Autoimmune Destruction of Islets Transplanted Into RT6-Depleted Diabetes-Resistant BB/Wor Rats

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We report a novel animal model of islet transplantation that distinguishes recurrence of autoimmunity from allograft rejection. In this study, diabetes-resistant (DR) BB rats, <1% of which develop spontaneous diabetes, were made hyperglycemic by either a single injection of streptozocin (STZ) or in vivo immune elimination of a regulatory T-lymphocyte subset that expresses the RT6 alloantigen. DR islet grafts were then transplanted into both groups. DR transplants into STZ-induced diabetic DR rats produced long-term normoglycemia. In contrast, DR transplants into DR rats that had been treated with anti-RT6 monoclonal antibody were all destroyed within an average of 4 days. Allogeneic islets transplanted into both STZ-induced and RT6-depleted diabetic DR rats were rejected within a mean of 3 days. We conclude that failure of DR islet grafts in RT6-depleted diabetic DR BB rats represents recurrent autoimmunity. *Diabetes* 39:643–45, 1990

Efforts to cure insulin-dependent diabetes mellitus (IDDM) by pancreas or islet transplantation are hampered by both allograft rejection and recurrence of the original autoimmune disease process. When monozygotic twins with IDDM receive pancreas transplants from discordant siblings in the absence of immunosuppression, the grafts are destroyed (1). This suggests that, long after disease onset, the immune system of IDDM subjects can recognize and destroy normal β-cells. To make transplantation a viable therapy for IDDM, recurrent autoimmunity must be prevented.

Until now, successful transplantation in humans has required general immunosuppression. In rodents, newer data suggest that allograft rejection can be overcome by better methods. Donor tissue can be depleted of antigen-presenting cells (2,3), or recipients can receive highly specific immune interventions that deplete populations of T lymphocytes (4,5). Unfortunately, these treatments do not prevent recurrent autoimmunity, or if they do, they also affect allograft rejection mechanisms and general immunocompetence, obscuring the relative importance of each process (6,7). Ideally, syngeneic transplantation of untreated tissue in an animal model should allow study of autoimmunity independent of allograft rejection, but this has not previously been feasible. We report an animal model of islet transplantation based on the BB rat model that distinguishes recurrence of autoimmunity from allograft rejection.

Diabetes-prone (DP) BB rats develop spontaneous autoimmune diabetes and are widely used as a model of IDDM (8,9). Most DP rats develop pancreatic insulitis, a mononuclear cell infiltrate of the islet, and 60–80% subsequently become diabetic. Transplantation with syngeneic grafts has not been investigated in the DP rat because of the likelihood of insulitis in the donor and uncertainty regarding possible autoantigenicity of the DP β-cell.

The diabetes-resistant (DR) BB rat was derived from DP forebears but bred for resistance to the disease. The cumulative incidence of spontaneous diabetes in the DR rat is <1%. However, administration of a cytotoxic anti-RT6.1 monoclonal antibody that depletes a population of mature T lymphocytes will induce diabetes in >50% of young DR rats (10). However, the reduction in $RT6^+$ T-lymphocyte numbers is transient, and $RT6^+$ peripheral T lymphocytes reappear after discontinuation of antibody treatment. These data have been interpreted to suggest that DR BB rats harbor populations of diabetogenic effector cells that are normally held in check by populations of regulatory cells, among them cells that express the $RT6^+$ phenotype (11). In this study, we investigated the survival of DR islet grafts into DR BB rats that had been rendered diabetic by in vivo immune elimination of regulatory $RT6^+$ T lymphocytes but allowed to repopulate their periphery with $RT6^+$ cells before transplantation.

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RESEARCH DESIGN AND METHODS

DP BB/Wor rats were obtained from the University of Massachusetts, Worcester (8). The cumulative incidence of diabetes in these rats averages ~60% in both sexes. Most cases (>85%) occur between 60 and 120 days of age. DR BB/Wor (RT1^u, RT6.1) and PVG rats (RT1^c) were also obtained from colonies at the University of Massachusetts Medical School. DR BB/Wor rats were derived from BA subline of DP BB/Wor rats after the 5th generation of brother-sister DP mating. Bred for disease resistance, DR BB/Wor rats were then brother-sister mated as a single line for 10 generations. Thereafter, 3 distinct sublines designated WA, WB, and WC were separately inbred (12). Minor histocompatibility differences may exist among the different sublines of DR BB/Wor rats. The DR BB rats used in this study were either the progeny of brother-sister DR matings or intercrosses between the inbred DR sublines. All rats were maintained in accordance with standard recommendations (13).

