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# Identification of T Cell Subsets and Class I and Class II Antigen Expression in Islet Grafts and Pancreatic Islets of Diabetic BioBreeding/Worcester Rats

ELORA J. WERINGER, PhD, and
ARTHUR A. LIKE, MD

From the Department of Pathology, University of Massachusetts Medical School Worcester, Massachusetts

**The BioBreeding/Worcester (BB/Wor) rat develops a spontaneous disorder that closely resembles human insulin-dependent (Type I) diabetes mellitus. The syndrome is preceded by lymphocytic insulitis that destroys pancreatic beta cells. The morphologic features of the spontaneous insulitis lesions are also observed within islets transplanted beneath the renal capsule of diabetes-prone and diabetic animals. This study reports the results of experiments in which immunohistochemical techniques were used to characterize the phenotype of the infiltrating mononuclear cells and detect the expression of class I and class II MHC antigens in native islets and islet transplants in diabetic and diabetes-prone BB/Wor rats. The infiltrates within native pancreatic islets and islet grafts were comprised predominantly of Ia⁺ cells (dendritic cells and macrophages) CD4⁺ cells (helper/inducer lymphocytes and macrophages), CD5⁺ (pan-T) cells and smaller numbers of CD8⁺ (cytotoxic/suppressor and NK) cells. Pancreatic and graft insulitis were accompanied by markedly enhanced class I antigen expression on islet and exocrine cells. Class II (Ia) antigens were not detected on normal islet cells, islets undergoing insulitis or on islet transplants subjected to immune attack. In islet grafts stained with polymorphic MAbs that distinguish Ia antigens of donor and host origin, Ia antigen expression was limited to infiltrating dendritic cells and macrophages of host origin. It is concluded that the phenotypes of infiltrating mononuclear cells that comprise the insulitis lesion in spontaneous BB/Wor diabetes, and the inflammatory attack on islets transplanted into diabetic BB/Wor rats are the same, that pancreatic islet and graft insulitis occur in the presence of enhanced class I antigen expression but in the absence of class II antigen expression, and that infiltrating Ia⁺ cells within islet grafts are exclusively of recipient (BB/Wor) origin and may explain the initiation of immune insulitis within grafts derived from donors of incompatible MHC. (Am J Pathol 1988, 132:292–303)**

THE SPONTANEOUSLY DIABETIC BioBreeding/Worcester (BB/Wor) rat develops IDDM in a manner similar to human Type I (insulin dependent) diabetes mellitus.[1] The human and BB/Wor syndromes each have an unknown cause and are hypothesized to be the result of a cell-mediated, autoimmune destruction of pancreatic beta cells. The pancreata of newly diabetic rats show lymphocytic insulitis with selective destruction of beta cells resulting in insulin deficiency and hyperglycemia.[1-3] These events are most probably mediated by NK cells and T lymphocytes of the helper subset.[4] Other evidence implicating immune factors in the pathogenesis of diabetes in the BB rat include: 1) the relationship between the susceptibility to diabetes and the MHC of the rat[5,6]; 2) the presence of circulating autoantibodies to thyroid colloid, smooth muscle, gastric parietal cells, and possibly islet cell surface antigens[7-9]; 3) the adoptive transfer of diabetes with Concanavalin-A stimulated diabetic spleen cells[10-12]; and, 4) the interruption or prevention of the syndrome by methods that eliminate or modify the effector cells responsible for beta cell destruction and/or enrich specific population(s) of cells that induce resistance to diabetes.[4,13-20]

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Address reprint requests to Elora J. Weringer, PhD, Department of Pathology, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655.

The authors reported that the mononuclear infiltrate observed in acutely diabetic BB/Wor rats also appeared within islets transplanted into diabetic or diabetes-prone recipients.[21] Furthermore, the immune attack on the transplanted islets did not appear to be MHC restricted because MHC incompatible islets also evidenced lymphocytic insulitis when grafted into diabetic and diabetes-prone recipients. In these studies, isolated islets and fragments of adrenal cortex from MHC compatible and incompatible donors were treated to prevent allograft rejection. Thus, the presence of islet graft inflammation could be attributed to the BB immune attack rather than allograft rejection because islet grafts in recipients without endogenous pancreatic insulitis or diabetes were free of infiltrating lymphocytes and the adrenal grafts were not rejected.

Presently, the phenotypic identification of the effector cells that mediate final destruction of the pancreatic beta cells has not been clarified. In the natural course of the BB/Wor diabetic syndrome, there is a striking infiltration of lymphocytes, $Ia^+$ macrophages, and dendritic cells into the pancreatic islets prior to and during the acute onset of hyperglycemia. To define more clearly the nature of this infiltrate and a possible functional role for these cells in the mediation of the BB immune attack, the pancreatic islets of spontaneously diabetic BB/Wor rats and cultured pancreatic islets transplanted into acutely diabetic BB/Wor recipients were examined. The objectives were to characterize the distribution and phenotype of the mononuclear cell infiltrate and to determine whether the infiltrating cells were the same in the pancreatic insulitis lesion and in the lesion observed in the islet grafts.

Recent evidence suggests that in certain autoimmune states, class II molecules may be expressed on target cells of affected organs. Hence, the expression of Ia antigens by thyroid follicular cells[22] and pancreatic beta cells[23,24] is believed to play a role in their specific immune destruction. There is controversy, however, concerning the reported presence of Ia antigens on the pancreatic beta cells of human islets undergoing immune attack[23,25-27] and on beta cells of spontaneously diabetic BB rats.[28-31] Because of this disagreement concerning the expression of Ia antigens on beta cells and the role of beta cell Ia expression in the mechanism of immune destruction, native pancreatic beta cells and transplanted islets were examined for the presence of Ia antigens during the process of acute insulitis. In addition, due to the availability of monoclonal antibodies that detect polymorphic rat class II antigenic determinants, the authors wished to determine the origin (host *vs.* donor) of Ia positive cells within islet grafts from MHC incompatible (non-$RT1^u$) donors.

Endocrine islet cells are known to express class I antigens.[32,33] Furthermore, the expression of class I and class II antigens may be induced and/or enhanced by factors released from activated T cells and macrophages during most inflammatory processes. It was therefore also of interest to examine endogenous islet cells and islet transplants for induction/enhancement of class I antigen expression before and during the early stages of insulitis.

## Materials and Methods

### Animals and Pancreatic Islet Grafts

BB/Wor diabetes-resistant (DB-RES), Lewis, ($RT1^l$, Harlan Sprague Dawley, Indianapolis, IN), and PVG ($RT1^c$, Bantin & Kingman, Fremont, CA) rats were used as islet and adrenal transplant donors. Spontaneous diabetes has not been reported in Lewis and PVG rats and occurs infrequently (<1%) among diabetes-resistant BB/Wor rats. Recipients were acutely diabetic BB/Wor male rats.

Diabetes-resistant and diabetes-prone BB/Wor rats are descendants of the same parental breeders during the fifth generation of sib-matings in the BB/Wor colony and are both MHC $RT1^u$. All BB/Wor rats are in the 25–28th generation of sib-mating and are therefore considered to be inbred. Plasma glucose levels and body weights were determined on the day of transplantation. All diabetic animals (BG > 250 mg/dl) were insulin dependent and treated with insulin for the duration of the experiments.

