EXPERIMENTAL METHODS FOR CLINICAL PRACTICE

Effects of Synthetic C-Terminal Fragment of Interferon-2α on Daudi Cells

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Synthetic fragment of human interferon-2α (124th-138th amino acid residue, laboratory code 2438) inhibits the growth of B lymphocytes (Daudi cell line) in a dose-dependent manner. Radiolabeled peptide 2438 binds to specific receptors on the cell surface and competes with interferon-2α for the common binding sites.

Key Words: *interferon; peptide; proliferation; binding; receptors*

Interferons (IF) are known to produce diverse biological effects. There is considerable evidence indicating that IF molecules has several active sites mediating certain biological activities. It was demonstrated that the C-terminal fragment is particularly important for realization of some biological effects of IF [7,11].

Several C-terminal peptides of human IF were synthesizes and characterized [4,5]. Similar to IF, some of these peptides exhibited proliferative activity in model systems. The peptide from the 124th to 138th amino acid residue (laboratory code 2438) exhibits the highest antiproliferative activity [1].

Peptides bind to specific cell receptors and compete with each other for the common binding sites. However, it is unclear whether antiproliferative activity of peptides is mediated by the IF receptors, i.e., are these peptides active sites of IF or act as independent biologically active substances with specific mechanism of action.

This study is an attempt to examine biological effects of peptide 2438 on Daudi cells which are highly sensitive to IF and to find out whether the peptide and IF bind to the same receptors on these cells.

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MATERIALS AND METHODS

C-terminal peptide of human IF-2α was synthesized by Dr. E. V. Makarov (Institute of Bioorganic Chemistry, Russian Academy of Sciences). The peptide is a sequence from the 124th to the 138th amino acid residue of human IF-2α: RITLYLKEKKYSPCA (a single-letter code). The peptide was purified by high-performance liquid chromatography on a reverse phase carrier. The preparation contained not less than 95% peptide. Human recombinant IF was a generous gift of Dr. G. Adolf (Bender Co., Austria). Daudi cells were grown in RPMI-1640 medium supplemented with fetal calf serum (Sigma).

The cells (10^4/well) were incubated in the presence of human IF-2α (10-10,000 U/ml) or peptide 2438 (10^{-4}-10^{-3} M) for 72 h. The proliferative response was assessed by ^{3}H-thymidine incorporation into DNA. Incorporated radioactivity was measured in a liquid scintillation counter.

Cells incubated without IF-2α and peptide served as the control.

Peptide 2438 was labeled with ^{125}I (0.5 mCi) in the presence of Iodogen [6]. Iodinated peptide was

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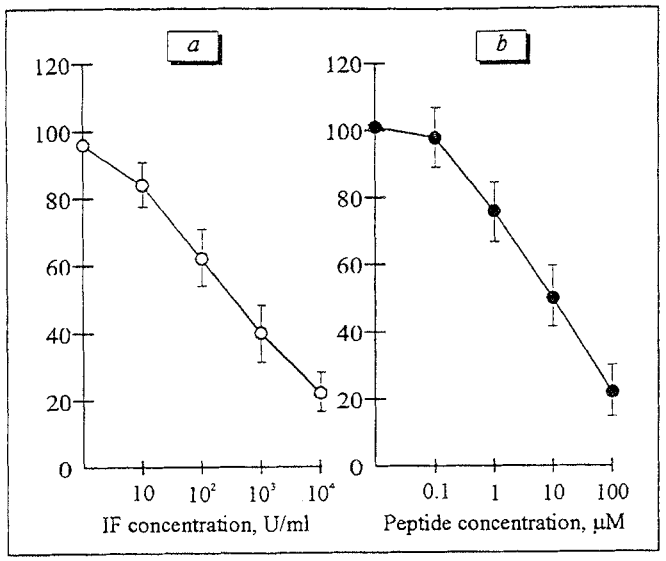

Fig. 1. Effect of IF-α (*a*) and synthetic peptide 2438 (*b*) on the proliferation of Daudi cells. Ordinate: cell proliferation, % of the control (proliferation without the peptide and IF-α).

separated by gel-filtration on Sephadex G-10. Its specific radioactivity was 2×10^{18} cpm/mol.

