Inhibition of IL-2-Dependent Proliferation of Human Lymphocytes and of the Expression of Interleukin-2 Receptors by Synthetic Peptide Fragments of α-2 Interferon

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It is shown that biologically active peptides inhibit the concanavalin A-induced generation of cells which may proliferate in response to exogenous recombinant interleukin-2. Determination of the number of CD25-containing cells showed that synthetic peptides are able to lower not only the number of CD25-containing cells but the level of its expression as well.

Key Words: *lymphocytes; interleukin-2; CD25; proliferation; inhibition*

Recent studies of the synthetic peptide fragments of the interferon (IF) α-2 C-terminal region showed that the peptide fragment of the amino-acid sequence from the 124th to the 138th amino-acid residue (2438) inhibits the proliferation of human peripheral blood lymphocytes *in vitro* [2]. Contrary to IF, this peptide does not possess antiviral activity and does not affect the functional activity of natural killers. Thus, this biologically active peptide fragment of human IF α-2 may be considered a potentially antiproliferative agent with a narrower spectrum of biological activity than IF.

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The aim of the present investigation was to study the biological activity of some synthetic peptide fragments of the C-terminal region of human IF α-2 using peripheral blood lymphocytes from healthy donors in an experimental system *in vitro*. The effect of these peptides was compared with the effect of IF under analogous experimental conditions. The capacity of peptides to inhibit the proliferation of mitogen-stimulated lymphocytes and their effect on interleukin-2-dependent proliferation of mitogen-activated T-lymphocytes were studied. The expression of membranous CD25 molecules, which are low-affinity receptors for interleukin-2 (IL-2), on mitogen-activated cells and the effect of synthetic peptides on the induction of CD25 expression were studied using flow cytofluorometry.

MATERIALS AND METHODS

A peptide fragment from the 124-138 region of the human IF α-2 molecule (2438) was synthesized previously. The full list of synthetic peptides

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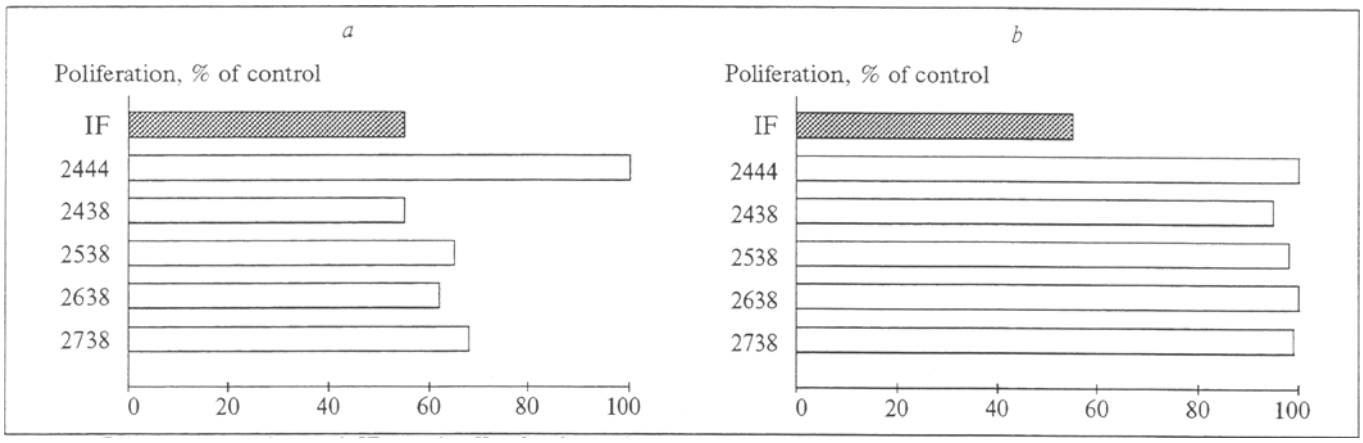

