

Increase of IFNAR1 Messenger RNA in Myxovirus-protein-A-induced Multiple Sclerosis Patients to Oppose Loss of Protein Receptor

a report by

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Type I Interferons and Their Receptor

Human interferons (IFNs), first recognised for their potent antiviral activity 50 years ago, are a group of naturally occurring cytokines with important immunomodulatory, antiviral, antiangiogenic, antiproliferative and antitumour activities.¹ They are classified into three major sub-families based on their biological and physical properties. Type I IFNs include IFN- α , IFN- β , IFN- ϵ , IFN- κ , IFN- ω , IFN- δ and IFN- τ ; among them, IFN- α and IFN- β are the main types of interest, since IFN- ϵ and IFN- κ are expressed only in the placenta and in keratinocytes and IFN- δ and IFN- τ are not found in humans.² There are more than 20 different IFN- α genes, of which 13

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encode functional polypeptides, whereas there is only one type of IFN- β . Type II IFNs include only IFN- γ , while the new family of type III IFNs has three subtypes of IFN- λ (also termed interleukin [IL]-28A, IL-28B and IL-29), which are co-produced with IFN- β .¹

As found for most cytokines and growth factors, the actions of IFNs are mediated by an interaction with specific cell surface receptors. All of the type I IFNs share the same receptor complex, whereas type II IFN binds to a distinct receptor, as do the interferon-like cytokines IL-28A, IL-28B and IL-29.³ The receptor complex for the type I IFNs consists of two chains, IFNAR1 and IFNAR2,^{4,5} whose genes are clustered on chromosome 21. Two splice variants of IFNAR1 have been identified in cell lines,⁶⁻⁸ but one is probably an artefact or an aberrant transcript found only in particular tumour cell lines.⁹ In contrast, IFNAR2 is expressed *in vivo* in three different isoforms, which are generated by alternative splicing, exon skipping and differential usage of polyadenylation sites and differ in the length of the carboxyterminal tail and in the signalling capacity.¹⁰ IFNAR2.2 full-length is the functional isoform, and is made up of 487 amino acids, 251 of which lie in the cytoplasmic portion.^{11,12} IFNAR2.1 short isoform has a truncated cytoplasmic tail of 67 residues, and is partially impaired in the signalling response¹¹⁻¹³ or incapable of complete signalling.¹⁴ IFNAR2.3, lacking both the transmembrane and intracytoplasmic domains, is a soluble receptor isoform that has been found in different body fluids.^{1,15} Depending on its relative concentration, the stability of its binding with the ligand and the rate of discharge, it may be regarded either as a competitive antagonist, acting as a molecular

decoy, or, indirectly, as an agonist, as it protects bound IFN- β from degradation and prolongs its half-life.¹⁶

The process of receptor activation involves the initial binding of type I IFNs to the IFNAR2 subunit¹⁷ followed by the recruitment of the IFNAR1 subunit, with subsequent commencement of a signalling cascade¹⁸ that leads to catalytic activation of associated tyrosine kinase 2 (Tyk2) and Janus tyrosine kinase 1 (Jak1), which in turn phosphorylate signal transducer and activator of transcription (STAT)-1 and STAT-2 (although activation of STAT-3, STAT-4, STAT-5 and STAT-6 has also been reported).¹⁹⁻²² All of these phenomena ultimately activate the IFN-stimulated response elements (ISRE) of the gene promoter,²³ which in turn regulate the transcription of the several genes responsible for the IFN-mediated effects.

Interferon- β Treatment in Multiple Sclerosis

Multiple sclerosis (MS) is an immune-mediated demyelinating disease of the central nervous system characterised by bouts of neurological symptoms (or relapses) and increasing disability. Although this disease was first described in the 1800s,²⁴ therapies have become commercially available only during the last 15–20 years. IFN- β was the first drug to be approved and, despite several novel therapies being tested and/or recently introduced, it still provides the mainstay of MS disease-modifying therapies. All of the three recombinant IFN- β preparations currently registered for MS therapy – Rebif (IFN- β -1a; Ares-Serono, Geneva, Switzerland), Avonex (IFN- β -1a; Biogen, Cambridge, MA, US) and Betaferon (IFN- β -1b; Schering AG, Berlin, Germany) – have been shown to positively modulate disease activity (relapses and active lesions apparent on magnetic resonance imaging), while therapy advantages on disease progression (disability and total lesion burden) are less consistent.

