

Monoclonal antibodies directed to human insulin-like growth factor I (IGF I)

Use for radioimmunoassay and immunopurification of IGF

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Mouse hybridomas secreting antibodies to human insulin-like growth factor I (IGF I) were produced by fusion of spleen cells of hyperimmunised mice with FO mouse-myeloma cells. Eight clones producing antibodies against human IGF I have been isolated, two of which have been characterised. One was used in a radioimmunoassay, the other for immunopurification of IGF.

<i>Insulin-like growth factor</i>	<i>Somatomedin</i>	<i>Monoclonal antibody</i>	<i>Radioimmunoassay</i>
<i>Immunopurification</i>	<i>High-performance liquid chromatography</i>		

1. INTRODUCTION

The insulin-like growth factors I and II (IGF I and II) are polypeptides which have been isolated from human serum [1]. Their amino acid sequences are homologous to the one of proinsulin [2,3]. IGF I and II act in vitro as growth factors on fibroblasts, chondrocytes and osteocytes [4]. In vivo IGF I induces growth in hypophysectomised rats and widening of the epiphyseal cartilage [4]. The concentration of IGF I and to a lesser extent that of IGF II in human serum have been shown to be under control of growth hormone [5]. Pygmies are deficient of IGF I in serum, whereas their levels of IGF II are within the normal range [6]. IGF I thus qualifies as somatomedin, whereas the physiological role for IGF II is less clear. Here, we describe the production of monoclonal antibodies

against IGF I by the hybridoma technique [7]. An unlimited amount of such antibodies would allow the preparation of IGF from serum by immunoadsorption and the determination of serum levels of IGF I in patients with growth disorders on a wider scale.

2. MATERIALS AND METHODS

Human IGF I was prepared as in [1]. It was conjugated to tetanus toxoid (Schweizerisches Serum- und Impfinstitut, Bern) using 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide·HCl (Serva, Heidelberg) as a coupling agent [8]. The molar ratio of peptide to carrier was 7:1. Two subcutaneous injections of 20 µg coupled antigen dissolved in complete Freund's adjuvant were given to 5 adult BALB/c mice in 3 week intervals. Three out of these 5 mice were injected intraperitoneally with 3 daily doses of 20 µg conjugated IGF I in saline. The next day, the spleens were used for cell fusion with FO myeloma cells [9]. Colonies were detectable in 568 of 1500 microplate wells (Dynatech, Sussex). Cultures producing antibodies to IGF I were cloned twice by limited

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Abbreviation: RIA, radioimmunoassay

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dilution. For the production of larger amounts of antibodies, cells were injected intraperitoneally into BALB/c mice (5×10^7 cells/mouse). A few days later serum and ascites fluid were collected. A solid-phase antibody-binding assay (see below) utilising subclass specific rabbit anti-mouse antibodies (Nordic, Tilburg) was employed to determine the IgG subclasses.

The chloramine T method was used for iodination (^{125}I , Eidgenössisches Institut für Reaktorforschung, Würenlingen) of IGF I [5] and protein A ($2.5 \mu\text{Ci}/\mu\text{g}$, Pharmacia, Uppsala) [10]. Sera of immunised animals and culture supernatants were tested for antibodies at several dilutions in a solid-phase antibody-binding assay [11] using polyvinyl chloride microtiter plates (Dynatech M25) coated with IGF I ($2 \mu\text{g}/\text{ml}$). ^{125}I -labelled protein A (50000 cpm/well) was added after rabbit anti-mouse antiserum (1:60, Nordic RAM/IgG (H + L)) used as a link to anti-IGF I. Supernatants giving a positive reaction were further tested by a radioimmunoassay (RIA) using polyethyleneglycol 6000 (Fluka, Buchs) to separate free and bound ^{125}I -IGF [12]. Levels of IGF I in sera of patients and recoveries of IGF I and II after immunopurification have been determined by a RIA using conventional antiserum to IGF I and II, respectively [5].

