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.

Insulin-like growth factor *Somatomedin* *Monoclonal antibody* *Radioimmunoassay* *Immunopurification* *High-performance liquid chromatography*

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.

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Abbreviation: RIA, radioimmunoassay

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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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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 μCi/μg, 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 M 25) coated with IGF I (2 μg/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 μl 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 IgG_1 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, ~10^9 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 μg/0.2 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

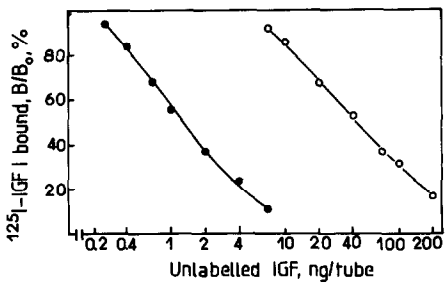

Fig. 1. Competitive inhibition of binding of ^{125}I-IGF I to cell supernatant 41/81 by unlabelled IGF I (●) and IGF II (○). All points are the mean of duplicates.

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

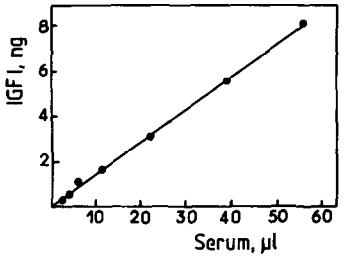

Fig. 2. Correlation of amounts of stripped serum with estimation of IGF-I by RIA using antibody 41/81.

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, $\sim 10^8$ 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

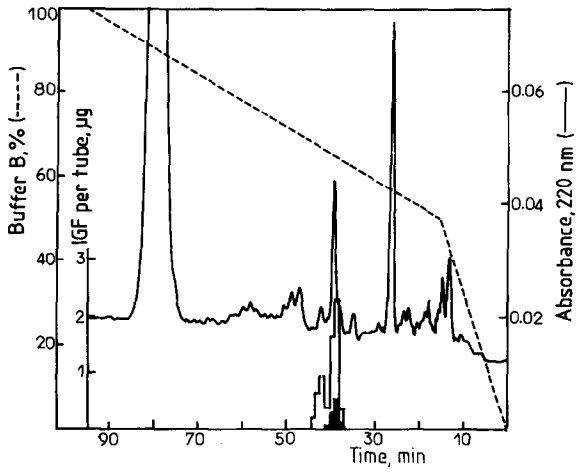

Fig. 3. High-performance liquid chromatography of IGF (1.4 µg IGF I and 6 µg IGF II) purified by immunoadsorption. IGF I (■) and II (□) concentration was measured by RIA.

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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REFERENCES

[1] Rinderknecht, E. and Humbel, R.E. (1976) Proc. Natl. Acad. Sci. USA 73 2365–2369.
[2] Rinderknecht, E. and Humbel, R.E. (1978) J. Biol. Chem. 253, 2769–2776.
[3] Rinderknecht, E. and Humbel, R.E. (1978) FEBS Lett. 89, 283–286.
[4] Schoenle, E., Zapf, J., Humbel, R.E. and Froesch, E.R. (1982) Nature 296, 252–253.
[5] Zapf, J., Walter, H. and Froesch, E.R. (1981) J. Clin. Invest. 68, 1321–1330.
[6] Merimee, T.J., Zapf, J. and Froesch, E.R. (1981) New Engl. J. Med. 305, 965–968.
[7] Köhler, G. and Milstein, C. (1975) Nature 256, 495–497.
[8] Skowsky, W.R. and Fisher, D.A. (1972) J. Lab. Clin. Med. 80, 134–144.
[9] Fazekas de St Groth, S. and Scheidegger, D. (1980) J. Immunol. Methods 35, 1–21.
[10] Hunter, W.M. and Greenwood, F.C. (1962) Nature 194, 495–496.
[11] Galfrè, G. and Milstein, C. (1981) Methods Enzymol. 73, 3–46.
[12] Desbuquois, B. and Aurbach, G.D. (1971) J. Clin. Endocrinol. Metab. 33, 732–738.
[13] Bradford, M. (1976) Anal. Biochem. 72, 248–254.
[14] Froesch, E.R., Bürgi, H., Ramseier, E.B., Bally, P. and Labhart, A. (1963) J. Clin. Invest. 42, 1816–1834.
[15] Wilson, K.J. and Hughes, G.J. (1981) Chimia 35, 327–333.
[16] Baxter, R.C., Axiate, S. and Raison, R.L. (1982) J. Clin. Endocrinol. Metab. 54, 474–476.