Rapid Publications

Complement-Fixing Islet Cell Antibodies in the Spontaneously Diabetic BB Rat

DIEGO R. MARTIN AND JOHN LOGOTHETOPOULOS

SUMMARY

Fourteen rats of the spontaneously diabetic BB line were bled from the retroorbital sinus approximately every 10 days. Sera taken from an early age up to 20 days after the onset of overt diabetes were assayed for complement-fixing antibodies against antigens of the surface of islet cells (CFA). Dispersed islet cells from normal Wistar rats prelabeled with ^{3}H-leucine were used as targets. Target cells in suspension were incubated with heat-inactivated rat sera and then, after washing, exposed to guinea pig complement. Cytolytic "injury" was measured by the percentage of labeled cellular proteins released into the medium. Sera from sequential bleedings from eight normal Wistar rats and three rats from a nondiabetic BB subline were assayed to establish basal control cytolytic activity. The mean response ± SD obtained with all control sera was 7.7 ± 1.7%. A response exceeding the mean + 3 SD (12.8%) was considered significantly different from the basal value. Thirteen of the fourteen BB rats developed strongly positive sera. The cytolytic activity preceded the onset of overt diabetes. In several rats CFA appeared 4–8 wk preceding diabetes while in other rats CFA appeared 1–2 wk preceding the manifestation of the disease. These results indicate that CFA may contribute to the destruction of pancreatic islets directly or by attracting mononuclear cells. DIABETES 33:93–96, January 1984.

The spontaneously diabetic BB rat has become an accepted model for insulin-dependent diabetes mellitus.[1] Immune mechanisms have been proposed to explain the loss of the pancreatic B-cells.[2–5] The presence of mononuclear infiltrations in the pancreatic islets (beginning 2–3 wk before diabetes[6]) indicated that a cell-mediated immune attack may play an important role in this destructive process.[2,3] Furthermore, neonatal thymectomy prevented the manifestation of overt diabetes.[4,5]

A humoral immune component directed against antigenic determinants on the surface of islet cells has also been implicated by recent experiments using ^{125}I-protein A to detect bound antibodies.[7,8] In order to investigate and further characterize the role of a humoral immune component we developed a novel, complement-mediated, antibody-dependent cytolysis assay. Dispersed islet cell suspensions that were prelabeled with ^{3}H-leucine were used as targets. Using this system we assayed sera obtained by serial bleedings of BB rats before and after the time of manifestation of overt diabetes.

MATERIALS AND EXPERIMENTAL PROCEDURES

Animals. The BB rats used in this study originated from a cross between two diabetic-prone BB sublines that had been inbred for seven generations at the University of Massachusetts. BB rats from random matings of this cross were obtained from the Hospital For Sick Children (Toronto, Ontario, Canada). Brother-sister matings (both parents diabetic) were reinitiated in our colony. Fourteen BB rats used in this study were obtained from seven litters from the third and fourth generations of our colony. The average age of onset of diabetes in the 14 animals was 107 ± 25 days (mean ± SD). The average age of onset for all diabetic rats in the seven litters was 92 ± 24 days. The incidence rate of diabetes in individuals of these litters (observed up to the age of 150 days) was 61% (N = 46). Eight Wistar rats from a commercial source and three rats from a nondiabetic subline derived from the BB rat (supplied by the Hospital For Sick Children) were used as controls.

Collection and storage of sera. Blood samples (1–2 ml) were obtained from the retroorbital sinus from rats mildly anesthetized with Penthrane (Abbott Laboratories Limited, Montreal, Canada). Samples were collected approximately every 10 days starting as early as 34 days of age and continuing up to 2–3 wk after the manifestation of diabetes.

From the Banting and Best Department of Research, University of Toronto, 112 College Street, Toronto, Ontario M5G 1L6, Canada.
Address reprint requests to Dr. John Logothetopoulos at the above address.
Received for publication 19 September 1983.

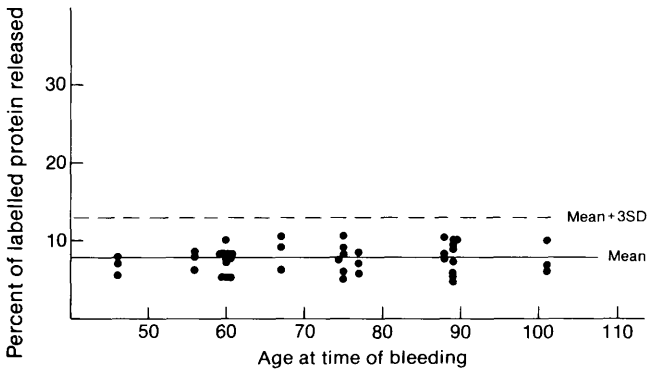

FIGURE 1. Cytolytic activity against pancreatic islet cells of sera from control rats. Nine Wistar rats and three rats from a nondiabetic subline of the BB rat were bled sequentially from an early age. The mean cytolytic activity and the mean + 3 SD for all control sera tested are indicated.

