Intratesticular Islet Allografts in the Spontaneously Diabetic BB/W Rat

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SUMMARY

Thirty-three male BB/W rats with diabetes of 11–145 days duration were divided into 3 groups: (1) six received abdominal, intratesticular islet allografts and no immunosuppression posttransplantation; (2) 15 were similarly grafted and in addition were given four injections of ALS regularly at 10-day intervals for 30 days after transplantation; and (3) 12 rats received scrotal, intratesticular islet allografts and four injections of ALS. The results: (1) in the absence of immunosuppression all six of the rats with abdominal, intratesticular islet allografts became normoglycemic within 2 days after transplantation but in none did graft survival exceed 17 days; (2) a marked prolongation of graft survival of >65–441 days occurred in 13 of the 15 animals with identical intratesticular allografts; (3) sustained immunosuppression was not needed for prolonged islet allograft survival in rats with cryptorchid islet allografts; and (4) only one of the 12 rats with scrotal, intratesticular allografts became normoglycemic whereas 11 remained severely glycosuric. However, on surgical translocation of the grafted testes from the scrotum into the abdominal cavity, the rats promptly became normoglycemic in the absence of any additional therapy. DIABETES 1985; 34:1019–24.

Islet cell transplantation for long-term control of streptozocin (STZ)-induced diabetes mellitus had been accomplished in the past, using either no immunosuppression[1] or the cryptorchid testes as an organ site for the injection of islet allografts into Wistar-Lewis rats.[2] These results encouraged us to extend our methodology to a much more difficult animal model: the spontaneous, autoimmune diabetes mellitus of the BB rat, akin to human type I diabetes, in which islet transplants would offer the potential of improved treatment.

It is now generally believed that type I diabetes is caused by an autoimmune disease process.[3,4] The BB/W rat has a diabetic syndrome that mimicks the human disease: the onset of hyperglycemia occurs spontaneously between the ages of 60 and 120 days,[5] the insulitis that leads to the destruction of the islets is caused by a cell-mediated autoimmune process,[6] and the animals are ketosis-prone, hypoinsulinemic, and hyperglucagonemic.[5] Although pancreatic islets have been successfully grafted into the BB/W rat, allograft survival has been achieved only with either rigorous and continuous ALS therapy[7] or with other immunosuppressive maneuvers.[8] Furthermore, on discontinuation of immunosuppression, the grafted rats reverted to the diabetic state and the islets were shown to be infiltrated by mononuclear cells and destroyed, apparently by the same autoimmune process that caused the primary lesion.[8] These observations have led to skepticism with regard to the feasibility of clinical islet transplantation for the control of type I diabetes.

We have recently shown that the abdominally located testis is an unusually effective organ site for the transplantation of insulin-producing islet allografts, compared with other organ sites such as the testis in its original, scrotal position, the liver, and the subcapsular renal space.[2] The testis is known to be an immunologically privileged site and there is histologic evidence for long-term viability of islets grafted into this organ.[9–11] The functional capacity of such grafted islets, however, had been in question and Gonet and Renold[10] noted that insulin secretion of fetal B-cells grafted into the scrotal testis was not sufficient to ameliorate the diabetic process.

This initial study is limited to two goals: (1) to determine whether the testis is also an appropriate organ site for the transplantation of islet allografts into BB rats with a spontaneous diabetes, and (2) to examine the effects of the position of the grafted testis, either in the scrotum or in the abdomen, on the functional integrity of islet allografts in these rats.

MATERIALS AND METHODS

Male BB/W rats (RT1-u) with spontaneous diabetes of 11–145 days duration were obtained from the Breeding Labo-

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ratories, Worcester, Massachusetts, courtesy of Dr. A. Like. On arrival, the rats were immediately placed in metabolic cages and the urine volume, urine glucose content, and body weights were recorded every 24 h. Plasma glucose levels were obtained at weekly intervals. The rats were given daily injections of 1–3 U of lente insulin, each, to prevent ketosis and to maintain their body weights.

