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Serum auto antibodies presence in multiple sclerosis patients treated with β-interferon 1a and 1b

Livianna Speciale[1], Marina Saresella[1], Domenico Caputo[2], Stefania Ruzzante[1], Roberta Mancuso[1], Maria Gaetana Calvo[1], Franca Rosa Guerini[1] and Pasquale Ferrante*[,1,3]

[1]Laboratory of Biology, Don C. Gnocchi Foundation, IRCCS, Milan, Italy; [2]Multiple Sclerosis Unit, Don C. Gnocchi Foundation, IRCCS, Milan, Italy and [3]Chair of Virology, Department of Preclinical Sciences, University of Milan, Italy

To verify the possible effect of IFN-β treatment on auto antibodies development in multiple sclerosis (MS) we studied 69 MS patients before and during the treatment with IFN-β 1b (*n*=35) and IFN-β 1a (*n*=20) for 27 and 12 months respectively, and, as controls, 14 untreated MS patients. The serum, collected every 3 months from all the patients, was investigated for the presence of antinuclear (ANA), anti-smooth muscle (ASMA), anti-mitochondrial (AMA), anti-native DNA (nDNA) anti-cardiolipin (aCL), anti-parietal cells (APCA), anti-microsomal (AMC) and anti-tireoglobulin (ATG) antibodies. Among the IFN-β 1b-treated MS patients an increase of the frequency and of the level of ANA, AMC and ATG was observed. ASMA and ANA antibodies were already present in about 45% of the MS patients before the treatment and fluctuated over the time. In one patient the treatment was interrupted after 6 months because of the occurrence of high ASMA level and of an autoimmune hepatitis. The data obtained in the smaller number of MS patients treated with IFN-β 1a were very similar. No increase in aCL level was observed during both the IFN treatments. Our results indicate that the treatment with IFN-β induces an increase of AMC and ATG antibodies in MS patients and confirm that, although rare, autoimmune diseases could be observed. The possible effect of these auto antibodies on the treatment efficacy and on MS clinical course need to be further investigated. *Journal of NeuroVirology* (2000) **6,** S57–S61.

Keywords: multiple sclerosis; ANA; ASMA; AMC; ATG; β-IFN treatment

Introduction

Multiple sclerosis (MS) is an inflammatory demyelinating disease of the central nervous system, induced in predisposed individuals by an immunomediated mechanism in which the proinflammatory cytokines seem to play a significant role (Ferrante *et al*, 1998). In the past, the therapeutic effect of IFN alpha (IFN-α) or beta (IFN-β) has been shown to reduce the frequency of clinical exacerbations and magnetic resonance (MRI) lesions in MS (The IFNB Multiple Sclerosis Study Group 1995; Jacobs *et al*, 1996). This therapeutic effect of IFN-β seems to be based more on an immunomodulatory than an antiviral action since in treated patients an immune imbalance can be observed (Conlon *et al*, 1990; Panitch, 1992; Arnason and Reder, 1994).

Besides the positive effects on MS clinical course and on MRI lesions, IFN-b has been shown to induce in some of the treated MS patients the production of auto antibodies and more rarely, of autoimmune disorders (Rotondi *et al*, 1998; Burman *et al*, 1986).

In the present study we investigated the frequency of antinuclear (ANA), anti-microsomal (AMC) and anti-tireoglobulin (ATG), anti-native DNA (nDNA), anti-smooth muscle (ASMA), anti-cardiolipin (aCL), anti-parietal cell (APCA) auto antibodies in the serum of 35 MS patients before and during the treatment with IFN-β 1b, in 20 MS patients treated with IFN-β 1a and in 14 MS patients not treated with IFN-β.

Results

The data so far obtained indicate that there are no significant peculiaries in the frequency and levels of AMA, nDNA, aCL and APCA antibodies, and thus we focused our attention on the other studied auto antibodies.

*Correspondence: P Ferrante, Laboratory of Biology, Don C. Gnocchi Foundation, IRCCS, Via Capecelatro, 66 I-20148, Milan, Italy

The percentages of MS patients showing the serum ANA antibodies in the serum before and during the treatment with IFN-β 1b or IFN-β 1a, and in those without IFN-β treatment are shown in Figure 1.