DR rats were made diabetic either with a single dose of streptozocin (STZ-D; 50–60 mg/kg i.v.) or by treatment 5 times/wk with DS4.23 anti-RT6 monoclonal antibody intraperitoneally from 30 days of age as previously described (10). Diabetes was defined as a plasma glucose ≥11 mM on 2 consecutive days. In the case of the RT6-depleted DR rats, monoclonal antibody treatment was stopped when diabetes was diagnosed, and the reappearance of RT6$^+$ lymph node cell populations was confirmed by previously described flow microfluorimetry 3–12 wk later (14). Percentages of RT6$^+$ cells are expressed as means ± SE. Experimental and control means were compared statistically with Student's *t* test. Diabetic anti-RT6–treated DR rats were treated daily with protamine zinc insulin until islet transplantation.

Uncultured DR (RT1^u) or PVG (RT1^c) islets were isolated from intact untreated donors by a modified collagenase-digestion technique (15). Recipients that had been anesthetized with chloral hydrate (0.012 mg/kg i.p.) were transplanted at a dose of 8–12 islets/g body wt into the portal vein. Transplanted rats were weighed and monitored for recurrence of diabetes. Normoglycemia was defined as a plasma glucose concentration <11 mM, and recurrence of diabetes was defined as plasma glucose >11 mM on 2 consecutive days.

Pancreas and liver specimens were obtained from all transplant recipients either at the time of recurrence of diabetes or at the conclusion of the study. Animals were killed in an atmosphere of 100% CO_2. The tissues were fixed in Bouin's solution and embedded in paraffin. Prepared sections were stained with hematoxylin and eosin and examined by light microscopy by a morphologist who was not aware of the treatment status of the specimens.

RESULTS

Anti-RT6 monoclonal antibody treatment was discontinued after the onset of diabetes in DR rats, and RT6$^+$ lymph node cell percentages, determined by flow microfluorimetry (14), returned to near normal levels at the time of islet transplantation. In 8 control rats 91 ± 13 days old, the mean level of RT6$^+$ lymph node cells was 37 ± 3%, whereas in 13 RT6-depleted diabetic rats 105 ± 4 days of age tested at least 4 wk after cessation of monoclonal antibody treatment, RT6$^+$ cells were 28 ± 3% (t = 2.05, NS).

Hyperglycemia recurred after a mean of ~4 days in RT6-depleted diabetic DR rats (n = 10) that were given DR islet grafts (Table 1). In contrast, transplantation of DR islets into STZ-D DR rats resulted in prolonged (>200 days) restoration of normoglycemia in six of eight cases. Allogeneic PVG islets failed to maintain normoglycemia in either STZ-D (n = 2) or RT6-depleted diabetic (n = 3) DR recipients for >2–4 days.

Histological examination of pancreases revealed end-stage islets lacking β-cells in all transplanted rats. An inflammatory infiltrate consisting of macrophages, natural killer cells, and lymphocytes was identified in the intrahepatic remnants of failed PVG and DR islet grafts by immunoperoxidase histochemistry (16). All destroyed islet grafts displayed comparable mononuclear cell infiltrates. In contrast, histological examination of islet grafts of normoglycemic syngeneically transplanted STZ-D DR rats revealed normal-appearing islets with hypertrophic β-cells in six of seven cases. An inflammatory infiltrate was noted in only one instance.

DISCUSSION

We have observed that DR BB/Wor islet grafts produce long-term normoglycemia in STZ-D DR recipients. Although minor histocompatibility differences between some of these DR donors and recipients could have been present, such differences generally did not lead to destruction of these islet grafts. We therefore interpret the failure of DR islet grafts in

TABLE 1
Islet transplantation in streptozocin-induced diabetic (STZ-D) and RT6-depleted diabetic diabetes-resistant (DR) BB rats

Graft donor	Graft recipient	Duration of islet graft survival	
		Mean (days)	Days
PVG	STZ-D DR	2.5	2,3
DR BB	STZ-D DR	220.0	20,*28,*222,242,257,258,359,372
PVG	Anti-RT6–treated DR	3.3	3,3,4
DR BB	Anti-RT6–treated DR	4.2	2,2,3,3,3,3,4,4,4,4,4,4,7,12