Pancreatic islets were isolated, cultured, and treated to prevent allograft rejection as described previously.[21] Briefly, islets were isolated using sterile techniques from the pancreata of groups of 7 male rats by collagenase digestion.[34] The islets were incubated with anti-$Ia^k$ alloantiserum (Cedarlane Laboratories, Ontario, Canada) followed by rabbit complement (Pel Freez, Rogers, AR) to destroy "passenger leucocytes" (dendritic cells). Groups of 50 islets were aggregated in U-shaped microtiter plates by incubation in 95% $O_2$ at 37 C for 48 hours. Islet clusters (mega-islets) were cultured for an additional 5 days in sterile petri dishes. The adrenals were prepared for transplantation by a modification of the technique of Lafferty et al.[35] Adrenal cortical fragments were separated from capsular and medullary tissues and cultured without Ia alloantiserum for 8 days before transplantation.

### Transplantation

Under pentobarbital anesthesia, each recipient received 4 to 5 mega-islets (equivalent to 200–250 indi-

vidual islets), positioned as a single aggregate under the kidney capsule. Adrenal grafts were transplanted into the same diabetic rat. Each animal therefore received both mega-islet and adrenal grafts in adjacent sites under the same renal capsule. Animals were killed 6–31 days after transplantation.

#### Pancreatic Tissues

To obtain examples of active insulitis, pancreata from acutely diabetic animals, aglycosuric littermates, or 90–100-day-old nondiabetic rats were frozen and 5-$\mu$ hematoxylin and eosin (H&E) stained cryostat sections examined for the presence of lymphocytic insulitis. Sections of pancreata with evidence of insulitis and surviving islet cells were selected for study. Previous studies[3] have documented the frequent presence of (early) insulitis with surviving pancreatic beta cells among aglycosuric BB/Wor rats killed during the age range (90–100 days) wherein most diabetics are detected.

#### Islet Grafts

To analyze sequentially the BB/Wor immune attack on the islet grafts, animals from each donor series were killed at specified time intervals after transplantation. The kidneys bearing the grafts were removed and divided to permit both the islet and adrenal grafts to be studied. The adrenal grafts and the recipients' pancreata were fixed in Bouins solution. Microscopic sections of paraffin-embedded tissues were stained with H&E and examined for the presence of adrenalitis and insulitis. The islet grafts with adjacent renal cortex were immersed in embedding medium (OCT compound, Tissue Tek II; Miles Laboratories, Inc., Naperville, IL) and snap frozen in liquid nitrogen-cooled isopentane. Cryostat sections (5 $\mu$) were stained for cell surface antigens using a variety of MAbs by the immunoperoxidase method.

#### Reagents

Avidin, biotin, avidin-horseradish peroxidase, and biotinylated goat anti-mouse antibody (IgG) were purchased from Vector Laboratories, Inc, Burlingame, CA. Diaminobenzidine was purchased from Aldrich Biochemical, Milwaukee, WI.

#### Monoclonal Antibodies

The MRC hybridoma cell lines producing the mouse anti-rat monoclonal antibodies used in these studies (Table 1) were obtained from Drs. A. F. Williams and D. W. Mason, Oxford University, Oxford, UK and from Dr. C. D. Dijkstra, Free University, Amsterdam, The Netherlands. OX4 and OX6 recognize nonpolymorphic determinants of rat MHC class II antigen[36]; OX3 binds polymorphic Ia determinants on BB/Wor ($RT1^u$) and Lew ($RT1^l$), but does not react with PVG ($RT1^c$)[36]; F17-23-2 (hybridoma cells obtained from Dr. J. W. Fabre) reacts with Ia antigens of LEW ($RT1^l$), but does not react with PVG ($RT1^c$) or BB/Wor ($RT1^u$)[37,38]; 151.21 reacts with PVG ($RT1^c$) Ia antigens but not LEW ($RT1^l$) or BB/Wor ($RT1^u$) (AAL, unpublished); OX18 reacts with nonpolymorphic class I antigens and OX27 recognizes polymorphic class I determinants specifically for the $RT1^c$ haplotype.[39,40] OX12 reacts with rat IgG-Kappa chain and was used to identify B lymphocytes in cryostat sections of the pancreas.[41] OX19 and OX52 react with thymocytes and all peripheral T cells.[42,43] W3/25, OX35, and OX38 react with thymocytes, helper/inducer cells, and macrophages.[40,44] OX8 and OX10 react with thymocytes, cytotoxic/suppressor cells, and NK cells.[45–48] ED1 recognizes cells of the monocyte-macrophage lineage in the rat.[49]

#### Immunohistochemistry

Antigen localization studies were performed on 5-$\mu$ cryostat sections of pancreas and islet grafts using a modification of the Warnke method.[50,51] Briefly, sections were mounted on gelatin-coated slides, fixed in cold (−20 C) acetone, and stored at −70 C until used. For staining, the slides were again immersed in acetone for 10 minutes, air dried for 5 minutes, ringed with vaseline, washed in phosphate-buffered saline + 1% normal goat serum (PBS/NGS), and immersed in 3% $H_2O_2$/1% BSA for 10 minutes. The slides were sequentially incubated with 0.5% avidin followed by 0.1% biotin for 30 minutes to quench endogenous biotin reactive sites.[51] Sections were incubated with the appropriate monoclonal antibodies (undiluted tissue culture supernatants) for 30 minutes, followed by biotinylated horse anti-mouse IgG and avidin horseradish peroxidase at 5 $\mu$g/ml for 30 minutes. The sections were developed by incubation with 3,3′-diaminobenzidine tetrahydrochloride at 2 $\mu$g/ml plus 0.3% $H_2O_2$. The sections were pretreated in a 2% solution of $CuSO_4$ to enhance reaction product and counterstained with methylene blue. All procedures were performed at room temperature. The slides were coded and examined without knowledge of the experimental status of the animal. The infiltrate was scored using a semiquantitative (1+ to 4+) estimation of the cells staining with each monoclonal antibody.

Table 1—Monoclonal Antibodies Used for Detection of Rat MHC Antigens and Lymphoid Cells

| Monoclonal antibodies used to detect rat MHC antigens | | | | Monoclonal antibodies: reactivity with lymphoid cells | | | |
|---|---|---|---|---|---|---|---|
| Antibody | MHC class | Haplotype specificity | Ref | Antibody | CD type | Cells labeled | Ref |
| MRC OX4, OX6 | Class II, (Ia) | Monomorphic | 36 | OX19, OX52 | CD5 | All T cells, thymocytes | 42, 43 |
| MRC OX3 | | $l^+, u^+, a^-, c^-$ | 36 | OX8 | CD8 | Cytotoxic/suppressor T cells | 45, 46 |
| | | | | | | NK cells, thymocytes | 47, 48 |
| F17.23.2 | | $l^+, a^+, u^-, c^-$ | 37, 38 | W3/25, OX35, OX38 | CD4 | T helper/inducer cells | 43 |
| 151.21 | | $a^+, c^+, l^-, u^-$ | Unpublished (AAL) | | | macrophages, thymocytes | 40, 44 |
| OX18 | Class I | Monomorphic | 39 | OX12 | | Rat Ig Kappa chain | 41 |
| OX27 | | $c^+, a^-, l^-, u^-$ | 40 | ED1 | | Rat monocytes/ macrophages | 49 |

## Results

### Routine Light Microscopy

There was a moderate to heavy mononuclear infiltrate in all of the islet grafts transplanted into BB/Wor diabetic recipients. This was observed in grafts derived from all 3 donor strains (Table 2) and in the absence of an inflammatory infiltrate in adjacent adrenal grafts. Graft insulitis was observed in diabetic recipients with ongoing active insulitis, as well as in rats with end stage islets characterized by the total destruction of pancreatic beta cells and resolution of islet inflammation. This observation was true for all time points examined regardless of donor strain or severity of diabetes.

