A β -cell glycoprotein of M_r 40 000 is the major rat islet cell immunogen following xenogenic immunisation

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Summary. An antiserum (R2) was raised in a rabbit against dispersed Sprague Dawley rat islet cells. The R2 antiserum contains islet cell surface antibodies, which mediate complement-dependent cytotoxicity against islet cells resulting in a block of glucose induced insulin release. Immunoprecipitation and gel electrophoretic analysis showed that R2 specifically recognizes an M_r 40 000 glycoprotein present in both rat islet and rat insulinoma cells. This glycoprotein is amphiphilic

in character and probably represents a pancreatic β cell specific plasma membrane component. The results support previous observations in mouse β cells that a plasma membrane glycoprotein of M_r 40K constitutes a major islet cell immunogen.

Key words: Glycoprotein M_r 40 000, islet cell immunogen, xenogenic immunisation, antiserum R2, rat.

Studies of islet cell surface antibodies (ICSA), both in antisera raised experimentally and in sera from insulin-dependent diabetic patients, indicate that the β cell may express cell specific surface determinants [1–3]. We have previously demonstrated that a mouse β cell plasma membrane protein of M_r 40 000 (M_r 40K) was the major antigen recognized by a rabbit antiserum (R4) raised against ob/ob mouse islet cells [1]. Another antiserum (R2) was raised in rabbits against dispersed Sprague Dawley rat islets [4]. In the presence of complement ICSA in this serum mediate cytotoxicity against rat islet cells in vitro and block glucose induced insulin release [5, 6]. In this study we describe the characterization of the antigen(s) in rat islets and insulinoma cells recognised by the R2 antiserum.

Materials and methods

Antiserum

A rat islet cell antiserum was raised by immunizing a rabbit with living dispersed rat islet cells as described previously [4]. Insulin antibodies have not been detected in this antiserum [4].

Isolation of islets, insulinoma cells and lymphocytes

Pancreatic islets were isolated from 100–150 g male Wistar rats (Møllegaards Breeding Laboratories, L.I. Skensved, Denmark) by collagenase digestion [7].

Rat insulinoma cell tumours were propagated in the New England Deaconess Hospital (NEDH) strain of rats using a tumour arising from an original X-ray induced insulinoma [8] (RIN-tumour). The insulin producing RIN-5AH cell line developed from the X-ray induced insulinoma [9] (kindly donated by Dr. H. Oie, National Cancer Institute, Washington, DC, USA) was also propagated as tumours in NEDH rats (RIN-5AH tumour).

Tumours, 0.5–5 g wet weight, were removed and the cells dispersed in Hanks' balanced salt solution containing 1% bovine serum albumin. Insulinoma cells were separated from erythrocytes by centrifugation (450 g, 5 min) on Ficoll Paque (Pharmacia, Uppsala, Sweden) and incubated in RPMI 1640 with 10% fetal calf serum for 3 h before radioactive labelling.

Labelling of cells

Islets, insulinoma cells and lymphocytes were biosynthetically labelled with ^{35}S methionine at 250 $\mu\text{Ci/ml}$ (islets) or 100 $\mu\text{Ci/ml}$ (insulinoma cells and lymphocytes) as described [10]. Islets (250/ml) were incubated at 37°C for 16 h, insulinoma cells and lymphocytes ($1 \times 10^6/\text{ml}$) for 6 h.

Lysis of cells and immunoprecipitation

Labelled islets ($10^3/\text{ml}$), insulinoma cells and lymphocytes ($10^7/\text{ml}$) were lysed in 20 mmol/l Tris HCl (pH 7.4) containing 150 mmol/l NaCl, 5 mmol/l methionine, 2 mmol/l phenylmethylsulphonylfluoride (PMSF) and either 1000 KIE trasyolol/ml and 1% NP-40 (NP40 buffer) or 15000 KIE trasyolol/ml and 1% Triton X-114 (Triton X-114 buffer). In some experiments cells were lysed by boiling in 0.1–0.2 ml 20 mmol/l Tris HCl (pH 7.4) containing 2% SDS followed by immediate dilution in 10 volumes of NP-40 buffer. Insoluble material was removed by centrifugation at 100,000 g for 30 min and the supernatants used for immunoprecipitation either after adsorption to normal rabbit

