Transplantation of Islets and Bone Marrow Cells to Animals with Immune Insulitis

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SUMMARY

The results of islet transplantation in an animal model of spontaneous immune insulitis were studied to see whether this disease process might damage transplanted tissue. Since the insulitis occurs only in "BB" rats (which are not genetically uniform) syngeneic grafts could not be used, therefore allograft rejection was avoided by rendering "BB" rats tolerant of WF transplantation antigens by inoculating them neonatally with WF bone marrow cells. Despite the resultant tolerant state, which permitted successful engraftment of WF skin and islets transplanted to artificially diabetic "BB" rats, tolerant "BB" rats with spontaneous diabetes accepted transplanted WF islets only briefly before they were destroyed by immune insulitis.

"BB" rats were found to have abnormalities in immune response (delayed skin graft rejection and decreased alloreactivity in mixed lymphocyte response). However, the immune response was more normal in "BB" rats that were treated neonatally with WF bone marrow. Moreover, "BB" rats inoculated with WF bone marrow neonatally were found less likely to become diabetic than untreated "BB" controls. It is suggested that the chimeric state (persistence of WF bone marrow cells) may be responsible for the improved immune response and perhaps for the decreased susceptibility to diabetes. DIABETES *31* (Suppl. 4):84–89, 1982.

In diabetic humans it has been demonstrated in a few instances that allografts of segmental vascularized pancreas can function normally for long periods of time, if immunosuppression is used.[1] Nevertheless, it is quite possible that if human diabetes is caused by an autoimmune process, the disease itself might sometimes damage or destroy transplanted pancreatic islets, especially in the absence of immunosuppression. The following experiments were designed to study this possibility in an animal model that closely resembles human type I diabetes and that appears to be caused by an immune process. Our studies may be particularly important to the efforts reported in this symposium to use graft pretreatment rather than immunosuppression as a means of overcoming islet allograft rejection since immunosuppression might be needed to prevent autoimmune destruction of islets even if rejection could be avoided without it.

MATERIALS AND METHODS

Animals. Two litters of "BB" rats were obtained from Bio-Breeding Laboratories of Ottawa, Canada, in October, 1977, and six additional litters from Sir Frederick Banting Research Center, Ottawa, in July, 1979. From these rats and their descendants we have raised about 275 additional litters, a total of more than 3000 rats. The rats were allowed free access to rat chow and tap water. Plasma glucose was determined weekly in animals over 60 days of age, to determine the time of onset of diabetes, which is defined as a plasma glucose of over 200 mg/dl (although it rarely remains less than 350 mg/dl unless insulin is administered).

The onset of diabetes in "BB" rats usually occurs abruptly when they are between 60 and 150 days of age. It is characterized by hypoinsulinemia and ketosis in association with lymphocytic infiltration of islet tissue (insulitis).[2] The diabetes is severe, and unless insulin is administered most of the affected animals are short-lived. Other "BB" rats never develop diabetes. The incidence of the syndrome usually ranges between 10% and 50% in "BB" rats of our colony but can be higher or lower in individual litters depending on the presence or absence of diabetes in the parents.[3]

In addition to "BB" rats, the following isogenic rat strains bred and maintained in our own colony were used: ACI ($RT1^a$); Wistar Furth (WF) ($RT1^u$), and BN ($RT1^n$).

Diabetic rats were maintained on 2–3 U PZI insulin daily. Although it is known that in a few "BB" rats a mild form of diabetes may occur that can be recognized only by the find-

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ing of insulitis, we did not kill rats routinely for histologic examination of the pancreas, and our definition of diabetes, as is the case in humans, was based on hyperglycemia.

Chemical diabetes was induced in some rats by i.v. injection of streptozotocin, 65 mg/kg body wt.

Pancreatic islet isolation and transplantation. Islets were isolated as previously described by collagenase digestion and centrifugation through Ficoll gradients.[4] Transplantation was by portal vein inoculation.