The proteins of 0.4 ml ascites fluid were coupled to 2 ml of CNBr-activated Sepharose (Pharmacia). Serum (15 ml) was gel-filtered on Sephadex G-75 in 0.5 M acetic acid to dissociate IGF from its carrier protein [5], lyophilised, dissolved in $300 \mu\text{l}$ 5 mM HCl and applied to the column in 3.5 ml phosphate-buffered saline (10 mM phosphate buffer, pH 7.4; 0.15 M NaCl). The column was washed with 20 ml 0.5 M NaCl, 25 mM Tris·HCl (pH 7.5), 0.2% Triton X-100 and with 10 ml 5 mM phosphate buffer, (pH 7.4). Elution was performed with 0.5 M acetic acid. Protein determinations were done according to [13] using Coomassie blue. Biological activity was determined in the fat cell assay [14]. The affinity purified IGF was characterised by reversed phase high-performance liquid chromatography [15]. A 4.6×250 mm Chromosorb LC-7 column (Brownlee Labs., Santa Clara, CA 95050), flow rate 1 ml/min at room temperature, was used. Buffer A was 10 mM NaClO_4 in 19 mM H_3PO_4 , buffer B 60% (v/v) acetonitrile in A.

3. RESULTS AND DISCUSSION

Eight of 568 hybrids (1.4% yield) produced antibodies of the IgG₁ type that are directed against IGF I as determined in the solid-phase antibody-binding assay. All showed a stable in vitro production of antibodies over 3 months and were successfully grown intraperitoneally in mice.

Five of the 8 antibody preparations showed a significant binding of ^{125}I -IGF I as determined by the RIA. The one with the highest affinity for IGF I (41/81, $\sim 10^9 \text{ M}^{-1}$) was used for determination of IGF I levels in human sera. Cell supernatant or ascites fluid 41/81 were used at a final dilution of 1:200 or 1:20000, respectively, at which they bound 50% of ^{125}I -IGF I. Fig. 1 shows the displacement of ^{125}I -IGF I by unlabelled IGF I. Half-maximal displacement was achieved by 1.3–1.4 ng IGF/0.2 ml. The lowest concentration detectable was 0.2 ng IGF/0.2 ml. IGF II showed a crossreactivity of 3%, whereas human insulin did not displace ^{125}I -IGF I even at $50 \mu\text{g}/0.2 \text{ ml}$. A linear relationship was observed between the amount of serum and the detectable IGF I (fig. 2). Sera of patients with abnormal IGF I levels and two serum pools of subjects with normal levels were analysed by RIA using both the monoclonal antibody 41/81 and the conventional antiserum. The two methods gave similar results (table 1), showing that both types of antibody are directed against the same antigen. Within-assay and between-assay standard deviations were 6% ($n = 10$) and 11% ($n = 7$), respectively. Sera from several species were tested for crossreactivity against IGF I tracer. The approximate relative potencies of

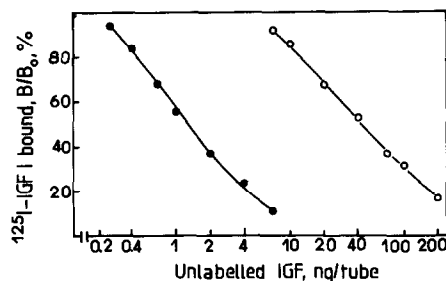


Fig. 1. Competitive inhibition of binding of ^{125}I -IGF I to cell supernatant 41/81 by unlabelled IGF I (●) and IGF II (○). All points are the mean of duplicates.

Table 1

Determination of serum levels of IGF I with conventional antiserum and cell supernatant 41/81

Serum source	Pool	RIA with conventional antiserum (ng/ml)	RIA with cell supernatant 41/81 (ng/ml)
Normals	(1)	160	175
	(2)	145	145
Acromegalics	(3)	580	560
	(4)	700	850
	(5)	785	930
	(6)	1060	1060
Patients with IGF deficiency	(7)	<40	<40
	(8)	<40	<40
	(9)	<40	<40

Results are the mean of duplicates at two different dilutions. Two pools of serum from subjects with normal IGF I levels (1,2), sera from 4 acromegalic patients (3-6), from two patients with extrapancreatic tumor hypoglycemia (7,8) and from one suffering from Leprechaunism (9) were tested

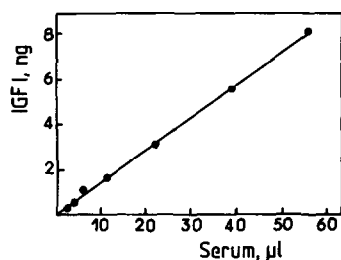


Fig. 2. Correlation of amounts of stripped serum with estimation of IGF-I by RIA using antibody 41/81.

guinea pig, rabbit, human, rat and mouse sera were 7:4:1: <0.01: <0.01.

