Distribution and Redistribution of Pancreatic Islet Cell Surface Antigen Reactive with Islet Cell Surface Antibody in the Rat

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Summary. Antigens on the rat pancreatic islet cell surface were redistributed into patch and cap formation when the cells were incubated in the presence of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine, in tissue culture medium 199 for 24 h, before addition of rat pancreatic islet cell surface antibody. In contrast, if the cells were cultured in tissue culture medium 199 supplemented with glucose (5.5 or 16.7 mmol/l) and 10% heat-inactivated fetal calf serum with-

out 3-isobutyl-1-methylxanthine, cap formation was not detectable. These results suggest that mobile antigen on the surface of pancreatic B cells can be induced to aggregate into patch and cap formations during conditions of increased cellular metabolism.

Key words: Rat, islet cell surface antigen, immunobeads, patch and cap formation.

The possibility that immunological reactions may be involved in the pathogenesis of insulin-dependent diabetes has been supported by the demonstration of islet cell cytoplasmic antibodies [1, 2] and islet cell surface antibodies (ICSA) [3] in the sera from insulin-dependent diabetic patients. Cytoplasmic islet cell antibodies and ICSA are most prevalent at the onset of the disease and decrease thereafter [4]. The role of these antibodies in the pathogenesis of diabetes and the nature of the antigens are still unknown.

Scanning electron microscopy has proved to be a useful technique for studying cell surface architecture and has gained wide application in cell biology. The surface architecture of the islet cell was found to change showing an increased number of villi or blebs after incubation in the presence of a high concentration of glucose [5].

The present investigation was designed to study the binding of antibodies to rat pancreatic islet cell surface antigen and the effect of the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine (IBMX) on the distribution of antigen using scanning electron microscopy and visualizing bound antibodies with immunobeads or indirect immunofluorescence.

Materials and Methods

Preparation of Dispersed Islet Cells

Adult rat pancreatic islets were prepared under sterile conditions by collagenase digestion of the pancreas [6]. Islets were separated from

the digests by discontinuous Ficoll gradient centrifugation, and washed by repeated low speed centrifugation in tissue culture medium 199 (TCM 199), before being selected individually by micropipette. The islets were placed in TCM 199 (10 ml) with IBMX (0.1 mmol/l), collagenase (30 mg), hyaluronidase (20 mg) and ethyleneglycol-bis-(β -aminoethyl ether) N, N'-tetra acetic acid (1.0 mmol/l) for 30 min at 37 °C in an atmosphere of 5% CO₂ and 95% air. The islet suspensions were then kept for 24 h in plastic petri dishes containing TCM 199, further supplemented with 10% heat-inactivated fetal calf serum, penicillin (400 U/ml), glucose (5.5 mmol/l) and IBMX (0.1 mmol/l) [7]. Finally, after resuspension in TCM 199 the islets were disrupted into free cells by mechanical shaking. The dispersed cells were washed by centrifugation in Krebs-Ringer-Henseleit-buffer. Viability of the cells as determined by Trypan blue exclusion was approximately 95%.

Preparation of Immunobeads

A 10% suspension of polystyrene latex particles (0.5 μ diameter, Dow Chemical, Midland, Michigan, USA) in 0.2 mol/l phosphate buffered saline (PBS, pH 7.4, 0.1 ml) was mixed with goat anti-rabbit-IgG (32 mg in 0.2 mol/l PBS, 20 ml) and incubated at room temperature for 6 h. The adsorption under these conditions was shown to be adequate for a monolayer formation of IgG on the polystyrene surface [8]. The beads adsorbed with anti-rabbit-IgG, designated as immunobeads, were separated by centrifugation (5000 rev/min; 20 min), washed three times in PBS (0.2 mol/l) and resuspended in 20 ml of the same buffer.

Incubation of Cell Suspensions

The dispersed islet cells were incubated at 37 °C for 24 h in TCM 199 containing either glucose (5.5 or 16.7 mmol/l) with or without IBMX (0.1 mmol/l) in an atmosphere of 5% CO₂ and 95% air. Aliquots (10⁴ cells) in Krebs-Ringer-buffer (100 μ l) were then incubated at 37 °C for 5 min either with or without 1.0% formaldehyde.

