International Immunopharmacology (2008) **8**, 881–886

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Treatment with type I interferons induces a regulatory T cell subset in peripheral blood mononuclear cells from multiple sclerosis patients

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Received 14 September 2007; received in revised form 7 February 2008; accepted 7 February 2008

KEYWORDS
Multiple sclerosis;
Autoimmune disease;
Type I interferons;
Regulatory T cells;
Metalloproteinases;
Cytokines

Abstract

Type I Interferon (IFN-α/β) therapy has altered the natural course of multiple sclerosis. In this paper we evaluate the possible molecular mechanisms involved in the *in vitro* effects of IFN-α/β on peripheral blood mononuclear cells from patients with clinically definite Relapsing-Remitting Multiple Sclerosis. The total RNA from IFN-α, IFN-β treated cells and untreated cells was extracted and amplified for CD86, CD28, CTLA-4, TNF-α, IFN-γ, CCL2, CCR5, IL-13, MMP-9, TIMP-1, CD25, TGF-β, IL-10 and the transcriptional factor Foxp3 by Reverse Transcription–Polymerase Chain Reaction and the CD4+CD25high subset was evaluated using flow cytometry. In general, there were no significant differences concerning the modulation of the genes studied in the response to IFN-α and IFN-β treatments, which suggest a similar mechanism of action for both interferons. However, we found a significant increment in IFN-γ expression after IFN-α but not after IFN-β treatments. The *in vitro* treatment of mononuclear cells from multiple sclerosis patients with both interferons significantly increased the CD25 mRNA. Furthermore, we observed a CD25/Foxp3 correlation and an increment of the CD4+CD25high subset, indicating that the induction of regulatory T cells could be a crucial mechanism involved in the type I interferon effects.

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doi:10.1016/j.intimp.2008.02.003

1. Introduction

Multiple Sclerosis (MS) is a chronic demyelinating disease of the central nervous system (CNS) primarily affecting young adults in their most productive age. While its ultimate etiology remains elusive, most evidence supports an autoimmune pathogenesis in individuals with a complex predisposing genetic trait, probably requiring an inciting environmental insult [1].

In recent years the family of IFN-α/β regained the interest of immunologists, due to the definition of novel immunomodulatory functions and signaling pathways and the observation that IFN-α/β are also relevant for autoimmune disease pathogenesis [2].

Human type I IFNs interact with the human IFN alpha receptor. The interaction of IFN-α/β with their receptor components results in the activation of a number of signaling pathways [3]. We hypothesize that IFN-α/β, type I IFNs binding to the same receptor, and have a common signaling pathway, so that it is possible to consider for them a similar molecular mechanism of action. Consequently, we evaluated the modulation of the expression of the genes involved in the different steps of the physio-pathogenesis of MS: antigen presentation, Th1/Th2 cytokines expression, brain blood barrier (BBB) integrity and natural and adaptive T regulatory (Tr) cells, which could explain the beneficial effect of these IFNs in therapeutic trials.

Although controversial, the identification and characterization of a CD4+CD25+ population highlight the critical roles of Tr cells in maintaining immunological self-tolerance. Experimental animal models for autoimmunity have demonstrated the crucial role of Tr cells in suppressing autoreactive T cells and promoting peripheral tolerance. Furthermore, the abnormality of natural Tr cells can be a primary cause of autoimmune and other inflammatory diseases in humans [4]. Since Tr cells have emerged as a dominant T cell population capable of mediating peripheral tolerance, we evaluated the effect of IFN-α/β on the modulation of this subset.

The role of IFN-α/β in the pathogenesis of autoimmune diseases remains at the forefront of scientific inquiry and has begun to reveal the mechanisms by which these molecules promote or inhibit systemic and organ-specific autoimmune diseases.

2. Materials and methods

2.1. Patients

Seventeen patients with clinically diagnosed Relapsing-Remitting MS (RRMS) according to McDonald's criteria [5] were studied. Patients did not receive immunosuppressive or immunomodulatory medications for at least 6 months before they entered in the study. The Scientific and Ethics Committee of the National Neurology Institute, Havana, Cuba approved the study and all subjects signed their informed consent. Brain and spinal cord magnetic resonance imaging (MRI) were performed as confirmatory diagnostic criteria.

2.2. Cells

Peripheral blood mononuclear cells (PBMC) were separated from the peripheral blood of patients by Ficoll gradient centrifugation (Ficoll paque TM plus. Amersham Biosciences. Endotoxin tested), and mononuclear cells were collected from the ring. PBMC were resuspended at 2×10^6 cells/mL in RPMI 1640 with 25 mM HEPES buffer with L-glutamine (GIBCO) and divided into three experimental groups: recombinant IFN-α2b (Heberon, CIGB, Havana, Cuba) treated cells (20 ng/mL), recombinant IFN-β1a (Avonex, Biogen, Cambridge, MA) treated (20 ng/mL) and untreated cells. The cells were incubated for 4 h at 37 °C and 5% CO_2. Then the cells of each group were lysed and the RNA extracted.

