Long-Term Study of Vascularized Free-Draining Intraperitoneal Pancreatic Segmental Allografts in Beagle Dogs

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ABSTRACT The purpose of the present study was to evaluate the significance of immunogenetic factors on the survival of pancreatic allografts in beagle dogs. Donors and recipients were leukocyte antigen (DLA)-typed and mixed lymphocyte culture (MLC)-tested. Recipients were made diabetic by total pancreatectomy and immediately implanted intraperitoneally with a vascularized, free-draining (duct unligated) pancreatic segmental (FDPS) allograft. Two groups of dogs were studied. In group I consisting of donor-recipient littermates, recipients were immunosuppressed with prednisone and azathioprine (n = 16 dogs), or not immunosuppressed (n = 4). In group II, recipients were made specifically unresponsive by total body radiation, autologous marrow implantation, and kidney transplantation from DLA-MLC identical donors, 1 yr before FDPS transplantation from the corresponding original kidney donors.

Survival of the FDPS grafts in group I was inversely related to pretransplant MLC reactivity, irrespective of DLA genotyped match between donor and recipient. Thus, immunosuppressed high MLC reactors (n = 8) rejected FDPS grafts between 7 and 14 d, whereas immunosuppressed low MLC reactors (n = 8) accepted grafts for 25 to 260+ days, and nonimmunosuppressed low MLC reactors (n = 4) accepted grafts for 9–55 d. Rejection (hyperglycemia) of FDPS grafts was sudden, permanent, and unpredictable despite weekly intravenous glucose tolerance tests with measurements of glucose disappearance rates and serum insulin responses. Nevertheless, serial in vitro cell-mediated lymphocytotoxicity (CML) assays revealed increases in CML before graft rejection in low MLC reactors, and decreases in both CML and MLC responses before graft rejection in high MLC reactors. FDPS graft survival was indefinite (>6 mo) in group II dogs, despite low-grade MLC reactivity (2:4 dogs) and CML responses (4:4 dogs). Biopsies of FDPS grafts at 6 mo in normoglycemic dogs showed disappearance of exocrine tissue and coalescence of islets in both groups I and II, but with less fibrosis in group I (immunosuppressed).

These results indicate that (a) pancreatic islets in vascularized grafts (FDPS) may survive indefinitely in the presence of a good tissue match best predicted by MLC testing, (b) tissue specific histocompatibility factors appear to be common enough between kidney and pancreas to allow for long-term survival of both organs transplanted from the same donor, at least in appropriate recipients (group II), and (c) immunosuppression is associated with less fibrosis in FDPS allografts.

INTRODUCTION

Transplantation of the endocrine pancreas has been under intensive study during the past several years (1–6). Although numerous approaches have been used, we have found that heterotopic intraperitoneal implantation of the immediately vascularized, free-draining distal pancreatic segment (FDPS)[1] is technically feasible (7–9), eliminating certain complications associated with attempts to anastomose the pancreatic duct (10, 11). However, because of strong immunogenetic

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[1] *Abbreviations used in this paper*: CML, cell-mediated lymphocytotoxicity; DLA, dog leukocyte antigen; FDPS, free-draining distal pancreatic segment; IVGTT, intravenous glucose tolerance test; MLC, mixed lymphocyte culture; PBL, peripheral blood lymphocyte.

disparities in randomly-bred or pure-bred strains of pigs (7, 8) and randomly-bred dogs (9), FDPS allografts rarely survived longer than 45 d, and, therefore, long-term observations were lacking.

The present experiments were performed in beagle dogs with defined histocompatibilities to permit long-term study of (*a*) the immunogenetic factors affecting pancreatic allograft survival, and (*b*) the evolution of immunologic, functional, and morphological changes in recipients of FDPS allograft that either undergo rejection or are indefinitely accepted.

METHODS

Animals. Two groups of beagles were studied. Group I consisted of 20 adult donor-recipient littermate pairs weighing between 10 and 15 kg. The animals were purchased from Marshall Laboratories in North Rose, N. Y. The five beagles in group II were older males and females of the Cooperstown colony, which had been inbred for many years (12). In group II, pairs of donors and recipients had been previously selected and used for kidney transplantation on the basis of extensive dog leukocyte antigen (DLA) haplotyping and pedigree-determined identity as previously described (13). A state of specific allogeneic unresponsiveness had been produced in the recipients by supralethal x irradiation, followed by reconstitution with stored autologous bone marrow and carefully timed kidney transplantation from a matched donor. Kidney allografts had survived in these recipients for more than 2 yr in the absence of immunosuppression. In the current study in group II, the original kidney donors also served as donors of FDPS allografts, and each pancreatic allograft was transplanted into the same dog that had been the recipient of a kidney from that donor. In conducting the research in this report, the investigators adhered to the Guide for Laboratory Animal Facilities and Care of the Institute of Laboratory Animal Resources, National Academy of Sciences-National Research Council.

Operative techniques. The methods of preparation of the FDPS graft and implantation in the recipient have been previously described (7–9). Briefly, in one operative procedure, the recipient animals were made diabetic by total pancreatectomy, immediately followed by intraperitoneal transplantation of the left pancreatic limb of the donor (about one-third of the pancreas). The vascular anastomoses were performed using the splenic artery and vein of the graft and the iliac artery and vein of the recipient. The pancreatic duct of the graft was left unligated to drain freely into the peritoneal cavity. Postoperatively, the animals received 1 g Sefadyl (Bristol Laboratories Div., Syracuse, N. Y.) twice daily for 5 d after the procedure. Feeding was resumed on the second postoperative day, using liver-fortified dog chow supplemented daily with 5 g of pork Viokase (VioBin Corp., Monticello, Ill.) and 5 g of beef Viokase to alleviate the nutritive exocrine deficiency effects of total pancreatectomy.

Immunosuppressive management. Immunosuppression consisted of intraoperative intravenous administration of 5 mg/kg of azathioprine and 2 mg/kg of methylprednisolone. The post-operative regimen consisted of prednisone 2 mg/kg on day 1, then decreasing doses every 3 d, to a daily maintenance dose of 0.4 mg/kg by day 30. Azathioprine was given in a dose of 5 mg/kg for 3 d, 4 mg/kg for 3 d, 3 mg/kg for 3 d, and then as a 2.5 mg/kg daily maintenance dose subsequently.

