NOTE

Production of Anti-Rat Islet Cell Surface Antibody in Guinea Pigs

SEIHO NAGAFUCHI^{*1}, TOSHIMITSU OKEDA¹, MINORU NAKAMURA¹,
KEISUKE YAMAGUCHI¹, JUNKO ONO² AND RYOSABURO TAKAKI²

¹The First Department of Internal Medicine, Faculty of Medicine, Kyushu University, Fukuoka, 812, ²Department of Medicine, Medical College of Oita, Oita, 879-56

Abstract

Anti-rat islet serum was prepared in guinea pigs by multiple subcutaneous inoculations of rat islets homogenates emulsified in complete Freund's adjuvant (CFA). The anti-rat islet serum was cytotoxic against rat spleen cells in the presence of complement and the nonspecific antibodies were absorbed with homogenates of rat livers and spleens. After absorption, the serum lost the cytotoxicity against the rat spleen cells yet showed specific cytotoxicity against the rat islet cells. The binding capacity of anti-rat islet antibody was determined by the indirect immunofluorescence test using FITC conjugated rabbit anti-guinea pig IgG serum.

As the guinea pig anti-rat islet serum contained anti-insulin antibody, the role of this antibody in this cytotoxic activity and surface immunofluorescence was studied. However, the anti-insulin antibody used as the control showed neither cytotoxicity nor surface immunofluorescence. After neutralizing the anti-insulin antibody in the antiserum with insulin, the serum remained cytotoxic to the rat islet cells and a surface immunofluorescence appeared.

These data show that specific anti-rat islet cell surface antibody can be produced in guinea pigs by multiple inoculations of rat islets homogenates with CFA.

There are reports on the anti-islet cell antibody in insulin-dependent diabetes mellitus with coexistence of autoimmune disease (Bottazzo *et al.*, 1974; MacCuish *et al.*, 1974) and on the anti-islet cell surface antibody in newly diagnosed patients with insulin-dependent diabetes mellitus (Lernmark *et al.*, 1978; Dobersen *et al.*, 1980; Eisenbarth *et al.*, 1981a). The anti-islet cell surface antibody was found to be organ specific and reacted with heterologous islet cells as assessed by the immunofluorescence test (Dobersen *et al.*, 1978; Lernmark *et al.*, 1978) and also showed a cytotoxicity against islet cells (Lernmark *et al.*, 1979; Do-

bersen *et al.*, 1980; Rittenhouse *et al.*, 1980) or against cloned insulinoma cells (Eisenbarth *et al.*, 1981b) in the presence of complement. These observations suggested the possible relationship between the production of anti-islet cell surface antibody and the pathogenesis of insulin-dependent diabetes mellitus.

Until recently, experimental production of antibodies to islet cells has been hindered by difficulties in obtaining a large number of islets. Nevertheless, it is now feasible to obtain successful collecting of large numbers of intact islets (Lernmark, 1974; Okeda *et al.*, 1979). Consequently, organ specific anti-rat islet cell surface antibody was generated in rabbits by immunizing dispersed rat islet cells with complete Freund's

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* to whom correspondence should be sent.

adjuvant (CFA) (Lernmark *et al.*, 1980).

In the present study, we attempted to produce anti-rat islet cell surface antibody in guinea pigs by injecting rat islets homogenates with CFA.

Materials and Methods

Animals

Islets were collected from six to 10 week old adult Wistar King A (WK-A) rats weighing 150 to 200 g. Adult guinea pigs weighing 250 to 300 g were used for immunization.

Collection of pancreatic islets

Pancreatic islets of WK-A rats or dispersed islet cells were obtained as described previously (Ono *et al.*, 1977, Okeda *et al.*, 1979). Pancreatic tissues were minced with scissors and washed with Hanks' balanced salt solution (HBSS). The minced pancreas was treated with 200 units/ml collagenase at 37°C for 15 min. Following centrifugation, the tissue pellet was resuspended in Ficoll-Conray discontinuous step gradient. After centrifugation of islets at 1900 rpm for 10 min, the majority were seen to be situated between 1072 and 1048 of specific gravity. To obtain free islet cells, isolated islets were treated with 5 ml of 0.02% EDTA for 5 min. at room temperature and collected by centrifugation. The islets were further treated with 4 ml of 1000 PU/ml Dispase with continuous stirring at 37°C for 15 min. Dispersed cells were collected by centrifugation and washed with HBSS twice.