Induction of specific immunologic tolerance. Within 24-h of birth, newborn rats were inoculated via the orbital branch of the anterior facial vein with 50×10^6 bone marrow cells suspended in 0.2-ml Hanks solution. Tolerance was assessed by a donor-strain, full-thickness, body-skin allograft performed at 4 wk of age. Recipients retaining a healthy skin allograft for >200 days were considered tolerant.[5]

Anti-T and Anti-B antibodies. The alloantisera, anti-pta (an alloantigenic system expressed on rat peripheral T-cells) is T-cell–specific.[6] The monoclonal mouse anti-mouse IaA^k antibody 10-36 (originally raised by Oi et al.) is considered to be B-cell–specific (7).

One-way mixed-lymphocyte reaction. Varying concentrations of responder (thoracic duct lymphocytes) and stimulator cell (lymph node cells irradiated with 1700 R) populations were co-cultured in RPMI-1640 containing L-glutamine, penicillin, streptomycin, 2, mercaptoethanol, and supplemented with 5% pooled normal rat serum. Cells were harvested and counted as described previously.[8]

RESULTS

Because "BB" rats are not genetically uniform, it is not possible to avoid rejection by employing grafts known to be genetically compatible. Thus interpretation of the failure of transplanted islets is complicated since it could theoretically be caused either by allograft rejection or by autoimmune destruction of the transplanted islets. In a previous study we demonstrated that the hyperglycemic state of "BB" rats could be reversed for many months by islet transplantation from allogeneic donors, if immunosuppressive therapy with anti-lymphocyte serum (ALS) was used.[9] The subsequent demonstration that treatment of acutely diabetic "BB" rats with ALS alone is capable of reversing diabetes (presumably by modulating the immune attack on islets) necessitated our reassessment of the islet transplant results.[10,11] Two approaches were employed: (1) comparing the results of closely histocompatible islet allografts in nonimmunosuppressed "BB" rats that had either spontaneous or chemically induced diabetes; (2) islet transplantation in diabetic "BB" rats that were immunologically tolerant of the donor strain.

Islets transplanted to artificially or spontaneously diabetic "BB" rats. Although "BB" rats are not genetically uniform, they are members of a closed colony, and their genetic disparity is not great. We found by serologic typing of lymphocytes from "BB" rats that they were identical ($RT1^u$) at the major histocompatibility complex (MHC) in all instances,[3,9] a finding that has been confirmed.[12] Furthermore we noted that "BB" rats have an impaired immune response to thymus-dependent antigens, probably on the basis of a reduction in T-cell numbers and function.[3,13] Not surprisingly, allograft rejection is delayed in these animals. A majority of skin allografts exchanged between "BB" rats in our colony are accepted for >100 days. It was therefore anticipated that islet allografts might also have prolonged survival in "BB" rats, unless they were damaged by autoimmunity. To determine the outcome of islet transplantation in the absence of host autoimmunity, an artificial hyperglycemic state was induced by streptozotocin (65 mg/kg) in previously normoglycemic "BB" rats of over 150 days of age. Since these rats had lived beyond the age of peak incidence of onset of diabetes without becoming hyperglycemic it was reasoned that they were unlikely either to have autoimmunity to islet cells or to subsequently develop it. Ten such streptozotocin-diabetic "BB" rats were given intraportal vein inoculations of isolated islets derived from "BB" donors. The donors were also normoglycemic rats over 150 days old and thus unlikely candidates for diabetes. A small biopsy of each donor pancreas was examined histologically to further exclude unrecognized insulitis in the donor as a possible cause of subsequent islet allograft failure. A cocktail of islets from eight donors was used for each transplant to improve the chances that at least some would be histocompatible with the recipient. No immunosuppression was used. In all 10 of these recipients the transplant brought about normoglycemia, which in 6 animals persisted for life (60–300 days). In the other 4 rats hyperglycemia recurred after 12–17 days and was assumed to be the result of rejection.

Twenty spontaneously diabetic nonimmunosuppressed "BB" rats received islet allografts from normoglycemic "BB" donors by a protocol identical to the one described for the streptozotocin diabetics. In 9 of these 20 instances the islets were transplanted to diabetic rats in the early stages of the disease (within 50 days of onset).