Histocompatibility studies and immunological monitoring. Serotyping of erythrocyte antigens and peripheral blood lymphocyte (PBL) antigens (DLA) was performed on all animals. Animals in group I were also DLA genotyped by testing the sires and/or dames of each litter (Dr. Robert Bull, Michigan State University, East Lansing, Mich.).

Primary mixed lymphocyte culture (MLC) assays were performed as previously described in our laboratory (14). Briefly, microcultures were prepared in micro-titer plates containing 1×10^5 PBL of the recipient dog as responding cells and 1×10^5 x irradiated PBL of the donor (or autologous x irradiated PBL) as stimulator cells in 0.2 ml of RPMI 1640 medium supplemented with 15% heat-inactivated "normal" dog serum, 1% L-glutamine, 1% penicillin-streptomycin, and 1% preservative-free heparin (complete medium). The cultures were incubated in a humidified atmosphere of 7% CO_2 for 3, 5, 7, and 9 d, then pulse-labeled with 1 μCi of [^{3}H]thymidine for 18 h before processing in the Mash II microharvester (15) for liquid scintillation counting (Packard Instrument Co., Inc., Downers Grove, Ill.).

For cell-mediated lymphocytotoxicity (CML) assays, cytotoxic effector cells were generated by culturing recipient PBL as responding cells with donor x irradiated PBL as stimulator cells in 8-d macro-MLC (16). Recipient PBL were also cultured in the presence of autologous x irradiated cells as controls. Donor PBL used as target cells were labeled with radioactive sodium chromate (^{51}Cr) (Amersham Corp., Arlington Heights, Ill.) by incubating 5×10^6 cells in 0.2 ml of RPMI 1640, containing 10% fetal calf serum with 50 μCi of ^{51}Cr at 37°C for 1 h. The cells were washed four times with medium at 4°C and suspended at a concentration of 10^6 cells/ml. The CML assays were performed in 4-ml tubes (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, Calif.) containing 2×10^6 recipient effector cells (or autologous controls) and 0.25×10^6 ^{51}Cr-labeled donor cells as targets in a total volume of 1.05 ml. After 18 h of incubation in a humidified 7% CO_2 atmosphere at 37°C, 1 ml of cold medium was added and the tubes were centrifuged. The decanted supernatants and pellets were then counted in a gamma counter (Packard Instrument Co., Inc.). The specific ^{51}Cr release from donor cells was calculated by the equation of Brunner (17).

Antibody-dependent cell-mediated cytotoxicity assays were performed employing ^{51}Cr-labeled donor lymphocytes as targets. The test was performed in a 4-ml tube (Falcon Labware) with 2×10^6 effector cells of either the recipient or a normal control and 0.25×10^6 ^{51}Cr-labeled donor target cells sensitized with an appropriate dilution of recipient serum (putative antibody). Target cells treated with fetal calf serum served as negative controls, and cells treated with rabbit anti-dog lymphocyte serum, or repeatedly frozen and thawed, served as positive controls. After 6 h, the incubation was terminated, the tubes centrifuged, and the radioactivity in the supernatants determined in a gamma counter. The specific ^{51}Cr release was determined (Brunner equation) with normal release values calculated from fetal calf serum-treated cells (18).

Pancreatic islet function tests. Postoperatively, serum glucose concentrations were determined (Beckman glucose analyzer, Beckman Instruments, Inc., Fullerton, Calif.) daily at 0900 after an overnight fast. Intravenous glucose tolerance tests (IVGTT) were performed weekly, after an overnight fast, and serum glucose and immunoreactive insulin (19) concentrations were measured just before (0 min) and at 5, 10, 15, 20, 30, and 60 min after intravenous injection of glucose, 0.5 g/kg body wt. Serum cholesterol, triglycerides, liver enzymes, and complete blood counts were also performed twice weekly.

Morphologic studies. Biopsies of the FDPS allografts were taken, at laparotomy, 6 mo after transplantation in long-term survivors. The tissue was fixed in buffered formalin and stained with hematoxylin and eosin, and also fixed in Bouin's solution and stained with aldehyde fuchsin. A small aliquot of tissue was weighed and assayed for insulin content after ex-

traction in acid-ethanol (20). In all other recipients, on the day that rejection was diagnosed (increase in fasting serum glucose concentration to >180 mg/dl) an immediate biopsy of the FDPS was obtained and similarly processed.

RESULTS

Mortality and morbidity in FDPS allograft recipients. The postoperative course in allograft recipients was characterized by friskiness, good appetite, and maintenance of body weight. When loss of pancreatic endocrine function occurred, this proceeded very rapidly, the fasting serum glucose concentration increasing from <120 mg/dl to >180 mg/dl within 24 h (Fig. 1). Once this occurred, hyperglycemia persisted, the dogs became anorectic, rapidly lost weight, and died within 5–24 d. Graft survival could, therefore, be defined as the last day that the fasting serum glucose was <180 mg/dl.

No animal in group I (n = 16:20 dogs), in which immunosuppression was administered, sustained a complication of peritoneal abscess, pancreatic ascites or technical failure. One animal of five specifically unresponsive recipients in group II, in which no immunosuppression was given, developed pancreatic necrosis, intraperitoneal abscess and peritonitis, and died 5 d postoperatively after sustained normoglycemia.

Histocompatibility and graft survival. Each donor-recipient littermate pair of group I was genotyped (sire or dame) to determine the DLA haplotype and preoperative MLC reactivity. All pairs were determined to be erythrocyte antigen disparate. The duration of graft survival could be clearly related to MLC reactivity. Thus, in genotypically defined littermate pairs, when MLC reactivity was present (n = 8 pairs of dogs), FDPS allograft rejection occurred within 14 d (Table I), whereas in MLC nonreactive pairs (n = 8), FDPS allograft survival was from 25 to >180 days (Table I). Histocompatibility as determined by DLA serology (either haplotype or total DLA identity) was unrelated to MLC reactivity, nor was it correlated with graft survival. The explanation for this dissociation of serologically-defined (i.e., DLA) and lymphocyte-defined (i.e., MLC) assays of genotyped littermates is unclear, but may possibly be associated with nonmajor histocompatibility complex locus related MLC reactivity and/or a moderately high recombination frequency.