ISLET ISOLATION

Islets were prepared from four different donor strains of rats, including three highly inbred, ACI (RT1-a), Wistar-Lewis (RT1-1), and Fischer (RT1-1), and an outbred strain, Sprague-Dawley. Young animals, weighing approximately 150–180 g, were used exclusively. The islets were isolated, purified on Ficoll gradients, and incubated at 37°C as previously described.[1]

ISLET TRANSPLANTATION

Thirty-three male BB/W rats of 11–145 days duration of diabetes were transplanted. The plasma glucose concentration of every rat equalled or exceeded 375 mg/dl on the day that therapy was initiated, regardless of duration of diabetes. Each male diabetic recipient received a total of 10, 3- to 5-day culture-maintained islets per gram of body weight, injected into the testes.[2] Of the 33, 14 rats received islets of only one of the four donor strains listed, 15 were given equal numbers of two different strains (for example, ACI in one testis and Wistar-Lewis in the other), and four rats received a mixture of three strains (for example, Fischer, Sprague-Dawley, and ACI, not necessarily in comparable numbers). A rat was considered cured of the diabetic process if the following criteria were met: plasma glucose concentration ≤150 mg/dl, aglycosuria, 24-h urine volume <15 ml, and steady weight gain. The 33 rats were divided into three different study groups:

TABLE 1
The duration of normoglycemia in BB/W recipients of abdominal, intratesticular islet allografts and four injections of ALS given at 10-day intervals after transplantation

Rat no.	Duration of diabetes at time of graft (days)	Source of islets	Duration of normoglycemia postgraft (days)
1	13	A	98 p
2	24	W	441 o
3	33	A+W	88 o
4	41	F+W	137 p
5	52	S	65 o
6	70	W	132 l
7	96	A+F+S	177 p
8	100	A+S	168 p
9	107	F+W	43 r
10	107	A+F	121 p
11	107	S	189 p
12	115	A	17 r
13	121	F+S+W	130 D
14	126	W	215 o
15	145	F+W	>228

Abbreviations: A, ACI; F, Fischer; S, Sprague-Dawley; W, Wistar-Lewis; p, killed because of pneumonia; o, orchiectomy; l, malignant lymphoma; r, reversal to hyperglycemia; and D, died during IVGTT.

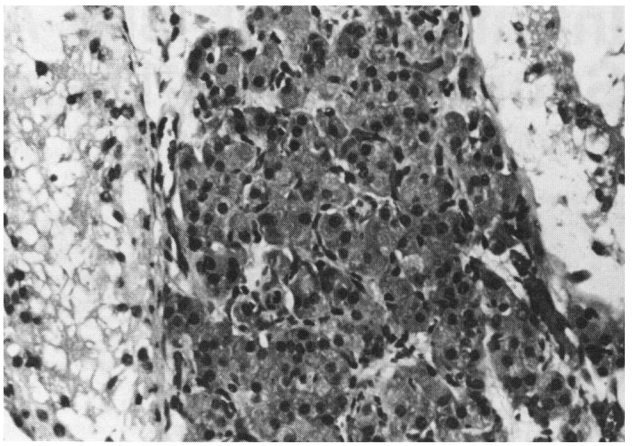

FIGURE 1. Photomicrograph of an abdominal, intratesticular islet allograft in a BB/W rat 88 days after transplantation and 58 days after the last ALS injection. Intact islet cells are visible within the intertubular space and in close association with the seminiferous tubules. The tubules are necrotic with an absence of spermatogenesis. There is no lymphocytic infiltration within either the testicular or endocrine tissues. Hematoxylin and eosin.

Group 1. Allograft survival in the absence of immunosuppression was examined in six males with abdominal, intratesticular islet grafts, with diabetes of 13–125 days duration at the time of transplantation. Neither ALS nor insulin therapy was given after surgery.

