Neutralising antibodies

Neutralising antibodies to interferon beta during the treatment of multiple sclerosis

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The significance of the generation of antibodies in response to interferon beta administration is discussed

Patients with multiple sclerosis (MS) receiving interferon beta may develop neutralising anti-interferon beta antibodies (NABs) during treatment. These NABs are clinically relevant and reduce the clinical efficacy of interferon beta. Although there is lack of consensus on how these antibodies should be measured, the relative prevalence of NABs induced by different interferon beta products seems to be consistent between studies. Subcutaneous interferon beta-1b (Betaferon) is the most immunogenic, followed by subcutaneous interferon beta-1a (Rebif), with intramuscular interferon beta-1a (Avonex) being the least immunogenic. Differences between the interferon beta products with regard to their structure/biochemistry, formulation, dose, route of administration, and dose frequency are likely to contribute to these observed differences in immunogenicity. This editorial highlights the consequences of NABs formation on the biological and clinical activity of interferon beta and the implications NABs have for the practicing neurologist and patient with MS.

BACKGROUND

Interferon beta is an established first line treatment in relapsing remitting MS.[1–5] As has been observed with other biological agents,[6] antibodies are sometimes generated in response to interferon beta administration.[7–10] A subset of these antibodies inhibit or neutralise (NABs) the biological activity of interferon beta. This editorial will attempt to clarify technical issues of NABs measurement, the clinical significance of NABs, differences between the currently available interferon beta products, and the clinical implications of NAB development.

ANTIBODIES ELICITED BY INTERFERON BETA

An immune response against protein based drugs is not unusual.[6] For example, neutralising antibodies have been reported during treatment with interferon alfa for viral hepatitis B and C, hairy cell leukaemia, and other types of cancer,[9 10] during treatment with bovine or porcine insulin for diabetes mellitus,[11] with human growth hormone [12] and factor VIII and IX therapy in haemophilia.[13]

Antibodies can be measured using a "binding assay", such as an ELISA. Only a subset of binding antibodies is neutralising. An in vitro or bioassay is required to identify NABs. A binding assay is usually used to screen patients for the presence of antibodies, before specifically screening for neutralising activity—that is, if the patient is negative for binding antibodies, there is no need to test for NABs.[14] NAB positivity is defined by the ability of a serum sample to neutralise an in vitro biological activity of interferon beta. Although there are many biological activities of type I interferon, the most common assays utilise its antiviral effects or its ability to induce the MxA protein (myxovirus-resistance protein). The antiviral assay is currently the standard method recommended by the World Health Organisation[15] to measure interferon activity and is based on the measurement of the virus induced cytopathic effect. Unfortunately, different laboratories often use different cell lines and viruses and hence these assays are not standardised. The MxA induction assay is becoming increasingly popular.[16] Of the usual biological markers of interferon beta activity in peripheral blood (neopterin, β-2-microglobulin, 2′5′ oligoadenylate synthetase, and Mx proteins (A and B)), Mx proteins have a relatively high dose dependent specificity for type I interferons.[9 14]

PROBLEMS ASSOCIATED WITH NABs ASSAYS

(1) NABs assays are not necessarily a measure of antibodies that bind interferon beta. This can lead to false positive readings because of non-antibody factors that inhibit the antiviral activity of the interferon.[17] To avoid this NAB quantisation should include serial sample dilutions along with controls for toxicity and endogenous interferon activity for each serum sample.[18]

(2) The NAB positivity rate varies depending on the selected sensitivity of the assay. This depends on the type of cells, the virus used, the amount of virus added, the initial dilution of the test serum, and the amount of interferon added to the assay that the antibodies must neutralise. In the case of the Mx assay, the method and reagents used to quantify Mx production are critical. The amount of interferon added to the bioassays is one of the more controversial aspects; adding too much interferon to the assay can lead to low NABs rates and adding too little interferon can result in identifying patients as positive when they have levels of NABs that are probably clinically irrelevant.[19 20]

(3) The interpretation of when a patient is NABs positive varies from study to study. Some regard positivity as being two consecutive positive results (Berlex/Schering) whereas others base positivity on a single positive result (Serono/Biogen). Furthermore, there is no consensus among the pharmaceutical industry with regard to the level of titre at which NABs become biologically relevant,[14 18] and therefore the proportion of patients developing NABs is reported using different titre cut off levels.

