Serum Interferon (IFN)-Neutralizing Antibodies and Bioactivities of IFNs in Patients with Severe Type II Essential Mixed Cryoglobulinemia

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The efficacy of alpha interferon (IFN-α) in the treatment of severe type II essential mixed cryoglobulinemia (EMC) has been reported previously. In some patients, the development of neutralizing antibodies to recombinant IFN-α (rIFN-α) can affect the clinical response achieved with rIFN-α; a second treatment with natural IFN-α preparations may reinduce the clinical response. In the present study, the ability of leukocyte IFN (LeIFN) to restore the response was investigated from a pharmacodynamic viewpoint. Specifically, the pharmacodynamic profiles of different IFN-α preparations were studied by measuring the serum neopterin levels and the levels of expression of protein MxA mRNA in in vivo peripheral blood mononuclear cells in two patients with EMC whose resistance to rIFN-α2a treatment increased concomitantly with the development of neutralizing antibodies. These markers were measured before injection and at 24 and 48 h after a single injection of rIFN-α2a, consensus IFN [(C)IFN], or LeIFN. No increase or only a slight increase in MxA mRNA levels was detectable after administration of rIFN-α2a or (C)IFN, whereas a significant increase (≥10-fold) in MxA mRNA expression was recorded following administration of LeIFN. The neutralizing antibodies to rIFN-α2a cross-react with (C)IFN. Sera from these patients neutralized most but not all of the subtypes present in the natural IFN-α (LeIFN) mixture, and no significant increase in neopterin levels was observed after these patients were switched to LeIFN treatment. In summary, the data demonstrate that the problem of neutralizing antibodies still exists and that LeIFN may induce an increase in the level of MxA mRNA expression but not an increase in neopterin levels in patients who are resistant to treatment with rIFN-α2a or (C)IFN.

It has been repeatedly reported that antibodies to interferon (IFN) may develop during alpha IFN (IFN-α) therapy (2). Of the antibodies that bind to different epitopes of the IFN molecule, some are neutralizing antibodies (NABs), as measured in antiviral neutralization assays.

The development of NABs against IFN-α has been correlated with a decline in therapeutic efficacy in patients with chronic myelogenous leukemia (39), hairy-cell leukemia (40), carcinoid tumors (33, 36), and chronic hepatitis C (7, 21, 31) treated with IFN-α and, more recently, in patients with multiple sclerosis treated with IFN-β (26, 27, 37). It has also been observed in patients with severe type II essential mixed cryoglobulinemia (EMC), for whom IFN-α is a well-established and widely used therapy (9, 10, 12, 32). Several studies have also demonstrated that second-line therapy with natural human IFN-α may be effective in restoring the therapeutic response in patients with chronic hepatitis C (5, 13, 31) and in those with cancer (8, 20, 38, 42) who relapse following the production of NABs to rIFN-α. However, the possibility that switching to alternative IFN-α preparations could overcome the NAB-induced fall in the biological and clinical activities of IFN has, so far, rarely been considered.

Furthermore, a clear cause-and-effect relationship between NAB production and the reduction in the biological effectiveness of IFN has not been proved conclusively.

One possible way of addressing these issues would be to study the effect of circulating antibodies on the pharmacodynamics of IFN in an attempt to establish whether they are capable of reducing the bioavailability and biological activity of the administered IFN-α.

The aim of the present study was thus to analyze the pharmacodynamic response to recombinant IFN-α2a (rIFN-α2a) in NAB-seropositive patients with EMC who, after initially responding to treatment with rIFN-α2a, demonstrated a subsequent lack of response. Specifically, the following markers were measured: (i) the expression in peripheral blood mononuclear cells (PBMCs) of the mRNA of a well-known IFN-induced protein, MxA, which is an accepted specific indicator of type I IFN (IFN-α and β) activity (22) and (ii) the levels of protein in serum, a marker of macrophage activity, which is a surrogate marker for IFN-α bioactivity (25).

In addition, the question was addressed whether IFN-α preparations that are different from rIFN-α2a, such as consensus IFN [(C)IFN] and leukocyte IFN (LeIFN), are able to...
overcome the decreases in the biological effects of IFN that developed concomitantly with the formation of NABS.