The use of a monoclonal antibody in a RIA for somatomedin-C/IGF I was reported in [16]. This antibody seems to have a higher affinity, but a lower specificity as judged by crossreaction with IGF II than the antibody 41/81. The limited availability of pure IGF I has so far prevented the large-scale production of conventional antiserum needed for radioimmunoassays on a wider scale. With the hybrid 41/81 we have now a theoretically unlimited source of antibodies to study IGF-I levels in sera of patients with growth disorders. The low crossreactivity of antibody 41/81 with IGF-II should prove of special value.

A monoclonal antibody with a lower affinity for IGF I (43/81, $\sim 10^8 \text{ M}^{-1}$) was used for immunopurification of IGF. Coupled to Sepharose, the 20 mg proteins of 400 µl ascites fluid bound 96% of the IGF I and 97% of the IGF II in the crude IGF-preparation derived from 15 ml serum (table 2). Of the total proteins 99% were detected in the neutral flow trough. Bound IGF I and II could be eluted with 0.5 M acetic acid with a recovery of 64% and 80%, respectively. A 200-fold purification was obtained. However, the affinity column does not discriminate between IGF I and II, using either antibody 43/81 or 41/81 (not shown). Analysis by HPLC of affinity purified IGF is illustrated in fig. 3. The elution points for IGF I and II were 65% buffer B, corresponding to the positions of conventionally purified IGF I and II. At 67% buffer B, a third component active in the RIA for IGF II was detected which has not been further characterised. The large peak at 90% buffer B is due to an impurity of non-protein origin as judged by Coomassie-blue protein determination and by the absorption spectra in the

Table 2

Immunopurification of IGF using antibody 43/81

Fraction	IGF I	IGF II	Protein	Purity of IGF	Biological activity
Applied to column	1.57 µg	8.1 µg	14.3 mg	0.068%	5.2 mU
Neutral flowthrough	0.07 µg	0.26 µg	14.2 mg	—	<0.22 mU
Acidic eluate	0.96 µg	6.3 µg	56 µg	13%	4.0 mU

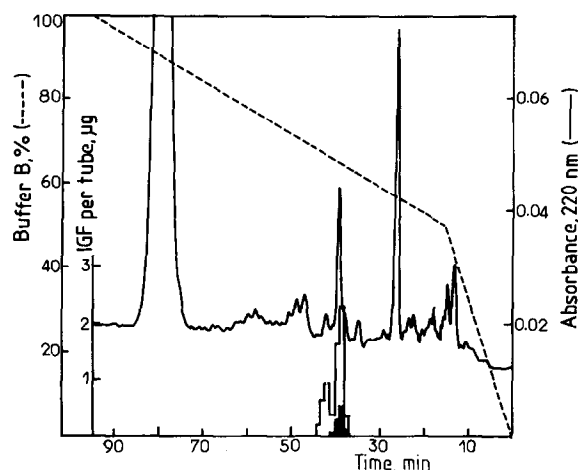


Fig. 3. High-performance liquid chromatography of IGF (1.4 μ g IGF I and 6 μ g IGF II) purified by immunoadsorption. IGF I (■) and II (□) concentration was measured by RIA.

ultraviolet range. Although it was not possible to separate IGF I from IGF II by immunopurification and high-performance liquid chromatography, the purity of IGF (IGF I and II combined) obtained by this procedure was found to be 0.75 ng RIA equiv./1.0 ng protein as determined by absorption at 220 nm. This three-step purification procedure for IGF with 75% purity of the end product and an overall yield of 60% represents an attractive alternative to the method in [1].

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