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Development of neutralizing and binding antibodies to interferon (IFN) in patients undergoing IFN therapy

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Abstract

Since the 1970's, people started to use interferon as a therapeutic agent against infectious and neoplastic diseases. Since the beginning of its therapeutical use, it was demonstrated that some patients produce, when treated with interferon, antibodies able to neutralize its biological activity. This paper will review and discuss what it is currently known about the technical, biological, and clinical aspects of these antibodies.

Key words: Interferon; Antibody; Interferon therapy; Viral hepatitis

1. Introduction

Several reports have been published describing the detection of antibodies (Abs) to interferon (IFN) in sera of normal individuals (De Maeyer-Guignard and De Maeyer, 1986; Ross et al., 1990; Caruso et al., 1990) or patients with autoimmune diseases or viral infections (Panem 1984; Prummer et al., 1989; Ikeda et al., 1991). Even more frequent is the observation of Ab production by patients receiving exogenous IFN as a therapeutic agent of viral or neoplastic diseases (for a review see Figlin and Itri, 1988; Antonelli and Dianzani, 1993).

At the beginning this finding was totally unexpected because IFN could be considered an autoantigen, i.e., a "self" molecule. However, since Abs responses have been reported in patients treated with human insulin or growth hormones (Fineberg et al., 1983; Gribben et al., 1990), and since autoantibodies to IL-1, IL-2, and TNF

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have been detected in normal individuals (Bendtzen et al., 1990), the presence in the serum of "naturally" occurring or induced Abs to cytokines or hormones started to be considered a general phenomenon that drew the attention of a number of scientists and in the last few years it has been proposed that these autoantibodies, by interacting with their own ligand, establish a physiological regulatory mechanism leading to the general homeostasis of the organism (Bendtzen et al., 1990; Avrameas, 1991).

However since most of such Abs, particularly when high titered, inhibit in vitro the biological functions of various mediators, it is thought that their production is undesirable when the autoantigen is exogenously administered to achieve therapeutical benefits. This is particularly true for IFN, since it has been shown that the development of such Abs is often associated with a decreased clinical response to therapy.

Here, we summarize what is currently known on the biological and clinical significance of the Abs to IFN with particular reference to Abs produced by chronic hepatitis patients undergoing IFN therapy. It should be now underlined that most of the discussion will be focused on Abs to IFN alpha since the data on therapeutically induced Abs to IFN beta and above all IFN gamma are as yet rather limited.

2. Biological and technical aspects of Abs to IFN

At least three assays are currently available for detecting Abs to IFN. These include an enzyme immunoassay (EIA), a radioimmunoassay (RIA), and an antibody-neutralizing bioassay (ANB).

Of the three, only ANB is capable of detecting Abs directed to functional epitopes involved in generating the biological activity of IFNs. Furthermore, the ANB assay permits the detection of neutralizing Abs directed against any type of IFN action. In fact besides the antiviral activity, these antibodies can neutralize activation of cell mediated cytotoxicity and cytostatic or antiproliferative activity (Antonelli et al., 1991; Prummer et al., 1991; Catani et al., 1992).

Conversely, EIA and RIA tests detect neutralizing as well as non-neutralizing or binding Abs, i.e., antibodies directed against epitopes that are not involved in the biological activity of IFN, but without permitting to distinguish between these two types of Abs.

Whatever is the test used, sera must be analyzed for Abs to IFN at least 48–72 h after the last injection, since, it has been demonstrated that the Ab titer falls after each IFN administration (Von Wussow et al., 1989). This is probably due to the formation of highly stable immunocomplexes.

It is also known that while neutralizing Abs are usually immunoglobulin of the IgG type, binding Abs, which develop before the appearance of neutralizing Abs, are usually of the IgM type (Von Wussow et al., 1989).

The time of appearance of Abs to IFN alpha is quite variable ranging from 1 to 30 months of treatment. Also the titer is very variable and unstable and, indeed, it has been shown that in some patients Ab titer may fall despite continuation of

therapy (Steis et al., 1991).

As it would be expected, the frequency of seroconversion measured by EIA or RIA is always higher than the seroconversion frequency revealed by ANB (Jacobs et al., 1989; Antonelli et al., 1992). Thus, one critical factor to be considered in calculating the incidence of seroconversion is the assay type that is being employed. Other factors such as IFN dosage or type, route of administration, duration of treatment, or underlying disease may affect the incidence of seroconversion.

