Rapid Publications

Isolation of T-Lymphocyte Lines with Specificity for Islet Cell Antigens from Spontaneously Diabetic (Insulin-dependent) Rats

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SUMMARY

T-lymphocyte lines specific for islet cell antigens were isolated from the spleen and pancreas of newly diabetic BB rats or from the related strain BBUF. These cell lines were grown in continuous culture with interleukin-2 (IL-2) containing medium for >60 days. Such T-lymphocytes responded by proliferation and IL-2 secretion in the combined presence of islet cell antigens and major histocompatibility (MHC)-matched antigen-presenting cells. By fluorescence-activated cell sorter (FACS) analysis the cells were W3/13$^+$, W3/25$^+$, and OX8$^-$. Thus, both functionally and by cell-surface-marker analysis they appear to be of the T-helper phenotype. The long-term growth and study of anti-islet T-lymphocyte lines will permit a detailed analysis of the role of T-lymphocytes in the pathogenesis of IDDM. DIABETES 33:801–803, August 1984.

The BB rat is prone to the spontaneous development of insulitis and insulin-dependent diabetes mellitus (IDDM).[1] Studies performed on diabetic rats suggest that T-lymphocytes play an important role in initiating islet cell injury. For example, concanavalin A (con A)-activated T-cell blasts derived from diabetic rats can transfer disease to nondiabetic BB rats,[2] or to major histocompatibility (MHC)-matched normal rats previously treated with cyclophosphamide.[3]

BB rats are characterized by severe abnormalities of the immune system, including a markedly decreased ability to produce interleukin 2 (IL-2).[4] As well, when BB rats are crossed with other strains, only offspring with at least one RT1^u haplotype develop IDDM.[5,6] Thus, this model of IDDM, like several other organ-specific autoimmune diseases, is dependent on the presence of a permissive MHC haplotype. By crossing BB rats with the Buffalo strain (prone to develop thyroiditis) we are in the process of deriving a new strain, the Buf x BB F7 (BBUF) (see MATERIALS), which is also prone to IDDM. BBUF rats are similar to BB rats except that their immune abnormalities are less severe and sometimes transient, and they more frequently develop thyroiditis.

In this study we have derived T-cell lines from the spleen and pancreas of newly diabetic BB and BBUF rats. These T-cell lines were maintained by continuous growth in IL-2–containing medium, and demonstrated proliferative responses in the combined presence of islet cell antigens and MHC-matched accessory cells. The isolation and characterization of autoreactive T-cells from rats with IDDM provides a tool for the study of the role that T-cells play in the pathogenesis of this disease.

MATERIALS

Rats. Male and female BB rats (RT1^u) were supplied by Dr. P. Thibert, Department of Animal Resources, Department of Health and Welfare, Ottawa, Canada. BBUF rats were initially derived by crossing a BB rat with a Buffalo rat, and also express the RT1^u haplotype. The genetic, endocrine, histopathologic, and immunologic characteristics of BB rats[1,4–6] and BBUF rats[7] have been described elsewhere.

METHODS

Preparation of cell populations. Splenectomies and pancreatic biopsies were performed as previously described.[4] Splenic lymphocytes were isolated by a standard technique. Islets of Langerhans were isolated by digestion of pancreatic tissue with collagenase (type V) (Sigma Chemical Co., St. Louis, Missouri), and centrifugation on a Percoll (Pharmacia, Uppsala, Sweden) density gradient as described by Buitrago et al.[8] Islet cell monolayers were prepared by digestion with trypsin (0.29%). Unless otherwise indicated, islet cells were cultured in RPMI 1640 supplemented by 10% fetal calf serum (FCS) (Flow Laboratories, Mississauga, Ontario, Canada), L-glutamine, penicillin-streptomycin mixture (Flow Lab-

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oratories), and 20 mM Hepes (Gibco Laboratories, Grand Island, New York).

