the introduction of $E_2$. These findings imply that the negative feedback component of the estrogenic influence is mediated at some regulatory loci other than the hormone-receptor complex.

The temporal pattern of responsiveness closely follows a change in the pituitary stores of LH. At any given time the content of LH in gonadotrophs is a function of the relative rates of synthesis, storage, and secretion (*15*). Exogenous $E_2$ may regulate the rate of one or more of these processes so as to make gonadotrophs refractory or hyperresponsive (*16*).

These findings (*17*) are consistent with the hypothesis that a portion of the estrogenic augmentation of pituitary responsiveness is mediated by an increase in the number of GnRH receptors on the surface of gonadotrophs. However, the negative feedback component of the $E_2$ effect is not a direct consequence of a corresponding change in GnRH receptor numbers.

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## Spontaneous Diabetes in Rats: Destruction of Islets Is Prevented by Immunological Tolerance

Abstract. *Spontaneous diabetes occurring in "BB" rats (derived from a colony of outbred Wistar rats) is the result of destruction of pancreatic islets by infiltrating mononuclear cells (insulitis) and may be a disease very similar to human juvenile onset diabetes. Both diseases probably have an autoimmune etiology. Evidence is presented that islets transplanted to diabetic BB rats are destroyed by the original disease process. Inoculation of bone marrow from normal (nondiabetes-susceptible) rat donors into neonatal BB recipients usually prevents the development of hyperglycemia.*

The spontaneous development of diabetes mellitus was observed in 1977 in several members of a colony of outbred Wistar rats (*1*). Selective breeding of these diabetic "BB" rats in our colony has resulted in an increase in incidence of the disease to about 30 percent. The onset of severe hyperglycemia (3 to 7 mg/ml) in affected animals is sudden and usually occurs between 60 and 180 days of age in previously normal animals and with equal frequency in both sexes. Nonaffected animals remain permanently normoglycemic. Pathophysiologic characteristics of the syndrome include hypoinsulinemia, hyperglucagonemia, and, if insulin therapy is not provided, ketoacidosis and death. Morphologically the pancreas from newly diabetic rats shows a striking mononuclear infiltration (insulitis) with selective β-cell destruction. Both the metabolic and histologic abnormalities closely resemble those of insulin-dependent juvenile onset diabetes of humans. As in the case of the human disease, the etiology is unknown. However, in both BB rats and human diabetics a cell-mediated, organ-specific autoimmune pathogenesis seems possible, based on the characteristic lymphocytic infiltration of the islets. In BB rats an autoimmune mechanism is also implicated by the finding that reversal of the insulitis and return of normoglycemia occurs in 36 to 60 percent of acutely diabetic rats treated with rabbit anti-rat lymphocyte serum (ALS) (*2*).

Since the BB rat may provide an animal model of human insulin-dependent diabetes, it was important to determine whether the pathogenetic process responsible for the disease was also capable of destroying the islets of a transplanted pancreas, an issue of utmost importance in the outcome of this therapy in humans. We previously reported that transplantation of allogeneic islets from Wistar Furth (WF) donors results in long-term correction of the hyperglycemia of BB recipients treated with ALS (*3*). However, when immunosuppression was stopped, hyperglycemia returned within a few days and transplanted islets were noted to be infiltrated by lymphocytes, a histological finding that can be indicative of either rejection or recurrence of the original disease. We have now conducted islet transplantation experiments in diabetic BB rats using procedures designed to exclude the possibly confusing influence of rejection or immunosuppression.

Although BB rats are not genetically uniform, serological lymphocyte typing, mixed lymphocyte culture reactions, and skin graft assays all indicate that inbreeding has significantly minimized the histoincompatibilities in our colony. Thus, not only do all these rats appear to have the same major histocompatibility


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Table 1. Outcome of islet transplantation in BB rats.

| Islet donor | Recipient | Type of diabetes* | Number of recipients | Cured | Rejecting transplants or with recurrent diabetes | Time showing normoglycemia after islet transplantation (days)‡ |
|---|---|---|---|---|---|---|
| BB† | BB | Streptozotocin | 11 | 6 | 5 (rejecting) | Cured: > 60, > 60, > 120, > 150, > 180, > 300; rejecting: 2, 12, 13, 14, 17 |
| BB† | BB | Spontaneous | 8 | 0 | 8 (recurrent diabetes) | 1, 1, 2, 2, 2, 3, 3, 4 |
| WF | BB tolerant of WF | Streptozotocin | 6 | 6 | | > 30, > 75, > 90, > 90, > 90, > 120 |
| WF | BB tolerant of WF | Spontaneous | 6 | 0 | 6 (recurrent diabetes) | 2, 2, 6, 6, 8, 11 |