Normoglycemia ensued in these animals but was very brief in every instance, with recurrent diabetes occurring within 4 days of grafting. Histologic examination of liver biopsies performed at the time of recurrent hyperglycemia revealed mononuclear infiltration of the transplanted islets similar in appearance to the original insulitis lesion in the pancreas of acutely diabetic "BB" rats.

In 11 other spontaneously diabetic "BB" rats, the islet transplant was delayed for at least 50 days after the onset of the diabetes, to evaluate the possibility that if an autoimmune process was causing the condition it might have diminished sufficiently by this time to allow for the survival of transplanted islet tissue. Indeed, this seemed to be the case (Table 1). In 5 of the 11 instances when islet implantation was not performed until 78–150 days after the onset of hyperglycemia, posttransplant normoglycemia persisted for 5, 9, 18, 110, and 165 days. Three other diabetic rats received islet transplants 60 days after the onset of the disease and 30 days after finishing a 30-day course of ALS (which in these instances had failed to reverse diabetes). Posttransplant normoglycemia persisted for 4–6 mo in these rats. In the final 3 cases of delayed islet transplantation the transplant was performed 60–120 days after the onset of diabetes in rats that had undergone another, possibly immunosuppressive, maneuver during the pretransplant period (a 24-h period of thoracic duct drainage). In these rats normoglycemia was prolonged for 75–210 days.

Islet transplantation in immunologically tolerant rats. The fact that the majority of islet allografts from closely histocompatible donors in streptozotocin diabetic "BB" recipi-

TABLE 1
Effects of transplantation delay on long-term function

Duration of diabetes at time of islet transplant (Days)		Duration of normoglycemia after islet transplant (Days)
2		3
5		3
6		2
6		1
6		2
7		4
23		1
28		2
47		2
78		18
95		5
100		9
105		165
150		110*
60		200*
60	†	120
60		120
60		80
120	‡	210
120		210

Results depicted here indicate that when closely histocompatible islets are transplanted to nonimmunosuppressed "BB" recipients soon after the onset of spontaneous diabetes, transplant failure occurs rapidly. If transplantation is delayed for more than 50 days, the chances of long-term function are improved.
* Death while normoglycemic.
† These animals had a 30-day course of ALS ending 30 days prior to islet transplantation.
‡ These rats had a 24-h period of thoracic duct drainage during the pretransplant interval.

ents were successful while islet transplants in spontaneous diabetics of recent onset were not constitutes strong evidence for autoimmune destruction of islets. However, islet damage by rejection could not be entirely excluded because of the outbred nature of the subjects. Therefore, to rule out this possibility islet transplantation was performed in "BB" recipients rendered immunologically tolerant by neonatal inoculation of 50×10^6 donor strain bone marrow cells from WF, ("BB" × ACI)F_1 or ("BB" × BN)F_1 rats. Presence of the tolerant state was confirmed in each islet recipient by acceptance of a WF, ACI or BN skin graft. Six "BB" rats tolerant of WF antigens that had failed to develop diabetes by 150 days of age were rendered artificially diabetic with streptozotocin and transplanted with WF islets. This resulted in permanent normoglycemia in all, indicating that the rejection of allogeneic islets could be consistently avoided by the induction of tolerance.

In eight "BB" rats tolerant of WF antigens, islet transplantation was carried out within 20 days of the onset of spontaneous diabetes. Following transplantation of WF islets these rats remained normoglycemic for only 1–11 days. Histologic examination of the transplanted islets at the time of recurrent hyperglycemia revealed mononuclear infiltration, which in these tolerant hosts could only be attributed to autoimmune damage of islets since the experimental design excluded the possibility of rejection. Indeed, in these animals not only did each of the previously transplanted WF skin grafts remain healthy but in two instances rats were rechallenged with a second WF skin graft after the islets were rejected and the second graft was also accepted, reconfirming the persistence of the tolerant state.

An interesting finding was that in one spontaneously diabetic "BB" rat (tolerant of RTI-incompatible ["BB" = BN]F_1 antigens) transplanted BN islets restored normoglycemia for >9 mo, the lifetime of the animal, a result quite different from the brief survival of RTI-compatible WF islets transplanted to tolerant recipients.

