A Major Histocompatibility Complex Class II Restriction for BioBreeding/Worcester Diabetes–inducing T Cells

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Summary

Inbred diabetes-prone (DP) BioBreeding/Worcester (BB/Wor) ($RT1^u$) rats develop spontaneous autoimmune diabetes, which, like human insulin-dependent diabetes mellitus, is mediated by autoreactive T lymphocytes. Breeding studies have shown an absolute requirement for at least one copy of the major histocompatibility complex (MHC) $RT1^u$ haplotype for spontaneous diabetes expression. Concanavalin A–activated spleen cells from acutely diabetic DP rats adoptively transfer diabetes only to recipients that express at least one $RT1^u$ haplotype. To investigate the basis for the MHC requirement in BB/Wor autoimmunity, diabetes-inducing T cell lines were derived from the spleens of acutely diabetic DP rats. Upon activation in vitro with islet cells, the T cell lines adoptively transfer insulitis and diabetes into young DP recipients and non–diabetes-prone RT1 congenic rat strains that are class II^u. Recipients that are $RT1^u$ at only the class I A or C locus, but not at the class II B/D loci, do not develop diabetes after T cell transfer. The adoptive transfer of diabetes by Concanavalin A–activated diabetic DP spleen cells also requires that donor and recipient share class II B/D^u gene products. Furthermore, the adoptive transfer of diabetes into MHC class II^u congenic rats is independent of the class I haplotype; i.e., it occurs in the presence of class I A^a C^u or A^u C^a gene products. BB/Wor T cells can be activated in vitro for the transfer of diabetes with islet cell antigens and class II–positive but not class II^u–negative antigen-presenting cells. The inductive phase of BB diabetes is therefore MHC class II restricted, and this appears to operate at the level of interaction between inducing T cells and class II^u antigen-presenting cells. These results may explain the well-documented, but not yet understood, MHC class II genetic contribution to insulin-dependent diabetes mellitus pathogenesis, and they may facilitate the development of protocols designed to prevent diabetes onset in susceptible individuals.

Diabetes-prone (DP)[1] BioBreeding/Worcester (BB/Wor) rats develop spontaneous autoimmune diabetes, in which the frequency of insulin-dependent, ketosis-prone hyperglycemia is 80–95% in both sexes. BB diabetes is characterized morphologically by a β cell–specific mononuclear cell infiltrate (insulitis) within the pancreatic islets of Langerhans. The autoimmune attack selectively destroys the insulin-producing β cells, with sparing of glucagon-, somatostatin-, and pancreatic polypeptide–synthesizing islet cells (1). These clinical features of BB diabetes are identical to those of human insulin-dependent diabetes mellitus (IDDM) (2).

BB/Wor rat diabetes is T cell dependent; the development of hyperglycemia is prevented by neonatal thymectomy (3) and in vivo treatment with mAbs directed against $CD5^+$ (pan T) or $CD8^+$ (cytotoxic) T cells (4). Spontaneous BB diabetes is an MHC-linked disease. Breeding studies have shown an absolute requirement for at least one copy of the MHC $RT1^u$ haplotype for the appearance of spontaneous diabetes in crosses between BB and non-BB strains of rat (5–7). Genetic susceptibility, however, has not been previously assigned to single genes of the MHC, nor have the immune effector mechanisms leading to β cell destruction been identified as being class I– or class II–restricted events.

BB/Wor diabetes can be adoptively transferred with acutely diabetic DP spleen cells that have been activated in vitro with Con A, a polyclonal mitogen (8), or staphylococcal enterotoxin E (SEE; Toxin Technology, Sarasota, FL) (9), a T cell receptor Vβ family–specific stimulus (10). Adoptive transfer has been demonstrated using young DP (8), cyclophosphamide-treated histocompatible $RT1^u$ non-BB (11) or athymic nude $RT1^u$ rats (12) as recipients. Transfer studies using young DP recipients have implicated both the $CD4^+$ (13) and $CD8^+$ (14) T cell subsets as being necessary for adoptive transfer. Since DP recipients possess

[1] *Abbreviations used in this paper:* BB/Wor, BioBreeding/Worcester; CAS, Con A-activated Lewis rat splenocytes; DP, diabetes prone; DR, diabetes resistant; IDDM, insulin-dependent diabetes mellitus; NOD, nonobese diabetic; SEE, staphylococcal enterotoxin E.