MATERIALS AND METHODS

Patient characteristics. In this study, attention was focused on two patients with type II EMC, in whom treatment with rIFN-α2a had failed and who therefore had to start a new cycle of IFN therapy. The clinical courses of these patients who were treated with different types of IFN are described below.

(i) Patient 1. Patient 1 is a 62-year-old woman with type II cryoglobulinemia, purpura, diffuse arthralgia, sicca syndrome, hypertension, moderate anemia, proteinuria (3 g/day), reduced creatinine clearance (54 ml/min), and a histopathological diagnosis of diffuse membranoproliferative glomerulonephritis with deposits of immunoglobulin M, immunoglobulin G, and complement. Tests for hepatitis C virus (HCV) antibodies and for plasma HCV RNA were repeatedly negative. Despite the lack of evidence of HCV infection, the patient was treated with rIFN-α2a (Roferon A; Hoffmann-La Roche, Basel, Switzerland) at 3 MU daily for 3 months, followed by 3 MU on alternate days (11). Her response was excellent overall: the cryoglobulin disappeared, as did the clinical symptoms. Some 8 months later, IFN therapy was stopped because of the reappearance of proteinuria that was attributed to IFN nephrototoxicity. The patient’s clinical condition then remained stable for about 5 years, at which point the purpura and proteinuria returned and her cryocrit level rose to 8%. The patient was again treated with rIFN-α2a at 3 MU daily; the purpura disappeared rapidly, but the proteinuria persisted (3 to 6 g/day). Her treatment was interrupted after 3 months when she was found to have NABs to rIFN-α2a. After a further 6 months, the purpura and weakness recurred, and she was treated with (C)IFN (Infergen; Yamanouchi Pharma S.p.A., Milan, Italy) at 9 g daily for 3 months and then on alternate days for a further 6 months. No changes in clinical symptoms or laboratory data were observed. The patient was then treated with LeIFN (Allater; Nuovo Istituto Sieroterapico, Luca, Italy) at 3 MU daily, and within a few weeks the purpura disappeared and the proteinuria level decreased to 0.8 g/day. At the time of this writing, in month 10 of treatment, the patient has no signs of vasculitis and her levels of proteinuria and cryocrit are below 0.1 g daily and below 3%, respectively.

(ii) Patient 2. Patient 2 is a 56-year-old man diagnosed with an HCV-associated type II cryoglobulinemia (cryocrit level, 34%) causing severe purpura, sicca syndrome, and mild nephropathy. He was treated with rIFN-α2a at 3 MU daily for 3 months, followed by 3 MU on alternate days. All clinical signs of vasculitis disappeared a few weeks after treatment began, but HCV RNA remained detectable and the level of cryoglobulins did not fall below 20% throughout treatment. During month 8 of therapy, the purpura returned and treatment with rIFN-α2a was therefore discontinued. The patient was treated with low-dose steroids for 3 years, until marked exacerbation of the purpura occurred. He was then retreated with rIFN-α2a at 3 MU daily for 2 weeks, without any improvement in symptoms. High-titer NABS to rIFN-α2a were then detected in his serum, but the patient refused treatment with other types of IFN. After about 4 years, both purpura and arthralgies had become markedly worse and he was treated with (C)IFN at 9 g daily for 3 months without any benefit. The patient was then treated with LeIFN at 3 MU daily; within 1 week of starting treatment, the purpura disappeared, and after 4 months, HCV RNA was undetectable.

Study design. A pharmacodynamic study of three different forms of IFN-α, namely, rIFN-α2a, (C)IFN, and LeIFN, was undertaken concomitantly with the switching of IFN-α preparations (see the scheme in Fig. 1) in the patients described above. Specifically, blood samples were taken from the patients at the baseline and at 24 and 48 h after a single injection of rIFN-α2a before the start of treatment with (C)IFN. The (C)IFN treatment was started 2 weeks after the injection of rIFN-α2a. Blood samples were also taken at the same time points after the first administration of (C)IFN and LeIFN, when the switch to (C)IFN and LeIFN treatment was about to be initiated.

An analysis was performed of serum interferon and the levels of all three IFN-α preparations, and changes in MxA mRNA and neopterin levels were evaluated at each time point in order to assess the in vivo biological activities of the IFN-α preparations.

Both patients gave their informed consent to participate in the pharmacodynamic study of IFNs.

