Neutralizing Antibodies to Multiple Sclerosis Treatments

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ABSTRACT

OBJECTIVE: This article reviews the incidence and clinical significance of neutralizing antibodies (NAbs) in patients with multiple sclerosis (MS) undergoing treatment with interferon beta (IFNβ). Implications for practice are also discussed in light of the currently available data on the clinical consequences of NAbs in patients with MS.

SUMMARY: As with other recombinant protein drugs used for the treatment of a number of diseases, antibodies commonly develop to IFNβ products during the treatment of patients with MS. Neutralizing antibodies (NAbs) are a subset of antibodies that reduce or diminish the biologic activity of IFNβ. Three formulations of IFNβ are currently available for the treatment of relapsing-remitting MS: IFNβ-1b (Betaseron, intramuscular (IM) IFNβ-1a (Avonex), and subcutaneous (SC) IFNβ-1a (Rebif). Individual phase III clinical trials and direct comparison studies have shown that NAbs develop more frequently during treatment with IFNβ-1b than IFNβ-1a and that between the 2 IFNβ-1a products, NAbs develop more frequently during treatment with SC IFNβ-1a than IM IFNβ-1a. Data from clinical trials of IFNβ products indicate that clinical efficacy of IFNβ is reduced in NAb-positive patients.

CONCLUSION: In light of these data, the immunogenicity of IFNβ products should be considered prior to initiating treatment with IFNβ. Also, ongoing laboratory monitoring of patients treated with higher-dose IFNβ is recommended for early detection of NAbs.

KEYWORDS: Interferon-beta, Neutralizing antibodies, Multiple sclerosis

THERAPEUTIC USE OF PROTEIN PRODUCTS IS FREQUENTLY ASSOCIATED WITH ANTIBODY DEVELOPMENT. ANTIBODIES DEVELOP, ALTHOUGH AT A REDUCED RATE, EVEN WITH THE WIDESPREAD USE OF RECOMBINANT DNA TECHNOLOGY TO PRODUCE PROTEIN DRUGS THAT ARE NEARLY IDENTICAL TO THEIR ENDOGENOUS HUMAN CONTPARTS. ANTIBODIES TO BIO THERAPEUTIC AGENTS ARE BROADLY CLASSIFIED INTO BINDING ANTIBODIES AND NEUTRALIZING ANTIBODIES (NABS). BINDING ANTIBODIES INCLUDE ALL ANTIBODIES THAT CAN BIND TO THE DRUG (AND MAY OR MAY NOT INHIBIT THE DRUG), WHEREAS NABS ARE A SUBSET OF BINDING ANTIBODIES THAT CAN INHIBIT OR NEUTRALIZE THE BIOLOGIC ACTIVITY OF THE PROTEIN DRUG.

NAbs have been shown to develop following treatment with a variety of recombinant human protein drugs, including insulin, erythropoietin, and coagulation factor VIII (Table 1).1-6 In most cases, the main clinical outcome of NAbs is loss of efficacy of the protein drug. For example, the use of interferon alpha (IFNα) for the treatment of hepatitis or cancer has been associated with NAb development resulting in nonresponsiveness to treatment, disease reactivation, and decreased response duration.7-10 Similarly, in patients treated for cervical dystonia with botulinum toxin type A, the development of NAbs rendered the treatment ineffective.11

In addition to reduced efficacy, more severe clinical effects may be observed when NAbs form against a protein that has an import-