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an endogenous cohort of autoreactive $CD4^+$ and $CD8^+$ T cells, these studies do not permit a precise determination of the cell type required for initiating the diabetogenic process. Experiments designed to discriminate between inductive versus effector events and to map each arm of the diabetogenic immune response to a specific MHC locus would require the use of non-BB, non–diabetes-prone $RT1^u$ class I or class II congenic recipients in adoptive transfer studies. In non-BB $RT1^u$ congenic recipients, one can measure de novo disease induction rather than the acceleration of a spontaneous disorder that would eventually occur in diabetes-prone BB recipients.

To investigate the basis for the MHC requirement in BB autoimmune diabetes, diabetes-inducing T cell lines were generated from the spleens of acutely diabetic DP rats. The diabetes transfer capabilities of the T cell lines were tested in syngeneic and class I or class II congenic non–diabetes-prone recipients. The ability of class I or class II congenic APC to activate the T cells in vitro for the adoptive transfer of diabetes in vivo was also assessed. The results presented herein explain, at least in part, the MHC genetic contribution to IDDM pathogenesis in the rat and shed light on the pathogenesis of β cell destruction.

Materials and Methods

Animals. BB/Wor DP and BB/Wor diabetes-resistant (DR) rats and inbred PVG.R8, PVG.R23, LEW1.AR2, and LEW1.WR1 rats were raised at the University of Massachusetts Medical Center (Worcester, MA) under viral antibody–free conditions. Viral antibody–free LEW1.AR2 and LEW1.WR1 breeding stock were obtained from the Central Institute for Laboratory Animal Breeding (Hannover, Germany).

Islet Cell Preparation. Islets were isolated from 90-d DR rats by the method of Gotoh et al. (15). Pancreatic tissue was digested with 4 mg/ml collagenase P (Boehringer Mannheim Corp., Indianapolis, IN) for 25 min at 37°C. Islets were enriched on Histopaque 1077 (Sigma Chemical Co., St. Louis, MO) (1,800 rpm, 30 min, 4°C) and purified by handpicking under a microscope. Islets were dispersed in trypsin/EDTA (12 min at 37°C) and immediately cryopreserved in 10% DMSO/45% RPMI 1640/45% FCS. Freshly thawed islet cells were γ irradiated (3,000 R) before use. To deplete residual islet-associated APC, freshly thawed islet cells at 10^6/ml were sonicated 2 × 20 s on ice with an ultrasonicator (Microson; Heat Systems Inc., Farmingdale, NY).

Generation of T Cell Lines. Spleens were taken from acutely diabetic DP rats (duration of diabetes <5 d), processed into single-cell suspensions, allowed to adhere to plastic for 2 h at 37°C, and plated at 6×10^6 nonadherent cells/ml in RPMI 1640, 2% autologous rat serum, and 2 μg/ml SEE at 37°C in 6.5% CO_2. After 3 d, the activated blasts were purified on Histopaque 1077 (2,000 rpm, 20 min, 22°C) and plated at 10^5/ml in RPMI 1640, 10% heat-inactivated FCS (Hyclone Laboratories, Logan, UT), and 5% supernatant from 48-h Con A–activated Lewis rat splenocytes (CAS). After two to three passages in CAS, the cells were restimulated for 3 d at 4×10^5/ml with whole 3,000-R irradiated DR islet cells (freshly thawed after cryopreservation), 5% CAS, and 1.25×10^6/ml irradiated nonadherent DP spleen cells (3,000 R) in RPMI 1640, 1% autologous rat serum. The ratio of islet cells to T cells was ~1:22. Antigen-activated blasts were again purified on Histopaque 1077 (2,000 rpm, 15 min, 22°C), plated at 10^5/ml in 10% CAS, and passaged several times before restimulation with antigen (islet cells or SEE) and APC. Culture medium consisted of RPMI 1640, 1 mM sodium pyruvate, 2 mM glutamine, 10 mM Hepes, 5×10^{-5} M 2-ME, and 1× antibiotic/antimycotic (GIBCO BRL, Gaithersburg, MD). Before adoptive transfer, T cell lines were activated for 3 d with antigen and APC. Blast-transformed cells were harvested on Histopaque 1077 and cultured for a further 2 d in 10% CAS. Resting (unstimulated) T cells were transferred after two passages in 10% CAS.

Adoptive Transfer of T Cell Lines into DP Rats. T cells were injected intraperitoneally into 21–28-d female DP rats, which were monitored for 21 d after transfer for the development of glycosuria (TesTape; Eli Lilly and Co., Indianapolis, IN) and hyperglycemia (blood glucose ≥13.8 mmol/liter; Beckman Glucose Analyzer II; Beckman Instruments, Inc., Fullerton, CA). The mean age at onset of spontaneous diabetes in the University of Massachusetts Medical Center DP colony is 75 d for females with an incidence of 0.04% at 45 d of age. Hyperglycemia detected before 45 d of age was considered to be the result of the injected T cells. Diabetic rats were killed on the day of detection, and nondiabetic rats at 21 d after transfer.