It is possible to see that already before the treatment (time 0), 54.3% of the IFN-β 1b and 30% of the IFN-β 1a treated patients had serum ANA antibodies, and that a similar percentage (42.8%) of the untreated MS patients had ANA antibodies already in the first serum. After the treatment, both the IFN-β 1b and IFN-β 1a MS patients had an increase of the frequency of the ANA positive sera (on months 3 and 6) that slowly decreased over the time. The percentage of MS patients with serum ANA was significantly higher IFN-β 1b than in IFN-β 1a treated MS patients, at 6, 9 and 12 months ($P<0.05$).

As observed for ANA, also the search of ASMA antibodies showed that, already in the first serum sample, all three groups of MS patients had a higher frequency of positivity (Figure 2), moreover the trend of the ASMA positive percentages is quite similar to that observed for the ANA without any peculiar aspect (Figure 2). On the other hand, it must be noted that one MS patient, after 6 months of treatment with IFN-β 1b, developed high titres of ASMA (1 : 160) and was forced to interrupt the IFN-β therapy because of the occurrence of an autoimmune hepatitis. Another IFN-β 1b-treated MS patient developed allergic dermatitis after 9 months of treatment and thus also in this case the IFN-β 1b therapy was discontinued.

None of the MS patients included in the study had ATG antibodies on time 0 (Figure 3). It is interesting to note that while the untreated MS patients did not develop ATG antibodies until 9 months from the enrolment, both the IFN-β 1a- and 1b-treated MS patients produced ATG antibodies. In particular, starting from the serum collected on month 6, both these groups had a small percentage of ATG antibody positive subjects. The percentage ranged from 5.2% (month 6) to 9.0% (month 12) in the IFN-β 1a-, and from 5.7 to 11.4% in the IFN-β 1b-treated MS patients. With regard to the AMC antibodies, as it is possible to see in Figure 4, even before treatment one of the IFN-β 1a- and two of the IFN-β 1b-treated MS patients resulted positive, while all the untreated MS cases were negative (Figure 4). During the follow-up, the frequency of positivity increased to 20% on month 3, to 22.8% on month 6 and to 28.6% on month 9 in

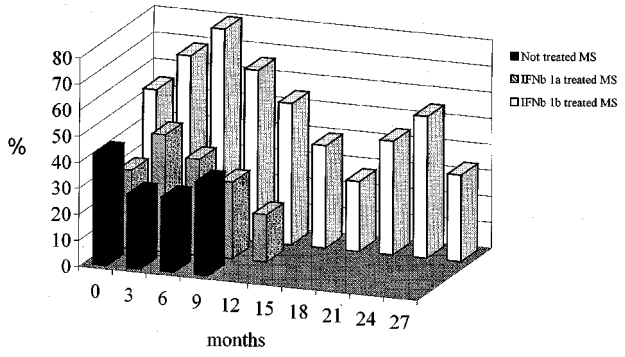

Figure 1 Frequency of ANA antibodies in MS patients untreated and treated with IFN-β 1b or IFN-β 1a at the various moments of the treatment.

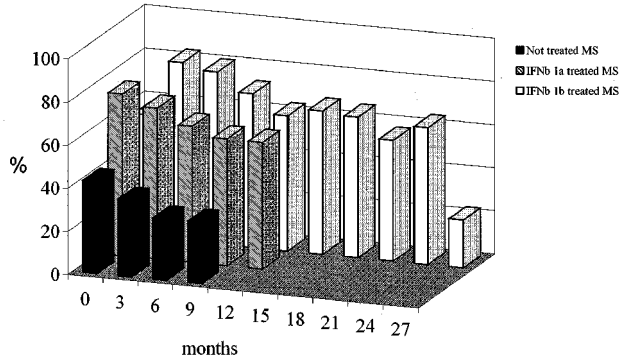

Figure 2 Frequency of ASMA antibodies in MS patients untreated and treated with IFN-β 1b or IFN-β 1a at the various moments of the treatment.