CURRENTLY LICENSED INTERFERON BETA PRODUCTS USED IN THE TREATMENT OF MS

Three interferon products have been marketed for the treatment of MS: Betaferon (Schering AG), which is marketed as Betaseron (Berlex Laboratories) in the United States, Avonex (Biogen), and Rebif (Ares-Serono). Product characteristics are compared in table 1. The immunogenicity of these three products has been examined in all of the phase 3 and phase 4 clinical trials. The lack of standardisation of assay techniques and definitions of seropositivity make it very difficult to compare the reported immunogenicity of the different products between clinical studies. However, a sufficient number of studies have now been performed to draw some conclusions. Among the licensed products, interferon beta-1b is more immunogenic than the interferon beta-1a products. [2–5 19–22] The difference in immunogenicity between interferon beta-1b and interferon beta-1a is not surprising given that interferon beta-1b has a cysteine to serine substitution at position 17, a deletion of the N-terminal methionine residue, and, unlike the natural protein is produced in *E coli* bacteria and is therefore non-glycosylated. Interferon beta-1a on the other hand is produced in mammalian cells, from the natural human gene sequence and is glycosylated.

Abbreviations: MS, multiple sclerosis; NAB, neutralising anti-interferon beta antibody

Table 1 Currently licenced interferon beta products used to treat RRMS

Characteristic	Betaferon/ Betaseron inteferon-1b	Avonex interferon-1a	Rebif interferon beta-1a
Manufacturer	Schering AG, Germany / Berlex, CA, USA	Biogen, France	Ares-Serono, UK
Approved	1995 in Europe 1994 in the US	1997 in Europe 1996 in the US	1998 in Europe 2002 in the US
Site of production	*E coli* bacteria cells	Chinese hamster ovary cells	Chinese hamster ovary cells
Amino acid sequence	Cysteine mutation at position 17	Identical to human inteferon beta	Identical to human IFNβ
N-terminal methionine	No	Yes	Yes
Glycosylated	No	Yes	Yes
Molecular weight	18.5 kDa	22–24 kDa	22–24 kDa
Excipients	Human serum albumin, di- and mono-basic sodium phosphate, sodium chloride final pH 7.2	Human serum albumin, di- and monobasic sodium phosphate, sodium chloride final pH 7.2	Mannitol, human serum albumin, sodium acetate, acetic acid, sodium chloride, final pH 3.8.
Therapeutic use	RRMS, secondary progressive MS	RRMS	RRMS
Therapeutic effect	Decreases frequency and severity of relapses Delay in time to progression of MS	Decreases frequency of relapses Slows progression of disability	Decreases frequency and severity of relapses Slows progression of disability
Therapeutic dose	250 µg	30 µg	22 µg and 44 µg
Specific activity	32 MIU/mg	>300 MIU/mg	>300 MIU/mg
Route of administration	Subcutaneous (SC) only	Intramuscular (IM) only	Subcutaneous (SC) only
Bioavailability	IM and SC effects similar in duration but different in effect	IM availability is threefold higher than SC	SC and IM produced equivalent exposure to IFNβ
Frequency of administration	Every other day	Once weekly	Three times per week
Average weekly dose	875 µg	30 µg	66 µg and 132 µg
NABs production reported in pivotal clinical trials conducted before drug approval	45% Reduction in clinical efficacy becoming evident at 18–24 months	24% in Phase III trial 3–5% in subsequent trials	12.5–24% after 24 months
Assay used for NABs analysis	CPE	CPE	CPE

References: The European Agency for the evaluation of medicinal products. Summary of product characteristics Avonex. Rev 1, 22 April 1999, CPMP/1063/96. The European Agency for the evaluation of medicinal products. Summary of product characteristics Betaferon (note: also Betaseron, Berlex, CA, USA). Rev 3 The European Agency for the evaluation of medicinal products. Summary of product characteristics, Rebif CPMP/0022/98.