### Class I Antigen Expression: Islet Grafts

In Lewis, PVG, and BB/Wor grafts stained with OX18 (nonpolymorphic), class I antigens were present on the transplanted islet endocrine cells, residual ducts, and mononuclear infiltrating cells. With increased intensity of cellular infiltration within the islet grafts, islet cells stained more intensely for class I antigens, with increased membrane and cytoplasmic staining on those cells adjacent to the infiltrate. With the nonpolymorphic OX18 MAb, islet and mononuclear cells in all grafts were stained positively (Figure 1C). In PVG ($RT1^c$) grafts incubated with the polymorphic MAb OX27, the islet cells only were stained positively (Figure 1A, B). The infiltrating mononuclear cells did not react with OX27, suggesting they were of recipient BB/Wor ($RT1^u$) origin, which does not react with OX27.

In the normal rat kidney, the renal tubular cells in the cortex were definitely but weakly positive for class I MHC antigens. With graft inflammation, there was an increase in the magnitude of class I renal tubular staining, notably in the area surrounding the islet graft (not shown).

### Class II Antigen Expression: Islet Grafts

The polymorphic specificity of MAb F17-23-2 for class II antigens has been previously described (positive for $RT1^{l,a,n}$ and negative for $RT1^{c,u}$)[37] and was demonstrated by the intense staining of dendritic cells and B lymphocytes in Lewis spleen sections as well as interstitial dendritic cells of the normal Lewis pancreas. Normal pancreatic ductal and exocrine cells, endothelium of large and small vessels, and islet endocrine cells do not express class II antigens.[32] However,

Table 2—Phenotype of Cells Infiltrating Islet Grafts Undergoing Immune Attack in BB/Wor Diabetic Rats

| Donor | Recipient | N | Age of graft (days) | Phenotype of infiltrating cells | | |
|---|---|---|---|---|---|---|
| | | | | CD5 | CD4 | CD8 |
| BB/Wor | Acute diabetic | 4 | 14 | 2+ | 2.5+ | 1+ |
| (diabetes-resistant) | | 2 | 18 | 2+ | 3+ | 1+ |
| | | 2 | 21 | 2+ | 3+ | 0.5+ |
| | | 2 | 27 | 1+ | 1+ | 0.5+ |
| Lewis | Acute diabetic | 2 | 17 | 2+ | 2+ | 1+ |
| | | 4 | 26 | 1.5+ | 2+ | 1+ |
| | | 4 | 28 | 2.5+ | 3+ | 1+ |
| | | 4 | 31 | 2.75+ | 2.5+ | 1+ |
| PVG | Acute diabetic | 2 | 28 | 2+ | 2+ | 1+ |

Semiquantitative results derived from the examination of 330 representative sections.

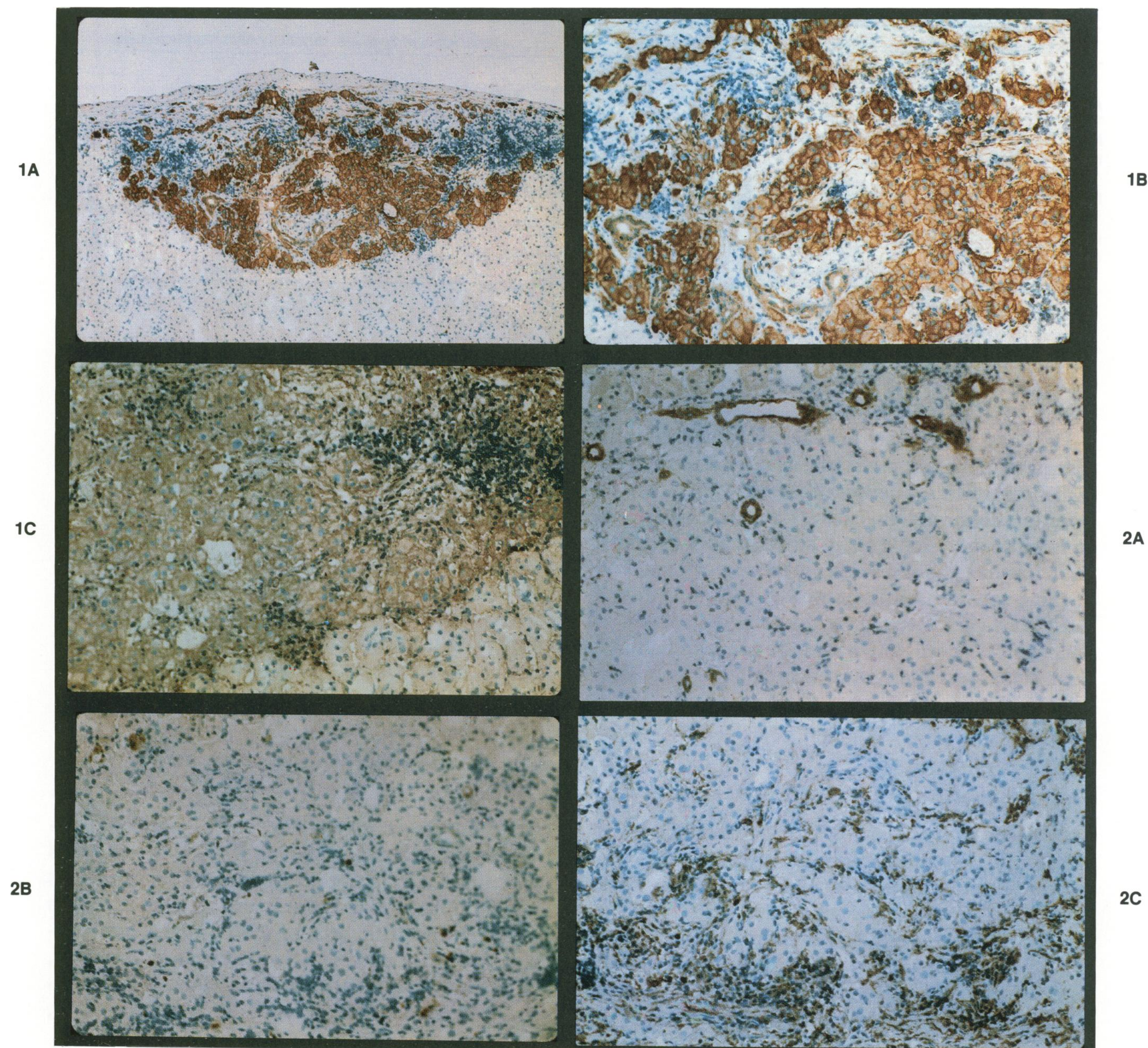


**Figure 1**—PVG islet graft, 28 days after transplantation into acute diabetic BB/Wor rat. **A and B**—section stained with OX27, a polymorphic MAb reacting with $RT1^c$ (PVG) donor but not $RT1^u$ (BB/Wor) recipient class I antigens. Only donor islet endocrine cells are positively stained (brown in micrograph). The infiltrating cells and surrounding renal tissue are unstained (blue in micrograph), indicating that they are of host origin. (A, ×70; B, ×140) **C**—Same field as B on an adjacent section, after staining with OX18, a monomorphic MAb for class I antigens. Both donor islet cells and recipient cells are positively stained (brown and black/blue in micrograph). (×140) **Figure 2**—Pancreatic islet grafts stained for class II antigens after transplantation into acute diabetic BB/Wor rats. **A**—Lewis graft stained with F17.23.2 (polymorphic, $RT1^{l+,u-}$). Only ductal epithelial cells are positively stained (brown in micrograph) for Lewis Ia antigens. Islet and interstitial cells are negative. (×140) **B**—PVG graft stained with 151.21 (polymorphic, $RT1^{c+,u-}$). Islet and infiltrating interstitial cells are negative for Ia antigens. (×140) **C**—PVG graft stained with OX3 (polymorphic, $RT1^{c-,u+}$). Infiltrating inflammatory cells are positively stained (brown and black/blue in micrograph) for Ia antigens. Islet cells are negative. (×140) Taken together, A, B, and C indicate that although infiltrating inflammatory cells express Ia antigens of recipient origin, grafted islet cells do not express Ia antigens. Donor Lewis ductal cells do stain for class II antigens.

a rare $Ia^+$ ductal cell was observed in normal Lewis pancreas stained with the above MAb. Dendritic cells of the BB/Wor ($RT1^u$) pancreas react positively with MRC OX3 but do not stain with F17-23-2 (not illustrated).

