Spontaneous diabetes mellitus in the Bio-Breeding/Worcester rat. Evidence in vitro for natural killer cell lysis of islet cells.

P MacKay, … , J Jacobson, A Rabinovitch

J Clin Invest. 1986;77(3):916-924. https://doi.org/10.1172/JCI112390.

Research Article

We sought direct evidence for anti-islet cellular cytotoxicity in diabetic Bio-Breeding/Worcester (BB/W) rats by comparing the effects of splenic lymphoid cells from BB/W diabetic (D), diabetes-prone (DP), and diabetes-resistant (DR) rats on the release of 51Cr from damaged islet cells in vitro. D and DP splenic lymphoid cells were cytotoxic to major histocompatibility complex (MHC)-compatible Wistar-Furth (WF) rat islet cells and also to MHC-incompatible Lewis rat islet cells and a rat islet cell line (RIN 5F), whereas WF and Lewis rat spleen cells and a rat pituitary cell line (GH3) were not lysed by lymphoid cells from D or DP rats. The cytotoxic cells were identified as natural killer (NK) cells since NK-sensitive cells (G1-TC and YAC-1 cell lines) were lysed by D and DP spleen cells, YAC-1 cells competed for the lysis of RIN islet cells by D spleen cells, lysis of RIN cells was increased by using D spleen cells from the low density fraction (large lymphocytes/monocytes) of a Percoll density gradient, and incubation of D spleen cells with an antiserum to NK cells (anti-asialo GM1 serum) and complement decreased monoclonal antibody-defined subsets containing NK cells (W3/13+ OX19- and OX8+), and this was accompanied by similar decreases in cytotoxicity to YAC-1, RIN, and WF islet cells. These studies demonstrate that NK cell activity is increased in BB/W diabetic […]

Spontaneous Diabetes Mellitus in the Bio-Breeding/Worcester Rat

Evidence In Vitro for Natural Killer Cell Lysis of Islet Cells

Peter MacKay, Jill Jacobson, and Alexander Rabinovitch
Department of Medicine, University of Miami School of Medicine, Miami, Florida 33101

Abstract

We sought direct evidence for anti-islet cellular cytotoxicity in diabetic Bio-Breeding/Worcester (BB/W) rats by comparing the effects of splenic lymphoid cells from BB/W diabetic (D), diabetes-prone (DP), and diabetes-resistant (DR) rats on the release of ^{51}Cr from damaged islet cells in vitro. D and DP splenic lymphoid cells were cytotoxic to major histocompatibility complex (MHC)-compatible Wistar-Furth (WF) rat islet cells and also to MHC-incompatible Lewis rat islet cells and a rat islet cell line (RIN 5F), whereas WF and Lewis rat spleen cells and a rat pituitary cell line (GH_3) were not lysed by lymphoid cells from D or DP rats. The cytotoxic cells were identified as natural killer (NK) cells since (*a*) NK-sensitive cells (G_1-TC and YAC-1 cell lines) were lysed by D and DP spleen cells, (*b*) YAC-1 cells competed for the lysis of RIN islet cells by D spleen cells, (*c*) lysis of RIN cells was increased by using D spleen cells from the low density fraction (large lymphocytes/monocytes) of a Percoll density gradient, and (*d*) incubation of D spleen cells with an antiserum to NK cells (anti-asialo GM1 serum) and complement decreased monoclonal antibody-defined subsets containing NK cells ($W3/13^+$ $OX19^-$ and $OX8^+$), and this was accompanied by similar decreases in cytotoxicity to YAC-1, RIN, and WF islet cells. These studies demonstrate that NK cell activity is increased in BB/W diabetic and DP rats, and that islet cells can serve as targets for these NK cells. The findings suggest that NK cells may participate in the islet-directed cellular cytotoxic response leading to beta cell destruction and diabetes.

Introduction

There is increasing recent evidence that the immune system may be involved in the pathogenesis of insulin-dependent diabetes mellitus (IDDM)[1] in humans as well as in certain animal models. The most extensively studied animal model is the Bio-Breeding (BB) rat (reviewed in reference 1). This rodent has a spontaneous form of diabetes that makes it a close counterpart of the human subject with IDDM. Obesity is absent, both sexes are affected equally, and the peak incidence of diabetes occurs around the age of sexual maturation (60–120 d). At this time, 30–60% of the rats become diabetic, manifesting severe hyperglycemia, glucosuria, hypoinsulinemia, and ketoacidosis that is lethal if insulin is not administered. Histologic study of acutely diabetic animals reveals intense infiltration of the islets (insulitis) by mononuclear cells (2, 3), mostly activated lymphocytes and macrophages (4), and chronically diabetic animals have islets devoid selectively of insulin-containing beta cells (2, 5).

The observation of pancreatic insulitis suggests a cell-mediated immune pathogenesis for diabetes in the BB rat. Furthermore, a variety of immune suppression or enhancement procedures have been reported to prevent and/or cure diabetes in this animal model. BB diabetes-prone (DP) rats have been protected from developing diabetes by neonatal thymectomy (6), neonatal bone marrow allografts (7, 8), sublethal whole body irradiation (9), total lymphoid irradiation (10), administration of antilymphocyte globulin (9), glucocorticoids (11), and cyclosporine (11, 12). Also, transfusions of whole blood (13), or peripheral T lymphocytes (14) from a diabetes-resistant (DR) line of BB rats have been reported to prevent the appearance of diabetes in DP rats. Conversely, concanavalin A (Con A)-activated splenic lymphocytes have been shown to adoptively transfer insulitis and diabetes upon injection into young DP BB/W (Worcester) rats (15), or into otherwise DR BB/W rats (16) and Wistar-Furth (WF) rats (17) that have been pretreated with cyclophosphamide.

These immunological manipulations of BB rats, therefore, provide strong evidence for the involvement of cellular immunity in the pathogenesis of diabetes in this animal. In a recent study (18) we demonstrated that splenic lymphoid cells from BB/W diabetic and DP rats could lyse cells and decrease insulin content in monolayer cultures of WF rat islet cells and a rat islet cell line (RIN). In the present study, we have characterized further this cell-mediated cytotoxicity (CMC) to islet cells in vitro, and have identified the cytotoxic cells to have the properties of natural killer (NK) cells.

Methods

Animals. BB/W female rats were obtained from the colony maintained at the University of Massachusetts Medical School, Worcester, MA (from Dr. Arthur A. Like). These animals have an expected frequency of diabetes of ~50% between 60 and 120 d of age. Rats were defined as diabetic if their urine glucose was 2–4+ with Tes-Tape (Eli Lilly & Co., Indianapolis, IN) and plasma glucose concentrations exceeded 200 mg/dl. Diabetic rats were treated with a single daily injection of 1–2 U of protamine zinc insulin and were studied within 12 d of diagnosis. DP rats were obtained from the same sublines as the diabetic animals (17–20 generations of brother-sister matings) and were studied at 100–130 d of age. DR rats were age-matched to the DP rats and obtained from BB/W sublines bred for resistance to diabetes. The BB/W diabetic, DP, and DR rats served

Address correspondence to Dr. Rabinovitch, Department of Medicine (R-134), U. of Miami School of Medicine, P.O. Box 016960.

Received for publication 19 June 1985 and in revised form 18 November 1985.

1. *Abbreviations used in this paper:* BB/W, Bio-Breeding/Worcester; CMC, cell-mediated cytotoxicity; Con A, concanavalin A; DP, diabetes prone; DR, diabetes resistant; FITC, fluorescein isothiocyanate; IDDM, insulin-dependent diabetes mellitus; LGL, large granular lymphocyte; MHC, major histocompatibility complex; NK, natural killer; RTI^u, MHC-compatible rats; RTI^b, RTI^s, RTI^l, and RTI^a, MHC-incompatible rats; $T_{c/s}$, T-cytotoxic/suppressor; WF, Wistar-Furth.

