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Induction of IL-1 receptor antagonist by interferon beta: implication for the treatment of multiple sclerosis

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IFNβ has been the first drug approved for the treatment of multiple sclerosis patients, but we still lack a full understanding of the mechanisms underlying its clinical effects and the great variability of its therapeutic efficacy among different patients. Serum levels of the anti-inflammatory cytokine IL-1 receptor antagonist increase after IFNβ administration in MS patients. We now report that IFNβ induced IL-1ra mRNA and mature protein in three myelomonocytic cell lines. The induction of IL-1ra was already visible after 2 h of stimulation and persisted at least for 24 h. The amounts of induced IL-1ra were equal or higher than those obtained using other IL-1ra stimuli (LPS, IL-1β, IFNγ, IL-4, dexamethasone). This prolonged and quantitatively elevated induction of IL-1ra may contribute to the anti-inflammatory effect of IFNβ and partially account for the reduction of exacerbation rate shown in most IFNβ-treated MS patients. *Journal of NeuroVirology* (2000) **6,** S33–S37.

Keywords: IFNβ; IL-1ra; myelomonocytic cell lines

Introduction

Multiple sclerosis (MS) is an autoimmune/inflammatory disease of the central nervous system (CNS) of unknown cause (Bell and Lathrop, 1996). Several pro-inflammatory cytokines contribute to the acute and chronic inflammatory phases of MS, including the two prototypical inflammatory cytokines IL-1: IL-1α and β (Dinarello, 1996). Both cytokines are induced in response to infection and to several inflammatory stimuli and trigger, among others, peripheral blood mononuclear cells recruitment and pro-inflammatory protein secretion. The production and activity of IL-1 are tightly regulated at different levels: gene expression, mature protein conversion and secretion, receptor binding and consequential intracellular response, production of a specific receptor antagonist (IL-1ra). The IL-1ra is a protein structurally related to IL-1β that forms a non-productive complex with IL-1 receptors (Eisenberg *et al*, 1990). IL-1ra occurs in alternatively spliced forms, one of which is secreted. IL-1ra is of potential therapeutic interest for MS and its administration has successfully inhibited autoimmune inflammatory demyelination in rats with experimental allergic encephalomyelitis (EAE) (Martin and Near, 1995). Moreover, although circulating serum levels of IL-1ra in patients with the relapsing remitting (RR) MS are normal during remission phases, they significantly increase during exacerbations or in response to IFNβ treatment (Nicoletti *et al*, 1996; Voltz *et al*, 1997).

The type I IFN family includes at least three proteins (IFNα, β, ω) all binding to the same receptor with different affinity, but (at least in the case of IFNα and IFNβ) triggering distinct signalling pathways (Abramovich *et al*, 1994). IFNβ exerts antiviral and anti-inflammatory properties and modulates repair mechanisms, T-cell activation, lymphocyte migration and immune suppression. More recently IFNβ has successfully altered the clinical course of relapsing-remitting (RR) MS. The mechanisms underlying this clinical effect, however, are still unclear. In this study we evaluated the production of IL-1ra by myelomonocytic cell lines in response to IFNβ at both mRNA and protein levels. We observed that, in our experimental system, IFNβ is one of the best inducers of IL-1ra when compared with other potent stimuli.

Results

We treated the three myelomonocytic cell lines [Monomac6 (MM6), U937 and THP-1] with IFNβ

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(10 000 u/ml), IFNγ (20 ng/ml), LPS (100 ng/ml), IL-1β (10 ng/ml) and IL-4 (10 ng/ml) for 2 h. We observed that IFNβ induced the production of a considerable amount of IL-1ra mRNA (Figure 1A) and soluble protein (Figure 1B). Compared to the other stimuli able to induce IL-1ra [IFNγ (Kline *et al*, 1995); LPS (Dinarello, 1996); IL-1β (Dinarello, 1996); IL-4 (Kline *et al*, 1995)], IFNβ was one of the most effective inducers of all three cell lines. The MM6 cell line was overall the best responder (see Figure 1).

The time-course of induction by IFNβ in these cell lines showed an increase of mRNA expression after 2 h of treatment with 10 000 u/ml of IFNβ that peaked at 8 h and decreased at 20 h (Figure 2).

