A sensitive radioimmunoprecipitation assay for assessing the clinical relevance of antibodies to IFN β

N Lawrence, J Oger, T Aziz, J Palace, A Vincent

Background: Some multiple sclerosis (MS) patients treated with interferon beta (IFN β) develop antibodies to the drug. Neutralising antibody (NAB) assays for IFN β are expensive and the clinical relevance of the results has been debated.

Objective: To establish a cheap, sensitive, and reliable assay for antibodies to 125I-IFN β, and to correlate levels of antibodies with clinical response to IFN β treatment.

Methods: We established a radioimmunoprecipitation assay (RIPA) using 125I-IFN β. We tested NAB positive sera, healthy control sera, and serial samples of 33 IFN β-1b treated MS patients from the Vancouver cohort of the Berlex pivotal trial who had a high incidence of NABs.

Results: We found that the RIPA was highly sensitive for the detection of antibodies to IFN β-1a and -1b, and that there was a strong correlation between reactivity of NAB positive sera for 125I-IFN β-1b and for 125I-IFN β-1a. The RIPA was more sensitive and consistent than the NAB. Moreover, there was a trend towards poorer MRI outcomes in RIPA positive patients, but not in NAB-positive patients.

Conclusions: The RIPA assay is sensitive and easy to perform. It should be of value in assessing the clinical impact of IFN β antibodies, and its use could help target expensive INF β treatments to those who will respond best.

Methods

Clinical samples
Sera came from two sources. NAB positive sera of known antibody titre were donated by Biogen UK and by Schering UK; no clinical data were available on these sera. Clinical data were available on sera from 50 individuals from the Vancouver cohort of the pivotal trial of IFN β-1b. Thirty three had received active therapy (Betaseron, Berlex, Richmond, CA, USA; 17 on 1.6 MIU and 16 on 8 MIU of IFN β-1b) subcutaneously on alternate days for 2 years; 17 were on placebo. Serum samples were taken at each visit, coded, and stored at −80°C. The total number of clinical attacks, the number of new or enlarging lesions, and the total lesion volume were recorded at baseline and every six weeks as previously reported.

RIPA for IFN β antibodies
10 µg of IFN β-1b (Berlex Biosciences, Richmond, CA, USA) or IFN β-1a (Ares-Serono International, Geneva) were iodinated with 125I (from Amersham, UK) using chloramine T, as previously described for botulinum toxin. The 125I-IFN βs were then separated from free 125I by gel filtration, filter sterilised, and stored at +4°C. Routinely, 10 µl of 125I-IFN β (50 000 total cpm) was incubated with 5 µl of serum in 50 µl 0.02 M phosphate buffer pH 7.2/0.1% triton X-100 (PTX buffer), for 2 hours at room temperature. 50-100 µl of sheep anti-human Ig G (The Binding Site, Birmingham, UK) was added (diluted 1:4 in PTX; the actual amount used was that required to form a visible precipitate with 5 µl of a range of human sera) and the precipitates centrifuged, washed in PTX, and counted on a gamma counter (Packard, Berkshire, UK). The mean value of three healthy sera was subtracted, and the results expressed as a percentage of the value obtained with a known high positive serum.

NABs were measured using the myxovirus associated antigen (MxA; by Berlex laboratories, CA, USA) on samples found to be positive by ELISA, as part of the IFN β-1b pivotal trial. The MxA is a bio-assay that measures IFN β-l of serum before performing the assay. The neutralisation titre is the reciprocal dilution of serum that reduces by 10-fold the MxA inducing activity of IFN β.

RESULTS

Establishment of a RIPA for antibodies to IFN β
We first tested healthy controls and known NAB positive sera (NAB >20 units) from IFN β-1b treated patients. Sera with high values showed a linear relationship of precipitation at serum that reduces by 10-fold the MxA inducing activity of IFN β. Positivity was defined as a NAB value of greater than 20.

Abbreviations: IFN β, interferon beta; MS, multiple sclerosis; MxA, myxovirus associated antigen; NAB, neutralising antibody; RIPA, radioimmunoprecipitation assay

Treatement with interferon beta (IFN β) reduces clinical attacks and MRI lesions in multiple sclerosis (MS). Some patients develop antibodies against IFN β, and an association between neutralising antibodies (NAB) and reduced efficacy has been reported. However, NABs have high intra-assay and inter-laboratory variability, they tend to oscillate from positive to negative, and do not detect all antibodies that will reduce the bioavailability of the drug in vivo. Moreover, there are difficulties in obtaining the serological and clinical data on individual cases, making it difficult to assess the relevance of the NABs. We established a radioimmunoprecipitation assay (RIPA) for binding antibodies to IFN β-1b and -1a, compared the results with those of the NAB assay, and correlated the results with clinical efficacy in a small cohort of patients for whom detailed clinical data were available. Preliminary results have been reported in abstract form.
positive serum (as in fig 1a). All seven NAB positive sera immunoprecipitated $^{125}$I-IFN $\beta$-1b (Fig 1b). We then tested NAB positive sera, from patients treated with IFN $\beta$-1b or IFN $\beta$-1a, against both $^{125}$I-IFN $\beta$-1b and $^{125}$I-IFN $\beta$-1a. There was a highly significant correlation of the results of the two assays ($p < 0.0001$), indicating that antibodies to one form of IFN generally bind well to the other (fig 1c).