In four MLC nonreactive pairs, no immunosuppression was administered to the recipients (Table I). Rejection occurred at 9, 20, and 28 d, and a fourth recipient was normoglycemic for more than 55 d. This range of graft survival was longer than in MLC-reactive recipients that were immunosuppressed (Table I).

Specific allogeneic unresponsiveness and FDPS allograft acceptance. In group II, four specifically unre-

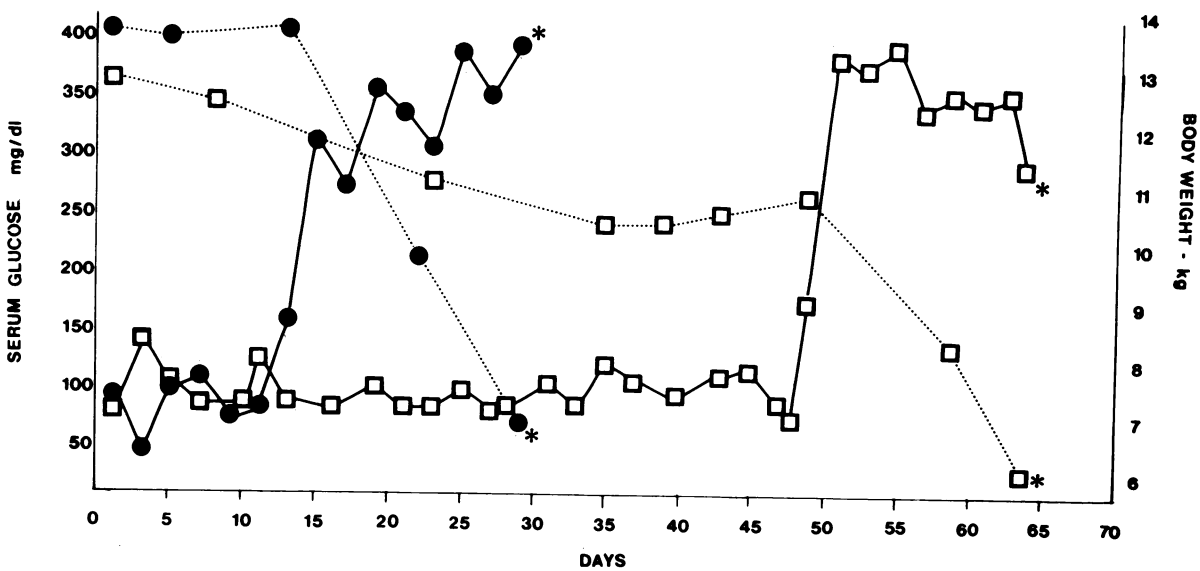

FIGURE 1 Fasting serum glucose concentrations (——) and body weights (-----) in two representative dogs in group I that received FDPS allografts on day 0. The recipient dog—42628 (●), which exhibited preoperative MLC reactivity against the donor (Table I), became hyperglycemic (>180 mg/dl) 13 d after transplantation and subsequently rapidly lost weight and died at 29 d (*). The recipient dog—42928 (□), which did not demonstrate significant MLC reactivity against the donor (Table I), became hyperglycemic 48 d after transplantation and subsequently rapidly lost weight and died at 64 d (*).

sponsive recipients of kidneys from donors that had been previously extensively genotyped and found to be DLA identical and MLC nonreactive before kidney transplantation (13) were tested in MLC against their specific donors again at the beginning of the present study. This group did not need immunosuppression due to the specific unresponsive state previously conferred upon them by x irradiation and autologous bone marrow transplantation (13), and therefore, provided a control for the effects of long-term immunosuppression (group I) on FDPS graft function. Also, study of this group tested the feasibility of transplanting kidney and pancreas in the same recipient. Skin grafts from the kidney donors had been known to be occasionally rejected, despite unaltered renal function in the recipients (13). Table II shows that MLC reactivity was definitely present in two of these four specifically unresponsive recipients at the onset of the present study, nevertheless graft survival was indefinite (>10 mo).

Morphologic changes in the pancreatic allografts. Once functional rejection of the FDPS allografts was detected, irreversible morphologic changes had already occurred. Thus, grafts biopsied within 24 h after hyperglycemia ensued, were, with one exception, completely necrotic. This finding was present in eight of eight MLC reactive pairs in which early rejection occurred (<14 d, Table I) and in four of the five MLC nonreactive pairs in which late rejection occurred (25–47 d, Table I).

At laparotomy in dogs (groups I and II) exhibiting normoglycemia at 6 mo, the pancreatic allografts were considerably shrunken in size, down to about one-fifth the size of the fresh implant. At this time, in group I, there was complete disappearance of pancreatic acinar cells, and the islets remained, having coalesced into large clusters of islet cells (Fig. 2b). This striking finding was not observed in previous short-term studies (7–9) in which the exocrine portion of nonrejecting grafts was still intact, and virtually unchanged from the appearance at the time of transplantation (Fig. 2a). Although similar predominance of islet tissue and absence of exocrine components was observed in the nonimmunosuppressed specifically unresponsive recipients (Fig. 2c), there was a more marked degree of fibrosis in these grafts (Fig. 3c and d) than in the immunosuppressed recipients (Fig. 3a and b).

In the two MLC nonreactive dogs in which the FDPS allograft survived for >180 d (Table I), biopsies of the grafts at 180 d revealed immunoreactive insulin concentrations of 212.5 and 426.7 μg/g tissue. These values were several-fold greater than the insulin concentration (95.8±8.1 μg/g, mean±SEM, $n = 6$ dogs) in the pancreatic segments originally serving as allografts, and reflects the loss of exocrine tissue and relative enrichment of the islet beta cell mass in the FDPS allografts after 180 d.