Blood was allowed to stand for 1 h at room temperature, then centrifuged at 2000 × *g* for 15 min at 5°C. Multiple aliquots of the serum were immediately frozen and stored at −70°C. Onset of diabetes was determined by directly testing the urine with Tes-Tape (Eli Lilly Canada Inc., Toronto, Ontario) and by assessing hyperglycemia in glycosuric rats with Chemstrips (Boehringer Mannheim, Germany).

Preparation of labeled dispersed islet cells as targets for cytolysis. The pancreata of adult female rats from a commercial source (Charles River, Quebec, Canada) were used. Pancreatic islets were isolated under sterile conditions by a modified collagenase method of Lacy and Kostianovsky.[9] Islets were transferred to a 13 × 100-mm pyrex tube containing 6 ml calcium-free RPMI 1640 medium,[10] 15 mM glucose, and 0.5 mM EGTA. This tube was placed on a rocking platform (Bellco Glass, Inc., New Jersey), kept at room temperature, and set to 10 cycles per minute. After 20 min the volume was reduced to 2 ml and the islets gently dispersed by repeated pipetting with a siliconized Pasteur pipette fitted with a rubber bulb. The dispersed cells were washed twice with leucine-free medium containing 7.5 mM glucose, 1.6 mM calcium, and 15% heat-inactivated calf serum (Gibco of Canada, Burlington, Ontario). The dispersed cells were centrifuged after each wash for 5 min at 50 × *g*. The pellet was finally resuspended in 5 ml of the same leucine-free medium containing in addition 20 μCi L-[4,5-^{3}H]leucine (52.3 Ci/mmol, New England Nuclear Corp., Boston, Massachusetts). The tube was sealed with a teflon-lined cap and placed on the rocking platform in an incubator at 37°C. All media used in these procedures and in the following cytolysis assay were equilibrated with air/CO_2 (95:5).

Cytolytic assay. After 20 h of labeling, the islet cell suspension was washed twice with serum-free RPMI 1640 containing 4 mM glucose (assay medium). The final volume was adjusted so that 0.2 ml contained 35–45 islet equivalents of dispersed cells. Small lymphocyte tubes (#72693, Sarstedt, St-Laurent, Quebec, Canada) were prepared containing 0.8 ml heat-inactivated (56°C for 30 min) rat sera to be tested, diluted in assay medium, and maintained at 5°C. To each of these tubes, 0.2 ml of the dispersed islet cell suspension was added. The final dilution of rat sera was 12.5%. After capping, the tubes were placed on a rotator (Labquake, Lab.

