Block in Insulin Release from Column-Perifused Pancreatic β-Cells Induced by Islet Cell Surface Antibodies and Complement

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SUMMARY

Dispersed rat pancreatic islet cells were mixed into a short column of Bio-Gel P-2 polyacrylamide beads and perifused with an antiserum containing islet cell surface antibodies. The release of radioactive chromium from prelabeled cells, as a measure of cell membrane permeability, was not affected by cell surface antibodies alone, but increased dramatically in the presence of complement. While there was an eightfold increase in glucose-stimulated insulin release from β-cells exposed to control serum and complement, insulin release was completely blocked from β-cells exposed to islet-cell-specific antibodies and complement. These findings suggest that islet cell surface antibodies can mediate complement-dependent cytotoxicity. DIABETES *30*:231–234, March 1981.

Homologous[1] and heterologous[2] islet cell surface antibodies can be generated in animals by immunization with islet cell suspensions. Many patients with newly diagnosed insulin-dependent diabetes[3,4] also have circulating antibodies reactive with the surface of pancreatic β-cells. To test whether antibodies reactive with surface determinants on islet cells are functionally significant, we used a technique of column perifusion[5] to study the effects of cell surface antibodies on the dynamics of insulin release and on cytotoxicity. The results demonstrate that serum containing islet cell surface antibodies can mediate complement-dependent cytotoxicity expressed as an increase in the release of either radioactive chromium or insulin from the perifused β-cells. Furthermore, the combined exposure of β-cells to cell surface antibodies and complement completely abolishes their ability to release insulin in response to a glucose stimulus.

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MATERIALS AND METHODS

In each experiment pancreases from four Sprague-Dawley rats were digested with collagenase.[3,6,7] Islets of Langerhans were separated from the digest by Ficoll gradient centrifugation and individually chosen by stereo microscopy in Swims' S 77 tissue culture medium, supplemented as described in detail elsewhere.[7]

Suspensions of cells were prepared by mechanical disruption of the isolated islets.[6] The cells were washed by centrifugation on a discontinuous gradient of bovine serum albumin[3,6] and carefully mixed with 1 ml Bio-Gel P-2 polyacrylamide beads (200–400 mesh, Bio Rad Laboratories, Rockville Center, New York) in a 5-ml plastic disposable syringe. Before use, the beads were first equilibrated overnight in Swims' S 77 tissue culture medium, supplemented as described in detail.[3] In each experiment, two columns were connected to reservoirs containing tissue culture medium, which was allowed to perifuse the cells at 37°C at a constant rate determined by a peristaltic pump (Minipuls HP4, Gilson Medical Electronics, Inc., Middleton, Wisconsin) placed after the column. The flow rate was 0.6 ml/min and 1–10-min fractions were collected. The column perifusion system was employed either to study the release of ^{51}Cr from prelabeled cells as a measure of islet cell viability[6,8] or the release of insulin. In experiments with ^{51}Cr-labeled cells, 7–8 × 10^5 islet cells were incubated at a cell concentration of 10^6/ml in Swims' tissue culture medium containing 4% bovine serum albumin (BSA, Fraction V, reagent grade, Miles Biochemicals, Elkhart, Indiana) and 100 cpm/cell of $Na_2{}^{51}CrO_4$ (New England Nuclear, Massachusetts) for 60–90 min. After incubation, the cells were washed twice by centrifugation (50 × *g* for 10 min at room temperature) in Swims' medium with 4% BSA, and the cell pellet was carefully resuspended and finally transferred to the perifusion columns in two equal portions. Chromium radioactivity present in the collected fractions was determined in a gamma scintillation

spectrometer. Results are expressed as %/min of the total cell radioactivity (3–4 × 10^5 cpm/column) added to the column.

In experiments to determine insulin release, about 5 × 10^5 cells were added to each of the two columns, which were perifused in parallel with Swims' medium containing 4% BSA, 2.5 mmol/L $CaCl_2$ and 0.25 mg/ml soybean trypsin inhibitor. Insulin in the collected fractions was determined by routine radioimmunoassay using a second antibody to separate bound and free hormone and rat insulin as a standard. The results are expressed as ng insulin released/min/5 × 10^5 cells.