Adoptive Transfer of T Cell Lines into $RT1^u$ Congenic Rats. Islet cell–activated T cells were injected intraperitoneally into 21–30-d male and female congenic rats treated 24 h earlier with cyclophosphamide (Cytoxan; Mead Johnson Laboratories, Princeton, NJ), 180 mg/kg body weight i.p. Rats were monitored for 21 d for the development of glycosuria and hyperglycemia (blood glucose ≥13.8 mmol/liter).

Con A Activation of Acutely Diabetic DP Spleen Cells. Spleens were taken from acutely diabetic DP rats (duration of disease <5 d), processed into single-cell suspensions, and cultured at 5×10^6 cells/ml in RPMI 1640, 2 mM glutamine, 10 mM Hepes, 5×10^{-5} M 2-ME, 5% FCS, and 4 μg/ml Con A (ICN Biomedicals, Inc., Costa Mesa, CA) for 3 d at 37°C, 6.5% CO_2. Activated cells were washed four times in RPMI 1640 before injection. One spleen equivalent of Con A–activated cells was injected intraperitoneally into recipient rats.

Morphologic Studies. Pancreata and thyroids were fixed in Bouin's solution and embedded in paraffin. Hematoxylin and eosin stained sections were examined for the presence of insulitis and thyroiditis. Selected sections were immunostained for insulin and glucagon (16) to evaluate the selective destruction of pancreatic α and β cells.

mAbs. OX-8 (anti-CD8) (17) and W3/25 (18, 19) (anti-CD4) mAbs were produced by hybridoma cell lines from Dr. A. Williams and Dr. D. Mason (Oxford University, Oxford, UK). The 3.2.3 hybridoma was obtained from J. C. Hiserodt. The 3.2.3 mAb recognizes rat NK cells and selectively depletes NK cells in vivo (20, 21). Undiluted tissue culture supernatants were used for flow cytometry as previously described (4).

Results

Generation and Characterization of BB/Wor Diabetes–inducing T Cell Lines. BB/Wor autoimmune diabetes can be adoptively transferred into syngeneic recipients with acutely diabetic DP spleen cells that have been activated in vitro by SEE, a TCR Vβ family–specific stimulus (9). T cell lines were generated from the spleens of acutely diabetic DP rats by first culturing them in vitro with SEE. After 3 d, SEE-reactive lymphoblasts (~9% of input spleen cells) were en-

riched on density gradients and recultured in CAS as a source of T cell growth factors. At the second round of antigen-specific selection, T cells were stimulated with whole BB/Wor islet cells and APC. Islet cell–reactive T lymphoblasts were density gradient purified and passaged further in CAS. At the third cycle of antigen activation, T cells lines were restimulated with either SEE and APC or with islet cells and APC. This protocol generates a population of T cells with potent diabetes transfer activity. A single intraperitoneal injection of 12–34 × 10^6 T cells adoptively transferred diabetes into 21–28-d-old DP recipients in as few as 5 d after cell injection (Table 1), with a mean time to hyperglycemia of 7.9 d (n = 48). DP rats also develop spontaneous thyroiditis, although at a low and variable incidence (22). Diabetic recipients of T cell lines, however, did not develop thyroiditis (n = 48). Hyperglycemia was always accompanied by a β cell–destructive insulitis.

Only antigen-activated T cells transfer disease; unstimulated (resting) T cells transferred >5 d after antigen activation (with islet cells or SEE) did not induce adoptive diabetes (Table 1, *line 4*) or insulitis. By single-color flow cytometry, the cell lines were comprised (at all time points tested) of 70–90% $CD4^+$ and 10–30% $CD8^+$ T cells (data not shown). Although DP rats have low, barely measurable levels of $CD8^+/CD5^+$ CTLs and increased numbers of $CD8^+/CD5^-/3.2.3^+$ NK cells (23, 24), the T cell lines did not contain $3.2.3^+$ NK cells at any time. The cell lines proliferate in vitro in response to both islet cells and SEE (data not shown). One cell line (A91-1), also derived from acutely diabetic DP spleens, was initially selected and then repetitively stimulated in vitro with whole islet cells and APC, but it did not transfer diabetes or insulitis into any of 28 DP recipients (21–25 d old) given 19–57 × 10^6 T cells (data not shown). The initial in vitro activation step with SEE appears to select for T cells with both islet cell reactivity and diabetes transfer capabilities.

