

Successful Long-Term Survival of Pancreatic Islet Allografts in Spontaneous or Pancreatectomy-induced Diabetes in Dogs

Cyclosporine-induced Immune Unresponsiveness

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SUMMARY

Nineteen pancreatectomized beagles and three spontaneously diabetic dogs were recipients of canine islet allografts from one or more unrelated donors. The islets, enriched 30–45-fold for endocrine cells and contained in a packed cell volume of <1.5 ml, were engrafted in the livers of recipient animals. Treatment of diabetic recipients with cyclosporine (CsA) was begun 3–5 days before islet transplantation and the initial dosage was adjusted to attain and maintain CsA serum trough levels between 400 and 600 ng/ml. Five dogs with CsA levels less than this (155 ± 35 SEM ng/ml) at the time of transplantation promptly rejected their grafts, whereas rejection was encountered in only 1 of 17 diabetic animals in which the initial level exceeded 400 ng/ml. CsA was discontinued 30, 60, or 90 days after continuous therapy in 10 animals. Graft failure was observed 2 mo after stopping CsA in 1 animal and 5 mo in the other. Eight other islet allograft recipients have sustained fasting euglycemia for 7 and 8 mo in 2 and for at least 2 mo in the remainder.

These results demonstrate that short-term CsA therapy prolongs survival of islet allografts and induces a state of immune unresponsiveness to islet alloantigens in dogs with experimental and spontaneous diabetes. The findings are unique for a nonrodent mammal and thus hold promise that similar results may be achieved for islet allografts of other mammalian species, including humans. DIABETES 1985; 34:825–28.

Immune rejection of fresh, unmodified pancreatic islet allografts has long appeared to present an intractable problem despite a variety of therapeutic maneuvers designed to achieve effective immunosuppression in recipients of these grafts. Thus even cyclosporine (CsA), a potent immunosuppressive agent of proven merit in kidney, heart, skin, and segmental pancreas allografts,^{1,2} has proven relatively ineffective in prolonging islet allograft survival in rats³ and dogs.^{4,5} However, in many of these studies, it is not possible to differentiate whether the functional failure was due

to problems with islet cell viability and engraftment or whether the extent of immunosuppression achieved was insufficient to interfere with immune rejection of the islet grafts.

The present study was made possible by the development of a procedure to isolate islets from a single canine pancreas in sufficient quantities and purity to allow for viable engraftment of islet autografts following their portal delivery to the livers of totally pancreatectomized beagle dogs. This uniform success with islet autografts allowed us to test directly for the immunosuppressive effectiveness of CsA using islet allografts in dogs with induced or spontaneous diabetes. Moreover, the experiments were also designed to evaluate whether interruption of CsA administration would reveal a state of immune unresponsiveness to islet alloantigens, as previously shown in rodents and rabbits treated with CsA for kidney, heart, and bone marrow allografts.^{6–10}

ANIMALS AND METHODS

Highly purified canine islets were isolated by minor modifications of a procedure previously described from this laboratory.¹¹ The average yield from a single donor was $30\text{--}40 \times 10^3$ islets with a packed cell volume of 1.5 ml or less. Islets so prepared were enriched 30–45-fold and comprised 60–90% endocrine cells, 20–35% endothelial, ductal, or unidentified cell types, and consistently <10% acinar ($N = 5$), as assessed by electron microscopy.¹² The mean number of endocrine cells isolated from a single donor pancreas was $57 \pm 6 \times 10^6$ ($N = 22$). When multiple donors were used, the number of endocrine cells was proportionately increased. The high degree of purity and small volume permitted implantation of such preparations in the liver, following infusion by gravity drainage into one of the branches of the superior mesenteric vein.

This work was presented in part at the meeting of the American Association of Physicians, Washington, D.C., May 1985.

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Received for publication 14 May 1985.