The authors have previously reported that graft insulitis occurs after transplantation into acute diabetic recipients.[52] In the present study, islet grafts were examined 14–31 days after transplantation. The endocrine cells of all islet grafts were negative for class II

Table 3—Distribution of Class I and Class II Antigen Expression on Cells of Islets Transplanted into BB/Wor Diabetic Recipients

| | Monoclonal Antibodies | | | | |
|---|---|---|---|---|---|
| | Class II | | | Class I | |
| Donor strains | OX6/OX3 | F17.23.2 | 151.21 | OX18 | OX27 |
| PVG ($RT1^c$) | | | | | |
| islet cells | – | – | – | + | + |
| infiltrating cells | + | – | – | + | – |
| (N = 2) | | | | | |
| BB/Wor ($RT1^u$) | | | | | |
| islet cells | – | – | – | + | – |
| infiltrating cells | + | – | – | + | – |
| (N = 6) | | | | | |
| Lewis ($RT1^l$) | | | | | |
| islet cells | – | – | – | + | NT |
| infiltrating cells | + | – | – | + | |
| ductal cells | + | + | NT | + | |
| (N = 9) | | | | | |

NT, not tested.

antigen expression as shown with the panel of MAbs used (Table 1 and 3); Ia antigens were not expressed, regardless of the donor strain, duration of transplantation, intensity of lymphocytic infiltrate, or synchrony with recipients' pancreatic insulitis (Figure 2). There was no induction of class II antigens on the vascular structures within the grafts. At 17 days after transplantation, however, class II antigens were detected on ductal epithelial cells located within several of the Lewis grafts. The ductal cells were distinctly and strongly positive with MAb F17.23.2 and therefore, were of donor ($RT1^l$) origin (Figure 2A). Ductal cells within PVG and BB/Wor grafts were not Ia positive.

Islet grafts from $RT1^u$ and non-$RT1^u$ donors incubated with OX6 to detect class II antigens were permeated by many positively stained mononuclear cells. To determine the origin of these infiltrating cells, Ia-specific polymorphic MAbs were used. Both 151.21 ($RT1^{c+,u-}$) and F17.23.2 ($RT1^{l+,u-}$) MAbs failed to stain the infiltrating mononuclear cells, which on adjacent sections stained intensely positive with OX6 and OX3 ($RT1^{u+}$) (Figure 2A, B, C). It was concluded, therefore, that these cells were of host rather than donor origin (Table 3).

The same MAbs were tested in normal PVG ($RT1^c$), Lewis ($RT1^l$), and BB/Wor ($RT1^u$) diabetes-resistant and diabetes-prone pancreata using identical staining procedures. The native endocrine islet cells in all pancreatic sections examined were negative for class II antigen expression after staining with the appropriate (polymorphic/monomorphic) monoclonal antibodies. Islet cell class II antigen staining was also not observed after incubation with nonpolymorphic MAb OX4 and OX6, in any BB/Wor pancreatic sections with a lymphocytic infiltrate.

**Phenotype of Infiltrating Cells in the Islet Grafts**

Cryostat sections were stained to establish the phenotype of mononuclear cells infiltrating the MHC-compatible and -incompatible islet grafts transplanted into acutely diabetic BB/Wor rats (Table 2). By day 7 after transplantation, grafts were diffusely infiltrated with an heterogeneous population of cells. By day 14, the infiltrates were localized to the areas at the margins of the graft and at the junction between the graft and the adjacent renal cortex. At 14–32 days, the infiltrate was more pronounced and the cells were dispersed throughout the grafts with areas of accumulation around the remaining endocrine cells. Most cells were stained with a mixture of MAbs W3/25, OX/35, and OX/38, all of which react with $CD4^+$ T cells of the helper/inducer subset and macrophages. A significant proportion of the infiltrating cells were $CD5^+$ T cells as determined by staining with the pan-T-cell MAbs OX19 and OX52 (Figure 3A). A smaller number were stained with OX8, the MAb for $CD8^+$ cytotoxic/suppressor cells and NK cells. A majority of the infiltrating cells were also $Ia^+$, and were stained with OX3 and OX6, suggesting a mixture of $Ia^+$ dendritic cells, macrophages and possibly B lymphocytes (Figure 2C).

To identify macrophages among the overlapping populations of $CD4^+$,$Ia^+$ cells, sections of representative grafts were stained with the ED1 monoclonal antibody that reacts with most rat macrophages.[49] Numerous ED1-positive cells were identified within the mononuclear infiltrate and were believed to coincide with the larger $CD4^+$ and $Ia^+$ cells, which possess abundant cytoplasm. There was a small but representative population of smaller cells that were W3/$25^+$ but ED1 negative. It is assumed that these small W3/$25^+$ cells belong to the helper/inducer T cell subset.

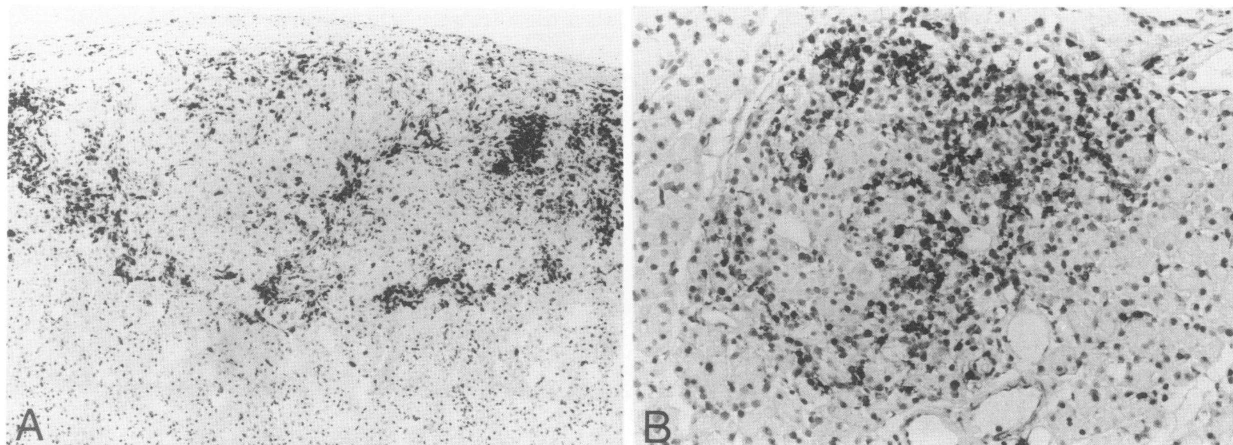

**Figure 3**—Staining for T cells with MAb OX19 (pan T cell). **A**—Lewis islet graft in BB/Wor acute diabetic rat. Numerous OX19$^+$ cells infiltrate and surround the cords of islet cells. (×70) **B**—Pancreatic islet from acute diabetic BB/Wor rat with insulitis, demonstrates the presence of OX19$^+$ T cells in the islet infiltrate. (×140)