*Both spontaneous and streptozotocin-induced diabetics had plasma glucose values of > 3 mg/ml prior to transplantation. †All donors were normoglycemic animals with histologically normal islets and were from the nondiabetes-prone BB stock. ‡The day of rejection or recurrent diabetes was defined as that day on which the plasma glucose increased to > 2 mg/ml, but in both types of islet failure the plasma glucose subsequently increased to pretransplantation levels within a few days.

genotype ($RTI^u$) (*4*) but 11 of 16 skin grafts exchanged between members of the colony survived for more than 100 days (*5*). We therefore reasoned that islets transplanted among BB rats would also frequently escape early rejection, especially if we used multiple donors so that, on the basis of chance, some islets would be compatible with the recipient. Indeed, this appeared to be the case when we transplanted islet allografts from BB donors to BB recipients that had reached adulthood without becoming spontaneously diabetic. We observed that BB rats with no diabetes in their ancestry for two generations show a less than 5 percent incidence of spontaneous diabetes. We induced hyperglycemia (plasma glucose > 3.5 mg/ml) in such rats by injecting them intravenously with streptozotocin (65 mg/kg), a specific β-cell toxin. Four to seven days later each recipient received islets from eight normoglycemic BB donors. The isolated islets were transplanted by embolization via the portal vein to the liver (*6*). Although no immunosuppressive agent was given, islet transplant survival was prolonged, with 6 of the 11 recipients remaining normoglycemic for 60 to 300 days before they were killed (Table 1). Histological examination of the rats revealed healthy islets in the liver but few if any viable β cells in the native pancreas. The remaining five animals also became normoglycemic after islet transplantation, but suffered recurrent hyperglycemia in 2, 12, 13, 14, and 17 days, apparently because the islets were rejected.

In a second series of experiments we used eight BB recipients with naturally occurring diabetes of recent onset (< 30 days). In these recipients of BB islets (also from eight normoglycemic BB donors) the normoglycemic period after transplantation did not exceed 4 days (Table 1). Histological examination of the liver showed islets heavily infiltrated by mononuclear cells. These results suggested that the β cells transplanted to naturally diabetic recipients were destroyed by the original disease process rather than by rejection.

Table 2. Prospective study of incidence of diabetes in seven litters of BB rats in which half of the members of each litter were inoculated with $50 \times 10^6$ bone marrow cells from normal WF donors.

| Group | Number of rats | Diabetics* | Percentage |
|---|---|---|---|
| Tolerant | 27 | 4 | 14.8† |
| Nontolerant littermates | 28 | 18 | 64.3† |

*All diabetic rats had plasma glucose values of > 3 mg/ml; normoglycemic rats had plasma glucose values of < 1.5 mg/ml. †$P < .001$.

To provide further evidence for this contention, we transplanted allogeneic, but RT1 compatible, WF islets to diabetic BB rats made immunologically tolerant by exposure at birth to WF bone marrow cells (*7*). Islet transplantation in six such rats that developed spontaneous diabetes resulted in normoglycemia which persisted for only 2 to 11 days prior to recurrence of diabetes. The islet failure could not be attributed to rejection since these rats were tolerant of WF antigens as confirmed by their acceptance of WF skin grafts. Biopsy revealed that the lymphocytic infiltration of the transplanted islets was similar to that of the islets in the native pancreas (*8*). In contrast, the same islet transplantation procedure permanently cured six other tolerant BB animals in which hyperglycemia was induced with streptozotocin (*9*) (see Table 1).

A serendipitous finding in the BB rats rendered immunologically tolerant of WF cells is also important. These rats proved to have a much lower than expected incidence of diabetes. Of 239 untreated (nontolerant) offspring of nondiabetic BB parents, 13 developed the disease (5.44 percent), whereas of 91 tolerant rats of similar parentage only one became diabetic (1.09 percent) ($P < .05$) (*10*). Of 151 nontolerant rats with one diabetic parent, 29 developed diabetes (19.2 percent); and of 58 tolerant animals, only four (6.89 percent) developed the disease ($P < .05$). To confirm this finding we undertook a controlled prospective study in which half of the newborn pups of seven BB litters were selected at random and rendered tolerant of WF (see Table 2) (*11*). The remaining pups were left untreated as controls. The incidence of diabetes was expected to be especially high in these litters since both parents were diabetic. The tolerant and nontolerant rats were raised together and cared for in an identical fashion, and blood samples were examined for glucose content at weekly intervals. Eighteen of 28 nontolerant rats (64.3 percent) became diabetic, whereas only 4 of 27 (14.8 percent) of the tolerant rats developed hyperglycemia ($P < .001$). Thus, there was a strikingly reduced incidence of diabetes in diabetes-prone BB rats tolerant of WF antigens.