In fact, the percentage of patients developing Abs during or after IFN treatment varies considerably, from total absence in a group of hairy cell leukemia (HCL) patients (Golomb et al., 1988) to as high as 56% seroconversion in one group of melanoma patients (Dummer et al., 1991). The seroconversion rate is not influenced by sex or age, while race seems to be a predisposing factor. In fact it has documented that chinese patients with chronic hepatitis B develop neutralizing Abs more frequently than caucasian patients (Lok et al., 1988). Also the duration of treatment and the route of administration are likely to influence the immunogenicity of IFN. For instance a higher incidence of Abs has been reported in HCL or chronic mieloid leukemia (CML) patients receiving long-term treatment with rIFN alpha 2 as compared to patients treated for a shorter period of time (Steis et al., 1988; Figlin and Itri, 1988; Von Wussow et al., 1989). In another study seroconversion was reported significantly higher in patients treated with IFN subcutaneously rather than intravenously (Larocca et al., 1989). Although no explanation has been provided, it has been reported that treatment at low dosage induces Abs more frequently than treatment at high dosage (Porres et al., 1989). As regard the type of disease, it has been reported that oncologic patients produced Abs to IFN to a lesser extent compared to patients suffering from infectious diseases (Jacobs et al., 1989).

Additionally, it was tempting to speculate that also the type of IFN administered could influence the frequency of both binding and non-neutralizing Ab development. Indeed, differences have been observed between the frequency of seroconversion to IFN in patients treated with rIFN alpha 2a, rIFN alpha 2b and IFN alpha N1, a natural mixture of different IFN alpha subtypes (Antonelli et al., 1989; Antonelli et al., 1991) (Table 1).

At this time it is difficult to explain the different immunogenicity of the types of IFNs used in that study. Several hypotheses can be considered. It is possible that natural IFN, which is a mixture of at least 18 homologous subtypes, may be less

Table 1
Frequency of anti-IFN Abs in hepatitis patients treated with rIFN alpha 2a, IFN alpha 2b, and IFN alpha N1

Type of IFN	Neutralizing Abs No positive/No tested	Titer of Neutralizing Abs	Binding Abs No positive/No tested
alpha 2a	15/74 (20.2%)	10–10.240	33/74 (44.6%)
alpha 2b	10/144 (6.9%)	10–160	21/144 (14.6%)
alpha N1	1/78 (1.2%)	N.A.	7/74 (9.4%)

Data from Antonelli et al. (1991) and Antonelli et al. (1992).

immunogenic than rIFN since any subtype is actually present at a lower concentration than in an equal dose of rIFN, which contains only a single molecular species (Finter, 1991). Since the rIFNs differ for one amino acid and both are not glycosilated, it is possible that the tertiary structure and thus immunogenicity of the molecule is influenced by specific molecular configuration. It is also possible that the carbohydrates present on native IFN may influence the antigenicity of the molecule by masking immunogenic sites (Finter, 1991). To answer some of the questions raised by the ANB, the same sera were tested by a solid phase EIA aimed to detect binding Abs to IFN alpha 2. The results are shown in Table 1. Thirty-three of the 74 (44.6%) patients treated with rIFN alpha 2a seroconverted, whereas 21 of the 144 (14.6%) patients treated with rIFN alpha 2b and 7 out of the 74 patients treated with (9.4%) IFN alpha N1 were positive by EIA (Antonelli et al., 1992).

These data confirm that rIFN alpha 2a is more immunogenic than the other IFNs used. They also suggest that, in case of IFN alpha N1, the development of binding Ab for the alpha 2a subtype occurs at a higher frequency than does neutralizing Ab. On the other hand, seroconversion rates for rIFN alpha 2b and IFN alpha N1 are not statistically different, suggesting that the subtype IFN alpha 2 may have similar immunogenic activity both in the recombinant product (IFN alpha 2b) and the "natural" product (IFN alpha N1).

Interestingly, most of the sera obtained by treated patients contain highly specific Abs. In fact, although cross-reactivity has been demonstrated between rIFN alpha 2a and 2b, Abs produced by rIFN alpha 2-treated patients fail to neutralize the antiviral activity of IFN alpha N1 (Table 2 and Antonelli et al., 1991). This suggests that the specificity is restricted to the single subtype used in the therapy and

Table 2
Neutralizing activity of sera derived from rIFN alpha 2-treated patients against different subtypes of IFN

Sera No.	Alpha					
	Titer (NU)[a] against IFN alpha:					
	2a	A	D	A/D[b]	B/D[c]	N1[d]
1	355	266	66	<5	<5	<5
2	266	166	22	<5	<5	<5
3	88	33	<5	<5	<5	<5
4	178	67	<5	<5	<5	<5
5	667	513	125	<5	<5	<5
6	167	128	22	<5	<5	<5
7	333	266	66	<5	<5	<5
8	167	166	<5	<5	<5	<5

[a]NU Neutralization Units.
[b]A/D Hybrid IFN alpha.
[c]B/D Hybrid IFN alpha kindly provided by Dr. H. Hockeppel (Ciba Geigy, Basel).
[d]N1 IFN alpha N1 is a mixture of different IFN alpha subtypes produced by Namalwa cells (see Finter, 1991).

possibly to other single subtypes, but it appears that such Abs fail to neutralize all subtypes present in the natural IFN alpha (Antonelli et al., 1991).