Monitoring of pancreatic endocrine function. In one MLC nonreactive dog of group I, FDPS allograft survival was indefinite (>260 d), and two dogs in this group were normoglycemic and died of pneumonia at 215 and 78 d after transplantation (Table I). IVGTT were performed on these three dogs at 192, 162, and 75 d, respectively after FDPS transplantation (Fig. 4). Glucose disappearance rates were similar to or only moderately reduced compared to values in the same recipients tested before pancreatectomy and transplantation. For this group of three dogs, the pretransplant glucose disappearance rate (mean±SEM) was 2.68 ±0.45%/min and the post-transplant glucose disappearance rate was 1.81±0.20%/min ($P < 0.1$, paired t test). Also, serum insulin responses were similar to, or only moderately less than the insulin responses observed in the same recipients tested before pancreatectomy and FDPS transplantation. Although maintenance of normal or near-normal intravenous glucose tolerances and accompanying serum insulin responses could be demonstrated in successful long-term FDPS allograft recipients, it was not possible to detect changes in pancreatic islet function before total graft necrosis occurred in the other dogs in group I. Thus, weekly IVGTT with measurements of serum glucose and insulin responses were not predictive of graft rejection in animals in which rejection occurred either early (<14 d) in MLC reactors, or later (25–47 d) in MLC nonreactors. Finally, in all long-term surviving animals (groups I and II), liver enzymes, serum cholesterol, and triglycerides remained within normal limits (not shown).

Immunologic monitoring. Immunosuppressed animals that were MLC nonreactive before FDPS transplantation exhibited no change in MLC reactivity after transplantation (Fig. 5a). However, CML responses, which were absent preoperatively, developed shortly before allograft rejection in each of the five recipients studied. After rejection, and as the animals became moribund with diabetes, CML reactivity declined. In MLC reactive recipients, both MLC and CML reactivity declined markedly in the perirejection period (Fig. 5b).

Table II shows the pre- and postoperative MLC and CML responses in the four specifically unresponsive, nonimmunosuppressed recipients (group II) with prolonged graft survival. Two of the four dogs that were MLC reactive preoperatively demonstrated continuing low-grade MLC responses after 6 mo. CML reactivity was present against donor target lymphocytes both before and after FDPS transplantation in all four recipients. The antibody-dependent cell-mediated cytotoxicity reactions, however, were negative before and after transplantation, and recipient MLC serum blocking

TABLE I

DLA Typing, MLC Reactivity, and FDPS Allograft Survival (Group I)

Animals*	Tissue type by DLA serotyping	Match by DLA genotyping‡	Preoperative MLC reactivity§ R + Rx	R + Dx	SI	Graft survival
MLC reactive immunosuppressed recipients						*d*
R-41580	bcegklno‖	—¶	472	23,662	50.1	7
D-41579	eg l o					
R-46190	(9,12) (10,6)	○	632	23,695	37.5	8
D-46189	(9,12) (10,6)					
R-42896	(2,12) (1,5)	○	513	3,925	7.7	9
D-42898	(2,12) (1,5)					
R-43198	(3,6) (10,12)	○	599	23,314	38.9	10
D-43199	(3,6) (10,12)					
R-45738	(2,6) (10,5)	◒	933	3,751	4.0	11
D-45731	(2,6) (9,4)					
R-42628	(2,—) (—,6)	◒	148	3,594	24.3	11
D-42629	(2,—) (7,12)					
R-48193	(10,12) (3,4)	○	344	9,087	26.4	11
D-48194	(10,12) (3,4)					
R-48198	(3,12) (10,4)	○	2,457**	22,125	8.7	14
MLC nonreactive immunosuppressed recipients						
D-48200	(3,12) (10,4)					
R-45734	(2,6) (9,4)	○	3,512**	1,566	0.4	25
R-45736	(2,6) (9,4)					
R-48191	(3,12) (9,6)	○	229	408	1.8	26
R-48192	(3,12) (9,6)					
R-41539	eglo‖	—¶	722	533	0.7	29
D-41538	eglo					
R-45733	(2,6) (10,6)	○	1,785	1,714	1.0	31
D-45735	(2,6) (10,6)					
R-42928	(3,12) (2,5)	●	5,283**	6,283	1.1	47
D-42929	(10,6) (1,5)					
R-46193	(9,12) (10,6)	○	106	113	1.1	78‡‡
D-46188	(9,12) (10,6)					
R-45674	(3,10) (—,—)	—¶	678	709	1.0	215‡‡
D-45672	(3,10) (—,—)					
R-48398	(3,6) (10,12)	◒	125	159	1.3	260+
D-48392	(3,6) (10,4)					
MLC nonreactive nonimmunosuppressed recipients						
R-52647	(2,6) (9,12)	○	53	61	1.2	9
D-52643	(2,6) (9,12)					
R-52625	(2,4) (10,13)	◒	808	213	0.3	20
D-52623	(—,4) (10,13)					

TABLE I (*Continued*)

Animals*	Tissue type by DLA serotyping		Match by DLA genotyping‡	Preoperative MLC reactivity§ R + Rx	R + Dx	SI	Graft survival
							d
R-52641	(2,6)	(9,12)	○	459	769	1.7	28
D-52642	(2,6)	(9,12)					
R-52645	(2,6)	(9,12)	○	280	453	1.6	55+
D-52644	(2,6)	(9,12)	○				

* R, recipient; D, donor.

‡ ○, DLA identical; ◒, haploidentical; ●, two haplotype mismatch.

§ Stimulation index (SI) = R + Dx ÷ R + Rx; i.e., allogeneic MLC response (R + Dx) of recipient (R) PBL cultured for 9 d (peak response) with donor x irradiated PBL (Dx), divided by the autologous MLC response of recipient PBL cultured for 9 d with recipient x irradiated PBL (R + Rx). MLC responses ([^{3}H]thymidine uptake in counts per minute) are shown as means for triplicate cultures with <15% variance.

‖ Typing performed at Mary Imogene Basset Hospital, Cooperstown, N.Y.

¶ Parents not available for direct genotyping.

** High MLC background.

‡‡ Died of pneumonia, normoglycemic.

factors could not be demonstrated (not shown). Thus, immunologic monitoring in this group II showed no change on long-term follow-up (6 mo), while functional stability of the FDPS allografts continued, as evidenced by maintenance of normal fasting serum glucose concentrations.

