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Interaction of a synthetic peptide of the interferon α-2 C-terminal part with human blood leukocytes

Binding to peripheral blood mononuclear cells

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A biologically active synthetic peptide, 2438, representing the 124–138 amino acid sequence of the human interferon α-2 (IFN α-2) molecule, which is known to possess IFN-like antiproliferative activity, specifically binds to human blood leukocytes. Scatchard plots reveal two different K_d values, for the 'low' and 'high' affinity binding. The interaction of the ^{125}I-labelled peptide 2438 with the cells is not impaired by human IFN α-2 or cholera toxin.

IFN α-2; Synthetic peptide; Specific binding; Human; Lymphocyte

1. INTRODUCTION

The family of IFN α is a group of related proteins manifesting multiple biological activities [1]. Numerous structure–functional studies of IFN molecules revealed several areas associated with different biological activities [2–4]. Synthetic peptides representing fragments of IFN polypeptide chains are a good tool for identification of functionally important regions of these macromolecules.

A study of a synthetic peptide corresponding to the 131–138 region of the human IFN α-2 molecule demonstrated its biological activity and specific binding to surface elements of mouse thymocytes. The binding of the labelled peptide 131–138 to the cells was impaired by IFN α-2 and thymosin α [5].

In an earlier work, we have shown that a synthetic peptide, 2438, corresponding to the amino acid residues 124–138 of the human IFN α-2 polypeptide chain, inhibited proliferation of human blood leukocytes [6] but did not affect the activity of NK-cells (our unpublished data) and, in contrast to native IFN, this peptide had no antiviral activity [7].

Abbreviations: ConA, concanavalin A; IFN, interferon; PBMC, peripheral blood mononuclear cells; peptide 2438, a synthetic peptide representing the IFN α-2 amino acid residues 124–138.

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In this study, we report on binding properties of the ^{125}I-labelled synthetic peptide 2438.

2. MATERIALS AND METHODS

2.1. *Synthetic peptides*

The peptides corresponding to the amino acid residues 124–138 (2438), 124–144 (2444), 129–138 (2938) and 129–144 (2944) of the human IFN α-2 molecule were synthesized as described [7].

2.2. *Iodination of peptide 2438*

50 μg of peptide 2438 was labeled by solid phase oxidation using Iodogen (Sigma) and Na[^{125}I] (0.5 mCi; Amersham Corp.) as described [8], and gel-filtrated on a column with Sephadex G-10 (Pharmacia). The specific activity of the labelled peptide was 2×10^6 cpm/μg.

2.3. *Separation of lymphocytes*

PBMC were obtained from blood of healthy donors according to Boyum [9]. Adherent cells were removed by incubation on plastic Petri dishes for 1 h at 37°C in an atmosphere of 5% CO_2. A suspension of lymphocytes was divided into adherent and non-adherent subsets on a nylon wool column [10]. A non-adherent fraction was eluted with RPMI 1640 medium supplemented with 5% heat-inactivated fetal calf serum. This fraction contained T-lymphocytes and null cells with small amounts of monocytes and B-lymphocytes.

2.4. *Culture conditions*

PBMC (2×10^6 cells/ml) were cultured in RPMI 1640 medium (Sigma) supplemented with 5% heat-inactivated fetal calf serum (Sigma), 100 mM L-glutamine, 50 μg/ml gentamicin. When necessary, concanavalin A (ConA) (2 μg/ml) or IFN α-2 (1000 IU/ml) were added to the culture medium. After culturing with ConA or IL2 for 20 h in a humid atmosphere of 5% CO_2 at 37°C the cells were harvested, washed three times with fresh medium and used in binding assays.

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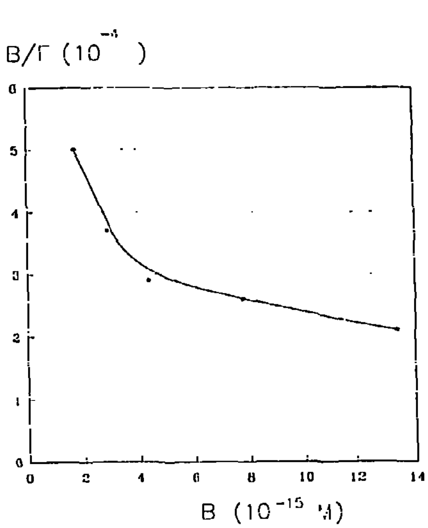

Fig. 1. Scatchard plot for the binding of the ^{125}I-labelled peptide 2438 to normal PBMC.

2.5. *Binding assay*

PBMC or separated T-lymphocytes (2×10^6 cells/tube) were incubated with the labelled peptide 2438 at concentrations of 10^{-12}–10^{-9} M for 40 min at 4°C in 100 μl of 199 medium (Serva) supplemented with 2% bovine serum albumin (Sigma). After the incubation, the cells were washed by centrifugation through 10% sucrose gradient as described [11], and the cell-bound radioactivity was measured in a Rack-Beta γ-counter (LKB, Sweden). In competitive binding assays PBMC were incubated with 10^{-11} M of the labelled peptide 2438 in the presence of 100-fold molar excess of unlabelled IFN α-2, synthetic peptides or cholera toxin (Calbiochem).

