ORIGINAL ARTICLE

Lifelong Prevention of Mesangial Enlargement by Whole Pancreas Transplantation in Rats With Diabetes Mellitus

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Background: Mesangial enlargement (ME) is one of the hallmark lesions of diabetic nephropathy and plays a major role in diabetic renal failure. Conventional treatment of type 1 diabetes mellitus with insulin injections, diet, and medications has often failed to prevent development and progression of ME, presumably because of difficulty in achieving tight metabolic control. Although many pancreas transplantations have been done in patients with type 1 diabetes mellitus, there is insufficient information about their influence on ME or other diabetic lesions that are responsible for its morbidity and mortality.

Hypothesis: Whole pancreas transplantation will prevent diabetic ME throughout the life of the rat with alloxan-induced diabetes mellitus.

Design: Mesangial enlargement was studied for 28 months by a highly reproducible quantitative morphologic method in 55 nondiabetic control rats, 57 control rats with alloxan-induced diabetes mellitus, 97 diabetic rats that received a pancreaticoduodenal isograft shortly after the induction of diabetes mellitus, and 126 diabetic rats that received a duct-ligated pancreas isograft shortly after the induction of diabetes mellitus. Mesangial enlargement was determined by measuring the area occupied by camera lucida tracings of the mesangium using an electronic planimeter connected to a computer.

Results: Monthly metabolic studies showed that whole pancreas transplantation maintained very tight metabolic control of diabetes mellitus. Alloxan-induced diabetes mellitus produced progressive accumulation of mesangial matrix and progressive enlargement of all elements of the mesangium during the study. The 2 types of whole pancreas transplants provided lifelong protection against abnormal ME ($P = .006$).

Conclusions: These results, combined with our previous finding of lifelong prevention of abnormal glomerular capillary basement membrane thickening, demonstrate that whole pancreas transplantation performed early in the course of alloxan-induced diabetes mellitus is capable of preventing diabetic kidney lesions. Moreover, these results suggest that whole pancreas transplants might be useful preventive therapy in patients with diabetes mellitus who undergo kidney transplantation for renal failure, in whom recurrence of nephropathy often develops in the transplanted kidney.

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THE MOST important criterion of efficacy of diabetes therapy is the effect of treatment on the widespread complications of type 1 diabetes mellitus (DM) that are responsible for its morbidity and mortality. Diabetic kidney disease is one of the most prominent of these complications; it is the most common cause of death in insulin-dependent (type 1) DM (IDDM) and the leading cause of end-stage renal disease in the United States, accounting for 35% of such patients.[1,2] Mesangial enlargement (ME) is one of the characteristic lesions of diabetic nephropathy.[3-5] Studies in patients with IDDM have shown a strong correlation of ME with the clinical manifestations of diabetic nephropathy, such as albuminuria, hypertension, and decreased glomerular filtration rate.[6-9]

It is difficult, for obvious reasons, to perform long-term studies in humans of the effects of any form of treatment on the kidney lesions of IDDM. Therefore, it is useful to perform such studies in animal models that mimic the human disease.[10] Rats with DM induced by alloxan or streptozotocin, which destroys the beta cells of the islets of Langerhans, develop kidney lesions that are similar to those found in humans with DM, particularly ME and glomerular capillary basement membrane thickening.[11-19] For this reason, the rat with alloxan-induced DM should be a suitable animal model for determining whether therapy can influence diabetic nephropathy.

DESIGN AND METHODS

EXPERIMENTAL GROUPS

Highly inbred Lewis strain rats aged 6 months (Simonsen Laboratories, Gilroy, Calif) and weighing 300 to 350 g were used for this study. These rats uniformly accepted tissue transplants from each other without rejection. The animals were housed 2 to 5 to a cage and fed standard rat chow and water ad libitum throughout the study. The animals were distributed among 4 groups. Group NC (nondiabetic controls) contained 55 unaltered rats. Group DC (diabetic controls) contained 57 untreated diabetic rats in which DM was induced by intravenous injection of alloxan monohydrate (Sigma-Aldrich Chemical Co, St Louis, Mo) at a dosage of 42 to 44 mg/kg. Only rats with plasma glucose levels above 11.1 mmol/L (200 mg/dL) on days 3 and 7 after alloxan injection were accepted into the study. More than half of the rats had plasma glucose levels above 16.7 mmol/L (300 mg/dL). Group PDT (pancreaticoduodenal transplant) contained 97 rats that received a heterotopic pancreaticoduodenal isograft from an age-matched donor rat 1 week after DM was induced by the same method used in group DC. Group DLPT (duct-ligated pancreas transplant) contained 126 rats that received a heterotopic whole pancreas isograft with its exocrine ducts ligated from an age-matched donor rat 1 week after DM was induced by the same method used in group DC.

