Influence of synthetic peptides, corresponding to fragments of the human α-2 interferon molecule, on the proliferation of lymphoblastoid cells in vitro. Growth inhibition and receptor binding

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Abstract Influence of nine synthetic peptides from the C-terminal part of human α-2 interferon (IFN) molecule on growth of a human T-lymphoblastoid cell line MT-4 was investigated. It was shown that some peptides inhibited the proliferation of MT-4 cells. It was also found that MT-4 cells expressed specific receptors on the outer surface of the plasma membrane. All studied peptides competed for binding to a common binding site. Antiproliferative activity of the peptides correlated with their length and affinity to receptors on the outer membrane of MT-4 cells.

Key words: Synthetic peptide; Interferon α; Tumor cell; Growth inhibition; Receptor

1. Introduction

Earlier it was shown that several synthetic peptides from the C-terminal part of the human α-2 IFN molecule possessed IFN-like antiproliferative activity on human blood lymphocytes in vitro [1,2]. In contrast to the native IFN molecule, these peptides were unable to protect cells from viral infection [3] and did not affect activity of natural killer cells [1]. The studied synthetic peptides were thus of limited biological activity as compared with the original IFN molecule. We considered it interesting to study antiproliferative properties of the peptides and their influence on tumor cell growth.

Nine synthetic peptides corresponding to the human IFN molecule region of amino acid residues 124–144 were prepared to investigate their effect on growth of an IFN-sensitive human lymphoblastoid cell line MT-4 in vitro. To elucidate the molecular mechanism of the peptide's action we studied their binding properties.

2. Materials and methods

2.1. Cell line

A human T-lymphoblastoid cell line MT-4 was kindly donated by Dr. B. Asjo (Dept. Virology, Karolinska Institute, Stockholm, Sweden).

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Abbreviations: IFN, interferon; [^{3}H]TdR, [^{3}H]thymidine; K_d, equilibrium dissociation constant; IC_{50}, concentration of competing ligand causing half-maximum displacement; K_i, inhibition constant.

2.2. Radioactive reagents

[^{3}H]thymidine ([^{3}H]TdR) was from Amersham Corp. (England), Na^{125}I (2×10^6 Ci/M specific activity) was from Russian Scientific Center 'Applied Chemistry' (St. Petersburg, Russia). All media, sera and other chemicals were from Sigma (USA).

2.3. Peptide synthesis

Peptides were synthesized essentially as described [4] using t-Boc protected amino acids. The protected amino acids were coupled according to the *N,N'*-diisopropylcarbodiimide/1-hydroxybenzotriazole (DIC/HOBt) method. The final deprotection was performed with an anhydrous hydrogen fluoride/m-cresol (10:1) at 0°C for 60 min.

All peptides have a HPLC purity not less than 95%. Mass values of the peptides were confirmed by fast atom bombardment mass spectral analysis. The peptides, their designation and location on the α-2 IFN molecule are shown in Table 1.

2.4. Cell culture

Cells were grown in RPMI 1640 medium supplemented with 10% fetal calf serum and gentamicin (50 μg/ml).

2.5. Cell proliferation

Cell proliferation was assayed by incorporation of [^{3}H]TdR into DNA. Briefly, 1×10^5 cells were incubated in 24-well plate in the presence or absence of the synthetic peptides (10^{-5} to 10^{-8} M) for 24–72 h, of them last 4 h with [^{3}H]TdR (1 μCi/well) added to the cell culture. The incubated cells were harvested and their radioactivity was measured in a liquid scintillation counter (Rack-Beta LKB-Wallac, Sweden).

2.6. Labeling of synthetic peptides

5 μg of peptides 124–138 and 127–138 were labeled by the solid phase oxidation method using Iodogen and Na^{125}I (1 mCi) as described [5]. The specific activities of the labeled peptides were 2×10^8 Ci/M.

2.7. Binding assay

5×10^5 to 2×10^6 cells per tube were incubated with labeled peptide 124–138 at a concentration of 10^{-6} to 10^{-10} M for 1 h at 4°C in 100 μl of 199 medium containing 20 mM of NaN_3 and 1% bovine serum albumin. After the incubation, the cells were applied to 10% sucrose gradient and centrifuged as described [6]. The cell-bound radioactivity was measured in a Rack-Beta gamma-counter (LKB, Sweden). Experimental data were analysed by the Scatchard method [7]. Nonspecific binding was estimated in the presence of 1000-fold molar excess of the unlabeled peptide 124–138.