J. Clin. Invest.

0021-9738/86/03/0916/09 $1.00
Volume 77, March 1986, 916–924

as sources of splenic lymphoid cells which were used as effector cells in the cytotoxicity assays. Pancreatic islet cells and splenic lymphocytes used as target cells in these assays were isolated from WF rats, major histocompatibility complex (MHC)-compatible ($RT1^u$) with the BB/W rats, and from MHC-incompatible ($RT1^l$) Lewis rats. The WF and Lewis rats were inbred, females, 125–150 g body weight, and purchased from Microbiological Assoc., Walkersville, MD.

Splenic lymphocyte isolation. Spleens were removed aseptically from anesthetized rats (sodium pentobarbital 50 mg/kg), then washed twice and disrupted in a glass homogenizer in RPMI-1640 medium (Gibco, Grand Island, NY) supplemented with 100 U/ml penicillin, 100 μg/ml streptomycin, and 10 mM Hepes (Gibco). The cell suspension was filtered through sterile gauze and transferred to a 50-ml polypropylene centrifuge tube in a final volume of 35 ml. 15 ml of Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ) was layered underneath the spleen cells and the cells were centrifuged at 400 *g* for 30 min. Lymphoid (mononuclear) cells were collected from the interface, washed twice, and resuspended in RPMI medium further supplemented (complete medium) with 10% heat-inactivated fetal calf serum (Gibco). The cells were counted and adjusted to the desired concentrations for direct use as effector cells in the cytotoxicity assays, or further purified on nylon wool or Percoll. Cell viability assessed by trypan blue exclusion was >95%.

Nylon wool purification. BB/W rat splenic lymphoid cells (50–150 $\times 10^6$ in 2 ml) were incubated in complete medium on nylon wool columns (0.6 g nylon wool per 5-ml syringe) for 1 h at 37°C in 95% humidified air/5% CO_2. The nonadherent population was eluted in 10 ml of warm medium, washed once, and resuspended to the desired concentrations for use as effector cells in the cytotoxicity assays. In the experiments where nylon wool adherent cells were assayed, these were forced from the columns (after first washing these through with another 10 ml of medium) by pressing the nylon wool with a Pasteur pipette while washing with cold medium.

Percoll purification. BB/W rat splenic lymphoid cells were separated into fractions enriched for either large lymphocytes and monocytes, or small lymphocytes, on discontinuous Percoll gradients. Dulbecco's 10 × phosphate-buffered saline (PBS) was diluted 1:10 with Percoll (Pharmacia Fine Chemicals) to make a 1 × Percoll-PBS solution. This solution was then diluted with 1 × PBS to form the 65, 55, and 40% vol/vol working solutions of Percoll for the discontinuous gradients. Splenic lymphoid cells, previously isolated on Ficoll gradients, were suspended in the 65% Percoll solution at a concentration of 10^7 cells/ml in 15-ml centrifuge tubes. Equal volumes of 55 and 40% Percoll and finally 1 × PBS were subsequently layered on top of each other and the cells centrifuged at 750 *g* for 15 min. A low density fraction enriched in large lymphocytes and monocytes was recovered from the 40–55% interface, and a high density fraction enriched in small lymphocytes was recovered from the 55–65% interface. Morphologic analysis of cytospin preparations of Wright's-stained cells revealed that, for diabetic and DP rats, large lymphocytes/monocytes were enriched from 40±6% before Percoll purification (total mononuclear cells) to 68±15% in the low-density Percoll fraction, and small lymphocytes were enriched from 54±5 to 65±2% in the high density fraction; and for DR rats, large lymphocytes/monocytes were enriched from 19±5 to 52±13% in the low density fraction, and small lymphocytes were enriched from 81±5 to 86±4% in the high-density fraction. Cells identified as large lymphocytes/monocytes included large granular lymphocytes (LGL), which were not counted separately since their characteristic granules could not always be identified definitively. The different cell fractions were washed twice by centrifugation and resuspended at 10^6 cells/ml in complete medium for use as effector cells in the cytotoxicity assays.

Immunofluorescence staining. Splenic lymphoid cells from BB/W rats were phenotyped by immunofluorescence assay, using mouse anti-rat monoclonal antibodies W3/13, OX19, W3/25, and OX8 (Accurate Chemical and Scientific Corp., Westbury, NY). W3/13 reacts with all T lymphocytes, NK cells, polymorphonuclear cells, haemopoietic stem cells, and plasma cells, but not with B lymphocytes. OX19 reacts with T lymphocytes only. W3/25 reacts with T-helper lymphocytes and macrophages. OX8 reacts with T-cytotoxic/suppressor lymphocytes ($T_{c/s}$) and NK cells. Aliquots of 1–5 × 10^6 splenic lymphoid cells from individual rats were incubated for 30 min at 4°C, separately, with monoclonal antibodies W3/13, OX19, W3/25, and OX8 (all at 1:20 dilution) in 100 μl PBS (pH 7.4) containing 10 mg/ml bovine serum albumin and 0.2 mg/ml sodium azide (Sigma Chemical Co., St. Louis, MO) (assay buffer). The cells were then washed twice and incubated for another 30 min at 4°C with fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG (Accurate Chemical and Scientific Corp.) at a 1:20 dilution in 100 μl assay buffer. Immunoglobulin (Ig)-positive rat B lymphocytes were labeled directly by incubation for 30 min at 4°C with FITC-conjugated rabbit anti-rat Ig (Cooper Biomedical, Malvern, PA) at a 1:20 dilution in 100 μl assay buffer. After incubation with the FITC-conjugated antibodies, the cells were washed twice and fixed in 4% paraformaldehyde, mounted on slides and scored for percent fluorescent cells by two different observers, using a Leitz fluorescence microscope. Background fluorescence was estimated by incubating cells with FITC-conjugated goat anti-mouse IgG only, and this revealed <1% fluorescent cells.

NK cell depletion. To assess the effects of NK cell depletion on the cytotoxic potential of the BB/W rat splenic lymphoid cells and to monitor this depletion by the immunofluorescence assay, a pool of splenic lymphoid cells from three diabetic rats and a pool from two DR rats were first depleted of adherent cells on nylon wool columns. A portion (20 × 10^6) of the nonadherent cells in each pool was maintained in serum-supplemented RPMI medium (complete) for later incubation with complement (control cells), while another portion (40 × 10^6) of cells from each pool was incubated for 30 min at 20°C in 1 ml of a 1:400 dilution (in serum-free RPMI) of anti-asialo GM1 rabbit serum, gammaglobulin fraction (10 mg/ml) (Wako Chemicals USA Inc., Dallas, TX). This antiserum contains antibody to a neutral glycosphingolipid (asialo GM1) present in high quantity on the surface of NK cells (19). The cells from both these pools and the control pools (no antiserum added) were then washed in serum-free RPMI and incubated in a 1:20 dilution of Low Tox H rabbit complement (Cedarlane Laboratories, Hornby, Ontario, Canada) in 3 ml for 45 min at 37°C in the CO_2 incubator. The cells were washed twice, viable cells counted and resuspended (5 × 10^6/ml) in complete medium for use as effector cells.

Target cells. Islets were freshly isolated from pancreases of WF ($RT1^u$) and Lewis ($RT1^l$) rats, by collagenase digestion (20). The islets were dissociated into single cells by incubation in Ca^{2+}/Mg^{2+}-free PBS with 3 mM EGTA for 10 min at 37°C, then in PBS with 1 mg/ml trypsin for 10 min at 37°C (21). Splenic lymphocytes were isolated from both WF and Lewis rats by using Ficoll-Hypaque as described above. Several cell lines were also used as targets. The RIN (clone 5F) is derived from the New England Deaconess Hospital ($RT1^s$) rat islet cell tumor, and synthesizes and secretes primarily insulin (22). RIN islet cells in monolayer culture were dissociated by incubation for 5 min at 37°C in 0.05% trypsin/0.02% EDTA in Ca^{2+}/Mg^{2+}-free PBS, then washed two times in complete medium before use as target cells. The GH_3 rat pituitary cell line (23), which also grows in monolayer culture, was treated similarly. Two NK-sensitive cell lines (24) were used as targets in the cytotoxicity assays: (*a*) YAC-1, a virus-induced mouse lymphoma cell line, and (*b*) G_1-TC, a WF ($RT1^u$) rat-derived thymoma line. These cell lines were maintained in suspension culture in complete RPMI medium.

