

Interaction of a synthetic peptide of the interferon $\alpha 2$ C-terminal part with human blood leukocytes. II. Effect on protein tyrosine phosphorylation

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Tyrosine phosphorylation in human blood lymphocytes was studied as a function of stimulation with concanavalin A (ConA) and treatment of the cells with interferon $\alpha 2$ (IFN $\alpha 2$) and/or an IFN-derived C-terminal synthetic peptide 2438 (amino acid residues 124–138). Both IFN $\alpha 2$ and the peptide 2438 decreased the level of protein tyrosine phosphorylation in the ConA-stimulated cells. In unstimulated cells, IFN $\alpha 2$ increased, and the peptide 2438 decreased the level of the tyrosine phosphorylation. A possible correlation of these effects with stimulation of cell proliferation is discussed.

Interferon $\alpha 2$; Synthetic peptide; Human; Lymphocyte; Tyrosine phosphorylation; Monoclonal antibody

1. INTRODUCTION

Activation of lymphocytes by antigens, mitogenic lectins and numerous hormones results in rapid phosphorylation of tyrosine, serine, and threonine residues of cellular proteins [1]. The role of protein tyrosine phosphorylation in regulation of cellular activation, and identification of endogenous substrates of TPKs still remain an unsolved and important problem.

In the present study we examined the effects of IFN $\alpha 2$ and the synthetic peptide 2438 on protein tyrosine phosphorylation in human blood lymphocytes, and probed cellular substrates of TPKs with monoclonal antibodies against P-Tyr [2].

It is known that IFN α may exert its biological activities using various pathways [3,4], but the detailed mechanisms of its action are still far from clear. Previously, we demonstrated that a short synthetic peptide (2438) representing the amino acid sequence 124–138 of the IFN $\alpha 2$ polypeptide chain, inhibited proliferation of human lymphocytes [5].

2. MATERIALS AND METHODS

2.1. Materials

Human recombinant IFN $\alpha 2$ (5×10^6 IU/mg) was a kind gift of Dr.

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Abbreviations: ConA, concanavalin A; IFN $\alpha 2$, interferon $\alpha 2$; PBMC, peripheral blood mononuclear cells; P-Tyr, phosphotyrosine; TPK, tyrosine protein kinase.

G. Chipens (Institute of Organic Synthesis, Riga, USSR). The peptide 2438 was synthesized as described [6].

2.2. Culturing and stimulation of the cells

PBMC were obtained from normal human blood according to Boyum [7]. The cells (2×10^6 cells/ml/well) were incubated in 24-well tissue-culture plates (Nunc) in a 5% CO₂ atmosphere for 1 h at 37°C in complete RPMI 1640 medium containing 5% heat-inactivated fetal calf serum. When necessary, ConA (2 mg/ml) and IFN $\alpha 2$ (1,000 IU/ml), or the peptide 2438 (10^{-6} M), or IFN $\alpha 2$ and the peptide were added to the culture medium. After incubation, the cells were collected and centrifuged. The pellet was lysed in the electrophoretic sample buffer [8].

2.3. Electrophoresis and Western blotting

Cellular proteins ($4 \cdot 10^5$ of lysed cells) were electrophoresed in denaturing 7.5–12.5% polyacrylamide gradient slab gels according to Laemmli [8], and electrophoretically transferred to nitrocellulose filters using the semi-dry Western blotting technique [9]. Bands of P-Tyr containing proteins were stained with monoclonal antibodies against P-Tyr (clone B4), conjugated with horse radish peroxidase, and 1-chloro-4-naphthol as a substrate [2]. Individual tracks with P-Tyr containing protein bands on nitrocellulose sheets were scanned on an UltroScan XL Laser densitometer (LKB, Sweden).

2.4. Proliferation assay

200 ml of PBMC suspension at a concentration of 10^6 cells/ml in a 96-well flat-bottom plate was cultured for 75 h in media containing ConA (2 mg/ml) in the presence of IFN $\alpha 2$ and/or the peptide 2438. For the last 15 h of the culturing, [³H]thymidine (0.5 mCi/well; Amersham Corporation) was added. Cells were harvested, and the radioactivity was measured in a liquid scintillation counter Rack-Beta (LKB-Wallac, Sweden).

3. RESULTS AND DISCUSSION

Several P-Tyr-containing proteins were detected in normal PBMC by Western blotting using anti-P-Tyr