2.3. RT—PCR

The total RNA was isolated using Tri reagent [6] (Sigma). Total RNA was reverse transcribed using the Perkin Elmer core kit [7] in reverse transcriptase buffer (5 mmol/L $MgCl_2$, 50 mmol/L KCl, and 10 mmol/L Tris—HCl, pH 8.3) with 2.5 μmol/L random hexamers, 1 mmol/L of each deoxynucleotide triphosphate, 1 U/μL of RNase inhibitor, and 2.5 U/μL of Moloney leukemia virus reverse transcriptase (Perkin-Elmer/Cetus, Norwalk, CT). Samples were incubated for 10 min at room temperature and then 42 °C for 15 min, 99 °C for 5 min. Then, each cDNA reaction was divided into two Eppendorf tubes and a specific pair of primers was used for the polymerase chain reaction (PCR). We used glyceraldehide-3 phosphate dehydrogenase (GAPDH) as the housekeeping gene in order to normalize the densitometric values of each PCR product obtained by the scanner. The following primers were used, GAPDH primers: for 5′-CCA TGG AGA AGG CTG GGG-3′ and back 5′-CAA AGT TGT CAT GGA TGA CC-3′. TNF-α primers: for 5′-TGC CAG GCA GGT TCT CTT CC-3′ and back 5′-GGT TAT CTC TCA GCT CCA CGC CA-3′; IFN-γ primers: for 5′-ATG AAA TAT ACA AGT TAT ATC TTG GCT TT-3′ and back 5′-GAT GCT CTT CGA CCT CGA AAC AGC AT-3′; CCR5 primers: for 5′-GGT GGA ACA AGA TGG ATT AT-3′ and back 5′-CAT GTG CAC AAC TCT TGA CTG-3′; MCP-1 primers: for 5′-AAC TGA AGC TCG CAC TCT CG-3′ and back 5′-TCA GCA CAG ATC TCC TTG GC-3′; IL-13 primers: for 5′-GAG TGT GTT TGT CAC CGT TG-3′ and back 5′-TAC TCG TTG GCT GAG AGC TG-3′; TGF-β primers: for 5′-CAA GCA GAG TAC ACA CAG CA-3′ and back 5′-GAT GCT GGG CCC TCT CAA GC-3′; IL-10 primers: for 5′-AAC AAG ACC CAG ACA TCA AG-3′ and back 5′-GAG GTA CAA TAA GGT TTC TCA AG-3′; MMP-9 primers: for 5′-TGG GCT ACG TGA CCT ATG ACA T-3′ and back 5′-GCC CAG CCC ACC TCC ACT CCT C-3′; TIMP-1 primers: for 5′-TTC TGC AAT TCC GAC CTC GT-3′ and back 5′-TCC GTC CAC AAG CAA TGA GT-3′; CD86 primers: for 5′-GGA AAA CTT GGT TCT GAA TGA GG-3′ and back 5′-ATA GTT GAA TTC TTG GTT CTT AGC-3′; CD28 primers: for 5′-ATA TCC TGT GTG AAA TGC TGC AGT-3′ and back 5′-GGT AGAATG TCA CTG ATT CAT TGC-3′; CTLA-4 primers: for 5′-AAG TCC TTG ATT CTG TGT GGG TTC-3′ and back 5′-GGA GAT GCA TAC TCA CAC ACA AAG-3′; CD25 primers: for 5′-CTA CAA GGA AGG AAC CAT GTT GAA C-3′ and back 5′-AGG TGA GCC CAC TCA GGA GGA GGA C-3′; Foxp3 primers: for 5′-CAG GCC ACA TTT CAT GCA CC-3′ and back 5′-ACA CCA TTT GCC AGC AGT GG-3′. Amplification was performed in 2 mmol/L $MgCl_2$, 50 mmol/L KCL, 10 mmol/L Tris—HCL, 0.2 mmol/L each deoxynucleotide triphosphate, 2.5 U/100 μL Taq DNA polymerase (Perkin-Elmer, Cetus), and 4 μg/mL of each specific primer with an automated thermal cycler (Eppendorf) at 94 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s. It was stopped at 35 cycles. PCR products were run in 1.5% agarose gel containing ethidium bromide and then the density of each band was quantified using the Molecular Analysis Program (Molecular Analysis software for Windows, version 1.4.1). The results were expressed as a ratio calculated from the optical density (OD) of a defined area of the amplified gene products divided by the corresponding OD of amplified GAPDH PCR product.

2.4. Flow cytometry analysis

All the flow cytometry experiments were performed the same day the blood was sampled. Two hundred microliters containing 5×10^5 PBMC/well were treated with 20 ng/well of recombinant IFN-α2b,