Recovery from Experimental Diabetes Mellitus in Mice after Pancreas Transplantation

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SUMMARY

Mice made diabetic with alloxan or streptozotocin received subcutaneous transplants of pancreases, which had been ligated six to eight weeks earlier, from three or four isogeneic donors. The course of diabetes in grafted mice was followed by determinations of glucose levels in blood and urine and changes in body weight. Five weeks after transplantation, blood glucose levels were substantially lower in grafted than in nongrafted mice. Recovery from hyperglycemia, defined as a drop in blood glucose below 150 mg./100 mL., was observed only in grafted mice and was greater in streptozotocin- than in alloxan-treated hosts. Normoglycemia was evident in over two thirds of the streptozotocin-treated animals from five weeks after transplantation to the termination of the study (fourteen weeks). The incidence of recovery in alloxan-treated grafted animals was variable, but nevertheless recovery could be demonstrated in comparison to nongrafted controls. Grafted animals returning to normoglycemia showed simultaneously a net gain in body weight and no glycosuria. DIABETES 23:183-88, March, 1974.

Several studies involving transplantation of fetal, neonatal, adult or cultured pancreatic tissue into alloxan-treated mice, rats or hamsters have failed to demonstrate a definite recovery from diabetes in the host.1-8 Even though histologically intact beta cells capable of releasing insulin were present in the grafts,5,7 the critical mass of beta cells was probably too small to be compatible with glucose homeostasis in the alloxan-treated animals. On the other hand, a clear remission from diabetes has been demonstrated in hereditarily obese hyperglycemic mice that received transplants of previously ligated pancreatic tissue or isolated islets obtained from normal mice.9,10 Other investigators have reported recovery from or amelioration of the diabetic state in New Zealand obese mice11 and streptozotocin-treated rats12-14 that received transplants of islets isolated by the collagenase digestion method of Lacy and Kostianovsky.15 We report here our results showing functional recovery from diabetes in alloxan- or streptozotocin-treated mice that received isogeneic transplants of previously ligated adult pancreas. Our interest in the transplantation of foreign tissues into mice rendered immunologically tolerant by irradiation and allogeneic bone marrow transplantation (termed a radiation chimera) prompted us to develop a method for transplanting pancreatic islet tissue in the mouse. This method would provide an organ transplant system in addition to the skin and tumor16 and ovarian17 transplant systems that have been used to study the immunologically tolerant state of allogeneic radiation chimeras. A functional graft of pancreatic islet tissue capable of reversing the diabetic state is unique in the sense that graft acceptance or rejection can be measured quantitatively by ascertaining the reversal or nonreversal of a given diabetic parameter, e.g. the glucose level in blood or urine.

MATERIALS AND METHODS

Female (C57BL/Cum 9 X C3H/Anf Cum d) F1 mice, five months of age, were used as donors or recipients of pancreatic tissue. Recipients were made diabetic by a single intravenous injection of either 80 mg. of alloxan or 230 mg. of streptozotocin per kilogram body weight. Alloxan was prepared in phosphate-buffered saline (PBS), pH 7.2, and streptozotocin was dissolved in citrate buffer, pH 4.5, immediately before injection. Spontaneous remission of alloxan diabetes in mice has been reported,18 and thus the time between diabetogenesis and demonstration of functional recovery from transplanted islets is critical. Alloxan was injected four days before transplantation. Since restrictions caused by spontaneous recovery do not apply to streptozotocin, this drug was given two to three weeks before transplantation. Mice received food and water ad libitum both before and after injection of diabetogenic drugs. The pancreas of
the donor animal was ligated six to eight weeks prior to transplantation by applying a ligature of silk around the pancreas approximately midway between the duodenum and the spleen. The atrophied portion of the pancreas between the ligature and the spleen was dissected from the decapitated donor and placed in a small Petri dish containing ice-cold PBS. The pancreatic tissue was minced with scissors into fragments about 1 to 2 mm. in diameter and immediately transferred to the host. The anesthetized host was prepared for transplantation by making a small incision over the sternum, inserting the tip of a pair of blunt-ended scissors through the incision and making small subcutaneous pockets in each axilla and over the abdomen. Each recipient received ligated pancreases from three or four donors. Control animals were treated in the same manner except that no pancreatic material was placed in subcutaneous pockets.