Comparative data across studies on different IFN- β preparations suggest that the optimal choice of IFN- β subtype, preparation and

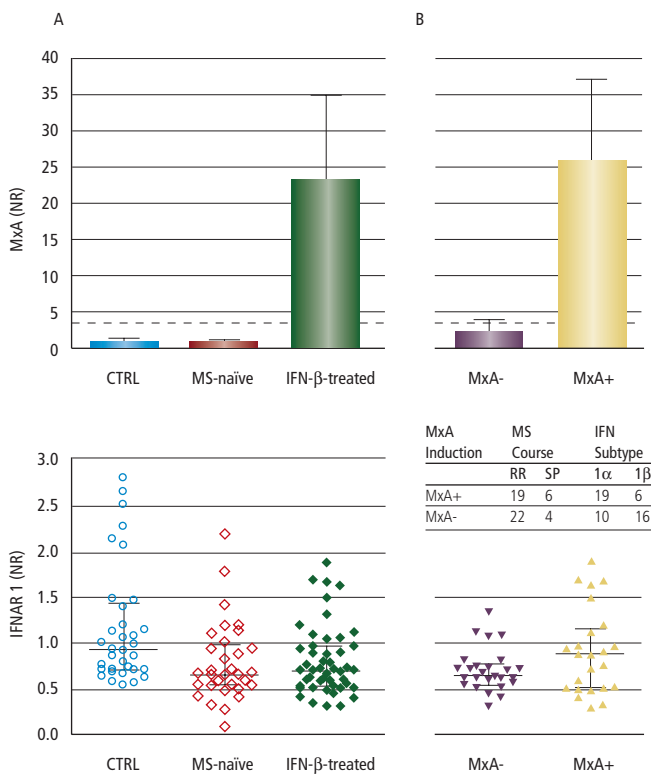


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allow more targeted interventions with patients, as well as benefits and costs containment. Dr Imberti received her MD from the University of Milan, and then undertook extensive training in tumour immunology in the US.

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Figure 1: Myxovirus Protein A and IFNAR1 Messenger RNA Expression



A: Myxovirus protein A (MxA) and IFNAR1 levels expressed as normalisation ratio (NR) in control subjects (CTRL), therapy-naïve multiple sclerosis (MS) patients and a group of MS patients undergoing interferon (IFN)- β treatment for at least 24 months. B: MxA and IFNAR1 messenger RNA (mRNA) levels in treated MS patients classified as MxA-induced (MxA+) or non-induced (MxA-) on the basis of their MxA level above or under the cut-off (99th percentile of controls, dotted line). Table shows characteristics of MxA-induced and non-induced patients.

dose regimen is an important determinant of drug efficacy. Indeed, IFN- β is effective in only a percentage of patients, since after six to 18 months of treatment some of them develop binding (BAb) and neutralising (NAb) anti-IFN- β antibodies, leading to loss of drug bioactivity.²⁵⁻²⁷ Because the response to IFN- β can be assessed on a clinical basis only after a relatively prolonged treatment, the quantification of NAb has for many years been the gold standard laboratory test for monitoring IFN- β therapy efficacy. Recently, alternative methods for measuring IFN- β biologic activity have been developed and, among these, the determination of the IFN- β -inducible gene product Myxovirus protein A (MxA) has proved to be the most reliable because this protein and its messenger RNA (mRNA) are induced in a dose-dependent manner by type I IFNs.²⁵ MxA induction loss, if measured exactly 12 hours after IFN- β injection, correlates well with the presence of anti-IFN- β antibodies; therefore, it can be considered an appropriate test to determine IFN- β bioavailability.²⁷⁻³⁰ However, some patients are unresponsive to treatment even in the absence of anti-IFN- β antibodies,³¹ and therefore other reasons for lack of therapy efficacy still have to be identified.

Involvement of IFNAR in Interferon- β Activity

It has been proposed that the biological response to IFN- β could be affected not only by the presence of BAb or NAb, but also by other competing serum factors,^{32,33} such as soluble IFNAR.³⁴ Furthermore, as

a body of arguments suggests that the differential affinities for IFNARs govern the diverse biological activities among members of type I IFN families sharing the same receptor,^{17,18} it is also likely that differences in the amount of IFN- β and IFNAR binding may account for the differential therapeutic activity of the cytokine in different patients. Finally, the cell surface concentration of IFNAR and lateral organisation of its two chains into microdomains might be other important cellular parameters that shape responsiveness to IFNs.³⁵ For example, the autocrine production of IFN- β from LPS- and poly I:C-matured dendritic cells can induce a marked decline in the level of the two IFNAR subunits,³⁵ and a modulation in receptor expression has been observed in the course of therapy with IFN- α in both HCV and chronic myelogenous leukaemia patients.^{36,37}