Derivation of T-cell lines: method 1. Spleen cells obtained from newly diabetic rats were depleted of macrophages by passage on a Sephadex G-10 column as previously described.[4] These cells were then incubated (3×10^6 cells/ml) with mitomycin-C–treated RIN-5F rat insulinoma cells (10^5 cells/ml) (a kind gift from Dr. H. K. Oie, National Naval Medical Center, Bethesda, Maryland). RIN-5F secretes insulin,[9] expresses $RT1^u$ class I (but not class II) MHC antigens, and expresses antigens cross-reactive with islet cells as detected by monoclonal antibodies. Spleen cells and RIN-5F cells were cultured for 3 days in medium prepared as described above except for the addition of 5×10^{-5} M 2-mercaptoethanol (henceforth called complete medium). Blast cells were then recovered by centrifugation on a Percoll density gradient, cultured in complete medium supplemented by 15% T-cell growth factor (TCGF), fed three times a week, with no further restimulation with antigen. All cell cultures were kept at 37°C, with 5% CO_2, in a humidified atmosphere.

Derivation of T-cell lines: method 2. Monolayers of pancreatic islet cells (10^5 cells/ml) were incubated in complete medium supplemented by 15% TCGF, and 5×10^5 mitomycin-C–treated autologous spleen cells. Dead cells were removed after 6 days on a Ficoll-Hypaque density gradient. Live cells were kept growing in 15% TCGF, and fed three times a week, without restimulation with antigen.

Preparation of TCGF. TCGF was prepared from concanavalin A (con A)-activated splenic lymphocytes as previously described.[4]

Preparation of cell lysates. Cell lysates were prepared by lysis of islet cells, or various rat tumor cell lines, in hypotonic buffer (10^6 cells/ml in 0.01 M Tris buffer, pH 8.0), and returned to isotonicity by dilution in 2X PBS. Cell lines used as a source of antigen included RIN-5F (see above), the rat myeloma line IR983 ($RT1^u$), and the rat fibroblast line LWP($RT1^l$).

Proliferative assay. T-cells (10^4 to 5×10^4) were added to mitomycin-C–treated spleen cells (5×10^5) (source of accessory antigen-presenting cells), in the presence of islet cells (10^5), RIN-5F cells, or control cells, or with a 10% (vol/vol) lysate of the same cells. These cultures were incubated in complete medium (200 μl per microwell, flat-bottom, 96 well plates) for 3 days and [3H]-thymidine uptake was then determined after an 18-h incubation with 1 μCi [3H]-thymidine per well.

Measurement of IL-2. IL-2 levels in culture supernatants were determined using the IL-2–dependent T-cell line CTLL-2 as described by Gillis et al.[10]

Determination of cell surface marker. The phenotype of cultured cells was determined by indirect fluorescence staining of cells and analysis with a fluorescence-activated cell sorter (FACS). The following monoclonal antibodies obtained from Sera-lab (Crawley Down, Sussex, England) were used: W3/13, a pan T-cell marker; W3/25, a marker found on T-helper cells; OX8, a marker found on T-suppressor/cytotoxic cells; OX6, an antibody that reacts against rat Ia; and anti-rat immunoglobulin (Ig).

TABLE 2
T-cell line derived from the pancreas of a diabetic BB rat

T-cell line BB.P.1C*	Accessory cells†	Islet cell antigen†	CPM†
−	+	+	672
+	−	−	502
+	+	−	809
+	+	+	8773

*Line BB.P.1C was derived from the pancreas of a newly diabetic BB rat. T-cells (10^4 per well) were added.
†See Table 1.

RESULTS AND DISCUSSION

Two methods were used to derive T-cell lines from diabetic rats. In the first approach T-cell lines (e.g., BFR.1C) were derived from the spleen of diabetic rats. Macrophages were partially depleted from the spleen cell population by passage on a Sephadex G-10 column. This procedure was performed to remove the suppressive effect that BB-derived macrophages exert on T-cell responses and IL-2 production, which we have previously described.[4] Nonadherent spleen cells were co-incubated with mitomycin-C–treated RIN-5F insulinoma cells (source of antigen) for 3 days. T-cell blasts (density <1.06 g/ml) were then recovered on a Percoll density gradient, and grown in the presence of 15% TCGF. These T-cell lines proliferated in the presence of RIN-5F or islet cell antigens, provided that these antigens were presented by