Adoptive Transfer of T Cell Line–mediated Diabetes is MHC Class II^u Restricted and Independent of Class I Haplotype. To exclude the possibility that the T cell lines were simply accelerating or costimulating an endogenous immune process genetically present in DP recipients, T cell lines were transferred into RT1 congenic non-BB strains of rat. These congenics contain non-BB $RT1^u$ genes, in different allelic combinations on the genetic background of parental Lewis ($RT1^l$) and PVG ($RT1^c$) strains. Neither the $RT1^u$ congenics nor their parental strains spontaneously develop insulitis or diabetes. To prepare congenic rats for BB/Wor T cell injections, recipients were treated with cyclophosphamide 24 h before transfer. Cyclophosphamide depletes T cells and enables RT1 congenic rats to accept the BB/Wor T cells, with which they share only partial genetic identity (11). Cyclophosphamide alone does not induce diabetes or insulitis in RT1 congenic rats (see Table 3).

Islet cell–activated T cell lines injected into PVG.R8 rats induced diabetes and insulitis in 14 out of 17 recipients, with a mean time to hyperglycemia of 7.4 d (Tables 2 and 3). Pancreatic tissue sections taken from diabetic and control cyclophosphamide-treated PVG.R8 rats were immunostained for the localization of islet cell peptide hormones. Islets of Langerhans from diabetic rats exhibited destructive lymphocytic insulitis with depletion of insulin-producing β cells and sparing of glucagon-producing α cells (Fig. 1 *C* and *D*) and somatostatin-producing δ cells (data not shown). Cyclophosphamide-treated rats revealed no insulitis and had normal numbers and distributions of α, β (Fig. 1, *A* and *B*), and δ cells. Islet cell–activated T cells also transferred diabetes and insulitis into 9 out of 10 LEW1.WR1 rats (Table 3), with a mean time to hyperglycemia of 9.7 d and a mean blood glucose of 20.4 mmol/liter. Thus, BB/Wor islet cell–activated T cell lines have an in vivo specificity for insulin-producing β cells, even when transferred into a non-BB genetic environment.

When islet cell–activated T cells were transferred into PVG.R23 recipients, none of the rats developed insulitis or diabetes (Table 3). T cells from the lines used in these experiments (at the same or a later passage) transferred diabe-

Table 1. *BB/Wor Diabetes–inducing T Cell Lines*

Cell line	Incidence of diabetes	Stimulus	Cell No.	Mean time to diabetes	Mean blood glucose
			× 10^6	*d*	*mmol/liter*
BF-1	6/6	Islet cells	16–20	7	26.4
BF-1	3/3	SEE	32	5	25.0
BB-3	4/4	Islet cells	19	6	19.8
BB-3	0/4	None	22	—	—
BB-3	2/2	Islet cells	12	8	15.7
BB-5	3/3	SEE	34	12	28.9
J193	2/3	Islet cells	17	7	27.9

T cell lines were activated for 3 d with islet cells/APC or SEE/APC as described in Materials and Methods. Blast-transformed cells were harvested on Histopaque 1077 and cultured for a further 2 d in 10% CAS. Resting T cells (no stimulus) were transferred after 2 passages in CAS. T cells were injected intraperitoneally into 21–28 d female DP rates. Recipients were monitored for 21 d for the development of hyperglycemia (blood glucose ≥13.8 mmol/liter). A total of 45 out of 48 DP rats injected with T cell lines developed diabetes, with a mean time to hyperglycemia of 7.9 d.

Table 2. *Adoptive Transfer of Diabetes into PVG.R8 Rats by Islet Cell–activated BB/Wor T Cell Lines*

Cell line	Cell No.	Incidence of diabetes	Mean time to diabetes	Mean blood glucose
	$\times 10^6$		*d*	*mmol/liter*
Ju92	31	3/4	7	23.8
BB-5	39	6/6	7.5	25.2
092	16	3/4	8	17.9
092	29	2/3	7	24.6

T cell lines were activated for 3 d with islet cells and APC as described in Materials and Methods. Blast-transformed cells were harvested on Histopaque 1077 and cultured for a further 2 d in 10% CAS. T cells were injected intraperitoneally into 21–30-d female PVG.R8 rats treated 24 h earlier with cyclophosphamide, 180 mg/kg body weight. None of eight female PVG.R8 rats given 180 mg/kg cyclophosphamide alone developed diabetes or insulitis. All rats were monitored for 21 d for the development of glycosuria and hyperglycemia (blood glucose ≥13.8 mmol/liter).

tes into 9 out of 10 DP recipients and six out of six PVG.R8 rats, confirming their in vivo diabetogenicity. Islet cell–activated T cells injected into LEW1.AR2 rats did not induce diabetes or insulitis in seven recipients (Table 3), even though the same line concurrently transferred diabetes into two out of two rats given 18×10^6 T cells, with a mean time to hyperglycemia of 8 d (data not shown). There were no signs of GVHD in PVG.R23 or LEW1.AR2 recipients.