In one series of experiments, dispersed islet cells (2–5 × 10^5 cells) were incubated at 37°C for 60 min in 150–200 μl Swims' medium with 4% BSA supplemented with 50% (v/v) heat-inactivated (56°C for 30 min) serum either from a normal rabbit or from a rabbit immunized with suspensions of rat islet cells.[2] After 60 min of incubation, guinea pig plasma, as a source of complement, was added to a final concentration of 10% (v/v) and the cells incubated for another 60 min. After suspending the cells by the aid of a Pasteur pipette, they were gently mixed with the Bio-Gel P-2 beads and perifused to determine the release of insulin. Cell counts in the presence of trypan blue indicated that 3.8 ± 1.0 (normal serum) and 2.3 ± 0.5 (test serum) × 10^5 cells (mean ± SEM for three experiments) trypan-blue negative cells were added to the columns.

A rat islet cell antiserum was raised by immunizing a rabbit with suspensions of living rat islet cells. The details of the preparation and characteristics of this antiserum are given elsewhere.[2] The antiserum was obtained by an initial injection of live, dispersed rat islet cells into the popliteal lymph nodes followed by three i.v. injections of live cells. Antibodies binding to the islet cell surface were demonstrated by indirect immunofluorescence or by a sensitive protein-A-binding assay.[2] Antibodies to insulin were not detected. In addition to organ-specific antibodies some non-organ-specific antibodies were present.[2] By extensive absorption of the antiserum to both rat spleen cells and hepatocytes, a specific antiserum was obtained.[2] This serum, which will be referred to as an islet-cell-specific antiserum, was used in some of the experiments described here. All rabbit sera were heat-inactivated (56°C for 30 min) and filtered through a 0.45 μm Millipore filter.

Results are presented as mean values ± SEM for the indicated number of experiments. Statistical significances were determined from the mean ± SEM of difference between the two perifusion columns run in parallel.

RESULTS

The dynamics of a complement-dependent cytotoxic reaction were studied in islet cells labeled with radioactive chromium, washed by centrifugation, and then perifused on the Bio-Gel columns (Figure 1). The rate of ^{51}Cr-release in the presence of 5.5 mmol/L glucose reached a steady baseline level after about 15 min and was not affected either when, after 30 min, the glucose concentration was increased to 30 mmol/L or after 60 min by introducing 5% (v/v) of either normal rabbit serum or a rabbit anti-rat islet cell antiserum. However, when normal fresh guinea pig plasma (10%, v/v) was introduced as a source of complement after 180 min for 5 min, there was a dramatic and immediate release of ^{51}Cr-

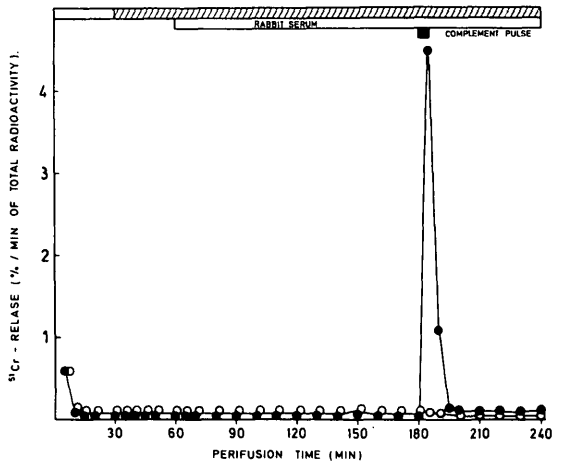

FIGURE 1. Effects of a rabbit anti-rat islet cell serum and complement on the release of radioactive chromium from prelabeled rat islet cells. In two experiments, 7.0 and 8.0 × 10^5 islet cells were first perifused at 37°C for 30 min in the presence of 5.5 mmol/L D-glucose (open bar) and thereafter in the presence of 30 mmol/L D-glucose (hatched bar). After 60 min of perifusion, the medium was supplemented with 5% (v/v) heat-inactivated normal rabbit serum (○) or the rabbit anti-rat islet cell antiserum (●). The rabbit sera (bar labeled rabbit serum) were present until the end of the experiment. After 180 min of perifusion, the perifusion medium was supplemented with 10% (v/v) guinea pig plasma as a source of complement (solid bar).

radioactivity only from the cells that had been exposed to the islet cell surface antibodies. Heat-inactivated guinea pig plasma did not produce this response. Following the pulse of complement the rate of ^{51}Cr-release decreased, but remained at a level elevated relative to that of the control cells.

