NAbs are likely to have lost the beneficial effect of IFN-β due to antibody-mediated decreased bioactivity, and therefore these patients are likely to become IFN-β nonresponders. Hence, awareness of the clinical consequences of NAbs should be incorporated into clinical practice.

The ultimate goal to overcome the problems with NAbs would be development of IFN-β preparations with very low immunogenicity. However, an adequate follow-up of IFN-β-treated patients with measurements of NAbs and in vivo IFN-β bioactivity can prevent disabling disease activity due to antibody-mediated decreased bioactivity.

As virtually all NAb-positive patients develop NAbs before 24 months on therapy it seems reasonable to screen a patient for NAbs after 12 and 24 months treatment with IFN-β. In patients who have been NAb-negative at 12 and 24 months, measurements of NAbs can be discontinued but should be resumed in the case of a relapse. Patients who develop NAbs should be followed with repeated NAb measurements.

It is not known exactly at which NAb titer antibody-mediated decreased bioactivity becomes significant and it is not known how much the bioactivity should be decreased before all beneficial effects of IFN-β are abrogated. However, there is substantial evidence indicating that high titers (>200 NU/ml with IFN-β-1b and >500 NU/ml with IFN-β-1a) are associated with abrogation or profound reduction in the effect of IFN-β. Persistent high titers should imply discontinuation of IFN-β therapy. A commonly adduced argument is that even in patients with persistently high NAbs titers, treatment decisions should be guided by disease activity and not by NAbs titers and, if NAbs-positive patients are doing well, there is no need for change of therapy. This is, however, a misperception. Patients with high titers do not any longer receive a biologically active MS therapy and IFN-β should be discontinued irrespective of the disease activity. At best, continued therapy is a waste of money and at worst, patients may experience a severely disabling relapse. The course of MS is unpredictable and even untreated patients may do well for long periods. Further, NAbs are only one among other causes of failure to IFN-β therapy. The response to IFN-β therapy is heterogeneous. Some patients are constitutive nonresponders and fail IFN-β therapy with absence of NAbs. Hence, some patients with high titers of NAbs will do well because they have a benign course while other NAbs-negative patients will experience severe disease activity [Chiu et al. 2007]. However, such observations do not change the fact that with high NAbs titers the therapeutic response to IFN-β is abolished.

Another argument for continued IFN-β therapy in NAb-positive patients has been that patients may revert to the NAb-negative state during continued therapy with IFN-β. However, patients with high titers usually remain NAb-positive for several years [Sorensen et al. 2005b]. As other therapies are available and patients with high titers are without protection from therapy IFN-β should always be discontinued and patients offered an alternative therapy, when NAbs are present persistently in high titres.

For many reasons it is not possible to define a cut-off titer above which NAbs severely reduce or abolish the therapeutic effect of IFN-β. Low and medium titers are ambiguous and their relevance should be checked by measurement of
percentage increase from baseline in T2-lesion volume compared with NAb-negative patients ($p = 0.0004$) [Polman et al. 2003a].

Although 48 weeks was far too short to provide an estimate of the negative effects of NAbs, the EVIDENCE trial comparing IFN-\(\beta\)-1a 44 \(\mu\)g s.c. with IFN-\(\beta\)-1a 30 \(\mu\)g i.m. showed higher MRI activity in NAb-positive compared to NAb-negative patients [Panitch et al. 2002].

The comparative study of IFN-\(\beta\)-1a i.m. 30 \(\mu\)g vs 60 \(\mu\)g once weekly for four years showed that disease activity measured on MRI either as gadolinium-positive lesions or new T2-lesions was negatively affected by NAbs [Kappos et al. 2005].

A three-year open follow-up study of 30 patients treated with IFN-\(\beta\)-1b i.m. for relapsing-remitting MS showed that NAb-positive patients had significantly more gadolinium-positive lesions and a higher T2-lesion load compared to NAb-negative patients [Frank et al. 2004].

**Implications for clinical practice**

Today, evidence of the detrimental effects of NAbs has accumulated and it is generally acknowledged that NAbs reduce the biological and clinical efficacy of IFN-\(\beta\). Patients with NAbs are likely to have lost the beneficial effect of IFN-\(\beta\) due to antibody-mediated decreased bioactivity, and therefore these patients are likely to become IFN-\(\beta\) nonresponders. Hence, awareness of the clinical consequences of NAbs should be incorporated into clinical practice.

The ultimate goal to overcome the problems with NAbs would be development of IFN-\(\beta\) preparations with very low immunogenicity. However, an adequate follow-up of IFN-\(\beta\)-treated patients with measurements of NAbs and *in vivo* IFN-\(\beta\) bioactivity can prevent disabling disease activity due to antibody-mediated decreased bioactivity.

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Binding antibodies: Vancouver’s perspective

J Oger and E Gibbs

Binding antibodies (BAbs) and neutralizing antibodies (NAbs) develop following the use of interferon beta (IFNβ) in patients with relapsing-remitting multiple sclerosis (RRMS). The appearance of anti-IFNβ antibodies has been associated with reduction of the therapeutic efficacy of IFNβ therapy; however, while BAbS and NAbs arise from exposure to IFNβ, they have different characteristics and different impacts on clinical outcomes. Not all patients develop BAbS and NAbs, and patients who do may revert to seronegative status for each of these antibodies, even with continued IFNβ treatment. This review examines the potential clinical and biological effect of BAbS and NAbs on therapeutic efficacy in the treatment of RRMS. Multiple Sclerosis 2007; 13:S36–S43. http://msj.sagepub.com

Key words: Multiple sclerosis, antibodies, binding antibodies, neutralising antibodies, Interferons, anti-interferons

Introduction

Interferon beta-1a (IFNβ-1a) and interferon beta-1b (IFNβ-1b) have some influence albeit limited on the course of multiple sclerosis (MS). However, a potential consequence of IFNβ therapy, as with other protein therapies, is the development of binding antibodies (BAbs) and neutralizing antibodies (NAbs). Both BAbS and NAbs bind to the IFNβ molecule. BAbS may bind to the IFNβ molecule at a variety of locations, and some of these interactions result in blocking interaction with the receptor resulting in NAbs [1,2]. Thus, NAbs appear to be a subset of BAbS. Through steric hindrance, high affinity, and possibly other mechanisms as yet unknown NAbs may prevent the IFNβ molecule from interacting with the interferon receptor, thereby decreasing the bioavailability of the therapeutic agent [1–3]. Some other mechanisms such as the presence of soluble receptors and interferon inhibitory activity may, however result in reduced activity of the interferons without NAbs being present [4]. The biological and clinical meaningfulness of BAb induction still needs to be defined with more precision, but the presence of NAbs has been associated with a reduction in the clinical effectiveness of IFNβ and a decrease in IFN-related side effects (fever, chills and myalgia) [5]. The overall result of BAb and NAb induction is therefore a reduction of the therapeutic response to IFNβ treatment. The purpose of this review is to discuss the clinical and biological effects that BAbS and NAbs have on the therapeutic efficacy of IFNβ in the treatment of relapsing-remitting MS (RRMS).

Determining the influence of BAbS and NAbs in patients with MS can be accomplished first by assaying for the presence of either or both anti-IFNβ antibodies, and by measuring the changes in the expression of specific pharmacologic markers that serve as indicators of IFNβ receptor activation [6]. Assays for BAb are relatively inexpensive but can generate a high level of false positives. False positives occur when the results suggest a high level of binding activity without evidence of NAb activity or loss of IFNβ bioactivity; false −, however are rare. Assays for BAb activity are often used as a screen for identifying patients with possible NAb activity [2]. The enzyme-linked immunosorbent assay (ELISA), the radioimmunoprecipitation assay (RIPA), and a column-based assay are common tests for determining BAb activity. The measurement of NAbs involves determining the antibody levels that will result in a reduced biological activity resulting from IFNβ receptor blockade (neutralization assays). Neutralization assays include the cytopathic effect (CPE) and myxovirus resistance protein A (MxA) assays. More recently a Luciferase reporter gene based assay has been developed [7]. As useful as these techniques can be in measuring the impact of BAb and NAb development, a consensus on how best to

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10.1177/1352458507076989
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interpret their results has not been reached. And there is no clear method to establishing a common titre for quantifying a NAb result or appreciating what high levels represent.

The biology of BAbs and NAbs

Determining whether NAbs and BAbs do indeed abrogate the clinical effects of IFNβ treatment in MS patients is challenging for a number of reasons. Several studies have examined the effect of NAbs, but there has not been a consistent approach to their evaluation. In addition, there is some evidence that production of NAbs and BAbs can cease despite continuous treatment with interferon [8]. In general, most studies have focused on the impact of NAbs on pharmacologic and clinical outcomes. Comparatively little attention has been placed on BAbs and their influence on IFNβ therapy, perhaps because of the difference in the molecular nature of the interaction of BAbs and NAbs with IFNβ; however, although BAbs may not directly affect IFNβ bioavailability, they may provide important clues for predicting clinical outcomes of IFNβ therapy in patients with RRMS.

The appearance of BAbs and NAbs follows a specific sequential development. The development of BAbs precedes that of NAbs, and both can often be detected in the same patient after some delay. BAbs also can be detected in patients who never express NAbs. NAbs, however, do not appear in patients who test − for Bab (BAb−) [9]. In a preliminary study, Gibbs et al. examined the time course of BAb development in MS patients treated with IFNβ-1b (Betaseron®) [10]. They showed that Bab levels could be detected as early as one to three months after initiation of treatment (Figure 1). This group also evaluated the difference in time course for Bab versus NAb expression (Figure 2). The expression of BAbs peaked at about four to six months, while the peak in NAb expression occurred at 13–18 months after treatment with IFNβ-1b began [10]. Perini and colleagues recently showed that of the total number of patients treated with one of three IFNβ preparations (IFNβ-1b 8 MIU subcutaneously [SC] every other day; IFNβ-1a 30 μg intramuscularly [IM] once weekly [QW]; or IFNβ-1a SC 22 μg three times weekly [TIW]), approximately 60% tested + for Bab (BAb+) after only three months of treatment [11]. In their study, in patients receiving IFNβ-1b, Bab levels peaked by the end of the first year and plateaued during the second year. The magnitude of the development of either Bab or NAb was dependent on the therapeutic agent used: anti-IFNβ antibody levels in patients treated with IM IFNβ-1a were significantly lower than those observed in patients treated with IFNβ-1b (P < 0.01). Perini and colleagues also showed that NAbs began to appear approximately six months after treatment initiation, and that nearly 40% of the patients treated tested + for NAb (NAb+) after 12 months of treatment. Both antibody concentrations peaked about 15 months after IFNβ treatment was started (Figure 3). Francis and colleagues reported that 60% of the patients receiving IFNβ-1a SC who developed BAbs eventually tested NAb+, and that NAb development tended to occur within 12

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![Figure 1](http://msj.sagepub.com)  
**Figure 1** Time course of binding antibody (BAb) expression in interferon beta-1b (IFNβ-1b; Betaseron®)-treated patients (n = 92) [9].

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Surprisingly, this proportion was slightly higher in patients receiving 22 μg IFNβ-1a TIW (67%) than in patients treated with 44 μg IFNβ-1a TIW (52%) there is currently no clear explanation for the difference in dose effect, and in our hands this difference is not clear.

In the above studies, the percentage of patients remaining BAAb or NAAb declined and stabilized at a much lower frequency after peak levels of both anti-IFNβ antibodies were reached (Figures 1–3). This is consistent with earlier results by Bellomi and colleagues, who showed that after six years of treatment with IFNβ-1a or IFN β-1b, most patients reverted to NAAb− (NAAb−) status [1,2,8]. In addition, patients who had high NAAb titres after two years of treatment remained NAAb+, but the titres reduced significantly ($P < 0.05$), even though treatment was still ongoing. In a study measuring the appearance and disappearance of NAAb, Sorensen and colleagues demonstrated that 34% of patients who became NAAb+ (neutralizing capacity $\geq 20\%$; the percentage of IFNβ that was neutralized due to the presence of NAAb) subsequently became NAAb over a period of 48 months [12]. They also demonstrated that the probability of reverting to NAAb status increased with continued treatment (range: 17–53%). Patients treated with IFNβ-1b reverted to NAAb status significantly more frequently and earlier than patients treated with IFNβ-1a SC ($P = 0.0048$). In the subset of patients with high levels of NAAb (neutralizing capacity $\geq 80\%$), Sorensen found that 23% reverted to NAAb− status over 48 months.
48 months. Although the probability of reverting to NAb− status in these patients also increased over the 48-month trial period (range: 9–42%), it was less than that which was found for the patients with 20% neutralizing capacity. Moreover, patients who remained NAb− during the first 24 months of treatment remained NAb-free for the remainder of the treatment.

Biological significance of BAbs and NAbs

The biological interaction of IFNβ with its receptor results in the up-regulation of several gene products, including neopterin, β2-microglobulin, and MxA [6]. These same byproducts are used to quantify the pharmacologic activity of IFNβ treatment in patients with RRMS. In patients with elevated NAb levels, several studies have shown reductions in the levels of neopterin, β2-microglobulin and MxA induced by IFN injections. In a follow-up study in which patients with RRMS were treated with 22 μg or 44 μg IFNβ-1a (Rebif®) SC TIW, and assessed over a two-year period [5], after 12 months of treatment, NAb+ patients showed lower mean levels of both neopterin and β2-microglobulin than did NAb− patients and patients receiving placebo (Figure 4). Mean neopterin values were significantly higher (P < 0.0001) in NAb− patients (mean increase 93% between 22 μg or 44 μg treatments) compared with NAb+ patients (mean increase 93% between 22 μg or 44 μg treatments). An earlier study by Cook and colleagues also showed that lower neopterin levels resulted with elevated NAb levels [13]. Indeed the presence of NAbs in these patients abolished the statistical difference between IFNβ-1a (Avonex®) and IFNβ-1b (Betaseron®) treatments (IFNβ-1a [Rebif®] was not included in this study). The development of NAbs is also associated with reduced levels of the MxA protein. Vallittu et al. showed that the development of NAbs correlated with a reduction in MxA expression (P < 0.001) [14]. More recently, Pachner and colleagues reported that in patients (n = 19) with mean NAb values between 20 and 150NU, MxA levels were 66% lower compared with those of NAb− patients [6]. In patients with values > 150NU (n = 9) MxA levels were 83% lower than those of NAb− patients. Interestingly, these investigators also discovered that MxA levels recovered following a substantial decrease in NAb levels [6,15]. Bertolotto and colleagues reported dramatic reductions in MxA levels in NAb+ patients [16]. In their study, the MxA values from NAb+ patients were approximately 78% lower than in NAb− patients. The mean (±SD) MxA messenger RNA (mRNA) expression level for the NAb− patients was 465 ± 250 fg MxA/pg GAPDH (glyceraldehyde-3-phosphate dehydrogenase) versus 102 ± 81 fg MxA/pg GAPDH for persistently NAb+ patients. The difference between the two groups of patients was statistically significant (P < 0.0001).