TABLE II

MLC and CML Reactions, and FDPS Allograft Survival in Specifically Unresponsive Recipients (Group II)

Animals*	Tissue type by DLA serotyping‡	MLC reactivity§ Preoperative R + Rx	R + Dx	SI	Postoperative R + Rx	R + Dx	SI	CML reactivity‖ Pre-operative	Post-operative	Graft survival
								%	%	*d*
R-2817	▲7/■4	263	2,550	9.7	859	2,620	3.1	20	28	330+
D-2804	▲7/■2 or 4									
R-2795	●1/▼1	354	1,181	3.3	318	1,307	4.1	6	67	322+
D-2797	●1/▼1									
R-3036	■4/▼X	405	250	0.5	762	1,158	1.5	9	24	270+
D-3035	■4/▼X									
R-3009	●1/▼X	113	468¶	4.1¶	362	620	1.7	40	24/0**	255‡‡
D-3010	●1/▼X									

* R, recipient; D, donor.

‡ Typing performed according to methods in reference 12.

§ R + Rx = autologous, and R + Dx = allogeneic MLC responses as defined in Table I, before (preoperative) and six mo after (postoperative) FDPS allograft transplantation. Stimulation Index (SI) = R + Dx ÷ R + Rx (Table I).

‖ Percent specific ^{51}Cr release from labeled donor PBL incubated as target cells with cytotoxic effector cells prepared by first culturing PBL of the recipient with x irradiated PBL of the donor for 8 d; means for triplicate cultures with <15% variance, before (preoperative) and 6 mo after (postoperative) FDPS allograft transplantation. A positive CML response is ≥5% specific ^{51}Cr release.

¶ Probably not significant.

** First CML response (24%) obtained 3 mo postoperatively, when allograft recipient was healthy; second CML response (0%) obtained at a time (6 mo) when the recipient was sick with pneumonia.

‡‡ Died of pneumonia, normoglycemic.

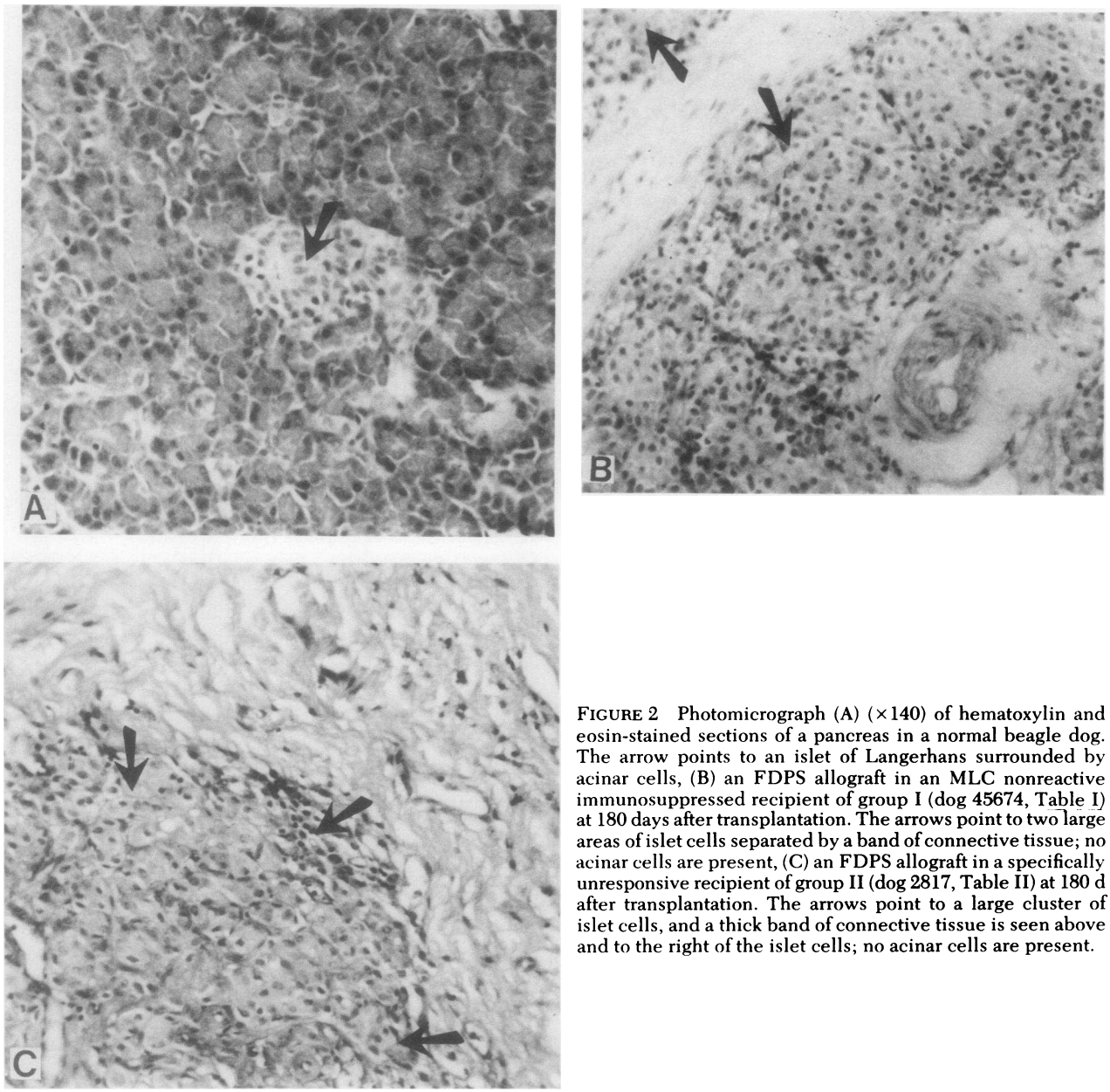

FIGURE 2 Photomicrograph (A) (×140) of hematoxylin and eosin-stained sections of a pancreas in a normal beagle dog. The arrow points to an islet of Langerhans surrounded by acinar cells, (B) an FDPS allograft in an MLC nonreactive immunosuppressed recipient of group I (dog 45674, Table I) at 180 days after transplantation. The arrows point to two large areas of islet cells separated by a band of connective tissue; no acinar cells are present, (C) an FDPS allograft in a specifically unresponsive recipient of group II (dog 2817, Table II) at 180 d after transplantation. The arrows point to a large cluster of islet cells, and a thick band of connective tissue is seen above and to the right of the islet cells; no acinar cells are present.