### Phenotype of Cells in Pancreatic Infiltrate

Cryostat sections of pancreata with evidence of insulitis demonstrated that Ia$^+$ cells (macrophages and dendritic cells) were most numerous, (Figure 4D) followed in decreasing numbers, by CD4, CD5, and CD8 cells. Only rare B lymphocytes (OX12$^+$) were identified. The distribution of the Ia$^+$ cells was not limited to the islets, with many positively stained cells localized around ducts and vessels and within the interstitium of the pancreatic parenchyma. The CD4 (W3/25) stain revealed islets infiltrated and surrounded by numerous positively stained cells, many with narrow cytoplasmic rims and presumably lymphocytes, and others with abundant positively staining cytoplasm consistent with macrophages. The exocrine tissue contained an occasional W3/25$^+$ lymphocyte and numerous positive cells of the macrophage or dendritic type. OX19$^+$ (Figure 3B) and OX8$^+$ cells were fewer in number and were clearly lymphoid in appearance. The distribution of these cells was predominantly peri- and intra-islet in location, with only an occasional cell seen in the pancreatic exocrine tissue.

### Expression of MHC Antigens in Pancreas

The distribution of class I and II MHC antigens in the normal pancreas has been described.[32,33] Class II antigens are restricted essentially to interstitial dendritic cells and rare mononuclear cells (macrophages) scattered at random throughout the exocrine and endocrine tissue (Figure 4C), with some increase in dendritic cell numbers in connective tissue surrounding ducts and blood vessels. Islet and acinar cells do not stain for class II antigens (Figure 4C). The distribution of class I MHC antigens in the normal pancreas appears to be restricted to duct epithelium, islet cells, and endothelium (Figure 4A). Pancreatic acinar cells do not stain for class I constitutively, and it is possible that these cells normally do not express class I antigens. The results with normal Lewis, PVG, and BB/Wor pancreata reacted with Mab OX18 and OX27 are consistent with these observations.

The pattern of class I and class II antigen staining in the pancreata of rats with insulitis was similar to that described for the islet grafts. Class II MAbs OX4 and OX6 stained interstitial dendritic cells and macrophages in the exocrine pancreas and islets exclusively, with no staining of other cellular components of the pancreas. Thus, Ia staining was absent on islets, acinar cells, ductal epithelium, and endothelial cells of small and large vessels, even in the presence of striking insulitis (Figure 4C, D). Concommitant with the influx of mononuclear cells, there was not only enhanced class I expression on islet cells, but also profound *de novo* expression of class I antigens on the surrounding pancreatic acinar tissue (Figure 4B). With resolution of the inflammatory infiltrate and progression towards end-stage islets, the areas of exocrine tissue that were class I positive receded to a "halo" of positively stained cells immediately around the affected islets. Finally, with the appearance of classical end-stage islets and absence of the mononuclear cell infiltrate, both the islets and the exocrine acinar tissue returned to the normal pattern of class I expression (Figure 4E, F).

## Discussion

In the present studies, cryostat sections of BB/Wor pancreas and islet transplants were stained with monoclonal antibodies directed against subsets of rat

