Inhibition of Mitogen-Stimulated Proliferation of Human Lymphocytes *in Vitro* by Synthetic Peptide Fragments of α-2 Interferon

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Human α-2 interferon and peptides representing parts of the amino-acid sequence 124-138 of the IF molecule inhibit the proliferative response of peripheral blood lymphocytes from healthy donors *in vitro* induced by ConA. It is shown that neither interferon α-2 nor biologically active peptides change the level of interleukin-2 ConA-induced production by human blood mononuclears.

Key Words: *interferon; peptides; lymphocytes; proliferation; inhibition*

The phenomenon of cell proliferation underlies such processes as the maturation of immunocompetent cells and the clonal expansion of antigen-recognizing lymphocytes in response to antigen stimulation. Selective regulation of proliferation in various types of immunocompetent cells being one of the main goals of immunosuppressive therapy, the quest for substances with antiproliferative activity remains on-going.

Interferon (IF) α-2 displays various kinds of biological activity including the inhibition of proliferation of normal and tumor cells *in vitro* and *in vivo*, the activation of natural killers, and antiviral activity [2].

Numerous experimental data testify that there are several functionally important parts in the IF molecule, each of which mediates a specific type of IF biological activity [6,8]. The experimental data obtained previously stress the importance of the COOH-terminal region of the IF α-2 molecule in the manifestation of its antiproliferative activity [7].

The aim of the present investigation was to study the antiproliferative activity of synthetic peptides, that are fragments of the C-terminal region of the human IF α-2 molecule in a model system *in vitro* on lymphocytes from the peripheral blood of healthy donors. The effect of the given peptides was compared with the effect of IF under analogous experimental conditions.

The capacity of peptides to inhibit the proliferation of lymphocytes stimulated by mitogens and their effect on interleukin-2 (Il-2) production by lymphocytes were studied.

MATERIALS AND METHODS

Synthesis of peptide fragments from the 124-144 region of the human IF α-2 molecule was described previously [1]. Recombinant Il-2 (Proleukin) was supplied by the firm Eurocetus B. V. (Holland) and recombinant human IF α-2 (reaf-

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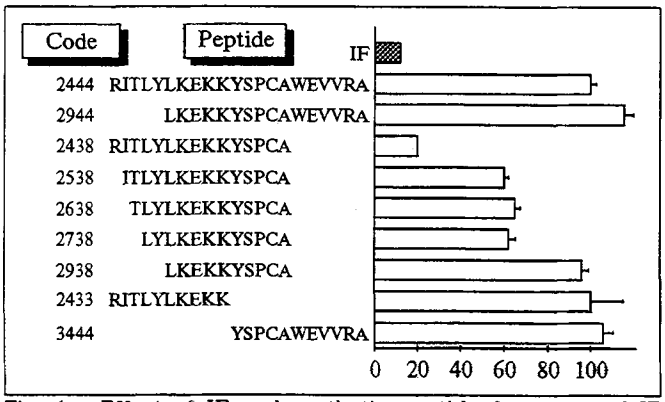

Fig. 1. Effect of IF and synthetic peptide fragments of IF on the proliferative response of human blood MNC induced by the polyclonal activator ConA *in vitro*. At left: amino-acid sequence of peptides and their laboratory code. At right: proliferative response of MNC affected by different peptides and IF, expressed in % of the control (control: MNC proliferation in the presence of ConA without the addition of compounds).

eron) by the Biopreparat State Concern. Culturing of cells was performed using the following reagents: RPMI-1640 medium, 5% fetal calf serum, L-glutamine (Sigma, USA) and plastic bottles and caps (NUNK, Denmark). Phytohemagglutinin (PHA) (Difco, USA), concanavalin A (ConA) (Pharmacia, Sweden), and radioactive labeled reagents (Amersham, UK) were used in the study. The other reagents used were produced by Sigma (USA) and Serva (Germany).

The peripheral mononuclear cells (MNC) were isolated by gradient centrifugation [3]. The proliferative response of cells in the reaction of blast transformation was detected by a micro method in 96-well plates for cell culturing in a 200 μl volume and the number of repeats for all variants was no less than 3 in each experiment. Cells were cultured in RPMI-1640 medium with the addition of 5% heat-inactivated fetal calf serum (FCS) at 37°C in a humid atmosphere. The suspension of MNC (10^6 cells/ml) was incubated in medium containing the polyclonal activator ConA (2 μg/ml) with synthetic peptides or IF for 75 h. Synthetic peptides were used in concentrations from 10^{-4} to 10^{-8} M and recombinant human IF α-2 in a concentration of 100 IU/ml. Cells nonstimulated by polyclonal activators and stimulated but untreated with synthetic peptide or IF α-2 were used as the control.