Recently, however, it has been reported that positive sera from rIFN alpha 2-treated patients can neutralize to some extent the IFN alpha N1 mixture (Ronnblom et al., 1992; Brand et al., 1993). In the same reports, it has been shown that the emergence of cross-neutralizing Ab coincided with the increasing titer to the rIFN used for the treatment. However such Abs neutralize 20–100-fold more effectively the rIFN alpha 2 than the other subtypes. Thus, one can speculate that the neutralizing Abs formed in patients treated with a single rIFN alpha: (i) have a variable specificity depending only on the subject who produces Abs; (ii) neutralize to some extent the subtypes present in natural mixture of IFN alpha; or, more likely, (iii) fail to neutralize some of the subtypes of IFN alpha mixture although displaying to some extent a cross-reactivity with other subtypes. Further studies are needed to dissect between the different hypotheses.

As far as Abs to IFN beta are concerned, it is common opinion that the incidence of seroconversion in IFN beta-treated patients is similar to that obtained in patients treated with IFN alpha (Larocca et al., 1991; Antonelli et al., unpublished data). However reports describing higher frequency of development of Abs in melanoma patients treated with natural IFN beta (56%; Dummer et al., 1991) or in multiple-sclerosis patients treated with IFN beta-Ser (above 40%; the IFNβ Multiple Sclerosis Study Group, 1993) have been recently published. Again, as stated in the case of IFN alpha, the discrepancy can be explained by the use of different type of assay or type of underlying disease.

A few papers are available on the immunogenicity of IFN gamma. Basically, they documented the absence of Ab formation in patients undergoing human IFN gamma therapy (Jaffe et al., 1987).

3. Clinical significance

The first observation of anti-IFN antibodies dates to 1981, when neutralizing Abs to IFN were identified in a patient treated with IFN beta (Vallbracht et al., 1981). Since then, many other reports have been published concerning development of anti-IFN antibodies in patients treated with IFN alpha and beta (Mogensen et al., 1981; Quesada et al., 1985; Figlin et al., 1986; Leavitt et al., 1987; Quesada et al., 1987; Golomb et al., 1988; Itri et al., 1988; Dianzani et al., 1989; Galton et al., 1989; Oberg et al., 1989; Weck et al., 1989; Liao et al., 1992).

Certainly, one of the key issues of the anti-IFN Ab development is to establish whether these Abs can affect the therapeutic efficacy of the administered IFN.

Resistance to the antitumor or antiviral effects of recombinant IFNs associated with development of neutralizing Abs has been found in CML (Von Wussow et al., 1987), HCL (Steis et al., 1988), or hepatitis patients (Porres et al., 1989; Lok et al., 1990), whereas other reports indicate that Abs to IFN do not appear to be associated with any adverse clinical sequelae (Craxi et al., 1988; Figlin and Itri, 1988). Furthermore, while some authors have recorded an improvement of flu-like syn-

drome concomitantly with the formation of Abs to IFN (Lok et al., 1990; Porres et al., 1989), others did not observe any effect of Abs on severity of the side effects (Spiegel et al., 1987; Steis et al., 1988).

The discrepancy of these results can be explained, at least in part, by taking into account that the failure of IFN therapeutic activity may not only be due to the "qualitative" presence of Abs to IFN but also to the amount and neutralizing efficacy of such Abs as well as the time of their appearance and their specificity. In regard, it is interesting to mention that patients who become resistant to rIFN alpha treatment can often be efficaciously treated with natural IFN alpha preparations (Von Wussow et al., 1988; Von Wussow et al., 1991; Casato et al., 1991; Catani et al., 1992; Colloredo-Mels et al., 1993). This is not unexpected if one considers that the results obtained in vitro indicate that natural IFN alpha can overcome the neutralizing activity displayed by positive sera derived from rIFN alpha 2 treated patients (Antonelli et al., 1991). Moreover recent results on the cross-reactivity between rIFN alpha 2 and some subtypes of IFN alpha N1 (Brand et al., 1993), may explain why a few patients who developed Ab-mediated resistance to treatment with rIFN alpha 2 are unable to respond when treated with IFN alpha N1.