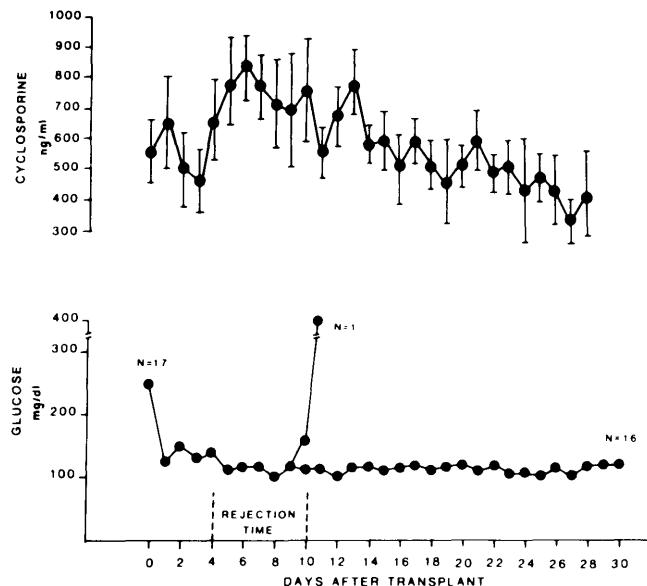


FIGURE 1. Trough serum cyclosporine levels (upper panel, mean \pm SEM) and fasting blood glucose levels in 17 animals (14 pancreatectomized and 3 spontaneously diabetic animals) after intrahepatic islet allografts. Cyclosporine was initiated 3–5 days before islet engraftment at dosages between 20 and 40 mg/kg.

Nineteen pancreatectomized beagles and 3 spontaneously diabetic dogs (poodle, Irish setter, mixed terrier) were recipients of islet allografts. Of these, 3 beagles received islet grafts from littermates and the remaining 19 were unrelated donors. Three of the littermate transplants were tissue typed according to standard protocols by Marshall Laboratories (North Rose, New York); 2 were mismatched by one haplotype, and 1 was mismatched by two haplotypes. Each of the transplanted littermate pairs exhibited high reactivity in a mixed-lymphocyte culture system.¹³

Donors were beagles obtained from Marshall Laboratories or mongrels obtained from local suppliers. In nine instances multiple donors (2–8) were used for a single recipient. The multiple donors were used as part of an ongoing study of the relationship between transplanted islet cell mass and the long-term survival and function of the allografts.

Serial overnight fasting serum glucose concentrations were measured daily for 30 days and weekly thereafter with a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, California). Serum CsA levels were determined with a radioimmunoassay kit (Sandoz Basle, Switzerland) on a daily basis for 4 days before and for 14 days after islet transplantation and biweekly thereafter. CsA (20 mg/kg) was admin-

istered intramuscularly each morning as a single daily dose to all recipients in the treated group except in the three spontaneous diabetic dogs. In two of the latter, 40 mg/kg CsA, divided into two equal doses, was administered by mouth. In the third dog with spontaneous diabetes, also presenting pancreatic insufficiency, CsA was given intramuscularly at the daily dose of 40 mg/kg. CsA administration was begun 3–5 days before islet transplantation and the dosage adjusted so as to attain and maintain CsA serum levels between 400 and 600 ng/ml. CsA was discontinued after 30 days in 5 animals, after 60 days in 3 animals, and after 90 days in 2 animals.

All pancreatectomized dogs and the spontaneously diabetic dog with pancreatic insufficiency received daily pancreatic enzyme supplements (Viokase, Viobin Corp., Ponticello, Illinois) in addition to their liver-fortified dog chow (Alpo Pet Foods, Allentown, Pennsylvania).

RESULTS

Of the 22 diabetic dogs (19 pancreatectomized and 3 with spontaneous diabetes) who were transplanted with an adequate number of islets (no less than 40×10^6 endocrine cells) obtained from one or more donors, all promptly reached fasting euglycemia, which was sustained for at least 30 days posttransplant in 16 recipient animals. Five beagle dogs with diabetes induced by pancreatectomy appeared to reject their islet allografts between 8 and 10 days after successful engraftment. The mean survival time of islet allografts in the absence of immunosuppression is 6.75 ± 0.7 days ($N = 8$, with a range of 4–10 days). The mean trough level of serum CsA (i.e., the level of CsA still present before the following injection) at the time we transplanted the islet in these five animals was 115 ± 35 ng/ml. By contrast, when immediate pretransplant CsA levels in serum exceeded 400 ng/ml, almost uniform success in prolonging islet allograft survival was achieved. The mean fasting blood glucose and trough serum CsA levels in these 17 animals are depicted in Figure 1. Only one pancreatectomized animal in this series appeared to reject the islets, on day 9 after transplantation.

CsA was discontinued in 10 animals (Table 1). In 5 the CsA was stopped after 30 days (group 1), in 3 after 60 days (group 2), and in 2 after 90 or more days (group 3) of continuous administration. Serum CsA levels were uniformly undetectable by RIA 7 days after discontinuation of the drug. Of the 5 transplanted animals treated with CsA for only 30 days, euglycemia was maintained for at least 60 days in 3, and for at least 230 days in another. In this group, a single graft was observed to fail functionally 60 days after discontinuation of CsA (90 days posttransplant). In groups 2 and 3

TABLE 1
Allotransplantation of canine pancreatic islets: CsA-induced unresponsiveness

| No. of dogs | CsA treatment (days) | Graft survival after discontinuation of CsA (days) |
|-------------|----------------------|---|
| Group 1 (5) | 30 | 1 \times 60; 3 \times >30; 1 \times >200 |
| Group 2 (3) | 60 | 3 \times >30 |
| Group 3 (1) | 99 | 1 \times >250 |
| 1 | 139 | 142 |
| 10 | | 60; 142; 6 \times >30; 1 \times >200; 1 \times >250 |

islet grafts continued to maintain fasting euglycemia for at least 30 days after discontinuation of CsA in all 5 animals. A graft failed to continue to function in 1 animal approximately 4.5 mo after CsA was discontinued. To date, the longest observed duration of graft survival in the absence of continuous immunosuppressive therapy is approximately 8 mo.