Antel et al. reported recently a study of the effect of NAbs on IFN regulated chemokine (CXCL-10) and IL-10. They found that in 4 NAbs+ sera the mean induced CXCL-10 level was 27713 ± 2747 pg/mL as opposed to 66059 ± 3936 for the NAb− sera (P < 0.001) [18].
In a study of 32 patients treated for more than one year, Sorensen et al. demonstrated that the normal response of β2 microglobulin to IFN injection was reduced in NAb+ patients [19].

The expression of MxA also appears to be influenced by the presence of BAbs. In preliminary experiments, Gibbs and colleagues demonstrated that MxA expression was reduced in BAb+ patients but remained high in BAb− patients following treatment with IFNβ-1b (Betaseron®) [9]. They also reported that once BAbs had disappeared, MxA protein induction reappeared. Since the incidence of BAbs typically precedes NAb by several months, it will be necessary for these investigators to fully establish the true nature of MxA protein inhibition, and whether it is due to BAbs or NAb. Although these studies provide valuable insights into the magnitude of the effect antibodies have on MxA mRNA expression, the results should be viewed with caution as the biologically relevant rate of MxA increase has not yet been determined [6,20]. It is clear, however, that the presence of NAb decreases the expression of IFNβ induced biological markers and most probably reflects a decrease in the pharmacologic efficacy of IFNβ.

Clinical implications of BAb and NAb expression

Several studies have investigated the influence of BAbs and NAbs on clinical outcomes. While most of these studies have linked the appearance of NAb to reductions in the clinical efficacy of IFNβ treatment, few have demonstrated that this reduction outweighs the continued clinical benefits of IFNβ therapy. It is also important to consider that not all patients develop BAbs or NAbs, and of the patients who do, some revert to seronegative status. Finally, as MS is a progressive, degenerative disease, the majority of patients are likely to experience breakthrough symptoms at some point. However, breakthrough symptoms are not necessarily an indication that the patient’s response to treatment has changed.

In assessing the effect of BAbs and NAbs on the change in IFNβ treatment response, outcomes that are typically included are relapse rate, disability progression, and change in the number of new or enlarging T2 lesions. In an analysis of NAb development and the impact on clinical outcomes from the PRISMS study in patients treated with 22 µg or 44 µg IFNβ-1a (Rebif®) SC, Francis and colleagues showed that during a four-year period, NAb− and NAb+ patients overall had similar relapse rates (0.74 versus 0.82; \( P = 0.98 \)) [4] (Figure 5). When examining years 1–2 and years 3–4 of the study separately, however, the investigators discovered that NAb+ patients had significantly more relapses than did NAb− patients (\( P = 0.004 \)) during years 3 and 4 where the relapse rate continued to decline in NAb− but remained stable in NAb+ patients [21]. Because the mean time to development of NAb was 14.5 ± 6.2 months, the annualized relapse rates were compared across months 12–48. In this study, the annual relapse rate of NAb+ patients was 39% higher than that of NAb− patients (\( P = 0.04 \)). These results are consistent with those of other studies. Sorensen and colleagues showed that NAb+ patients experienced more relapses than did NAb− patients [22]. These authors also showed that the time to first relapse was significantly longer for patients who were NAb− rather than NAb+ at 12 months after beginning IFNβ treatment (605 days versus 361 days, respectively; \( P = 0.009 \)).

The Expanded Disability Status Scale (EDSS) is generally used to measure disability and tracking dis-
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testing should then be done in those patients testing + for BAbs, and should continue for up to 24 months if the patient continues to be BAb+ but remains NAb−. Testing for NAb− is not recommended in BAb− patients, as the likelihood of NAb development is low in the absence of BAbs. This is consistent with the course of testing suggested by Giovannoni and Goodman [24].

Discussion

The data outlined in this and other reviews clearly suggest that the presence of NAb s in patients treated with IFNβ agents influences clinical outcomes. Although some of these studies suggest that the influence of NAb s on IFNβ treatment may be short lived, and that only a percentage of IFNβ-treated patients develop NAb s, it is difficult to measure the true impact of NAb induction on treatment outcomes in patients with RRMS.

MS is a variable disease in which IFNβ has been shown to be of limited efficacy in reducing relapses and MRI activity; however, the criteria for assessing the overall risk of treatment failure, with respect to the development of anti–IFNβ antibodies, have not yet been established. The clinical significance of BAb s and NAb s in the treatment of MS is becoming clearer. Several investigators have demonstrated that the appearance of NAb s results in lower efficacy of IFNβ treatment [5,6,24,25]. Also, as shown by Sorensen and colleagues, patients who do not develop NAb s after 24 months of treatment are unlikely to develop them with further treatment. Future studies are needed to increase understanding of which risk factors may be most important in considering how patients will react to IFNβ therapy. As MS is also a degenerative disease and interferons appear to treat only the inflammatory component, the majority of patients would be expected to have a breakthrough in disease activity at some time, according to Giovannoni [25]; thus, the direct impact of BAb s and NAb s may be impossible to separate from the disease course. The issue then becomes whether reductions in efficacy of IFNβ therapy due to NAb s outweigh the benefits of IFNβ therapy in patients with RRMS. Although many investigations have examined the former, a clear consensus on the latter is still forthcoming. Finally, the reduction in the expression of NAb s over time with continual IFNβ treatment suggests that while NAb s may represent an unwelcome result of IFNβ therapy, it! is possible that the impact of NAb s is only transient, and that some patients may require longer treatment to receive the benefits that NAb− patients enjoy. Long-term studies are required to better understand the dynamics of BAb s and NAb biology. Studies designed to examine these issues will shed additional light on this perplexing biological phenomenon.

References

testing should then be done in those patients testing + for BAbs, and should continue for up to 24 months if the patient continues to be BAb+ but remains NAb−. Testing for NAb is not recommended in BAb− patients, as the likelihood of NAb development is low in the absence of BAbs. This is consistent with the course of testing suggested by Giovannoni and Goodman [24].

Discussion

The data outlined in this and other reviews clearly suggest that the presence of NAb in patients treated with IFNβ agents influences clinical outcomes. Although some of these studies suggest that the influence of NAb on IFNβ treatment may be short lived, and that only a percentage of IFNβ-treated patients develop NAb, it is difficult to measure the true impact of NAb induction on treatment outcomes in patients with RRMS.

MS is a variable disease in which IFNβ has been shown to be of limited efficacy in reducing relapses and MRI activity; however, the criteria for assessing the overall risk of treatment failure, with respect to the development of anti–IFNβ antibodies, have not yet been established. The clinical significance of BAbs and NAb in the treatment of MS is becoming clearer. Several investigators have demonstrated that the appearance of NAb results in lower efficacy of IFNβ treatment [5,6,24,25]. Also, as shown by Sorensen and colleagues, patients who do not develop NAb after 24 months of treatment are unlikely to develop them with further treatment. Future studies are needed to increase understanding of which risk factors may be important in considering how patients will react to IFNβ therapy. As MS is also a degenerative disease and interferons appear to treat only the inflammatory component, the majority of patients would be expected to have a breakthrough in disease activity at some time, according to Giovannoni [25]; thus, the direct impact of BAbs and NAb may be impossible to separate from the disease course. The issue then becomes whether reductions in efficacy of IFNβ therapy due to NAb outweigh the benefits of IFNβ therapy in patients with RRMS. Although many investigations have examined the former, a clear consensus on the latter is still forthcoming. Finally, the reduction in the expression of NAb over time with continual IFNβ treatment suggests that while NAb may represent an unwelcome result of IFNβ therapy, it is possible that the impact of NAb is only transient, and that some patients may require longer treatment to receive the benefits that NAb− patients enjoy. Long-term studies are required to better understand the dynamics of BAb and NAb biology. Studies designed to examine these issues will shed additional light on this perplexing biological phenomenon.

References


Multiple Sclerosis 2007; 13: S36–S43 http://msj.sagepub.com

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anti-IFNβ BAB
anti-interferon beta Binding Antibodies
ELISA

EK-IFNB 96 tests

Revision date: 2006-07-28
β IgG antibodies to therapeutically administered Interferon-β (IFNβ) in human serum (1-10)

**INTENDED USE**

The BUHLMANN anti-IFNβ ELISA kit is intended for the direct and quantitative in vitro diagnostic determination of IgG antibodies to therapeutically administered Interferon-β (IFNβ) in human serum.

**PRINCIPLE OF THE ASSAY**

Serum from Multiple Sclerosis (MS) patients treated with interferon-β and suspected to contain antibodies (Ab) to the substance administered, calibrators, and controls are incubated in microtiter wells coated with a mix of different IFNβ molecules (natural human IFNβ, IFNβ-1a and IFNβ-1b). After removal of unreacted material by washing, a horseradish peroxidase (HRP)-labelled antibody to human IgG is added to the wells.

After a second washing step, the TMB Substrate (tetramethylbenzidine) is added to the wells and color develops in proportion to the amount of anti-IFNβ binding antibodies bound in the initial step. The reaction is terminated by the addition of stop solution and the color absorbance is measured in a microtiter plate reader at a wavelength of 450nm.

**REAGENTS SUPPLIED AND PREPARATION**

<table>
<thead>
<tr>
<th>Reagents</th>
<th>Quantity</th>
<th>Code</th>
<th>Reconstitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microtiter Plate</td>
<td>12 x 8 wells</td>
<td>B-IFNB-MP</td>
<td>Wash twice before use</td>
</tr>
<tr>
<td>Plate Sealer</td>
<td>3 pieces</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wash Buffer Concentrate</td>
<td>1 vial 100 ml</td>
<td>B-IFNB-WB</td>
<td>Dilute with 1:100 ml of deionized water</td>
</tr>
<tr>
<td>Incubation Buffer With preservatives</td>
<td>1 vial 100 ml</td>
<td>B-IFNB-IB</td>
<td>Ready to use</td>
</tr>
<tr>
<td>Calibrators A to D</td>
<td>4 vials 1 ml</td>
<td>B-IFNB-CASET</td>
<td>Reconstitute each vial with 1 ml of Incubation Buffer; vortex</td>
</tr>
<tr>
<td>Control Low / High</td>
<td>2 vials 1 ml</td>
<td>B-IFNB-CONSET</td>
<td>Reconstitute each vial with 1 ml of Incubation buffer; vortex</td>
</tr>
<tr>
<td>Enzyme Label</td>
<td>1 vial 11 ml</td>
<td>B-IFNB-ELG</td>
<td>Ready to use</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>1 vial 11 ml</td>
<td>B-TMB</td>
<td>Ready to use</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>1 vial 11 ml</td>
<td>B-STS</td>
<td>Stable up to 24 hours or until completion of assay.</td>
</tr>
</tbody>
</table>

**MATERIALS REQUIRED BUT NOT PROVIDED**

- Precision pipettes with disposable tips: 20 μl, 100 μl and 1 ml pipettes.
- Disposable polystyrene or polycarbonate tubes for the preparation of sample dilutions.
- 1000 ml cylinder for the dilution of the Wash Buffer Concentrate.
- Microtiter plate washer or squeeze bottle for the Wash Buffer.
- Blotting paper.
- Refrigerator.
- Microtiter plate rotator.
- Microtiter plate reader for the measurement of absorbance at 450 nm.

**SPECIMEN COLLECTION AND STORAGE**

The procedure calls for 20 μl of serum per duplicate determination. Collect blood into plain tubes, avoid hemolysis, mix by inverting sample tube several times and leave to clot for 45 minutes at room temperature (18-28°C) protected from light. Centrifuge at 2000 x g for 15 minutes at room temperature (18-28°C) and collect the serum.

Lipemic, hemolytic, and icteric samples should not be used in this assay. Lipemic samples can be avoided by asking patients to fast for at least 12 hours prior to the sample being taken.

**STORAGE AND SHELF LIFE OF REAGENTS**

**Unopened Reagents**

<table>
<thead>
<tr>
<th></th>
<th>Store at 2-8°C. Do not use past kit expiration date.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microtiter Plate</td>
<td>Return unused strips immediately to the foil pouch containing the desiccant packs and reseal along the entire edge of zip-seal. Store until expiration date at 2-8°C.</td>
</tr>
<tr>
<td>Wash Buffer</td>
<td>Store for up to 2 months at 2-8°C after dilution.</td>
</tr>
<tr>
<td>Calibrators</td>
<td>Store for up to 2 months at -20°C.</td>
</tr>
<tr>
<td>Controls</td>
<td>Store at 2-8°C until expiration date printed on the labels.</td>
</tr>
<tr>
<td>Incubation Buffer</td>
<td>Store at 2-8°C until expiration date printed on the labels.</td>
</tr>
<tr>
<td>Enzyme Label</td>
<td>Stable up to 24 hours or until completion of assay.</td>
</tr>
<tr>
<td>TMB Substrate</td>
<td>Stable up to 24 hours or until completion of assay.</td>
</tr>
<tr>
<td>Stop Solution</td>
<td>Stable at 2-8°C until expiration date printed on the label.</td>
</tr>
</tbody>
</table>

**WARNINGS AND PRECAUTIONS**

The Microtiter Plate (B-IFNB-MB), Calibrators (B-IFNB-CASET) and Controls (B-IFNB-CONSET) of this kit contain components of human origin. Each serum donor unit used in the preparation of the kit components was tested by an FDA approved method and found negative for HBV surface antigen, so as for HCV and HIV/12 antibodies. Although these methods are highly accurate, there is no guarantee that this material cannot transmit Hepatitis or AIDS. Therefore, all patient specimens and kit components should be handled as if capable of transmitting infections. All products containing human source material should be handled in accordance with good laboratory practice using appropriate precautions.

**PROCEDURAL NOTES**

- The enzymatic used as the label is inactivated by oxygen and is highly sensitive to sodium azide, thimerosal, hypochlorous acid and aromatic chlorohydrocarbons often found in laboratory water supplies. Therefore, use only deionized high quality water.
- The sample dilution (1:50) is considered in the concentration specification of the Calibrators. Therefore, the amount of BTU present in the unknown sample can be directly read by interpolation with the standard curve.
- If the initial concentration of an unknown sample is greater than the highest Calibrator, the serum sample should be further diluted with Incubation Buffer and assayed again according to the assay procedure. The additional dilution must be considered when calculating the actual concentration of BTU present in the unknown sample.
- If the microtiter plate reader is not capable of reading absorbance greater than 2 or greater than the absorbance
of the Calibrator A, a second reading at a wavelength of 490 or 492 nm is recommended (reference filter at 600 to 620 nm if available). In this case, proceed to construct a second standard curve with the absorbance readings of all calibrators at 490 or 492 nm. The concentration of the off-scale samples at 450 nm are then read from the new standard curve as described above. The readings at 490 or 492 nm should not replace the on-scale readings at 450 nm.