The cytotoxic reaction occurs without delay, and within a few minutes, leads to a marked depletion of radioactivity from the perifused cells (25–30% of the total initial radioactivity).

The experiments shown in Figure 2 demonstrate that the rate of insulin release was not affected when a 5% (v/v) rabbit anti-rat islet cell serum was added alone after 30-min perifusion in the presence of 5.5 mmol/L D-glucose. It should be noted that heat-inactivated normal rabbit serum was without effect. Following perifusion of cells for 2 h with antiserum, the glucose concentration was increased after 150 min to 30 mmol/L, which resulted in an almost fivefold increase in the rate of insulin release. The presence of islet cell surface antibodies had no effect on the insulin-releasing action of glucose. Addition of 10% (v/v) guinea pig plasma to the perifusion system caused an immediate release of insulin, whether the perifused β-cells had been exposed to membrane antibodies or not. During the period 210–220 min, 1147 ± 345 ng/10^5 cells was released from β-cells exposed to islet cell surface antibodies, compared with 767 ± 335 from the control cells ($P < 0.02$). Since each β-cell contains an estimated 25–30 pg insulin,[6] the antibody-complement-induced release of insulin would correspond to a total lysis of only 30–40 × 10^3 cells or about 8% of the total number of cells added to the column. Thus, the decreased rate of insulin release after exposure to antibodies and complement (perifusion period 220–270 min in Figure 2) probably does not reflect simply a diminished number of cells, but rather a block in the ability of the β-cells to release insulin.

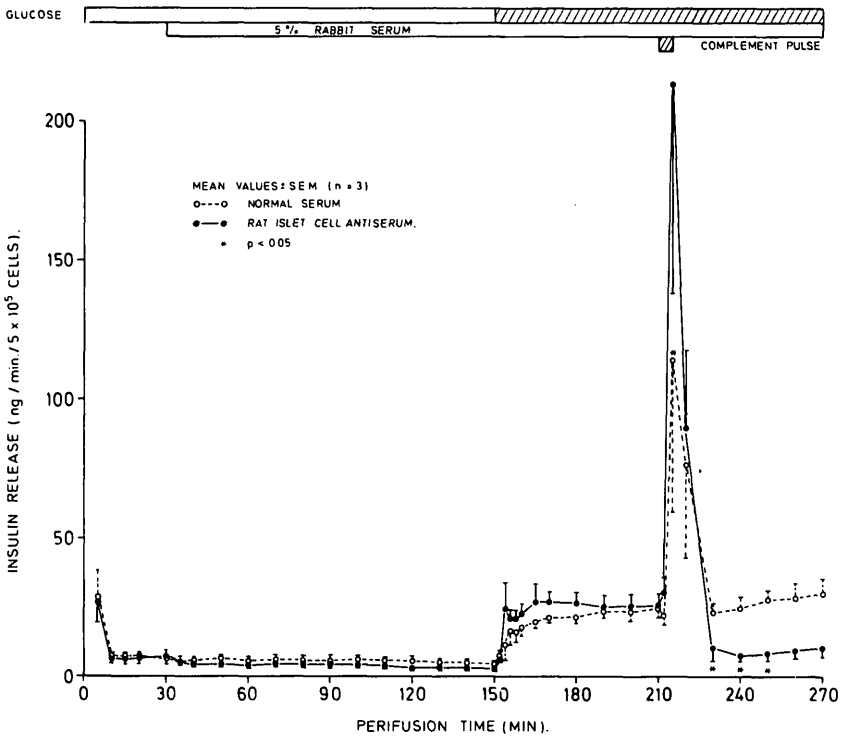

FIGURE 2. Effect of rabbit anti-rat islet cell serum and complement on insulin release from column-perifused, dispersed islet cells. The cells were first perifused for 150 min in the presence of 5.5 mmol/L D-glucose (open bar). Thirty minutes from the start of the experiment until the end of the experiment, the cells were perifused with either 5% (v/v) normal rabbit serum (○) or 5% anti-rat islet cell antiserum(●). The glucose concentration was 30 mmol/L (hatched bar) from 150 min until the end of the experiment. During the perifusion with 30 mmol/L glucose and 210 min after the start of the experiments, the cells were perifused with medium further supplemented with 10% (v/v) guinea pig plasma as a source of complement. Mean values ± SEM for three experiments.