TABLE 1

<table>
<thead>
<tr>
<th>Disease/Condition</th>
<th>Consequence of Antibody</th>
<th>Biotherapeutic Agent</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes</td>
<td>Loss of efficacy</td>
<td>Insulin</td>
<td>Meager et al.7</td>
</tr>
<tr>
<td>Acute myocardial infarction</td>
<td>Streptokinase, Staphylokinase</td>
<td>Rosenschein et al.11</td>
<td>Vanderven et al.12</td>
</tr>
<tr>
<td>Adenosine deaminase deficiency</td>
<td>Adenosine deaminase</td>
<td>Chaffee et al.43</td>
<td></td>
</tr>
<tr>
<td>Cervical dystonia</td>
<td>Botulinum toxin</td>
<td>Lusher, 2000.3</td>
<td></td>
</tr>
<tr>
<td>Hemophilia A</td>
<td>Factor VIII</td>
<td>Freund et al.7, Quesada et al.10</td>
<td></td>
</tr>
<tr>
<td>Malignant carcinoma tumors</td>
<td>Interferon alpha-2</td>
<td>Sorensen et al.13</td>
<td></td>
</tr>
<tr>
<td>Multiple sclerosis</td>
<td>Interferon beta</td>
<td>IFNβ MS Study Group, PRISMS Study Group, Sorensen et al.13</td>
<td></td>
</tr>
<tr>
<td>Cancer</td>
<td>Interleukin-2 (IL-2)</td>
<td>Prümmer et al.14</td>
<td></td>
</tr>
<tr>
<td>Hypogonadotropic azoospermic men</td>
<td>Gonadotropin-releasing hormone</td>
<td>Blumenfeld et al.45</td>
<td></td>
</tr>
<tr>
<td>Cutaneous T-cell lymphoma</td>
<td>Denileukin difitox</td>
<td>Olsen et al.15</td>
<td></td>
</tr>
<tr>
<td>Hypogonadotropic hypogonadism</td>
<td>Human chorionic gonadotropins</td>
<td>Clauser et al.16</td>
<td></td>
</tr>
<tr>
<td>Carcinoma</td>
<td>GM-CSF*</td>
<td>Raghavan and Wadhua17</td>
<td></td>
</tr>
<tr>
<td>Anemia</td>
<td>Neutralization of native protein</td>
<td>Casadevall et al.18, Prabhakar and Muhlfelder.19</td>
<td></td>
</tr>
</tbody>
</table>

* GM-CSF = granulocyte-macrophage colony-stimulating factor.
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Incidence of Neutralizing Antibodies to Interferon Beta

The incidence of NABs (titer ≥20) among IFNβ products varies widely, as reported in phase III trials (Table 2). In the pivotal phase III trial of IFNβ-1b, 47% of patients who received IFNβ-1b 1.6 million international units (MIU) and 45% of patients who received 8 MIU SC every other day developed NABs. In a study conducted by the European Study Group on IFNβ-1b in secondary progressive MS, approximately 28% of patients (100 of 360) who received SC IFNβ-1b 8 MIU every other day tested positive for NABs at some time during the study, with most patients becoming NAB positive in the first 6 months of treatment.

Phase III studies with IM IFNβ-1a have shown consistently lower levels of immunogenicity, with incidences of NABs ranging from 2% to 5.8%. Interestingly, in the earlier pivotal phase III trial of IM IFNβ-1a, NABs were detected in 14% of patients who received IM IFNβ-1a 30 mcg once weekly at week 52, 21% at week 78, and 22% at week 104. The reduction in immunogenicity of IM IFNβ-1a after the pivotal phase III trial is thought to be due to improvements in manufacturing, purification, and storage processes of the now commercially available IM IFNβ-1a product. In the pivotal phase III trial of SC IFNβ-1a (Prevention of Relapses and Disability by Interferon β-1a Subcutaneously in Multiple Sclerosis [PRISMS] study), NABs were observed in 23.8% of patients who received SC IFNβ-1a 22 mcg and 12.5% of patients who received SC IFNβ-1a 44 mcg. In PRISMS-4, which was the extension study of the phase III trial, 23.7% of patients who received SC IFNβ-1a 22 mcg and 14.3% of patients who received SC IFNβ-1a 44 mcg had a positive test result for NABs. However, in patients who had been on placebo during the first 2 years of the trial and received SC IFNβ-1a 22 mcg or 44 mcg during years 3 and 4, the incidence of NABs was 27.7% and 24.4%, respectively.