Influence of induction of immunologic tolerance on the incidence of autoimmune diabetes. The above experiments on islet transplantation to immunologically tolerant rats suffering from spontaneous diabetes were performed in a relatively small number of animals because only a few tolerant "BB" rats ever became diabetic, though many newborn rats were inoculated with bone marrow. This phenomenon, which slowed the progress of the islet transplant studies, was initially attributed to coincidence since the incidence of diabetes in litters born in our "BB" colony is highly variable. However, the finding was intriguing and a prospective study was therefore designed to determine whether neonatal inoculation with bone marrow from rats of normal strains might influence the incidence of the disease. At birth, individual members of each "BB" litter were randomly separated into two equal groups. Members of one group received 50×10^6 bone marrow cells from RT1-compatible WF donors or from RT1-incompatible (ACI × "BB")F_1 or (BN × "BB")F_1 donors; members of the other group remained untreated. Inoculated and noninoculated animals were marked for identification, housed together, and given identical care. After 30 days they were grafted with donor-strain skin grafts, which were accepted by all of the inoculated rats. After 60 days, plasma glucose values were obtained at weekly intervals to determine the onset of diabetes. Preliminary results of this treatment have been reported.[14]

There have been 14 litters of "BB" rats in which one-half of the rats from each litter have now been inoculated with WF bone marrow cells and monitored for diabetes for at least 12 mo. The results are summarized in Table 2. The overall incidence of diabetes in the noninoculated members was 25 of 61 (40.9%), while in the tolerant rats only 5 of 61 (8.19%) ever became diabetic, a highly significant difference ($P < .001$). In 3 litters in which both parents were diabetic, a situation usually associated with a very high incidence of diabetes, all of 14 noninoculated rats became diabetic while none of 14 inoculated ones became diabetic. In the case of "BB" rats inoculated neonatally with (ACI × "BB")F_1 cells the results were not as clear. Six litters have been followed for a year. The incidence of diabetes is somewhat higher in the noninoculated rats ($^5/_{22}$) than in the inoculated littermates ($^3/_{30}$). This difference is not, however, statistically significant.

In the case of litters inoculated with ("BB" × BN)F_1 bone marrow, no difference was apparent in inoculated and noninoculated members of four litters followed for 12 mo ($^1/_{23}$ diabetics in inoculated rats compared to $^0/_{21}$ in noninoculated ones). However, the number of diabetics in this group was small, and early mortality in these litters was high, making interpretation of these preliminary results impossible.

Abnormalities of the immune system in unmanipulated "BB" rats compared with that of "BB" rats receiving bone marrow. It seemed unlikely that the decreased sus-

TABLE 2
Incidence of diabetes (diabetic/nondiabetic) in 14 litters of BB rats in which half of the members were inoculated with 50×10^5 WF bone marrow cells within 24 h of birth (incidence was significantly less ($p < .001$) in inoculated than noninoculated rats ($P < .001$)).

Parental background	Tolerant "BB" rats	Nontolerant littermates
D × D	0/4	4/4
D × D	0/6	6/6
D × D	0/4	4/4
D × D	0/3	0/1
D × D	1/2	0/1
D × D	0/3	2/5
D × D	3/6	2/7
D × D	0/3	0/1
D × ND	0/5	3/7
D × ND	0/4	1/4
D × ND	0/5	2/6
D × ND	0/4	0/4
ND × ND	0/6	1/6
ND × ND	1/6	0/5
	5/61 (total)	25/61 (total)

D = Diabetes, ND = Nondiabetes.

ceptibility of "BB" rats to diabetes was the specific result of their becoming tolerant of foreign transplantation antigens, especially since a small number of rats known to be tolerant did in fact become diabetic. Thus, we investigated other possible consequences of bone marrow inoculation of neonatal rats which might be responsible for altering susceptibility to the disease. A particularly attractive hypothesis was that "BB" rats have an abnormality of immunoregulation (since the disease onset is signaled by autoimmune inflammation of the islets) that is corrected in tolerant rats by the persistence of normal donor bone marrow cells.