To compare the kinetic of stimulatory effect of IFNβ with the other most efficient stimuli, we treated the MM6 cell line with 1000 or 10 000 u/ml IFNβ, 10^{-7} M dexamethasone (Barnes, 1998), 100 ng/ml LPS, 10 ng/ml IL-1β; we then measured IL-1ra mRNA and protein levels at different time points (Figure 3). Induction of IL-1ra by IFNβ was quantitatively equivalent to that obtained by LPS, but with a slower kinetic: the induction of IL-1ra mRNA by LPS was appreciable after 2 h of treatment, peaked at 4 h and was already decreasing by 8 h (Figure 3A); protein levels of IL-1ra started to be induced by LPS at 4 h and grew thereafter, peaking approximately at 24 h (Figure 3B). The induction of IL-1ra mRNA by both doses of INFβ was detectable after 2 h, peaked at 8 h and was still evident at 24 h (Figure 3A); protein levels were induced by IFNβ at 4–8 h and grew thereafter, reaching the levels induced by LPS at 24 h (Figure 3B).

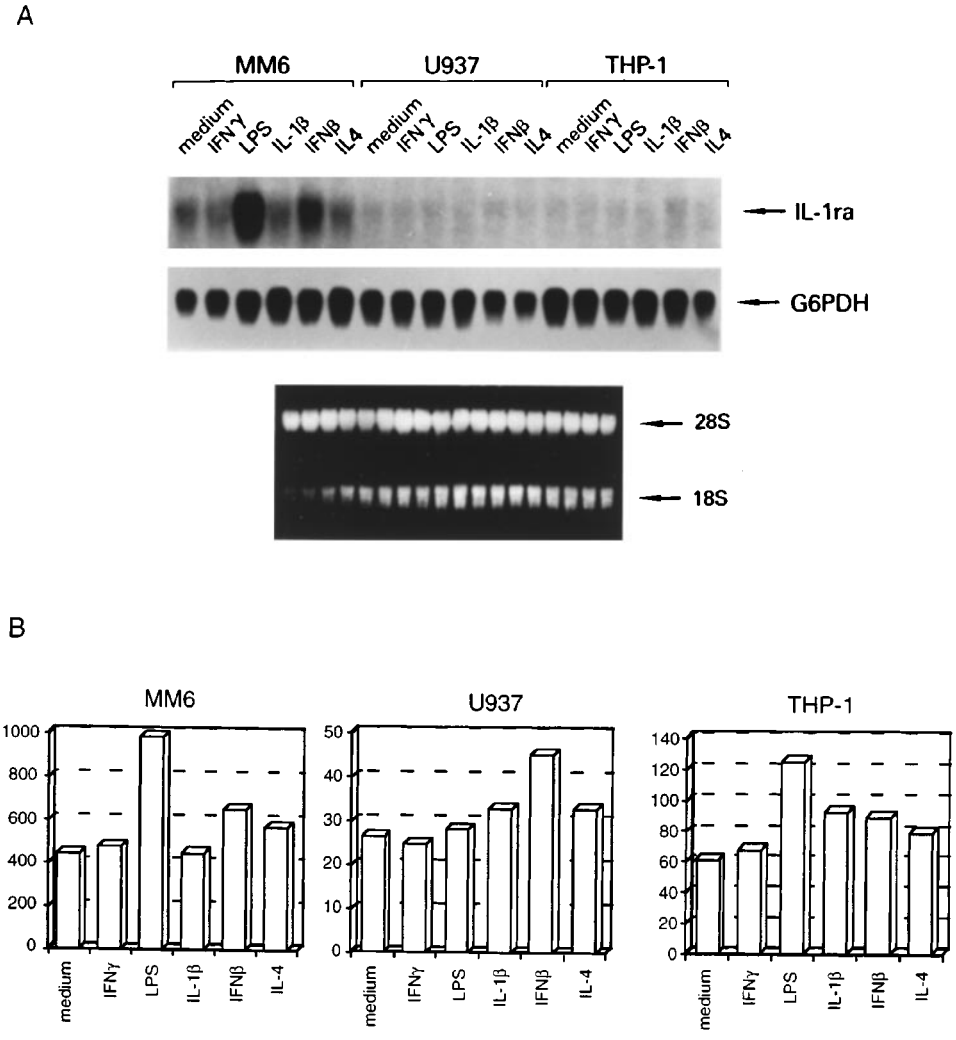

Figure 1 Expression of IL-1ra mRNA (**A**) and soluble protein (**B**) in three myelomonocytic cell lines treated for 2 h with medium or one of the indicated stimuli. Densitometric analysis revealed that IFNβ was able to induce expression of IL-1ra mRNA in all three cell lines (**A**). IL-1ra was measured at the same time point in cell culture supernatants confirming that IFNβ was able to induce the release of IL-1ra mature protein after a short time of stimulation (**B**).