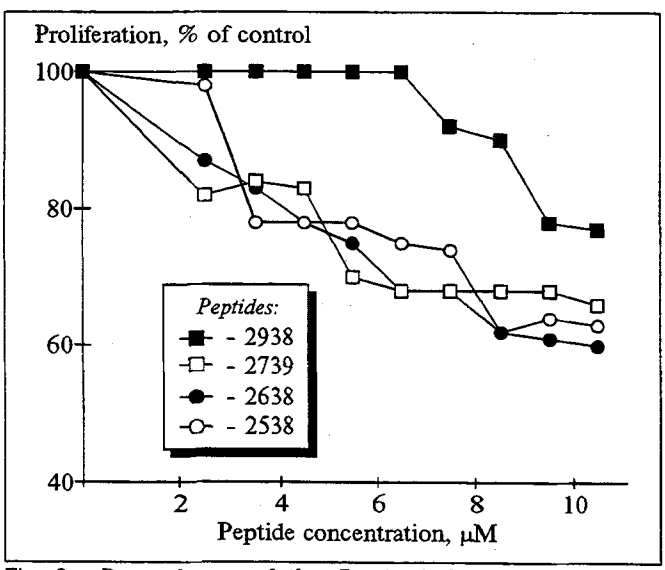

Fig. 2. Dependence of the ConA-induced proliferative response of human MNC on the concentration of peptides. Control: proliferation of MNC in the presence of ConA without peptides. The standard error of mean is less than 10%.

The proliferative response of lymphocytes was assessed by the incorporation of the labeled precursor, ^{3}H-thymidine, in DNA added during the last 15 h of culturing. The cells were then transferred to GF-C fiberglass filters (Watman, England) and radioactivity was detected using a RACK-BETA liquid scintillation counter (LKB-WALLAC, Finland).

The induction of IL-2 synthesis in the MNC culture of human peripheral blood (2×10^6 cells/ml) was prepared in 24-well-plates at 1 ml volume. Cells were incubated for 40 h with ConA (2 μg/ml) and peptides (10^{-5} M) or IF α-2 (1000 IU/ml). The content of IL-2 in culture supernatants was determined by the proliferation of ConA-activated mouse splenocytes. To obtain ConA-activated cells, splenocytes from BALB/c mice (5×10^6 cells/ml) were placed in cell culture flasks and incubated in medium with 5% FCS containing 2 μg/ml ConA for 48 h later after which the cells were sedimented by three-trial centrifuging with subsequent resuspending in a fresh portion of medium containing 100 mM α-methylmannoside. The supernatants were tested by the micro method described above. ConA-activated mouse cells (50,000 cells/well) were cultured with 50% of the test supernatants or without them for 24 h. The culture medium contained 100 mM α-methylmannoside for a blockade of the proliferative response of mouse cells to residual ConA. ^{3}H-thymidine (2 μCi/well) was added to the wells during the last 4 h and the radioactive labeling of cells was measured as described above. The Student *t* test was used to assess the reliability of differences of mean values ($p<0.05$).

RESULTS

Chevalier and co-workers synthesized 6 peptides from the 124-144 region of the human IF α-2 molecule and showed that not one of the synthe-

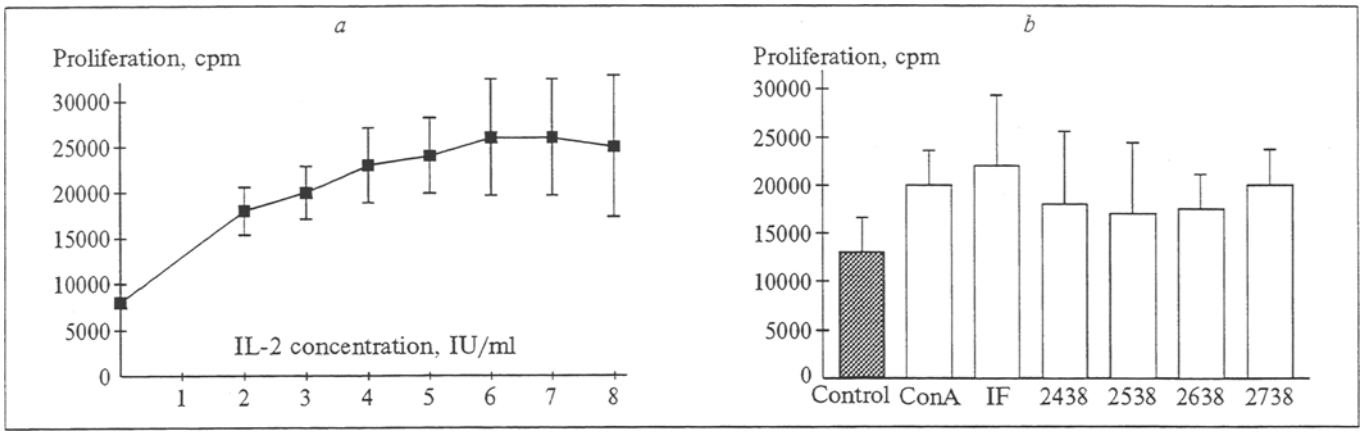

Fig. 3. Effect of synthetic peptides and IF on L-2 production in a culture of human peripheral blood MNC under the influence of ConA. The presence of IL-2 in culture supernatants was determined by their capacity to intensify the proliferation of ConA-stimulated mouse splenocytes. *a*) calibration curve for the quantitative determination of IL-2 contained in culture supernatants; *b*) effect on the proliferation of ConA-activated mouse splenocytes of culture supernatants from human MNC incubated with ConA and peptides or IF.