Immunization

Approximately 1000 islets were homogenized and mixed with 1 ml of CFA. A half ml of the mixture of islets and CFA was given subcutaneously to each guinea pig. The immunization was repeated 4 times every one week. Seven days after the last immunization, the animals were bled, the sera separated, heat inactivated at 56°C for 30 min and then stocked at -20°C until use.

Absorption of antiserum with livers and spleens

One milliliter of the anti-rat islet serum was treated with the same volume of rat liver homogenates at 37°C for 60 min and the mixture was centrifuged at 3000 rpm for 30 min. The supernatant was repeatedly treated with liver homogenates. Following centrifugation, the supernatant fluid was further mixed with rat spleen homogenates in the same manner. The final supernatant was stocked at -20°C as absorbed antiserum until use.

Cytotoxicity test

Dispersed rat islet cells and spleen cells were

suspended in 0.2 ml of diluted unabsorbed antiserum or absorbed antiserum and incubated at 37°C for 60 min. Following centrifugation, the pellet was resuspended in 10 times diluted guinea pig serum and kept at 37°C for 60 min. After being spun down, the pellet was suspended in 0.5% trypan blue dye and assessed for the viability as dye exclusion test under light microscope. The viability was expressed as the mean of the contents of two test tubes.

$$\% \text{ cytotoxicity} = \frac{\frac{\% \text{ dead cells by test serum} - \% \text{ dead cells by normal serum}}{100 - \% \text{ dead cells by normal serum}} \times 100}{\text{normal serum}}$$

Immunofluorescence test

Rat islet cells were treated with 1:8 diluted antiserum at 37°C for 60 min. After washing three times with the antibody coated rat islet cells were further treated with 1:20 diluted Fluorescein isothiocyanate (FITC) conjugated anti-guinea pig IgG serum (Fujizoki Pharm. Co. Ltd.) at 37°C for 60 min. After the cells were washed three times with phosphate buffered saline (PBS), surface immunofluorescence was observed under a fluorescent microscope.

Preparation and assay of anti-insulin antibody

Anti-insulin antibody was prepared in guinea pigs by giving multiple subcutaneous inoculations of swine insulin with CFA. The anti-insulin serum showed the anti-insulin antibody titer of 1:1,000,000, as assessed by ¹²⁵I-insulin binding assay. The titer of antibody was expressed as the serum dilution which caused a significant binding to ¹²⁵I-insulin. To neutralize the anti-insulin antibody, 0.6 ml of swine insulin (40 U/ml) was added to 0.2 ml of antiserum. The mixture of antiserum and insulin did not show binding with ¹²⁵I-insulin.

Results

Cytotoxic activity of anti-rat islet serum against rat spleen cells.

Anti-rat islet serum was reacted with rat spleen cells in the presence of guinea pig complement. The serum showed cytotoxicity at the serum dilution of 1:4, indicating that the serum contained non-organ specific cytotoxic antibody. After absorption of the serum with homogenates of rat livers and spleens, the serum lost the cytotoxic activity against rat spleen cells (Fig. 1). The absorbed serum was used for the following experiments.

Fig. 1. Cytotoxic effect of guinea pig anti-rat islet serum on rat spleen cells.

Fig. 2. Cytotoxic effect of guinea pig anti-rat islet serum on rat islet cells after absorption with rat livers and spleens.

Fig. 3. Cell surface immunofluorescence on dispersed rat islet cells exposed to 1:8 diluted guinea pig anti-rat islet serum and FITC conjugated rabbit anti-guinea pig IgG serum, $\times 400$.

Cytotoxic activity of anti-rat islet serum against rat islet cells.

The anti-rat islet serum was cytotoxic against rat islet cells at the serum dilution of 1:32 by 50% (Fig. 2). Therefore, the anti-rat islet serum contained the anti-rat islet cell specific cytotoxic antibody.