Group 2. The influence of minimal immunotherapy on islet allograft survival in the cryptorchid testis was examined in 15 rats with diabetes of 13–145 days duration at the time of grafting. Each rat was given a single intraperitoneal injection of 1 ml ALS immediately after transplantation and then regularly at 10-day intervals for 30 days. Each rat thus received a total of only four, 1-ml injections of ALS. Immunosuppression was then stopped. Insulin injections were discontinued a day before transplantation.

Group 3. Twelve rats with diabetes of 11–120 days duration were given scrotal, intratesticular islet allografts and four injections of ALS posttransplantation as outlined under group 2. They were immediately returned to their cages and the metabolic data, as outlined above, were obtained at daily intervals. At intervals between 5 and 10 days after transplantation, six of the still-hyperglycemic rats were reoperated and the grafted testes were anchored into the abdominal cavity. They were then returned to the metabolic cages for further daily examination of body weights, urine glucose content, urine volume, and plasma glucose levels. In the remaining six animals, the grafted testes were left in the scrotum and metabolic measurements were obtained every day.

HISTOLOGIC EXAMINATION OF THE GRAFTED TESTES

A bilateral orchiectomy was performed under light ether anesthesia on four normoglycemic rats, 65, 88, 215, and 441 days, respectively, after transplantation (Table 1). The grafted gonads of three other cured rats were also studied: one was killed on day 121 because of the onset of pneumonia, one developed a malignant lymphoma and was killed on day 132, and one animal expired unexpectedly during a glucose tolerance test on day 130 after transplantation. The testes of a

rat that had reverted to hyperglycemia on day 17 after grafting were also examined.

The tissues were fixed in formalin, sectioned, and stained with hematoxylin and eosin. Beta cells were identified by immunoperoxidase staining for insulin and by aldehyde-fuchsin.

RESULTS

Group 1. Induction of normoglycemia occurred promptly within 24–48 h in four of the six rats that received an abdominal, intratesticular islet allograft and no ALS posttransplantation. The other two remained glycosuric. In the four normoglycemic animals, a return to hyperglycemia took place equally abruptly on days 12, 15, 15, and 17 after transplantation. The source of the donor islets was irrelevant (data not shown).

Group 2. Induction of euglycemia occurred within 24–48 h in all 15 of the grafted rats. As shown in Table 1, two of these rats reverted to hyperglycemia on days 17 and 43, respectively, after transplantation. Of the remaining 13, all had graft survivals in excess of 65 days. Of these rats, four were orchiectomized, six developed pneumonia, one developed a malignant lymphoma, and one rat is still surviving and normoglycemic for >228 days. Again, the source of transplanted islets was irrelevant to the outcome of the graft (Table 1).

Group 3. Only one of the 12 rats with scrotal, intratesticular allografts became aglycosuric within 4 days after transplantation. It remained normoglycemic for 42 days and then suddenly reverted to a hyperglycemic state. None of the five rats that were left untreated became normoglycemic. All of the six rats that had their grafted testes translocated from the scrotum into the abdominal cavity on days 5–12 promptly became normoglycemic within 24–48 h. Of these, four were killed on days 22, 40, 160, and 175 because of the onset of pneumonia. The other two have remained normoglycemic for more than 118 and 124 days, respectively, after the translocation of the testes. The source of the islets was irrelevant (data not shown).

The four rats that were orchiectomized on days 65, 88, 215, and 441, respectively, after transplantation, almost immediately became ill with symptoms of an acute diabetic ketoacidosis. This was characterized by a watery diarrhea, a marked loss of body weight, and a scanty, dark urine a day before death. All four of these rats were dead within 2–4 days after surgery.

