Neutralizing antibodies in interferon beta treated patients with multiple sclerosis: knowing what to do now

Commentary to: 10.1007/s00415-010-5844-5 “One-year evaluation of factors affecting the biological activity of interferon beta in multiple sclerosis patients”

by S. Malucchi et al.

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Longterm treatment of parenterally administered interferon beta (IFNb) is one of the mainstays of multiple sclerosis (MS) therapy [1]. The first trials were conducted in the early 1980s when human fibroblast derived IFNb was administered intrathecally in an open-label fashion [2]. In ten patients treated monthly for 6 months a significant reduction of the annualized relapse rate was noted compared to the relapse rate at baseline. This effect was not observed in ten control patients with MS that, for ethical reasons, did not undergo repeat sham lumbar punctures. This prompted a larger multicentre trial in which again human IFNb purified from cultured fibroblasts was administered intrathecally [3]. After the advent of recombinant techniques to produce larger amounts of IFNb, the first pivotal trial of IFNb-1b administered subcutaneously every other day in 372 patients with relapsing-remitting MS was published in 1993 [4], and the drug was approved by the FDA for use in MS the same year. Since then two additional IFNb-1a products were evaluated in pivotal trials that yielded positive results and have been marketed subsequently [5, 6]. Treatment with IFNb was also shown to delay disease progression and halt disease activity in patients with clinically isolated syndromes [7–9]. This observation along with neuropathological and MR imaging studies emphasized the importance of early diagnosis and treatment initiation of patients [10]. Overall the effect size of the IFNb preparations is comparable and moderate [1] and the effects of these drugs have been proven to be sustained for more than 15 years [11]. Additionally the

long-term safety profile of IFNb treatment is excellent [12, 13].

However, with a so called *biological*, i.e. a proteinaceous therapeutic compound, the issue of IFNb immunogenicity is inherent and has remained a matter of debate [14]. Antibodies against IFNb can be detected in up to 47% of patients undergoing treatment with IFNb (Table 1) [14]. These antibodies are either purely binding, and then termed binding antibodies (BAb) or inhibit IFNb activity and then termed neutralizing antibodies (NAb) (Fig. 1). The proportion of NAb is variable and differs between available IFNb products (table 1) [14]. Once the bioactivity of injected IFNb is blocked by persistent and high-titer NAb the beneficial clinically detectable treatment effects of IFNb seem to vanish, albeit with a latency of some one to four years. A diminution of the downregulatory effect on MR disease activity becomes apparent earlier, i.e. 6–18 months after development of high titer NAb. [15]. Immediate negative effects have not been sufficiently demonstrated. It is of note that most analyses were done in a post-hoc fashion on data from trials originally designed and powered to show treatment effects of IFNb rather than detrimental effects of NAb [16]. Thus, there is ongoing controversy regarding the clinical impact of NAb on the management of MS patients [17]. Current European guidelines recommend switching therapy in patients with persistently high-titer NAb with suspected clinical or MRI activity [16, 18]. Importantly, NAb may also affect endogenous IFNb as cross-reactivity between antibodies against IFNb-1a or IFNb-1b and endogenous, human fibroblast derived IFNb was shown only recently by us [19].

Antibodies to IFNb are measured in a two-step procedure. First, BAb are detected by standard antibody assays. In a second step the neutralizing capacity of these

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Table 1 Prevalences of NAbS in prospective IFNb treatment trials

	<i>n</i>	NAbS positive patients (%)	References
IFNb-1b s.c.			
1.6 MU qod	125	42	[4]
		47	[25]
8.0 MU qod	124	38	[4]
		45	[25]
	277	32	[26]
	69	22	[27]
8.0 MU qod (MxA assay)	360	23/28	[28, 29, 38]
IFNb-1a s.c.			
22 µg			
q wk	95	5	[30]
t.i.w.	189/167	24	[6, 31]
	209	21	[32]
44 µg			
q wk	98	16	[30]
t.i.w.	184/167	13/14	[6, 31]
	204	15	[32]
	374	27	[33]
	336	21	[34]
	259	17	[35]
IFNb-1a i.m.			
30 µg q wk	193	2	[7]
	402	2	[36, 37]
	338	2	[34]
	67	6	[27]
Assay not known	150	22	[5]
60 µg q wk	400	6/5	[36, 37]