2.8. Competition assay

MT-4 cells (1.5×10^6 per tube) were incubated at a constant concentration of ^{125}I-127–138 peptide (8×10^{-8} M) and at various concentrations of unlabeled peptides (10^{-4} to 10^{-9} M) as described above (Section 2.7.). The inhibition constant (K_i) was calculated as described [8] according to the equation:

$$K_i = IC_{50} \times (1 + F/K_d),$$

where F is a molar concentration of peptide ^{125}I-127–138, K_d is the dissociation constant of the peptide ^{125}I-127–138-receptor complex, and

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IC_{50} is the concentration of the competing ligand causing half-maximum displacement of ^{125}I-127–138.

2.9. Peptide conjugation with dextran

Conjugation of radiolabeled and unlabeled peptide 127–138 with dextran 500 (Pharmacia) (Dx) was carried out by sodium periodate method as described [9]. Dextran/peptide molar ratio was 1/1000 (as determined using radioactive peptide ^{125}I-127–138). MT-4 cells were incubated with the ^{125}I-127–138-Dx conjugates obtained at a peptide concentration of 1.8×10^{-8} M in the absence or presence of 100-fold molar excess of unlabeled 127–138-Dx conjugates or free peptide 127–138 as described above in Sections 2.7. and 2.8.

2.10. Treatment of cells with trypsin

MT-4 cells (2×10^6/ml) were incubated with trypsin (5 mg/ml) (Sigma) in 199 medium (Sigma) for 30 min at 37°C. Trypsin activity was then blocked by adding a large volume of medium 199 supplemented with fetal calf serum. The cells were washed thrice by centrifugation and used in binding experiments. As judged from a Trypan blue exclusion test, the cell viability after the trypsin treatment was above 95%.

3. Results and discussion

Earlier it was demonstrated that at least four synthetic peptides corresponding to fragments of the human α-2 IFN amino acid sequence between residues 124 and 138 did inhibit the proliferation of lymphocytes from normal human blood [1,2]. Interestingly, other similar and partially overlapping peptides did not affect the cell proliferation [1].

Among the growth inhibiting peptides the most pronounced effect was observed for peptide 124–138. Trimming of this peptide from the N-terminus resulted in decreased antiproliferative activity (Fig. 1). Growth inhibition effect of the synthetic peptides was time- and dose-dependent: maximum inhibition of cell growth was observed after 72 h of cell culturing at a peptide 124–138 concentration of 10^{-5} M (not shown). Moreover, it depended also on peptide length, the longer the peptide the more pronounced was the effect (Fig. 1).

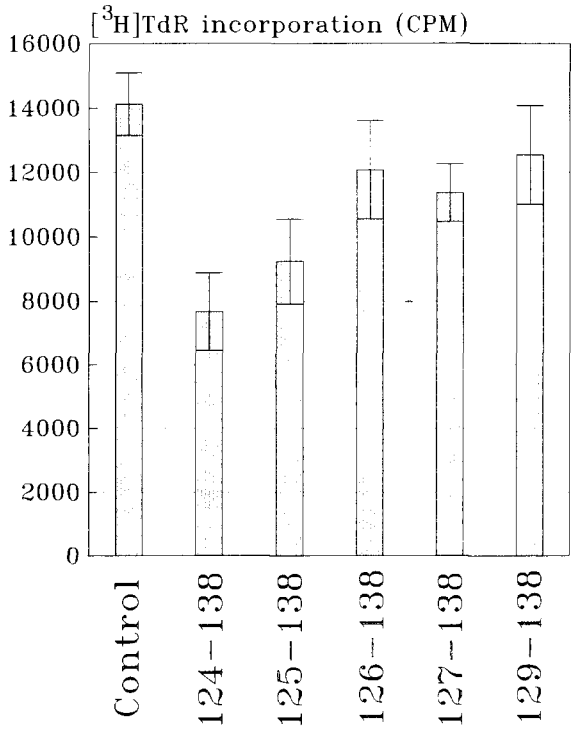

Fig. 1. Inhibition of MT-4 cell proliferation by synthetic peptides corresponding to fragments of the human α-2 IFN molecule. No peptides were added to culture media in control. Where added, the concentration of peptides was 10^{-5} M. [^{3}H]Thymidine was incorporated during last 4 h of 3-day culturing. S.E.M. was calculated from 4 experiments.