Fig. 1. Effect of peptides and IF on the IL-2-dependent proliferation of activated human blood MNC. Proliferation of activated cells is expressed in % of the control (abscissa). Cells generated in the presence of only ConA were the control. *a*) proliferative response of activated human blood MNC generated in the presence of ConA and synthetic peptides or IF to recombinant IL-2; *b*) proliferative response of ConA-stimulated MNC from human peripheral blood to IL-2 in the presence of synthetic peptides or IF. Statistical error of mean is no more than 10%.

used and their codes are listed in Table 1. Recombinant human IF α-2 (Reaferon, Biopreparat State Concern) and recombinant Il-2 (Proleukin, Eurocetus B. V., Holland) were used in the study. Culturing of cells was performed using the following reagents: RPMI-1640 medium, 5% fetal calf serum (FCS), L-glutamine (Sigma, USA), plastic culture apparatus (NUNK, Denmark), phytohemagglutinin (PHA) (Difco, USA), and concanavalin A (ConA) (Pharmacia, Sweden). The other reagents used were supplied by Sigma (USA) and Serva (Germany). Radioactive labeled reagents (Amersham, UK) were used.

The peripheral mononuclear cells (MNC) were separated by ficoll-verograffin centrifugation [1]. Cells were cultured in RPMI-1640 medium with the addition of 5% FCS at 37°C in a humid atmosphere.

Two-step experiments were performed for a study of the effect of peptides on the IL-2-dependent proliferation of activated cells. Lymphocytes were preincubated with the polyclonal activator ConA (2 μg/ml) for 48 h, after which their ability to proliferate in response to exogenous IL-2 was determined. In such experiments the ability of peptides both to inhibit ConA-induced generation of cells capable of responding to exogenous recombinant IL-2 and to block the IL-2-dependent proliferation for the combined addition of IL-2 and peptide to cells preactivated without peptide or IF was studied. For this purpose in the second experimental stage the proliferative response of ConA-activated cells was assayed with a micro method in 96-well plates (volume 200 μl) in triplets for all variants in each test. IL-2-dependent proliferation of ConA-activated cells was determined in the presence of 100 mM α-methylmannoside for the removal of residual ConA. Lymphocyte proliferation was judged by ^{3}H-thymidine incorporation in DNA (0.5 μCi/well for 15 h). Cells nonstimulated by polyclonal activators and cells stimulated, but untreated with synthetic peptide or IF α-2 were used as the control. The total number of experiments was 9. The reliability of differences between mean values was assessed using the Student *t* test ($p<0.05$).

Recombinant IL-2 in a concentration of 20 IU/ml, synthetic peptides in a concentration of 10^{-5} M, and human recombinant IF α-2 at 1000 IU/ml were used.

The expression of CD25 on the membrane of mitogen-activated MNC in human peripheral blood was assayed with flow cytofluorometry. Mouse monoclonal antibodies against CD25 were kindly made available by Dr. Vaclav Horejsi, Institute of Molecular Biology, Prague. MNC were cultured for

TABLE 1. Synthetic Peptides, Fragments of Human IF α-2 Molecule

Position of amino acids in IF α-2 molecule	Peptide code	Amino-acid sequence
129-138	2938	L-K-E-K-K-Y-S-P-C-A
127-138	2738	L-Y-L-K-E-K-K-Y-S-P-C-A
126-138	2638	T-L-Y-L-K-E-K-K-Y-S-P-C-A
125-138	2538	I-T-L-Y-L-K-E-K-K-Y-S-P-C-A
124-138	2438	R-I-T-L-Y-L-K-E-K-K-Y-S-P-C-A