^{51}Cr release assay. Lysis of target cells was detected by an assay for the release of ^{51}Cr from the damaged cells. Targets (2–3 × 10^6 cells) were labeled with 100 μCi ^{51}Cr-sodium chromate (New England Nuclear, Boston, MA) in 0.3 ml complete RPMI medium for 90 min at 37°C, then washed three times, resuspended to 0.5 × 10^6 cells/ml, and seeded at 25,000 cells/well in a 96-well ½ area microculture plate (Costar, Cambridge, MA). Effector splenic lymphoid cells were seeded in the wells (50,000–500,000 cells in 100 μl) in quadruplicate. The plates were centrifuged (100 *g* for 3 min) and incubated at 37°C for either 8 h with RIN, GH_3, YAC-1, and G_1-TC cells, or for 14 h with WF and Lewis islet or spleen cells. An aliquot (100 μl) was collected from each well and counted in a Beckman gamma counter. Total ^{51}Cr release was determined by adding 100 μl 4% Triton X-100 to a set of target cell wells. Release of ^{51}Cr in the absence of effector cells (spontaneous release) was <20% of total ^{51}Cr release. Specific cytotoxicity was calculated from the formula:

% lysis = 100 × (test cpm-spontaneous cpm) ÷ (total cpm-spontaneous cpm).

Statistical analyses. Statistical significance levels were evaluated by *t* test for unpaired samples or for paired samples if so stated. Results are expressed as mean±SEM.

Results

Differential cytotoxic effects of splenic lymphoid cells from BB/W diabetic, DP, and DR rats. Splenic lymphoid cells from BB/W diabetic, DP, and DR rats showed different degrees of CMC in a ^{51}Cr release assay (Table I). At a 1:20 target/effector cell ratio, cytolysis of MHC-compatible WF (RTI^u) rat islet cells by diabetic spleen cells (9.7±1.1%, $P < 0.001$) and by DP cells (7.0±1.0%, $P < 0.005$) was significantly greater than by DR cells (3.5±0.7%). Similarly, cytolysis of MHC-incompatible Lewis (RTI^l) rat islet cells by diabetic cells (10.7±2.0%, $P < 0.005$) and by DP cells (8.2±1.0%, $P < 0.005$) was significantly greater than by DR cells (4.1±0.7%). No appreciable cytotoxicity was observed when WF or Lewis rat spleen cells (Table I), or Con A-activated WF rat splenic lymphoblasts (not shown), were used as targets.

Using an islet cell line (RIN) as target, the dose-dependent cytotoxic effects of BB/W rat splenic lymphoid cells were investigated (Fig. 1). At a target/effector ratio of 1:20, lysis of RIN cells by diabetic spleen cells (21.4±1.8%, $P < 0.001$) and by DP cells (14.0±1.7%, $P < 0.001$) was significantly greater than by DR cells (6.0±0.8%), and the cytotoxic effect of diabetic spleen cells was significantly greater than that of DP cells ($P < 0.01$). At a target/effector ratio of 1:8, lysis of RIN cells by diabetic cells (11.2±1.4%, $P < 0.001$) and by DP cells (8.4±0.9%, $P < 0.001$) was significantly greater than by DR cells (2.0±0.8%). At a target/effector ratio of 1:2, the effects of diabetic, DP, and DR cells did not differ. In contrast to RIN islet cells, rat pituitary cells (GH_3 cell line) exhibited low (<6%) and not significantly different levels of lysis by diabetic, DP, and DR splenic effector cells, at all target/effector ratios tested (Fig. 1).

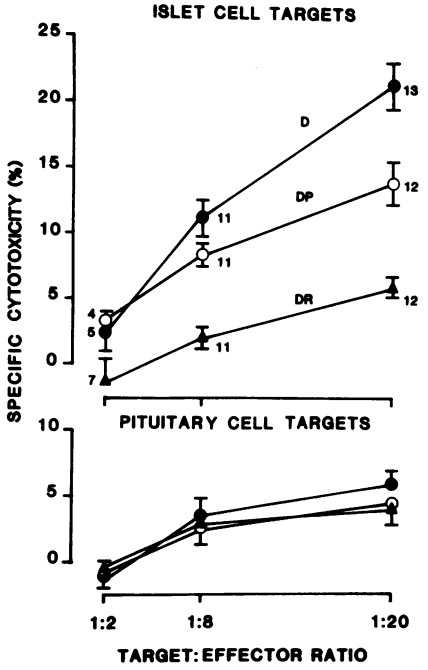

Figure 1. Specific lysis (cytotoxicity) of ^{51}Cr-labeled target cells (25,000/well) from a rat islet (RIN, *top*) and a rat pituitary (GH_3) cell line (*bottom*) by splenic lymphoid cells (effectors, 500,000/well) from BB/W diabetic (D), DP, and DR rats as a function of the target/effector cell ratio. Mean±SEM percent specific ^{51}Cr release values are shown for the numbers of animals indicated. Significant differences are described in Results.

Identification of cytotoxic cells in BB/W diabetic and DP rats. To determine the nature of the cytotoxic cells from BB/W diabetic rats, Ficoll-isolated splenic cells were incubated on nylon wool columns. The cytotoxic activity against RIN cells, and against WF islet cells, was found mainly in the nylon wool nonadherent population (Table II). This would exlude macrophages as signficant contributors to islet cell cytotoxicity. In addition, nonadherent splenic cell fractions from diabetic and DP rats were significantly more cytotoxic to RIN islet cells than were the total splenic lymphoid (mononuclear) cells (Table III). After passage through nylon wool, cytotoxicity to RIN islet cells increased by 51±13% ($P < 0.005$) for diabetic effector cells, and by 48±14% ($P < 0.02$) for DP effector cells, while no increase

Table I. CMC by BB/W Rat Spleen Cells

	Effector spleen cells from BB/W rats		
Target cells	Diabetic	DP	DR
WF rat islet	9.7±1.1* (26)	7.0±1.0‡ (20)	3.5±0.7 (27)
WF rat spleen	2.6±1.4 (9)	2.3±1.5 (11)	2.8±0.9 (13)
Lewis rat islet	10.7±2.0‡ (20)	8.2±1.0‡ (15)	4.1±0.7 (19)
Lewis rat spleen	0.1±1.3 (8)	0.8±1.0 (9)	1.0±0.9 (11)

BB/W rat splenic lymphoid cells were isolated by centrifugation on Ficoll density gradients, then incubated as effector cells (500,000/well) with ^{51}Cr-labeled WF or Lewis rat islet or spleen cells as targets (25,000/well) for 14 h. Values are mean±SEM percent specific ^{51}Cr release for numbers of BB/W rats shown in parentheses.

* $P < 0.001$.

‡ $P < 0.005$ vs. corresponding value for DR rats.

Table II. Effects of Nylon Wool Purification of BB/W Diabetic Rat Spleen Cells on CMC

	Effector cells	
Target cells	Nonadherent	Adherent
RIN	15.1±1.3	5.2±1.8
WF rat islet cells	8.1±2.5	0.3±0.3

Splenic lymphoid cells from BB/W diabetic rats were isolated by centrifugation on Ficoll density gradients and then incubated on nylon wool columns. Nylon wool nonadherent and adherent cells were collected separately and used as effectors in a cytotoxicity assay. Values are mean±SEM percent specific ^{51}Cr release after an 8-h incubation of effector cells (500,000/well) with ^{51}Cr-labeled target cells (25,000/well) for $n = 3$ diabetic BB/W rats in three separate experiments.