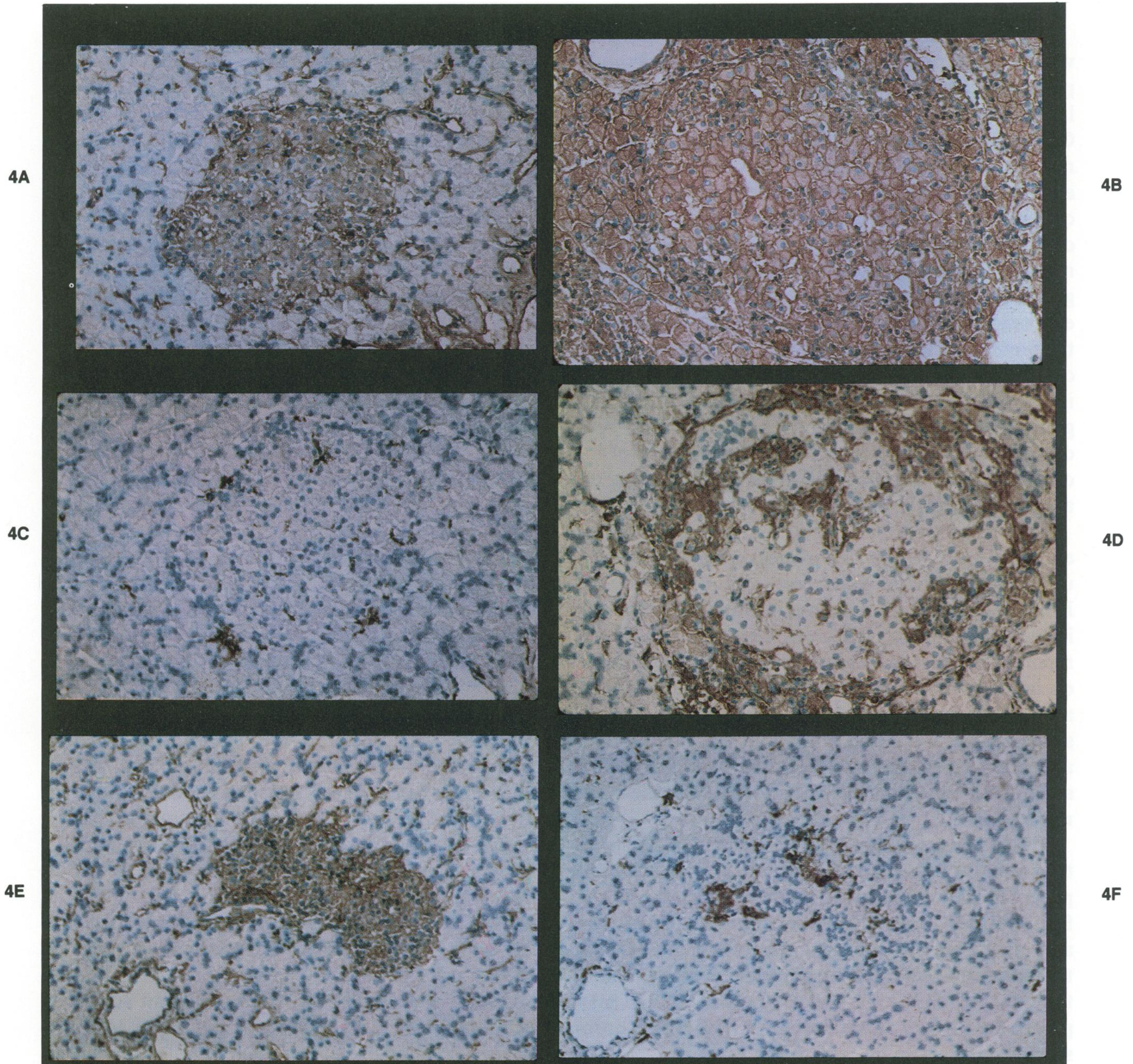


**Figure 4**—BB/Wor pancreatic sections stained for class I and class II antigen expression with OX18 and OX6 MAbs. **A**—Normal pancreas stained with OX18: Islet endocrine cells, endothelium and ductal cells are positively stained for class I antigens. Exocrine cells are negative. (OX18, ×140) **B**—Acute insulitis: pancreatic islet is infiltrated with mononuclear inflammatory cells. Class I antigen expression is markedly enhanced in islet cells and induced in surrounding exocrine cells. (OX18, ×140) **C**—Normal pancreas stained with OX6: class II antigen expression is limited to scattered interstitial dendritic cells and rare macrophages. Endocrine and exocrine cells are negative. Microscopic fields illustrated in A and C are from adjacent cryostat sections. (OX6, ×140) **D**—Acute insulitis: endocrine and surrounding exocrine cells are negative for Ia antigens. Infiltrating macrophages and dendritic cells are deeply stained after exposure to OX6. B and D are from adjacent cryostat sections. (OX6, ×140) **E and F**—Adjacent sections illustrate "end-stage" islets after staining with OX18 (E) and OX6 (F). **E**—With the disappearance of insulitis, class I antigen expression is once again restricted to the islet cells, endothelium and ductal cells. Exocrine cells are negative. **F**—Class II expression in end-stage islets is limited to residual clusters of cells resembling dendritic cells and macrophages. (e, OX18, ×140; f, OX6, ×140)

lymphocytes and macrophages to characterize phenotypically the mononuclear infiltrate within the pancreatic islets and islet transplants of diabetic and diabetes-prone BB/Wor rats. In addition, monoclonal antibodies specific for MHC antigens were used to determine if class I and class II (Ia) antigen expression on pancreatic islets, islet grafts, and exocrine cells were induced or enhanced by the inflammatory process that precedes and accompanies destruction of pancreatic beta cells.

The results suggest that the inflammatory infiltrate within native pancreatic islets and islet transplants is phenotypically similar and comprised of $Ia^+$ cells and lymphocytes. Of the CD5 ($OX19^+$) T cells labeled, CD4 helper/inducer ($W3/25^+$) cells were most numerous, with smaller numbers of CD8 ($OX8^+$) NK and cytotoxic/suppressor cells identified. $OX12^+$ B cells were infrequent. $Ia^+$ macrophages and dendritic cells that stained intensely with OX4/OX6 also surrounded and permeated the native islets and islet grafts. These findings also indicate that class I antigen expression on islet and exocrine cells was enhanced in the presence of insulitis. Ia antigen expression was not detected in transplanted islet cells, native pancreatic islets or acinar cells. In both the native islets and grafts, lymphocytic infiltration and pancreatic beta cell destruction were observed in the presence of increased class I expression, but in the absence of detectable class II antigen expression.

The presence of class II antigen expression in ductal epithelial cells within Lewis mega-islets is presently unexplained in view of its absence in ductal cells of BB/Wor rats with spontaneous insulitis. Although the induction of ductal cell Ia antigen expression has been reported in Lewis pancreatic allografts,[33] the authors do not believe that the mega-islet grafts have elicited an alloresponse because $Ia^+$ ductal cells were not observed in the PVG islet grafts that are MHC $RT1^c$, and previous studies[21] rule out the likelihood of an allograft response to mega-islets prepared in the manner described.

The process mediating pancreatic beta cell destruction remains uncertain, but 2 possibilities have been proposed: 1) the synthesis of specific anti-beta cell surface antibodies that may precede the onset of diabetes[9] and may target the islet beta cells for specific destruction, and 2) T cell-mediated specific beta cell killing that may be MHC class I/class II restricted and may proceed with or without the help of MHC-unrestricted destruction by natural killer cells.

The presence of $W3/25^+$ cells in the mononuclear infiltrate within the native pancreatic islets and islet transplants suggests that CD4 (helper/inducer) cells contribute to the recognition, initiation, and most likely the stimulation of a cellular effector limb mediating beta cell destruction. It has been reported that rat dendritic cells and macrophages are both $CD4^+$ and comprise the antigen presenting cell population necessary for the induction of most immune responses[40,53,54] by providing antigenic signalling for T cell activation. Recent evidence also indicates that CD4 cells consist of 2 functional subsets: one that plays a role in NK cell activity, which is believed to be involved in BB/Wor diabetes[4,55] and cell-mediated immunity, and another that stimulates B lymphocyte antibody synthesis. It is not known if and how CD4 cells induce disease, or if they may serve as functional cytotoxic effector cells.[56] Morphologic studies suggest that there may be a functional compartment within the transplanted islets and native islets wherein $Ia^+$ antigen-presenting cells and $CD4^+$ cells are in close enough approximation to promote the T cell proliferation and lymphokine production necessary for activation of effector cells.

The mononuclear cells infiltrating both Lewis and PVG islet grafts were stained positively with OX3 but stained negatively after incubation with polymorphic MAbs F.17.23.2 and 151.21, indicating that the $Ia^+$ cells were of MHC $RT1^u$ (host) origin and not of donor $RT1^l$ and $RT1^c$ derivation. Morphologic studies also revealed that host $Ia^+$ cells and macrophages (ED1) were found in close proximity to sites of aggregated host lymphoid elements. It has been reported that accumulations of $Ia^+$ cells may be the *in vivo* counterpart of dendritic cell-lymphocyte clustering, which is a requirement for *in vitro* class II sensitization in the mouse and rat.[57] Thus, host $Ia^+$ antigen-presenting cells may be the source of class II antigens necessary for the activation of effector T lymphocytes. In this way, in spite of the discordant MHC antigens on Lewis and PVG grafts, host $Ia^+$ cells may populate the MHC-incompatible grafts and present beta cell antigens in conjunction with compatible (host) Ia antigens. These antigens will then recruit and activate the T lymphocytes necessary for BB immune attack. It is widely believed that T helper cells are class II MHC-restricted in their interaction with accessory cells.[58] The data presented in this manuscript and a previous report that the BB immune attack on transplanted islets is not MHC restricted[21] suggest that activated BB effector cells are not MHC restricted in their interaction with target beta cells. Hence, afferent activation or initiation of CD4 cells may require an MHC-restricted priming event, but efferent (effector cell) action may not need further MHC self-recognition. Thus, $CD4^+$ cells may function in both restricted and nonrestricted effector roles.

Precedent for this speculative hypothesis can be derived from the review by Singer and Hodes[59] and from the studies of Shih et al,[60] wherein $CD4^+$ primed T cells interacted with $H_2$-unrelated B cells. More recently, Prud'homme reported experiments in which T cell hybrids prepared by the fusion of diabetic BB rat T cell blasts with mouse thymoma cells responded to $RT1^g$ RIN-5F tumor cells after *in vitro* stimulation by $RT1^u$ antigen-presenting cells.[61] (Prud'homme assumed that the RIN-5F cells that were derived from $RT1^g$ NEDH rats were $RT1^u$ and therefore incorrectly

concluded that the response of BB rat T cell blasts was MHC restricted.)

The results reported above clearly show that endocrine islet cells in the transplants and in the endogenous pancreata were negative for Ia$^+$-antigen expression, and further that there was no induction of class II expression at the onset of insulitis or during the observed time course of the BB/Wor immune attack. The reports by Savino[62] and Issa-Chergui[31] confirm the absence of Ia expression by beta cells in the native endocrine islets of BB/Wor rats. Dean et al[28] reported that class II MHC molecules were detected on beta cells during the late stages of diabetes in the BB/E rat model. These conflicting results may be due to technical differences (immunofluorescence *vs.* immunoperoxidase localization) in the present study. It is also possible that the observation of both Ia$^+$ and insulin staining in the same cell may result from macrophage phagocytosis of beta cells and/or insulin granules from degenerating beta cells.[29,63] This phenomenon would not be resolved by light microscopy alone.