IFN $\beta$-1b antibodies in patients in the pivotal trial
We examined the NAB and RIPA levels in sera from patients at one year into the pivotal trial. All patients on placebo remained negative throughout (Fig 2a and 2b). With both assays, the antibodies were a little higher, and there were more positive results, in the patients given the lower dose of IFN $\beta$, as in other studies, but the difference was not significant. Only 5/33 of the patients treated in this cohort of betaseron treated patients were negative with both assays at one year. There was a weak correlation between the values in the two assays ($r^2 = 0.42$, $p < 0.001$; fig 2c), but 12 IFN $\beta$-1b treated patients who were persistently NAB negative were
RIPA positive, whereas none of the RIPA negative sera were NAB positive. Thus, the RIPA is more sensitive than the NAB assay.

Positive RIPA values were first detected between one and four months after starting treatment; 26/33 were positive by six months, 29/33 by 12 months; and 27/33 were still positive at 24 months. RIPA values did not oscillate markedly over time (fig 3a and 3b), although there was a tendency for values to decrease in some patients (eg fig 3a). Positive NAB values were first detected between 1.5 and 13 months after starting treatment, but in some cases they oscillated between positive and negative (fig 3a and 3b), and only 8 sera were positive on two consecutive occasions by six months of treatment.

Because the distribution of positive results with the RIPA was broadly bimodal (fig 2b), we set an arbitrary level of 25% as a cut off between high and low/negative RIPA values (dotted line in fig 2b). With this cut off 21, 18, and 14 sera out of 33 treated patients had high values at 6, 12, and 24 months, respectively. The two year clinical outcomes of the pivotal trial patients were assessed in the placebo and IFN β-1b treated patients, dividing patients according to their one year antibody results (fig 4). There was the expected reduction in new or extending MRI lesions in the treated patients, but no difference between RIPA and NAB positive and negative patients (fig 4a). There was no expected reduction in new or extending MRI lesions in the treated patients, but no difference between RIPA and NAB positive and negative patients (fig 4a). There was no expected reduction in new or extending MRI lesions in the treated patients, but no difference between RIPA and NAB positive and negative patients (fig 4a). There was no expected reduction in new or extending MRI lesions in the treated patients, but no difference between RIPA and NAB positive and negative patients (fig 4a).

DISCUSSION

We established a simple radioimmunoprecipitation assay that is highly sensitive and can be used for measuring antibodies to either IFN β-1b or IFN β-1a. When we studied sera from patients who were treated with IFN β-1b as part of the pivotal trial, we found that 21/33 sera had high levels of antibody by RIPA at six months into the trial (at which time only eight were positive by MxA) and remained positive thereafter, showing none of the oscillations that have been reported for various bioassays, and found here for the MxA assay. Nevertheless, the values did decrease with time in some patients, confirming results reported by others using ELISA assays.9–11

The MxA assay12 has been widely used, but it is very expensive and there are problems in determining the appropriate cut off level; moreover, inhibition by non-antibody serum factors could be misinterpreted as being due to specific antibody. In addition, although in vitro bioassays indicate the ability of the antibodies to prevent drug efficacy if applied at a target site, they are not informative about the ability of the antibodies to prevent the drug from accessing the site of action. Any antibody that binds to IFN β before it reaches its target could lead to clearance of the drug from the circulation and reduce its bioavailability, irrespective of whether the antibody inhibits drug receptor interactions. Thus there are good reasons for looking for binding antibodies as we have done here.
The RIPA is quick, technically easier than the bioassay, and should be relatively cheap. It detects antibodies earlier than the NAB assay and, because the results do not oscillate over time, it could be performed at just one time point (eg at six months or one year after starting treatment). Moreover, although our study was not powered to examine the effects of antibodies on the clinical outcome measures, the results in this small cohort of patients suggest that the RIPA may be of greater clinical relevance than the NAB.

Antibodies that neutralise the in vitro effects of a drug are expected to reduce its efficacy and yet there has been controversy regarding the clinical relevance of IFN β-b NABs. However, if one examines the results of the most robust cohorts, that is the large pivotal IFN β trails, NABs do appear to attenuate efficacy: MRI outcomes were affected within one to two years of treatment, with an earlier effect on lesion volume increase than on lesion activity (explaining why we might not see a difference in lesion activity between antibody negative and positive patients at one year in our small series); the effects of NABs on relapse rate were delayed beyond 18–24 months; and the effects on disability outcomes showed a trend in favour of an effect in the PRISMS study over 4 years, reaching significance in the high dose group. Therefore, underpowering, lack of treatment effect, and short follow up periods are the likely explanations for difficulties in demonstrating an effect of IFN β antibodies on the treatment efficacy.

It is difficult to assess the clinical relevance of antibodies from studies using different assays, directed against a drug that is only partially effective, in a disease that has a high level of biological variability and unpredictability. But in addition, the antibody tests have often been performed in house or sponsored by the pharmaceutical companies. Independent analysis of the antibody results with the clinical data has not been forthcoming. Previous reports suggest that there is a lower antibody rate with the use of IFN β-1a products, and this is supported by our own unpublished observations (Lawrence, Palace, Vincent, 2000).

It is important that simple, cheap, and standardised antibody assays, like the RIPA, are set up by independent laboratories and that the effect on disability outcomes is clarified. One could then define those patients who are unlikely to benefit from treatment, at the same time targeting those who will benefit the most. This would allow these expensive treatments to be used in a more cost effective and ethical way.

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REFERENCES