Table III. Effects of Nylon Wool Purification of BB/W Rat Spleen Cells on CMC

BB/W rat effector spleen cells	RIN targets	GH_3 targets
Diabetic		
Total mononuclear cells	22.6±3.0 (7)	5.8±1.6 (6)
Nonadherent cells	32.7±3.5 (7)*	9.9±1.9 (6)
DP		
Total mononuclear cells	11.4±1.1 (7)	3.3±1.2 (6)
Nonadherent cells	16.3±1.6 (7)‡	5.6±0.6 (6)
DR		
Total mononuclear cells	9.0±1.7 (7)	3.4±1.6 (4)
Nonadherent cells	8.7±0.6 (5)	4.9±1.0 (4)

BB/W rat splenic lymphoid cells were isolated by centrifugation on Ficoll density gradients (total mononuclear cells); a portion of these were purified further on nylon wool (nonadherent cells). The two cell fractions were incubated, separately, as effector cells (500,000/well) with ^{51}Cr-labeled cells from a rat islet (RIN) or pituitary (GH_3) cell line as targets (25,000/well) for 8 h. Values are mean±SEM percent specific ^{51}Cr release for numbers of BB/W rats shown in parentheses.
* $P < 0.005$ vs. effects of total mononuclear cells from corresponding rats (paired t test).
‡ $P < 0.02$.

was seen for DR cells. In contrast, passage of the splenic cells through nylon wool did not signficantly increase the small cytotoxic effects of diabetic, DP, and DR spleen cells on GH_3 target cells.

Enrichment for islet-directed cytotoxicity in the nylon wool nonadherent splenic lymphoid cells of diabetic and DP rats suggested that the cytotoxic effector cells were T lymphocytes and/or NK cells. The latter possibility was explored by using an NK-sensitive cell line (G_1-TC rat thymoma) as a target. G_1-TC cells were lysed by diabetic and DP spleen cells in a dose-dependent manner (Fig. 2). Furthermore, the cytotoxic effects of individual rat splenic cell preparations on RIN islet cells were proportional to their effects on G_1-TC cells, suggesting that the cells cytotoxic to RIN cells might be NK cells. This possibility was explored further by using another NK-sensitive cell line (YAC-1 mouse lymphoma) as target, and by comparing the cytotoxic effects of splenic cells from diabetic, DP, and DR rats on RIN and YAC-1 cells. There was a highly significant correlation between the cytotoxic effects of splenic cells from diabetic, DP, and DR rats on RIN and YAC-1 cells (Fig. 3). In addition, unlabeled YAC-1 cells competed proportionally for the lysis of ^{51}Cr-labeled YAC-1 and RIN cells by diabetic effector cells (Table IV). This indicated that RIN cells shared a common determinant(s) with the NK-sensitive YAC-1 cells, and implied that the cells cytotoxic to RIN islet cells might be NK cells.

Since NK cell cytotoxic activity has been attributed to LGL (25), we separated the Ficoll-isolated total splenic mononuclear cells further on Percoll density gradients into fractions enriched in small lymphocytes or large lymphocytes/monocytes (containing LGL), then tested the separate cell fractions in cytotoxicity assays (Table V). The large lymphocyte/monocyte cell fractions were significantly more cytotoxic to RIN islet cells than were the total (unfractionated) mononuclear cells from the same animals, for diabetic rats (21±2.4 vs. 7.0±2.3%, $P < 0.001$), DP rats (15.2±2.4 vs. 8.5±0.7%, $P < 0.05$), and DR rats (8.0±1.0 vs. 0.2±1.5%, $P < 0.05$). Also, the cytotoxic effects of the large lymphocyte/monocyte fractions from diabetic (21.8±2.4%) and DP (15.2±2.4%) rats were significantly higher than that of the large lymphocyte/monocyte fraction from DR rats (8.0±1.0%). In contrast to the significantly increased cytotoxic effects of the large lymphocyte/monocyte fractions using RIN islet cells as targets, cytotoxicity to GH_3 pituitary cells remained negligible.

Effects of an anti-NK cell serum on cytotoxicity of BB/W rat spleen cells. In order to demonstrate further the NK cell identity of the cytotoxic spleen cells, nylon wool-purified (nonadherent) splenic mononuclear cells from a BB/W diabetic rat were incubated with an antiserum to NK cells (anti-asialo GM1 serum) and complement, to lyse NK cells, and cytotoxic activity of the cells remaining was assayed (Table VI). The cytotoxic effects of the diabetic spleen cells were reduced in a dose-dependent fashion after incubation with anti-asialo GM1 serum and complement, and the reductions in cytotoxicity were similar for YAC-1 and RIN targets. A 1:100 dilution of antiserum reduced cytotoxicity to both YAC-1 and RIN cells to 12% of that effected by diabetic spleen cells treated with complement alone, and this was associated with the removal of ~20% of the effector cells, indicating that the cytotoxic cells accounted for only a small proportion of the diabetic rat splenic lymphoid cells, even after enrichment in a nylon wool nonadherent cell fraction.

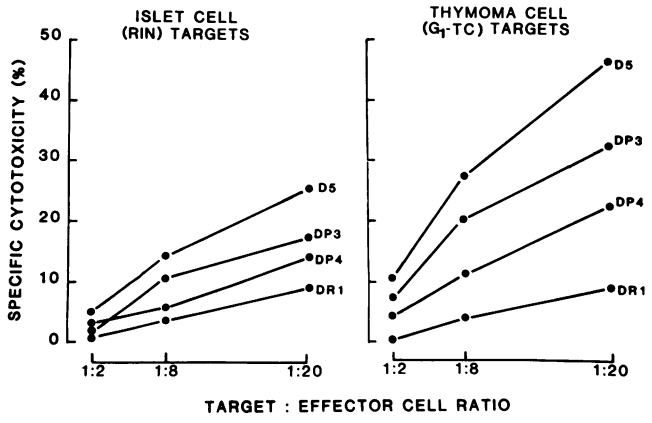

Figure 2. Specific lysis (cytotoxicity) of ^{51}Cr-labeled target cells (25,000/well) from a rat islet (RIN) and an NK-sensitive rat thymoma cell line (G_1-TC) by splenic lymphoid cells (effectors, 500,000/well) from a BB/W diabetic rat (D5), two DP rats (DP3 and DP4), and a DR rat (DR1), as a function of the target/effector cell ratio.

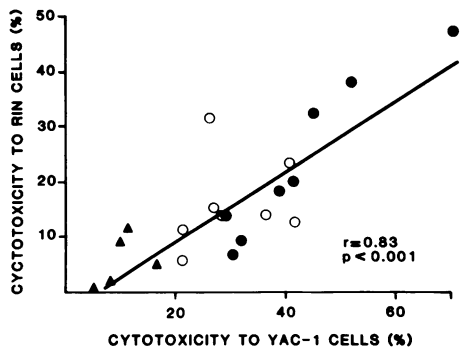

Figure 3. Relationship between cytotoxic effects (percent specific ^{51}Cr release) to cells from a rat islet (RIN) and an NK-sensitive mouse lymphoma line (YAC-1) by splenic lymphoid cells from BB/W diabetic (●), DP (○), and DR (▲) rats.