NAbS status was determined by CPE unless stated otherwise. NAbS were considered positive if titers >20 TRU/mL were measured

s.c. subcutaneously, *i.m.* intramuscularly, *qod* every other day, *q wk* once weekly, *t.i.w.* three times weekly

Fig. 1 IFNb-induced signal transduction and possible interferences of anti-IFNb antibodies. Binding of IFNb to its receptor (IFN α/β receptor) assembles an active signalling complex resulting in signal transduction through phosphorylation of tyrosine kinases (e.g. Tyk-2, Jak-1). Phosphorylated tyrosine kinases may activate certain transcription factors, that lead to IFNb-mediated gene transcription. Gene translation to proteins, e.g. neopterin, myxovirus resistance protein A (MxA), results in IFNb-mediated cellular function. BAbS may not

interfere with the interaction of IFNb with its receptor, leaving the downstream pathways unaltered (middle panel). In contrast, NAbS hinder the engagement of IFNb with its receptor, thus inhibiting receptor activation. Intracellular downstream responses will not be turned on and IFNb response proteins not be secreted (right panel). Therefore, the assessment of the MxA response either by measuring the MxA protein or its mRNA gene transcript is a sensitive and validated marker for bioactivity of IFNb in humans

antibodies is demonstrated, for which the in vitro cytopathic effect (CPE) assay can be regarded as the gold standard [20]. Recently it was demonstrated that measuring the mRNA response of the IFNb-induced antiviral MxA

protein ex vivo by quantitative PCR yields a good correlate of the bioavailability of IFNb in a given subject [16, 20]. However, since there are inter-individually different response curves of the MxA mRNA expression, a small

proportion of patients may be classified false-positively [21–23]. To minimize this potential error it would be ideal to compare individual MxA mRNA responses on IFN β therapy with the respective responses at treatment initiation of the same individual [24].

In this issue of the Journal S. Malucchi et al. have set out to evaluate BAbs and NAbs in 167 patients with MS after 1 year of treatment with IFN β . The proportions of patients treated with either IFN β product were roughly identical. Twenty-two percent of patients showed a pathological, i.e. negative, MxA mRNA response, indicative of reduced bioavailability. Twenty-two of those 37 patients were both positive for BAbs by capture ELISA and for NAbs by CPE; therefore, in these patients the presence of NAbs as detected by CPE resulted in a reduced in vivo bioactivity of IFN β . Eleven of all 167 patients, i.e. 6.6%, had NAbs at high-titers (>100 TRU/mL), ten of these completely abrogated the MxA response. There were also 9.6% samples with a borderline diminished MxA response. Notably, in three of the 37 MxA-negative patients, i.e. in 1.8% of the entire cohort studied, neither BAbs nor NAbs were detected. The reasons for this may be treatment incompliance, presence of soluble IFN β receptors that capture the injected IFN β [23] or a false-negative result as discussed above.

This study is very helpful and interesting for several reasons and lets us participate in challenging the NAbs issue by one of the leading NAbs research groups in Europe: It demonstrates a good, albeit incomplete, link between the presence of high-titer NAbs (as measured by CPE) and the loss of bioactivity in vivo, and it identifies a small ($\sim 2\%$), yet substantial number of patients with loss of IFN β bioactivity not explainable by the presence of NAbs. It is worthwhile to assess these patients in greater detail, as suggested by the authors, given the availability of alternative treatment options, although these may have potential side effects not seen with IFN β . Thus, before switching a patient to glatiramer acetate or escalating the patient to, e.g., natalizumab, a false-negative MxA result and noncompliance should be ruled out. Lastly, this study reproduces independently in a well-defined cohort of patients the frequency of NAb-positivity and high-titer NAb-positivity after exactly one year of treatment reported earlier. It should be rewarding to follow up those 6.6% of patients with a reduced, not yet negative MxA responses in order to quantify the proportion of patients that will eventually completely lose their IFN β bioactivity, i.e. become NAb positive.

Emergence, frequency and origin of NAbs remain challenging issues while to date consensus seems to have been achieved regarding their immediate impact on IFN β bioactivity and their delayed impact on disease activity. The paper by S. Malucchi et al. does not attempt to settle the matter — a task too vast for one group anyway. But the careful and detailed expert analysis of the antibody status

helps to further our understanding of this complicated picture of treatment induced humoral immunity.

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