It seemed likely that this defect in immunoregulation was also affecting protective immunity since the mortality of "BB" rats was very high as compared to other inbred strains in our animal colony, the usual cause of death being pulmonary infection. Therefore, we examined several aspects of the lymphoid system and immune response in "BB" rats.

Isolation of lymphocytes from various lymphoid compartments revealed a marked paucity of lymphocytes in "BB" rats. The mean number of lymphocytes harvested from the spleen of four diabetic "BB" rats ($75 \pm 33.4 \times 10^6$ per spleen) was significantly less than from five normal rats of non-"BB" strains ($205.5 \pm 66.0 \times 10^6$ per spleen) ($P < 0.001$). The reduction in lymphocytes was also reflected in 24-h thoracic duct lymphocyte (TDL) output collected via a free-draining thoracic duct cannula. The mean number of TDL per 24 h was $630 \pm 134 \times 10^6$ in five rats of normal inbred (non-"BB") strains but significantly less ($157 \pm 59.9 \times 10^6$) in 16 nondiabetic "BB" rats ($P < .005$) and even less in nine diabetic "BB" rats ($73.6 \pm 61.5 \times 10^6$).

When the percentage of T- and B-lymphocytes in TDL were measured in a microcytotoxicity assay using antiserum pta and monoclonal laA^k as anti–T- and anti–B-cell reagents, respectively, a preferential decrease in T-cells was noted. Rats of normal strains have approximately equal numbers of T- and B-cells.[15] However, in five nondiabetic "BB" rats the T-cells represented 19% of the lymphocyte count and in diabetic "BB" rats only 17%. Since total numbers of lymphocytes were also reduced in "BB" rats, a profound T-lymphopenia existed.

Histologic examination of lymph nodes and spleens of "BB" rats revealed marked depletion of T-cell–dependent zones. Thus in the spleen the periarteriolar lymphocyte sheaths (PALS) and in the lymph nodes the paracortical zones were markedly underpopulated by small lymphocytes. However, the thymuses of "BB" rats were normal in appearance, containing normal densities of cortical and medullary T-cells. In contrast, B-cell compartments such as splenic marginal zone follicles and cortical follicles of lymph nodes appeared to be of normal or increased cellularity. Alloreactivity of "BB" rats was measured in vitro by mixed lymphocyte culture responses (MLR) and in vivo by skin allograft rejection. Abnormal lymphocyte function of TDL from "BB" rats was observed in MLR when responder cells from "BB" rats were cultured with stimulator cells from normal rat strains of several different haplotypes. In over 60 separate experiments the consistent finding was that of either an absent or significantly reduced proliferative response as compared with the MLR of lymphocytes from normal rats. The loss of reactivity occurred regardless of the source of the allogeneic stimulator cells. Representative experiments are depicted in Table 3. Because a decrease in the proportion of T-cells in the TDL of "BB" rats (and thus in the number of proliferating cells) could be responsible for the observed decreased alloreactivity, additional experiments were carried out in which nylon-wool separation of T- and B-cells was done and the proportion of T-cells in re-

TABLE 3
Responses of "BB" and WF TDL to MHC-compatible and -incompatible stimulator cells in primary one-way mixed lymphocyte culture. Note that both diabetic and nondiabetic "BB" cells have a decreased alloreactivity to stimulator cells of all haplotypes

Strain of origin of responder cells	Unstimulated cultures (background)	Strain of origin of stimulator cells					
		L*	WF†	BN*	DA*	AUG*	BB†
WF (N = 5)	477.2 ± 260.2	28,494 ± 10,988	254.3 ± 197.6	35,022 ± 24,003	17,127 ± 2691.2	23,290 ± 2154	543 ± 47.2
Diabetic BB (N = 7)	242 ± 200	564 ± 347	215 ± 98	815 ± 647	805 ± 541	375 ± 59	210 ± 63
Nondiabetic BB (N = 9)	374 ± 330	1344 ± 1350	427 ± 121	729 ± 819	1429 ± 1185	875 ± 577	409 ± 425

* These rats all differ from WF and "BB" at the MHC. All responses are expressed as mean ± SD of ^{3}H-thymidine uptake (counts per minute).
† These rat strains are identical at the MHC and would not be expected to stimulate each other in MLR.

sponder populations enriched to normal levels. This, however, did not lead to an increase in the MLR of cells from "BB" rats, confirming that the lack of responsiveness was on a functional basis rather than being a simple decrease in numbers of T-cells.