Table IV. Competitive Effects of NK-sensitive Cells (YAC-1) on Cytotoxic Effects of BB/W Diabetic Rat Spleen Cells

BB/W diabetic effector cells	YAC-1 competing cells	YAC-1 targets	RIN targets
		%	%
500,000	0	27.9 (100)	26.8 (100)
500,000	50,000	10.2 (37)	11.8 (44)
500,000	200,000	4.8 (17)	3.1 (12)

Nylon wool purified (nonadherent) splenic lymphoid cells from a BB/W diabetic rat (effectors) were incubated for 8 h with ^{51}Cr-labeled YAC-1 or RIN target cells (25,000/well), in the absence or presence of unlabeled (competing) YAC-1 cells. Values for percent specific ^{51}Cr release from target cells are shown for a representative experiment; values in parentheses compare cytotoxicity in the presence of competing YAC-1 cells with that found in the absence of competing cells.

Effects of an anti-NK cell serum on monoclonal antibody-defined lymphocyte subpopulations and cytotoxicity. In order to assess the effects of the anti-NK cell serum used (anti-asialo GM1) on the phenotypic composition of the splenic lymphoid cells from BB/W rats, nylon wool-purified (nonadherent) splenic lymphoid cells were incubated with this antiserum and complement, or complement alone, and then with monoclonal antibodies to the different lymphocyte subsets (Table VII). The percentages of NK cells were calculated by subtraction of OX19+ (T) cells from W3/13+ (T + NK) cells and expressed as W3/13+ OX19− cells. Treatment of diabetic spleen cells with anti-asialo GM1 serum and complement resulted in a significant decrease (65±18%, $P < 0.05$) in the W3/13+ OX19− population containing NK cells. Also, OX8+ ($T_{c/s}$ + NK) cells were significantly decreased (53±12%, $P < 0.05$) after treatment with the antiserum. The decreases in W3/13+ OX19− cells and in OX8+ cells after treatment of the diabetic spleen cells with anti-asialo GM1 serum were accompanied by increases in OX19+ (T) cells and Ig+ (B) cells. In the DR rats, the population of W3/13+ OX19− cells was very small (3.7±2.2%) compared with that in the diabetic rats (31.1±8.6%), and this small population was completely eliminated by treatment with anti-asialo GM1 serum (−0.2±1.3%). The smallness of the NK cell population in the DR rats was also evident from the small (insignificant) decrease

Table V. Effects of Percoll Purification of BB/W Rat Spleen Cells on CMC

Targets	BB/W rat effector spleen cell fractions	Total mononuclear	Small lymphocytes	Large lymphocytes and monocytes
RIN cells	Diabetic (6)	7.9±2.3	8.2±2.0	21.8±2.4*§
	DP (4)	8.5±0.7	11.2±3.5	15.2±2.4‡‖
	DR (8)	0.2±1.5	3.0±1.5	8.0±1.0‡
GH_3 cells	Diabetic (4)	0.9±1.0	3.1±0.9	4.2±1.4
	DP (3)	1.8±2.0	3.2±1.6	1.5±1.9
	DR (6)	1.3±1.0	2.2±0.6	1.1±0.6

BB/W rat splenic lymphoid cells were isolated by centrifugation on Ficoll density gradients (total mononuclear cells); a portion of these were separated further on Percoll density gradients into a high density fraction enriched in small lymphocytes, and a low density fraction enriched in large lymphocytes plus monocytes (see Methods). The different cell fractions were incubated as effector cells (100,000/well) with ^{51}Cr-labeled cells from a rat islet (RIN) or pituitary (GH_3) cell line as targets (25,000/well) for 8 h. Values are mean±SEM percent specific ^{51}Cr release for numbers of BB/W rats shown in parentheses.
* $P < 0.001$.
‡ $P < 0.05$ vs. effects of total mononuclear cells from corresponding rats (paired t test).
§ $P < 0.001$.
‖ $P < 0.02$ vs. effects of large lymphocytes and monocytes from DR rats.

Table VI. Effects of an Anti-NK Cell Serum on the Cytotoxic Effects of Diabetic BB/W Rat Spleen Cells

Anti-NK cell serum	Cell recovery × 10^{-6}	YAC-1 targets	RIN targets
	%	%	%
0	3.6±0.3 (100)	22.2±6.2 (100)	16.7±0.7 (100)
1:1,600	3.4±0.5 (94)	15.0±4.5 (68)	14.5±1.8 (87)
1:400	3.1±0.6 (86)	7.1±2.9 (32)	6.0±1.2 (36)
1:100	2.9±0.3 (81)	2.7±1.2 (12)	2.0±0.8 (12)

Nylon wool purified (nonadherent) splenic lymphoid cells from a BB/W diabetic rat were incubated with anti-asialo GM1 (anti-NK cell) serum and complement. Viable cells were counted (cell recovery) and incubated as effectors (500,000/well) with ^{51}Cr-labeled YAC-1 or RIN cells as targets (25,000/well) for 8 h. Mean values±SEM for percent specific ^{51}Cr release from YAC-1 and RIN targets are shown for three experiments; values in parentheses compare effects of diabetic cells treated with anti-NK cell serum and complement to those of the cells treated with complement alone.

Table VII. Effects of an Anti-NK Cell Serum on the Splenic Lymphocyte Subsets in BB/W Rats

Lymphocyte subsets	Diabetic spleen cells: Control	Diabetic spleen cells: Anti-NK cell serum	DR spleen cells: Control	DR spleen cells: Anti-NK cell serum
W3/13+	53.6±4.9	46.1±2.7	64.4±4.0	62.6±4.5
OX19+	22.5±4.7	33.3±7.9	60.6±5.6	62.8±3.7
W3/13+ OX19−	31.1±8.6	12.8±6.9*	3.7±2.2	−0.2±1.3
OX8+	29.8±5.6	15.0±5.2*	29.9±4.9	26.2±6.4
W3/25+	25.9±3.7	25.2±12.8	36.2±4.5	37.4±6.4
Ig+	14.3±6.4	18.7±7.9	6.7±4.2	12.2±3.9

Nylon wool purified (nonadherent) splenic lymphoid cells from BB/W rats were incubated with anti-asialo GM1 (anti-NK cell) serum (1:400) and complement, or complement alone (control). Viable cells were counted and incubated, separately, with mouse monoclonal antibodies to the different rat lymphocyte subsets shown, washed, then incubated with FITC-conjugated goat anti-mouse antiserum, washed, and fixed in 4% paraformaldehyde. Immunoglobulin-positive (Ig+) cells (B lymphocytes) were identified directly, by using FITC-conjugated rabbit anti-rat Ig. The W3/13+ OX19− subset is calculated as the difference between W3/13+ and OX19+ cells. Mean values±SEM for percent fluorescent cells are shown for four separate experiments.
* $P < 0.05$ vs. control value (paired t test).

in OX8+ ($T_{c/s}$ + NK) cells after the antiserum treatment (29.9±4.9–26.2±6.4%).

The effects of depletion of monoclonal antibody-defined subsets containing NK cells (W3/13+ OX19− and OX8+) by anti-asialo GM1 serum on the cytotoxic activity of the BB/W rat spleen cells is shown in Table VIII. Diabetic spleen cells treated with the antiserum and complement were significantly less cytotoxic to YAC-1 cells (42.4±4.4 vs. 18.7±4.8%, $P < 0.001$), RIN cells (15.7±4.9 vs. 6.9±2.3%, $P < 0.05$), and WF islet cells (9.3±1.4 vs. 3.1±0.9%, $P < 0.05$). Moreover, these decreases in the cytotoxic effects of the diabetic spleen cells to YAC-1 (56%), RIN (57%), and WF islet cells (67%) were similar to the decreases in proportions of W3/13+ OX19− cells (59%) or OX8+ cells (50%) observed in these same antiserum-treated splenic cell preparations (Table VII). Table VIII also shows that splenic cells (control) from diabetic rats were significantly more cytotoxic to YAC-1 ($P < 0.001$), RIN ($P < 0.05$), and WF islet cells ($P < 0.005$) than splenic cells (control) from DR rats. Also, the low levels of cytotoxicity delivered by DR spleen cells were completely eliminated by anti-asialo GM1 serum (Table VIII), and this was accompanied by the total removal of W3/13+ OX19− cells by this antiserum (Table VII).