A third series of experiments was therefore carried out, in which the cell number was determined after the treatment with an islet-cell-specific antiserum and complement in batch-type incubations, but before the cells were added to the perifusion system (Figure 3). While there was an eight-fold increase in glucose-stimulated insulin release from β-cells previously exposed to normal rabbit serum and complement, insulin release was completely blocked from β-cells exposed to the islet-cell-specific antibodies and to complement. The total amounts of insulin released from control cells during min 30–80 (Figure 3) were 1085 ± 264 ng/5 × 10^5 cells and 732 ± 287 from cells exposed to the islet-cell-specific antiserum (mean ± SEM for three experiments, $P < 0.05$).

DISCUSSION

These experiments demonstrate that antibodies against surface components of rat pancreatic β-cells can mediate a complement-dependent cytotoxic reaction. The use of perifused islet cells supported in a bed of polyacrylamide

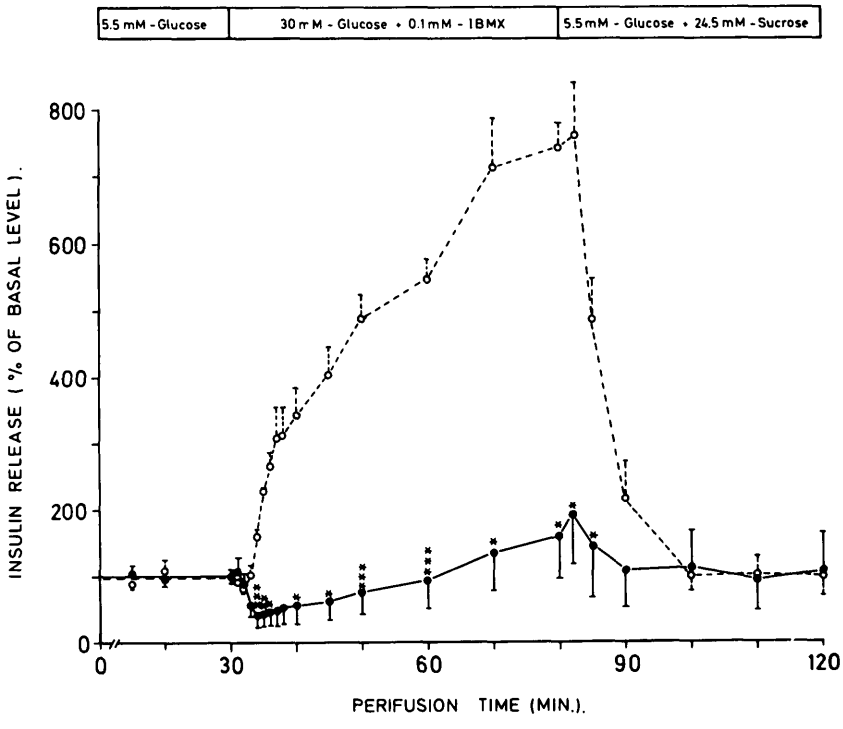

FIGURE 3. Insulin release from column-perifused dispersed islet cells incubated before perifusion with either normal rabbit serum (○) or a rabbit islet-cell-specific antiserum (●) as well as complement. The cells were first perifused in Swims' medium with 5.5 mmol/L D-glucose. After 30 min of perifusion, the glucose concentration was increased to 30 mmol/L and 0.1 mmol/L isobutyl-methylxanthine (IBMX) was present as well. The cells were exposed to this medium for 50 min before the medium was changed to 5.5 mmol/L D-glucose and 24.5 mmol/L sucrose. The results (mean values ± SEM for three experiments) are expressed as % change of insulin release relative to the basal secretion in the presence of 5.5 mmol/L glucose. A steady baseline was obtained 10 min after the initiation of the experiment and amounted to 4.1 ± 1.1 (○) and 13.9 ± 0.7 (●) ng/min/5 × 10^5 cells. Effect of the rat islet cell antiserum (Student's *t* test): *$P < 0.05$, **$P < 0.02$, and ***$P < 0.001$ (asterisks are shown vertically in the figure).

beads made it possible to study some dynamic aspects of the cytotoxic reaction. The immediate release of ^{51}Cr-radioactivity from antibody-treated cells following a complement pulse suggests that an antibody-dependent complement-mediated cytotoxic reaction rapidly renders the islet cells leaky.