DISCUSSION

In a review of studies of experimental islet allografts associated with CsA administration, Gray and Morris³ concluded that CsA can prolong somewhat islet allograft survival in rodents, but that the drug rarely produces long-term survival. Moreover, these workers were unable to demonstrate a beneficial effect of CsA on the survival of islet allografts in the dog.

The available but limited experience thus contrasts sharply with the data obtained in the present study. Figure 1 shows that in animals with CsA levels of 400 ng/ml or better, we were able to achieve acceptance of islet allografts for 30 days in 16 of 17 diabetic dogs. The results of sequential glucose tolerance testing, islet secretory response measurements, and long-term functional assessment of these 17 animals will be the subject of a subsequent detailed report.

There are several differences in the design of the studies reported here and those of previous workers. Our islet preparations were substantially free of acinar cell contamination and, by virtue of the degree of enrichment for endocrine cells and the resultant low packed cell volume, the liver could be successfully used as a transplant site. By contrast, the usual canine islet preparations preclude this site because of the large packed cell volume associated with islet preparations heavily contaminated with nonendocrine pancreatic cells.¹⁴ Also, the recipient animals were pretreated with CsA for 3–5 days before the transplant, to be confident that the inhibitory effects of the agent on lymphocyte activation would be fully present during the critical period immediately surrounding islet cell engraftment. Since CsA inhibits lymphocyte activation maximally, only after exposure to the stimulating antigen, it is probable that the favorable response reported here was due to the level of immunosuppression achieved rather than to the duration of CsA pretreatment. In the dosages used, CsA did not interfere with engraftment or discernibly alter islet cell function, although fasting serum glucose levels did seem to fall in some animals after discontinuation of CsA. In view of reports of CsA-related islet cytotoxicity,^{15,16} however, this issue still needs to be studied in greater detail. There was no evidence of nephrotoxicity in dogs that carried successful islet allografts, despite these high serum levels of CsA, and this has been the general experience with CsA in this species.¹⁷

To determine whether a state of immune unresponsiveness to islet alloantigens can be induced in dogs, we discontinued administration of the drug after 30–139 days. Hyperglycemia recurred in only 2 animals in this series (Table 1). Recurrence of hyperglycemia in these animals, 3 and 9 mo after islet transplantation and 2 and 4 mo after discontinuation of CsA, seems unlikely to represent immunologic rejection. The observation is more likely to represent late failure of islet graft function, since such failure has also been repeatedly observed in autografted pancreatectomized dogs.¹⁸

CsA has been reported to induce a state of tolerance to kidney, heart, and bone marrow allografts in rats and rabbits through a suppressor cell mechanism. During the inductive phase of CsA-associated tolerance, IL-1, IL-2, and IL-3 production is suppressed, and allograft rejection is inhibited. However, after CsA is withdrawn, IL-1 and IL-2 release returns to normal, and spontaneous release of IL-3 is heightened.¹⁹ This is thought by some investigators to play a role in the induction of active suppression.¹⁹ Whether this is also the case in the studies reported here needs to be further clarified.

At present, our results clearly demonstrate that short-term CsA therapy prolongs survival of islet allografts and induces a state of immune unresponsiveness to islet alloantigens in dogs with experimental and spontaneous diabetes. The findings are unique for a nonrodent mammal and thus hold promise that similar results may be achieved for islet allografts of other mammalian species, including humans.

ACKNOWLEDGMENTS

The authors are indebted to Dr. Albert Renold (University of Geneva) for his thoughtful criticism of the manuscript and to Dr. David Winter (Sandoz, Inc., East Hanover, New Jersey) for his encouragement and support during the conduct of these studies. We gratefully thank the staff of the Diabetes Research Institute for their dedicated technical and secretarial assistance.

This work was supported by National Institutes of Health grants AM25802 and AM07346, and by donations from Alpo pet food (Allentown, Pennsylvania), Abbott Laboratories (North Chicago, Illinois), and DuPont Pharmaceuticals (Wilmington, Delaware).

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