Discussion

In a recent study (18) we demonstrated that splenic lymphoid cells from BB/W diabetic and DP rats could lyse cells and decrease insulin content in monolayer cultures of WF rat islet cells and a rat islet cell line (RIN). In the present study, we demonstrate that splenic lymphoid cells from BB/W diabetic rats, and to a lesser extent DP rats, can lyse MHC-compatible WF (RTI^u) rat islet cells, as well as MHC-incompatible Lewis (RTI^l) rat islet cells and RIN cells derived from an islet tumor in a New England Deaconess Hospital rat (RTI^g). Neither WF or Lewis splenic lymphocytes nor rat pituitary cells (GH_3 line) were lysed by splenic cells from diabetic or DP rats. These studies provide data in vitro to support the hypothesis that a cell-mediated anti-islet immune response is involved in the destruction of islet beta cells resulting in diabetes in the BB rat.

Table VIII. Effects of an Anti-NK Cell Serum on the Cytotoxic Effects of BB/W Rat Spleen Cells

	Diabetic spleen cells		DR spleen cells	
Target cells	Control	Anti-NK cell serum	Control	Anti-NK cell serum
YAC-1 cells	42.4±4.4	18.7±4.8*	6.0±2.3	1.0±0.7
RIN cells	15.7±4.9	6.9±2.3‡	4.0±1.5	0.1±0.1
WF rat islet cells	9.3±1.4	3.1±0.9‡	2.4±0.6	0.6±0.4

Nylon wool purified (nonadherent) splenic lymphoid cells from BB/W rats were incubated with anti-asialo GM1 (anti-NK cell) serum and complement or complement alone (control). Viable cells were counted and incubated (effectors, 500,000/well) with ^{51}Cr-labeled YAC-1, RIN, or WF rat islet cells (targets, 25,000/well) for 8 h. Mean values±SEM for percent specific ^{51}Cr release from target cells are shown for the same four experiments shown in Table VII.
* $P < 0.001$ vs. control (paired t test).
‡ $P < 0.05$.

The lack of MHC restriction for the islet-directed cytotoxic response detected in BB/W diabetic rats in the present study has precedents. In another study (26), both MHC-compatible (RTI^u) and MHC-incompatible (RTI^l, RTI^b, and RTI^n) rat isolated islet grafts revealed lymphocytic insulitis after transplantation into DP BB/W rats. Insulitis in the islet grafts was almost always limited to recipients that became diabetic or in which insulitis appeared in the recipient's endogenous islets. Adrenal grafts from the same donors were free of inflammation. The authors concluded that BB/W rat immune insulitis is not MHC-restricted, and may be directed against otherwise normal islet cells. Similarly, in another animal model of diabetes mellitus, the mouse made diabetic with multiple low doses of streptozotocin, islet-specific cytotoxic cells in the spleen of diabetic mice were demonstrated in a xenogeneic cytotoxicity assay (27) by using rat RIN cells as targets, as were used in the present study.

The results of our experiments in vitro, therefore, support these recent reports suggesting that cell-mediated islet cell destruction may not be MHC-restricted. We extend the observations by demonstrating that the islet-directed cytotoxic activity in BB rats may be attributed to cells with NK-like properties. The evidence for this conclusion is based on the findings that (1) two different NK-sensitive targets (G_1-TC rat thymoma cells and YAC-1 mouse lymphoma cells) were lysed by splenic lymphoid cells from diabetic and DP BB/W rats; (2) NK-sensitive cells (YAC-1) competed effectively for the lysis of RIN islet cells by diabetic spleen cells; (3) lysis of RIN islet cells was amplified by enrichment of the effector cells for large lymphocytes/monocytes on Percoll density gradients (NK cells are large granular lymphocytes) (25); (4) an anti-NK cell serum (anti-asialo GM1) reduced cytotoxicity to RIN and YAC-1 cells in a proportional dose-dependent fashion; and (5) the decreases in the islet-directed cytotoxic effects of diabetic spleen cells, treated with anti-asialo GM1 serum, were accompanied by proportional decreases in monoclonal antibody-defined subsets containing NK cells (W3/13+ OX19− and OX8+).

The anti-asialo GM1 serum we used to remove NK cells is not entirely specific for NK cells; there is also some binding to monocytes, immature thymocytes, and polymorphonuclear leukocytes in the rat (28). In our experiments, however, anti-asialo GM1 serum and complement did not reduce total T lymphocytes (OX19), T-helper cells (W3/25), or B lymphocytes (Ig+) from splenic cell preparations of BB/W rats (Table VII). Only the population containing NK cells (W3/13+ OX19− and OX8+) were decreased by this antiserum. Indeed, the OX8+ population includes a high percentage of large granular lymphocytes (NK cells) in the rat (28, 29), and it has been demonstrated recently that virtually all OX8+ cells in BB/W DP and acute diabetic rats are phenotypic NK cells (30).

Although these data provide the first evidence that NK cell numbers and activity are increased in BB DP and diabetic rats, this observation is supported by earlier studies. Since the now well-documented generalized T-lymphopenia (in blood, lymph nodes, spleen, and thymus) was first described in BB DP and diabetic rats, it has been recognized that these rats possess a significantly greater subpopulation(s) of lymphoid cells not identified by available monoclonal antibodies for T and B lymphocyte subsets. Recently, an increase in a subpopulation of large thymocytes has been reported in BB DP rats (31). This cell population was identified to have the monoclonal antibody-defined phenotype W3/13+ OX8+ OX19− W3/25− (31), and

this phenotype identifies NK cells in rats of different strains (28, 29), including BB/W rats (30, 32).

The reason(s) for the increases in NK cells in BB diabetic and DP rats are not clear. This could be associated with the T cell-deficient state existent in the BB DP rat, by analogy to the findings of increased NK activity in athymic nude mice (33) and rats (26). A defect in thymocyte maturation has been suggested as a cause of the T lymphopenia observed in BB DP rats (1). This may then be associated with an increase in immature T cells. Indeed, we observed that many of the splenic cells from diabetic and DP animals identified as large lymphocytes/monocytes included, in addition to LGL (NK cells), immature lymphoid and/or lymphoblastic cells. Immature mouse T cells have been reported to bear asialo GM1 determinants (19) and, therefore, removal of such cells by treatment with anti-asialo GM1 serum in our experiments may have contributed to the decrease in islet-directed cytotoxicity observed. Therefore, we must consider the possibility that the W3/13+ OX19− population identified to contain NK cells may include immature T cells, and that the latter may have participated in the islet-directed cytotoxicity observed.

Previous studies have shown that activation of BB/W rat spleen cells with Con A is required for the effector cells to passively transfer diabetes in vivo (15–17). Similarly, we have demonstrated that cytotoxic effects of BB/W diabetic spleen cells against RIN islet cells in vitro is increased after preincubation of the diabetic effector cells with Con A (18). Although these observations implicate activated T cells in the cytotoxic process, it should be noted that NK cells as well as T cells can be activated by, and proliferate in response to, the T cell growth factor interleukin 2 (34), a byproduct of Con A stimulation.