Daudi cells (10^6/vial) were incubated with the radiolabeled peptide (10^{-12}-10^{-6} M) for 40 min at 4°C in medium 199 containing 2% bovine serum albumin. After incubation cells were centrifuged in 10% sucrose gradient, and their radioactivity was measured. Nonspecific binding was measured in replacement experiments with radiolabeled and native (a 100-fold excess) peptide 2438.

In the competitive binding experiments, Daudi cells were incubated with radiolabeled peptide (8×10^{-8} M) and varied concentrations of IF-α and native peptide 2438.

The dissociation constant and the number of binding sites were determined in Scatchard plots [10].

The results were analyzed by Student's *t* test ($p<0.05$). Each value is the mean of at least three determinations.

RESULTS

Interferon IF-α is known to suppress proliferation of various cells [8,9]. In this study we have shown that synthetic peptide 2438 and human recombinant IF-2α inhibit proliferation of Daudi cells in a dose-dependent manner (Fig. 1, *a*, *b*). It should be noted that antiproliferative effects of IF and peptide 2438 also depended on the time of incubation, reaching the maximum after 48 h.

Figure 2 illustrates the binding of peptide 2438 to the plasma membranes of Daudi cells in Scatchard plots. The dissociation constant was 5.9×10^{-8} M and the number of binding sites was 5000.

In the competitive binding experiments, radiolabeled peptide was replaced by both intact peptide (Fig. 3, *a*) and by human IF-2α (Fig. 3, *b*). This indicates that IF-2α and its C-terminal synthetic peptide compete for the common binding sites.

It has been recently reported [2] that the IF-2α receptor consists of two subunits (α and β), β-subunit providing a low-affinity binding, while the receptor represents a high-affinity binding site.

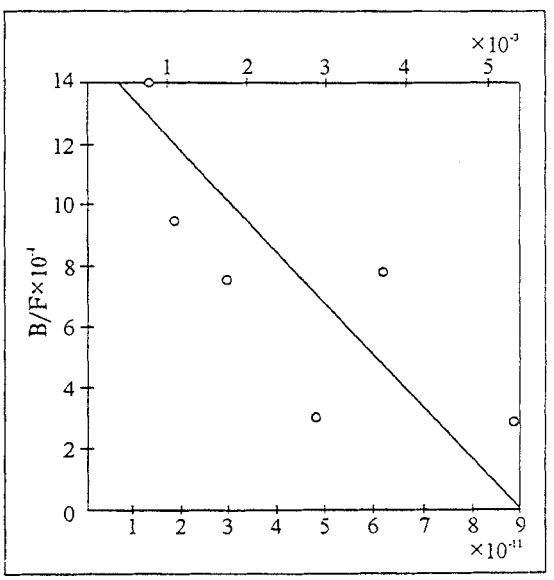

Fig. 2. Binding of ^{125}I-labeled synthetic peptide 2438 to Daudi cells. Scatchard plots. Abscissa: concentration of bound peptide, M; ordinate: bound-free peptide ratio, M. Dissociation constant is 5.9×10^{-8} M. Number of binding sites per cell is 5400.