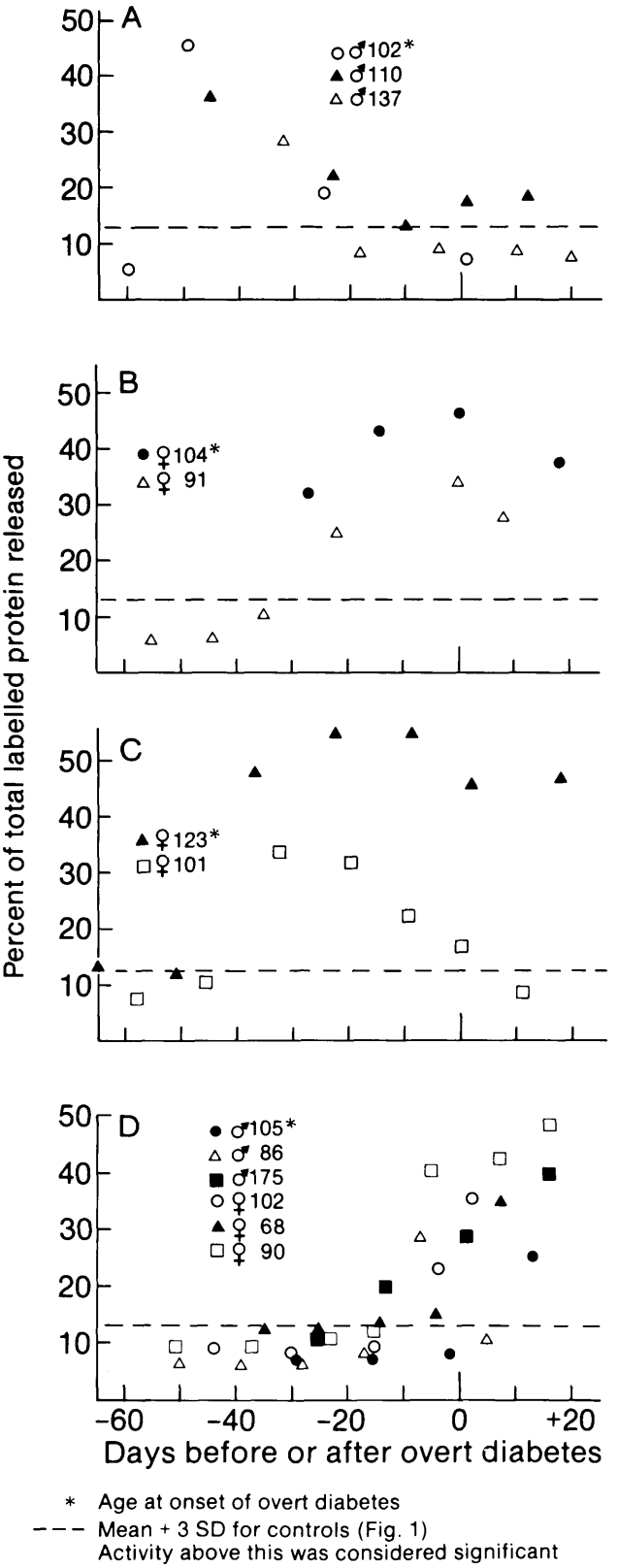

FIGURE 2. Cytolytic activity against pancreatic islet cells of sera from 13 BB rats. Rats were bled sequentially approximately every 10 days from a young age up to 2–3 wk after overt diabetes (day 0). Grouping of rats according to profiles of cytolytic activity in their sera (panels A; B plus C; D) is discussed in RESULTS. Serum samples resulting in cytolytic activity above the mean + 3 SD of control sera (see Figure 1) were considered positive.

Industries, Berkeley, California) for 1 h in a cold room (5°C). After centrifugation (50 × *g* for 5 min at 5°C) and decanting, the cell pellet was washed once with 2 ml cold assay medium. The washed cell aggregates were finally suspended in 1 ml of a complement solution (1 part guinea pig serum to 10 parts assay medium), then placed on the rocking platform in an incubator (37°C). After a 1.5-h incubation, tubes were centrifuged as before. Supernatants were decanted with Pasteur pipettes and saved. The cell pellets were solubilized with 0.3 ml 0.25 N NaOH and transferred into larger glass tubes. Duplicate 0.1-ml aliquots of supernatant were also transferred into similar tubes. An appropriate amount of albumin solution was added to each sample in order to increase protein bulk (7 mg albumin to solubilized cells, 3.5 mg to supernatants). Proteins were precipitated with cold 12% trichloroacetic acid (TCA) containing 3 mM cold leucine. The centrifuged precipitates were washed once with 7.5% TCA and recentrifuged. The pellets were then dissolved in 0.5 ml formic acid, transferred to scintillation vials containing 10 ml Aquasol (New England Nuclear), and counted for radioactivity.

Expression of results. TCA-precipitable radioactivity released into the supernatant was expressed as a percentage of total precipitable activity (islets + supernatant). This percentage represented "cytolytic activity" in a given rat serum sample. All serum samples were assayed in duplicate and had a standard deviation not exceeding 1%.

RESULTS

The range of cytolytic activity in sera from control animals was small (4.5–10.5%) and is shown in Figure 1. The mean for all control sera assayed was 7.7% and the standard deviation 1.7%. Any sample giving a value above 12.8% (mean + 3 SD) was considered positive for cytolytic antibodies.

Of 14 sequentially bled rats that became diabetic, 13 yielded one or more positive samples. The appearance of CFA in these 13 rats followed broadly one of three patterns: (1) Three rats developed CFA 30–50 days before overt diabetes (Figure 2A). However, at the time of diabetes cytolytic activity in the sera had approached or attained control values. (2) Four rats had positive sera 22–40 days before diabetes (Figure 2B and C). In this group, cytolytic activity persisted at the time of overt diabetes. (3) Finally, six animals yielded positive sera no earlier than 14 days before diabetes (Figure 2D). All but one of these rats had positive sera 2–3 wk after diabetes.

The following additional points regarding the assayable activity should be made: (1) if complement in the second incubation was heat inactivated, no cytolytic activity of positive sera could be demonstrated; and (2) the cytolytic activity of positive sera was associated with the 40% ammonium sulfate precipitable γ-globulin fraction.

DISCUSSION

Our cytolytic assay using islet cells prelabeled with ^{3}H-leucine as targets proved a convenient procedure to study antibody-dependent, complement-mediated cytotoxicity in BB rats. The dispersion of islets into small cell aggregates and their maintenance in suspension culture permitted homogeneous labeling and accessibility of cell surfaces to macromolecules.