Somewhat surprising is the reported differences in immunogenicity between the two interferon beta-1a preparations, Avonex and Rebif. This may be attributable to differences in the manufacturing, storage, and formulation of these products. For example, the difference in the immunogenicity between the closely related interferon alfa-2a and interferon alfa-2b (interferon alfa-2a was approximately 10 times more immunogenic than interferon alfa-2b[23 24]) was attributed to a oxidation and aggregation of the protein during purification and storage.[10] Effects of manufacturing on the immunogenicity of interferon beta-1a has been observed for Avonex. The interferon beta-1a Avonex preparation used in the pivotal phase 3 trial resulted in 24% of the treated patients developing NABs.[3] However, the immunogenicity has subsequently decreased fivefold to between 2% and 5%, presumably as a result of the introduction of a new manufacturing process for the commercial product.[19 22] In comparison, 12.5%–24% of patients treated with a the other interferon beta-1a formulation (Rebif) develop NABs.[4 25] This difference may be explained by the route, dose, and frequency of protein administration.[18 20] In the OWIMS study 5.3% of patients receiving interferon beta-1a (Rebif) 22 µg subcutaneously weekly developed NABs compared with 16.3% receiving 44 µg subcutaneously weekly.[26] Similarly, in a dose comparison study of interferon beta-1a patients receiving 30 µg by intramuscular injection weekly had a lower rate of NABs formation than the group receiving 60 µg by intramuscular injection weekly, 2.2% compared with 5.8% (Professor M Clanet, platform presentation ENS 2001). In comparison in the PRISMS study of interferon beta-1a (Rebif) and its extension phase, about 14% of patients receiving 44 µg three times a week developed NABs compared with 24% receiving 22 µg three times a week.[25] The lower incidence of NABs in the high dose Rebif group may be a spurious finding as a result of persistent circulating interferon beta-1a quenching or artificially lowering NABs titres. At least 10% of serum samples from patients receiving Rebif 22 µg thrice weekly have detectable levels of interferon beta-1a up to 48 hours after a subcutaneous injection.[18] You would expect this figure to be higher with Rebif 44 µg thrice weekly. Another comparison of the immunogenicity of the interferon beta-1a products comes from the recently completed 12 month head to head EVIDENCE study, 25% of patients receiving Rebif 44 µg thrice weekly developed NABs compared with 2% of Avonex treated patients (http://www.fda.gov/cber/review/ifnbser030702r1.pdf). Although these results are preliminary and incomplete they are not consistent with the PRISMS study and need clarification. However, they do provide further evidence that there are differences between the two interferon beta-1a products with regard to their ability to induce NABs.

CLINICAL SIGNIFICANCE OF NABs TO INTERFERON BETA

Efficacy

The kinetics of NABs formation varies depending on the product and dose regimen. NABs become detectable between 3 and 18 months after the start of treatment.[19 27 28] They appear sooner with interferon beta-1b, with the majority of patients becoming positive six months after starting treatment, compared with interferon beta-1a, in which it takes 9–15 months for the NAB positive rate to reach a plateau.[18] Negative effect of NABs on efficacy, particularly for interferon beta-1a, are delayed and not detectable in trials of less than a duration of two years. In the PRISMS study there were no reported difference in the clinical and MRI end points between NAB positive and NAB negative patients at two years.[4] However, in the four year extension phase of the study the relapse rate was 62% higher (0.81 compared with 0.50, $p=0.002$), the median number of T2 active lesions was nearly five times greater (1.4 compared with 0.3, $p<0.01$) and the median change from baseline in the MRI burden of disease was three times greater (+17.6% compared with −8.5%, $p<0.001$) in NAB positive compared with NAB negative patients.[25] The +17.6% median change from baseline in the burden of disease equates to +4.4%/year is similar to the +5.5%/year median increase noted in the placebo treated patients within the first two years of the study.[4] These data are the strongest evidence yet that interferon beta

Table 2 Incidence of NABs to interferon beta in MS patients treated with interferon beta