DISCUSSION

There is increasing evidence that transplantation of the endocrine pancreas may prevent the tissue changes attributable to diabetes mellitus, both in kidney and in other tissues affected by microangiopathy (21, 22). Although allografts of both isolated islet tissue and whole pancreas have been effective in experimental animals (primarily rodents), there are recent studies suggesting that the whole organ pancreas transplant is associated with better graft survival than can be obtained with isolated islet tissue, at least using the present methods of islet isolation and transplantation (23, 24). This may be due to a greater susceptibility to immune damage of isolated islets than of islets situated in the vascularized pancreas. Indeed, we have recently found evidence for tissue specific islet antigens, demonstrable in vitro (25), and the immunogenicity of these islet antigens may be amplified by currently used enzymatic isolation techniques.

Whereas attempts to transplant islets in the human in an allogeneic setting have been routinely unsuccessful (26–29), a technique employing intraperitoneal transplantation of a vascularized, FDPS has been used clin-

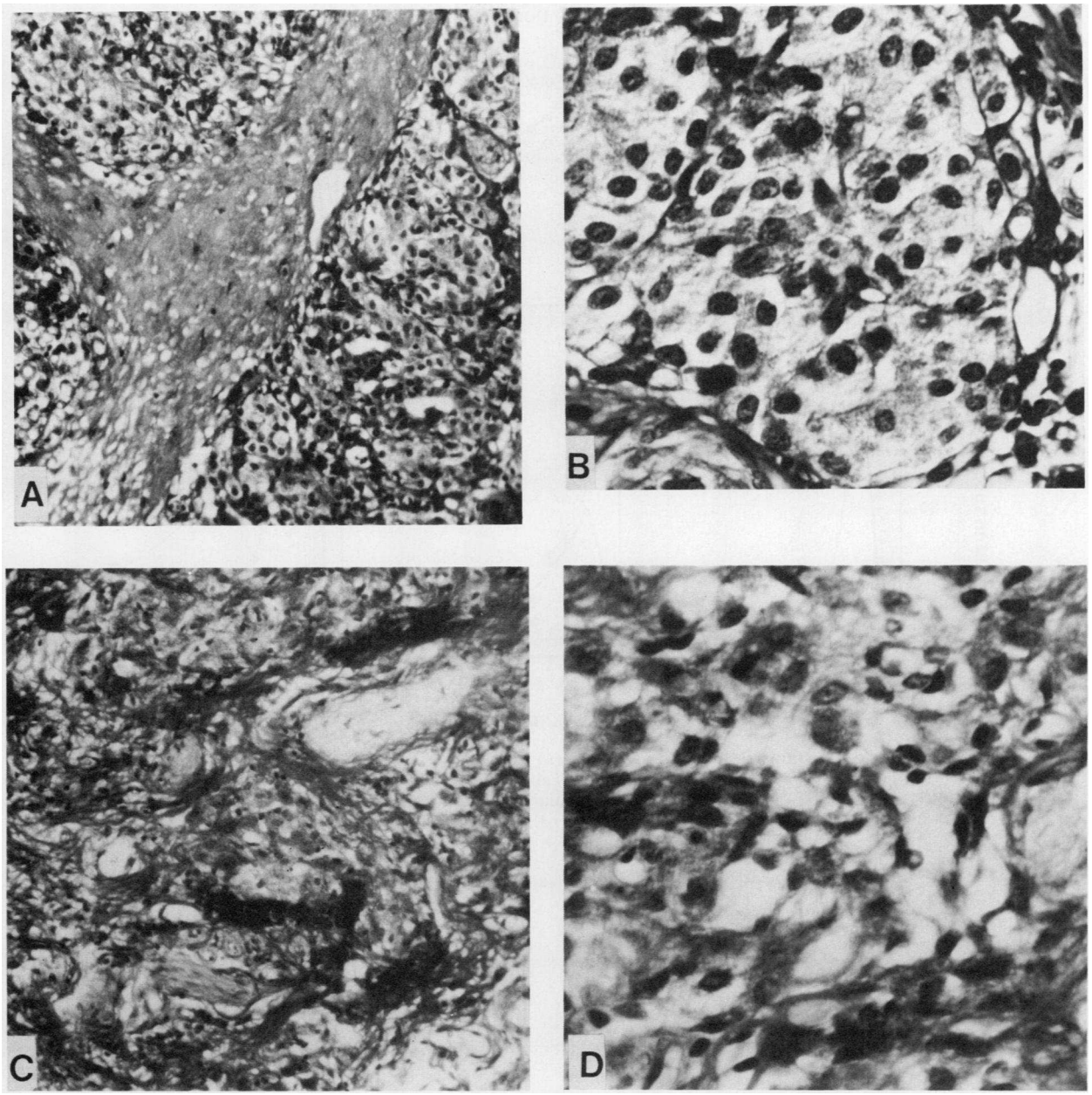

FIGURE 3 Photomicrographs of Masson trichrome stained sections of (A and B) an FDPS allograft in an MLC nonreactive immunosuppressed recipient of group I (dog 45674, Table I) at 180 d after transplantation. (A) Large clusters of islet cells are separated by a connective tissue stroma (×120). (B) Clusters of islet cells with interspersed fine bands of connective tissue staining faintly positive for collagen (×274). (C and D) An FDPS allograft in a specifically unresponsive recipient of group II (dog 2795, Table II) at 180 d after transplantation. (C) Islet cell clusters are separated by numerous bands of connective tissue (×120). (D) Islet cell clusters invaded by thick bands of connective tissue staining strongly positive for collagen (×274).

ically in 13 patients by Sutherland et al. (30, 31), with few technical problems and with encouraging results. We had previously demonstrated, in animal studies, that the FDPS technique unexpectedly was not associated with autoenzymatic tissue damage and that long-term graft survival was possible (7–9). However, a definitive study to examine the immunogenicity of allografts using this technique in experimental animals had not been performed. Therefore, in the present work, we examined various immune mechanisms that might be