The islet cells from a nondiabetic, commercial Wistar strain were effective targets in our assay. This suggests that the islet cell proteins that are recognized by the aberrant immune responses in the BB rat are not "abnormal" islet cell proteins unique to the BB rat. This is also born out by studies of ^{125}I-protein A binding to islet cells mediated by sera from BB rats[7,8] and by studies with sera from human diabetic subjects.[11–13] In all these studies homologous or heterologous normal islet cells were used.

The antibody-dependent, complement-mediated cytolytic activity against islet cells in several BB rats clearly preceded the manifestation of overt diabetes by many weeks. We have previously shown by studying pancreatic biopsies that mononuclear infiltrations of pancreatic islets start 2–3 wk before the onset of overt diabetes in the young adult BB rat.[6] These data taken together with those of the present study indicate that the cytolytic activity detected was not the consequence of "self-immunization" by degenerating pancreatic islet cells.

There is no easy immunologic answer as to why in some rats the cytolytic activity appeared 4–8 wk before overt diabetes and in other rats 1–2 wk before onset. It is difficult to rationalize each individual pattern in terms of immune regulation. Cell damage can be induced by multiple effector arms of an immune response. It is uncertain whether the antibody-dependent, complement-mediated islet cell lysis obtained with our assay in vitro is playing an important role in the destructive process of islet cells in vivo. Other simultaneously activated immune responses in vivo, involving direct cellular cytotoxicity or antibody-dependent, cell-mediated cytotoxicity, may be the major culprits. A chronologic study of these effector responses in the BB rat in relation to the onset of overt diabetes and to the evolution of the insular lesions will increase our insight into the mechanism of islet cell loss and may help us in the development of preventive treatments.

ACKNOWLEDGMENTS

This work was supported by the Medical Research Council of Canada (MT-2925) and the C. Best Foundation.

REFERENCES

[1] Chappel, C. I., and Chappel, W. R.: The discovery and development of the BB rat. Metabolism 1983; 32 (Suppl. 1):8–9.

[2] Nakhooda, A. F., Like, A. A., Chappel, C. I., Wei, C.-N., and Marliss, E. B.: The spontaneously diabetic Wistar rat (the "BB" rat). Diabetologia 1978; 14:199–207.

[3] Seemayer, T. A., Tannenbaum, G. S., Goldman, H., and Colle, E.: Dynamic time course studies of the spontaneously diabetic BB Wistar rat. III. Light-microscopic and ultrastructural observations of pancreatic islets of Langerhans. Am. J. Pathol. 1982; 106:237–49.

[4] Like, A. A., Williams, R. M., Kislauskis, E., and Rossini, A. A.: Neonatal thymectomy prevents spontaneous diabetes in the Bio Breeding Worcester (BB/W) rat. Clin. Res. 1981; 29:542A.

[5] Like, A. A., Butler, L., Williams, M. R., Appel, M. C., Weringer, E. J., and Rossini, A. A.: Spontaneous autoimmune diabetes mellitus in the BB rat. Diabetes 1982; 31 (Suppl. 1):7–13.

[6] Logothetopoulos, J., Valiquette, N., Madura, E., and Cvet, D.: The onset and progression of pancreatic insulitis in the overt, spontaneously diabetic, young adult BB rat studied by pancreatic biopsy. Diabetes 1984; 33:33–36.

[7] Dyrberg, T., Lernmark, A., Oie, H. K., and Gazdar, A. F.: Islet cell surface antibodies in spontaneously diabetic BB rats. Acta Biol. Med. Ger. 1982; 41:1105–1109.

[8] Dyrberg, T., Poussier, P., Nakhooda, A. F., Marliss, E. B., and Lern-

mark, A.: Humoral immunity in the spontaneously diabetic BB rat. Metabolism 1983; 32 (Suppl. 1):87–91.

[9] Lacy, P. E., and Kostianovsky, M.: Method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 1967; 16:35–39.

[10] Moore, G. E., Gerner, R. E., and Franklin, H. A.: Culture of normal human leukocytes. JAMA 1967; 199.519–29.

[11] Baekkeskov, S., Nielsen, J. H., Marner, B., Bilde, T., Ludvigsson, J., and Lernmark, A.: Autoantibodies in newly diagnosed diabetic children immunoprecipitate specific human pancreatic islet cell proteins. Nature 1982; 298:167–69.

[12] Botazzo, G. F., Florin-Christensen, A., and Doniach, D.: Islet cell antibodies in diabetes mellitus with autoimmune polyendocrine deficiencies. Lancet 1974; 2:1279–83.

[13] Dobersen, M. H., Scharff, J. E., Ginsberg-Fellner, F., and Notkins, A. L.: Cytotoxic autoantibodies to beta-cells in the serum of patients with insulin-dependent diabetes mellitus. N. Engl. J. Med. 1980; 303:1493–98.