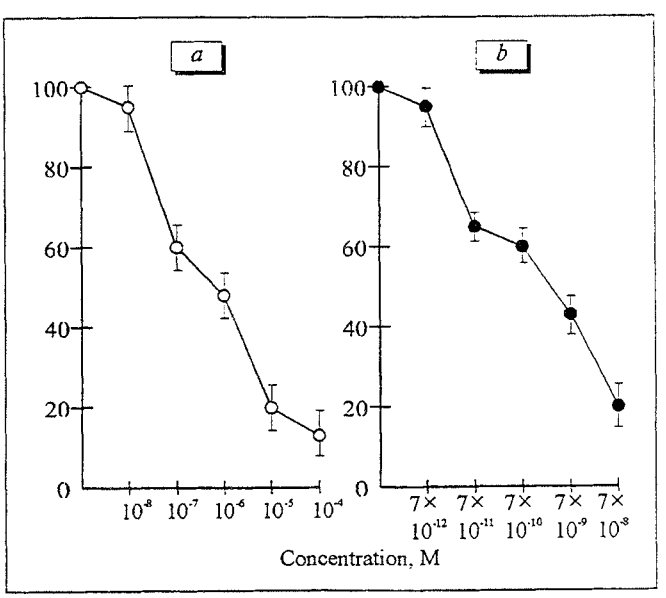

Fig. 3. Replacement of ^{125}I-peptide 2438 on the plasma membrane receptors by native peptide 2438 (*a*) and IF-α (*b*). Ordinate: percent of specific binding. Concentration of radiolabeled peptide is 8×10^{-8} M.

On the basis of the binding parameters of peptide 2438 it can be hypothesized that the peptide receptor is a low-affinity binding site for IF-α. This hypothesis will be tested in further investigations.

REFERENCES

1. A. V. Danilkovich, K. V. Frese, O. Targoni, *et al.*, *Byull. Eksp. Biol. Med.*, **117**, No. 1, 75-79 (1994).
2. O. R. D. Colamonici and L. M. Pfeffer, *Pharmacol. Ther.*, **52**, 227-231 (1992).
3. A. V. Danilkovich, K. V. Freze, J. A. Romashkova, *et al.*, *FEBS Lett.*, **363**, 121-124 (1995).
4. A. V. Danilkovich, K. V. Freze, A. F. Shevalier, *et al.*, *Immunol. Lett.*, **31**, 15-20 (1992).
5. A. V. Danilkovich, A. I. Kharitonenkov, K. V. Freze, *et al.*, *FEBS Lett.*, **295**, 70-72 (1991).
6. J. S. Dooley, J. Vergalla, J. H. Hoofnagle, *et al.*, *J. Lab. Clin. Med.*, **113**, 623-631 (1989).
7. E. N. Fish, K. Banergee, and N. J. Stebbing, *Interferon Res.*, **9**, 97-114 (1989).
8. L. M. Meadows, D. J. George, and R. E. Kaufman, *J. Biol. Response Mod.*, **9**, 212-220 (1990).
9. S. Miscia, A. Cataldi, and P. M. Grimlly, *Exp. Cell Res.*, **187**, 197-202 (1990).
10. P. J. Munson and D. Rodbard, *Anal. Biochem.*, **107**, 220 (1980).
11. T. Senda, T. Shimazu, S. Matsuda, *et al.*, *EMBO J.*, **11**, 3193-3201 (1992).

Pathogenetic Factors of Menstrual Function Disturbances in Women with Pathological Puberty

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A correlation is shown between the peculiarities of puberty and clinical and laboratory parameters during reproductive period. Differences in the state of receptor apparatus in ovaries of examined patients attest to different pathogenesis of menstrual function disturbances with and without mild virilization in anamnesis. The patients with mild virilization and changed pattern of menstrual disturbances probably reflecting desensitization of the endometrium to hormone stimulation have the worst prognosis.

Key Words: *puberty; menstrual function*

The majority of endocrine gynecological disorders is formed during puberty [1,3]. There are several reports on the state of the reproductive system in women with menstrual disturbances at puberty [2,4]. However, in the available literature we found practically no data on the pathogenesis of gynecological disorders as an outcome of pathological puberty. Hence, the objective of the present study was to determine the clinicolaboratory parameters allowing one to reveal the peculiarities of the pathogenesis gynecological disorders depending on the course of puberty.

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MATERIALS AND METHODS

We analyzed the data on complex dynamic examination of 32 women aged 22-34 with a history of menstrual disturbances at puberty. Group 1 comprised 21 patients with disorders of menstrual function: 12 patients with oligomenorrhea-amenorrhea and 9 with dysfunctional uterine bleeding with signs of mild virilization. Group 2 consisted of 11 patients with dysfunctional uterine bleeding without virilization.

The examination included analysis of anamnestic and clinical data, measurement of plasma steroid hormones, endometrial hysteroscopy and biopsy, laparoscopy with ovarial biopsy, and morphological examination of the obtained specimens. The contents of steroid hormone receptors in the specimens were

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