An evaluation of the relative immunogenicity of the different IFNβ products by comparing incidences among phase III trials is not ideal because of differences in the methods used for detecting and reporting NABs. However, results from individual phase III

<table>
<thead>
<tr>
<th>IFNβ Product</th>
<th>Dose and Frequency of Administration</th>
<th>Incidence of NABs (% of Patients)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>IFNβ-1b (Betaseron)</td>
<td>SC 3.5 times weekly 1.6 MIU*</td>
<td>47.0</td>
<td>IFNB MS Study Group</td>
</tr>
<tr>
<td></td>
<td>8 MIU</td>
<td>45.0</td>
<td>European Study Group</td>
</tr>
<tr>
<td></td>
<td>8 MIU</td>
<td>27.8</td>
<td></td>
</tr>
<tr>
<td>SC IFNβ-1a-RA (Rebif)</td>
<td>SC 3 times weekly 22 mcg</td>
<td>23.8</td>
<td>PRISMS Study Group</td>
</tr>
<tr>
<td></td>
<td>44 mcg</td>
<td>12.5</td>
<td>SPECTRIMS Study Group</td>
</tr>
<tr>
<td></td>
<td>22 mcg</td>
<td>20.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>44 mcg</td>
<td>14.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>44 mcg</td>
<td>25.0</td>
<td></td>
</tr>
<tr>
<td>IM IFNβ-1a-Avonex</td>
<td>IM once weekly 30 mcg</td>
<td>22.0</td>
<td>Jacobs et al</td>
</tr>
<tr>
<td></td>
<td>30 mcg</td>
<td>2.0</td>
<td>Jacobs et al</td>
</tr>
<tr>
<td></td>
<td>30 mcg</td>
<td>2.3</td>
<td>Clanet et al</td>
</tr>
<tr>
<td></td>
<td>60 mcg</td>
<td>5.8</td>
<td>Cohen et al</td>
</tr>
<tr>
<td></td>
<td>60 mcg</td>
<td>3.3</td>
<td>Panitch et al</td>
</tr>
<tr>
<td></td>
<td>30 mcg</td>
<td>2.1</td>
<td>Panitch et al</td>
</tr>
</tbody>
</table>

* SC = subcutaneously. † MIU = million international units. ‡ IM = intramuscularly.

FIGURE 1 Factors Affecting Immunogenicity of Biotherapeutic Agents

The ability of a biologic protein product to trigger formation of antibodies is influenced by its structural properties as well as other factors, including formulation, presence of contaminants and impurities, route of administration, length of treatment, and dose.


TABLE 2 Incidence of Neutralizing Antibodies (NABs; Titer ≥20) in Phase III Clinical Trials of Interferon Beta (IFNβ)
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**Factors Affecting Immunogenicity of Interferon Beta Products**

All 3 IFNβ products are recombinant protein drugs. Some factors affecting the immunogenicity of IFNβ products are shown in Figure 1.1 These include the protein sequence and molecular structure of the drug, manufacturing and storage conditions, and route and frequency of administration. Unlike IFNβ-1a formulations, which are both identical in protein sequence to the natural human IFNβ, IFNβ-1b has a serine-to-cysteine substitution at position 17 and a deletion of the N-terminal methionine residue.20 Also, IFNβ-1b is produced in *Escherichia coli* bacteria, whereas IM IFNβ-1a and SC IFNβ-1a are produced in mammalian cells (Chinese hamster ovarian cells). Because it is produced in bacterial cells, IFNβ-1b is not glycosylated. The lack of glycosylation is thought to result in an increased tendency of IFNβ-1b to form aggregates that can trigger the formation of antibodies.25 The differences in protein sequence and molecular structure between IFNβ-1b and IFNβ-1a likely account for the observed greater incidence of NAbS with IFNβ-1b compared with either of the IFNβ-1a products.