If we consider the rather low number of IFNAR molecules on the cell surface,¹² especially on lymphocytes,³⁸ providing a limited safety margin for response, it is evident that even a minimal modulation of IFNAR component expression may interfere with the biological activity of IFN- β in MS patients. Up to now, only a few studies have investigated the role of all IFNAR components in human diseases or under conditions of chronic receptor stimulation, and only a few studies have analysed the expression of all of the different isoforms of IFNAR2 at the same time.³⁹⁻⁴¹ A quantitative realtime polymerase chain reaction (PCR) assay that measures concomitantly IFNAR1 and IFNAR2 subunit mRNA and the three IFNAR2 isoforms is now available.⁴⁰ Using this method we have previously demonstrated that IFNAR1 and total IFNAR2 subunits are both expressed at high levels, and that IFNAR2.2 is significantly the most represented isoform, while IFNAR2.3 is found, on average, at barely detectable levels. However, the distribution of IFNAR2.3 isoform values is rather wide, indicating that it is possible to find higher levels of soluble receptor in the sera of a few samples.⁴⁰ We then used this laboratory test to quantify mRNA expression for all IFNAR components in a group of long-term IFN- β -treated MS patients selected among

Comparative data across studies on different interferon (IFN)- β preparations suggest that the optimal choice of IFN- β subtype, preparation and dose regimen is an important determinant of drug efficacy.

those undergoing routine MxA determination in our laboratory. Their IFNAR mRNA levels were compared with those of a control group and of therapy-naïve MS patients in order to ascertain receptor modulation during prolonged receptor stimulation by IFN- β . Our results indicated that the overall pattern of expression of the various subunits and isoforms in peripheral blood cells of MS patients is similar to that observed in healthy controls and in HIV patients.⁴²

IFNAR1 Subunits in Interferon- β -treated Patients

We also found that naïve and IFN- β -treated MS patients showed a significantly decreased expression of mRNA for the IFNAR1 but not the IFNAR2 subunit in comparison with healthy controls (see Figure 1). Notably, the expression of IFNAR1 and IFNAR2 also appears to be

independently regulated in dendritic cells,⁴³ where the two subunits show a different turnover.³⁵ When MS patients under IFN- β therapy were divided according to IFN- β bioavailability into the two subgroups of MxA-non-induced and MxA-induced, IFNAR1 mRNA reached the values observed in controls only in MxA-induced samples, remaining significantly lower in the others.⁴² Our data differ from those of Oliver et al.,⁴⁴ who reported that MS patients with a good clinical response to IFN- β treatment had a significant decrease in IFNAR1 (as well as IFNAR2) expression compared with non-responders, untreated patients and healthy controls. This discrepancy may be due to the different selection of patients (all of our MS patients had an active phase of the disease), different methods of IFN- β response monitoring (our IFN- β -treated patients were divided into two groups on the basis of their MxA mRNA status rather than with respect to their clinical response) and the different methodological approach used for IFNAR quantification.

At present, we cannot fully explain the reason for the decreased expression of IFNAR1 in our MS patients. Leyva et al.⁴⁵ showed a significant association between an IFNAR1 gene polymorphism and MS

susceptibility, thus suggesting a possible role of IFNAR1 alteration in the development of MS. Since IFNAR1 has been described as a short-lived protein that is internalised and degraded rapidly via lysosomes upon ligand binding,⁴⁶ we hypothesise that the increase in IFNAR1 mRNA might serve as a mechanism for rapid counterbalancing of the receptor loss. The net effect of mRNA increment might be a restoration of IFNAR surface protein levels and, consequently, cell responsiveness to IFN- β , leading to MxA induction, at least in MxA-induced patients. Recent functional studies in cell lines have directly related the binding affinity of IFN- β towards IFNAR1 with the specificity of its biological effects.¹⁸ A possible higher efficiency of IFNAR1 restoration on the cell surface could be related to better IFN- β bioactivity.

Further studies are necessary to clarify whether the diminished IFNAR1 expression, restored only in the MxA-induced patients, can be somewhat correlated to the pathogenesis of the disease, or whether it is a mere consequence of chronic immune system activation or of its altered regulation. Whatever the situation, studies to detect these receptors may help in monitoring treatment response in MS patients. ■

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