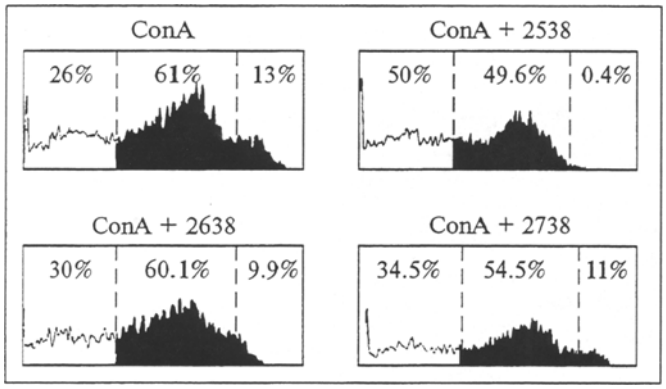

Fig. 2. Effect of synthetic peptides, fragments of IF, on expression of α-chain of IL-2 receptor (CD25) in lymphocytes from human blood treated with ConA. Abscissa: intensity of fluorescence (in relative units); ordinate: number of cells. Subpopulation of fluorescent (CD25 positive) cells is colored dark. The percent correlation of CD25-negative, CD25-positive, and CD25-strongly positive is shown.

72 h in the presence of ConA (2 μg/ml) and peptides (10^{-5} M) or IF α-2 (1000 IU/ml) as described above. Cultures of untreated cells were the control. Identification of CD25 on cells was performed by an indirect method using (Fab)2-fragments of goat antibodies against mouse immunoglobulins labeled with fluorescein isothiocyanate. Detection of $CD25^+$ (fluorescent) and $CD25^-$ (nonfluorescent) cells was performed with an ARGUS 100-5 flow cytophotometer (Scatron, Norway) at 480 nm wavelength of excitation and 520 nm of emission. The number of experiments was 8.

RESULTS

It was previously shown that synthetic peptides, fragments of human IF α-2 which include amino-acid residues from the 124th to the 138th (2438, 2538, 2638, and 2738), inhibit *in vitro* the proliferative response of lymphocytes from human peripheral blood induced by polyclonal activators. However, the preincubation of human blood MNC with PHA (or with other polyclonal activators) during 18-24 h leads to a blockade of the antiproliferative action both of IF α-2 and of the peptides [2]. At the same time, cell culturing with mitogen does not result in the disappearance of the membrane receptors responsible for binding with 2438 peptide [3]. In both cases, for ConA- and for IF-treated cells, the character of 2438 peptide interaction with the receptors remained more or less unchanged. Therefore, the resistance to the antiproliferative effect of 2438 peptide cannot be related to the alteration of 2438 reception.

These results enable us to assume that biologically active fragments of the IF α-2 molecule may inhibit the generation of mitogen-activated cells proliferating in response to such lymphokines as autocrine growth factor of T lymphocytes, or IL-2.

In fact, in the studies of IL-2-dependent proliferation biologically active peptides inhibited the ConA-induced generation of cells proliferating in response to exogenous recombinant IL-2 (Fig. 1, *a*), but did not block the IL-2-dependent proliferation itself when IL-2 and peptides were added simultaneously to cells preactivated in the absence of peptides (Fig. 1, *b*). Thus, the inhibitory effect of the peptides manifests itself in the stage of generation of activated IL-2-sensitive cells, but not in the stage of IL-2-dependent proliferation. On the other hand, the effect of IF was different and proliferation was inhibited in the IL-2-dependent stage as well.

It is known that the proliferation of T lymphocytes in response to IL-2 is related to the expression of receptive structures on their membrane. The low-affinity IL-2 receptor, or CD25 molecule (also called TAC antigen), is a marker of activated T and partially B lymphocytes [4]. We assume that the antiproliferative effect of the peptides studied may be related to the inhibition of IL-2 receptor expression.

In fact, determination of the number of cells containing the low-affinity receptor for IL-2, whose generation is induced by ConA, showed that biologically active peptides may inhibit CD25 expression (Fig. 2). There is a decrease not only in the relative number of CD25-containing cells, but also in the level of receptor expression, which manifests itself in a decrease of the number of high-expressing (strongly positive) cells. Such an effect diminishes as the peptides become shorter from the NH_2 end. Thus, the findings attest that synthetic peptide fragments of human IF α-2 block *in vitro* the accumulation of activated cells which express IL-2 receptors and can proliferate in response to IL-2. Such peptides may not merely serve as probes for examination of the molecular mechanisms of regulation of the activation of various types of immunocompetent cells, but are also of interest in clinical practice.

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