**ASSAY PROCEDURE**

1. Dilute all patient samples 1:50 with Incubation Buffer (e.g. 20 µl of serum + 980 µl of Incubation Buffer). Allow diluted samples to set for 30 minutes at 18-28°C prior to pipetting in step 4d.

2. Prepare a plate with sufficient strips to test the desired number of Calibrators, Controls and samples. Remove excess strips from the holder and re-seal them in the plastic foil bag containing the desiccant bags without delay. Store refrigerated.

3. Wash the coated strips twice using at least 300 µl of Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper.

4a. Pipet 100 µl of Incubation Buffer in duplicate into the wells A1+A2 as reagent blank.


4c. Pipet 100 µl of Low Control in duplicate into wells F1+F2.

4d. Pipet 100 µl of each diluted sample in duplicate into the subsequent wells.

5. Cover with a Plate Sealer and incubate for 2 hours (± 5 minutes) at 2-8°C.

6. Remove and discard the Plate Sealer. Empty the wells and wash three times using at least 300 µl of Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper.

7. Add 100 µl of Enzyme Label to all wells.

8. Cover the plate with a Plate Sealer and incubate for 2 hours (± 5 minutes) at 2-8°C.

9. Remove and discard the Plate Sealer. Empty the wells and wash three times using at least 300 µl of Wash Buffer per well. Empty the wells and strike the plate firmly onto blotting paper.

**IMPORTANT:** Allow the TMB Substrate Solution to reach 18-28°C before use in step 10.

10. Add 100 µl of the TMB Substrate Solution to each well.

11. Cover the plate with a Plate Sealer, place the plate on a plate mixer set at 800-1000 rpm, protect the plate from direct light and incubate for 30 minutes (± 5 minutes) at 18-28°C.

12. Add 100 µl of Stop Solution to all wells. Remove air bubbles with a pipette tip. Proceed to step 13. within 30 minutes.

13. Read the absorbance at 450 nm in a microtiter plate reader.

**RESULTS AND CALCULATION**

**Standard Curve:** Record the absorbance at 450 nm for each Calibrator and Blank (NSB) well. Average the duplicate values, subtract the average of the blank wells and record the averages (± corrected average absorbance). Plot the corrected average absorbance (vertical axis) versus the Bühlmann Titer Units (BTU) of the standards (horizontal axis) using a lin/log graph paper. Draw the best fitting curve or calculate the standard curve using a smoothed spline fitting algorithm.

**Samples and Controls:** Record the absorbance at 450 nm for each sample and control well. Average the duplicate values, subtract the average of the blank wells and record the averages (± corrected average absorbance). Locate the corrected average absorbance value of the samples and controls on the vertical axis, draw a horizontal line intersecting the standard curve and read the Bühlmann Titer Units (BTU) from the horizontal axis.

**Standardization:** The standards of the anti-IFNβ BAB ELISA kit were calibrated against an internal reference Bühlmann Titer Units (BTU) were established as follows:

- Normal donor samples as well as samples from MS patients before IFNβ treatment were assayed according to the anti-IFNβ BAB ELISA assay procedure (cf. table below).
- Serially diluted samples of the reference pool were assayed in the same run.
- The dilution at which the reference pool falls short of the cut-off of the control samples, mentioned in first step, corresponds to the titer of the reference pool, expressed in Bühlmann Titer Units (BTU).

**QUALITY CONTROL**

- THROUGH UNDERSTANDING OF THIS INSTRUCTION FOR USE IS NECESSARY FOR THE SUCCESSFUL USE OF THE PRODUCT. RELIABLE RESULTS WILL BE OBTAINED ONLY BY USING PRECISE LABORATORY TECHNIQUES (CURRENT GLP GUIDELINES) AND ACCURATELY FOLLOWING THIS INSTRUCTIONS FOR USE.

- SINCE THERE IS NO CONTROL SERUM FOR ANTI-INFβ-BAB COMERCIALY AVAILABLE, WE RECOMMEND USING A POSITIVE SERUM POOL FOR INTERNAL QUALITY CONTROL. THE CONFIDENCE LIMITS FOR THE BÜHLMANN CONTROLS ARE LOT-SPECIFIC AND PRINTED ON THE ADDITIONAL QC DATA SHEET.

- THE REPRODUCIBILITY OF STANDARD CURVE PARAMETERS AND CONTROL VALUES SHOULD BE WITHIN ESTABLISHED LIMITS OF LABORATORY ACCEPTABILITY.

- IF THE PRECISION OF THE ASSAY DOES NOT CORRELATE WITH THE ESTABLISHED LIMITS AND REPETITION EXCLUDES ERRORS IN TECHNIQUE, CHECK THE FOLLOWING ISSUES: I) PIPETTING, TEMPERATURE CONTROLLING AND TIMING DEVICES II) ELISA READER SETTINGS III) EXPIRATION DATES OF REAGENTS IV) STORAGE AND INCUBATION CONDITIONS V) TMB SUBSTRATE SOLUTION SHOULD BE COLORLESS VI) PURITY OF WATER.

**LIMITATIONS**

- THE REAGENTS SUPPLIED WITH THIS KIT ARE OPTIMIZED TO MEASURE ANTIBODIES DIRECTED AGAINST INJECTED INFα, INFβ, INFγ-HUMAN SERUM.

- ANTI-INFβ ANTIBODY TITER VALUE SHOULD BE USED AS SUPPLEMENTARY DATA AVAILABLE TO THE PHYSICIAN IN MONITORING INFα-TREATMENT.

- SERUM TITER VALUES DEPEND ON THE ASSAY METHOD AND, IN PARTICULAR, ON THE SPECIFICITY AND THE CUT-OFF VALUES ESTABLISHED WITH AN ASSAY METHOD. TITER VALUES OBTAINED WITH DIFFERENT ASSAY METHODS CANNOT BE COMPARED DIRECTLY.
Neutralizing antibodies to interferon beta-1a and interferon beta-1b in MS patients are cross-reactive

Omar A. Khan MD
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Department of Neurology, University of Maryland School of Medicine, Neurology & Research Service, Veterans Affairs Medical Center, Baltimore, MD.

Presented in part at the annual meeting of the American Academy of Neurology; Minneapolis, MN; April 25 to May 2, 1998.

Received June 3, 1998.
Accepted in final form August 22, 1998.

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Article abstract--

Objective: To determine whether neutralizing antibodies (NABs) to interferon beta (IFNbeta)-1a (Avonex) and IFNbeta-1b (Betaseron) cross-react. Background: A total of 38% of MS patients treated with IFNbeta-1b and 22% of those treated with IFNbeta-1a were reported to develop NABs, which could reduce the clinical efficacy of the drug. Methods: Blood from 10 MS patients was collected before and at 3 and 6 months after initiating treatment with IFNbeta-1a. ELISA was performed to detect binding antibodies to IFNbeta-1a. Sera from patients who tested positive for binding antibodies to IFNbeta-1a were then screened for NABs to IFNbeta-1a in a biologic assay based on neutralization of antiviral activity. These serum samples were subsequently tested for cross-reactivity with IFNbeta-1b both in the ELISA and the biologic assay. In the second part of the study, sera from patients who participated in the phase III IFNbeta-1b trial at the University of Maryland were examined for cross-reactivity with IFNbeta-1a in the ELISA and the biologic assay. Results: Of the 10 patients treated with IFNbeta-1a, three developed binding as well as NABs to IFNbeta-1a 6 months after treatment, and these antibodies cross-reacted with IFNbeta-1b both in the binding and the biologic assay. Similarly, sera from six patients with NABs to IFNbeta-1b showed cross-reactivity with IFNbeta-1a in the binding assay. Three of these six serum samples tested for neutralizing activity against IFNbeta-1a demonstrated the presence of NABs to IFNbeta-1a. Conclusions: NABs to IFNbeta-1a (Avonex) and IFNbeta-1b (Betaseron) cross-react, both in the binding and the biologic assays. This suggests that switching to alternate IFNbeta preparation in patients who develop NABs may not be clinically beneficial. Studies examining cross-reactivity between NABs to IFNbeta-1a and IFNbeta-1b in a large number of patients are indicated.

Currently, two types of recombinant human interferon beta (IFNbeta) preparations are approved by the Food and Drug Administration for the treatment of relapsing MS in the United States. Recombinant human IFNbeta-1b (Betaseron; Berlex Laboratories, Richmond, VA) administered as 8 million international units (MIU) subcutaneously (SC) on alternate days was approved in 1993 following the results of a pivotal phase III trial demonstrating its efficacy in reducing the frequency of relapses by 34%. Interferon beta-1b is produced as a nonglycosylated molecule in Escherichia coli with a genetically engineered serine substitution at position 17. Recombinant human IFNbeta-1a (Avonex; Biogen Inc., Cambridge, MA) injected intramuscularly at a dose of 6 MIU once a week was approved in 1996 based on the results of a phase III trial demonstrating efficacy by slowing disease progression as well as frequency of relapses by 32% in patients with relapsing MS. Interferon beta-1a, produced in a Chinese hamster ovarian cell line, is glycosylated at position 80 and contains the natural human
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Differential effects of three interferon betas on neutralising antibodies in patients with multiple sclerosis: a follow up study in an independent laboratory

A Bertolotto, S Malucchi, A Sala, G Orefice, P B Carriero, M Capobianco, E Milano, F Melis, M T Giordana

Objective: To evaluate the incidence and the prevalence of neutralising antibodies (NABs) to three interferon betas (IFNβ) products in patients with multiple sclerosis (MS).

Methods: Sera were tested from 125 patients with relapsing-remitting MS. Patients were treated with IFNβ-1b (Betaferon, n = 29) 8 MIU subcutaneously every other day, IFNβ-1a (Avonex, n = 44) 30 µg intramuscularly once weekly, or IFNβ-1a (Rebif, n = 36) 22 µg subcutaneously three times weekly for 6 to 18 months. An additional 16 patients were treated with Rebif 22 µg intramuscularly once or twice weekly. NABs were assessed using the cytopathic effect assay before treatment and every three months during treatment. Patients with two or more consecutive positive samples were considered to be persistent NAB positive (NAB+).

Results: At baseline, no patients were NAB+. NABs developed during the first three months of treatment and continued to develop until month 18. Over 18 months of treatment, the risk of being persistent NAB+ was 31% for Betaferon, 15% for Rebif, and 2% for Avonex. The reported percentages of NAB+ patients observed in these studies is not possible because of differences in type of assay used to detect NABs; methods used to represent neutralisation potency; criteria for determining NAB positivity; three month versus six month time points for measurement; and treatment duration.

Conclusion: The three IFNβ preparations have different degrees of immunogenicity, with Betaferon producing the highest incidence of NABs and Avonex the lowest. These differences should be considered by neurologists when selecting treatment for their patients with MS because NABs can reduce both bioavailability and clinical efficacy of IFNβ.

Patients with multiple sclerosis (MS) can develop neutralising antibodies (NABs) during treatment with interferon beta (IFNβ) products: IFNβ-1b (Betaferon; Schering AG, Berlin, Germany), IFNβ-1a (Avonex; Biogen, Inc, Cambridge, UK), and IFNβ-1a (Rebif; Ares-Serono, Basel, Switzerland). The presence and concentration of NABs may be clinically important in the management of patients treated with IFNβs because NAB-positive (NAB+) patients have low or undetectable serum concentrations of IFNβ. Furthermore, NABs reduce or abolish IFNβ bioavailability and NABs have been shown to reduce the therapeutic efficacy of IFNβ.

The reported percentages of NAB+ patients observed in studies of individual IFNβs vary considerably. However, a direct comparison of the percentages of NAB+ patients reported in these studies is not possible because of differences in assay methods in different laboratories, including differences in the following parameters: type of assay used to detect NABs; methods used to represent neutralisation potency; criteria for determining NAB positivity; three month versus six month time points for measurement; and treatment duration.

The present study was conducted to determine the incidence and prevalence of NABs in serum samples from patients with MS who were treated with Betaferon, Avonex, or Rebif for 6 to 18 months. NABs were quantified every 3 months using the cytopathic effect (CPE) assay in a single laboratory in our MS centre. Hence, NABs were directly compared for the three IFNβ products under controlled conditions in a single laboratory using the same assay procedure.

METHODS

Patients
Patients with clinically definite MS were enrolled in the study from May 1995 to July 2000. Patients were included in the study if they were between 17 and 65 years of age, had a relapsing-remitting or secondary progressive course of MS, and had an expanded disability status scale (EDSS) score of ≤5.5. Patients were excluded from the study if they had received prior treatment with an IFNβ product, immunosuppressive treatment during the 12 months before the study, or corticosteroids four weeks before the initiation of the study. Patients who were pregnant or breastfeeding, had other neurological or autoimmune diseases, or had infectious conditions were excluded from the study. Before enrolment, all aspects of the study protocol were reviewed with each patient and informed consent was obtained.

Procedure
Eligible patients were screened for the presence of NABs before (baseline) and every 3 months during IFNβ treatment for up to 18 months. Patients were treated with one of the

Abbreviations: CPE, cytopathic effect; EDSS, expanded disability status scale; IFNβ, interferon beta; LU, laboratory units; MS, multiple sclerosis; NAB, neutralising antibody; PRISMS, prevention of relapses and disability by interferon β-1a subcutaneously in multiple sclerosis; WHO, World Health Organization
Differential effects of three interferon betas on neutralising antibodies in patients with multiple sclerosis: a follow up study in an independent laboratory

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Conclusion: The three IFNβ preparations have different degrees of immunogenicity, with Betaferon producing the highest incidence of NABs and Avonex the lowest. These differences should be considered by neurologists when selecting treatment for their patients with MS because NABs can reduce both bioavailability and clinical efficacy of IFNβ.
Review of interferon beta-1b in the treatment of early and relapsing multiple sclerosis

Abstract: Multiple sclerosis (MS) is the most common autoimmune illness of the central nervous system. For many years the inflammatory manifestations of MS were treated using only corticosteroids. Since the 1990s the results of several clinical trials with immunomodulatory agents have changed the therapeutic approach to this disease. Interferon beta (IFNβ)-1b represents the pioneer of those therapies. There is growing evidence from clinical trials on relapsing-remitting MS and clinically isolated syndromes suggestive of MS that IFNβ-1b reduces the frequency and severity of relapses and the development of new and active brain lesions as assessed by magnetic resonance imaging. Long-term data suggest a persistent efficacy of IFNβ-1b on disease activity and a positive effect in slowing disability worsening. Furthermore a reduction of relapse rate and a slight positive effect on the progression were demonstrated when IFNβ-1b was administered to still-active secondary progressive MS. IFNβ-1b therapy is well tolerated and relatively free of long-term side effects. In spite of the emergence of new agents for the treatment of MS, IFNβ-1b still remains a first-line therapy with a fundamental role in all stages of the disease.