Blood sampling. Venous peripheral blood from each patient was drawn into tubes containing EDTA and into anticoagulant-free tubes. PBMCs were separated by Ficoll-Hypaque gradient sedimentation; 5 x 10⁹ PBMCs were collected, pelleted, and frozen at −80°C until required. Serum samples, obtained after centrifugation, were also stored at −80°C until required.

Detection of NABs to IFN-α. Antibody titers were determined as described previously (3) by a neutralization test against 10 IU of IFN-α. Different IFN-α preparations were used: rIFN-α2a, (C)IFN, LeIFN, and subtypes of IFN-α (PBL Biomedical Laboratories, New Brunswick, N.J.). The sera were routinely inactivated at 56°C for 30 min before titration. Serial dilutions (starting from 1:10 and increasing twofold) of sera from patients or controls were incubated at 37°C with 60 μl containing 20 IU of each type of IFN-α per ml. After 1 h, 100 μl of each of the individual mixtures was added to duplicate monolayers of human lung carcinoma (A549) cells in 96-well microtiter plates. After 18 to 24 h of culture and after extensive washing, the cells were challenged with the encephalomyocarditis virus and incubated at 37°C for 24 h. The control cells included a titration of the IFN-α preparations used in the respective assays and a reference standard antibody to IFN-α (code CD037-501-572; National Institutes of Health, Bethesda, Md.). Antiviral activity and its neutralization were assessed on the basis of the virus-induced cytopathic effect, and to quantify this, the cells were stained with crystal violet in 20% ethanol. The dye taken up by the cells was eluted with 33% acetic acid, and its absorbance was measured in a microdensitometer at 540 nm. The extent of the virus-induced cytopathic effect, its inhibition by IFN-α, and the reversal of this by NABs were demonstrated by determination of the amount of dye that had eluted from each well. Titers were calculated by the method of Grossberg and Kawade (24) and Kawade (28), and the titers were expressed as 1/10, that is, the dilution of serum that reduces 10 laboratory units of IFN per ml to 1 laboratory unit/ml. The serum samples were routinely assayed for and were found to be free of endogenous or residual IFN activity.

Analysis of MxA transcript expression by a QC RT-PCR technique. Expression of the human MxA gene transcript in PBMCs from the patients was quantified by a quantitative-competitive (QC) reverse transcription (RT)-PCR procedure that was recently set up in our laboratory. The principles and the application of the method have been described in detail elsewhere (4).

(i) RNA extraction. Total RNA was extracted from the PBMCs with TRIZOL (Life Technologies, Milan, Italy), which is based on the acid phenol guanidine thiocyanate method.

(ii) RT-PCR for detection of β-actin mRNA. For RT, 5 μl of each of the RNA samples (25 μg/ml) was heated for 5 min at 80°C, put on ice, and combined with 5 μl of the RT mixture containing a final concentration of 0.5 mM dNTPs, 1 U of RNase inhibitor (Roche) per μl, 0.012 μg of antisense primer (CGT CAT ACT CCT GCT TGC TGA TCC ACA TCT GC) per μl, and 0.8 μl of Moloney murine leukemia virus (M-MuLV) reverse transcriptase (Roche) per μl in a reaction buffer containing 50 mM Tris-HCl, 40 mM KCl, 6 mM MgCl₂, and 10 mM dithioerythritol (Roche). After 1 h at 42°C and 15 min at 65°C, serial dilutions of cDNA were made. Then, 5 μl of each dilution was added to each PCR mixture (45 μl) containing a final concentration of 0.003 μl of each primer (sense primer, TAC CAC TGG CAT GAT GGA CTC CGG TGA CG; antisense primer, CGT CAT ACT CCT GCT TGC TGA TCC ACA TCT GC) per μl each dNTP at a concentration of 200 μM, and 0.04 U of Taq DNA polymerase (Roche) per μl in a reaction buffer containing 10 mM Tris-HCl (pH 8.3), 1.5 mM MgCl₂, and 50 mM KCl. Amplification was performed in a programmable thermal cycle apparatus (Whatman Biometra GmbH, Göttingen, Germany), as follows: 1 cycle at 94°C for 3 min; 30 cycles at 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min; and 1 cycle at 72°C for 10 min. The length of the amplicon obtained was 649 bp. An aliquot of each reaction mixture was subjected to electrophoresis on 1.5% agarose gels, and the gels were stained with ethidium bromide (1 μg/ml) (Bio-Rad Laboratories, Milan, Italy).