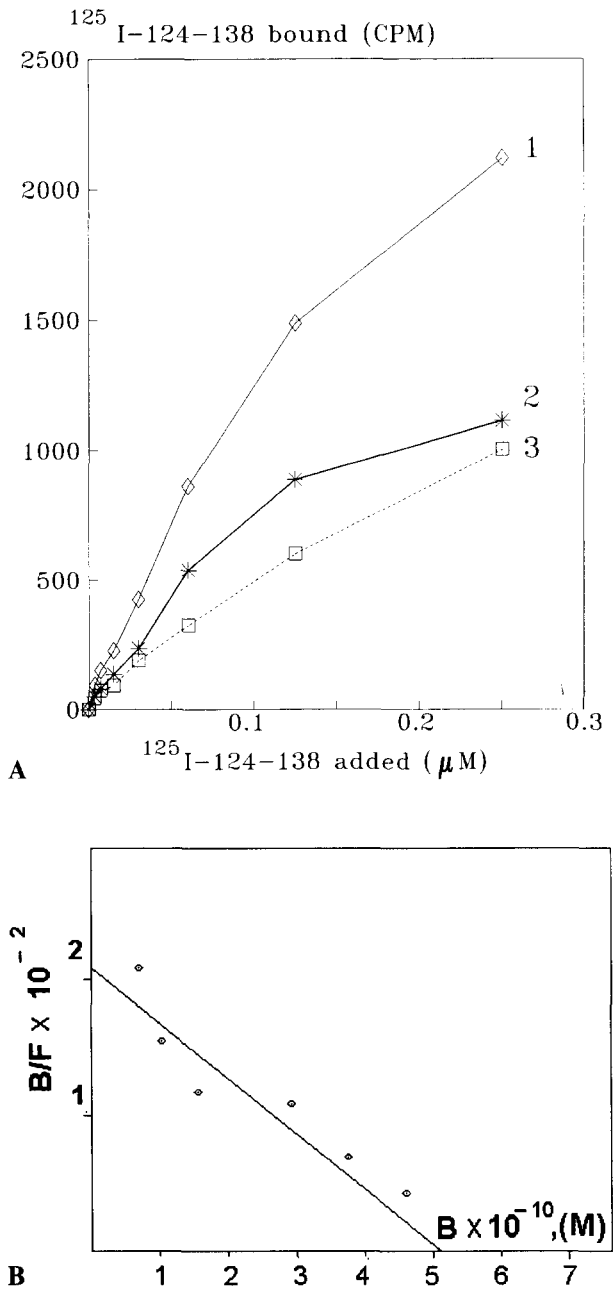

Fig. 2. Binding of ^{125}I-124–138 peptide to MT-4 cells. (A) Saturation curve. Specific binding (line 2) is presented as the difference between the total (line 1) and unspecific (line 3) values. (B) Scatchard plot.

Thus, some of the studied synthetic peptides are characterised by antiproliferative activity as to both normal human lymphocytes [1,2] and tumor lymphocytes of a lymphoblastoid cell line MT-4.

In our previous work [11] it was shown that lymphocytes separated from normal human blood express specific receptors of peptide 124–138. Binding characteristics of iodinated peptide

Table 1

Peptide code	Amino acid sequence (one-letter code)	K_i (M)
134–144	YSPCAWEVVRA	2.7×10^{-7}
124–144	RITLYLKEKKYSPCAWEVVRA	5.3×10^{-7}
129–144	LKEKKYSPCAWEVVRA	6.2×10^{-7}
129–138	LKEKKYSPCA	5.8×10^{-8}
124–133	RITLYLKEKK	5.0×10^{-5}
124–138	RITLYLKEKKYSPCA	4.2×10^{-9}
125–138	ITLYLKEKKYSPCA	5.8×10^{-9}
126–138	TLYLKEKKYSPCA	5.8×10^{-8}
127–138	LYLKEKKYSPCA	4.0×10^{-8}

124–138 to MT-4 cells (Fig. 2A) supports specific character of the interaction. Scatchard analysis [7] of the data suggested the K_d value of 2.02×10^{-8} M and the number of binding sites of 5.01×10^4 per cell (Fig. 2B).