Keywords: interferon beta-1b, relapsing-remitting multiple sclerosis, clinically isolated syndromes, efficacy, safety, neutralizing antibodies

Introduction
Interferon beta (IFNβ)-1b was the first immunomodulatory therapy approved for the treatment of relapsing-remitting (RR) multiple sclerosis (MS)1–2 and currently is the only IFNβ licensed for use in secondary progressive (SP) MS.3 Moreover, recent studies4–5 have defined IFNβ-1b efficacy in treating patients with clinically isolated syndrome (CIS).6

In this review we focus on biologic activity, clinical and magnetic resonance imaging (MRI) evidence of efficacy, and safety of IFNβ-1b in RRMS and patients with CIS.

Pharmacokinetics and pharmacodynamics of IFNβ-1b
Human IFNβ-1b (Betaferon®/Betaseron®; Bayer HealthCare) is a lyophilized protein produced by DNA recombinant technology by Esecheria coli. As bacteria lack the ability to glycosylate proteins, the recombinant protein was not glycosylated. The cysteine residue at position 17 was replaced with a serine residue to ensure the stability of the molecule and the N-terminal methionine residue was deleted.7 It is combined with mannitol and human albumin to reach a neutral pH of 7.2.
In CIS patients included in the BENEFIT study the incidence of positive NAb titers ranged from 16.5% to 25.2% of the treated patients.\textsuperscript{4} Neutralizing activity was detected at least once in 75 out of 251 (29.9%) IFNβ-1b patients who provided samples during the treatment phase; of these, 17 (22.7%) converted to negative status later in the study. No significant effect of NAb status on time to CDMS in IFNβ-1b treated patients was found; in this analysis there was a trend toward a lower risk of progressing to CDMS in patients with at least one positive NAb titer. When analyses were performed at the end of the study (at least 180, 270 or 360 days after start of treatment) no differences were observed between NAb+ and NAb– patients.

The clinical impact of NAbbs on treatment efficacy in 6698 MS patients receiving IFNβ-1b was investigated by Goodin et al.,\textsuperscript{7} suggesting that NAbbs are not responsible for poor clinical responses and NAb status is of little clinical value.

In conclusion the results derived from existing studies are conflicting and have to be interpreted with caution. The differences might be due to the different duration of follow-up, assays and definitions of NAb positivity used. The impact of NAbbs seems to be more frequently evident on proper inflammatory clinical events of MS (relapse rate) but the influence of NAbbs on the progression of the disease remains uncertain. Therefore the decisions to discontinue IFNβ therapy are still based mainly on the patient’s clinical response to the treatment.

**IFNβ-1b and adverse events**

The administration of IFNβ is associated with the risk of a variety of adverse effects.\textsuperscript{53-54} The most common, compared with placebo, are flu-like symptoms and, in SC-treated patients, injection-site reactions.\textsuperscript{55} In the pivotal IFNβ-1b trial,\textsuperscript{1} flu-like symptoms in the high-dose group were initially observed in 52% of patients. However, by the end of the first year, these had decreased to 8%, only 3% to 8% of patients experiencing symptoms throughout the study. Injection-site reactions in the same pivotal trial were initially reported by 80% of those receiving 250 µg IFNβ-1b, a figure that declined to between 44% and 50% at years 4 and 5.

During the first year of the BEYOND study,\textsuperscript{55} flu-like symptoms were reported significantly more frequently in the IFNβ-1b 250 µg group compared to the GA group. Although initially high, the frequency of flu-like symptoms in IFNβ treated group declined very quickly over time. For injection-site reactions, pain and pruritus were significantly more frequent in the GA group than in the IFNβ-1b 250 µg group, but in the same way, the incidence of this reactions decreased over the study. The incidence of other adverse events such as fatigue, depression, arthralgia and paresthesia was comparable between treatment groups. The evaluation of laboratory safety as elevations in liver enzymes abnormalities, in blood lipids and markers of thyroid function, as well as leukocytopenia were more frequently detected in patients treated with IFNβ-1b than in those treated with GA. Adherence to treatment was also assessed demonstrating that the adherence to treatment was high in all three treatment groups.\textsuperscript{56,57} The proportion of patients completing the anticipated treatment period ranged from 73% in the IFNβ-1b 500 µg group through 78% in GA group to 82% in the IFNβ-1b 250 µg group. No significant differences in discontinuation rates were observed among the groups.

The management of IFNβ-related side effects is of great importance to improve the patients’ treatment adherence. General precautions to reduce or avoid this side effect include the correct preparation of the injection solution and a proper injection technique. In all studies frequency and severity of adverse effects depend on the duration of treatment and they are at a maximum during the first weeks of treatment.

**Conclusions**

Results from rationally planned and statistically convincing studies in RRMS and CIS patients demonstrated that IFNβ-1b treatment has very favorable efficacy on clinical and MRI measures of disease activity and progression since very early stages of disease.

Early and continuous treatment with IFNβ-1b maintains persistent effectiveness and safety in the long term. A reduction in attack rate and a slight positive effect on the progression of disability were also demonstrated when IFNβ-1b was administered to patients in SP phase. Therefore IFNβ-1b still represents a key therapeutic option for MS patients, playing a fundamental role in all stages of the disease. No definitive relationship between development of NAbbs and response to treatment has been demonstrated.

**Disclosures**

DP and VD declare no conflicts of interest. MT has received honoraria for speaking from Sanofi-Aventis, Biogen and Bayer Schering, and research grants from Merck Serono.


Antonio Bertolotto
Florian Deisenhammer
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Per Sölberg Sørensen

Immunogenicity of interferon beta:
differences among products

Abstract
Protein-based therapies are useful in a variety of diseases; however, their potential for immunogenicity is a disadvantage. Neutralizing antibodies (NAbs) that develop to interferon beta (IFNβ) products (IFNβ-1b, IFNβ-1a-Avonex®, or IFNβ-1a-Rebif®), which are first-line therapies for the treatment of multiple sclerosis, are reported to reduce the clinical efficacy of these agents. In individual clinical studies of each commercially available IFNβ product, 28% to 47% of patients develop NAbs to IFNβ-1b, 12% to 28% to IFNβ-1a-Rebif, and 2% to 6% to IFNβ-1a-Avonex. Problems exist in comparing the incidence of NAbs among IFNβ products across studies because of differences in study methodology, including assay methods, treatment duration, and the definition of NAb positive. Results from studies that have directly compared these products are consistent with results from the respective clinical trials of IFNβs. Both the clinical trials and the independent studies have shown that NAbs develop more frequently with IFNβ-1b treatment than with IFNβ-1a treatment and that, among IFNβ-1a products, NAbs develop more frequently with IFNβ-1a-Rebif treatment than with IFNβ-1a-Avonex treatment. Factors that may affect the immunogenicity of IFNβs, including the dosing regimens and the biochemical properties of the products, are discussed.

Key words
multiple sclerosis · interferon beta · immunogenicity · neutralizing antibodies · binding antibodies

Introduction
Advances in gene cloning have facilitated the production of biologically active proteins to treat a variety of human diseases. One problem associated with the use of therapeutic proteins, however, is their potential for immunogenicity [38]. The use of porcine insulin for diabetes [20], factor VIII for hemophilia [20], and growth hormone for growth hormone deficiency [21] has been associated with the production of antibodies directed against the administered drug. Interferon (IFN) products also have been associated with the development of both binding antibodies (BAbs) and neutralizing antibodies (NAbs). A number of studies have reported the development of NAbs to IFNα in patients with hepatitis or cancer [2, 8, 9, 19, 26, 30, 43]. NAb formation has been reported with the use of IFNβ in the treatment of MS, and NAbs reportedly reduce the clinical efficacy of these drugs [12, 28].

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Neutralizing antibodies to interferon beta: Assessment of their clinical and radiographic impact: An evidence report

Report of the Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology

D.S. Goodin, MD; E.M. Frohman, MD, FAAN; B. Hurwitz, MD; P.W. O’Connor, MD; J.J. Oger, MD, FRCPC, FAAN; A.T. Reder, MD; and J.C. Stevens, MD, FAAN

Abstract—The clinical and radiologic impact of developing neutralizing antibodies (NAbs) to interferon beta (IFNβ) while on this therapy for multiple sclerosis (MS) is assessed. On the basis of Class II and III evidence, it is concluded that treatment of patients with MS with IFNβ (Avonex, Betaseron, or Rebif) is associated with the production of NAbs (Level A). NAbs in the serum are probably associated with a reduction in the radiographic and clinical effectiveness of IFNβ treatment (Level B). In addition, the rate of NAb production is probably less with IFNβ-1a treatment than with IFNβ-1b treatment, although the magnitude and persistence of this difference is difficult to determine (Level B). Finally, it is probable that there is a difference in seroprevalence due to variability in the dose of IFNβ injected or in the frequency or route of its administration (Level B). Regardless of the explanation, it seems clear that IFNβ-1a (as it is currently formulated for IM injection) is less immunogenic than the current IFNβ preparations (either IFNβ-1a or IFNβ-1b) given multiple times per week subcutaneously (Level A). However, because NAbs disappear in some patients even with continued IFNβ treatment (especially in patients with low titers), the persistence of this difference is difficult to determine (Level B). Although the finding of sustained high-titer NAbs (>100 to 200 NU/mL) is associated with a reduction in the therapeutic effects of IFNβ on radiographic and clinical measures of MS disease activity, there is insufficient information on the utilization of NAb testing to provide specific recommendations regarding when to test, which test to use, how many tests are necessary, or which cutoff titer to apply (Level U).

The development of neutralizing antibodies (NAbs) to proteins administered therapeutically is often associated with a reduction in the biologic actions that these proteins exert. It is therefore surprising that the clinical and radiographic impact of NAbs to interferon beta (IFNβ) in the treatment of multiple sclerosis (MS) is controversial. This assessment evaluates the clinical and radiographic impact of NAbs in this setting and considers some of the difficulties in this research area that may explain the ongoing controversy. In this regard, it is useful for readers to appreciate the complexity of this particular biologic system.1-9 Thus, a brief overview of IFNβ biology is provided in the supplementary material to this article.

From the University of California, San Francisco (D.S.G.); University of Texas Southwestern (E.M.F.), Dallas; Duke University Medical College (B.H.), Durham, NC; St. Michaels Hospital (P.W.O.), Toronto, Ontario, Canada; University of BC (J.J.O.), Vancouver, British Columbia, Canada; The University of Chicago (A.T.R.), Oak Park, IL; and Lutheran Medical Office (J.C.S.), Fort Wayne, IN.

Disclosure: The authors report no conflicts of interest.

Received August 10, 2006. Accepted in final form December 7, 2006.

Approved by the Therapeutics and Technology Subcommittee on July 28, 2006; by the Practice Committee on November 11, 2006; and by the AAN Board of Directors on January 4, 2007.

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P. S. Sørensena, F. Deisenhammerb, P. Duda, R. Hohlfeldd, K.-M. Myhre, J. Palacef, C. Polman, C. Pozzillih and C. Ross for the EFNS Task Force on Anti-IFN-β Antibodies in Multiple Sclerosis

Keywords: antibodies, binding antibodies, guidelines, immunogenicity, interferon-beta, multiple sclerosis, neutralizing antibodies

Therapy-induced binding and neutralizing antibodies is a major problem in interferon (IFN)-β treatment of multiple sclerosis. The objective of this study was to provide guidelines outlining the methods and clinical use of the measurements of binding and neutralizing antibodies. Systematic search of the Medline database for available publications on binding and neutralizing antibodies was undertaken. Appropriate publications were reviewed by one or more of the task force members. Grading of evidence and recommendations was based on consensus by all task force members. Measurements of binding antibodies are recommended for IFN-β antibody screening before performing a neutralizing antibody (NAB) assay (Level A recommendation). Measurement of NABs should be performed in specialized laboratories with a validated cytopathic effect assay or MxA production assay using serial dilution of the test sera. The NAB titre should be calculated using the Kawade formula (Level A recommendation). Tests for the presence of NABs should be performed in all patients at 12 and 24 months of therapy (Level A recommendation). In patients who remain NAB-negative during this period measurements of NABs can be discontinued (Level B recommendation). In patient with NABs, measurements should be repeated, and therapy with IFN-β should be discontinued in patients with high titres of NABs sustained at repeated measurements with 3- to 6-month intervals (Level A recommendation).

Background and objectives

Interferon (IFN)-β is a first-line therapy for relapsing–remitting multiple sclerosis (MS). In recent years, several publications have concordantly reported that binding antibodies (BABs) and neutralizing antibodies (NABs) occur during treatment with recombinant IFN-β products. The frequencies and titres of anti-IFN-β antibodies vary considerably depending on the IFN-β preparation, the frequency and route of administration, and the type of assay being used. There is no generally accepted standardized assay for measuring BABs and NABs. Clinical studies in patients with MS have demonstrated that when NABs to IFN-β develop, the therapeutic benefits of IFN-β are reduced or abolished.

The objectives of our task force were to: (i) evaluate differences in immunogenicity of IFN-β products, (ii) evaluate the reliability and give recommendations on BABs and NABs assays, (iii) evaluate the impact of NABs on clinical efficacy and give recommendation on the clinical use of measurement of IFN-β antibodies and (iv) review the evidence on prevention of NAB development and the management of patients with NABs.