			Betaferon (Betaseron)			Avonex			Rebif		
Author/year	Duration of follow up	Assay	N	(%)	Reduced response	N	(%)	Reduced response	N	(%)	Reduced response
Fernandez *et al.*[49]	12 months	CPE	31	24	No	22	14	No	—	—	—
Ross *et al.*[18]	24 months	ANB	311	60	NR	140	NR	NR	143	about 50	NR
Jacobs *et al.*[48]	Up to 30 months	CPE	—	—	—	141	<1 (18 m) 2 (24–30 m)	NR	—	—	—
Myhr *et al.*[9]	Mean = 11 months	ANB MxA	10	80	NR	9	22	NR	—	—	—
Rudick *et al.*[19]	24 months	CPE	43 23	23 (12–18 m) 26 (>18 m)	Yes	70 33	6 (18 m) 3 (24 m)	Yes	—	—	—
Jacobs *et al.*[3]	24 months	NR	—	—	—	158 85	14 (1 y) 22 (2 y)	NR	—	—	—
Giovannoni *et al.* (Unpublished data, 2001)	Mean = 31 months (range = 12–48 m)	CPE	32	38	No	18	0	No	23	43	No
Kivisakk *et al.*[21]	Mean = 8–11 m (range 1–46 m)	CPE	48	44	No	20	5	No			
Deisenhammer *et al.*[14]	17 months	MxA	59	15 (1–31 months)	Yes	—	—	—	—	—	—
Rice *et al.*[45]	8 years	MxA	28	50 (1 y) 11 (8 y)	—	—	—	—	—	—	—
Cook *et al.*[49]	16 months	MxA	64	39 (>1:20) 22 (>1:60)	Yes	98	9 (>1:20) 7 (>1:60)	Yes	—	—	—
European Study group[5]	36 months	MxA	360	27.8	Yes	—	—	—	—	—	—
IFNβ MS Study Group[27]	36 months	AVA	91	38 (3 y)	Yes	—	—	—	—	—	—
Antonelli *et al.*[7]	24 months	CPE	—	—	—	—	—	—	35	16.7 (11 μg sc 3×/week)	No
									33	15.1 (33 μg sc 3×/week)	
OWIMS[26]	48 weeks	CPE	—	—	—	—	—	—	95	5.3 (22 μg sc/week)	No
									98	16.3 (44 μg sc/week)	
PRISMS-2[4]	24 months	CPE	—	—	—	—	—	—	189	24 (22 μg sc 3×/week)	No
									184	13 (44 μg sc 3×/week)	
PRISMS-4[25]	48 months	CPE	—	—	—	—	—	—	167	24 (22 μg sc 3×/week)	Yes
									167	14 (44 μg sc 3×/week)	
INCOMIN[52]	24 months	CPE	96	30 (1 y) 22 (2 y)	No	88	7 (1 y) 6 (2 y)	No	—	—	—
SPECTRIMS[53]	36 months	CPE	—	—	—	—	—		209	21 (22μg sc 3×/week)	Yes
									204	15 (44μg sc 3×/week)	
Bertolotto[54]	6–18 months	CPE	29	31	—	44	2	—	52	15	—
EVIDENCE*	12 months	CPE	—	—	—	294	5 (>1:5) 2 (>1:20)	No**	298	33 (>1:5) 25 (>1:20)	No**

ELISA = enzyme linked immunosorbent assay, ANB = antiviral neutralisation bioassay, CPE = cytopathic effect, AVA = anti-viral activity. *EVIDENCE Study, 2002, preliminary data, http://www.fda.gov/cber/review/ifnbser030702r1.pdf. **In the EVIDENCE study p values were not presented, but the memorandum mentions that there were differences between NAB+ve and NAB-ve patients receivig Rebif 44 μg sc thrice weekly.

has little if any clinical and MRI efficacy in the presence of NABs.

In both neutralising and binding assays antibodies elicited in response to one interferon beta product cross reactive with other interferon beta products.[29 30] Because of the cross reactivity of the antibodies, a switch from one preparation to the other will not benefit patients while they are NAB positive.