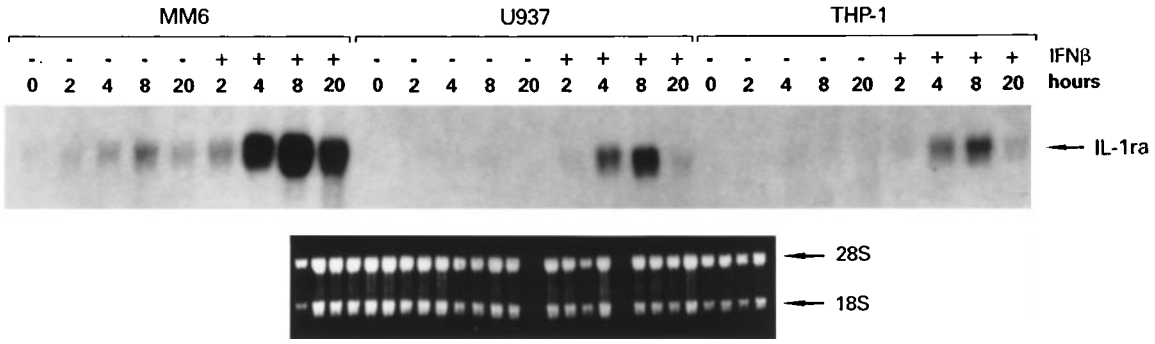

Figure 2 Time course of expression of IL-1ra mRNA in three myelomonocytic cell lines treated or not with 10 000 u/ml IFNβ. In all three cell lines, IFNβ was able to increase the expression levels of IL-1ra at all time points. Higher levels of IL-1ra expression were visible after 8 h of stimulation.