(iii) Quantification of MxA transcript expression by a QC RT-PCR technique. For quantification of the MxA-specific transcript, an internal competitor was constructed that contained a deletion of 40 bp (thus distinguishing it from the
TABLE 1. Neutralizing activities of sera from two patients with severe type II EMC resistant to rIFN-α2a

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Neutralization titer against:</th>
<th>IFN-α subtype</th>
<th>Neutralizing activity (t₁/₂) against subtypes of natural IFN-α</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>rIFN-α2a</td>
<td>(C)IFN</td>
<td>LeIFN</td>
</tr>
<tr>
<td>1</td>
<td>950 ± 190</td>
<td>63 ± 10</td>
<td>ND</td>
</tr>
<tr>
<td>1</td>
<td>486 ± 80</td>
<td>30 ± 5</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>1,124 ± 11</td>
<td>189 ± 8</td>
<td>36 ± 7</td>
</tr>
<tr>
<td>3</td>
<td>14 ± 5</td>
<td>108 ± 3</td>
<td>43 ± 4</td>
</tr>
<tr>
<td>4</td>
<td>1379 ± 472</td>
<td>136 ± 74</td>
<td>&lt;10</td>
</tr>
<tr>
<td>5</td>
<td>112 ± 64</td>
<td>49 ± 20</td>
<td>&lt;10</td>
</tr>
<tr>
<td>6</td>
<td>83 ± 55</td>
<td>84 ± 7</td>
<td>&lt;10</td>
</tr>
<tr>
<td>7</td>
<td>1,124 ± 11</td>
<td>136 ± 76</td>
<td>&lt;10</td>
</tr>
<tr>
<td>8</td>
<td>37 ± 2</td>
<td>43 ± 4</td>
<td>&lt;10</td>
</tr>
<tr>
<td>9</td>
<td>22 ± 13</td>
<td>&lt;10</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

a Sera for detection of neutralizing activity were collected before the injection of the different IFN-α preparations (see text for details). The data are expressed as the means ± standard deviations for three separate determinations.

b Serum was analyzed before the injection of rIFN-α2a.

c ND, not done.
d Serum was analyzed before the first injection of (C)IFN.

e Serum was analyzed before the first injection of LeIFN.

α2a, both patients had NABs to rIFN-α2a in their sera, with the titer in patient 1 being higher than that in patient 2.

To determine whether there was any cross-reactivity between the different types of IFN-α, the neutralizing activities of positive sera from both patients against rIFN-α2a, (C)IFN, and LeIFN were compared. The antibodies neutralized (C)IFN, although the patients had not previously been treated with this type of IFN. However, a higher concentration of antibody was required to neutralize rIFN-α2a than to neutralize (C)IFN. Table 1 shows that these NABs were specific to both recombinant forms of IFN-α but were completely ineffective against LeIFN (as analyzed before the switch to treatment with this type of IFN). Interestingly, the serum sample from patient 2 that was collected before treatment with (C)IFN contained a higher titer of NABs than that collected before treatment with rIFN-α2a.

Further analysis allowed us to evaluate the IFN-α subtype specificities of the NABs in the patients’ sera before the LeIFN treatment cycle was started. Table 2 shows the results of the cross-reactivity experiment, in which the levels of neutralizing activity against several recombinant subtypes of IFN-α were measured in the sera of the patients with EMC. It can be seen that the titer against rIFN-α2a, the type of IFN originally administered, was high in both patients. Several other subtypes of IFN-α were neutralized by the same sera, but for some subtypes, the antiviral activity was only marginally affected. The inhibitory activities of the NABs were overcome by two subtypes of IFN-α (subtypes 8b and 14a) in patient 1 and by three subtypes (subtypes 1, 8b, and 21a) in patient 2. Interestingly, a low titer (t₁/₂ <20) against subtypes 7a and 10a was seen in NAB-positive patient 1.