In the control groups, 2 rats were killed every month; in the transplant groups, 3 to 5 rats were killed every month for 28 months after the onset of diabetes. Complete autopsies were performed and the kidneys were removed for quantitative morphologic studies. The normal life span of the Lewis rat in our laboratory ranges from 24 to 30 months, so that the studies of ME extended over nearly the entire life span of the animals. Animal use protocols were approved by the Animal Care Committee of the University of California, San Diego, and conformed to the standards for animal care and usage of the National Institutes of Health, Bethesda, Md.

TECHNIQUE OF WHOLE PANCREAS TRANSPLANTATION

The microvascular surgical technique of heterotopic whole pancreas transplantation in the rat originated in our laboratory in 1971.[29,30] We developed 2 types of transplants, the pancreaticoduodenal graft and the duct-ligated whole pancreas graft, shown in **Figure 1**. Both grafts were harvested from the donor rat by a similar technique, except that the pancreas for group DLPT was dissected free from the duodenum and its excretory ducts were meticulously ligated and divided at their junction with the bile duct. Both grafts were removed with a segment of aorta containing the celiac and superior mesenteric arteries supplying the graft and a segment of portal vein providing venous drainage of the graft. Both types of grafts were transplanted to the recipient rat by performing end-to-side microvascular anastomoses using continuous 9-0 nylon sutures between the graft aortic segment and the host aorta and between the graft portal vein and the host inferior vena cava. In the pancreaticoduodenal transplantation, the proximal end of the graft duodenum was closed with sutures and the distal end was anastomosed end-to-side to the third portion of the host duodenum using a continuous 7-0 silk suture. The mean ± SD graft ischemia time was 25 ± 5 minutes.

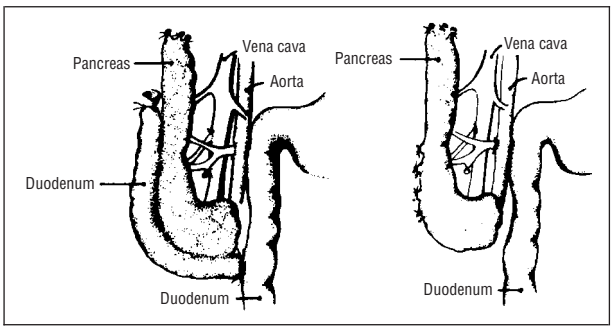

Figure 1. *Microvascular surgical technique of heterotopic pancreaticoduodenal transplantation (left) and duct-ligated whole pancreas transplantation (right), showing the procedures performed on the recipient rats.*

The Diabetes Control and Complications Trial, conducted for almost 10 years in 1441 patients with IDDM, showed that the incidence of clinical nephropathy could be reduced by intensive medical treatment.[20-22] Nevertheless, substantial evidence indicates that conventional therapy of IDDM with insulin injections, diet, and medications often has not prevented nephropathy and the other diabetic complications, presumably because of failure to achieve tight physiologic control of the metabolic abnormalities.[22-25] Interest in various forms of endocrine pancreas replacement therapy, including pancreas transplantation, is based on the hope that such treatment will provide physiologic beta cell function and precise metabolic control. However, it remains to be established that endocrine pancreas replacement therapy can prevent, stabilize, or reverse the lesions of IDDM that occur in the kidney and other organs.

Previous studies in our laboratory showed that whole pancreas transplantation performed early in the course of DM in rats produced lifelong metabolic control[26,27] and prevented abnormal glomerular capillary basement membrane thickening.[28] Because of the important role played by ME in diabetic nephropathy, our present investigation was undertaken to determine by a quantitative morphologic method if whole pancreas transplants could provide lifelong protection against the abnormal expansion of the mesangium that occurs in rats with DM.