Given that the 2 IFNβ-1a formulations have an identical protein sequence, differences in their immunogenicity are likely due to manufacturing, purification, and storage conditions. It is conceivable that differences in these conditions can lead to the production of recombinant proteins with different glycosylation patterns. Also, under differing conditions, oxidation and deamidation of amino acids may vary. Indeed, in the case of IM IFNβ-1a, improving the manufacturing process resulted in a less immunogenic product. Other factors affecting the immunogenicity of IFNβ may include dose, route of administration, length of treatment, and frequency of administration. Both IFNβ-1b and SC IFNβ-1a are administered 3 to 3.5 times weekly by SC injection. In contrast, IM IFNβ-1a is administered once weekly by IM injection.

Although Ross et al. showed that SC administration of IFNβ is more immunogenic than IM administration,22 Bertolotto et al. showed no differences in immunogenicity between SC and IM administration of IFNβ.23 Hence, the route of administration is a potential factor influencing immunogenicity.

**Impact of Neutralizing Antibodies on Biologic Activity and Clinical Efficacy of Interferon Beta Products**

**Biologic Activity**

In general, NAbS are detected based on their ability to diminish the biologic activity of IFNβ in vitro. The ability of NAbS to interfere with the in vivo biologic activity of IFNβ has also been demonstrated.13-15 Typically, the biologic activity of IFNβ is determined by measuring levels of biologic markers of IFNβ activity, such as neopterin, myxovirus resistance protein A (MxA), and β2-microglobulin.

Measurements of serum neopterin and β2-microglobulin levels before and 48 hours after IM IFNβ-1a injection were conducted in...
the open-label, safety-extension study of the pivotal phase III trial of IM IFNβ-1a. The levels of serum NAb titers correlated with measurements of neopterin levels (Figure 2), such that the mean increase in neopterin level after IFNβ-1a injection was significantly lower in NAb-positive patients (titer 5 to 19 and titer ≥20) compared with NAb-negative patients (P = 0.012 and P = 0.001, respectively). Similar results were also observed with serum levels of β2-microglobulin. Another study used MxA as a marker for the biologic activity of IFNβ in patients with MS who received IFNβ-1b and a healthy control group. MxA levels were significantly lower in NAb-positive patients compared with NAb-negative patients (P<0.001). Furthermore, the levels of MxA in NAb-positive patients were similar to those of the untreated healthy control group, suggesting that the biologic activity of IFNβ-1b in these patients was completely inhibited by NAbs. Inhibition of MxA expression by NAbs has also been reported at the level of messenger ribonucleic acid (mRNA), with MxA mRNA levels being significantly lower in persistent NAb-positive (≥2 consecutive positive samples titer ≥20) patients compared with NAb-negative (P<0.0001) and isolated NAb-positive (1 positive titer ≥20) patients (P<0.005).

Clinical Efficacy

NAbs have been shown to reduce the clinical efficacy of IFNβ in MS patients, based on increased relapse rate and lesion activity on magnetic resonance imaging (MRI). In the pivotal phase III trial of IFNβ-1b, the mean relapse rate during 18 to 36 months of treatment was significantly greater in NAb-positive patients (1.16 per year) compared with NAb-negative patients (0.50 per year) (Figure 3). In fact, the relapse rate in NAb-positive patients was similar to that observed in patients given placebo (1.02 per year, P < 0.05). Similarly, in the 2-year extension study of the phase III trial of SC IFNβ-1a (PRISMS-4), in patients treated with SC IFNβ-1a 44 mcg, NAb-positive patients experienced a significantly higher mean relapse rate during years 3 and 4 (0.81) compared with NAb-negative patients (0.50, P = 0.002).

