REPORT

Contributions to the study of the role of IFN gamma and its receptor on the physiopathology of disorders involving the immune system

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ABSTRACT

Interferon gamma (IFNγ) is a Th1-type cytokine. The study of the IFNγ system and its receptor is essential for increasing the efficacy of this drug for clinical settings. Here we describe the role of IFNγ and its receptor on the physiopathology of disorders involving the immune system. Several molecular forms of IFNGR1, from 84 to 13 kDa, and soluble receptor (60-67 kDa) with the capacity to bind IFNγ arising from proteolytic processing events are shown. These forms bind IFNγ. A new type of interaction between the IFNα and IFNγ receptors that depends on the presence of IFNα is also described. There are high levels of soluble IFNGR1 in the plasma of rheumatoid arthritis (RA) patients. The role of IFNγ as a negative modulator for CCR-4, a chemokine receptor up-regulated significantly in juvenile rheumatoid arthritis (JRA) patients, is described. A recombinant anti-IL-2-IFNγ antagonist was developed. This molecule inhibits the biological actions of IL-2 and IFNγ, turning this protein into a Th1 antagonist (AnTh1) potentially useful for the treatment of autoimmune disorders.

*I*ntroduction

The interferon (IFN) system is one of the defense mechanisms of the immune system involved in both its innate and adaptive arms. It is stimulated by infection with a microorganism, and works at restricting the propagation of the infectious process. The outcome of this response can be either beneficial or detrimental to the host, depending on characteristics of the invading agent and of the host itself.

The immunomodulatory properties of IFN play a key role in the regulation of antigen presentation, in the differentiation of T-lymphocytes into a Th1 or Th2 phenotype, and in the regulation of cellular migration to inflammatory sites. Interleukin 2 (IL-2) is another cytokine which, together with IFNγ, participates in the regulation of the immune system as well. IL-2 is also involved independently as a crucial player in the activation, growth and differentiation of T and B lymphocytes, as well as in the activation of natural killer (NK) cells and lymphokine-activated killer cells (LAK) [1, 2].

The immunomodulatory functions of IFN are mediated by the interaction of this molecule with the IFNGR1 receptor, which is found on the surface of almost all cell types. This receptor not only transduces, but also regulates the signal given by IFNγ. The fact that the soluble ectodomains of cellular receptors often retain the capacity of binding their cognate ligands has encouraged the use of these molecules for the therapy of disorders in which these ligands play a pathogenic role.

Taking into account its biological functions, a beneficial effect of IFNγ for the treatment of asthma and certain infectious diseases is to be expected. However, it is also known that IFNγ and IL-2 are involved in the pathogenesis of several disorders. Certain autoimmune diseases, such as systemic lupus erithematosus (SLE), myasthenia gravis (MG), relapsing-remitting multiple sclerosis (RRMS), type I diabetes mellitus and rheumatoid arthritis (RA), are based on a malfunction of the IFNγ and IL-2 system.

The above underlines the importance of a thorough understanding of the system formed by IFNγ and IFNGR1 and its potential therapeutic applications, as well as of the possibility of neutralizing its actions, and those of other cytokines, during pathological states in which they may be involved.

*M*aterials and methods

Classical immunochemical techniques were used in this study (immunoaffinity chromatography, protein radioiodination, ligand affinity measurements, *dot blot*, *western-blot*) for the purification of IFN receptors, their identification and characterization. The evaluation of gene expression profiles was based on the use of gene amplification methods, combining reverse transcription reactions with the polymerase chain reaction (RT-PCR). The measurements of cytokine concentrations in serum were performed with commercial ELISA-based kits. Conventional recombinant DNA techniques were used to clon the chains of the IL-2 fragment and the extracellular domain of IFNGR1, as well as biological assays to assess the activity of IL-2 using the IL-2-dependent murine cell line CTLL-2, for the antiproliferative activity of IFNγ on the Hep-2 line (from a larynx carcinoma) and to inhibit the expression of HLA-DR in the Colo 205 cell line by AnTh1.