In vivo alloreactivity was also decreased. MHC-compatible WF skin allografts survived 36–210 days in "BB" recipients (median 74 days) as compared to the usual rejection time of 10–12 days when skin grafts are exchanged between rats of other MHC-compatible strains such as Lewis and Fischer.

Several of the abnormalities found in the lymphoid system of diabetic and nondiabetic "BB" rats as well as their impaired immune response have been found to be either corrected or improved in animals treated neonatally with inoculations of bone marrow from normal rat strains. There was an increase in both the total numbers of lymphocytes and in the proportion of T-lymphocytes in the thoracic duct lymph of tolerant as compared to unmodified "BB" rats. In most instances the T-dependent zones of the lymph nodes and spleen were more densely populated with small lymphocytes in tolerant than in unmodified "BB" rats. Alloreactivity to skin grafts was also more normal in the tolerant "BB" rats. In six "BB" rats tolerant of ACI antigens MHC-compatible WF skin allografts were rejected in 17–21 days as compared to 36–210 (median 74.0) days in 18 unmodified "BB" rats. Preliminary evidence also indicates that MLC responses are improved in tolerant "BB" animals.[16]

DISCUSSION

Although considerable information exists regarding islet transplantation in experimentally induced diabetes, almost nothing is known about the possible outcome of this treatment in natural diabetic states.[17] It appears that virtually all attempts at transplantation of isolated islets to human diabetics have either failed to correct hyperglycemia or have functioned only very briefly, either because of inadequate islet dosage or early rejection.[1] Thus, the studies reported here are the only ones known to us in which a reasonable interpretation is possible of the outcome of isolated islet transplantation in a naturally occurring insulin deficiency state. Since the etiology of diabetes is not known in either "BB" rats or humans, these can hardly be assumed to be identical conditions. However, the abrupt onset of insulinopenia in previously normal, nonobese individuals, the genetic predisposition for the disease, which appears to be linked to the MHC, and the insulitis lesion are all common to the two syndromes.[18] Thus the diabetes of "BB" rats is at present the animal model most closely resembling type I diabetes of humans.

Because "BB" rats are not genetically uniform, syngeneic grafts are not available. Nevertheless significant conclusions can be drawn from the results of transplanting islets into either closely related or immunologically tolerant recipients suffering from artificially induced or naturally occurring diabetes. When islets are transplanted to streptozotocin-diabetic "BB" rats, they have prolonged or permanent survival in most instances. However, if islets are similarly transplanted to "BB" rats that have recently become spontaneously diabetic, failure of the transplanted islets occurs within a few days.

Somewhat unfortunately the evidence presented here indicates that rapid destruction of transplanted islets in "BB" rats early in the course of their diabetes is likely to occur on the basis of damage by the original disease process. This argues against the success of islet transplantation in the human disease if it has a similar etiology. There is ample precedent for recurrence of autoimmune diseases in transplanted tissue. Transplanted kidneys from human identical twin donors often fail if the original disease process was an autoimmune one such as glomerulonephritis.[19] Conceivably one human segmental pancreas graft that was transplanted from a living donor to her diabetic identical twin sister also failed on this basis (though fibrosis from the ductal obliteration technique employed may be a more likely explanation of islet damage).[20]