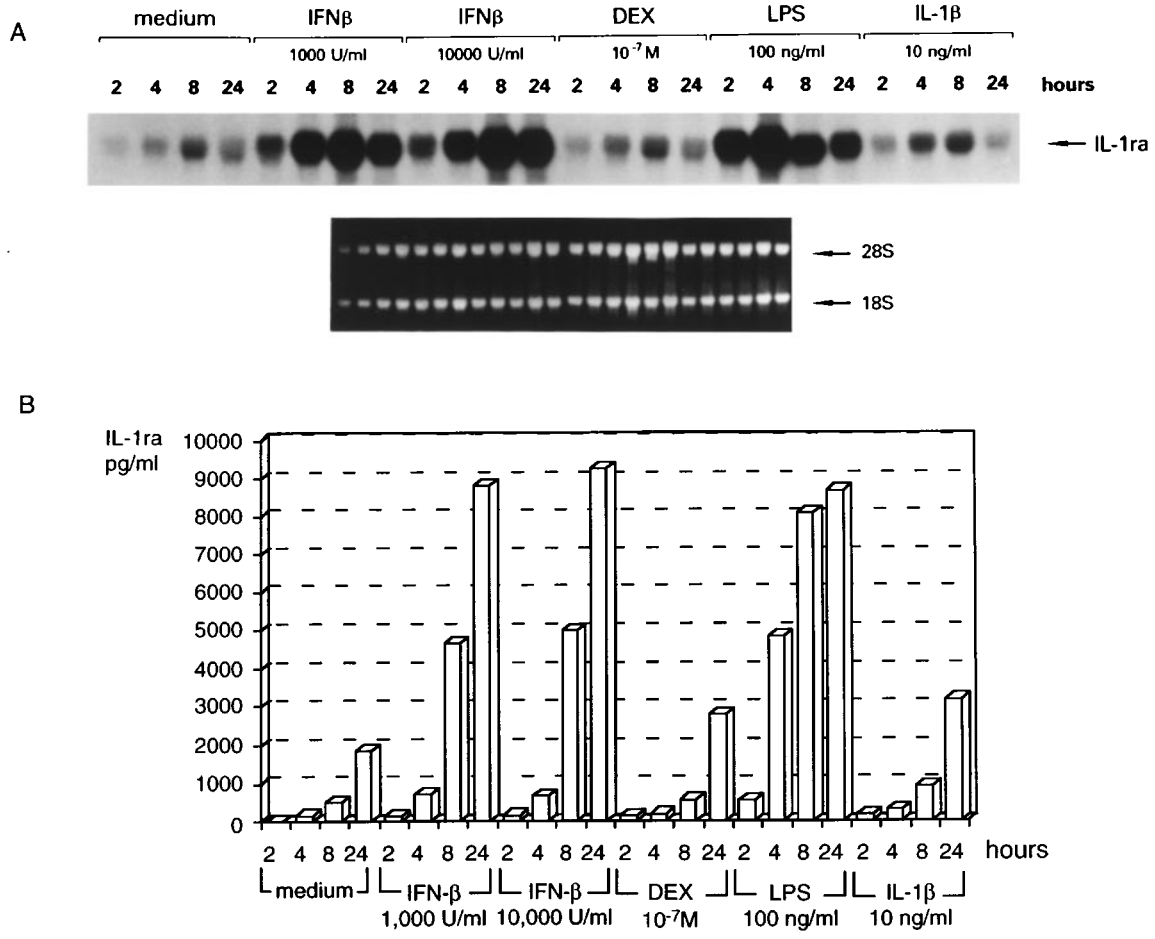

Figure 3 Time course of expression of IL-1ra mRNA (**A**) and soluble protein (**B**) in MM6 cell line cultured in the absence of stimuli (medium) or in the presence of the indicated stimuli. IFNβ was able to induce IL-1ra amount comparable to those obtained with LPS stimulation, but after a longer incubation time.

IFNβ-dependent IL-1ra mRNA induction was abolished by an anti-IFNβ antibody and was agonized by IFNα (Figure 4), suggesting that the IL-1ra induction observed was type I IFNs-specific.

Discussion

MS is an immune-mediated disease of the CNS in which selective destruction of myelin and oligoden-

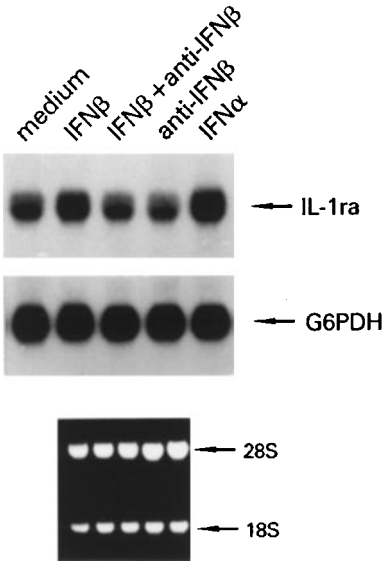

Figure 4 IFNβ induction of IL-1ra in MM6 cell line: inhibition by specific antibody and agonistic activity of IFNα.

drocytes occurs as a result of inflammation (Traugott, 1996). Several epidemiological, pathological, and immunological evidences indicate that an autoimmune process against myelin components in a pro-inflammatory environment is potentially able to perpetuate the recurrent demyelination seen in MS (Martino *et al*, 1998). Pro-inflammatory cytokines, such as IL-1, represent potentially noxious molecules to the myelin sheet and/or oligodendrocytes. In acute MS, IL-1β is expressed predominantly by microglial cells throughout the white matter within and around the lesions (Cannella and Raine, 1995). Furthermore, IL-1 can enhance the *in vitro* activation of encephalitogenic T lymphocytes and worsen adoptive EAE (Mannie *et al*, 1987). The IL-1ra has the potential to block the noxious effect of IL-1, as exemplified by its protective role in EAE (Martin and Near, 1995). This cytokine is the natural antagonist of the prototypical inflammatory cytokine IL-1 and is able to block the IL-1-induced inflammatory burst by sequestering IL-1 receptors.

In the present report we show that IL-1ra is actually induced by INFβ, the most important drug currently used in MS therapy. The induction of IL-1ra by IFNβ occurs both at mRNA and protein levels; it is rapid, occurring after 2–4 h of stimulation, conspicuous in amount, being comparable to those obtained with the potent LPS stimulation, and specific, being abolished by an anti-IFNβ antibody and agonized by IFNα. This effect is potentially able to play a role in the *in vivo* situation: the high production of IL-1ra by monocytic cell type has the potential to suppress the inflammatory effect of IL-1 in the peripheral circulation. The IL-1ra induction by IFNβ could also play an important anti-inflammatory role in the central nervous system (CNS) of MS patients, were the damage of the blood-brain barrier allows the infiltration of peripheral blood leukocytes. This mechanism is likely to play a role also in the CNS microenvironment, since IFNβ induction of IL-1ra has also been demonstrated in LPS or IL-1α pre-activated microglia (Liu *et al*, 1998).