Fig. 1A–C. SDS-PAGE and autoradiography of immunoprecipitates with a rabbit anti-rat islet cell serum (R2) or normal rabbit serum (NRS). **A** Rat islet cell proteins solubilized in 2% SDS followed by 10-fold dilution in NP-40 buffer (see Materials and Methods). **B** Rat RIN-tumour cell proteins solubilized in NP-40 buffer. Immunoprecipitations were performed on total cell lysate and on the glycoprotein fraction eluted from a lentil lectin affinity column. **C** Rat RIN-5AH tumour cell proteins solubilized in Triton X-114 and separated in amphiphilic (detergent phase) and hydrophilic (aqueous phase) proteins before immunoprecipitation. SDS-page was carried out on either 10% uniform slab gels (A, B) or 7.5%–15% gradient gels (C). M_r of molecular weight markers expressed in Kdaltons is indicated to the right

serum [10], after purification of glycoproteins on a lentil lectin-Sepharose 4B affinity column (NP-40 lysate) [11] or after purification of amphiphilic membrane proteins by phase separation in Triton X-114 (Triton X-114 lysate) [12]. Aliquots (100 μ l) were incubated with 5 μ l of R2 or normal rabbit serum for 16 h and the immune complexes were adsorbed to 100 μ l of pre-swollen protein A-Sepharose CL-4B (Pharmacia). The protein A-Sepharose was washed four times in 0.5% NP-40 or Triton X-114 buffer and once with ice-cold water.

The immunocomplexes were analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) on 7.5%–15% gradient or 10% uniform slab gels using the discontinuous buffer system of Laemmli [14]. The gels were stained and processed for autoradiography as described [10] except that Enlightning (New England Nuclear) was used as an autoradiographic enhancer.

Results

Analyses of proteins recognised by the R2 antiserum in whole lysates of rat islets, insulinoma cells and lymphocytes

The R2 antiserum specifically immunoprecipitated a major protein of M_r 40K from 35 S methionine labelled rat islets, RIN-tumour and RIN-5AH tumour cells (Fig. 1). Several additional components were precipitated from whole NP-40 lysates of rat islet cells (not shown) while few weak additional bands were observed in immunoprecipitates of RIN-tumour cells lysed in NP-40 (Fig. 1B, before lentil lectin chromatography). To

reduce the background of non-specific or low affinity binding rat islet cell proteins, rat islets were first lysed in 2% SDS and then diluted 10 times in NP-40 buffer. The major protein immunoprecipitated specifically from such lysates was an M_r 40K component (Fig. 1A). Additional components of M_r 75K, 32K, and 14K were also observed (Fig. 1A). No proteins were specifically immunoprecipitated from lysates of rat spleen lymphocytes analysed in parallel (not shown). Accordingly extensive adsorption of the R2 antiserum to rat spleen cells did not affect the ability of this antiserum to detect the M_r 40K component in islet cells (data not shown).

Analyses of glycoproteins recognized by the R2 antiserum

NP-40 lysates of RIN-tumour cells were subjected to Lens culinaris lectin affinity column chromatography. A glycoprotein fraction, eluted by 3% α -methyl mannoside was used for immunoprecipitation. The R2 antiserum immunoprecipitated glycoprotein components of M_r 14K, 40K, 50K and a smear of bands at M_r >90K (Fig. 1B). The 50K and 14K proteins were tentatively identified as major histocompatibility (MHC) class I antigen heavy chain and β_2 microglobulin, respectively, since components of similar M_r were immunoprecipitated by a rabbit antiserum raised against purified mouse MHC class I antigens (kindly donated by Dr.

P. Peterson, Department of Cell Research, The Wallenberg Laboratory, Uppsala, Sweden). This antiserum cross-reacts with rat MHC class I components [10].