Search strategy and consensus

The task force systematically searched the Medline database for available information published in English up to September 2004. Key words included: interferon-

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The measurement of antibodies binding to IFNβ in MS patients treated with IFNβ

Andrew R. Pachner, MD; Joel Oger, MD, FRCP; and Jacqueline Palace, MD

The intensity of the antibody response to injected interferons (IFNαs) depends on many factors: route of injection, dose injected, frequency of injections, and duration of treatment. The optimal assay or approach for testing for anti-IFN antibodies has not been determined. Neutralizing antibody (NAb) determinations are most commonly used but have intrinsic problems when used as clinical assays. They are time consuming, expensive, and only indirectly measure antibodies. Thus, false-positive results can occur because of other serum factors. In contrast, binding assays measure antibodies directly; they are mainly used as an initial screen to detect NAbs. ELISA methodologies for binding antibodies have been used, but direct adhesion of the antigen (i.e., IFNβ) to the plate has resulted in false-negative and false-positive results, presumably because of changes in antigenicity.1 This limitation is circumvented by using the capture ELISA in which a first antibody is used to capture the antibody and hold it in an antigenic position, mimicking that of IFNβ in its natural state.

Radioimmunoprecipitation assays (RIPAs) are used routinely to measure pathogenic autoantibodies, including antibodies to the acetylcholine receptor and voltage-gated calcium and potassium channels, with good sensitivity and specificity. The RIPA also has been used to measure antibodies to another biologic substance, botulinum toxin, in patients treated with this toxin.

The experience of groups in the United States (University of Medicine and Dentistry of New Jersey) and Canada (University of British Columbia) with improved ELISAs and in the United Kingdom (Oxford University) with RIPA is summarized below.

Materials and methods. Direct ELISA. The assay has been previously described.2,3 In brief, IFNβ was directly coated onto an ELISA plate, followed by serial incubations with serum, conjugate, and substrate, with washes in between.

Capture ELISA. United States. The assay has been previously described.4 Experience with this assay in a broad spectrum of patients with MS has recently been published.4 In brief, 96-well microtiter plates were coated overnight with 50 μL/well of the monoclonal antihuman IFNβ immunoglobulin (IgG) G antibody BO2 (Yamasa-Shoyu Co. Ltd., Tokyo, Japan). After plate washing and blockade with nonfat dry milk, wells were coated with either buffer or IFNβ (1a as Avonex or 1b as Betaseron) at a dilution of 1.5 μg/mL. Subsequent incubation with serum samples, conjugate, and development was the same as the direct ELISA outlined previously. Calculation of units was identical with the direct ELISA outlined previously, except optical densities (ODs) were calculated by subtracting ODs of the wells lacking IFN from the ODs of the IFNβ-treated wells.

Canada. Binding antibodies (BAb) were detected using a sandwich ELISA based on the capturing of IFNβ with an anti-IFNβ monoclonal antibody.

Radioimmunoprecipitation assay. United Kingdom. This assay was performed as previously described.5 In brief, 10 μL iodinated IFN (approximately 50,000 cpm) was added to 5 μL serum in 0.02 phosphate buffer with 0.1% triton X100 and incubated for 2 hours at room temperature. Fifty microliters sheep antihuman IgG (The Binding Site, Birmingham, UK) was added and centrifuged, and the precipitate was washed in P TX buffer and counted on a gamma counter (Packard, Berkshire, UK). Three healthy control sera and a high-positive serum were tested in each assay. The mean of the healthy control values was subtracted from every test value, and the results were expressed as a percentage of the value obtained with the high-positive serum.

Results. United States. Four hundred fifty-three serum samples were obtained from patients with MS or control subjects and were then aliquoted and stored at −70 °C. The number of patients taking each medication, with the total samples in parentheses, was as follows: Betaseron, 145 (168); Avonex, 189 (214); and Rebif, 17 (17). In 30 patients taking IFNβ, the preparation was not noted. In 23 serum samples, the source of the specimen was a patient about to begin medication. Sixty-nine percent of the patients with MS were women. The mean age of the patients was 37 years, and the mean duration of IFNβ therapy was 1.3 years.

Two hundred eighty-five serum samples from patients with MS taking IFNβ had cELISA values of less than 2.3 U. Most patients with high antibody levels began to develop antibodies within the first 3 months after initiation of therapy. Because no sera with cELISA values of less than 8 U had neutralizing activity, the range of 2.3 U to 8 U was termed “moderate;” there were 52 serum samples in this range. In sera with cELISA values in the range of 8 to 55 U, called “high” levels, the frequency of NAbs was 53% (39/74). The 19 serum samples with cELISA values greater than 55 U were NAb positive, and these were labeled “very high.” These data are summarized in the table.

The Pearson correlation coefficient was 0.750; the correlation was significant at 0.01 (two tailed). The mean cELISA values for Avonex, Betaseron, and Rebif were 2.5, 18.4, and 48.4 U; the percentages of NAb positivity for these therapies were 6, 34, and 14. Only 17 samples were available from Rebif-treated patients.

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Supported by grants from Berlex Canada/Shering-AG Canada (Canada) and from the Foundation of UMDNJ (United States).

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The measurement of antibodies binding to IFNβ in MS patients treated with IFNβ

Andrew R. Pachner, MD; Joel Oger, MD, FRCP; and Jacqueline Palace, MD

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article in this supplement, needs to be determined.

Discussion. These studies demonstrate that bind-
ing assays can serve as excellent assays to screen for
anti-IFNβ antibodies. This is an important finding
because binding assays are relatively inexpensive,
fast, and easily performed by most clinical laborato-
ries. Whether the assays described previously can serve
as stand-alone assays or whether they need to be
followed with either NAb assays or bioactivity
studies, such as the MxA assays described in another
article in this supplement, needs to be determined.

The capture ELISA format allowed the native
structure of the IFN to be maintained; this is in
contrast to the direct ELISA, in which the IFNβ is
directly stuck to the plate and the native structure is
deformed. When these two ELISAs are compared,
direct-binding ELISA underreports BAb, and be-
cause it is frequently used as a screening test before
assaying for NAb, NAb themselves may be missed.
The US and Canadian groups found that the capture
ELISA significantly outperformed the direct ELISA.
The capture ELISA and the RIPA should thus be
considered as “second-generation” BAb assays, much
improved over the “first-generation” BAb assay, in
which the IFNβ is directly stuck to the plate.

There were a number of other findings that were
consistent in the three countries. The US and Cana-
dian groups found that using the same IFNβ prepa-
ration in the assay as was used in treating the
patient improved test reliability. The consensus of
the groups was that IFNβ-1b was considerably more
immunogenic than IFNβ-1a, either given once a
week or three times a week. The US and Canadian
groups also found that BAb might have effects on
bioactivity independent of their NAb status, the lat-
ter defined as the levels of neutralization high
enough to be detected by NAb assays. These data are
consistent with Dr. Bendtzen’s theory, as described
in his presentation: NAb activity is a continuum, and
all sera that have significant BAb activity have NAb
activity if the sensitivity of the assays is set appro-
priately. Thus, the Canadian group demonstrated for
the first time that BAb-positive patients had a
greater chance than BAb-negative patients of being
treatment nonsuccesses, having more frequent re-
lapses, and increasing EDSS.

Another important finding by the Canadian group
was that there was a difference in persistence of
BAb between Betaseron and Rebif. Despite the rel-
atively lower incidence of BAb in the Rebif group,
anti-IFNβ antibodies appeared to persist more in the
Rebif group than in the Betaseron group.

In conclusion, the data from the three groups
demonstrate that BAb assays can be used clinically
to detect anti-IFNβ antibodies. At a minimum, the
role of BAb can be that of a screening assay for
NAb. However, they may be able to function as
stand-alone assays for antibody, especially when
combined with an IFNβ bioactivity assay, such as
mRNA or protein assays for MxA.

Acknowledgment
The authors thank Donna Dail and Marie Hurd (United States)
and Ebrima Gibbs and Tariq Aziz (Canada).

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Effect of neutralizing antibodies on biomarker responses to interferon beta

The INSIGHT study

Neurology® 2009;73:1493–1500

ABSTRACT

Background: Interferon beta (IFNβ) effectively reduces disease activity in patients with multiple sclerosis (MS). Neutralizing antibodies (NAbs) can diminish or abolish the clinical efficacy of IFNβ therapies. Biomarkers of the IFNβ response, such as myxovirus resistance protein A (MxA), viperin, and interferon-induced protein with tetratricopeptide repeats 1 (IFIT-1), may be used to measure the in vivo effects of NAbs on IFNβ bioactivity.

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Results: Treatment with IM IFNβ-1a was associated with a lower rate of Nab formation among 718 patients screened ($p < 0.0001$ vs SC IFNβ-1a $22 \mu g$, $44 \mu g$, and IFNβ-1b). At baseline, patients who were binding antibody positive (BAb+)/neutralizing antibody positive (NAb+) had lower MxA, viperin, and IFIT-1 response compared with BAb-negative (BAb−)/NAb-negative (NAb−) patients (all $p < 0.0001$). Analyses stratified by NAb titer level among BAb+/NAb+ patients showed diminished biomarker response in patients with NAb titers from 20 to 99 tenfold reduction units (TRU) and abolished response in patients with NAb titers ≥100 TRU compared with BAb−/NAb− patients. A majority of patients BAb+/NAb+ at screening remained BAb+/NAb+ throughout the study, and biomarker responses remained consistently depressed in these patients at month 6.

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BAb = binding antibody; cELISA = capture ELISA; IFIT-1 = interferon-induced protein with tetratricopeptide repeats 1; IFNAR = interferon α/β receptor; IFNβ = interferon beta; INSIGHT = Impact of Neutralizing Antibodies on Interferon Responsive Genes Highlights Biomarker Response; MS = multiple sclerosis; MxA = myxovirus resistance protein A; NAb = neutralizing antibody; NR = normalization ratio; OR = odds ratio; SC = subcutaneous; TRU = tenfold reduction unit.

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* A list of investigators appears in the appendix at the end of this article.
From the University of Medicine and Dentistry of New Jersey (A.R.P.), New Jersey Medical School, Newark, NJ; and Biogen Idec, Inc. (J.D.W., A.P., S.G.), Cambridge, MA.
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Disclosure: Author disclosures are provided at the end of the article.
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came BAb+/NAb+ at month 6. Thus, a majority (77.6%) of the patients BAb+/NAb+ at screening remained BAb+/NAb+ throughout the duration of the study. The majority of BAb+/NAb+ patients who converted to NAb+ by month 6 were on IFNβ-1b (14 of 26 patients, 53.8%).

Analyses of the impact of NAbs/BAb on the biomarker response in these patients using antibody status at the time the samples were drawn (i.e., either baseline or month 6) are shown in figure 5. Persistently NAb+ patients (+/+/++) showed a depressed response as compared with NAb− patients, whereas patients with fluctuating NAb+ status demonstrated a variable response.

**Associations of baseline patient characteristics with NAb status.** After controlling for other variables in the model, those aged 50 years or older were more likely to be NAb+ compared with patients aged $\leq 50$ years (odds ratio [OR] = 1.76, $p = 0.0067$). Similarly, patients whose IFNβ therapy was IFNβ-1b (OR = 20.57, $p < 0.0001$), SC IFNβ-1a 22 μg (OR = 12.49, $p = 0.0012$), or SC IFNβ-1a 44 μg (OR = 18.22, $p < 0.0001$) were more likely to be NAb+ compared with those whose initial therapy was IM IFNβ-1a. In contrast, patients treated for $\leq 30$ months were less likely to be NAb+ compared with patients treated for $<30$ months (OR = 0.564, $p = 0.0041$). It should be noted, however, that patients who are NAb− are likely to be IFNβ responders and therefore are more likely to remain on IFNβ therapy for a longer period of time. Patients also were less likely to be NAb+ when treated previously with IM IFNβ-1a before switching to SC IFNβ (OR = 0.524, $p = 0.0128$).

**DISCUSSION** NAbs have been shown to reduce the clinical efficacy of IFNβ, presumably as a result of the reduction of IFN binding to its receptor. In this study, IM IFNβ-1a showed the least immunogenicity compared with SC IFNβ-1a or IFNβ-1b, which is consistent with previous studies that reported differences in the rate of NAb positivity among IFNβ preparations.

In the present study, to our knowledge the largest study to date of the effect of NAbs on IFNβ bioactivity, NAbs significantly reduced expression of MxA, IFIT-1, and viperin, 3 highly induced and specific IFNβ-responsive genes that are good surrogates for all IFNβ-induced biologic responses. In previous studies, NAbs have been shown to reduce expression of several IFN-induced products, including MxA, neopterin, oligoadenylate synthetase, 2'-5' oligoadenylate synthetase, β2-microglobulin, tumor necrosis factor–related apoptosis-inducing ligand, and Stat-1.7,9,15,27,30,33,34 The observation that all 3 IFN-response genes evaluated in this study had significantly reduced expression in the presence of NAbs confirms previous studies in demonstrating the broad-based effects of NAbs in blocking IFNβ binding to IFNAR.

The effect of NAbs on biomarker response was correlated with NAb titer. Patients with lower NAb titers (20–99 TRU) showed a biomarker response...
that was detectable but markedly reduced when compared with BAb−/NAb− patients. The biomarker responses for BAb+/NAb− patients were somewhat lower than those for BAb−/NAb−, though not markedly so. This may be due to a direct effect of high-titer BAbs on the biomarker response, but more likely is due to the “NAb−” population being defined by a cutoff of 1:20, which is a relatively arbitrary but well-accepted cutoff for determining NAb positivity. However, some of the BAb+/NAb− patients in the study likely had low levels of NAb (e.g., 1:5 or 1:10), and it has been documented that subsets of such patients have lower IFNB biomarker responses than those with completely negative NAb responses.15 Patients with NAb titers ≥100 TRU showed no measurable biomarker response. These results are consistent with previous studies, which showed similar correlations between NAb titer and biomarker response.7,9,15,27,30,33,34 Furthermore, in previous studies NAb titers also have been shown to correlate with MRI outcomes. Patients with high titers of NAb have significantly more MRI activity compared with patients who were NAb−.20 Another study reported that MxA and NAb measurements may predict the risk of new relapses.19 Given this evidence, measuring NAb titers, rather than simply measuring the presence or absence of NAb, may provide a more precise estimate of the effects of NAb on IFN response. This observation also provides evidence that, when analyzing results of clinical trials of IFNβ, a simple definition of populations as NAb− or NAb+ is not optimal because the latter population has patients who have IFNβ bioavailability ranging from near normal to absent. Thus, in future clinical trials, bioactivity measurements would be ideally included at the time clinical measures are being taken to validate use of biomarkers to ascertain the in vivo activity of IFNβ in individual patients.

These data confirm the variability in incidence of NAb among the IFNβ products and demonstrate that high titers of NAb abolish the biologic response to IFNβ as ascertained by 3 robust biomarkers.

AUTHOR CONTRIBUTIONS

Statistical analysis for this article was conducted by Amy Pace, ScD, Biogen Idec, Inc.