According to our and previous reports, we conclude that the induction of IL-1ra could be one of the mechanisms by which IFNβ exerts its beneficial effects in patients with MS. We are currently verifying whether such activation occurs in patients under IFNβ treatments and whether there is a correlation between the variable clinical response and the actual induction of IL-1ra by IFNβ in these patients.

Materials and methods

Cell cultures

U937 and THP-1 cell lines were cultured in RPMI 1640 medium (Bio Whittaker, Walkersville, MD, USA) supplemented with 2 mM glutamine (Bio Whittaker), 50 u/ml penicillin/streptomycin (Bio Whittaker) and 10% aseptically collected fetal calf serum (FCS, Biological Industries, Kibbutz BeitHaemek, Israel); MM6 cell line was cultured in RPMI 1640 medium supplemented with 2 mM glutamine, 50 u/ml penicillin/streptomycin, 20% FCS, 1 mM oxalacetic acid (Sigma, St. Louis, MO, USA), 1 mM piruvic acid (Sigma) and 1 × non essential aminoacids (Sigma). The supplemented cultured media are referred as complete media. For all experiments, the same number of exponentially growing cells was precipitated and resuspended in fresh medium at 1×10^6 cells/ml (time 0), then incubated at 37°C in 5% CO_2 for different times with and without stimuli [IL-1β (Genzyme, Cambridge, MA, USA), LPS (Sigma), IFNγ (Genzyme), IFNβ (IFNβ_{1b}, Shering, Berlin, Germany), IL-4 (Genzyme), dexamethasone (Sigma), anti-IFNβ antibody (Genzyme)]. At each time point, an aliquot of the cell suspension was obtained from all groups (both resting and stimulated MM6, U937 and THP-1 cells). Cells were then centrifuged: cellular pellets were lysed for RNA extraction and Northern analysis and supernatants collected for the ELISA measurement of secreted IL-1ra protein content.

Northern blot analysis

Total RNA was isolated from myelomonocytic cell lines by the guanidine isothiocyanate method or, alternatively, by the RNeasy kit (Qiagen, Hilden, Germany). Northern blot analysis was carried out according to standard procedures (Maniatis *et al*, 1982). Briefly, 5–8 μg total RNA were analysed by electrophoresis through 1% agarose formaldehyde gels in the presence of ethidium bromide (Sigma), followed by transfer to Gene Screen Plus membranes (New England Nuclear, Boston, MA, USA). A

259 bp DNA fragment obtained by PCR amplification (forward primer: 5′-TTGCTGCAGTCACA-GAAT-3′; backward primer: 5′-TAATGGACATCC-TCTCCA-3′) of IL-1ra gene was used as probe and random primed with ^{32}[P]dCTP (5000 Ci/mmol; Amersham, Buckinghamshire, UK). Membranes were pretreated and hybridised in 50% formamide (Merck, Darmstadt, Germany) with 10% dextran sulphate (Sigma) and washed twice with $2 \times$ SSC, then twice with $2 \times$ SSC plus 1% SDS (Sigma) at 63°C for 30 min and finally rinsed several times with $0.1 \times$ SSC at room temperature. Autoradiography of the membranes onto X-ray films was performed for 8–24 h at −80°C with intensifying screens. RNA loading and transfer to membrane were checked by examination of filters under UV light. The relative amount of mRNA was quantified when necessary by comparison with the expression of the glucose-6-phosphate dehydrogenase (G6PDH) housekeeping gene (Persico *et al*, 1986): after autoradiography filters were re-hybridized with a G6PDH probe and re-exposed to X-ray films. The optical density (OD) of IL-1ra and G6PDH expression signals were measured by densitometry (Computing Densitometer; Molecular Dynamics, Sunnyvale, CA, USA) and analysed with the Image Quant 3.3 software. A ratio between OD values of IL-1ra and G6PDH expressed in arbitrary units was obtained for each sample.

IL-1ra protein ELISA measurements
The levels of secreted IL-1ra were measured by a double antibody ELISA kit (R&D System, Oxon, UK) in cell culture supernatants of myelomonocytic cell lines. Data were analysed by the Microplate Manager software (BioRad, Hercules, CA, USA).

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