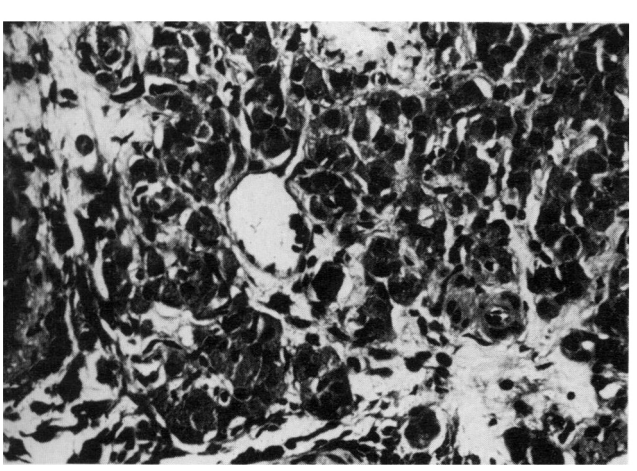

FIGURE 2. Photomicrograph of an islet allograft in the abdominal testis of a BB/W rat 441 days after transplantation. The endocrine tissue is arranged in small clusters of cells. Fibrous bands are also visible within the allograft. There is no lymphocytic infiltration within either the testicular or the endocrine tissues. Hematoxylin and eosin.

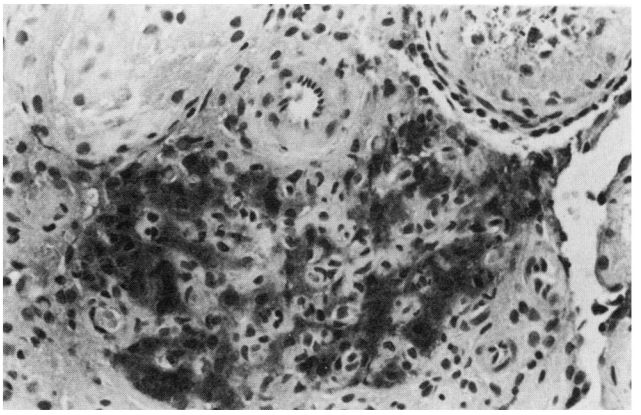

FIGURE 3. Photomicrograph of an islet allograft in the abdominal testis of a BB/W rat 65 days after transplantation. An abundance of insulin-producing cells is evident within the allograft as indicated by the shaded areas. An immunoperoxidase stain specific for insulin was used.

Histologic examination of the testes of the four orchiectomized animals and of the other three cured rats at autopsy on days 130, 132, and 215 revealed the presence of well-defined, pancreatic islet cells in the intertubular spaces. The grafted cells were closely associated with, but never within, the seminiferous tubules. In the allografts of shorter duration, i.e., 65 and 88 days, the endocrine tissue was arranged as large, globular masses (Figure 1). By contrast, in allografts of longer duration, i.e., 130 days and more, a different pattern was seen and the grafted endocrine tissue was rearranged into small clusters of cells. Fibrous bands were interspersed between the cells. The "rearrangement" of the grafted cells was particularly obvious in the allograft of 441 days duration (Figure 2). Although the size of these allografts was not quantitated by means of the determination of insulin contents, an abundance of insulin-producing cells was found as determined by two different staining techniques: an immunoperoxidase stain specific for insulin (Figure 3) and by an aldehyde-fuchsin stain (data not shown). Similar observations to those in Figure 3 were made by electron microscopic examination of islets grafted into the abdominal testis (personal communication, Dr. R. Margolis). A special stain for somatostatin-producing cells was also used, and this illustrated the presence of single cells spread in a random manner throughout the endocrine tissue (data not shown). None of the testes of the cured rats had a lymphocytic infiltration, either in the vicinity of the grafts or elsewhere within the testicular tissue (Figures 1, 2, and 3). In contrast to normal-appearing endocrine tissues, the seminiferous tubules were atrophic, necrotic, and with a complete absence of spermatogenesis. These features were particularly striking in the rat that had cryptorchid testes for 441 days (Figure 4). A careful search for neoplastic changes, as are sometimes found in the undescended testis,[12] was made, but none were found.