Analyses of amphiphilic proteins recognized by the R2-antiserum

The phase separation technique of Bordier [12] was used to separate amphiphilic and hydrophilic islet cell proteins in Triton X-114 lysates. Integral membrane proteins with an amphiphilic nature are concentrated at 30°C in a confluent detergent phase which is isolated by centrifugation allowing hydrophilic proteins to remain in the aqueous phase. Certain bulky transmembrane proteins have been found to remain in the aqueous phase together with the hydrophilic proteins, while hydrophilic proteins are not found in the detergent phase [13].

The R2 antiserum specifically immunoprecipitated a M_r 40K protein from the detergent phase of RIN-5AH tumour cells (Fig. 1C) and rat islet cells (not shown). Additional components at M_r 14K and 50K (probably MHC class I components) and a smear of bands at M_r > 80K were also specifically precipitated. In the aqueous phase no proteins except a very faint band at M_r 40K were specifically immunoprecipitated by the R2 antiserum.

Discussion

The major protein detected in immunoprecipitates of rat islet cell lysates with the R2 antiserum, but not with normal rabbit serum had a M_r of 40K as determined by SDS-PAGE and autoradiography. The detection of the M_r 40K amphiphilic glycoprotein as a major rat β cell antigen recognized by an antiserum raised in a rabbit is in agreement with the earlier demonstration of a β cell specific plasma membrane protein of M_r 40K being a major antigen in a xenogenic immunisation with β cell rich mouse ob/ob islet cells [1]. The detection of this protein in rat islet tumour cells whether passaged from the original tumour [8] or a cloned insulin-producing cell line [9] indicates that it is expressed in pancreatic β cells. The present analysis does not exclude that other endocrine islet cells also express this component; however, the lack of expression of this protein by rat spleen cells was evidenced by both immunoprecipitation and adsorption experiments. The glycoprotein character of the M_r 40K component was indicated by its binding to Lens culinaris lectin affinity column and its amphiphilic nature, characteristic for integral membrane proteins was reflected by its ability to be concentrated in the Triton X-114 detergent phase at temperatures above the clouding point of this detergent.

The R2 serum is a xenogenic antiserum raised against dispersed rat islet cells. It is therefore expected to recognize not only antigenic determinants present in

the different types of endocrine pancreatic cells, but also determinants common to many rat cells. This is illustrated by the ability of the R2 antiserum to detect rat insulinoma components (M_r 50K and 14K) tentatively identified as MHC class I antigen heavy and light (β_2 microglobulin) chain, previously shown to be present in islet cells with specific antisera [10]. In rat islets the 14K but not the 50K component was specifically detected by the R2 antiserum. However, the heavy chain component may be covered by proteins at a similar M_r , immunoprecipitated by both R2 and normal rabbit serum. Some of the components immunoprecipitated by the R2 antiserum from whole rat islet cell lysates, and not detected in immunoprecipitates of insulinoma cells, could either originate from islet cells other than the β cells or be expressed solely in normal islet cells. In addition to the M_r 40K protein, it was furthermore observed that the R2 antiserum detected a smear of bands at high M_r in the glycoprotein fraction (> 90K) and amphiphilic fraction (> 80K) of rat islet tumour cells.

Although some degree of homology could be expected between the mouse and rat β cell protein of M_r 40K, the cross reactivity of the mouse protein with the R2 antiserum and the rat protein with the R4 antiserum [1] is weak, judged from immunoprecipitation experiments (unpublished results). The interspecies variations in antigenic epitope structure of this protein may therefore be considerable. Neither the R2 nor the R4 antiserum was found to immunoprecipitate an M_r 40K component in human islet cells (unpublished data). ICSA-positive sera from diabetic BB-rats immunoprecipitate an M_r 64K islet cell protein from detergent lysates of rat islets and insulinoma cells [15]. The M_r 40K component has not been detected in such immunoprecipitates. Thus although the M_r 40K component is a major immunogen evoking a xenogenic immune response it does not seem to play a role in an autoimmune response against islet cells.

Acknowledgements. The authors thank Dr. Ole Bjerrum for valuable discussions and advice regarding the phase separation technique. The expert technical assistance of H. Richter Olesen and A. Borch is gratefully acknowledged. Steinunn Bækkeskov is a recipient of a Juvenile Diabetes Foundation Research Fellowship. This study was supported by the National Institute of Health (grant AM 26190).

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