Fortunately the transplantation experiments in "BB" rats also allow some room for optimism about the outcome of clinical islet transplantation. When transplantation of rat islets was delayed for 50–100 days, failure of the transplanted islets was either delayed or avoided. Damage to islets transplanted even early in the course of the disease could be prevented by the use of immunosuppression. Since in the case of human transplantation both delay of the transplant (usually for many years after the onset of the disease) and immunosuppression (since identical twin donors are seldom available) would both be necessary, it seems unlikely that autoimmune destruction of transplanted islets will be the major obstacle to the success of this procedure. However, methods for avoiding rejection without immunosuppression (such as pretransplant tissue culture)[21,22] may not obviate the need for immunosuppression, since this might be necessary to prevent recurrent autoimmune disease. Also on the basis of very preliminary evidence cited here, such recurrence might conceivably be more likely to occur in MHC-compatible than -incompatible islets. The anomalous success noted above in one instance of an MHC-incompatible islet transplant in a tolerant "BB" rat (whereas MHC-compatible islets always failed) is possibly coincidental but raises the question of MHC restriction for the islet cell targets of autoimmunity.[23]

These studies may also have implications beyond predicting the outcome of clinical islet transplantation. Recurrence of diabetes following islet transplantation, even when rejection is excluded, may constitute the strongest evidence available of an autoimmune etiology of the disease. The defects found in the immune response of "BB" rats, although possibly coincidental, seem likely to be related to the propensity for diabetes. The unexpected finding that inoculation of bone marrow from normal donors into newborn "BB" rats decreases their incidence of diabetes seems likely to have some correlation with the finding that the various parameters of the immune response are more normal in marrow recipients than in their untreated littermates. The induction of tolerance to transplantation antigens of the donor strain seems an unlikely explanation of this. A more plausible reason for the protective effect of bone marrow is it re-equips the "BB" host with a clone of cells ordinarily missing from its repertoire. Such a clone might, for example, allow normal elimination of a yet-to-be-identified diabetogenic virus. Alternatively a clone of cells capable of suppressing an abnormal cellular immune response to islet cells might be absent in "BB" rats and replaced by marrow cells from normal donors.

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Discussion

Dr. Clark: I suppose in a sense MLR is a delayed-type hypersensitivity reaction, at least it measures proliferation of what we would call in mice the Ly1-type T-cells, which may be involved in delayed hypersensitivity reactions. Looking at your slides, it appears that the lesions are sort of an allergic hypersensitive type reaction with infiltration. Apparently other cells are gettng attracted to the site of the lesion—monocytes, granulocytes, and so forth.

Dr. Naji: Yes. But locally on a histological basis that is ruled out because in the pancreatic islets, what you have is small lymphocytes, macrophages, and very few eosinophils. You don't have the phenomena of recruitment.

Dr. Clark: What is the proportion of lymphocytes and monocytes, for example?

Dr. Naji: The monocytes are normal. Basically it is a T-cell deficiency.

Dr. Clark: And yet your lesions are probably T-cell generated?

Dr. Naji: Right. It seems to me you have very few T-cells but, unfortunately, you have the wrong type. You don't have regulatory T-cells.

Dr. Barker: How about the regulatory T-cells? Do you have information on that?

Dr. Naji: What does bone marrow provide to the diabetes-prone BB's? Does it supply them with a normal population of suppressors? It is not clear.

Dr. Clark: That is one interpretation, certainly.

Dr. Naji: I don't have any slides here, but from the raw data I can tell you that I cannot induce suppressor cells in a classic mitogen-induced suppressor assay in these rats.

Dr. Clark: Can you induce a delayed hypersensitivity reaction?

Dr. Naji: I haven't done that.

Dr. Sutherland: Dr. Naji, these rats are basically immunodeficient animals, at least the diabetic and the diabetic-prone ones. Presumably the immunodeficiency that you have is not just secondary to the diabetes per se. We know that diabetes can induce some immune defects. We do know that autoimmune phenomena are associated with immunodeficiency that either occurs naturally in both animals and humans, or with induced immunodeficiency by thymectomy or some other method.

So as you study these rats further, they do become different from the type I diabetic human. What you are looking for is a model that may be akin to humans and may have some predictive power as to what will happen with islet transplants. So to me it looks like this model is becoming less and less like the human. You have a genetically immunodeficient animal that may be complicated by autoimmunity.