The combined data from Tables 2 and 3 demonstrate that donor and recipient must share class II $RT1^u$ gene products for the successful transfer of BB/Wor diabetes. $RT1^u$ congenicity at only the class I RT1A or RT1C locus is not sufficient for T cell–mediated transfer of diabetes. Of importance is the observation that the adoptive transfer of autoimmune diabetes into class II^u congenic rats is independent of the class I haplotype; i.e., diabetes transfer occurs in the presence of class I A^a C^u (PVG.R8) or A^u C^a (LEW1.WR1) gene products.

Adoptive Transfer of Diabetes by Con A–activated Acutely Diabetic DP Spleen Cells Also Requires that Donor and Recipient Share Class II^u Gene Products. To confirm the in vivo MHC restriction pattern obtained with islet cell–activated T cell lines, Con A–activated acutely diabetic DP spleen cells were also injected into cyclophosphamide-treated RT1 congenic rats. One spleen equivalent of Con A–activated cells transferred diabetes and insulitis into PVG.R8 and LEW1.WR1 but not PVG.R23 and LEW1.AR2 rats (Table 4). 13 out of 14 LEW1.AR2 recipients developed fulminant GVHD (manifested by wasting, anemia, and massive splenomegaly) 10 d after transfer. Two out of three DP rats concurrently receiving the same Con A–activated spleen cells developed diabetes at 10–11 d after transfer. Thus, cells with diabetogenic potential survived in LEW1.AR2 recipients, but without inducing hyperglycemia or insulitis.

As assessed by adoptive transfer, the cognitive or inductive phase of BB diabetes is MHC class II^u restricted and can proceed in the presence of either class I A^u or C^u gene products. Thus, the induction of transferred diabetes is dependent first upon $CD4^+$ T cell recognition of β cell autoantigen in the context of class II^u in recipient target tissue (islet of Langerhans). These experiments do not, however, rule out a role for $CD8^+$ CTL in the autoimmune attack leading to β cell destruction.

Class II^u Restriction of BB Diabetes Operates at the Level of Interaction between Inducing T Cells and APC. To examine the APC as a potential locus for the class II^u restriction, BB T cell lines were activated in parallel with sonicated BB islet cells and BB or RT1 congenic APC (irradiated spleen

Table 3. *Adoptive Transfer of Diabetes into MHC Congenic Rat Strains by Islet Cell–activated BB/Wor T Cell Lines*

Strain	RT1 haplotype	Cell No.	Incidence of diabetes	Incidence of insulitis
		$\times 10^6$		
PVG.R8	A^aB/D^uC^u	16–39	14/17	14/17
PVG.R8	A^aB/D^uC^u	0	0/8	0/8
PVG.R23	A^uB/D^aC^{avl}	27–36	0/14	0/14
PVG.R23	A^uB/D^aC^{avl}	0	0/11	0/11
LEW1.AR2	A^aB/D^aC^u	36	0/7	0/7
LEW1.AR2	A^aB/D^aC^u	0	0/10	0/10
LEW1.WR1	A^uB/D^uC^a	28	9/10	9/10
LEW1.WR1	A^uB/D^uC^a	0	0/7	0/7

21–30-d-old male and female RT1 congenic rats received cyclophosphamide, 180 mg/kg body weight intraperitoneally, 24 h before cell transfer. Control rats received cyclophosphamide alone. Rats were monitored for 21 d for glycosuria and hyperglycemia (blood glucose ≥13.8 mmol/liter).

Table 4. *Adoptive Transfer of Diabetes into MHC Congenic Rat Strains by Con A–activated Spleen Cells from Acutely Diabetic Diabetes-prone BB/Wor Rats*

Strain	RT1 haplotype	Diabetes	Insulitis
PVG.R8	A^aB/D^uC^u	19/23	21/23
PVG.R23	A^uB/D^aC^{avl}	0/17	0/17
LEW1.AR2	A^aB/D^aC^u	0/14	0/11
LEW1.WR1	A^uB/D^uC^a	5/5	5/5
BB/Wor DP	A^uB/D^uC^u	24/27	25/27

Recipients were 21–30-d-old rats of both sexes. Each rat received one spleen equivalent of Con A–activated acutely diabetic DP spleen cells intraperitoneally or intravenously. Congenic rats received cyclophosphamide, 150–180 mg/kg body weight i.p., 24 h before spleen cell injections. For each experiment, cells were concurrently transferred into both congenic and BB rats. Recipients were monitored for 3 wk (BB rats) or 4 wk (congenic rats) for the development of hyperglycemia (blood glucose ≥13.8 mmol/liter).