*R*esults and discussion

Isolation, identification and characterization of membrane-bound and soluble IFNGR1

Figure 1A shows a competition chromatography of IFNGR1 labeled with I^{125}, which is chromatographed on an affinity matrix with covalently bound IFNγ in the presence (1) or absence (2) of an excess of IFNγ as a competitor. The excess IFNγ competes during the procedure with the matrix-linked IFNγ in binding to the labeled IFNGR1, thereby displacing the 27, 17

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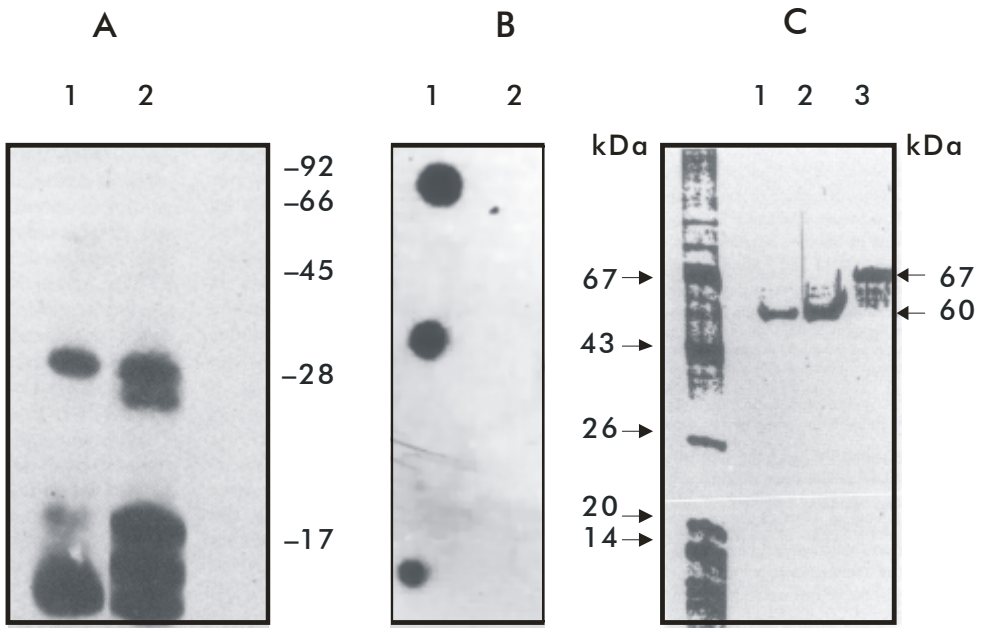

Figure 1. Competition chromatography (A), *Dot blot* (B) and electrophoresis and *Western-blot* of soluble IFNGR1 isolated from serum (C).

and 13 kDa bands. Figure 1B shows a *dot-blot* identifying the soluble IFNγ receptor from different sources, which binds I^{125}-labeled IFNγ in the absence (1), but not in the presence (2) of excess cold IFNγ. Figure 1C shows the identification by *Western-blot* under non-reducing conditions (1) and the electrophoretic profiles (Coomassie Blue staining) under non-reducing (2) and reducing (3) conditions of soluble IFNGR1 purified by ligand affinity chromatography [3, 4].

Cross-interactions between the chains binding IFNα and IFNγ

Figure 2 shows that radioiodinated p100 and p70 (previously purified by binding to IFNα2b) are recognized in a second chromatography by the monoclonal antibody (mAb) A6 (mAbA6 competes with IFNγ in binding to IFNGR1), but only if IFNα is present during the chromatographic run. This indicates that the proteins purified with IFNα immobilized on a solid support contain elements of IFNGR1, and that these two types of components are associated.

High levels of soluble IFNGR1 in rheumatoid arthritis patients

This study was performed on Cuban and Swiss RA patients together with their healthy controls. The levels of the soluble IFNγ receptor were measured in 19 RA patients and 24 controls, using soluble preparations of IFNGR1 obtained from the plasma. As a result, the concentrations of the soluble receptor were higher in the RA patients (mean = 2415 arbitrary OD units) than in controls (mean = 1411 arbitrary OD

