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.

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 Impfinsitut, 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
dilution. For the production of larger amounts of antibodies, cells were injected intraperitoneally into BALB/c mice (5 × 10⁷ 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 (¹²⁵I, 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). ¹²⁵I-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 ¹²⁵I-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 NaClO4 in 19 mM H3PO4, 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 ¹²⁵I-IGF I as determined by the RIA. The one with the highest affinity for IGF I (41/81, ~10⁹ 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 ¹²⁵I-IGF I. Fig. 1 shows the displacement of ¹²⁵I-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 ¹²⁵I-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 ¹²⁵I-IGF I to cell supernatant 41/81 by unlabelled IGF I (●) and IGF II (○). All points are the mean of duplicates.](image-url)
Table 1

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

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

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.
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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