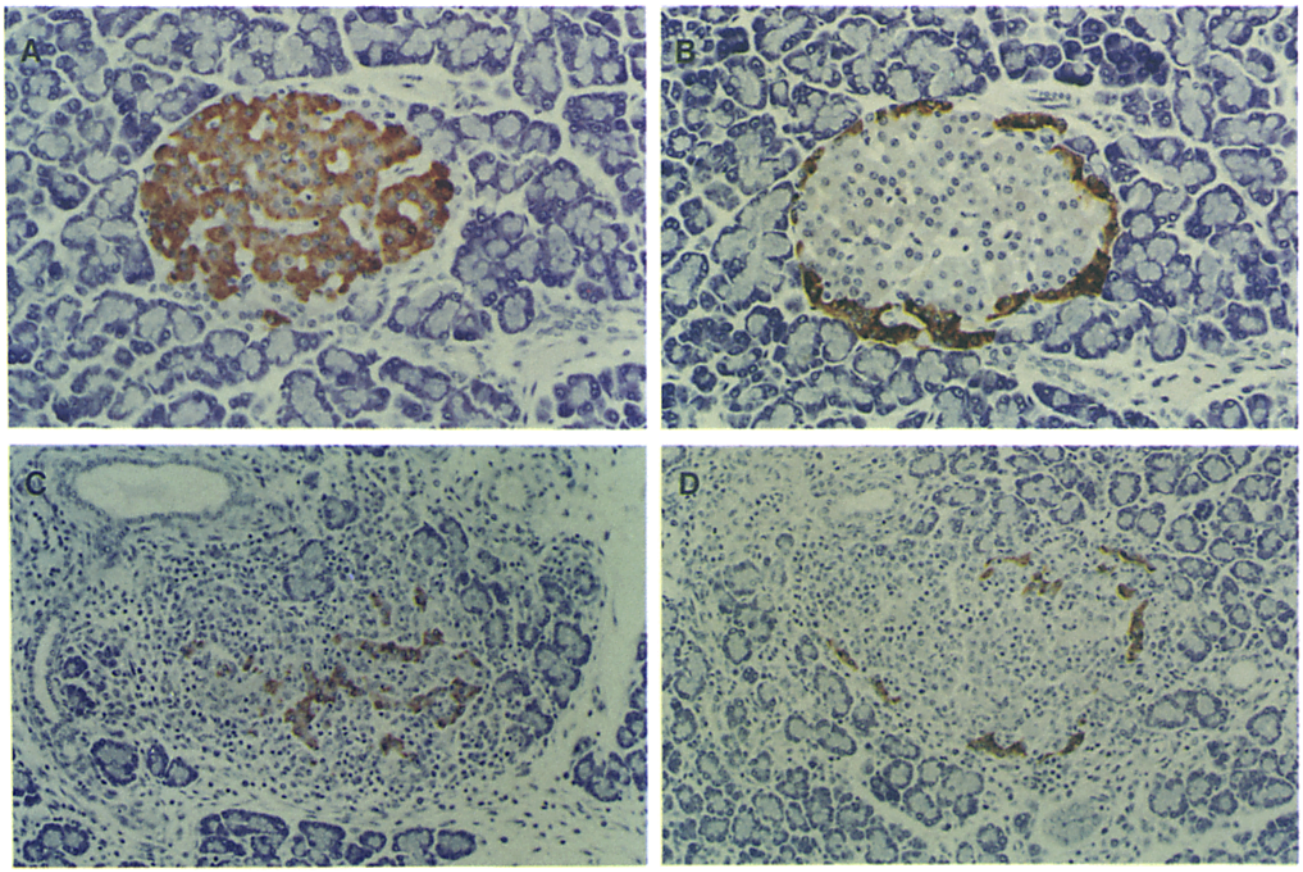

Figure 1. Photomicrographs show adjacent pancreatic islet sections taken from PVG.R8 rats treated with cyclophosphamide alone (*A*, *B*) or cyclophosphamide followed 24 h later by an injection of islet cell–activated BB/Wor T cells (*C*, *D*). Tissues were fixed in Bouin's solution, and an immunoperoxidase technique was used for identification of insulin (*A*, *C*) and glucagon (*B*, *D*). The islets of cyclophosphamide control (*A*, *B*) reveal no evidence of insulitis. Insulin-positive β cells (*A*) and surrounding glucagon-positive α cells (*B*) are normal in appearance. The islets of the diabetic rat reveal an intraislet mononuclear cell infiltrate with almost complete destruction of the pancreatic β cells (*C*). Peripheral glucagon-positive α cells (*D*) are preserved in the diabetic PBV.R8 rats. *A* and *B*, ×200; *C* and *D*, ×170.