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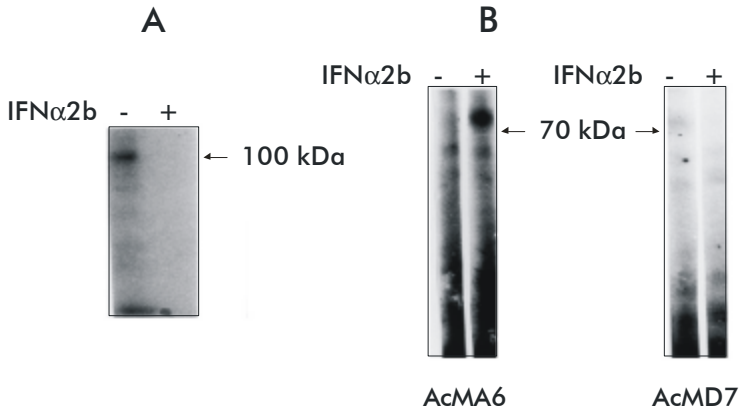

Figure 2. (A) Autoradiograph of the eluate from affinity chromatography columns in precense or not of IFNα2b. (B) Autoradiographs of proteins that bind IFNα2b in a matrix with AcM against IFNGR1 wich compete (AcMA6) or not (AcMD7) for the binding to IFNGR1.

Table 1. Relative IFNGR1 levels in RA patients and healthy controls. The measurements are expressed in arbitrary optical density units.

Subjects		N	Levels of soluble (Mean ± SD)
Control	Cubans	17	1 533 ± 135
	Swiss	7	1 116 ± 255
	All	24	1 411 ± 123
RA patients	Cubans	5	2 263 ± 650
	Swiss	14	2 470 ± 344
	All	19	2 415 ± 297

units), and this difference was statistically significant ($p < 0.05$) according to the non-parametric Mann-Whitney U test. There were no differences between the Cuban and Swiss samples [4].

Differential CCR-4 regulation in JRA and RA patients

Furthermore, the experiments described below show a decrease in the expression levels of CCR-4 when PBMN cells from children afflicted with juvenile rheumatoid arthritis (JRA) are treated with IFNγ, either during *in vivo* or *in vitro* therapy. It should be highlighted that this decrease was statistically significant when the cells of the patients were treated with IFNγ *in vivo* and then *in vitro*. This finding is important, since it suggests that there may be even higher reductions *in vivo* by using higher doses of the drug. The decrease in CCR-4 expression due to IFNγ treatment in cells of JRA, but not of RA patients, is congruent with the positive clinical response to IFNγ therapy in children afflicted with JRA [6, 7], but not in adult RA patients [8].

Development of an IL-2 and IFNγ antagonist

In order to develop an antagonist against IL-2 and IFNγ, the recAnTH1 protein was expressed as inclusion bodies in *Escherichia coli* (*E. coli*) to a level of approximately 30% of the total protein, in order to facilitate its purification. This protein is formed by amino acids 1-60 from the N-terminus of IL-2, followed by an Ala-His-Met-Met peptide and then by the 231 aminoacids of the extracellular region of IFNGR1 [9].

The activity of recAnTH1 was evaluated with *in vitro* biological assays, such as a test for measuring the capacity to inhibit the human IL-2-mediated proliferation of T cells. Probably, the first 60 amino acids of the N-terminus of IL-2 interfere with the high affinity binding of IL-2 to the receptor present in these cells. This hypothesis is sustained by the fact that all amino acids that contact the RαIL-2 chain (Lys 35, Arg 38, Phe 42, and Lys 43) are localized in this region, together with other amino acids that interact with the RβIL-2 [10] subunit; therefore, an N-terminal fragment from IL-2 should interfere with the binding of full-length IL-2 to its high-affinity receptor (RαβγIL-2).

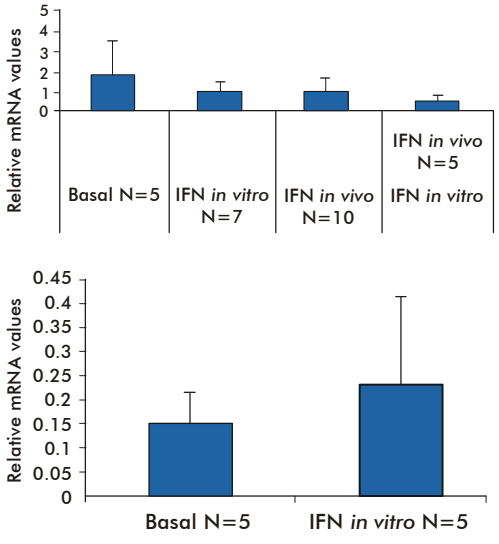

Figure 3. Evaluation of the effect of the IFNγ treatment on the CCR-4 mRNA levels in JRA (A) and RA (B) patients. The differences were statistically significant ($p < 0.05$) according to Dunn's multiple comparisons test.