The data currently available do not allow definite conclusions to be made, but some recent results strongly support the view that, at least in some patients, the development of neutralizing Abs directly correlates with the loss of the therapeutic effect.

For instance, neutralizing Abs to rIFN alpha could be detected in 3 out of 4 cryoglobulinemia patients who relapsed during maintenance therapy while none of the patients with persistent response showed detectable amounts of Ab to IFN (Casato et al., 1991).

Furthermore in a trial conducted in Germany on HCL patients, neutralizing Abs have been found in all relapsing patients while none of the Ab-negative patients relapsed (Von Wussow et al., 1991).

In our recent study on hepatitis C patients, the seroconversion rate was significantly lower in responder patients than in non-responders. Specifically, the therapeutic failure due to antibody formation might be attributed to hepatitis patients who after an initial response showed disease reactivation (breakthrough) concomitantly with anti-IFNAb appearance (Fig. 1), while no clinical significance could be attributed to the Ab development in patients who never showed ALT-reduced levels during treatment (Milella et al., 1993; Colloredo-Mels et al., 1993). The finding that breakthrough of hepatitis patients can be efficaciously overcome by changing the type of IFN administered (Colloredo-Mels et al., 1993; Milella et al., unpublished data) supports the view that neutralizing Abs to IFN, are profoundly involved in inducing the non-responsiveness during IFN therapy. Obviously other factors, such as subtype of HCV, natural hyporesponsiveness to IFN, down regulation of IFN receptors, etc., could be taken into account to explain the breakthrough phenomenon in hepatitis C patients.

As far as binding Abs are concerned, it should be pointed out that although the clinical significance has yet to be established, some preclinical evidence suggests that this type of antibody can prevent normal clearance and degradation of administered

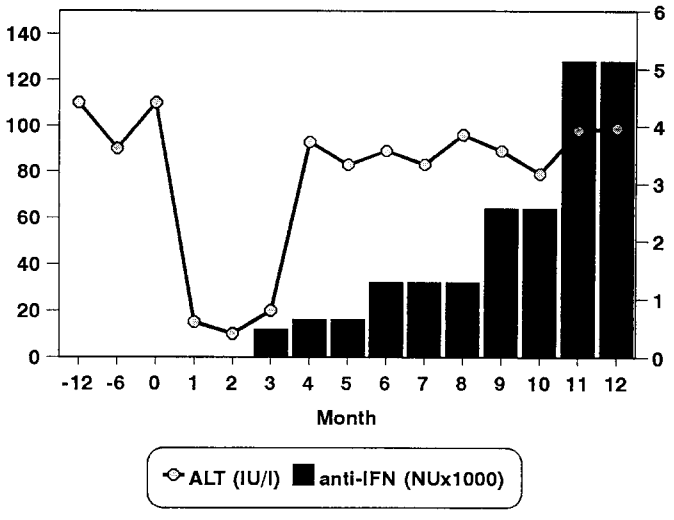

Fig. 1. Typical case of hepatitis C reactivation concomitantly with anti-IFN Ab development in one patient who had an initial response to therapy. ALT levels and neutralizing antibodies IFN were assayed monthly since the beginning of the IFN therapy (Time 0). (Data from Milella et al., 1993.)

IFN alpha, therefore modifying its pharmacokinetics (Rosenblum et al., 1985). Furthermore, we could show that: (i) although the percentage of binding Abs positive hepatitis C patients is not different between responders and non responders, the production of binding Abs in non responders patients occurs significantly earlier than in responder patients (Giannelli et al., 1994); (ii) most of the neutralizing Ab-negative patients experiencing a breakthrough developed binding Abs (Colloredo-Mels, 1993). These findings suggest that also this type of Abs to IFN can in some way influence the outcome of the therapy. In this respect it should be considered that at the moment: (i) it is not possible to exclude the existence of different types of such Abs, which might be specific for different epitopes of the IFN molecule; (ii) binding Abs can be functionally different, from a subject to another, depending on their affinity for IFN molecule.

In conclusion the current literature demonstrates that, at least in some patients, anti-IFN Abs development can affect the response to IFN but raises new and important questions, whose answers still have to be elucitated.

For instance, it is hard to explain why only a minority of patients develop Abs to IFN, or why some types of IFN induce a higher percentage of seroconversion. Furthermore, it remains to be ascertained why in some patients these Abs are produced only transiently during therapy and why they have a well-defined specificity. It is our firm opinion that further studies are needed to give new insights into the phenomenon of this "atypical" humoral response.

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