NABs have been shown to reduce clinical efficacy of other type I interferons. It is accepted that when interferon alfa has been used to treat thrombocytosis, chronic hepatitis B and C and certain types of cancer, NABs are associated with loss of clinical effectiveness.[8 10 31–33] Although the impact of NABs on the clinical effect of interferon beta initially seemed less clear than that for interferon alfa, several studies have now shown a consistent correlation between the presence of NABs and decreased efficacy (table 2).[2 9 14 19 25 27] The effect of NABs on clinical efficacy is probably not an all or nothing phenomenon with the avidity, a measure of both titre and antibody affinity, as well as the dose of interferon playing a part. Rudick *et al* showed that the development of NABs to interferon beta-1a (Avonex) resulted in a titre dependent reduction in neopterin and β-2 microglobulin induction.[19] Others have reported similar findings with Mx protein.[14] The beneficial shift in immune cell populations has been shown to be inhibited by NABs. Kastrukoff *et al* reported that MS patients who are NABs positive do not exhibit the changes in NK cell activity that interferon beta treatment normally induces.[34] Perini showed that interferon beta treatment of MS patients results in a decrease in the CD16+, CD3+ cell population.[35] Patients that become NAB+ revert to pre-treatment levels of these cells.[35] All these studies indicate that the biological effects of interferon beta are inhibited in patients with NABs.

In the pivotal interferon beta-1a (Avonex) trial, a strong trend towards reduced treatment benefit on MRI disease activity in NABs positive patients was seen.[19] The PRISMS four year,[25] but not two year,[4] data provide the clearest correlation between positive NAB status and loss of therapeutic benefit. With interferon beta-1b (Betaseron), where

Table 3 Clinical effect of NABs in MS patients treated with interferon beta

	Study	Study period	Placebo	NABs–	NABs+	p Value
Betaferon/Betaseron[27] Attack rate:	Phase III RRMS	2 years	1.06	0.56	1.08	0.001
Avonex[19] MRI–Gd lesions: mean (n)	Phase III RRMS	2 years	1.6 (82)	0.5 (63)	1.7 (18)	0.062
Rebif[25]	PRISMS Extension	4 years				
Attack rate			NA	0.5	0.81	0.002
T2–active lesions			NA	0.3	1.4	<0.001
Change in T2 volume from baseline			NA	–8.5%	+17.6%	<0.001

the incidence of NABs is sufficiently high and develops earlier,[18] clinical effects have been seen in two year studies (see table 3).[27] As expected NAB positive patients have less systemic side effects or flu-like symptoms compared with NAB negative patients.[27] No differences with regard to local or cutaneous reactions between the NABs positive and negative patients were noted.[27]

Strategies to reduce or reverse the development of NABs

Patients with low titres of NABs tend to become NAB negative and occasionally titres oscillate between low positive and negative over time.[17] Whether these conversions or oscillations are attributable to technical aspects related for example to the timing of the sample collection in relation to treatment or represent "B cell tolerance" needs further clarification. In the case of interferon beta-1b some NAB positive patients revert to NAB negative status over two to five years of follow up.[27 36–40] Similarly, it has been reported that NAB positive interferon beta-1a (Rebif) treated patients can also revert to negative status.[37] In the PRISMS four year study the proportion of patients who were NAB positive at least once but not at the last visit was 0% with 22 μg thrice weekly and 13% with 44 μg thrice weekly.[37] This second observation suggests a dose effect and may explain why the reversion from NAB positive to negative may be more commonly observed with interferon beta-1b (Betaferon) in which the actual quantity of interferon beta protein administered is greater—that is, 875 μg/week for Betaferon compared with 30 μg/week for Avonex and 66 or 132 μg/week for Rebif. In our experience patients with high titres of NABs seldom revert to being negative.