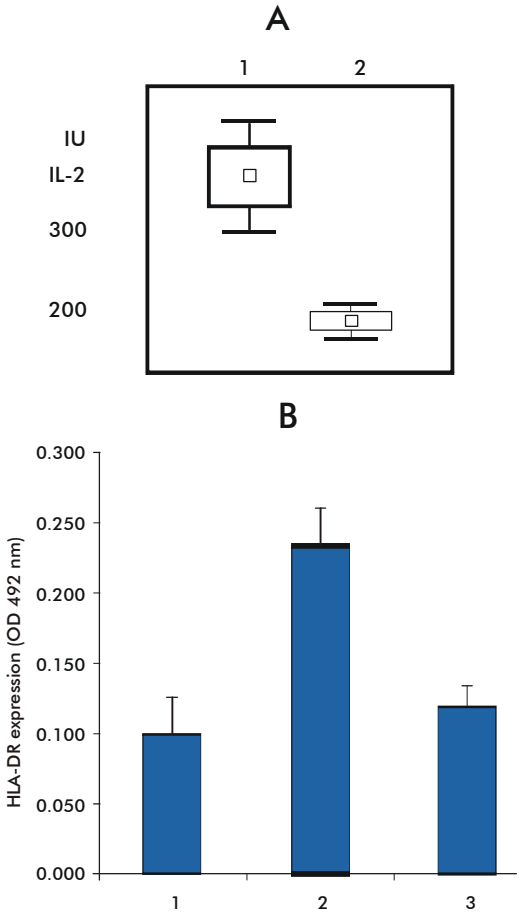

Figure 4. (A) Inhibition, mediated by recAnTH1, of the stimulation of the proliferation of CTLL-2 cells by human IL-2. Results were obtained using three experimental replicates, and the inhibition was statistically significant ($p < 0.05$) according to the Kruskal-Wallis test. (B) Inhibition, was mediated by recAnTH1, of HLA-DR induction. Bar 1: Basal level of HLA-DR, bar 2: IFNγ 500 IU/mL (71 ng/mL), bar 3: IFNγ 500 IU/mL + 1.5 μg/mL of AnTH1. The results were obtained using three experimental replicates, and the difference between stimulation (bar 2) and inhibition (bar 3) was statistically significant (p 0.05) according to the Kruskal-Wallis test.

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6. Coto C y cols. Use of recombinant interferón gamma in pediatric patients with advanced juvenile chronic arthritis. Biotherapy 1998;11:15-20.

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The recAnTH1 protein also inhibits the stimulation and antiproliferative activities of HLA-DR that are induced by IFNγ, probably by binding the latter in the extracellular milieu and, therefore, blocking or interfering with its binding to IFNGR1 on the cell membrane.

Conclusions

The differential regulation of chemokine receptors by IFNγ allow the selection of JRA patients with a favorable prognosis to the treatment with this product. The cross-interaction between IFNα and IFNγ via their receptors is a new finding on the cellular communication routes of these IFN types that provides further explanations about the synergistic biological activities observed when they are combined. Also, the presence of high levels of IFNγ receptor in diseases such as RA contributes to the definition of the physiopathological role of this system in autoimmune disorders. Based on the existence of soluble IFNγ receptors, and on the evidence suggesting that IFNγ may use other receptors to mediate its biological activities, we were posible to define that the development of IFNγ antagonists had to be based on drugs directed not against the receptor, but against the ligand; and that these antagonists could be formed by variants of IFNGR1 itself. This study describes a new therapeutic candidate obtained against a group of autoimmune disorders characterized by the pathological role played by Th1-type cytokines.

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