In addition to data from phase III studies of IFNβ, a recent study by the Danish Multiple Sclerosis Study Group also provides evidence of diminished clinical efficacy of IFNβ in NAb-positive patients. The study involved 541 patients randomly selected from all patients in Denmark who started treatment with IFNβ between 1996 and 1999. Yearly measurements of NAbs in these patients revealed that relapse rates were significantly higher during NAb-positive periods (0.64 to 0.70) than they were during NAb-negative periods (0.43 to 0.46, P<0.03). Furthermore, the proportion of relapse-free patients was significantly lower (P = 0.0064), and the median time to first relapse was significantly reduced (by 244 days; Kaplan-Meier analysis, log rank test 6.83, P = 0.009) in NAb-positive patients compared with NAb-negative patients.

Decreased efficacy of IFNβ due to NAbs has also been noted using MRI measures. Patients who developed NAbs to IFNβ-1b had significantly more enlarging T2 lesions than NAb-negative patients (0.41 versus 0.19 between years 1 and 2 [P≤0.03], 0.589 versus 0.26 between years 3 and 4 [P = 0.011]). Furthermore, NAb-positive patients also showed an increased tendency to form new lesions (mean values of 1.03 in NAb-positive patients versus 0.40 in NAb-negative patients, P = 0.067). Data from the open-label extension study of the pivotal phase III trial of SC IFNβ-1a (PRISMS-4) provide even stronger evidence of diminished therapeutic effects of IFNβ on MRI due to NAbs (Table 3). After 4 years of treatment with SC IFNβ-1a, disease burden on MRI was decreased by 8.5% from baseline in NAb-negative patients compared with a 17.6% increase in disease burden in NAb-positive patients (P<0.001). The values in NAb-positive patients approached those of patients treated with placebo during the first 2 years of the study. Furthermore, the median number of T2 active lesions was 1.4 in NAb-positive patients compared with 0.3 in NAb-negative patients (P≤0.001).

<table>
<thead>
<tr>
<th>TABLE 3</th>
<th>Decrease in Efficacy of SC IFNβ-1a (Rebif) 44 mcg in the Presence of Neutralizing Antibodies (NAbs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of T2 active lesions</td>
<td>NAb−</td>
</tr>
<tr>
<td>Burden of disease</td>
<td>−8.5%</td>
</tr>
</tbody>
</table>

Data from the open-label extension study of the pivotal phase III trial of SC IFNβ-1a (PRISMS-4) provide even stronger evidence of diminished therapeutic effects of IFNβ on MRI due to NAbs (Table 3). After 4 years of treatment with SC IFNβ-1a, disease burden on MRI was decreased by 8.5% from baseline in NAb-negative patients compared with a 17.6% increase in disease burden in NAb-positive patients (P<0.001). The values in NAb-positive patients approached those of patients treated with placebo during the first 2 years of the study. Furthermore, the median number of T2 active lesions was 1.4 in NAb-positive patients compared with 0.3 in NAb-negative patients (P≤0.001). Although only a small number of patients develop NAbs to IM IFNβ-1a, results in these patients are similar to those noted with IFNβ-1b and SC IFNβ-1a. Patients who developed NAbs to IM IFNβ-1a have
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![Figure 4](image_url)

**Figure 4** Time Course for Development and Clinical Consequences of Neutralizing Antibodies (NAbs)

- **NAb Triggered**
- **NAb Appears and MRI Impacts**
- **6-Month Persistent Effect**
- **When Clinical Effects May Become Apparent**

**Duration of Phase III Trials**

- **Extension Studies**

In MS patients undergoing treatment with IFNβ, NAb can be detected as early as 3 months. Once formed, the effect of NAb on MRI measures of disease burden is apparent by 12 months. The effect of NAb on clinical outcomes is not apparent until after 18 to 24 months of treatment.

been shown to have an increased number of gadolinium-enhanced lesions on MRI compared with NAb-negative patients (mean values of 1.7 versus 0.6; P = 0.062). The number of lesions in NAb-positive patients were similar to that seen in patients who received placebo (mean of 1.6 lesions).