Whereas the present study demonstrates that BB/W diabetic and DP rats have increased NK cell-mediated cytotoxicity, it is difficult to explain the islet-directed cytotoxicity within the classical definition of NK cell target specificities. The WF and Lewis rat islet cells are nontumor and presumably not carrying viral antigens on their plasma membranes. Nevertheless, NK cells are attractive as possible islet-directed cytotoxic effector cells for a number of reasons. First, NK cells are not MHC-restricted. Likewise, in the present study, as well as in recent studies of islet transplantation in BB/W rats (26) and streptozotocin-induced insulitis in mice (27), the immune attack against pancreatic islets was demonstrated to be not MHC-restricted. Second, the cytotoxic population in the present study was nylon wool nonadherent, making it unlikely that the lysis of islet cells was caused by macrophages. Whereas monocytes and macrophages are known to exhibit spontaneous cytolytic activity against many tumor cell lines, only NK cells are characterized as causing rapid cell lysis in a 4–8-h ^{51}Cr release assay using YAC-1 targets (35). Third, if the cytotoxic cells in the BB/W rats (RTI^u) responsible for killing WF (RTI^u) and Lewis (RTI^l) islet cells were classical cytotoxic T cells, then these would be either MHC-restricted and capable of reacting to islet antigens (in the case of WF target cells) or they would be non–MHC-restricted allospecific cells capable of recognizing alloantigen but not islet antigen. It seems improbable, however, that BB/W rats have T cells presensitized to alloantigens. This is supported by the well-documented diminished alloreactivity of lymphocytes from BB diabetic and DP rats in mixed lymphocyte cultures (36, 37), and by the fact that we did not detect lysis of allogeneic (Lewis) lymphocytes by splenic cells from BB/W diabetic or DP rats. These observations suggest that the cells responsible for the killing of WF and Lewis islet cells are one and the same. The islet-directed cytotoxic cells are likely to be NK cells, since the killing of WF islet cells, RIN islet cells, and YAC-1 cells were similarly reduced after treatment with anti-asialo GM1 serum, and since this was associated with proportional reductions in monoclonal antibody-defined populations containing NK cells (W3/13+ OX19− and OX8+). Lastly, it is becoming apparent that NK activity measured in vitro reflects the participation of several distinct subpopulations with different target specificities, and evidence for nonlymphoreticular targets for NK cells is beginning to emerge (38).

Some evidence for cell-mediated immunity has been obtained also in human subjects with IDDM. Lymphocyte mediation of islet cell destruction has been suggested by cytotoxicity assays in vitro (39). These studies in human IDDM require confirmation, however, and further identification of the putative islet cytotoxic cell(s) remains to be completed. In contrast to the BB rat, human subjects do not demonstrate lymphopenia or defects in proportions of T and B lymphocytes (40). Abnormalities of peripheral blood lymphocyte subpopulations have been reported, however, and these include decreased T-suppressor cell numbers and activity (41–43), the presence of activated (Ia+) T cells (44, 45), and increased killer cells (46) and killer cell activity (47). Killer cells are responsible for antibody-dependent cell-mediated cytotoxicity. NK cells, however, can also mediate this function, and NK and killer activities appear to be two manifestations of the same cell (48).

In summary, we have demonstrated that NK cell activity is increased in BB/W acutely diabetic and DP rats, and that islet cells can serve as targets for these NK cells in vitro. Clearly, we have no direct evidence that diabetes mellitus is an NK cell-mediated disease in the BB rat. Since NK activity and islet-directed cytotoxicity were detected in nondiabetic, DP rats, although at lower levels than in acutely diabetic rats, it appears that NK cells per se, may be insufficent to cause islet beta cell destruction and diabetes. However, BB DP rats, although not hyperglycemic and glucosuric, may manifest varying degrees of glucose intolerance and insulitis (49). Therefore, it remains to be determined whether the present findings of increased NK cell cytotoxicity to islets in vitro may contribute to the development of insulitis and diabetes in the BB rat in vivo. That this may occur is suggested by the recent reports that virtually all OX8+ cells in DP and acutely diabetic BB/W rats are phenotypic NK cells (30), and treatment of young DP BB/W rats with OX8 monoclonal antibody can prevent the appearance of diabetes in these animals (50).

Acknowledgments

The authors thank Ms. Carol Quigley and Mr. Walter Sumoski for their skillful technical assistance.

This work was supported by a grant from the U. S. Public Health Service, National Institute of Arthritis, Diabetes, and Digestive and Kidney Disease (AM-25832).

References

1. Yale, J.-F., and E. B. Marliss. 1984. Altered immunity and diabetes in the BB rat. *Clin. Exp. Immunol.* 57:1–11.

2. Seemayer, T. A., G. S. Tannenbaum, H. Goldman, and E. Colle.

1982. 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.* 106:237–249.

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

4. Like, A. A., R. M. Forster, B. A. Woda, and A. A. Rossini. 1983. T-cell subsets in islets and lymph nodes of BioBreeding Worcester (BB/W) rats. *Diabetes.* 32:51A. (Abstr.)

5. Nakhooda, A. F., A. A. Like, C. L. Chappel, F. T. Murray, and E. B. Marliss. 1976. The spontaneously diabetic Wistar rat: metabolic and morphologic studies. *Diabetes.* 26:100–112.

6. Like, A. A., E. Kislauskis, R. M. Williams, and A. A. Rossini. 1982. Neonatal thymectomy prevents spontaneous diabetes mellitus in the BB/W rat. *Science (Wash. DC).* 216:644–646.

7. Naji, A., W. K. Silvers, H. Kimura, D. Belgrau, J. F. Markham, and C. F. Barker. 1983. Analytical and functional studies on the T-cells of untreated and immunologically tolerant diabetes-prone BB rats. *J. Immunol.* 130:2168–2172.

8. Naji, A., W. K. Silvers, D. Belgrau, A. O. Anderson, S. Plotkin, and C. F. Barker. 1981. Prevention of diabetes in rats by bone marrow transplantation. *Ann. Surg.* 194:328–338.

9. Like, A. A., A. A. Rossini, M. C. Appel, D. I. Guberski, and R. M. Williams. 1983. Spontaneous diabetes mellitus: reversal and prevention in the BB/W rat with antiserum to rat lymphocytes. *Science (Wash. DC).* 206:1421–1423.

10. Rossini, A. A., S. Slavin, B. A. Woda, M. Geisberg, A. A. Like, and J. P. Mordes. 1984. Total lymphoid irradiation prevents diabetes in the Bio-Breeding/Worcester (BB/W) rat. *Diabetes.* 33:543–547.

11. Like, A. A., M. Anthony, D. I. Guberski, and A. A. Rossini. 1983. Spontaneous diabetes mellitus in the BB/W rat. Effects of glucocorticoids, cyclosporin-A, and antiserum to rat lymphocytes. *Diabetes.* 32:326–330.

12. Laupacis, A., C. R. Stiller, C. Gardell, P. Keown, J. Dupre, A. C. Wallace, and P. Thibert. 1983. Cyclosporin prevents diabetes in BB Wistar rats. *Lancet.* I:10–12.

13. Rossini, A. A., J. P. Mordes, A. M. Pelletier, and A. A. Like. 1983. Transfusions of whole blood prevent spontaneous diabetes mellitus in the BB/W rats. *Science (Wash. DC).* 219:975–977.

14. Rossini, A. A., D. Faustman, B. A. Woda, A. A. Like, I. Szymanski, and J. D. Mordes. 1984. Lymphocyte transfusions prevent diabetes in the Bio-Breeding/Worcester rat. *J. Clin. Invest.* 74:39–46.

15. Koevary, S., A. A. Rossini, W. Stoller, W. Chick, and R. M. Williams. 1983. Passive transfer of diabetes in BB/W rats. *Science (Wash. DC).* 220:727–728.

16. Like, A. A., E. J. Weringer, A. Holdash, P. McGill, and A. A. Rossini. 1985. Adoptive transfer of autoimmune diabetes in BioBreeding Worcester (BB/W) inbred and hybrid rats. *J. Immunol.* 134:1583–1587.

17. Koevary, S. B., D. E. Williams, R. M. Williams, and W. Chick. 1985. Passive transfer of diabetes from BB/W to Wistar-Furth rats. *J. Clin. Invest.* 75:1904–1907.

18. MacKay, P., A. Boulton, and A. Rabinovitch. 1985. Lymphoid cells of BB/W diabetic rats are cytotoxic to islet beta cells *in vitro*. *Diabetes.* 34:706–709.