3. RESULTS AND DISCUSSION

We analysed the binding of the labelled synthetic peptide 2438 to normal PBMC and T-lymphocytes. Fig. 1 presents a Scatchard plot which is obviously curvilinear. As judged from the Scatchard analysis of the binding to normal PBMC, there are two distinct binding sites characterized by different dissociation constant values (K_{d1} = 1.2×10^{-11} M, and K_{d2}= 1.2×10^{-10} M, for 'high' and 'low' affinity sites, respectively).

The number of high- and low-affinity binding sites for PBMC is 2.01×10^3 and 1.12×10^4 per cell, respectively.

As expected, the unlabelled peptide 2438 effectively competes with the labelled one for binding to PBMC. About 20–35% of the peptide was estimated to bind nonspecifically (Fig. 2). Qualitatively and quantitatively similar results were obtained with separated T-lymphocytes (data not shown).

The results shown in Figs. 1 and 2 suggest that the peptide 2438 can specifically bind to human blood leukocytes.

According to Dooley et al. [12], Scatchard plots for the binding of IFN α-2 to normal PBMC are also curvilinear. Bearing in mind this analogy, we examined the ability of IFN α-2, cholera toxin and a number of IFN-derived peptides of the 124–144 region to compete with

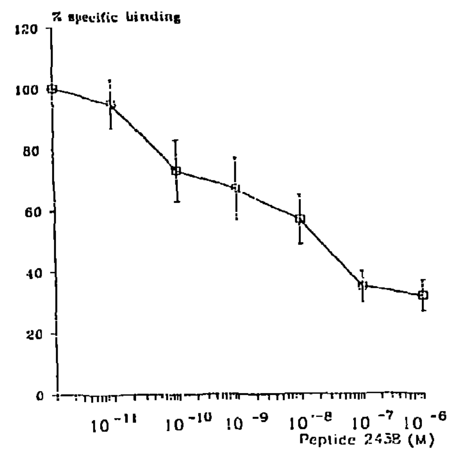

Fig. 2. Competition between the ^{125}I-labelled and unlabelled peptide 2438 for binding to PBMC. Concentration of the ^{125}I-labelled peptide is 10^{-11} M.

the peptide 2438 for PBMC binding sites. All of these molecules are known to contain amino acid tracks of high local homology [13]. The results of the competition experiments (Fig. 3) clearly demonstrate that neither the whole IFN α-2 molecule nor the aforementioned peptides nor cholera toxin interfered with the binding of the labelled peptide 2438 to PMBC.

The pretreatment of PBMC with ConA was found earlier to completely block biological effects of both IFN α and the peptide 2438 (our unpublished data). On the other hand, the expression of the IFN-receptor is inhibited by pre-incubation of cells with IFN [14,15]. Therefore we characterized the effects of ConA and IFN on the peptide 2438 binding. To this end, prior to binding measurements, PBMC were pre-incubated for 20 h with 2 μg/ml ConA or 1000 IU/ml recombinant IFN α-2. In both cases, the binding of the peptide 2438 was unaffected (data not shown).

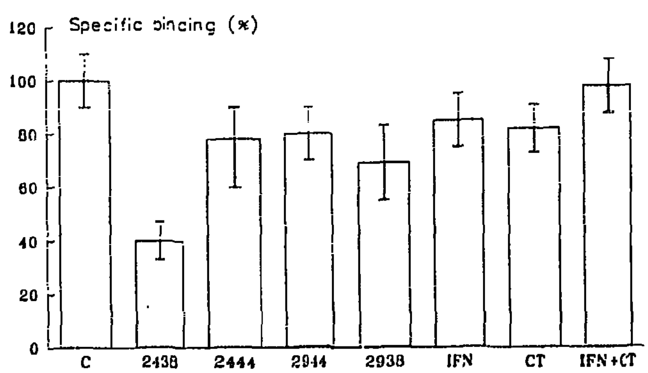

Fig. 3. Effects of different competitors, added at a 100-fold molar excess, on binding of the ^{125}I-labelled peptide 2438 to PBMC. Competitors: 2438, 2444 and 2944 are peptides corresponding to the 124–138, 124–144 and 129–144 amino acid sequences of the human IFN α-2 molecule; IFN, human interferon α-2; CT, cholera toxin; C, control, without competitors.

These findings suggest that (i) non-responsiveness of ConA-activated PBMC to the peptide 2438 seems not to be associated with the lack of appropriate binding sites on the cell membrane, and (ii) pretreatment of PBMC with IFN or ConA does not influence the binding of the peptide to the cells.

Although the present results may be interpreted as an indication that the peptide 2438 binds to membrane targets different from those for IFN α-2, this conclusion needs additional support. The question of identity of cell surface binding sites for IFN α-2 and the peptide 2438 remains open and awaits further investigation.

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