Dr. Barker: Isn't it possible that humans prone to diabetes are immunodeficient?

Dr. Sutherland: Well, they may be. I don't know if they have been studied as well as they should be.

Dr. Barker: I think that all we know is that this is the closest thing in animals, except maybe Dr. Lafferty's mouse is closer—I don't know—but the closest thing that we know about in animals to human juvenile-onset diabetes. You wouldn't expect it to be the same disease. But I think that it is very likely that there are principles that can be learned from this that will help us know what human diabetes is. It is an odd syndrome. They are immunodeficient. Furthermore, the immunodeficiency is there from the beginning. It does not follow the hyperglycemia and it doesn't follow the immune insult, whatever that is. It is there well before all of this. It is there at a time when Dr. Naji's biopsies showed the pancreas to be absolutely normal. The predisposing factor is there.

Suppose there is a clone of cells in yourself or in a non-diabetes-prone BB rat that can handle whatever virus it is that causes diabetes. Suppose that is absent from these animals but present in the chimeric population of cells that goes in with that bone marrow. That is just one of a number of possibilities.

We don't have the explanation, obviously. It may be a complete and total coincidence that the tolerant animals

lack this immunodeficiency and also have a decreased incidence of diabetes. But I don't think that that is as likely as that those two things are related in some way that we don't yet understand.

Dr. Lafferty: This profound sort of hyporeactivity in the MLC could easily be looked for in human diabetics.

Dr. Sutherland: I think studies like that have been done.

Dr. Lafferty: And what comes out?

Dr. Barker: That it is decreased.

Dr. Mandel: In nondiabetics?

Dr. Naji: No, in human diabetics.

Dr. Barker: That is a little harder to be sure of. We are looking at that and we are looking at siblings of diabetics, a population that might correspond to the diabetes-prone population here. That answer is not known. But the MLC reactivity is not decreased to the same extent. Human diabetics and BB rats do not have the same disease, Dr. Sutherland. I agree with you there. It is obviously not the same disease. But it may be something which, in the BB rat, happens to take this form but which in other species has similar defects.

Dr. Sutherland: Well, it seems more profound than in humans.

Dr. Barker: Oh, it is much more profound

Dr. Scharp: In the diabetes-prone immunodeficient animals that you now identify and tag at the start, what is the percentage incidence of diabetes developing? Do you have some animals who will live out their entire life and if so, what percentage are getting diabetes and what aren't?

Dr. Naji: It's hard to say. Some of them remain normoglycemic throughout the course of their lives. It is hard for me to say because some of these have been manipulated for various reasons. If they have had thoracic duct drainage or ALS, there is a problem because of the experimental procedure.

Dr. Scharp: You've not looked at that as a separate entity?

Dr. Naji: No.

Dr. Scharp: They could have histologic evidence of disease and not have clinical disease.

Dr. Sutherland: Your genetic information actually is very interesting. That does fit the human pattern. All your genetic information does. Most HLA identical siblings of diabetics don't become diabetic, in fact, so maybe the incidence is a little higher in the BB rat than it would be in the human situation, but basically it doesn't entirely sort out with the haplotype. It would be nice if you could actually tissue-type the rats for the class I and class II antigens as you can in humans. It would sort out whether the association is, for instance, a class I or a class II. You are not quite able to do that because it isn't defined as well as it is in humans. But I guess progress is being made in rat immunogenetics and you might be able to do that eventually.

Dr. Mandel: If you assume that this is at least initiated by a virus or triggered by a virus, is there any evidence whether it is vertically transmitted or whether it is an environmental virus?

Dr. Naji: We looked extensively over two years for "virus." We didn't find it, we didn't detect it, we didn't isolate any virus. But your point is very relevant that if it is a virus, we don't know the strain of it or whether it is vertically transmitted.

Dr. Lafferty: You don't see anything in the EM like what you see in. . . .

Dr. Naji: No.

Dr. Barker: We've looked, we've done that.

Dr. Sutherland: Even in human diabetics it is hard.

Dr. Barker: Negative results with regard to looking for a virus don't mean anything.