The analysis of class I antigen distribution in the endogenous pancreas and the pancreatic islet grafts demonstrates that MHC antigen expression in these tissues during BB/Wor diabetes is a dynamic process. In the normal pancreatic islet, endocrine cells are class I positive while exocrine pancreatic acinar cells are class I negative.[32,33] Class I antigen expression was both enhanced in pancreatic islet cells and induced in exocrine cells surrounding islets infiltrated with mononuclear cells. Islet grafts infiltrated with mononuclear cells also evidenced enhancement of class I expression within endocrine cells and the adjacent renal cortex. These tissues thus have the ability to synthesize class I antigens and to respond to the inducing signal(s) provided by the cells of the insulitis lesion. Infiltrating macrophages, which are known to release beta interferon(s) upon activation, are likely to be involved in the induction phenomenon. This raises the question of whether the induction of class I MHC antigens has any influence on the course of the insulitis process, ie, by either limiting or localizing the mononuclear infiltrate in some way, or primarily as a response to the presence of inflammatory cells and their mediators.

Immune responses in which MHC plays a pivotal role generally require the collaborative efforts of class I and class II antigen molecules for cellular mediated reactions. The inability to demonstrate MHC class II antigens on native islet cells or islet grafts may reflect either the absence or low density of these molecules, and as such, their expression may be below the detection limit of the immunoperoxidase methods employed. It is possible however, that class I antigen expression by the pancreatic islet cells and acinar tissue in BB/Wor diabetes may be a singular response to the release of cytokines and lymphokines by infiltrating macrophages and lymphocytes. This effect may possibly inhibit or mask class II antigen production and/or expression. The clarification of this process might reveal important information on the mechanism of islet beta cell destruction.

## References


1. Nakhooda AF, Like AA, Chappel CI, Murray FT, Marliss EB: The spontaneously diabetic Wistar rat: Metabolic and morphologic studies. Diabetes 1977, 25:100–112
2. Seemayer TA, Tannenbaum GS, Goldman H, Colle E: Dynamic time course studies of the spontaneously diabetic BB Wistar rat. III. Light microscopic and ultrastructural observations of pancreatic islets of Langerhans. Am J Pathol 1982, 106:237–249
3. Logothetopoulos J, Valiquette N, Madura E, Cvet D: Onset and progression of pancreatic insulitis in the overt, spontaneously diabetic, young adult BB rat studied by pancreatic biopsy. Diabetes 1984, 33:33–36
4. Like AA, Biron CA, Weringer EJ, Byman K, Sroczynski E, Guberski DL: Prevention of diabetes in Biobreeding/Worcester rats with monoclonal antibodies that recognize T lymphocytes or natural killer cells. J Exp Med 1986, 164:1145–1159
5. Colle E, Guttman RD, Seemayer T: Spontaneous diabetes mellitus in the rat. I. Association with the major histocompatibility complex. J Exp Med 1981, 154: 1237–1242
6. Jackson RA, Buse J, Rifai R, Pelletier D, Milford E, Carpenter C, Eisenbarth GS, Williams RM: Two genes required for diabetes in BB rats: Evidence from cyclical intercrosses and backcrosses. J Exp Med 1984, 159: 1629–1636
7. Elder M, Maclaren N, Riley W, McConnell T: Gastric parietal and other autoantibodies in the BB rat. Diabetes 1982, 31:313–318
8. Like AA, Appel MC, Rossini AA: Autoantibodies in the BB/W rat. Diabetes 1982, 31:816–820
9. Dyrberg T, Nakhooda AF, Baekkeskov S, Lernmark A, Poussier P, Marliss EB: Islet cell surface antibodies and lymphocytic antibodies in the spontaneously diabetic BB Wistar rat. Diabetes 1982, 31:278–281
10. Koevary S, Rossini AA, Stoller W, Chick WL, Williams RM: Passive transfer of diabetes in the BB/W rat. Science 1983, 220:727–728
11. Like AA, Weringer EJ, Holdash A, McGill P, Atkinson D, Rossini AA: Adoptive transfer of autoimmune diabetes mellitus in Biobreeding/Worcester (BB/W) inbred and hybrid rats. J Immunol 1985, 134:1583–1587
12. Koevary S, Williams DE, Williams RM, Chick WL: Passive transfer of diabetes from BB/W to Wistar-Furth rats. J Clin Invest 1985, 75:1904–1907
13. Like AA, Rossini AA, Guberski DL, Appel MC, Williams RM: Spontaneous diabetes mellitus: reversal and prevention in the BB/W rat with antiserum to rat lymphocytes. Science 1979, 206:1421–1423
14. Like AA, Kislauskis E, Williams RM, Rossini AA: Neo-