Fig. 1. Time course of ligand-dependent patch and cap formation. After prolonged incubation in TCM 199 supplemented with or without 3-isobutyl-1-methylxanthine (0.1 mmol/l), cells were incubated with islet cell surface antibodies at 37 °C for 30–180 min in an atmosphere of 5% CO₂ and 95% air, then ligand-dependent patch or cap formation of surface IgG was determined. ●—●, ○---○: cells were cultured in the medium with and without 3-isobutyl-1-methyl xanthine for periods up to 24 h respectively. > 200 cells were observed at each time point shown

Fluorescence Microscopy

The cells were washed with Krebs-Ringer buffer and incubated at 37 °C in an atmosphere of 5% CO₂ and 95% air for 30–180 min in TCM 199 (100 µl) containing glucose (16.7 mmol/l) and either anti-rat ICSA positive sera (dilution 1:100) or normal rabbit serum (1:100 dilution). The cells were then washed with Krebs-Ringer buffer and incubated for 60 min at 37 °C in fluoresceinated goat anti-rabbit-IgG (1:10 dilution), and finally washed with cold Krebs-Ringer buffer and mounted without fixation. Using an Olympus fluorescence microscope (Model BH-RFC), photographs were taken with Kodak Tri-X film (exposure time 30 s).

The rabbit anti-rat islet cell serum, extensively adsorbed with rat spleen cells, was kindly given by Dr. Å. Lernmark (Hagedorn Research Laboratory, Copenhagen, Denmark); its preparation and characterization for ICSA have been described elsewhere [9].

Scanning Electron Microscopy

Islet cells in suspension were incubated at 37 °C for 90 min in Krebs-Ringer buffer (100 µl) with anti-rat ICSA positive sera (dilution 1:100), or with normal rabbit serum (dilution 1:100). After washing by centrifugation in Krebs-Ringer buffer, the cells were resuspended in PBS containing anti-rabbit-IgG immunobeads (100 µl) at 37 °C for 60 min and again washed in cold Krebs-Ringer buffer. The cells were fixed at 4 °C for 24 h with 1.25% glutaraldehyde in physiological saline, washed by centrifugation in Krebs-Ringer buffer and adsorbed onto poly (L-lysine)-coated glass coverslips [10].

The cells on the coverslips were rapidly frozen at –70 °C and freeze-dried for 48 h. The coverslips were mounted on sample stages and coated with gold in a vacuum. The cells were examined in a Hitachi-Akashi MSM-101 scanning electron microscope.

Results

Islet cells, incubated with rabbit anti-rat ICSA positive serum at 37 °C for 30–180 min, were fixed and labelled

with fluoresceinated goat anti-rabbit IgG. Patch and cap formation was determined as a function of time after the TCM 199 was supplemented with the antibodies. The ligand-dependent patch and cap formation reached a plateau within 90 min (Fig. 1). Approximately 50% of the cells formed patches after incubation with 3-isobutyl-1-methylxanthine, and occasional caps were also observed during the 3-h incubation period. IBMX induced patch and cap formation at the same rate whether the cells were incubated with either low (5.5 mmol/l) or high (16.7 mmol/l) concentrations of glucose (data not shown). In contrast, when the cells were incubated for 24 h in a medium without IBMX, patch and cap formation was markedly reduced (Fig. 1). Cells which were incubated with normal rabbit serum instead of rabbit anti-rat ICSA positive serum at 37 °C for 90 min, showed neither patch nor cap formation. Fixation of cells in 1.0% formaldehyde inhibited redistribution of surface-bound fluorescent antibodies.

Figure 2 shows the differential mobilities of surface IgG observed on the islet cells incubated with IBMX and antibodies. During a 3-h incubation period, 30 cells were examined at 30, 90, 120 and 180 min. This procedure was repeated eight times. Almost all the cells showed a diffuse distribution of fluorescent IgG before starting the 3-h incubation period with ICSA. Patch and cap formation was observed during the incubation period with antibodies. When the cells were incubated in a medium without IBMX, patch and cap formation was markedly reduced.