Complete lysis of β-cells seems an unlikely consequence of immune cytotoxicity, as the amounts of insulin released were not sufficient to account for a massive cell rupture and, in fact, glucose-induced insulin release was suppressed by antibody and complement. The release of insulin from the control β-cells in response to guinea pig plasma agrees with our previous finding that whole serum from different species is able to induce a prompt insulin release that can be prevented by heating (50°C or 56°C for 30 min).[8,9] An extended series of experiments suggested, furthermore, that islet cells can activate complement via the alternative pathway.[9] In the present study, the data in Figure 2 demonstrate that heat-inactivated rabbit serum did not affect the rate of insulin release. However, as shown in Figures 2 and 3, the possible complement-mediated adverse effects of plasma or serum do not prevent the β-cells from responding to glucose, as is the case with β-cells exposed to islet cell surface antibodies in addition to a source of complement. Care was taken in the latter experiments to determine the number of cells added to the perifusion columns and to assess their viability by the exclusion of trypan blue. Thus, the lesion induced by β-cell surface antibodies and guinea pig plasma may have some degree of specificity for a component, or components, of the stimulus coupling pathway leading to secretion.

As human islet cells become more generally available, the technique used here may be sufficiently sensitive to test whether islet cell surface antibodies in serum from human diabetics[3,4] can also affect human β-cell function, and if so, under what circumstances. If antibodies and complement indeed can reach their target cells in vivo, their combined action would very probably be detrimental to the function and survival of pancreatic β-cells.

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REFERENCES

[1] Kromann, H., Lernmark, Å., Vestergaard, B. F., Egeberg, J., and Nerup, J.: The influence of the major histocompatibility complex (H-2) on experimental diabetes in mice. Diabetes *16*:107–14, 1979.

[2] Lernmark, Å., Kanatsuna, T., Patzelt, C., Diakoumios, K., Carroll, R., Rubenstein, A. H., and Steiner, D. F.: Antibodies directed against the pancreatic islet cell plasma membrane: detection and specificity. Diabetologia *19*:445–51, 1980.

[3] Lernmark, Å., Freedman, Z. R., Hofmann, C., Rubenstein, A. H., Steiner, D. F., Jackson, R. L., Winter, R. J., and Traisman, H. S.: Islet cell surface antibodies in juvenile diabetes mellitus. N. Engl. J. Med. *299*:375–80, 1978.

[4] Lernmark, Å., Hägglöf, B., Holmgren, G., and Ludvigsson, J.: Antibodies against the pancreatic β-cell plasma membrane in insulin-dependent diabetic children. Acta Endocrinol. (Kbh) *277* (Suppl.):48–49, 1979.

[5] Schrey, M. P., Brown, B. L., and Ekins, R. P.: Studies on the control and dynamics of thyrotropin secretion from isolated adenohypophyseal cells. Mol. Cell. Endocrinol. *8*:271–82, 1977.

[6] Lernmark, Å.: The preparation of, and studies on, free cell suspensions from mouse pancreatic islets. Diabetologia *10*:431–38, 1974.

[7] Lernmark, Å., Nathans, A., and Steiner, D. F.: Preparation and characterization of plasma membrane-enriched fractions from rat pancreatic islets. J. Cell Biol. *71*:606–23, 1976.

[8] Lernmark, Å., Sehlin, J., Täljedal, I.-B., Kromann, H., and Nerup, J.: Possible toxic effects of normal and diabetic patient serum on pancreatic β-cells. Diabetologia *14*:25–31, 1978.

[9] Idahl, L.-Å., Sehlin, J., Täljedal, I.-B., and Thornell, L.-E.: Cytotoxic activation of complement by mouse pancreatic islet cells. Diabetes *29*:636–42, 1980.