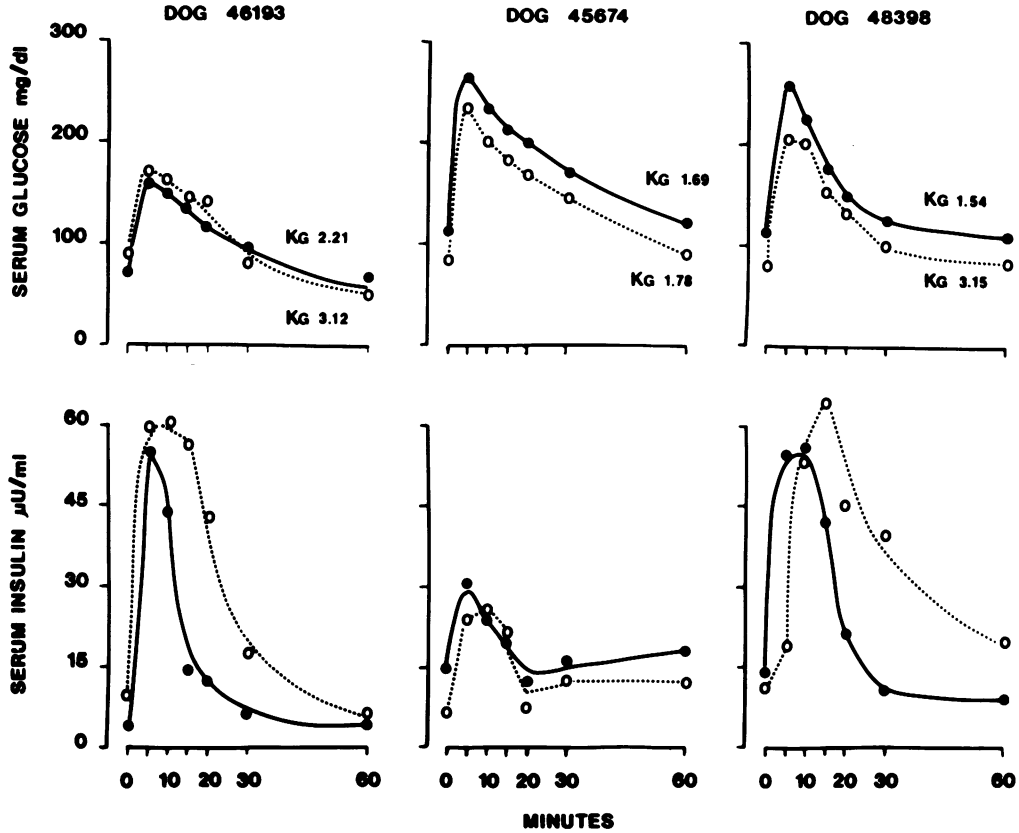

FIGURE 4 Serum glucose and insulin responses to intravenous injection of glucose (0.5 g/kg/body wt) in three MLC nonreactive dogs of group I (Table I) at 74 d (dog 46193), 162 d (dog 45674), and 192 days (dog 48398) after FDPS allograft transplantation (——) are compared to the corresponding responses in the same dogs before (-----) total pancreatectomy and transplantation with a FDPS allograft. K_G, intravenous glucose disappearance rate.

involved in graft rejection in long-term FDPS allografts in beagle dogs with characterized histocompatibilities, with and without immunosuppression.

It appears clear, from the present study, that immunogenetic disparities remain the predominant factors in the outcome of FDPS allograft transplantation. These findings support previous findings (32) of a close relationship between lymphocyte-defined reactivity as assessed in the MLC, and renal graft survival. Furthermore, we demonstrate in this study, that the MLC is a significant determinant of FDPS allograft survival, even between littermate donor-recipient pairs of dogs genotypically identical, at least as determined by presently available reagents for serologically-defined specificities of the major histocompatibility complex.

Although MLC nonreactivity was associated with prolonged FDPS survival, nonetheless, cytotoxic cells, demonstrable by donor-directed CML reactions in vitro, did appear after transplantation. The CML assay, therefore, may have heralded rejection in recipients of MLC nonreactive pairs (Fig. 5a). This finding differs from observations in humans and mice, in that CML reactivity should not be generated in lymphocyte-defined and serologically defined identical pairs, although there are isolated observations to the contrary (33, 34). Also, tight controls for other DLA specificities were not included in the present study. Nevertheless, the results suggest that nonmajor histocompatibility complex-immune determinants could play a more important role in transplant rejection in the canine species. Detection of cytotoxic cells in the transplant recipient may provide a useful immunologic assay to monitor attempts at controlling rejection of FDPS transplants, at least in the dog.

In MLC reactive recipients, MLC reactivity disappeared during FDPS allograft rejection (Fig. 5b), in support of observations in our previous studies (35, 36). In addition, cytotoxic cells also decreased during this period (Fig. 4b). This could not be attributed to immunosuppressive therapy per se, since in the MLC nonreactors, cytotoxic cells actually appeared in the presence of the same therapy (Fig. 5a). Also, we have