ACKNOWLEDGMENT

The authors thank Sabrina Maurer and Matthew Hasson, Scientific Connections, Newtown, PA, for technical editing, copyediting, and word processing assistance in preparing the manuscript for submission. Their work was supported by Biogen Idec.

DISCLOSURE

Dr. Pachner has served on a scientific advisory board for MediciNova, Inc.; has received speaker honoraria from Biogen Idec, EMD Serono, Inc., Novartis, Pfizer Inc.; Teva Pharmaceutical Industries Ltd., and Xored Molecular Corp.; serves on speakers bureaus for Biogen Idec, Inc., EMD Serono, Inc., and Pfizer Inc.; and has received research support from Biogen Idec, EMD Serono, Inc., Novartis, MediciNova, Inc., Neutrekio Ltd., the New Jersey Commission on Spinal Cord Research, and the National Multiple Sclerosis Society. Dr. Warth, Ms. Pace, and Dr. Goelz are employees of and own stock in Biogen Idec.

APPENDIX

Investigators and coordinating sites for the INSIGHT Study Group were as follows: Khurram Bashir, University of Alabama at Birmingham; Stanley Brod, University of Texas Health Science Center at Houston; Mark Cascione, South Tampa Multiple Sclerosis Center; Bruce Cohen, Northwestern University; Dennis Garwacki, University of Illinois, College of Medicine at Peoria; Doug Goodin, University of California, San Francisco; John Huddleston, MultiCare Neurosciences Center of Washington; Bruce Hughes, Mercy Ruan Neurology Clinic; Samuel Hunter, Brain and Nerve Neurology, Advanced Neurosciences Institute; George
Effect of anti-IFNβ antibodies on MRI lesions of MS patients in the BECOME study


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Background: Interferon beta (IFNβ) administered subcutaneously is immunogenic in some patients with multiple sclerosis (MS) and leads to the development of neutralizing antibodies (NAbs). Considerable evidence has accumulated that NAbs diminish or abolish IFNβ bioactivity, but there is less evidence that NAbs impact clinical efficacy of the drug.

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Results: 147 patients were assessed at 16 centres. Predictivity parameters (with confidence intervals) were as follows: active scan, sensitivity (SN) 52% (34–69%), specificity (SP) 80% (65–91%), negative predictive value (NPV) 73% (58–77%), positive predictive value (PPV) 62% (42–79%), p = 0.002; NAb positivity, SN 71% (45–88%), SP 66% (55–76%), NPV 92% (82–97%), PPV 29% (16–45), p = 0.01; active scan and NAb positivity, SN 71% (38–91%), SP 86% (73–94%), NPV 94% (86–98%), PPV 50% (29–70%), p = 0.0003.

Conclusions: MRI activity and NAb occurrence during the first 6 months of interferon β treatment were reliable predictors of long term clinical response, particularly when combined. Patients with negative predictors showed a less than 10% risk of developing clinical activity. Patients with positive predictors showed a 50% risk of further clinical activity. These patients need to be followed carefully with further MRI and NAb tests.

Several randomised clinical trials have demonstrated that immunomodulatory drugs (human recombinant interferon β (IFNβ) and glatiramer acetate) are more effective than placebo in the treatment of relapsing–remitting multiple sclerosis (RRMS).14 It is, however, accepted in clinical practice that not all patients with RRMS experience a satisfactory treatment response, even when the most effective treatments are used. Patients who demonstrate a suboptimal response to immunomodulatory treatment might require a treatment change. This is a very important clinical decision that, when made, must be based on a reliable indicator of treatment response. However, identifying patients with a suboptimal response remains a problem for neurologists.

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MRI is an effective tool used in the diagnosis and prognosis of MS and, in particular, in monitoring the clinical efficacy of pharmacological treatments.16 The number of lesions detected by MRI is substantially higher than the number of clinical events observed, rendering MRI a sensitive index of disease activity.12 Clinical exacerbations are associated with the appearance of gadolinium (Gd) enhancing lesions or new or enlarging proton density (PD/T2) lesions.13 MRI activity is predictive of the subsequent evolution of clinical disease activity, particularly of the frequency of relapses in the following 12 months.14 The effects of IFNβ-1b on the frequency of MRI enhancing lesions are detectable very early, even after only a few weeks of treatment.15

During treatment, some patients develop antibodies against IFNβ.17 Neutralising antibodies (NAb) affect the clinical and radiographic efficacy of IFNβ, particularly in the years after their appearance.18 The occurrence of NAb might, therefore, identify patients whose treatment will later fail.

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**Supplementary tables 1 and 2 are published online only at http://jnnp.bmj.com/content/vol78/issue6**

**Members of the Italian Multiple Sclerosis Study Group are listed in the appendix.**

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Pediatric multiple sclerosis

E. Ann Yeh, Tanuja Chitnis, Lauren Krupp, Jayne Ness, Dorothee Chabas, Nancy Kuntz and Emmanuelle Waubant for the US Network of Pediatric Multiple Sclerosis Centers of Excellence

Abstract | Pediatric multiple sclerosis (MS) accounts for up to 5% of all MS cases. Work conducted over the past 5 years has provided new information about the treatment, pathogenesis, demographics, and natural history of this disorder. Genetic and environmental factors seem to exert critical influences on its development. Clinical, MRI and laboratory data from prepubertal and postpubertal children suggest differences between the immune response and/or CNS environment in younger compared with older children and adults with MS. Randomized, controlled treatment trials for pediatric MS have not yet been performed, but therapies used in adult MS have been evaluated in this population, and their use seems to be safe. This article provides a comprehensive review of current knowledge regarding pediatric MS, highlighting new advances in the field.


Introduction

Over the past 5 years, interest in and knowledge about pediatric multiple sclerosis (MS), including its treatment, pathogenesis, demographics, natural history, and MRI and laboratory features, have increased considerably. In this article, we review the currently available literature on this disorder, including the most recent advances in research. In general, studies of pediatric MS in North America have focused specifically on the population under the age of 18 years, although many other studies include only those under the age of 16 years. We will review studies of MS with onset under 18 years of age, including those studies using an earlier cut-off point.

Definitions

According to consensus definitions from the International Pediatric MS Study Group (IPMSSG), pediatric MS can be diagnosed after two clinical episodes of CNS demyelination that are separated by at least 30 days. No lower age limit is specified. According to these definitions, the Barkhof adult brain MRI criteria can be used to meet the requirement for lesion dissemination in space. Three of the following four features should be demonstrated: first, nine or more white matter lesions or one gadolinium-enhancing lesion; second, three or more periventricular lesions; third, one juxtacortical lesion; and fourth, an infratentorial lesion. These adult MRI criteria have not, however, been validated in children. The combination of an abnormal cerebrospinal fluid (CSF) test and two lesions on MRI, of which one must be in the brain, can also meet the dissemination in space criteria. The CSF must show either at least two oligoclonal bands (OCBs) or an elevated IgG index.

MRI might also be used to satisfy criteria for dissemination in time following the initial clinical event, even in the absence of a new clinical demyelinating event. New T2-bright or gadolinium-enhancing foci must develop 3 months or more after the initial clinical event.

Importantly, an episode consistent with the clinical features of acute disseminated encephalomyelitis (ADEM) cannot at present be considered to be the first event of MS, although our experience over the past 4 years is challenging this idea, as detailed below (see Figure 1 for a diagnostic algorithm). Limited diagnostic criteria are available to clarify the clinical distinction between ADEM and a first attack of MS, emphasizing the need for a long interval between clinical events.
weekly for intramuscular IFN-β1a, and 22 mcg three times weekly or 44 mcg three times weekly for subcutaneous IFN-β1a. Children over the age of 10 years seem to be able to tolerate full doses of IFN-β, although tolerance might be reduced in the younger population. In the US Pediatric MS Network series of children with MS receiving therapy (mean follow-up 3.5 years, n = 264), of the children started on intramuscular IFN-β1a therapy, 42 of 97 (42%) required change to another therapy, 24 owing to breakthrough disease and 18 owing to adverse effects and/or compliance problems. Of those who were initially started on subcutaneous IFN-β1a therapy, 21 of 74 (28%) required a change to another therapy, 10 owing to breakthrough disease and 11 owing to adverse effects and/or compliance problems. Of the 31 patients started on subcutaneous IFN-β1b, 15 of 31 (48%) required a change to another therapy, 10 owing to breakthrough disease and 5 owing to adverse effects and/or compliance problems.

Little information is available regarding neutralizing antibodies to IFN-β in the pediatric MS population, although one small study has suggested that positive neutralizing antibodies might be less commonly seen in pediatric MS than in the adult population (E. A. Yeh et al., unpublished work).

Glatiramer acetate
Glatiramer acetate is the acetate salt of a mixture of synthetic polypeptides composed of L-alanine, L-glutamic acid, L-lysine and L-tyrosine. This drug is designed to mimic human MBP, and is postulated to induce a myelin-specific response mediated by suppressor T lymphocytes and to inhibit specific effector T lymphocytes, as well as affecting the function of antigen-presenting cells. Glatiramer acetate was found to reduce the number of relapses by 29% in adults with RRMS over a period of 2 years. One small retrospective study describing the use of glatiramer acetate in seven children with MS suggested that the medication is well tolerated. In the US Pediatric MS Network series of 56 children with MS who were initially started on this medication, 12 of 58 (21%) required change to another therapy—9 owing to breakthrough disease and 3 owing to adverse effects—over a mean follow-up period of 3.5 years.

Treatment failure
First-line treatment failure is a concern in both adult and pediatric MS. The currently available first-line DMTs are accepted to be only partially effective, resulting in a reduction in relapse rate of ~30% in the adult population. Treatment failure can also arise because of intolerable adverse effects or the presence of an unacceptable level of breakthrough disease (either in terms of severity or frequency), continued presence of gadolinium-enhancing lesions on MRI, or progression of disability and/or disease despite adherence to medication. The biological mechanisms underlying poor response to therapy have not been elucidated, but could involve heterogeneity of disease processes between individuals owing to genetic, immunological or environmental variability.

Compliance is a key issue in the treatment of children with MS, as daily to weekly injections of medication are required, and are frequently associated with adverse effects. 15% of children followed at the six centers participating in the US Network of Pediatric MS Centers of Excellence changed therapies owing to compliance issues.

Definition of treatment failure is a challenging issue, and has been the subject of considerable debate among clinicians treating adult-onset and pediatric-onset MS. Consensus criteria for the adult MS population, which were proposed by Cohen et al. in 2004, include the presence of more than one relapse per year, no decrease in relapse rate, incomplete recovery from relapses and/or accumulation of disability, new brainstem and/or spinal cord lesions on MRI, polyregional disease, and worsening motor and/or cognitive impairment. In general, these criteria are reserved for patients who have been on therapy for at least 6 months.

Consensus definitions of breakthrough disease in the pediatric MS population are not available, and the use of second-line therapies has not been well studied in this population. At present, many practitioners adhere to the guidelines that are used for the adult population. Under these guidelines, approximately one-quarter of children with MS experience breakthrough disease, prompting a switch to a second-line therapy an average of 1.5 years after starting a first-line therapy.

Second-line agents
The use of second-line agents, such as cyclophosphamide, mitoxantrone, mycophenolate mofetil, daclizumab, rituximab or natalizumab, in pediatric MS has been described in retrospective case series and reports with limited follow-up (E. A. Yeh et al., unpublished work). Owing to the retrospective, open-label nature of the studies, no firm conclusions regarding the efficacy and safety of these agents can be drawn. A retrospective study of cyclophosphamide use in 17 children with MS suggested a temporary reduction in relapses and disease progression. Use of this drug was, however, associated with secondary bladder cancer in one case, and with amenorrhea and infertility in several patients. Further studies evaluating the short-term and long-term safety as well as the efficacy of these agents are needed. For ethical reasons, randomized, placebo-controlled trials of second-line agents are unlikely to be performed in pediatric patients.

Conclusions and future directions
Pediatric MS represents a relatively rare but important entity, as it provides unique insights into disease processes related to MS. Studies have suggested substantial variability in presenting symptoms and laboratory and imaging features between children with prepubertal disease and those with postpubertal disease. Given the
Paola Perini  
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The clinical impact of interferon beta antibodies in relapsing-remitting MS

Introduction

The clinical efficacy of interferon beta (IFNβ) therapy has been demonstrated in relapsing-remitting and secondary-progressive MS (RRMS and SPMS). However, it also has been shown that administration of IFNβ may induce the development of anti-IFNβ antibodies [1–9].

Abstract  We analysed the kinetics and clinical impact of binding antibodies (BAbs) and neutralizing antibodies (NAbs) to three interferon beta (IFNβ) products in patients with relapsing-remitting MS (RRMS). Patients with RRMS received IFNβ-1b 8 MIU subcutaneously (SC) every other day, intramuscular (IM) IFNβ-1a 30 mcg once weekly, or SC IFNβ-1a 22 mcg three times weekly for up to 4 years. The changes of BAbs and IFNβ were measured using enzyme-linked immunosorbent assay (ELISA), and positive BAb samples were then analysed for neutralizing activity using an antiviral cytopathic effect assay. Patients were considered BAb+ if they had a positive sample with an optical density (OD) > mean + 3SD of the OD of the control sample; high BAb titers were defined as > 1:500. Patients were considered NAb+ if they had titers ≥20 LU/mL, with high NAb titers defined as > 1:100. The impact of BAbs and NAbs on relapses and Expanded Disability Status Scale (EDSS) score also was evaluated. Thirty patients were enrolled in each treatment group. Over the course of the study, 83 % of patients developed BAbs to IFNβ-1b, 13 % to IM IFNβ-1a, and 47 % to SC IFNβ-1a. Forty percent of patients developed NAbs to IFNβ-1b, 6.7 % to IM IFNβ-1a, and 26.7 % to SC IFNβ-1a. Of 22 NAb+ patients, 10 patients (45.5 %) demonstrated high titers of both NAbs and BAbs (20 % IFNβ-1b, 3.3 % IM IFNβ-1a, 10 % SC IFNβ-1a). The relapse rate significantly increased after the appearance of high NAb titers (p = 0.03); however, an even higher significance level (p < 0.001) was observed in patients with high titers of both NAbs and BAbs. In 10 patients with high titers of both NAbs and BAbs, an increase in mean EDSS score from 2.2 ± 0.8 at baseline to 3.6 ± 1.2 at year 2 (p < 0.01) was observed. NAb-negative patients showed no significant change in EDSS score at year 2. These findings demonstrate that high titers of both BAbs and NAbs reduce the clinical efficacy of IFNβ in patients with RRMS, which is important for the long-term efficacy of these drugs.