Surface immunofluorescence test.

To determine whether or not anti-islet cell antibody binds the cell surface of islet cells, the islet cells were treated with antiserum at 37°C for 60 min, as described above, and were further reacted with FITC conjugated anti-guinea pig IgG serum of

rabbit at 37°C for 60 min. After being spun down, the cells were washed three times with PBS, and the surface immunofluorescence was observed under a fluorescent microscope. The surface immunofluorescence was in the form of a ring and a patchy pattern on the islet cell surface (Fig 3). There was no immunofluorescence on the living cells exposed to normal rabbit serum and FITC conjugated anti-guinea pig IgG serum.

The role of anti-insulin antibody in cytotoxicity and surface immunofluorescence.

The anti-rat islet serum contained 1:1,000 of anti-insulin antibody. Therefore, experiments were conducted to determine the role of anti-insulin antibody in the cytotoxicity and immunofluorescence tests. As shown in table 1, the anti-insulin antibody prepared in guinea pigs showed no cytotoxicity to rat islet cells in the presence of complement, nor did they show surface immunofluorescence, indicating that the anti-insulin antibody did not bind to the surface of the islet cells. Furthermore, after neutralizing the anti-insulin antibody in the anti-rat islet serum with insulin, the cytotoxic activity and surface immunofluorescence were not abolished. Therefore, the possible participation of anti-insulin antibody in the cytotoxicity test and surface immunofluorescence test was ruled out in this experiment.

Table 1. Cytotoxicity and surface immunofluorescence by anti-rat islet serum and anti-insulin antibody.

	Cytotoxicity	Surface immunofluorescence
Anti-rat islet serum	83%	++
Anti-rat islet serum+insulin	73%	++
Anti-insulin antibody	7%	—

Discussion

Recent studies have suggested that anti-islet cell surface antibody, which is organ specific but non-species specific, was detected in the serum of insulin-dependent diabetes mellitus patients soon after the onset of the disease (Lernmark *et al.*, 1978, Dobersen *et al.*, 1980). Experimentally, anti-rat islet cell surface antibody was generated in rabbits by injecting the dispersed islet cells with complete Freund's adjuvant (Lernmark *et al.*, 1980) and anti-mouse islet homogenates (Leiter *et al.*, 1981) as well. The anti-islet cell surface antibodies showed cytotoxicity in the presence of complement.

In the present study, experiments were conducted in an attempt to produce anti-rat islet cell surface antibody in guinea pigs by subcutaneous immunization of rat islets homogenates with CFA. Anti-islet cell surface antibody was successfully produced, as assessed by cytotoxicity and surface immunofluorescence tests. Although non-specific antibody to spleen cells was produced, the antibody was successfully absorbed with homogenates of livers and spleens of rats. As the anti-insulin antibody was also generated in the serum, the role of the anti-insulin antibody was examined. However, the possible influence of anti-insulin antibody in the cytotoxic activity and surface immunofluorescence of the antiserum was negated by using control anti-insulin antibody and by neutralizing the anti-insulin antibody in the antiserum with insulin.

We successfully produced anti-rat islet cell surface antibody by injecting homogenates of rat islets plus CFA into guinea pigs and the data obtained are consistent with the results of Lernmark *et al.* who produced anti-rat islet cell surface antibody in rabbits (Lernmark *et al.*, 1980). Thus, heterologous organ specific antigens are

expressed on the surfaces of rat islet cells.

It is uncertain whether the organ specific antigens are related to the specific metabolism of the islet cells and whether the cytotoxic antibody to islet cells plays a role in the pathogenesis of insulin-dependent diabetes mellitus. Some reports have indicated that the antibody found in patients with juvenile diabetes mellitus was cytotoxic to B cells, resulting in the inhibition of B cell metabolism (Lernmark *et al.*, 1979, Kanatsuna *et al.*, 1981). Furthermore, islet cells may possibly be destroyed by the mechanism of antibody-dependent cell-mediated cytotoxicity (Lernmark *et al.*, 1981). Further studies are underway in an attempt to clarify these problems.

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