Reducing or reversing the development of NABs to recombinant therapeutic proteins in potentially life threatening conditions is a high priority, for example, in haemophiliac patients intensive immunosuppression is used to reverse NABs formation to factor VIII.[41] In an open labelled study of 161 MS patients, receiving interferon beta-1b (Betaferon, 8 MIU subcutaneously on alternate days), randomised to receive either intravenous methyl-prednisolone 1 g monthly for 12 months compared with no corticosteroids the prevalence of NABs at 15 months in the prednisone treated group was 12.1% compared with 26.8% in untreated group, a relative reduction of 54.9%.[42] Interestingly, in one study in which NAB positive Betaferon patients were directly switched to Avonex, 53% and 75% reverted to NAB negative after one and two years, respectively.[43] Combining other immunosuppressive therapies with interferon beta, for example, azathioprine or mitoxantrone, to reduce the incidence of NABs is another strategy worthy of investigation. The induction of tolerance is the proposed mechanism that underlies the observed reduction in NABs to recombinant factor VIII when haemophiliac patients are transferred from intermittent to continuous replacement therapy[44] and may also explain the disappearance of NABs in some patients treated with higher doses of interferon beta administered more frequently.

IMPLICATIONS FOR THE PRACTICING NEUROLOGIST

The following conclusions and/or recommendations can be made:

(1) The evidence that NABs abrogate the biological and clinical effects of interferon beta is beyond reasonable doubt.

(2) NABs are cross reactive between different interferon beta products and interferon beta-1b is more immunogenic than interferon beta-1a.

(3) The immunogenicity of the different interferon beta preparations should be one of the factors that need to considered when starting treatment.

(4) Ideally patients taking interferon beta who have ongoing disease activity—that is, frequent disabling relapses—should be screened for NABs, particularly if the clinician is considering switching preparations and/or increasing the dose of interferon beta. If positive another treatment such as glatiramer acetate or mitoxantrone hydrochloride should be considered. Interferon therapy can only be reconsidered if the patient becomes NAB negative.

(5) Once high titre NABs have developed they tend to persist. If reversal of NABs positivity does occur it tends to be in patients with low titres.

(6) In the UK routine screening for NABs cannot be performed at present in view of the poor availability of validated assays, the lack of assay standardisation, and the lack of clinical data regarding the significance of low titre NABs.

(7) If routine screening becomes available the optimal time to test for NABs is between 6–12 months for interferon beta-1b and 12–24 months for interferon beta-1a.

(8) Whether interferon beta therapy should be stopped in all patients who are NAB positive, irrespective of their disease activity, requires further study. This question can only be answered using standardised clinical protocols and well validated assays.

CONCLUSION

There are accumulating data that indicate that NABs are clinically relevant in MS patients receiving interferon beta therapy. Neurologists need to consider this when starting treatment and assessing treatment failures. At the same time neurologists need to keep the issue of NABs in perspective. NABs are clearly not the only reason for treatment failures. Not all patients respond to interferon beta treatment and the reasons for this are still unknown. Unfortunately, no criteria have yet been identified that reliably predict responsiveness. The issue of NABs has particular relevance in the UK in which interferon beta therapy has been deemed by the National Institute of Clinical Excellence (NICE) not to be cost effective. If interferon beta treatment were to be stopped in all patients who became NAB positive this would clearly have a positive impact on the long term cost effectiveness of interferon beta treatment.

Conflicts of interests

All authors have participated in meetings sponsored by, and received travel grants and honorariums from, pharmaceutical companies marketing treatments for multiple sclerosis; our departments have received financial support for participation in randomised controlled trials of interferon beta-1b (Betaferon, Schering), interferon beta-1a (Avonex, Biogen; Rebif, Serono), glatiramer acetate (Copaxone, Teva), and mitoxantrone (Novatrone, Immunex) in multiple sclerosis. All authors have received honorariums for acting in an ad hoc capacity as advisors to various pharmaceutical companies who have drug development programmes for multiple sclerosis. GG is the principal investigator at the National Hospital for Neurology and Neurosurgery in a trial of Natalizumab (Antegren) sponsored by Biogen Inc; GG is chairman of the UK Medical Advisory Board of Biogen and is an ad hoc member of the European and UK Advisory boards for Biogen and Teva respectively. GG is also a member of the editorial board of a MS related publication sponsored by Serono. FD is currently a

treating physician in the Antegren trial, sponsored by Biogen, the EVIDENCE trial, sponsored by Serono, and the BENEFIT trial, sponsored by Schering. FEM serves as an ad hoc consultant to Biogen.

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