Key words  multiple sclerosis · interferon beta · neutralizing antibodies · relapse rate · EDSS

Although data from the IFNβ trials seem to indicate that patients who develop neutralizing antibodies (NAbs) to IFNβ show a reduction in the clinical and radiological (i.e., MRI) benefits associated with therapy [2, 4, 6–8], the clinical significance of both NAbs and binding antibodies (BAbs) is still debated [10–12]. The purpose of this report is to evaluate the kinetics and clinical effects of BAbs and NAbs to three commercially available IFNβ...
The clinical impact of interferon beta antibodies in relapsing-remitting MS

**Introduction**

The clinical efficacy of interferon beta (IFNβ) therapy has been demonstrated in relapsing-remitting and secondary-progressive MS (RRMS and SPMS). However, it also has been shown that administration of IFNβ may induce the development of anti-IFNβ antibodies [1–9].

Although data from the IFNβ trials seem to indicate that patients who develop neutralizing antibodies (NAbs) to IFNβ show a reduction in the clinical and radiological (i.e., MRI) benefits associated with therapy [2, 4, 6–8], the clinical significance of both NAbs and binding antibodies (BAbs) is still debated [10–12]. The purpose of this report is to evaluate the kinetics and clinical effects of BAbs and NAbs to three commercially available IFNβ products in patients with relapsing-remitting MS (RRMS). Patients with RRMS received IFNβ-1b 8 MIU subcutaneously (SC) every other day, intramuscular (IM) IFNβ-1a 30 mcg once weekly, or SC IFNβ-1a 22 mcg three times weekly for up to 4 years. The changes of BAbs and IFNβ were measured using enzyme-linked immunosorbent assay (ELISA), and positive BAbs samples were then analysed for neutralizing activity using an antiviral cytopathic effect assay. Patients were considered BAb+ if they had a positive sample with an optical density (OD) > mean + 3SD of the OD of the control sample; high BAb titers were defined as > 1:500. Patients were considered NAb+ if they had titers ≥20 LU/mL, with high NAb titers defined as > 1:100. The impact of BAbs and NAbs on relapses and Expanded Disability Status Scale (EDSS) score also was evaluated. Thirty patients were enrolled in each treatment group. Over the course of the study, 83% of patients developed BAbs to IFNβ-1b, 13% to IM IFNβ-1a, and 47% to SC IFNβ-1a. Forty percent of patients developed NAbs to IFNβ-1b, 6.7% to IM IFNβ-1a, and 26.7% to SC IFNβ-1a. Of 22 NAb+ patients, 10 patients (45.5%) demonstrated high titers of both NAbs and BAbs (20% IFNβ-1b, 3.3% IM IFNβ-1a, 10% SC IFNβ-1a). The relapse rate significantly increased after the appearance of high NAb titers (p = 0.03); however, an even higher significance level (p < 0.001) was observed in patients with high titers of both NAbs and BAbs. In 10 patients with high titers of both NAbs and BAbs, an increase in mean EDSS score from 2.2 ± 0.8 at baseline to 3.6 ± 1.2 at year 2 (p < 0.01) was observed. NAb-negative patients showed no significant change in EDSS score at year 2. These findings demonstrate that high titers of both BAbs and NAbs reduce the clinical efficacy of IFNβ in patients with RRMS, which is important for the long-term efficacy of these drugs.

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**Methods**

**Patients**

Patients 18 to 44 years of age with clinically definite RRMS, a baseline Expanded Disability Status Scale (EDSS) score of 1.0 to 3.5 inclusive, and 2.2 documented clinical exacerbations within the 2 years prior to enrolment were included in the study. Patients must have been free of clinical exacerbations at the time that IFNβ therapy was initiated. Treatment with either immunosuppressive/immunomodulatory drugs or corticosteroids was not permitted during the month prior to enrolment.

Before enrolment, all aspects of the study protocol were reviewed with each subject and informed written consent was obtained. The study protocol was approved by the university’s Institutional Review Board and was carried out according to the Declaration of Helsinki.

**Study design**

According to the treatment modality, patients with RRMS were divided into three groups: 1) patients treated with IFNβ-1b 8 MIU SC every other day; 2) patients treated with IM IFNβ-1a 30 mcg once weekly; and 3) patients treated with IFNβ-1a 22 SC mcg three times weekly. Peripheral blood was collected before initiation of therapy (baseline) and then every 3 months during treatment. Samples were allowed to clot at room temperature for 1 hour prior to centrifugation. Serum was subsequently stored at –40°C in small aliquots, which were thawed prior to testing.

**Demonstration of anti-IFNβ antibodies**

BAbs and NAbs were assayed using procedures previously described [12, 13]. BAbs to IFNβ were measured using enzyme-linked immunosorbent assay (ELISA), and positive BAb samples were subsequently analysed for neutralizing activity using an antiviral cytopathic effect assay. Patients were considered BAb positive (BAb+) if they had a positive sample with an optical density (OD) that was higher than the mean + 3SD of the OD of the control sample. Patients were considered NAb positive (NAb+) if they had a titer ≥20 LU/mL. High BAb titers were arbitrarily defined as > 1:500, and high NAb titers were defined as > 1:100.

**Effect of IFNβ antibodies on clinical outcome**

The effect of IFNβ antibodies on clinical outcome measures, including clinically documented relapses and EDSS score, was analysed. A relapse was defined as the appearance of a new symptom or the worsening of a pre-existing symptom, lasting more than 24 hours and producing a modification in the corresponding functional system of the EDSS. All relapses were treated with a high dose of methylprednisolone (1 g/day for 6 days).

**Statistical methods**

The Wilcoxon test was used to assess the association between antibody titers and clinical parameters (i.e., relapses and EDSS score), and the Spearman rank correlation test was used to assess the relationship between antibody titers and time. Differences in antibody titers were analysed using nonparametric tests. All reported p values are based on two-tailed statistical tests, with a significance level of 0.05.

**Results**

Thirty patients received IFNβ-1b 8 MIU SC every other day, 30 patients received IM IFNβ-1a 30 mcg once weekly, and 30 patients received SC IFNβ-1a 22 mcg three times weekly for 4 years. Although the patients were not randomized to treatment, no significant differences were observed among the three groups with regard to age, gender, age at disease onset, disease duration, and number of relapses during the 2 years before therapy initiation (Table 1).

Fig. 1 shows the time course of development of BAbs in each treatment group over 48 months of treatment. Fig. 2 shows the time course of development of NAbs in each treatment group. The kinetics displayed by BAbs and NAbs were similar, with the exception that BAbs developed much earlier than did NAbs. In IFNβ-1b-treated patients, BAb levels peaked by the end of the first year and plateaued during the second year. Titer slowly but progressively declined throughout the remainder of the treatment period and were detected in 30% of the patients at month 48 (3 positive out of 10 tested, p = 0.09 vs. baseline). Over the course of the study, 83.3% (25/30) of IFNβ-1b-treated patients developed detectable levels of BAbs and 40% (12/30) developed NAbs. Of the 12 patients who developed NAbs, seven had high titers (titers > 1:100) and five had low titers (titers ≤ 1:100); 10 of these patients (including six patients with high NAb titers) also had high BAb titers (titers > 1:500). Seventy-five percent of NAb+ patients (9/12) discontinued therapy within the first 2 years, and 20 (66.7%) patients discontinued treatment prior to the final follow-up visit at 4 years. Within the latter group, 10 patients terminated therapy because of disease activity, and 10 discontinued because of side effects (e.g., skin necrosis, autoimmune thyroiditis, increased liver en-

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>SC IFNβ-1a (n = 30)</th>
<th>IM IFNβ-1a (n = 30)</th>
<th>IFNβ-1b (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex, n, F/M</td>
<td>22/8</td>
<td>22/8</td>
<td>21/9</td>
</tr>
<tr>
<td>Age, years, mean ± SD</td>
<td>30 ± 6</td>
<td>30 ± 6</td>
<td>32 ± 8</td>
</tr>
<tr>
<td>Age range years</td>
<td>18–36</td>
<td>18–36</td>
<td>20–42</td>
</tr>
<tr>
<td>Disease duration, years, mean ± SD</td>
<td>5.5 ± 3.8</td>
<td>6.0 ± 3.6</td>
<td>6.4 ± 2.9</td>
</tr>
<tr>
<td>EDSS at study entry, mean ± SD</td>
<td>1.2 ± 0.8</td>
<td>1.0 ± 0.8</td>
<td>1.4 ± 1.1</td>
</tr>
<tr>
<td>Relapses in last 2 years, mean ± SD</td>
<td>2.2 ± 1.2</td>
<td>2.2 ± 1.0</td>
<td>2.5 ± 1.2</td>
</tr>
</tbody>
</table>

EDSS Expanded Disability Status Scale; IM intramuscular; SC subcutaneous; SD standard deviation
zymes, increased spasticity) or poor compliance. No correlations were found between the time of appearance of IFNβ antibodies and any of the following measures: IFNβ antibody titer, age of patient, gender, disease duration, and relapse rate before therapy.

Of the 30 patients treated with IM IFNβ-1a, none had BAbs after 1 month of therapy, one patient (3.3%) was slightly positive for BAbs at month 3, and four patients (13.3%) were slightly positive at months 9, 12, and 15. Two BAb+ patients (6.7%) also were NAb+, but only one (3.3%) had high NAB (> 1:100) and BAb (> 1:500) titers. At month 24, however, only two patients had detectable IFNβ antibodies, and only one was NAb+, but at a very low titer [1:20]. At month 36 and month 48, no detectable NABs or BAbs were found in any patient. In general, anti-IFNβ antibody levels in patients treated with IM IFNβ-1a were significantly lower than those observed in patients treated with IFNβ-1b (p < 0.01).

In patients treated with SC IFNβ-1a, 14 patients (46.7%) developed BAbs and 8 (26.7%) developed NABs during the first 2 years of therapy. Five patients (16.7%) had high NAB (> 1:100) titers, three (10%) of whom also had high BAb (> 1:500) titers. The timing of presentation and kinetics of both BAbs and NABs in these patients were similar to those of IFNβ-1b-treated patients. At month 36, a decline in both the titers and prevalence of anti-IFNβ antibodies was observed: eight patients (26.7%) had detectable levels of BAbs and five (16.7%) had detectable levels of NABs, with only two patients (6.7%) demonstrating high NAB titers.

Overall, 33 of the 90 MS patients randomized to the three treatment groups developed BAbs (25/30 IFNβ-1b, 4/30 IM IFNβ-1a, 14/30 SC IFNβ-1a) whereas 22 of the patients became NAb positive (12/30 IFNβ-1b, 2/30 IM IFNβ-1a, 8/30 SC IFNβ-1a). However, only 10 of 22 (45.5%) NAb-positive patients (6/30 [20%] IFNβ-1b, 1/30 [3.3%] IM IFNβ-1a, 3/30 [10%] SC IFNβ-1a) had high titers of both NABs and BAbs. Three or 4 years after therapy initiation, the prevalence rate of anti-IFNβ antibodies was reduced by 50%.

The relapse rate before and after the appearance of anti-IFNβ antibodies was analysed in relation to antibody appearance/titers (Table 2). Because patients had relapse rates during the 2 years preceding therapy that

Table 2 Relationship between relapse rate and either presence or titers of anti-IFNβ antibodies in IFNβ-treated patients* with relapsing-remitting MS

<table>
<thead>
<tr>
<th>p Value</th>
<th>Presence of BAbs</th>
<th>High BAb titers (&gt;1:500)</th>
<th>Presence of NABs</th>
<th>High NAB titers (&gt;1:100)</th>
<th>High BAb and NAB titers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of BAbs</td>
<td>&lt; 0.1</td>
<td>0.08</td>
<td>0.07</td>
<td>0.03</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>High BAb titers (&gt;1:500)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presence of NABs</td>
<td></td>
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<td></td>
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</tr>
</tbody>
</table>

* Patients with ≥ 2 relapses after NAb appearance

BAbs binding antibodies; NABs neutralizing antibodies
ranged from 2.2 to 2.5, “non-responders” were defined as those patients who had ≥ 2 relapses during the first 18 months after therapy initiation (i.e., the period in which the majority of IFNβ-treated patients develop high BAb and NAb titers). Although the presence of NAb (all titers – low and high – considered) did not correlate with increased relapse rate ($p = 0.07$), high NAb titers alone were significantly correlated with a higher number of relapses ($p = 0.03$). Furthermore, the significance level of the correlation with relapse rate was higher ($p < 0.001$) when high titers of both NAbs and BAbs were considered. In addition, the 10 patients (six IFNβ-1b patients, one IM IFNβ-1a patient, and three SC IFNβ-1a patients) who had both high NAb and BAb titers showed a significant increase ($p < 0.01$) in EDSS score, which increased from 2.2 ± 0.8 at baseline to 3.6 ± 1.2 at 24 months after therapy initiation. In contrast, in NAb-negative patients, there was no significant change in EDSS score (from 2.3 ± 0.9 to 2.6 ± 0.6) at the same time points.

No patients entered the secondary progressive phase of the disease during the first two years of the study. At the end of the follow-up, 6 NAb-positive patients and 5 NAb-negative patients were classified as having secondary progressive MS.

Discussion

The present study confirms and extends previous observations on the immunogenicity of IFNβ and the clinical significance of anti-IFNβ antibodies in patients with RRMS. Consistent with the results of other studies [13–17], we found that IFNβ-1b was more immunogenic than IFNβ-1a, and SC IFNβ-1a was more immunogenic than IM IFNβ-1a. Furthermore, our findings demonstrated that the simultaneous presence of high levels of NAbs and BAbs was correlated with higher levels of disease activity and progression.

In general, approximately 50% of BAb+ patients developed NAbs following administration of IFNβ therapy. In NAb+ patients, the development of BAbs is an earlier event, and BAb titers are usually higher than in NAb-negative patients. Therefore, the early detection of high BAb titers may predict the development of NAbs.

The results of the present study indicate that a period of at least 4 years was necessary to achieve a state of “tolerance” to IFNβ in the majority of patients who were positive for IFNβ antibodies. However, as previously described by Rice et al. [18] patients with high titers of IFNβ antibodies (the majority were lost during the present follow-up) may require several years before becoming tolerant.

From a clinical perspective, our findings may help clarify a currently debated aspect of IFNβ therapy. As previously discussed, data from clinical trials suggest that the development of NAbs may reduce the therapeutic efficacy of IFNβ therapy [4, 6, 8]. However, there is no consensus on the immunomodulatory role of anti-IFNβ antibodies, and some authors have published findings that refute their reported antitherapeutic effect [19–21].