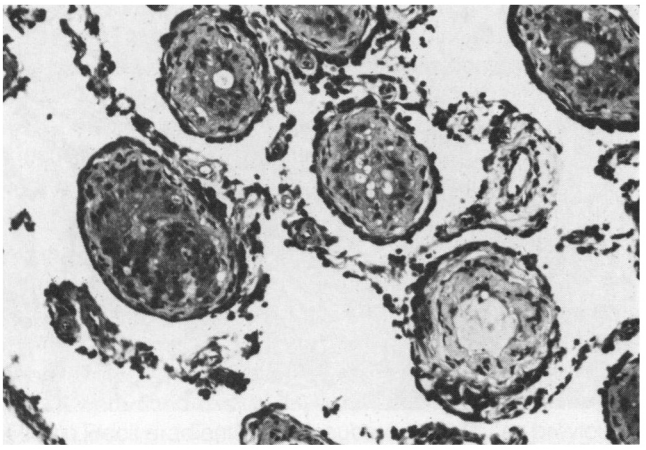

FIGURE 4. Photomicrograph of the cryptorchid testis of a BB/W rat 441 days after it had been anchored into the abdominal cavity. The seminiferous tubules are completely necrotic and replaced by fibrous material. Hematoxylin and eosin.

The histologic appearance of the testis of the rat that suddenly reverted to a hyperglycemic state 17 days after transplantation is shown in Figure 5. The islet morphology was distorted and the allograft appeared to be destroyed by a heavy infiltration of small lymphocytes. The inflammatory reaction was most prominent in the periphery with a few remaining islet cells still visible in the center of the graft.

In Figure 6 a characteristic metabolic response of a rat that was made cryptorchid 5 days after transplantation, instead of immediately afterward, is shown. The urine glucose content 1 day before transplantation was 3480 mg; 1 day after transplantation the level dropped to 1575 mg but rapidly increased thereafter and attained levels comparable to pretransplant concentrations by day 5. The grafted gonads were then elevated into the abdominal cavity without any additional therapy. Within 24–48 h afterwards the rat was aglycosuric. Similar metabolic responses were found in the other five reoperated rats (data not shown).

DISCUSSION

We have previously described the methodology of islet cell transplants across major histocompatibility barriers, without immunosuppression, into the abdominal testes for the cure of STZ-induced diabetes mellitus in rats.[2] These experiments were now expanded to the grafting of spontaneous, autoimmune, type I diabetes in the BB rat in which transplants of isolated islets had been difficult to achieve without rigorous and continuous immunosuppression.[7]

Several aspects of islet survival and function were unexpected. Allograft survival in the cryptorchid testis was independent of the duration of the diabetes at the time of transplantation. Animals with hyperglycemia of variable duration were selected in view of the report that BB rats with hyperglycemia of prolonged duration are more amenable to transplantation than are rats with a more recent onset of disease.[8] Apparently, the autoimmune process diminishes with time and the grafted islets in the more chronically ill rats are therefore less susceptible to immunologic destruction than are those in the acutely diabetic animals.[8] The median duration of diabetes in our 33 transplanted rats was 60 days with a range from 11 to 145 days at the time of grafting. In the critical second group, i.e., the rats that were given abdominal, intratesticular islet allografts, with minimal immunosuppression, there was no difference in either the time to restoration of normoglycemia (24–48 h) or in the number of rats that achieved permanent allograft survival in those with a subacute diabetes (<70 days) versus those with a more chronic hyperglycemia (>90 days) at the time of grafting. Moreover, the two animals that spontaneously reverted to hyperglycemia on days 17 and 43 after transplantation had diabetes of 115 and 107 days duration, respectively, on the day of transplantation. If the autoimmune disease process is indeed responsible for the destruction of the grafted islets in BB rats, then our data would indicate that either the use of the testis as an organ site and/or the immunosuppression used protected the islets against the immunologic attack regardless of the severity of the reaction. The testis is known as an immunologically privileged organ site and a variety of cell types, including parathyroid,[13] tumor cells,[14] and pancreatic islets[9–11] have been successfully grafted into the gonad. The mechanisms of protection of intratesticular allografts against rejection have not yet been elucidated, although the most likely factor responsible is the elevated, local concentrations of the steroid hormones, testosterone and progesterone.[15] The latter hormone, in particular, has been shown to exert a marked inhibitory effect on lymphocyte blastogenesis in vitro.[16]