The surface architecture of rat pancreatic islet cells undergoing patch and cap formation was studied in more detail by scanning electron microscopy (Fig. 3). During the 3-h incubation period with ICSA positive serum, surface microvilli on the islet cells were replaced by lamellae, and subsequently an almost completely smooth surface appeared. These cells were then labelled with immunobeads.

When the islet cells were incubated with normal rabbit serum instead of rabbit anti-rat islet cell surface serum, goat anti-rabbit IgG coated beads did not attach to the cells. The results shown in Figure 3 also suggest that goat anti-rabbit IgG immunobeads exert a stimulatory effect on patch or cap formation of related receptors.

Discussion

It is well known that lymphocyte surface immunoglobulin can be redistributed into patch and cap formation, a phenomenon which is thought to require the cross-linking of receptors by the antibody [11–13]. We have reported here that rat pancreatic islet cell surface antigens can also be redistributed into patch and cap formations by binding with specific rabbit anti-rat ICSA after exposure of the cells to a medium containing IBMX for at least 24 h. Maximum patch formation occurred within 90–180 min under these conditions. In the standard me-

Fig. 2. Differential mobilities of surface IgG on individual rat pancreatic islet cells. After prolonged incubation in TCM 199 supplemented with 3-isobutyl-1-methylxanthine, the cells were incubated with islet cell surface antibody for 120 min as described in Fig. 1 and then incubated with fluoresceinated goat anti-rabbit IgG. (a-d): Bright field illumination; (a'-d'): Fluorescence micrographs of the same fields of cells. (a') Cell showing diffuse distribution of IgG fluorescence as observed in the majority of cells incubated with ICSA. (b') Cell showing patch formation of IgG. (c') Cell showing transition from patch formation to capping. (d') Cell showing distinct cap formation. (magnification $\times 4000$)

Fig. 3. After prolonged incubation in TCM 199 supplemented with 3-isobutyl-1-methylxanthine (0.1 mmol/l), the cells were incubated with islet cell surface antibody for 120 min as described in Fig. 1 and then labelled with anti-rabbit IgG immunobeads. (a–d) Control cells of the same incubation period fixed with glutaraldehyde before addition of anti-rabbit IgG immunobeads. During the incubation period the cell surface gradually developed lamellae (a–c) but later developed a smooth appearance (d). (e–h) The distribution of immunobeads on an individual islet cell. Random distribution of immunobeads on an islet cell (e). Patchy distribution of immunobeads and lamellar-appearance of the cell surface (f, g). Clearly aggregated beads showing cap formation on the cell and marked flattening of the cell surface (h). Magnification: ($\times 9000$) a, b, d, f, h; ($\times 6300$) c, e, g

dium without IBMX, ligand-dependent patch and cap formation was minimal.

The data in Figure 1 demonstrate the stimulatory effect of IBMX on the appearance of patch and cap formation on B cells. Before starting the 3-h incubation period, almost all of the cells showed diffuse distribution of fluorescent IgG. Patch and cap formation occurred during the incubation with antibody. The possibility that lymphocytes were involved in the patch and cap formation was excluded as no formation was seen during the first 30 min of incubation. (Lymphocytes form patches and caps within 10–15 min of incubation with antibody.)

Patch and cap formation has been discussed in terms of interactions between surface receptors and cytoplasmic structure [11, 16, 17]. There is some evidence to suggest that cap formation involved a contractile system consisting of actin, myosin and regulating protein [18, 19]. In several studies, it was reported that cap formation took place even in the absence of a specific ligand [14, 15], but we did not observe this in our study. In our experiments, we demonstrate that antibodies bound to the rat islet cell surface can redistribute into patch and cap formations. The patch formation was predominant on islet cells that reacted with ICSA. In particular, in the medium with IBMX, it occurred almost simultaneously to the addition of the ICSA positive sera.

It has been reported that microvilli are developed to a greater extent on metabolically inactive cells when compared with active cells [22, 23], and also it was suggested that microvilli and lamellae may be composed mainly of microtubules and microfilaments [20, 21]. Yahara et al. reported that surface lamellae, not microvilli, are responsible for the translocation of receptors during cap formation [24]. These observations imply that the islet cell surface possesses antigens which are able to move and that this mobility is stimulated by IBMX.

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