The discrepancies among the published data may be explained by differences in both methodology and numbers of patients analyzed. Moreover, in the majority of the published studies, only NAb+ patients were considered; BAbs were not determined because they were considered “irrelevant antibodies” a priori, and patients were not stratified based on antibody titers. Instead, patients were usually defined as NAb+ if they had titers > 1:20 LU/mL in two consecutive samples taken over a period of 3 months. Our data suggest that the presence of high titers of both types of antibodies correlates with disease activity and progression. Therefore, the magnitude of the polyclonal anti-IFNβ (BAb + NAb) response may be responsible for the diminution in therapeutic benefit of IFNβ. These findings are not surprising given the presence of several immunogenic epitopes on the IFNβ molecule and the possibility that circulating IFNβ/IFNβ-antibody immunocomplexes can be easily removed by the reticuloendothelial system. In agreement with the results of our study, a titer-related effect of interferon antibodies was suggested in the recently published analysis of the relationship between NAb and disease activity observed in the European trial on IFNβ in Secondary Progressive MS [22].

In summary, the data in this study suggest that patients may become unresponsive to IFNβ therapy owing to the presence of elevated levels of BAbs and NAbs.

References

Heterogeneity in Response to Interferon Beta in Patients With Multiple Sclerosis

A 3-Year Monthly Imaging Study

Annie W. Chiu, BS; Nancy Richert, MD, PhD; Mary Ehrmantraut, MS; Joan Ohayon, MSN; Shiva Gupta, MD; Giuseppe Bomboi, MD; Deyce Gaindh, AB; Fredric K. Cantor, MD; Joseph A. Frank, MS, MD; Henry F. McFarland, MD; Francesca Bagnato, MD, PhD

Objectives: To investigate the heterogeneity in magnetic resonance image (MRI) patterns of response to interferon beta across patients with multiple sclerosis or within an individual patient over time.

Design, Setting, and Patients: Fifteen patients with relapsing-remitting multiple sclerosis underwent monthly MRIs and clinical examinations (6-month pretherapy phase and 36-month therapy phase) and bimonthly neutralizing antibody tests. On each MRI, the total number of contrast-enhancing lesions was noted. Therapy MRI responders were defined as those with a reduction of 60% or more in the total number of contrast-enhancing lesions during each semester of therapy.

Intervention: Subcutaneous administration of interferon beta-1b, 250 µg, every other day for 3 years.

Main Outcome Measure: Reduction in the number of contrast-enhancing lesions.

Results: Eight patients (53.3%) were MRI responders and 7 (46.7%) were nonresponders. Of those 7, 3 (20.0%) had only an initial optimal reduction of the total number of contrast-enhancing lesions, 2 (13.3%) never reached an optimal response, and 2 (13.3%) had a delayed optimal response. No clear association between neutralizing antibody profile and MRI response was evident.

Conclusions: Multiple MRI evaluations disclose that approximately only half of the patients treated with interferon beta achieve and maintain a full response to the drug over time, although an additional small number of individuals may still restore an optimal response to the drug after an initial failure.


MAGNETIC RESONANCE imaging (MRI) allows for unique visibility of inflammatory plaques, namely contrast-enhancing lesions (CELS), in patients with multiple sclerosis (MS).1 The CELs precede the occurrence of clinical relapses,2 which in turn are presumed to lead disease progression. Many clinical studies have demonstrated the ability of interferon beta to reduce CELs (for review, see the article by Clerico et al). However, little is known regarding the heterogeneity of the MRI response profiles between patients or within an individual patient over time. While important for clinicians to tailor appropriate therapeutic intervention, this information is still missing probably because there is no uniform consent as to how to assess recombinant interferon beta responsiveness. Highly variable proportions of responder patients were observed depending on the definition of responders used, the study design, and the duration.4-12

In addition to the observed interpatient variability in the profile of response to interferon beta, it is unknown whether MRI responsiveness to interferon beta changes with time and disease progression and, if so, whether this is a patient-dependent phenomenon.

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In this study, the MRI profile’s responses to interferon beta were analyzed and described in detail in a cohort of 15 patients with relapsing-remitting MS who were imaged monthly for 3 years on therapy onset. The novel aspect of the study is the unique number of monthly
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NAb ACTIVITY

Details of NAb activity and the profile of the total number of CELs in NAb-positive patients were previously reported,17 and only data complementary to the previously reported work are presented here (Table 2).

The NAbs appeared in 5 of the patients (33.3%) as early as the third month of therapy. One responder (6.7% of the study cohort and 12.5% of responders; patient 7, who corresponds to patient 3 in previously reported data17) became NAb positive but persisted as a responder even in the presence of high titers of NAbs (≥1:400). Of the 7 nonresponders, 4 (26.7% of the study cohort and 57.1% of the nonresponders) exhibited some NAb activity. Patients 5 (corresponding to patient 1 in previously reported data17) and 8 (patient 2 in previously reported data17) had transient and low NAb titers. The remaining 2 patients (13.3% of the cohort; patients 10 and 14) had high NAb titers for some time during the study period. Patient 10 (corresponding to patient 4 in previously reported data17) exhibited high NAb titers only throughout months 13 to 24 of the TP but persisted as a nonresponder throughout the entire TP. Similarly, patient 14 (corresponding to patient 5 in previously reported data17) exhibited high NAb titers as early as the ninth month of the TP until the end of the second year, but his total number of CELs never decreased to an optimal response level.

COMMENT

To our knowledge, our descriptive study provides for the first time a detailed long-term analysis of MRI patterns of patients undergoing long-term interferon beta-1b therapy. The results show that on a close monthly MRI inspection, approximately half of the patients fail therapy from an MRI perspective. Also, we show that an additional small proportion of patients may not be necessarily recognized as MRI nonresponders during the first semester of therapy, and frequent radiological monitoring is advised during the first year of therapy. Multiple MRIs, beyond the first 6 months of therapy, also disclose a small proportion of patients with a delayed but eventually sustained response to interferon beta and provide compelling information regarding the clinical outcome of patients during the course of a longer trial.

Neither MRI nor clinical parameters at the beginning of the study could segregate responders vs nonresponders. Because the number of patients is small, any definitive conclusion is precluded. However, it is noteworthy that contradictory results were obtained when examining the power of baseline characteristics in predicting outcome to therapy.6,7,9,10,18,19 In our cohort of patients, a trend was visible in that responders presented a higher disease activity in terms of the total number of CELs during the PTP. One might argue that changes due to interferon beta-1b administration may be more easily identified in patients with a higher total number of CELs during the PTP. However, on close inspection of the data, one can see that although a few responders had quite a higher total number of CELs, overall the total number of CELs among different responder types demonstrated a heterogeneous distribution.

The NAbs appeared in 5 of the patients (33.3%) as early as the third month of therapy and decreased in titers during the third year of therapy. The occurrence of NAbs was generally low in MRI responders and more prominent in nonresponders. However, no clear association between the NAb profile and MRI activity could be clearly identified within each NAb-positive patient.17

Possible limitations of this study need to be addressed before drawing conclusions. Besides the small number of patients, this was an open-label study that lacked a systematic analysis of potential effects of steroids given for clinical relapses. However, it was shown previously that steroids given for acute relapse likely do not affect the long-term response to interferon beta10,23 and that while persistently low enhancement is seen in the follow-up scans of patients treated with steroids and interferon beta, a rebound increase in the number and volume of CELs may be observed in patients who are not receiving interferon beta.20 Finally, care needs to be taken with respect to patient 7. This patient experienced 2 clinical relapses during the PTP. It is likely that part of the optimal MRI response to interferon beta-1b might be the result of some regression to the mean associated with the relative increase of the total number of CELs during the PTP. While worth mentioning, we do not think the data per se would form a bias in the interpretation of our results.

Accepted for Publication: February 18, 2008.
Published Online: November 10, 2008 (doi:10.1001/ archneur.66.1.noc80047).
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Author Contributions: Study concept and design: Gupta and Bagnato. Acquisition of data: Richert, Ehrmantraut, Ohayon, Frank, and McFarland. Analysis and interpretation of data: Chiu, Richert, Gupta, Bomboi, Gaindh, Cantor, and Bagnato. Drafting of the manuscript: Chiu, Gupta, Cantor, McFarland, and Bagnato. Critical revision of the manuscript for important intellectual content: Chiu, Richert, Ehrmantraut, Ohayon, Bomboi, Gaindh, Frank, McFarland, and Bagnato. Statistical analysis: Chiu, Gupta, McFarland, and Bagnato. Obtained funding: McFarland. Administrative, technical, and material support: Ehrmantraut, Gaindh, and Cantor. Study supervision: Frank, McFarland, and Bagnato.
Financial Disclosure: None reported.
Funding/Support: This work was supported by the Intramural Research Program of the National Institute of Neurological Disorders and Stroke, National Institutes of Health. Dr Bomboi’s contribution was sustained by a public-private partnership supported jointly by the University La Sapienza, Rome, Italy, and a grant from the Bayer-Schering Pharmaceuticals Group.
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Safety and immunogenicity of a new formulation of interferon β-1a (Rebif® New Formulation) in a Phase IIIb study in patients with relapsing multiple sclerosis: 96-week results

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Background  A new formulation of subcutaneous (s.c.) interferon-β-1a has been developed (Rebif® New Formulation, RNF), produced without fetal bovine serum and without human serum albumin as an excipient, with the aim of improving injection tolerability, and reducing immunogenicity.

Objectives  This article reports 96-week analyses of a Phase IIIb, open-label study of the safety and immunogenicity of RNF compared with historical (EVIDENCE study) and recent ( REGARD study) data on the original formulation.

Methods  Patients with relapsing multiple sclerosis (McDonald criteria) and an Expanded Disability Status Scale score < 6.0 received RNF, 44 μg s.c. three times weekly.

Results  The proportion of neutralizing antibody-positive (NAb+) patients (serum NAb status ≥ 20 neutralizing units/mL) at week 96 (last observation carried forward; primary endpoint) was 17.4% (exact 95% confidence interval [CI]: 13.0–22.5), compared with 21.4% (95% CI: 17.2–26.2) in the EVIDENCE study, and 27.3% (95% CI: 22.8–32.1) in the REGARD study. The proportion of patients NAb+ at any time during the 96 weeks was 18.9% (95% CI: 14.4–24.2), compared with 27.1% (95% CI: 22.4–32.2) and 33.7% (95% CI: 28.9–38.7), respectively. Most pre-specified categories of adverse events were reported by patients in the RNF study at a similar or lower proportion than in the EVIDENCE and REGARD studies. Injection-site reactions were experienced by fewer patients than in the EVIDENCE and REGARD studies.

Conclusions  RNF has improved overall immunogenicity and safety profiles compared with the original formulation. Multiple Sclerosis 2009; 15: 219–228. http://msj.sagepub.com

Key words: immunogenicity; injection-site reactions; interferon-β-1a; multiple sclerosis; Rebif® New Formulation; safety

Introduction  As has been observed with other therapeutically administered recombinant proteins, treatment with interferon (IFN)-β in patients with multiple sclerosis (MS) can be associated with the development of neutralizing antibodies (NAbs) [1–4]. Although the full clinical impact of NAbs is still to

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Received 5 June 2008; accepted 6 August 2008
Interferon beta-1b in secondary progressive MS

Results from a 3-year controlled study

The North American Study Group on Interferon beta-1b in Secondary Progressive MS

Dr. Hillel Panitch, Member of the North American Study Group

Abstract—Objective: To evaluate the efficacy and safety of interferon beta-1b (IFNβ-1b) in subjects with secondary progressive multiple sclerosis (SPMS). Methods: This 3-year, multicenter, double-blind, placebo-controlled, randomized trial of IFNβ-1b included 939 subjects from the United States and Canada with SPMS and Expanded Disability Status Scale (EDSS) scores ranging from 3.0 to 6.5. Subjects were randomly assigned to receive either placebo or IFNβ-1b (250 μg or 160 μg/m² body surface area), administered subcutaneously every other day. The primary outcome was time to progression by ≥1.0 EDSS point (0.5 point if EDSS score was 6.0 to 6.5 at entry) confirmed at 6 months. Secondary outcomes included mean change in EDSS score from baseline, relapse-related measures, MRI activity, and a standardized neuropsychological function test. Results: There was no significant difference in time to confirmed progression of EDSS scores between placebo-treated patients and either of the IFNβ-1b treatment groups. However, IFNβ-1b treatment resulted in improvement on secondary outcome measures involving clinical relapses, newly active MRI lesions, and accumulated burden of disease on T2-weighted MRI. Effects were similar for both IFNβ-1b treatment groups. Neutralizing antibodies to IFNβ-1b were detected in 23% of 250-μg and 32% of 160-μg/m² recipients, but their presence did not consistently affect clinical or MRI outcomes. IFNβ-1b was also well tolerated at both doses. Conclusions: Although no treatment benefit was seen on the time to confirmed progression of disability, relapse- and MRI-related outcomes showed significant benefit with both dosing regimens tested, a result consistent with the outcomes of earlier clinical trials.

NEUROLOGY 2004;63:1788–1795

Most subjects with multiple sclerosis (MS) initially experience recurrent symptoms, accompanied by transient disability, that reflect partially reversible focal inflammation and demyelination.1 Subjects who remain clinically stable between these recurrent attacks (relapses) are classified as having relapsing–remitting (RR) MS.² Within 10 years, approximately 50% of RR subjects will transition to the secondary progressive (SP) phase of MS. This phase is distinguished from RRMS by gradual progression of disability, either between acute relapses or in the absence of relapses.³ In general, focal inflammation is considered to be less pronounced during the SP phase of MS, and increasing disability presumably reflects cumulative and irreversible axonal loss.⁴

Interferon β (IFNβ) has been shown to benefit both patients with an initial demyelinating episode who are at high risk to develop MS and patients with relapsing forms of MS (either RRMS or SPMS with ongoing relapses). For example, IFNβ-1a administered once weekly delays the onset of a second demyelinating event.⁵,⁶ In addition, IFNβ has been consistently shown to reduce clinical relapses and MRI activity in patients with relapsing forms of MS.⁷-¹² the results of these trials leading to the approval of IFNβ-1b and IFNβ-1a for use as disease-modifying therapies for RRMS in North America, Europe, and other parts of the world. Subsequent trials¹³,¹⁴ and reviews of these and other studies¹⁵ suggest that higher-dose or more frequently dosed IFNβ or both offers greater and more robust clinical benefits than once-weekly IFNβ.

Four clinical trials of IFNβ in subjects with SPMS have been conducted to date.¹⁶-¹⁸ The European trial of IFNβ-1b in SPMS included 360 patients allocated to receive 250 μg of IFNβ-1b and 358 allocated...
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