sized peptides displayed the antiviral activity, in contrast to the whole molecule [1]. It is interesting to note that rabbit antiserum against synthetic peptides containing the amino-acid sequences 124-138 and 129-144 of the IF α-2 molecule are able to bind with the whole IF molecule and to block its antiviral effect [1]. Moreover, it was found that those synthetic peptides, unlike the IF molecule, do not affect the activity of natural killers [4]. We showed previously that only one such synthetic peptide, fragments of human IF α-2 including amino-acid residues from the 124th to the 138th (2438), inhibits the proliferative response of lymphocytes from human peripheral blood induced by polyclonal activators *in vitro* and is able to bind specifically with receptor structures of the lymphocyte membrane [4,5]. There are now other synthetic peptide analogs, fragments of the sequence 125-138 (2538), 126-138 (2638), and 127-138 (2738) amino-acid residues, which differ from those previously studied in the length of the amino-acid sequence (Fig. 1).

The effect of these peptides, fragments of the human IF α-2 molecule, on the proliferative response of lymphocytes from the peripheral blood of normal donors was studied *in vitro*. It was found that IF and peptides 2438, 2538, 2638, and 2738 inhibit *in vitro* the ConA-induced proliferative response of peripheral blood lymphocytes from healthy donors (Fig. 1).

An analogous result was obtained using the experimental model of a nonseparated population of mononuclears with PHG and IL-2 as polyclonal activators. There was no direct cytotoxic effect of IF and the peptides in the range of concentrations used.

The antiproliferative effect of peptide 2438 tested *in vitro* is dose-dependent and manifests itself in a concentration no lower than 10^{-7} M [4]. Synthetic peptides 2538, 2638, and 2738 also dose-dependently inhibit the proliferative response to ConA (Fig. 2), but the optimum concentration for their effect is different, namely 10^{-5} M. Moreover, a weak antiproliferative effect of peptide 2938 (129-138 amino-acid residues) is noted when the concentration rises to 10^{-4} M.

Next it was shown that neither IF α-2 nor biologically active peptides changed the level of IL-2 production by MNC of human blood induced by ConA (Fig. 3, *b*). The concentration of IL-2 in MNC supernatants cultured with IF α-2 or with peptides was determined by the calibration curve for recombinant IL-2 (Fig. 3, *a*) and did not differ significantly from that in the absence of the compounds.

The antiproliferative effect of the synthetic peptides, manifested in the absence of a down-regulation of IL-2 production, may be related to an inhibition of the expression of the receptors for IL-2 or/and other lymphokines which can maintain the growth of lymphocytes *in vitro*.

The findings attest that synthetic peptides that are IF fragments have, just like IF itself, a negative effect on the proliferation of lymphocytes in response to the mitogen, but, at the same time, possess a narrower spectrum of biological activity. Further study of the antiproliferative effect of synthetic human IF α-2 fragments may be useful not only for clarifying the structural and functional characteristics of the organization of the IF molecule, but also for creating new selective immunoregulatory drugs for clinical use. Thus, synthetic peptides may be considered as potential antiproliferative drugs for use in transplantology and therapy of autoimmune disorders.

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ONCOLOGY

Effect of Extract from a Transplant for Eyelid Plasty (series ALLOPLANT™) on DNA Synthesis in Cultures Cells

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Extract isolated from a collagen-containing transplant (series ALLOPLANT™), which is used in the treatment of benign and malignant neoplasms of the eyelid, inhibits DNA synthesis in the cell *in vitro*. This effect is nonspecific, reversible, and dose-dependent. The extract is thermostable and resistant to proteolytic enzymes.

Key Words: *eyelid plasty; transplant extract; DNA synthesis; cell culture; ALLOPLANT™*

During the last decade special attention has been focused on the effect of the extracellular matrix (EM) and its components on shape modulation, proliferation and differentiation of various cell types [11,12,14], and cell adhesion to the EM [3]. As is well known EM is a stable complex of macromolecules which can be assigned to 4 classes: collagens, elastins, proteoglycans, and glycoproteins. Proteoglycans and glycoproteins are involved in the regulation of cell proliferation. For example, the proteoglycan heparan sulfate inhibits cell growth; inhibition and stimulation of cell growth have been demonstrated for a number of glycoproteins and glycoconjugates.

Synthesis of artificial analogs of the EM and their use as substrate for cell culturing is one of the major approaches to the elucidation of the role of EM. Such models are convenient tools in the study of the behavior of various cell types [8] and

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