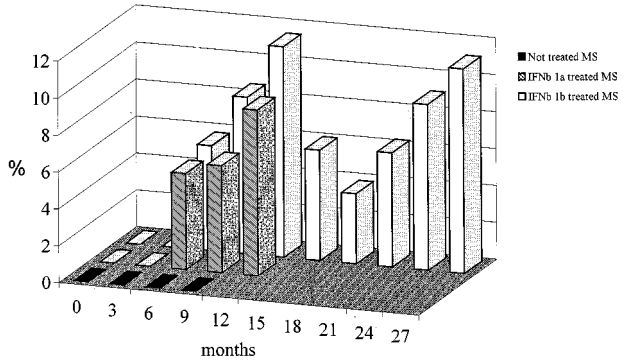

Figure 3 Frequency of ATG antibodies in MS patients untreated or treated with IFN-β 1b or IFN-β 1a at the various moments of the treatment.

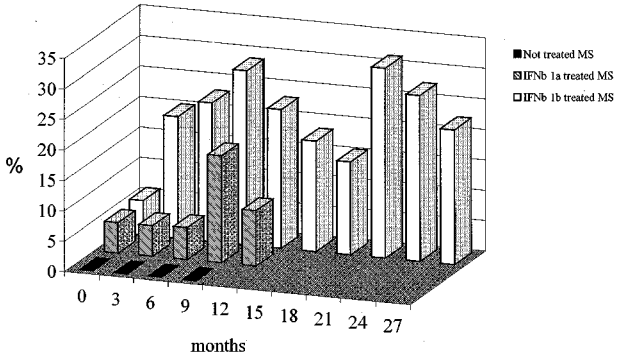

Figure 4 Frequency of AMC antibodies in MS patients untreated or treated with IFN-β 1b or IFN-β 1a at the various moments of the treatment.

IFN-β 1b-treated MS patients. After this time a fluctuation of AMC antibodies was observed in this group. The frequency of AMC antibodies in IFN-β 1a-treated MS cases on months 3 and 6 was lower than that observed before the treatment with an increased frequency (17.6%) on month 9.

Discussion

The development of autoimmune abnormalities and clinical disorders during IFN-α therapy in patients with tumours or chronic hepatitis has largely been studied with some contradiction as regard to the incidence, the severity, and the causative mechanism of such manifestations (Burman *et al*, 1986; Carella *et al*, 1995; Preziati *et al*, 1995; Roti *et al*, 1996; Marazuela *et al*, 1996). The most accepted idea is that, beside its immunomodulatory effect, IFN-α can induce these autoimmune phenomena, in some treated patients, by exacerbating a pre-existing subclinical autoimmune disorder (Ronnblom *et al*, 1991; Conlon *et al*, 1990).

The effects of long term IFN-β therapy on the production of auto antibodies and on the development of clinical symptoms have not been extensively investigated (Kivisäkk *et al*., 1998). The data obtained in our study indicated that IFN-β treatment has some effect on the serum positivity for ANA antibodies that have an increase (not statistically significant) during the follow up in both groups of patients treated with the two different types of IFN-β. Although the percentage of MS patients positive for ANA antibodies are higher in IFN-β 1b group than in IFN-β 1a-treated MS patients, the overall trend and the comparison with the control MS cases seem to suggest that the impact of the therapy on the development of ANA antibodies is minimal. The same observation could be made for ASMA antibodies, however, in this case it must be remembered that one MS patient had an autoimmune hepatitis during the IFN-β 1b treatment with high titres of ASMA. In this regard it is important to note that this patient, and another case also treated with IFN-β, interrupted the treatment because of the occurrence of adverse effects. Based on these observations IFN-β 1b could be suspected of inducing autoimmune clinically relevant complications more easily than IFN-β 1a, but, due to the limited number of patients studied so far and the different length of the follow up period, further investigations are needed to fully clarify this point.