The number of islets transplanted was critical, as was shown by others[17] and by us.[18] Surprisingly, however, the source of rat islets appeared irrelevant to the outcome of the graft. Thus, mixtures of islets from several donor strains, rather than from a single strain of rat, were often used to graft the BB rats. This was done for practical reasons because of the limitations in the supply of suitable donors and because it had been shown that the source of islets did not affect the end results under the conditions of our experimental procedures.[1] For example, we have previously shown that the purification of islets on several Ficoll gradients, done in tan-

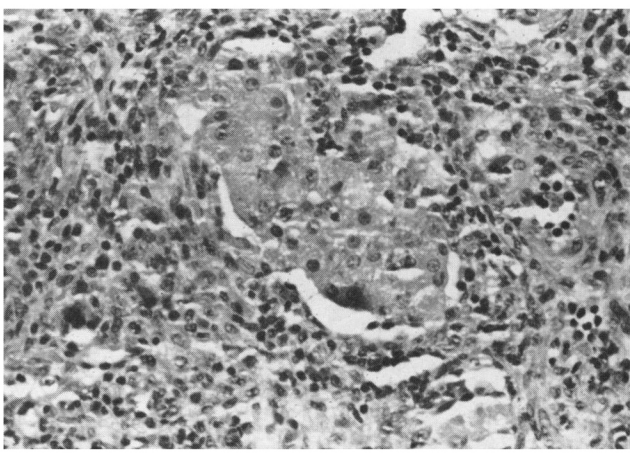

FIGURE 5. Photomicrograph of an abdominal, intratesticular islet allograft of a BB/W rat that suddenly reverted from a normoglycemic to a diabetic state 17 days after transplantation. The islet morphology is distorted by a heavy infiltration of small lymphocytes. The inflammatory reaction is most prominent in the periphery, with some intact cells still remaining in the center of the graft. Hematoxylin and eosin.

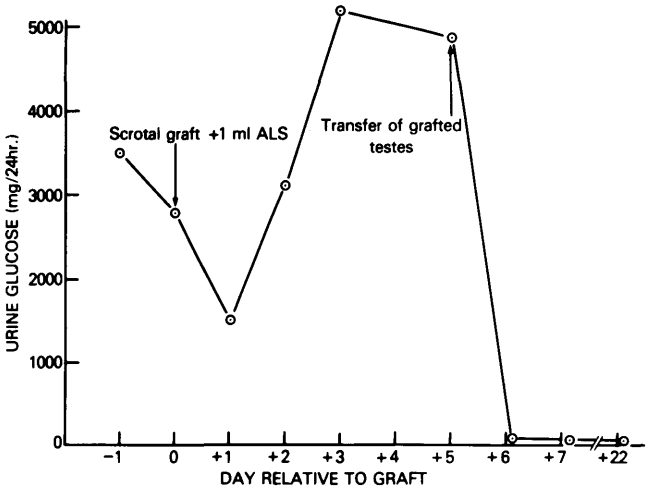

FIGURE 6. A typical urinary glucose response pattern in a BB/W rat that was given a scrotal, intratesticular islet allograft on day zero plus a single intraperitoneal injection of ALS. On day 5, the grafted gonads were surgically anchored into the abdominal cavity. No other therapy was given.

dem, combined with a brief period of culturing at 37°C, produced unusually clean preparations that could be grafted successfully in nonimmunosuppressed rats.[2] It was also determined that MHC disparity, as reflected by in vitro mixed-lymphocyte reactions, was not a reliable predictor of islet allograft survival in the rat.[1] Islet allograft acceptance in the previous study,[1] confirmed by the present data, was thus independent of the degree of histoincompatibility between different rat strains.