Results are of islet transplantation into DR BB/Wor rats made diabetic either by STZ injection or treatment with a cytotoxic anti-RT6.1 monoclonal antibody. RT6-depleted diabetic DR rats were treated 5 times/wk with DS4.23 anti-RT6 monoclonal antibody from 30 days of age until onset of diabetes (10). Monoclonal antibody treatment was then stopped, and reappearance of RT6$^+$ lymph node cell populations was confirmed by flow microfluorimetry 3–12 wk later (14). Four of the DR islet transplants into RT6-depleted DR recipients were carried out with donors and recipients from the WA subline obtained at the 30th generation of brother-sister mating.
*Two DR islet transplants into STZ-D DR recipients failed early. Histological examination of their grafts revealed histologically intact-appearing islets in 1 case and inflammatory infiltrates and destruction of islets in the other. The STZ-D DR rat that remained normoglycemic for 258 days was found dead and not studied histologically.

the RT6-depleted diabetic DR BB rat to represent recurrence of the primary autoimmune disease process. This finding is consistent with results of human pancreas transplantation between discordant monozygotic twins in which insulitis and β-cell destruction occurred in isografts implanted up to 27 yr after initial disease presentation (1). Our data confirm studies of Naji et al. (17,18), which also suggest that allograft rejection and recurrence of autoimmunity may be different processes in IDDM. In their studies, diabetic DP BB rats that had been made tolerant neonatally with Wistar-Furth bone marrow cells later destroyed islet grafts but not skin grafts from Wistar-Furth donors.

There are several aspects of the RT6-depleted DR BB rat model of IDDM that make its use in the study of transplantation for autoimmune diabetes advantageous. First, neither prior treatment of donor tissue nor immunosuppression of the recipient to avoid allograft rejection is required. Second, the DP BB rat is chronically immunodeficient (19), and transplantation studies performed with this model are difficult to interpret due to its intrinsically abnormal immune environment. In contrast, the anti-RT6–treated DR rat repletes its $RT6^+$ T-lymphocyte population soon after discontinuation of monoclonal antibody treatment, is not lymphopenic, and does not appear to be overtly immunocompromised. Third, the problems of insulitis and islet autoantigenicity that have precluded syngeneic transplantation studies in the DP BB rat and have raised concerns in studies of the spontaneously diabetic NOD mouse, another commonly used model of IDDM (20), are minimized in the RT6-depleted DR rat model.

Our data demonstrate that repopulation of $RT6^+$ T lymphocytes to near-normal numbers does not prevent subsequent destruction of DR islet grafts in diabetic RT6-depleted DR rats. This is a seemingly paradoxical effect, because we have previously shown in DP rats that rat spleen cell transfusions that result in the engraftment of $RT6^+$ T lymphocytes prevent diabetes (21). The transfusion data indicate that $RT6^+$ T lymphocytes may act as regulators of the initiation process, leading to subsequent autoimmune β-cell cytotoxicity. Our data suggest that once the autoimmune process has been initiated and diabetes has occurred, regulatory T lymphocytes can no longer prevent autoreactive effector cell activity. We previously suggested that the initiation of autoimmunity is the result of an imbalance between autoreactive (effector) and regulatory ($RT6^+$) cells (8). The simplest explanation for our results is that, once the balance has been shifted and effector activity is initiated, these autoreactive cells no longer respond to regulatory controls. Alternatively, the destruction of DR grafts in RT6-depleted DR rats could be due to the failure of a critical subpopulation of regulatory $RT6^+$ T lymphocytes to regenerate after cessation of monoclonal antibody treatment. On the other hand, if the RT6 molecule itself is important for immune regulation, then treatment with anti-RT6 monoclonal antibody may permanently alter its function. Experiments are under way to distinguish among these possibilities.

In conclusion, the destruction of DR islet grafts in anti-RT6–treated diabetic DR recipients provides an excellent model in which to study the recurrence of autoimmune processes free of the confounding effects of allograft rejection. Further study of this model may improve understanding of the cell mechanisms that underlie autoimmune recurrence and allow it to be treated without compromising normal immune function. This model also provides a tool for dissecting the regulatory network that normally prevents initiation of autoimmunity and, more specifically, the role of T lymphocytes of the $RT6^+$ phenotype in this process.

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