The course of diabetes was followed by determinations of glucose levels in blood and urine and changes in body weight. Whole blood was collected by inserting a 25-μl. capillary pipette into the retro-orbital blood sinus. A Somogyi filtrate was then prepared and analyzed for glucose by an ultramicro test with a glucose oxidase reagent (Glucostat, Worthington Biochemical Corp.). Urinary glucose was determined semiquantitatively using Tes-Tape (Eli Lilly Co.).

Since urinary glucose can be simply and rapidly determined with Tes-Tape, this assay was used frequently to approximate the incidence of recovery from diabetes and was used also as an indicator of when to sample blood for quantitative glucose analysis. The initial test for blood glucose after transplantation was performed when at least half of the animals in a group showed either negative urine glucose or a trace amount (< 0.1 gm./100 ml. urine).

RESULTS

The histologic appearance of the pancreas eight weeks after ligation is depicted in figure 1. Many intact pancreatic islets containing both alpha and beta cells were present in the ligated portion in addition to ductal and vascular elements. The complete disappearance of the acinar compartment and its replacement by adipose tissue were evident.

Four experiments were performed in which three or four ligated pancreases were transplanted to both alloxan- and streptozotocin-diabetic hosts. Results are shown in table 1. Before transplantation, blood glucose levels in experimental animals were similar to those in controls in each experiment (p ≥ 0.32). Beginning at five weeks after transplantation and at each time interval thereafter, blood glucose levels of grafted mice were significantly lower than those of control animals (p < 0.005). Although blood glucose levels gradually declined after transplantation in alloxan-treated hosts, mean values were still in the hyperglycemic range (231 or 208 mg./100 ml.) at nine weeks. In contrast, an early return toward normoglycemia was noted in transplanted mice injected with streptozotocin, and from five to fourteen weeks after transplantation blood glucose concentrations were below 150 mg./100 ml.

The diabetic state produced by alloxan or streptozotocin, as assessed by the degree of hyperglycemia one day before transplantation, varied among the four experiments. Before transplantation, animals injected with alloxan had higher blood glucose levels than animals injected with streptozotocin. The alloxan-diabetic state combined with sham transplantation in experiment 1 progressively worsened, whereas control animals in experiment 2 appeared to be somewhat improved at nine weeks. For the two experiments involving streptozotocin, mice of experiment 4 were more severely hyperglycemic initially and throughout the study than animals of experiment 3.

The normoglycemic range for the hybrid mice used in our study is 94 to 150 mg./100 ml.; recovery from hyperglycemia can be considered as a drop in blood glucose below 150 mg./100 ml. The incidence of recovery, based on individual blood glucose values, is shown in table 2 for each experiment. The incidence of recovery was greater in streptozotocin- than in alloxan-diabetic animals. In addition, a higher incidence of recovery was apparent in animals receiving four grafts than in those receiving three grafts, particularly in alloxan-treated animals at five and seven weeks after transplantation. As related in the footnote to table 2, some grafted, streptozotocin-treated hosts showed a transient reduction of blood glucose below 150 mg./100 ml., but in no case did these animals revert to the hyperglycemic levels observed before transplantation. Recovery from hyperglycemia in control animals was not observed.

Islets containing beta cells which stained specifically with aldehyde fuchsin (AF+) were present in subcutaneous transplants taken from alloxan- or streptozotocin-treated mice that had reverted to normoglycemia after transplantation. Islets in the host pancreas of alloxan-treated mice were essentially devoid of AF+ cells, indicating that massive destruction of beta cells had occurred before transplantation. Marked beta cell destruction was also noted in the
FIG. 1. (A) Normal pancreas of mouse fasted overnight. X 50. (B) Pancreas eight weeks after ligation in mouse fasted overnight. X 50. Note the complete obliteration of acinar tissue, involution of fat and persistence of islets (arrows). (C) Islets of ligated pancreas showing distinct alpha cells (a) and beta cells (b). X 160. Tissues were fixed in Bouin's fixative and stained with aldehyde fuchsine trichrome stain.
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TABLE 1
Blood glucose in alloxan- or streptozotocin-treated hosts receiving transplants of ligated pancreatic tissue