Pharmacodynamic study. In the pharmacodynamic study, the levels of MxA mRNA expression in PBMCs and serum neopterin levels were measured under ex vivo conditions before and after a single injection of rIFN-α2a, (C)IFN, or LeIFN when the change to (C)IFN or LeIFN was about to be initiated (see the scheme illustrated in Fig. 1). Figures 2 (patient 1) and 3 (patient 2) show the results of one representative experiment of the three performed.
Induction of MxA mRNA expression. No increase in MxA mRNA levels was detectable in patient 1 after administration of rIFN-α2a. A similar result was obtained after the administration of (C)IFN, whereas a significant increase (≥10-fold) in the level of MxA mRNA expression was noted after the administration of LeIFN. The induction peak for MxA mRNA was observed 24 h after administration of LeIFN.

In patient 2, who had only a low NAB titer, a slight response was observed in terms of induction of MxA mRNA expression after administration of rIFN-α2a, with the peak of activity occurring at 24 h. There was no increase in the MxA mRNA level in this patient after the first administration of (C)IFN. This lack of increase coincides with the increase in the NAB titer against IFN-α2a and (C)IFN (Table 1). However, after the injection of LeIFN, a significant increase (≥10-fold) in the level of transcription of the human MxA gene was observed at 24 and 48 h.

Induction of serum neopterin. The results indicated that in patient 1 there was no induction of neopterin production after administration of IFN-α, this being independent of the type of commercial preparation (Fig. 4).

A similar finding also applied to patient 2, although a slight increase in the neopterin level was observed 48 h after the rIFN-α2a injection (Fig. 4).

To verify whether this lack of induction of neopterin is due to the presence of NABs and not to the inability of these patients to produce neopterin in response to IFN treatment, the serum neopterin levels were measured in five NAB-negative EMC patients before and at 24 and 48 h after rIFN-α2a administration. The results are shown in Fig. 5, from which it can be seen that neopterin levels increased significantly in NAB-negative patients after injection of rIFN-α2a (P < 0.05).

DISCUSSION

A key issue in anti-IFN antibody development is establishment of whether these antibodies can affect the bioactivity of administered IFN.
In this study, we demonstrated that NABs inhibit the in vivo biological activity of IFN-α in EMC patients receiving treatment with rIFN-α by measuring the levels of expression of the human MxA gene transcript in PBMCs. This finding indicates the in vivo biological significance of NABs against rIFN-α2a and (C)IFN and confirms the results of previous investigations, in which depressed levels of surrogate markers of IFN activity were seen in patients suffering from several diseases in which NABs developed during rIFN therapy (14–16, 42).

Furthermore, when treatment was switched to LeIFN, the biological response was restored in rIFN-α2a- and (C)IFN-seropositive treatment-resistant patients, with the administration of a natural preparation of IFN-α being able to induce a significant increase in MxA mRNA expression at 24 and 48 h postinjection.

We consider the restoration of the biological responses in these patients to be due to their ability to overcome the activities of anti-IFN-α NABs. Indeed, NABs that were specific to the earlier rIFN-α2a preparations cross-reacted with (C)IFN but not with all the subtypes that comprise LeIFN. Indeed, at least four subtypes of IFN-α (i.e., subtypes 1, 8b, 21a, and 14a) retained their antiviral activities in the presence of NAB-containing sera from the two patients.

Consistent with our observation, other investigators have reported that most of the sera obtained from patients treated with rIFN-α2 contain antibodies that are capable of neutralizing recombinant forms of IFN-α2 but that are generally incapable of significantly neutralizing the antiviral activity of natural IFN-α (1). However, we also demonstrated that the NABs that developed during rIFN-α2a therapy had a well-defined spectrum of specificity. Indeed, the sera from these patients neutralized most, but not all, of the subtypes present in the

FIG. 3. QC PCR analysis of MxA mRNA in PBMCs from patient 2. PBMCs were collected before and at 24 and 48 h after a single injection of rIFN-α2a, (C)IFN, and LeIFN. Serial logarithmic dilutions of extracted RNA, normalized by using β-actin mRNA as a control, were mixed with 2 × 10⁶ copies of the internal competitor and subjected to RT-PCR (for details, see Materials and Methods). The arrows indicate the equivalence points.
subtypes are not neutralized by antibodies to rIFN-α/H9251 which it has been indicated that natural forms of IFN-α/H9251 natural mixture of IFN-α/H9251 2a, (C)IFN, and LeIFN. Bars represent means ± standard deviations for three separate determinations.