cells). The sonication step was added to deplete the islet cells of intact class II^+ APCs of BB origin, as such cells are a normal component of the islets of Langerhans. After activation, equal numbers of T cells were injected into 21–25-d DP recipients, which were monitored for hyperglycemia for 15 d after cell injection. BB T cells activated in vitro with islet cell antigens and BB, PVG.R8 (B/D^u), or LEW1.WR1 (B/D^u) APC, but not PVG.R23 (B/D^a) or LEW1.AR2 (B/D^a) APC, adoptively transferred diabetes (Table 5). Thus, BB diabetes–inducing T cells are class II^u restricted both in vivo and in vitro. The class II restriction appears to operate at the level of the interaction between inducing T cells and class II^u APC.

Discussion

BB autoimmune diabetes is an MHC-linked disease with a requirement for both $CD4^+$ (13) and $CD8^+$ (4, 14) T cells. To investigate the basis for the MHC requirement, diabetes-inducing T cell lines were generated from the spleens of acutely diabetic DP rats. When activated in vitro with whole islet cells and APC, the T cell lines have potent diabetes transfer activity, wherein a single injection of T cells adoptively transferred hyperglycemia into 21–28-d-old DP recipients in as few as 5 d after injection. Hyperglycemia was always accompanied by a β cell–destructive insulitis with sparing of the glucagon- and somatostatin-secreting islet cells. Although DP rats may also develop spontaneous thyroiditis (22), diabetic recipients of T cell lines never manifested any signs of intrathyroid lymphocytic infiltrates. Interestingly, the T cell lines can also be activated for diabetes transfer with the superantigen, SEE. The ability of a superantigen to activate diabetes-inducing T cells is reminiscent of a recent report suggesting a role for superantigen in human IDDM etiology (25).

To determine which type of T cell initiates the development of diabetes, BB/Wor T cell lines were injected into non–diabetes-prone $RT1^u$ class I or class II congenic recipients. Upon islet cell activation in vitro, the T cell lines rapidly transferred insulitis and diabetes into class II^u congenic rats. Recipients that are $RT1^u$ at only the class I A or C locus, but not at the class II B/D loci, did not develop diabetes after T cell transfer. The adoptive transfer of dia-

Table 5. *BB/Wor Diabetes–inducing T Cells Require Activation with MHC Class II^u–positive APC and Islet Cell Antigens for Disease Transfer*

Experiment No.	Source of APC	Class II loci	Incidence of diabetes	Mean time to diabetes
				d
1	BB/Wor	B/D^u	7/8	11
	PVG.R23	B/D^a	0/8	—
2	BB/Wor	B/D^u	6/7	9
	LEW1.WR1	B/D^u	4/5	10
3	BB/Wor	B/D^u	7/8	9
	PVG.R8	B/D^u	8/9	9
4	PVG.R8	B/D^u	1/2	11
	LEW1.AR2	B/D^a	0/3	—
5	BB/Wor	B/D^u	4/4	12
	LEW1.AR2	B/D^a	0/2	—

BB/Wor T cells (4×10^5/ml) were activated in parallel with DP or RT1 congenic 3,000-R spleen cells (2×10^6/ml), 5% FCS, 5% CAS, and sonicated BB islet cells. The sonicates were always checked microscopically for the absence of whole cells. Islet cell sonicates were used at a ratio of 1 cell equivalent per 21 T cells. After 3 d, blasts were harvested on Histopaque 1077 and cultured for 2 d in 10% CAS. Equal numbers of T cells (usually 25×10^6) were then injected intraperitoneally into 21–25-d female DP rats, which were monitored for 15 d for the development of glycosuria and hyperglycemia (blood glucose ≥13.8 mmol/liter).

betes by Con A–activated, acutely diabetic DP spleen cells also requires that donor and recipient share class II B/D^u gene products. Therefore, the induction of BB diabetes is restricted by class II^u gene products and is $CD4^+$ T cell mediated. Of importance is the fact that the adoptive transfer of diabetes into class II^u congenic rats is independent of the class I haplotype; i.e., it occurred in the presence of class I A^a C^u (PVG.R8) or A^u C^a (LEW1.WR1) gene products. In contrast, the adoptive transfer of antiviral cytotoxic $CD8^+$ T cell effector activity is restricted by recipient class I, but not class II, genes (26).