19. Kasai, M., M. Iwamori, Y. Nagai, K. Okamura, and T. Tada. 1980. A glycolipid on the surface of mouse natural killer cells. *Eur. J. Immunol.* 10:175–180.

20. Lacy, P. E., and M. Kostianovski. 1967. Method for the isolation of intact islets of Langerhans from the rat pancreas. *Diabetes.* 16:35–39.

21. Rabinovitch, A., T. Russel, F. Shienvold, J. Noel, N. Files, Y. Patel, and M. Ingram. 1982. Preparation of rat islet B-cell enriched fractions by light-scatter flow cytometry. *Diabetes.* 31:939–943.

22. Gazdar, A. F., W. L. Chick, H. K. Oie, H. L. Sims, D. King, G. C. Weir, and V. Lauris. 1980. Continuous, clonal, insulin- and somatostatin-secreting cell lines established from a transplantable rat islet cell tumor. *Proc. Natl. Acad. Sci. USA.* 77:3519–3523.

23. Bancroft, R. C., and A. A. Tashjian. 1971. The growth in suspension culture of rat pituitary cells which produce growth hormone and prolactin. *J. Exp. Cell Res.* 64:125–128.

24. Oehler, J. R., L. R. Lindsay, M. E. Nunn, and R. B. Herberman. 1978. Natural cell-mediated cytotoxicity in rats. I. Tissue and strain distribution and demonstration of a membrane receptor for the Fc portion of IgG. *Int. J. Cancer.* 21:204–209.

25. Reynolds, C. W., T. Timonen, and R. B. Herberman. 1981. Natural killer (NK) cell activity in the rat. I. Isolation and characterization of the effector cells. *J. Immunol.* 127(1):282–287.

26. Weringer, E. J., and A. A. Like. 1985. Immune attack on pancreatic islet transplants in the spontaneously diabetic BioBreeding/Worcester (BB/W) rat is not MHC restricted. *J. Immunol.* 134(4):2383–2386.

27. McEvoy, R. C., J. Andersson, S. Sandler, and C. Hellerstrom. 1984. Multiple low-dose streptozotocin-induced diabetes in the mouse: evidence for stimulation of a cytotoxic cellular immune response against an insulin-producing beta cell line. *J. Clin. Invest.* 74:715–722.

28. Reynolds, C. W., S. O. Sharrow, J. R. Ortaldo, and R. B. Herberman. 1981. Natural killer activity in the rat. II. Analysis of surface antigens on LGL by flow cytometry. *J. Immunol.* 127:2204–2208.

29. Cantrell, D. A., R. A. Robins, C. G. Brooks, and R. W. Baldwin. 1982. Phenotype of rat natural killer cells defined by monoclonal antibodies marking rat lymphocyte subsets. *Immunology.* 45:97–103.

30. Woda, B. A., A. A. Like, C. Padden, and M. L. McFadden. 1986. Deficiency of phenotypic cytotoxic-suppressor T lymphocytes in the BB/W rat. *J. Immunol.* 136:856–859.

31. Jackson, R. A., J. Buse, A. L. Goldstein, and G. S. Eisenbarth. 1984. BB rat thymus: increase of large W313+, 6B2+, OX19− thymocytes. *Diabetes.* 33(Suppl. 1):60A. (Abstr.)

32. Woda, B. A., M. L. McFadden, R. M. Welsh, and K. M. Bain. 1984. Separation and isolation of rat natural killer (NK) cells from T cells with monoclonal antibodies. *J. Immunol.* 132(5):2183–2184.

33. Kaminsky, S. G., I. Nakamura, and G. Cudkowicz. 1985. Genetic control of the natural killer cell activity in SJL and other strains of mice. *J. Immunol.* 135:665–671.

34. Henney, C. S., K. Yuribayashi, P. E. Kern, and S. Gillis. 1981. Interleukin-2 augments natural killer cell activity. *Nature (Lond.).* 291: 335–338.

35. Tagliabue, A., A. Mantovani, M. Kilgallen, R. B. Herberman, and J. L. McCoy. 1979. Natural cytotoxicity of mouse monocytes and macrophages. *J. Immunol.* 122:2363–2370.

36. Elder, M. E., and N. K. MacLaren. 1983. Identification of profound peripheral T lymphocyte immunodeficiencies in the spontaneously diabetic BB rat. *J. Immunol.* 130:1723–1731.

37. Bellgrau, D., A. Naji, W. K. Silvers, J. F. Markmann, and C. F. Barker. 1982. Spontaneous diabetes in BB rats-evidence for a T-cell dependent immune response defect. *Diabetologia.* 23:359–364.

38. Vanky, F., and E. Klein. 1982. Alloreactive cytotoxicity of interferon-triggered human lymphocytes detected with tumor biopsy targets. *Immunogenetics.* 15:31–39.

39. Charles, M. A., M. Suzuki, N. Waldeck, L. E. Dodson, L. Slater, K. Ong, A. Kershnar, B. Buckingham, and M. Golden. 1983. Immune islet killing mechanisms associated with insulin-dependent diabetes: *in vitro* expression of cellular and antibody-mediated islet cell cytotoxicity in humans. *J. Immunol.* 130:1189–1194.

40. Hahn, S., R. Kaye, and B. Falkner. 1976. Subpopulations of peripheral lymphocytes in juvenile diabetes. *Diabetes.* 25:101–103.

41. Buschard, K., C. Ropke, S. Madsbad, J. Mehlsen, and J. Rygaard. 1983. T lymphocyte subsets in patients with newly diagnosed type I (insulin-dependent) diabetes: a prospective study. *Diabetologia.* 25:247–251.

42. Ledermann, M. M., J. J. Ellner, and H. M. Rodman. 1981. De-

fective suppressor cell generation in juvenile onset diabetes. *J. Immunol.* 127:2051–2055.

43. Fairchild, R. S., J. L. Kyner, and N. I. Abdou. 1982. Specific immunoregulation abnormality in insulin-dependent diabetes mellitus. *J. Lab. Clin. Med.* 99:175–186.

44. Jackson, R. A., M. A. Morris, B. F. Haynes, and G. S. Eisenbarth. 1982. Increased Ia-antigen bearing T cells in type I diabetes mellitus. *N. Engl. J. Med.* 306:785–788.

45. Alviggi, L., P. J. Hoskins, D. A. Pyke, C. Johnson, D. E. H. Tee, R. D. G. Leslie, and D. Vergani. 1984. Pathogenesis of insulin-dependent diabetes: a role for activated T lymphocytes. *Lancet.* II:4–6.

46. Pozzili, P., M. Sensi, A. N. Gorsuch, G. F. Botazzo, and A. G. Cudworth. 1979. Evidence for raised K cell levels in Type I diabetes. *Lancet.* II:173–175.

47. Sensi, M., P. Pozzili, A. N. Gorsuch, G. F. Botazzo, and A. G. Cudworth. 1981. Increased killer cell activity in insulin dependent (Type I) diabetes mellitus. *Diabetologia.* 20:106–109.

48. Herberman, R. B., and J. R. Ortaldo. 1981. Natural killer cells: their role in defenses against disease. *Science (Wash. DC).* 214:24–30.

49. Marliss, E. B., A. F. Nakhooda, P. Poussier, and A. A. F. Sima. 1981. The diabetic syndrome of the "BB" Wistar rat: possible relevance to type I (insulin-dependent) diabetes in man. *Diabetologia.* 22:225–232.

50. Weringer, E. J., K. Byman, V. Biggs, D. L. Guberski, and A. A. Like. 1985. Monoclonal antibodies to rat OX-8 and OX-19 reactive T-cells prevent BB/W diabetes. *Diabetes Res. Clin. Practice.* (Suppl. 1)1: S599. (Abstr.)