**Temporal Aspects of the Development of Neutralizing Antibodies to Interferon Beta**

The time course of the development of NAb is an important facet of monitoring and assessing the clinical effects of NAb during treatment with IFNβ. In general, NAb becomes detectable at any time between 3 and 18 months following initiation of treatment with IFNβ (Figure 4).22-34,36,37 An 18-month study that compared the immunogenicity of the 3 IFNβ products showed that, although patients continued to develop NAb to each of the 3 products throughout the study period, 76% developed NAb during the first 9 months and a further 14% developed NAb by 12 months. Thus, 90% of the NAb-positive patients in the study developed NAb during the first year of treatment with IFNβ.22 Results from clinical trials indicate that in NAb-positive patients undergoing treatment with IFNβ, the effects of NAb on MRI measures of disease burden become apparent at approximately 1 year and effects on clinical outcomes after 18 to 24 months of treatment.20,34 Thus, short-term studies (<2 years) cannot adequately assess the impact of NAb on the clinical efficacy of IFNβ.

An unresolved question with regard to the clinical relevance of NAb is how long NAb persist once they are formed. Available data indicate that once formed, NAb can persist for several years.22-34 In a recent study by the Danish Multiple Sclerosis Study Group, 45% of patients were NAb-positive to IFNβ-1b at 1 year, 35% at 3 years, and 28% at 4 years.25 Thus, approximately 80% of patients who were NAb-positive to IFNβ-1b remained positive over 3 years and approximately 70% remained NAb-positive over 4 years. However, these data are difficult to interpret because a large proportion of patients dropped out of the study, and no information was provided regarding the number of NAb-positive and NAb-negative patients who discontinued the study.25 There is evidence that the persistence of NAb is dependent on both NAb titer (higher-titer NAb persist longer) and IFNβ product.26

**Implications for Practice**

The Consortium of Multiple Sclerosis Centers recently published a list of consensus statements (>70% agreement) regarding the issue of NAb in patients with MS; this list was developed based on the opinions of 33 researchers in the area of NAb.22 Of note, this group of experts believes that NAb should be one of the factors that clinicians consider in the ongoing management of MS patients and that future studies should be conducted to determine how best to counteract NAb.22 Specific recommendations for NAb testing and the management of NAb-positive patients are provided in the article in this supplement by Sheldon J. Rich et al.

As discussed in the preceding sections, data from a number of clinical studies have shown that NAb can develop in MS patients undergoing treatment with IFNβ, but there is no way to predict which patients will develop NAb. Key evidence from these studies that should be considered when making treatment decisions relating to IFNβ treatment in MS patients include the following:

- NAb (titer ≥20) can reduce the bioavailability and clinical efficacy of IFNβ.
- The incidence of NAb varies with the 3 IFNβ preparations. IFNβ-1b treatment is more immunogenic than IFNβ-1a treatment, and between the 2 IFNβ-1a products, SC IFNβ-1a treatment is more immunogenic than IM IFNβ-1a treatment. Prior to initiating treatment with an IFNβ product, these differences in immunogenicity of IFNβ products should be considered.
- IFNβ-treated patients who experience worsening in clinical status should be tested for the presence of NAb. For patients who have a positive test result for NAb, switching to another IFNβ product is not recommended because antibodies are cross-reactive among IFNβs.27
- Given that the clinical effects of NAb are manifested several months after they develop, ongoing monitoring and early detection of NAb in patients at higher risk (i.e., those on higher-dosing, more frequently administered, SC IFNβs) will likely improve the quality of treatment received by MS patients undergoing treatment with IFNβ.

**Conclusions**

Data from a number of clinical trials indicate that NAb can reduce the therapeutic benefits of IFNβ treatment in patients with MS. Loss of clinical efficacy has been observed in these studies in the form of increased relapse rates and disease burden on MRI in NAb-positive patients. Another important issue is the persistence of NAb once they are formed. Available data indicate that once...
formed, NABs tend to persist for several years. In light of these data, the immunogenicity of IFNβ products should be considered prior to initiating treatment with IFNβ. Also, ongoing laboratory monitoring of patients treated with higher-dose IFNβ is recommended for early detection of NABs.

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