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Host treatment*</th>
<th>Blood glucose (mg./100 ml.)† before and at intervals after transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-1 day 5 weeks 7 weeks 9 weeks 14 weeks</td>
</tr>
<tr>
<td>1</td>
<td>Alloxan + 4 l.p.</td>
<td>374 ± 6 (20) 260 ± 25 (20) 257 ± 27 (20) 231 ± 34 (19) –</td>
</tr>
<tr>
<td></td>
<td>Alloxan + sham operation</td>
<td>371 ± 4 (29) 528 ± 12 (28) 485 ± 14 (25) 530 ± 21 (23) –</td>
</tr>
<tr>
<td>2</td>
<td>Alloxan + 3 l.p.</td>
<td>375 ± 6 (19) 303 ± 26 (19) 302 ± 29 (18) 208 ± 17 (18) –</td>
</tr>
<tr>
<td></td>
<td>Alloxan + sham operation</td>
<td>377 ± 7 (28) 400 ± 15 (27) 410 ± 16 (25) 371 ± 18 (25) –</td>
</tr>
<tr>
<td>3</td>
<td>Streptozotocin + 4 l.p.</td>
<td>274 ± 12 (18) 137 ± 9 (18) – 147 ± 7 (13) 125 ± 5 (13) –</td>
</tr>
<tr>
<td></td>
<td>Streptozotocin + sham operation</td>
<td>260 ± 18 (7) 222 ± 20 (7) – 209 ± 14 (7) 248 ± 23 (7) –</td>
</tr>
<tr>
<td>4</td>
<td>Streptozotocin + 3 l.p.</td>
<td>326 ± 13 (15) 145 ± 8 (15) – 135 ± 5 (14) § 137 ± 6 (14) –</td>
</tr>
<tr>
<td></td>
<td>Streptozotocin + sham operation</td>
<td>352 ± 21 (10) 319 ± 19 (10) – 280 ± 23 (9) 331 ± 33 (9) –</td>
</tr>
</tbody>
</table>

* l.p. = ligated pancreas.
† Mean ± standard error (sample size).
‡ Five normoglycemic animals were killed for histologic evaluation.
§ One normoglycemic animal was killed for postmortem examination.

Only one death, which occurred in a control group of ten animals, was recorded for streptozotocin-treated mice. One streptozotocin-diabetic animal that received three pancreatic grafts had a bloated abdomen at nine weeks. Postmortem examination revealed a greatly enlarged renal capsule of the left kidney, which contained a large amount of serosanguineous fluid.

Judging from general appearance and measurements of body weight, grafted animals fared much better than those of control groups. The animals in the control groups injected with alloxan had a mortality of 21 per cent (6/29) at nine weeks, whereas only one death occurred in each of the implanted groups, giving mortality rates of 5 per cent (1/20 and 1/19).

TABLE 2
Recovery from hyperglycemia in alloxan- or streptozotocin-treated hosts receiving transplants of ligated pancreatic tissue

<table>
<thead>
<tr>
<th>Experiment no.</th>
<th>Host treatment*</th>
<th>No. of animals showing normoglycemia (&lt;150 mg./100 ml.) per total tested at intervals before and after transplantation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>-1 day 5 weeks 7 weeks 9 weeks 14 weeks</td>
</tr>
<tr>
<td>1</td>
<td>Alloxan + 4 l.p.</td>
<td>0/20 5/20 7/20 8/19 –</td>
</tr>
<tr>
<td></td>
<td>Alloxan + sham operation</td>
<td>0/29 0/28 0/25 0/23 –</td>
</tr>
<tr>
<td>2</td>
<td>Alloxan + 3 l.p.</td>
<td>0/19 0/19 1/18 5/18 –</td>
</tr>
<tr>
<td></td>
<td>Alloxan + sham operation</td>
<td>0/28 0/27 0/25 0/25 –</td>
</tr>
<tr>
<td>3</td>
<td>Streptozotocin + 4 l.p.</td>
<td>0/18 15/18 – 8/13† 13/13</td>
</tr>
<tr>
<td></td>
<td>Streptozotocin + sham operation</td>
<td>0/7 0/7 – 0/7 0/7 –</td>
</tr>
<tr>
<td>4</td>
<td>Streptozotocin + 3 l.p.</td>
<td>0/15 10/15 – 12/14† –</td>
</tr>
<tr>
<td></td>
<td>Streptozotocin + sham operation</td>
<td>0/10 0/10 – 0/9 0/9 –</td>
</tr>
</tbody>
</table>

* l.p. = ligated pancreas.
† Five normoglycemic animals were killed for histologic evaluation. Blood glucose values of two animals that were <150 mg./100 ml. at five weeks were slightly above 150 mg./100 ml. at nine weeks.
‡ One normoglycemic animal was killed for postmortem examination.
§ Blood glucose values of two animals that were <150 mg./100 ml. at nine weeks were slightly above 150 mg./100 ml. at fourteen weeks.
beter than nongrafted animals. Nongrafted mice usually appeared weak, scruffy, hunched, and emaciated. The majority of grafted mice, however, showed a gain in weight and healthy coat color and were generally vigorous.