These findings are consistent with several clinical reports in which it has been indicated that natural forms of IFN-α may be useful in reestablishing responses in patients who have become resistant to recombinant preparations of IFN-α (5, 8, 13, 20, 30, 31, 38, 42). Indeed, the treatment of our patients with LeIFN also resulted in a restoration of the clinical response.

Some questions, however, remain unanswered. For instance, it is not clear why the neutralizing titer for rIFN-α2a was higher than that for (C)IFN. Several hypotheses can be considered.

It is possible that the amino acid sequence of (C)IFN, which represents a consensus IFN sequence (the most commonly appearing amino acids at each locus) of most naturally occurring human type I IFNs (29), may have a conformational structure different from that of rIFN-α2a, leading to more or more accessible antigenic epitopes. Alternatively, the lower NAB titer may be explained by the fact that the (C)IFN molecule has been shown to have in vitro biological activity different from those of other type I IFNs (6, 35), and this may mean that different amounts of antigen or molecule must be neutralized in the bioassay; consequently, there are different ways in which NABs may neutralize it. Further studies into this phenomenon are needed.

FIG. 4. Neopterin protein levels in the sera of patients 1 (A) and 2 (B). Serum neopterin concentrations were measured by enzyme-linked immunosorbsent assay before and at 24 and 48 h after a single injection of rIFN-α2a, (C)IFN, and LeIFN. Bars represent means ± standard deviations for three separate determinations.

Moreover, an increase in the NAB titer was observed in patient 2 approximately 2 weeks after the single injection of rIFN-α2a, together with a lack of induction of MxA after the first administration of (C)IFN concomitant with the increase in NAB titers to rIFN-α2a and (C)IFN, as described above. A possible explanation for the latter is that the single administration of an antigen (i.e., rIFN-α2a) stimulated a secondary humoral response. However, because no further data on this topic are available, such a consideration can be only speculative because the serum of patient 1 seems to have behaved in a different way, with the NAB titer being slightly lower after the administration of rIFN-α2a. Further studies are needed to verify this hypothesis. It is also worth noting that both patients showed resistance to rIFN-α2a from the clinical point of view, although the concentration of NAB in patient 1 was significantly higher than that in patient 2. It can be speculated that in EMC patients, in contrast to what is observed in hairy-cell leukemia patients (42), the pharmacokinetic and pharmacodynamic properties of rIFN-α2a may also be affected by a relatively low concentration of NAB.

It is both interesting and intriguing that the increase in the level of MxA expression was not in parallel with the increase in the serum neopterin concentration. A possible reason for the lack of increase in the serum neopterin concentration in patients after administration of LeIFN could be that, in contrast to MxA transcript expression, neopterin is not induced solely by type I IFN but is induced by a broader spectrum of other cytokines, such as IFN-γ (25), tumor necrosis factor alpha (25), and interleukin-10 (41), such that it therefore represents a less specific parameter in IFN-treated patients and, above all, in NAB-positive patients. One can also speculate that the IFN-α subtypes that are not neutralized by NABs to rIFN-α2a are unable to induce the expression of such a marker. It has already been reported that some subtypes of IFN-α have peculiar activities (17–19, 23, 34, 43), and this observation could be a further confirmation that the existence of different subtypes of IFN-α may be of biological significance. In future work, a better understanding of IFN signaling pathways will enable us
to explain the different behaviors of MxA and neopterin seen here.

Because LeIFN restored the clinical responses of these patients, the results strongly indicate that MxA represents a surrogate marker for the clinical outcome, while other markers, such as neopterin, that are significantly affected by INF-α treatment, may be only indirect markers of the biological activity of IFN. From this it may be concluded that neopterin is a less specific marker, particularly in NAB-positive INF-α-treated patients, during monitoring of IFN-treated patients.

In conclusion, although the results presented here relate to only two patients, they provide evidence that NABs have in vivo biological significance against INF-α in patients with EMC and that the problem of NABs still exists. In addition, the data demonstrated that the effects of NABs on changes in the bioactivity of INF-α and the restoration of biological and clinical responses to IFN can be monitored by measuring the expression of MxA in patients undergoing IFN therapy.

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