The low intensity and short duration of immunotherapy were likewise surprising: (1) very minimal immunosuppression, consisting of four evenly spaced, 1-ml injections of ALS given over a 30-day period posttransplantation, led to extended islet allograft survival in 11 of 13 such-treated animals; chemotherapeutic immunosuppression was not necessary; and (2) continuous immunosuppression was not required, and with the cessation of the ALS injections on day 30 only 1 of the 13 rats reverted to hyperglycemia 43 days after transplantation. As discussed earlier, the special hormonal environment of the testis may have played a major role in ensuring extended allograft survival, but some immunosuppression was likewise essential for graft survival. It is not clear from the available data whether the ALS was required to suppress the BB immune attack or whether over the 30-day period its action prevented rejection of the islets, thus allowing the islet allografts to survive a certain "critical" period and to become "established,"[19] and therefore no longer susceptible to immunologic destruction.

That the grafted islets were responsible for the maintenance of normoglycemia was supported by the observation that the orchiectomized, transplant-cured rats promptly reverted to a diabetic state and died with symptoms characteristic of acute DKA within 4 days after surgery. In addition, there was histologic evidence of intact, well-granulated islets in the testes of these four rats and in the gonadal tissues of the other three transplant-cured rats that were either killed or died on days 121, 130, and 132 days after transplantation.

Islet cell transplants in the scrotal testes had been done in the past.[9,10,14] While the islets were shown to survive for extended periods in this position, they were rarely functioning.[10] The present data show that the functional integrity was restored to normal by the autotransplantation of the grafted testis into the abdominal cavity. The rapid rate at which the metabolic values were restored to normal strongly suggested that the grafted islets were viable, but apparently had a diminished capacity to release insulin, while the testes were in the original scrotal position.

The mechanisms responsible for the inhibition of insulin secretion from scrotal, intratesticular islets are not known. It is conceivable that the cooler temperature of the scrotum may have resulted in an impaired insulin release from the grafted beta cells. Whether temperature alone, however, played the critical role in influencing islet cell function is rather doubtful, since it is generally known that insulin secretion, at least in vitro, is intact at 34°C, the average temperature of the scrotal testis in the rat. On the other hand, the heterotopic position of the testis may have resulted in the induction of other biochemical changes; in turn, these factors may have, either in conjunction with or independently of the temperature, restored to normal the insulin secretory responses of the grafted beta cells.

Also apparent from these studies was the observation that, despite a complete reversal and correction of the metabolic abnormalities associated with diabetes, the rats remained unusually susceptible to upper respiratory tract infections. Although our BB/W rats were housed in a separate room and were never in contact with other rat strains, we recently had a small epidemic of pneumonia that necessitated the killing of 10 animals with long-term islet allografts. None of the rats responded to antibiotic therapy after the onset of breathing difficulties. Susceptibility to bacterial and mycoplasmic infections in these rats is probably caused by the T-cell-dependent immune pathogenesis characteristic of these animals,[19] evidenced by a marked lymphopenia[20] and defective T-cell function in in vitro studies.[19] The altered cell-mediated immunity precedes the onset of hyperglycemia and is not caused by the metabolic derangements associated with diabetes.[20] Our observations are in agreement with these findings and strongly suggest that a reversal of the diabetic process by means of islet transplantation does not restore to normal the abnormal immune effector cells.

In conclusion, we have demonstrated that indefinite islet allograft survival is feasible in BB/W rats with the injection of a sufficient quantity of very clean islets into the cryptorchid testis. Furthermore, the duration of diabetes at the time of grafting did not influence graft survival, and the origin of the donor islets was irrelevant. Most importantly, allograft survival was accomplished with only four injections of ALS given posttransplantation, and sustained and/or rigorous immunosuppression was not required for the protection of the allografts against destruction by the autoimmune disease process.

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