These data are surprising, because, in the rat, the insulin-producing β cell targets do not express detectable MHC class II gene products in vivo (27–29), even during the course of spontaneous BB/Wor insulitis and diabetes (30). BB rat β cells do, however, express class I products in vivo, with class I hyperexpression and infiltrating $CD8^+$ T cells being invariant concomitants of the autoimmune attack (24, 28, 31). It is likely that β cells in situ shed or secrete a protein that is taken up by class II^+ intraislet APC, which process and then present antigen to β cell peptide–specific $CD4^+$ T cells in a class II-restricted manner. The activated $CD4^+$ T cells would, in turn, activate β cell peptide–specific $CD8^+$ T cells, which would serve as the final effectors of β cell cytolysis. $CD8^+$ T cells are known to be critically important in BB diabetes. Anti-CD8 mAb treatment removes $CD8^+$ CTL and decreases the incidence of spontaneous BB diabetes (4, 24). In vitro–activated acutely diabetic BB spleen cells, when depleted of $CD8^+$ cells, do not adoptively transfer disease into CD8-depleted DP recipients (14). $CD8^+/3.2.3^+$ NK cells are not the final effectors of β cell cytolysis, because 3.2.3 mAb treatment of DP rats removes NK cells but does not reduce the frequency of BB diabetes (24).

Although the induction of BB diabetes is clearly an MHC class II–restricted $CD4^+$ T cell–mediated event, the data do not rule out a role for $CD8^+$ CTL as the final effectors of β cell destruction. In the case of the diabetes-inducing T cell lines described above, it has not been shown that the transferred $CD8^+$ T cells actually play a role in adoptive diabetes. It is possible that the $CD4^+$ T cells (which make up 70–90% of the diabetes-inducing T cell lines) are recruiting β cell–specific $CD8^+$ CTL from within the recipient T cell compartment. Alternatively, the transferred $CD4^+$ T cells may be homing to the islets of Langerhans and inducing the production of inflammatory cytokines in situ that ultimately lead to β cell cytosis (32). A formal demonstration of a role for $CD8^+$ CTL in BB diabetes would depend upon the ability of islet cell–activated $CD8^+$ T cells to adoptively transfer insulitis and hyperglycemia into class I^u congenic recipients, irrespective of the animals' class II haplotype. Finally, if $CD8^+$ CTL are the final effectors of β cell cytolysis, then the data presented above suggest that they can recognize β cell autoantigen in the context of either class I A^u (LEW1.WR1) or C^u (PVG.R8) gene products.

In the nonobese diabetic (NOD) mouse model of IDDM, both $CD4^+$ and $CD8^+$ spleen cell populations are also required for the transfer of diabetes into young NOD (33–35) or NOD-scid/scid recipients (36). However, the matter has not been further clarified by transferring spleen cells into other inbred strains of mice that are class I ($H\text{-}2K^dD^b$) or class II (NOR/Lt [37], B10.H-2^{g7} [38]) congenic with NOD mice. Diabetes-accelerating $CD4^+$ T cell clones have been described in the NOD mouse (39), but their ability to induce (as opposed to accelerate) diabetes in non-NOD MHC class II congenic recipients has not been reported. Furthermore, the acceleration of diabetes in NOD mice given $CD4^+$ T cell clones is effective only if the recipients are <19 d of age (40). In contrast, BB T cells efficiently transfer diabetes into 21–30-d-old BB or class II^u congenic recipients.

The MHC contains the predominant genetic susceptibility factors for IDDM in humans (41, 42), the BB rat (7), and the NOD mouse (43, 44). In particular, MHC class II genes are associated with disease susceptibility in all three species (45–47). The manner in which the products of the IDDM-associated MHC genes influence the pathogenesis of diabetes is still unknown. The mechanism could be either at the level of thymic T cell selection or during peripheral immune response activation, both of which require appropriate peptide presentation by MHC molecules. Our data indicate that, in the BB rat, the MHC class II ge-

netic contribution to IDDM pathogenesis may be explained by binding of β cell peptide to permissive class II^u molecules, resulting in the activation of diabetes-inducing T cells. Specifically, BB T cells can be activated in vitro for the transfer of diabetes with islet cell antigens and class II^u–positive, but not class II^u–negative, APC (Table 5). The MHC class II restriction of BB diabetes thus operates at the level of interaction between inducing T cells and class II^u APC. These data lend support to the peptide affinity model for the class II genetic contribution to IDDM susceptibility: Susceptibility is caused by peptide presentation by a class II gene product that binds diabetogenic peptide, resulting in the activation of β cell–specific autoreactive T cells (48).

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