The studies reported here may be pertinent to the therapy or prophylaxis of diabetes as well as to our understanding of its pathogenesis. Inasmuch as the hyperglycemic syndrome in BB rats seems remarkably similar to human diabetes, the finding that the disease recurs in transplanted pancreatic tissue, independently of rejection, could portend badly for the success of human pancreatic transplantation. However, since we have previously demonstrated that islet allografts can survive and function in ALS-treated BB rats, and since a few human pancreas allografts have been successful for more than a year (*12*), recurrent disease seems unlikely to occur in heavi-

ly immunosuppressed patients. Nevertheless, the results of our experiments, which strengthen the evidence of autoimmune factors in diabetes, indicate that recurrent disease could be a potential problem, especially if optimal histocompatibility, improved drugs, or other protocols in the future allow avoidance of rejection with no or minimal immunosuppression. A precedent for such a problem is provided by the results of renal transplantation in victims of another, probably autoimmune, disease, glomerulonephritis. Recurrence of the original disease occurred in normal kidneys taken from identical twins in 11 of 17 instances (*13*).

The finding that neonatal BB rats inoculated with WF bone marrow cells failed to develop the expected incidence of diabetes in later life suggests an entirely new approach to therapy or prophylaxis of diabetes. The mechanism by which allogeneic bone marrow protected these rats from diabetes is not obvious; it seems unlikely to be tolerance of WF histocompatibility antigens per se, since a few tolerant rats did become diabetic and their disease was capable of destroying transplanted WF islet tissue, despite the fact that transplanted WF skin was accepted. Another possibility is destruction by the WF cells of a "forbidden clone" of BB cells responsible for autoimmune diabetes in unmodified BB rats. Perturbations in the immunological network may be the underlying cause of diabetes if autoimmunity is involved. In this regard there is experimental evidence that T cell immunoregulatory malfunction is a dominant factor in the etiology of autoimmune disease (*14*). Persistence in BB rats of a population of lymphohematopoietic cells from normal nondiabetes-prone WF donors could play a role in keeping an autoimmune process in abeyance. The chimeric cells might, for example, correct possible deficiencies in BB lymphocyte subpopulations—such as suppressor cells. Alternatively, if the syndrome is triggered by a yet to be detected virus, the chimeric cells could reequip the BB hosts with competent antiviral clones which they may lack. Study of these possible mechanisms in this animal model should provide fruitful avenues for future investigations of etiology and therapy of diabetes.

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8. Two of these recipients that suffered recurrent diabetes were challenged with a second WF skin graft which they permanently (more than 100 days) accepted, again confirming the persistence of the tolerant state.
9. All of these rats remained normoglycemic through a 1- to 4-month observation period after which they were killed and found histologically to have healthy transplanted WF islets.
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# Retrograde Amnesia: Possible Role of Mesencephalic Reticular Activation in Long-Term Memory

Abstract. *A patient with cerebral trauma recovered considerably from the resulting anterograde but not retrograde amnesia. The persistence of retrograde amnesia is attributed to a lesion in the ventral tegmental region, which suggests a role for mesencephalic reticular activation in long-term retrieval.*

Two types of memory deficits are commonly identified: anterograde amnesia (AA), an inability to learn and retain new information after the onset of pathology; and retrograde amnesia (RA), an inability to retrieve information acquired prior to the onset of pathology, usually involving loss of remote memory (*1*). Parallel recovery from AA and RA is a common clinical finding (*2*), as is RA recovering first with AA recovering later or not at all (*3*).

Double dissociation between the two types of amnesia and the presence of RA without AA have been described (*1, 4*), but cases are rare and sparsely documented. We now report a case of considerable recovery from AA but not RA, which may offer insights into mechanisms of long-term retrieval.

A 36-year-old, right-handed, college-educated male had an open skull fracture in the right parieto-occipital and temporal areas and herniation of the right hemisphere with compression of the left mesencephalon at the tentorial notch. In surgery, a small portion of macerated brain was removed, the dural laceration was sewn over, and the bone-plate replaced. Six weeks later, a ventriculoatrial shunt was performed. There was hemiparesis with spasticity of the distal upper right extremity, a right Babinski's sign, and a left superior quadrantanopsia. The patient was disoriented, his speech anomic and agrammatic. There was profound AA: his recall of seeing a person did not exceed 2 to 3 minutes, and his ability to retain names was still shorter. There was also profound RA. The patient maintained that he was 16 to 18 years old and mentioned his parents' address as his residence. He revealed no knowledge of his subsequent life history, his marriage, children, or past employment. His command of general information was equally impaired.

During the 2-year course of recovery the patient became fully oriented; linguistic and motor deficits virtually disappeared, but the quadrantanopsia remained. Recent memory was improving. A continuity of experience became possible; he could keep track of weekly and monthly events and retain information from newspapers and television. There was no parallel recovery from the 20-year deep RA. The patient's past history was reconstructed for him, but lacked a sense of authenticity. Command of general information did not improve beyond what he had been taught during this time; he could not answer questions like, "Who wrote *Hamlet*?" or "What is the capital of France?"

Table 1 summarizes the patient's per-


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