To decide if the peptides were bound to the same binding sites, displacement experiments werer carried out where radioactively labeled peptide 127–138 competed with unlabeled peptides for binding sites on MT-4 cells. Iodinated peptide 127–138 was found specifically interacted with MT-4 cells (Fig. 3). The results of the displacement assay confirmed that all studied peptides competed for the same binding sites (Fig. 3), although their inhibition constant values were different (Table 1). Growth-inhibiting peptides were characterised by smaller K_i values as compared with their inactive counterparts. The K_i values correlated with peptide-receptor affinity and inhibiting potential of the peptides.

To confirm that the peptide receptors are located on the outer membrane of MT-4 cells, bulky conjugates of radiolabeled or unlabeled peptide 127–138 with dextran (^{125}I-127–138-Dx and 127–138-Dx, respectively) were used. The dimensions of the conjugates prevented their penetration into the cell through the plasma membrane. To avoid the uptake of the conjugate by cells the incubation medium was supplemented with sodium azide. It was found that radiolabeled conjugate ^{125}I-124–138-Dx was displaced both by unlabeled conjugate 127–138-Dx (51 ± 5% of total ^{125}I-124–138-Dx binding) and free peptide 127–138 (47 ± 5% of total ^{125}I-124–138-Dx binding). These results clearly demonstrate that conjugated and free peptide 127–138 interact with the same binding sites exposed on the outer cell membrane.

As a first attempt to characterize the peptide receptors which probably mediate the inhibitory effect, MT-4 cells were trypsinized followed by a peptide-binding assay. Trypsin was shown to completely destroy the peptide binding sites on MT-4 cells, supporting that the sites are trypsin-sensitive. This finding suggests that the receptors are represented by protein or modified protein molecules expressed on the surface of MT-4 cells.

Further characterization of the peptide receptors and their relation to IFN receptors is a subject of separate studies.

We believe that the synthetic peptides studied in the present work may serve as a useful tool for investigation of tumor cell growth and also may be of interest as potential nontoxic antiproliferative agents.

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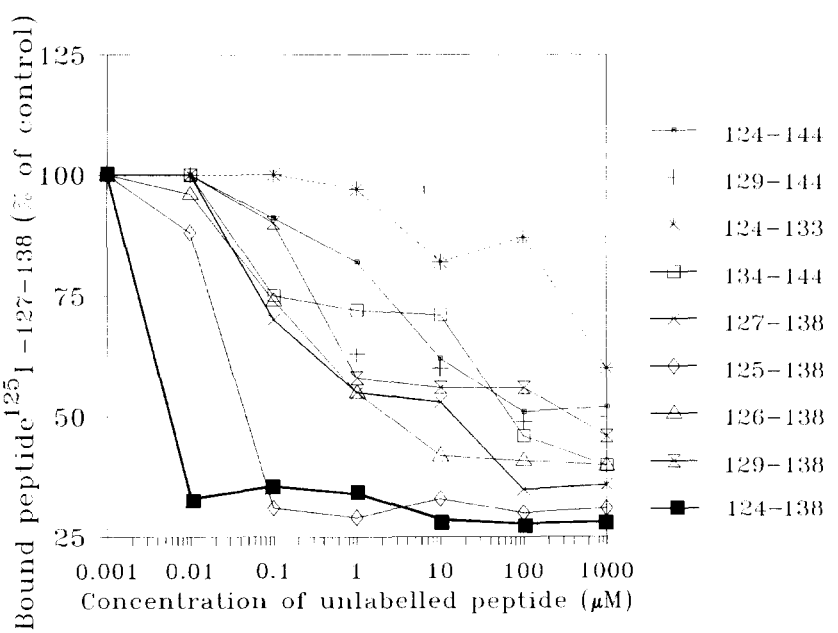

Fig. 3. Binding competition between peptide ^{125}I-127–138 at a concentration of 8×10^{-8} M and different unlabeled peptides.

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