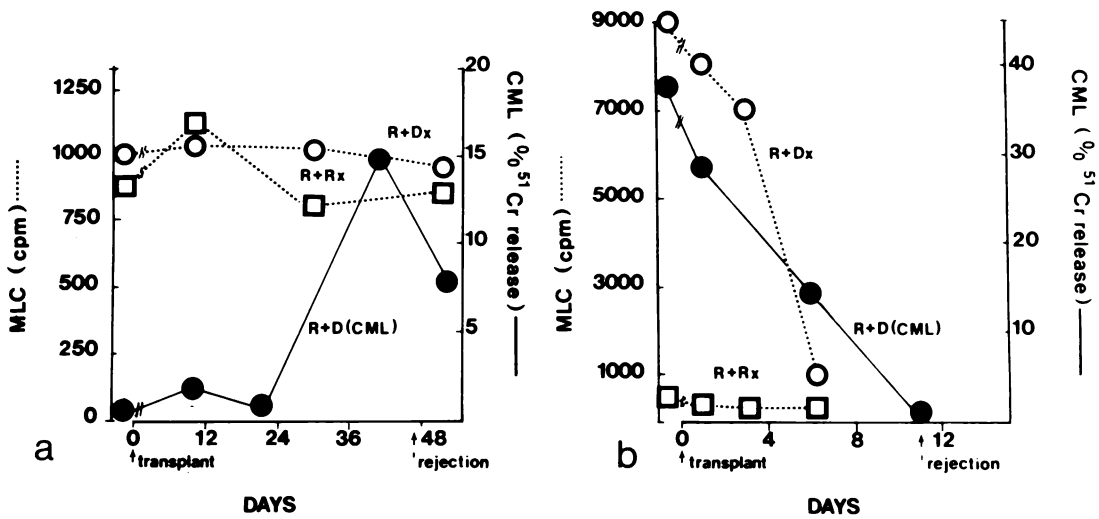

FIGURE 5 Effects of FDPS allograft transplantation in dogs on their MLC and CML responses in vitro. MLC (-----) and CML (——) responses (means for triplicate cultures with <15% variance) in a donor-recipient pair representative of five pairs tested are shown in MLC nonreactive recipients (*a*) and MLC reactive (pretransplant) recipients (*b*) before (within 10 d) transplantation, and at 1–2-wk intervals thereafter. For MLC reactions, PBL of the recipient (R) were cultured with either autologous (Rx) or allogeneic donor (Dx) x irradiated PBL as stimulator cells and lymphoproliferative responses ([^{3}H]thymidine incorporation in counts per minute) are shown at 9 d of culture (peak response). For CML reactions, cytotoxic effector cells were prepared by culturing PBL of the recipient (R) with x irradiated PBL of the donor (D) for 8 d, and these cells were then added to ^{51}Cr-labeled donor PBL as target cells and the percent specific ^{51}Cr release from the donor cells is shown [R + D (CML)].

previously reported decreases in MLC reactivity in MLC reactive recipients in the absence of immunosuppression (35). Among other explanations, entrapment of both MLC and CML reactive lymphocytes might occur simultaneously, as had been postulated during strong rejection reactions in kidney transplant recipients (37). In any event, immunosuppression, in the dosages used, did not alter the development of these processes and vigorous rejection of the FDPS allograft ensued.

An unexpected finding was that the four animals made specifically unresponsive by x irradiation, followed by autologous marrow transplantation, and then kidney transplantation, possessed CML reactivity before FDPS transplants were performed from the original kidney donors (Table II). Also, two of these animals had low-grade MLC reactivity, and this had not been present during the earlier study in these dogs (13). Differences in sensitivity of the techniques might account for the disparate MLC findings between the two studies. The other two "adoptively unresponsive" dogs in group II were MLC nonreactive and exhibited CML reactivity. CML reactivity has not previously been observed in untreated MLC nonreactor animals, either in previous studies in our laboratory, or in animals in group I before transplantation in the present study (Fig. 5a). The findings of CML reactivity (4:4 dogs) and MLC reactivity (2:4 dogs) do not support suppressor cell mechanisms as an explanation for host adaptation to kidney and/or pancreas allografts in the specifically unresponsive animals. Also, since recipient MLC serum blocking factors were not present in this group, enhancement did not appear to be operative as a mechanism of immunoregulation (38). Although not shown here, all four recipients rejected skin allografts from their respective donors at 6 mo, whereas FDPS and renal function continued for more than 9 mo. The specific unresponsiveness was therefore incomplete. Taken together, the findings suggest that mechanisms other than suppressor cells or enhancement may exist for adaptation of the host to tissue allografts, and that tissue specific antigens may be important in graft rejection (13, 39).

A discouraging observation in the present study was that allograft rejection and resumption of the diabetic state appeared to be an "all or none" phenomenon (Fig. 1). Pancreatic grafts biopsied within 24 h after hyperglycemia ensued were, with one exception, completely necrotic. Also, weekly IVGTT with measurements of serum glucose and insulin responses did not forewarn of imminent rejection of the pancreatic allografts. This is in contrast to observations in kidney transplant recipients, in which changes in the serum creatinine concentration permit detection of rejection episodes which can be successfully treated. Generation

of cytotoxic cells may precede pancreatic rejection in low MLC donor-recipient pairs (Fig. 5a), and allograft rejection might potentially be reversible by increasing immunosuppressive therapy at the appropriate times. Also, assays that monitor humoral immunity (40, 41) may detect changes before irreversible rejection of a FDPS allograft has occurred, and these studies are in progress.

An encouraging finding was that when FDPS allografts were accepted in immunosuppressed recipients, intravenous glucose tolerances and insulin secretory responses several months after transplantation were similar to those in the corresponding animal before pancreatectomy (Fig. 4). In agreement with earlier reports of pancreatic transplantation (1, 2) exocrine elements virtually disappeared from the graft, whereas islet tissue survived (Fig. 2b). In our earlier work, we found that administration of steroids in high doses would inhibit fibrosis and prevent inflammatory degeneration of both segmental pancreatic autografts and allografts during the first 30 d (4, 42). This was also found in the immunosuppressed dogs (group I) in the present study (Fig. 3b). In contrast, despite similar loss of exocrine elements in the non-steroid treated dogs (group II) made unresponsive, fibrosis of the FDPS was not avoided (Fig. 3d). Similar long-term morphological findings of a sclerosing reaction have been seen in animals and patients in which neoprene was injected into the pancreatic duct to completely ablate the exocrine elements (6, 9, 43). Although most of these subjects were treated with steroids, the addition of the sclerosing agent may have been enough to allow the fibrotic process to proceed and confuses interpretation of the histologic appearance, since rejection mechanisms are difficult to dissociate from the inflammatory response set up by neoprene. Thus, paradoxically, the use of diabetogenic steroids, which might be predicted to doom pancreatic allotransplantation in diabetic recipients to failure, has not proven deleterious, at least to date in the present long-term experiments in dogs, or in human recipients of vascularized, free-draining pancreatic segmental allografts (31, 32).

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Tissue typing (Table I) performed at Mary Imogene Basset Hospital, Cooperstown, N. Y.

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