TABLE 1
Spleen cell-derived anti-islet T-cell line

T-cell line BFR.1C*	Accessory cells†	Islet cell antigen‡	CPM§
−	+	+	1146
+	−	−	2138
+	+	−	1918
+	+	+	11,600

*BFR.1C is a T-cell line derived from the spleen of a diabetic BBUF rat. T-cells (10^4 per well) were added.
†WF ($RT1^u$) mitomycin-C–treated spleen cells (5×10^5 per well) were added.
‡Islet cell lysate of WF rat origin. Similar results were obtained with RIN-5F insulinoma lysates.
§[3H]-thymidine uptake was determined on day 3 of assay. Results of a representative experiment are shown.

TABLE 3
Specificity of T-cell lines

Accessory cell origin	Source of antigen	BFR.1C* (SI)†	BB.P.1C* (SI)
WF	Islet cells	+	+ (15)‡
WF	IR983 (myeloma)	ND§	− (<1)
WF	LWP (fibroblast)	−	− (<1)
ACI	Islet cells	−	− (<1)
Lewis	Islet cells	−	− (<1)

*T-cells (10^4 per well) were added.
†SI (stimulation index) = [3H]-thymidine uptake (cpm) experimental/medium control. +, SI > 3; −, SI < 3.
‡Numbers in parentheses represent U/ml of IL-2 in 24-h supernatants (5×10^4 cells per well).
§ND, not determined.

appropriate accessory cells. These results obtained with a representative line, i.e., BFR.1C (obtained from a BBUF rat), are presented in Table 1.

In the second approach, pancreatic tissue was recovered from newly diabetic BB rats. This pancreatic tissue was treated with collagenase, and islets of Langerhans were recovered on a Percoll density gradient. These islets were then treated with trypsin to yield a cell monolayer. Cells isolated by this method were cultured with 15% TCGF and mitomycin-C–treated autologous splenic filler cells. After 6 days dead cells were removed on a Ficoll-Hypaque gradient. After 2 wk incubation with TCGF the growth of T-cells became apparent. The results obtained with a representative T-cell line, BB.P.1C, isolated by this method reveal that these T-cells proliferated in the combined presence of islet cell antigens and accessory cells (Table 2).

BFR.1C and BB.P.1C were antigen specific, and only responded in the presence of MHC-matched accessory cells (i.e., antigen-presenting cells) (Table 3). Analysis of cell surface markers revealed that BFR.1C and BB.P.1C were W3/13$^+$, W3/25$^+$, and OX8$^-$ (data not shown). We could not demonstrate direct killing of islet cells or RIN-5F cells by these T-cell lines in a chromium release assay (data not shown). In addition, BB.p.1C produced detectable levels of IL-2 when stimulated (Table 3). The above findings all suggest that these are T-helper cells. Interestingly, other authors have demonstrated T-helper cell lines capable of responding to autoantigens in experimentally induced autoimmune diseases such as experimental autoimmune thyroiditis.[11] Our findings in the study of IDDM, as well as those of other authors in organ-specific autoimmune diseases, suggest that T-helper lymphocytes play an important role in the pathogenesis of these diseases.

BFR.1C and BB.P.1C (as well as similar T-cell lines) were maintained in continuous culture for >60 days, with conserved antigen-specific proliferative responsiveness. However, after this time these lines underwent a period of crisis with slow growth and extensive cell death. Cells that survived this crisis period proliferated rapidly, sometimes in the absence of IL-2. Although these cells continued to express T-cell markers they no longer responded to islet cell antigens.

The results of this study reveal that anti-islet cell antigen T-cell lines can be isolated from the spleen or pancreas of newly diabetic rats. The study of such T-cell lines will provide an important tool for the study of IDDM. An obstacle to future studies is the relatively short lifespan of these T-cell lines under the culture conditions we have used. Other authors have reported similar problems with the culture of T-helper cell lines.[12] Potentially, this problem can be overcome by frequent restimulation with antigen, or the use of T-cell hybridomas.[13,14] We are presently assessing the value of various T-cell cloning strategies in an attempt to increase the in vitro lifespan of anti-islet cell antigen T-cell lines.

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