DISCUSSION

In the present series of experiments, remission of experimentally induced diabetes mellitus occurred in mice after pancreatic transplantation. Remission from diabetes in grafted mice was characterized by a decrease in the mean blood glucose level and individual reversions to normoglycemia. Transplants of pancreatic tissue were obtained from mice in which the pancreas had been ligated six to eight weeks earlier. Ligation of the pancreas produced a tissue that was completely free of acinar tissue but included histologically intact islets. Similar morphologic results, showing degeneration of acinar cells and persistence of islets after ligation, have been reported in rats.20-22 The ligated pancreas has been considered by others as suitable material for transplantation. Hultquist6 transferred to the eye of diabetic rats fragments of adult pancreas four to six weeks after ligation, and found persistence of islets in grafts but no effect on hyperglycemia in the host. Strautz9 was the first to demonstrate functional recovery from diabetes after intraperitoneal transplantation of ligated pancreas from normal donors to obese hyperglycemic mice. In his study the pancreas was ligated two weeks before transplantation.

The alloxan-diabetic animal has been used frequently in studies of pancreatic transplantation in small laboratory rodents.6-8 Histologic evidence provided by these studies has shown that islets of transplanted pancreatic tissue survive and grow in alloxan-treated animals. Although a relative improvement of hyperglycemia or glycosuria was sometimes observed,1-3,8 marked remission of diabetic symptoms due solely to functionally active transplanted islets was not demonstrated. Since the hyperglycemic state produced by alloxan is often spontaneously reversible in rodents18,23 and particularly unstable in hamsters,24 it was not clear what the contribution of such reversion was in ameliorating diabetes in some of the studies.1-3 In our experiments, a large number of sham-operated controls were studied concurrently and under the same conditions as transplanted animals. The significant and substantial decrease in blood glucose and higher incidence of recovery in transplanted mice compared to controls provide assurance that the transplants were functional and effective. Moreover, these improvements in the alloxan-diabetic state occurred between five and nine weeks after alloxan injection, whereas spontaneous recovery from alloxan-diabetes in mice is usually manifested after twelve weeks.25

Spontaneous remission from diabetes does not occur in animals given streptozotocin, providing a suitable dose of the drug is administered.18,26 Thus, streptozotocin-treated hosts provide a reliable means of assessing the capacity of grafted pancreas to effectively control experimental diabetes. Ballinger and Lacy12 reported that intraperitoneal isografts of isolated islets reversed the diabetic state of streptozotocin-treated rats, as assessed by mortality figures, weight gains, and urinary glucose and excretion volumes. A decrease in mean blood glucose levels was not demonstrated in grafted rats, although individual cases of reversion to normoglycemia were apparent. Other investigators13,14 have reported a similar remission of diabetes in streptozotocin-treated rats that received intraperitoneal transplants of isolated islets. Our blood glucose data show that recovery from streptozotocin diabetes can also be achieved in the mouse after pancreatic transplantation.

Transplantation with functional recovery from diabetes has been demonstrated in hereditarily obese hyperglycemic mice. Strautz9,10 and Gates et al.11 reported that blood glucose levels in obese hyperglycemic mice decreased to levels comparable to those in nonobese siblings five to six weeks after intraperitoneal implantation with islets isolated from nonobese mice. It is notable that within the same period of five to six weeks after transplantation, streptozotocin-treated mice in our study also returned to normoglycemia.

Our results indicate that four transplants might produce a higher incidence of recovery than three transplants; conclusions cannot be made until additional experiments are done, since the duration and degree of hyperglycemia after drug treatments were different in each experiment. Presumably, the severity of hyperglycemia produced by each diabetogenic drug and the number of grafted islets that survive and become functional are two factors that must be considered in any dose-response relationship between grafted islets and glucose homeostasis in diabetic mice.

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REFERENCES