natal thymectomy prevents spontaneous diabetes mellitus in the BB/W rat. Science 1982, 216:644–646
15. Like AA, Anthony M, Guberski DL, Rossini AA: Spontaneous diabetes mellitus in the BB/W rat: Effects of glucocorticoids, Cyclosporin-A, and antiserum to rat lymphocytes. Diabetes 1983, 32:326–330
16. Laupacis A, Stiller CR, Gardell G, Keown P, Dupre J, Wallace AC, Thiebert P: Cyclosporin prevents diabetes in BB Wistar rats. Lancet 1983, 1:10–12
17. Like AA, DiRodi V, Thomas S, Guberski DL, Rossini AA: Prevention of diabetes mellitus in the BB/W rat with Cyclosporin-A. Am J Pathol 1984, 117:92–97
18. Naji A, Silvers WK, Bellgrau D, Barker CF: Spontaneous diabetes in rats: destruction of islets is prevented by immunological tolerance. Science 1981, 213:1390–1392
19. Rossini AA, Mordes JP, Pelletier A, Like AA: Transfusions of whole blood prevent spontaneous diabetes mellitus in the BB/W rat. Science 1983, 219:975–977
20. Rossini AA, Faustman D, Woda BA, Like AA, Szymanski I, Mordes JP: Lymphocyte transfusions prevent diabetes in the BioBreeding/Worcester rat. J Clin Invest 1984, 74:39–46
21. Weringer EJ, Like AA: Immune attack on pancreatic islet transplants in the spontaneously diabetic BioBreeding/Worcester (BB/W) rat is not MHC restricted. J Immunol 1985, 134:2383–2386
22. Pujol-Borrell R, Hanafusa T, Chiovato L, Bottazzo GF: Lectin-induced expression of DR antigen on human cultured follicular thyroid cells. Nature 1983, 303:71–73
23. Bottazzo GF, Dean BM, McNally JM, McKay EH, Swift PGF, Gamble DR: In situ characterization of autoimmune phenomena and expression of HLA molecules in the pancreas in diabetic insulitis. N Engl J Med 1985, 313:353–360
24. Pujol-Borrell R, Todd I, Doshi M, Bottazzo GF, Sutton R, Gray D, Adolf GR, Feldman M: HLA class II induction in human islet cells by interferon-$\gamma$ plus tumor necrosis factor or lymphotoxin. Nature 1987, 326:304–306
25. Foulis AK, Farquharson MA, Hardman R: Aberrant expression of class II major histocompatibility complex molecules by B cells and hyperexpression of class I major histocompatibility complex molecules by insulin containing islets in Type I (insulin-dependent) diabetes mellitus. Diabetologia 1987, 30:333–343
26. Sibley RK, Sutherland DER, Goetz F, Michael AF: Recurrent diabetes mellitus in the pancreas iso- and allograft. Lab Invest 1985, 53:132–144
27. Sibley RK, Sutherland DER: Pancreas transplantation: An immunohistologic and histopathologic examination of 100 grafts. Am J Pathol 1987, 128:151–170
28. Dean BM, Walker R, Bone AJ, Baird JD, Cooke A: Prediabetes in the spontaneously diabetic BB/E rat: Lymphocyte subpopulations in the pancreatic infiltrate and expression of rat MHC class II molecules in endocrine cells. Diabetologia 1985, 28:464–466
29. Like AA: Spontaneous diabetes in animals, The Diabetic Pancreas. Edited by BW Volk, ER Arquilla. New York, Plenum Press, 1985, p 385
30. Weringer EJ, Like AA: T cell subsets and class II antigens in islets transplanted into BioBreeding/Worcester (BB/Wor) rats. Diabetes 1986, 35(Suppl 1):273A
31. Issa-Chergui B, Yale JF, Vigeant C, Seemayer TA: Major histocompatibility complex gene product expression on pancreatic beta cells in acutely diabetic BB rats after passive transfer. Am J Pathol 1988, 130:156–162
32. Hart DNJ, Newton MR, Reece-Smith H, Fabre JW, Morris PJ: Major histocompatibility complex antigens in the rat pancreas, isolated pancreatic islets, thyroid and adrenal. Transplantation 1983, 36:431–435
33. Steiniger B, Klempnauer J, Wonigeit K: Altered distribution of class I and class II MHC antigens during acute pancreas allograft rejection in the rat. Transplantation 1985, 40:234–239
34. Lacy PE, Kostianovsky MA: A method for the isolation of intact islets of Langerhans from the rat pancreas. Diabetes 1972, 16:35–39
35. Lafferty KJ, Bootes A, Dart G, Talmage DW: Effect of organ culture on the survival of thyroid allograft in mice. Transplantation 1976, 22:138–149
36. McMaster WR, Williams AF: Identification of Ia glycoproteins in rat thymus and purification from rat spleen. Eur J Immunol 1979, 9:426–433
37. Hart DNJ, Fabre JW: Demonstration and characterization of Ia-positive dendritic cells in the interstitial connective tissues of rat heart and other tissues, but not brain. J Exp Med 1982, 154:347–361
38. Hart DNJ, Fabre JW: Endogenously produced Ia antigens within the cells of convoluted tubules of rat kidney. J Immunol 1981, 126:2109–2113
39. Fukumoto T, McMaster WR, Williams AF: Mouse monoclonal antibodies against rat major histocompatibility antigens: Two Ia antigens and the expression of Ia and class I antigens in rat thymus. Eur J Immunol 1982, 12:237–243
40. Jeffries WA, Green JR, Williams AF: Authentic T-helper CD4 (W3/25) antigen on rat peritoneal macrophages. J Exp Med 1985, 162:117–127
41. Hunt SV, Fowler MH: A repopulation assay for B and T lymphocyte stem cells employing radiation chimeras. Cell Tissue Kinetics. 1981, 14:445–464
42. Dallman MJ, Thomas ML, Green JR: MRC OX19: A monoclonal antibody that labels rat T lymphocytes and augments in vitro proliferative responses. Eur J Immunol 1984, 14:260–267
43. Robinson AP, Puklavec M, Mason DW: MRC OX-52: A rat T-cell antigen. Immunology 1986, 57:527–531
44. Williams AF, Galfre G, Milstein C: Analysis of cell surfaces by xenogeneic myeloma-hybrid antibodies: differentiation antigens of rat lymphocytes. Cell 1977, 12:663–673
45. Brideau RJ, Carter PB, McMaster WR, Mason DW, Williams AF: Two subsets of rat T lymphocytes defined with monoclonal antibodies. Eur J Immunol 1980, 10:609–615
46. Dallman MJ, Mason DW, Webb M: The roles of host and donor cells in the rejection of skin allografts by T cell-deprived rats injected with syngeneic T cells. Eur J Immunol 1982, 12:511–518
47. Cantrell DA, Robins RA, Brooks CG, Baldwin RW: Phenotype of rat natural killer cells defined by monoclonal antibodies marking rat lymphocyte subsets. Immunology 1982, 45:97–103
48. Woda BA, Like AA, Padden C, McFadden M: Deficiency of phenotypic cytotoxic-suppressor T-lymphocytes in the BB/W rat. J Immunol 1986, 136:856–859

49. Dijkstra CD, Dopp BA, Joling P, Kraal G: The heterogeneity of mononuclear phagocytes in lymphoid organs: distinct macrophage subpopulations in the rat recognized by monoclonal antibodies ED1, ED2 and ED3. Immunology 1985, 54:589–599
50. Warnke R, Levy R: Detection of T and B cell antigens with hybridoma monoclonal antibodies: A biotin-avidin-horseradish peroxidase method. J Histochem Cytochem 1980, 28:771–776
51. Wood GS, Warnke R: Suppression of endogenous avidin-binding activity in tissues and its relevance to biotin-avidin detection systems. J Histochem Cytochem 1981, 29:1196–1204
52. Weringer EJ, Like AA: Diabetes mellitus in the BB/W rat: Insulitis in pancreatic islet grafts after transplantation in diabetic recipients. Am J Pathol 1986, 125:107–112
53. Steiniger B, Klempnauer J, Wonigeit K: Phenotype and histological distribution of interstitial dendritic cells in the rat pancreas, liver, heart and kidney. Transplantation 1984, 38:169–175
54. Mason DW, Dallman MJ, Arthur RP, Morris PJ: Mechanisms of allograft rejection: the roles of cytotoxic T cells and delayed-type hypersensitivity. Immunol Rev 1984, 77:167–184
55. MacKay P, Jacobson J, Rabinovitch A: Spontaneous diabetes mellitus in the Biobreeding/Worcester rat: Evidence *in vitro* for natural killer cell lysis of islet cells. J Clin Invest 1986, 77:916–924
56. Sun D, Wekerle H: Ia-restricted encephalitogenic T lymphocytes mediating EAE lyse autoantigen presenting astrocytes. Nature 1986, 320:70–72
57. Green JR, Joffe R: Interactions between T helper cells and dendritic cells during rat mixed lymphocyte reaction. J Exp Med 1985, 162:1546–1555
58. Rosenthal AS, Shevach EM: Function of macrophages in antigen recognition by guinea pig T lymphocytes. I. Requirement for histocompatible macrophages and lymphocytes. J Exp Med 1973, 138:1194–1212
59. Singer A, Hodes RJ: Mechanisms of T cell–B cell interaction. Ann Rev Immunol 1983, 1:211–241
60. Shih WH, Matzinger PC, Swain SL, Dutton RW: Analysis of histocompatibility requirements for proliferative and helper T cell activity. J Exp Med 1980, 152:1311–1328
61. Prud'homme GJ, Fuks A, Guttman RD, Colle E: T-cell hybrids with specificity for islet cell antigens. J Immunol 1986, 136:1535–1536
62. Savino W, Timsit J, Bach JF: Ia-bearing cells in pancreatic islets of the diabetic BB rat. Diabetes 1985, 34(Suppl 1):287A
63. Pipeleers DG, In't Veld PA, Pipeleers-Marichal MA, Gepts W, Van De Winkel M: Presence of pancreatic hormones in islet cells with MHC-class II antigen expression. Diabetes 1987, 36:872–876

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