More interesting are the results obtained by monitoring the trend of ATG antibodies in the two groups of treated MS patients and in the untreated MS patients. In this case none of the MS cases had ATG antibodies before enrolment in the study, and, while the untreated patients remained negative, both the groups treated with IFN-β 1b and 1a developed auto-antibodies with a relatively important frequency. These observations are in agreement with previous reports and with the reported finding of thyroid dysfunction during IFN-β therapy (Schwid *et al*, 1997; Rotondi *et al*, 1998). In our study, during the IFN-β treatment the MS patients developed, or had an increase of the frequency of AMC antibodies. Also in this case, as already observed for ANA and ASMA antibodies, the overall picture suggests that IFN-β 1b induces the production of AMC antibodies earlier and more frequently than IFN-β 1a.

On the whole, the data of the present study confirm the capability of IFN-β treatment to modulate *in vivo* the production of auto antibodies, however further investigations are needed to elucidate the possible pathogenic role of these auto antibodies. It should also be remembered that we did not observe significant variations in the trend of positivity for AMA, nDNA, aCL and APCA antibodies, thus indicating that the effect of IFN-β treatment on the production of auto antibodies is in some way selective.

In conclusion our data confirm the absolute need of serum auto antibody survey during IFN treatments also in MS patients to verify the presence of autoimmune asymptomatic disorders and their potential role on the effectiveness of this therapy.

Materials and methods

Subjects

Sixty-nine patients with relapsing remitting MS diagnosed according to Poser's criteria (Poser *et al*, 1983) were enrolled in this study. Thirty-five patients (ten males and 25 females) were treated with IFN-β 1b (Betaferon, Schering AG, Berlin, Germany) at dose of 8×10^6 IU every other day injected subcutaneously. Their age ranged from $21-49$ (mean 34.2 ± 7.8) and the mean disease duration was 9.6 ± 6.5 years (range $2-24$). At the time of the first examination the EDSS (expanded disability status scale) (Kurtzke, 1983) averaged 2.8 ± 0.6 points (range $1-3.5$). The number of clinical relapses during the treatment period averaged 0.5 ± 1.1 (range $0-5$).

Twenty patients (eight males and 12 females) were treated with IFN-β1a (Avonex, Biogen B.V., Amsterdam) at dose of 6×10^6 IU injected intramuscularly once a week. Their ages ranged from $17-47$ (mean 32 ± 8.5) and the mean disease duration was 9.9 ± 6.8 years (range $2-26$). At the time of the first examination the EDSS averaged 2.7 ± 0.8 points (range $1.5-3.5$). The number of clinical relapses during the treatment period averaged 0.5 ± 0.8 (range $0-3$). Fourteen patients (four males and ten females) with comparable disease duration and EDSS have not been treated.

The patients were examined before and periodically until 27 months after treatment with IFN-β 1b or until 18 months after treatment with IFN-β1a.

The patients not treated with IFN were examined over 9 months. In the case of relapses, short courses of corticosteroids were allowed. None of the MS patients had any history of autoimmune diseases.

Methods

The serum was collected and auto antibodies were evaluated every 3 months during IFN-β or IFN-β1a therapy. Antinuclear (ANA), anti-native DNA (nDNA), anti-smooth muscle (ASMA), anti-mitochondrial (AMA), anti-parietal cell (APCA) antibodies were assayed using standard indirect immunofluorescence methods on fixed Hep-2 cells, *Crithidia Luciliae* and rat stomach, kidney and liver tissue (Sanofi Diagnostic Pasteur, Milan, Italy). ANA were considered positive at the titre $\geqslant 1:40$; nDNA were considered positive at the titre $\geqslant 1:10$ and ASMA or AMA or APCA were considered positive at the titre $\geqslant 1:20$. Anti-tireoglobulin (ATG), anti-microsomal (AMC) were performed using microtiter particle agglutination test kits (Serodia-ATG and AMC, Fujirebio Inc., Tokyo, Japan). Titres $\geqslant 1:100$ were considered positives. Anti-cardiolipin (aCL) IgG and IgM were performed using Cardiolipin-Isotyping ELISA (Fresenius Gull Laboratories, SLC, UT, USA). The upper normal limit of aCL was 10 GPL and 10 MPL.

Statistical analysis

The frequencies were compared using χ^2 test. For comparing numbers of auto antibody positive *versus* negative patients during treatment, Fischer's exact test was used. A P-value < 0.05 was considered statistically significant.

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