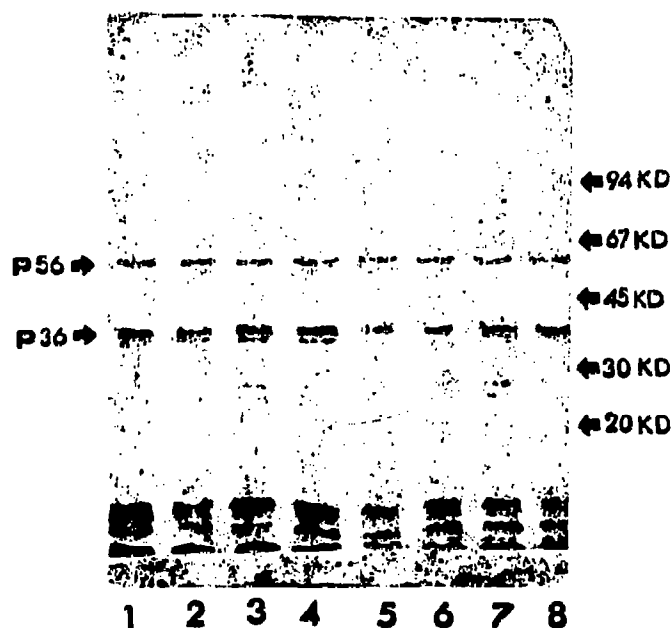


Fig. 1. Western blot of separated polypeptides from human PBMC. Individual tracks demonstrate changes in tyrosine phosphorylation level after treatment with different agents during 1 hour. Lanes: (1) IFN α 2, (2) 2438, (3) IFN α 2 + 2438, (4) ConA, (5) ConA + IFN α 2, (6) ConA + 2438, (7) ConA + IFN α 2 + 2438, and (8) control (medium alone). Left arrows, p36 and p56 respectively; right arrows, molecular weight markers.

antibodies (Fig. 1), the dominant bands corresponding to molecular mass values of 56, 36, and between 10–20 kDa.

The 56-kDa protein may represent an autophosphorylated form of tyrosine protein kinase pp56^{lck}.

Src-related tyrosine protein kinase p56^{lck} is detected in both human and murine T-cells. The activation of lymphocytes induces a rapid increase in the tyrosine-specific protein kinase activity of p56^{lck} and is associated with autophosphorylation and phosphorylation of p56^{lck} endogenous substrates [10].

The 36-kDa protein has the same molecular mass

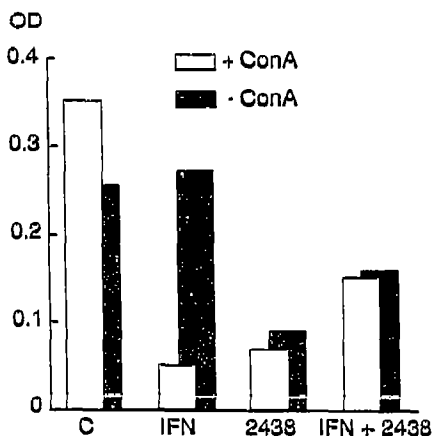


Fig. 2. Absorption of scanned area in individual tracks for p36 phosphorylated in presence of different agents.

value as p36 (p35), which is known to be an endogenous protein substrate for several TPKs [11].

Incubation of the cells with ConA, IFN α 2, and the peptide 2438 in various combinations resulted in significant changes of the level of the 36-kDa protein tyrosine phosphorylation, while the level of tyrosine phosphorylation for the 56-kDa protein was unchanged (Figs. 1 and 2). In particular, incubation of the cells with ConA alone leads to an essential increase in the 36-kDa protein P-Tyr content. If the cells were incubated with ConA and IFN α 2 simultaneously, the level of the 36-kDa protein tyrosine phosphorylation was markedly decreased. In the absence of ConA, IFN α 2 had no effect on tyrosine phosphorylation. Irrespective of the presence of ConA, the peptide 2438 reduced the level of P-Tyr content in the 36-kDa protein, the effect being partially compensated for by addition of IFN α 2.

IFN α 2 and/or the peptide 2438 inhibit the stimulation of PBMC proliferation by ConA (Fig. 3). In the absence of ConA, the proliferation is insensitive to IFN α 2 and/or the peptide 2438 (not shown).

It appears that the effects of IFN α 2 and the peptide 2438 on cellular proliferation (Fig. 3) and P-Tyr content in the 36-kDa protein (Fig. 2) in the presence of ConA could be interrelated, although the simultaneous addition of IFN α 2 and the peptide leads to a synergistic effect on proliferation (Fig. 3), and antagonistic one on the 36-kDa protein tyrosine phosphorylation level (Fig. 2). The decline of tyrosine phosphorylation of the 36-kDa protein under treatment of the cells with IFN α 2 and/or the peptide 2438 could be explained by inhibition of TPKs and/or activation of P-Tyr specific phosphatases.

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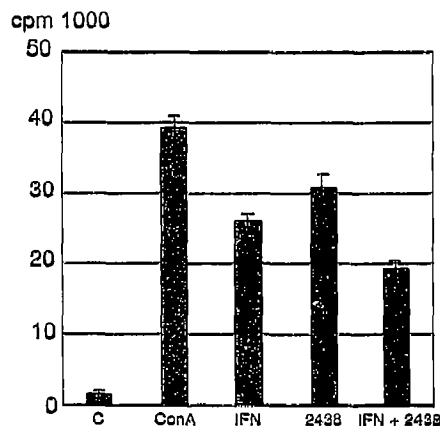


Fig. 3. Proliferation of human PBMC cultured with IFN α 2 and/or synthetic peptide 2438 in the presence of ConA. The column of control cells (C) refers to culturing without stimulation. Concentrations: ConA, 2 mg/ml; IFN α 2, 1000 IU/ml; peptide 2438, 10⁻⁶ M. S.E.M. was calculated from 5 experiments.

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