RESULTS

METABOLIC STUDIES

Plasma glucose concentrations in the 4 groups of rats over a 2-year period are shown in **Table 1**. Diabetes mellitus in the untreated diabetic control rats (group DC) was associated with plasma glucose concentrations that were

METABOLIC STUDIES

The results of detailed metabolic studies are the subject of a separate report. In all groups, monthly measurements were made of body weight and plasma concentrations of glucose, insulin, and glucagon; glucose tolerance tests were also done monthly. Plasma glucose concentrations were was measured by a modification of the toluidine blue O method, and plasma insulin and glucagon concentrations were measured by specific radioimmunoassays.

QUANTITATIVE MORPHOLOGIC STUDIES OF ME

Kidney specimens obtained at autopsy were minced into 1-mm cubes, fixed in 2.5% glutaraldehyde for 24 hours, and then rinsed in 0.1-mol/L phosphate buffer. The tissue was postfixed in 1% osmium tetroxide, then dehydrated in acetone and embedded in Araldite 502 resin. Sections 0.2 µm thick were cut on an LKB ultratome V and stained with toluidine blue. Quantitative morphologic measurements of the glomerular mesangium were made on camera lucida tracings by Hägg application[13] of the point-counting method of Iidika et al.[31] The sections were examined under a ×10 oil immersion lens of a Leitz-Wetzlar light microscope with a ×12.5 drawing arm attachment, giving a total magnification of ×1250. Camera lucida tracings were made of 5 glomeruli from each kidney. First, the inner surface of Bowman capsule was traced, and then the toluidine blue–positive mesangial areas and the mesangial nuclei were traced (**Figure 2**). Care was taken to differentiate mesangial nuclei from endothelial and epithelial nuclei. Measurements of the camera lucida tracings were performed with an electronic planimeter (Hewlett-Packard digitizer model 9874A; Hewlett-Packard Co, Palo Alto, Calif) equipped with an electrosensitive cursor that was connected to a Hewlett-Packard 9875A calculator-computer that analyzed all measurements. The following measurements were made or automatically calculated: total glomerular area (in square micrometers), determined by circumferentially measuring the inner surface of Bowman capsule; total mesangial area (in square micrometers), determined by measuring the tracing of toluidine blue–positive material; nuclear area (in square micrometers), determined by measuring the area occupied by mesangial nuclei; nuclear-free mesangial area (in square micrometers), calculated by subtracting the nuclear area from the mesangial area (it represented the mesangial matrix and mesangial cell cytoplasm and was called the *absolute mesangial area*); and relative mesangial area, calculated as the percentage of the total glomerular area occupied by the nuclear-free mesangial area.

To eliminate observer bias, the histologic sections were coded during preparation to mask their identification by those making the camera lucida tracings and those performing the morphologic measurements. The code was not broken until the study was completed. To determine the reproducibility of the morphometric method, 5 glomeruli in each of 50 kidney specimens were measured independently by 2 examiners. In addition, 2 examiners repeated their own measurements of 5 glomeruli in each of 50 kidney specimens without knowing the results of their initial measurements. The maximum variation in these 2 tests of reproducibility was 2.3%.

STATISTICAL ANALYSES

A 2-tailed *t* test was used to examine differences between mean values, and linear regression analysis was used to evaluate mesangial changes over time.

3 to 4 times those observed in the nondiabetic controls (group NC) ($P<.001$). Both types of pancreas transplants (groups PDT and DLPT) prevented hyperglycemia throughout the study and maintained tight metabolic control. Plasma glucose levels in the transplanted rats were not significantly different from those in the nondiabetic controls at any time. Plasma insulin concentrations were markedly depressed in the untreated diabetic controls, but were within the normal range in the rats with pancreas transplants of both types.

QUANTITATIVE MORPHOLOGIC STUDIES OF ME

Total Glomerular Area

Table 2 shows the results of measurements of total glomerular area during 28 months of study in the 4 groups of rats. In group NC, total glomerular area increased slightly with time. Total glomerular area was significantly larger in group DC than in group NC at all intervals ($P<.01$), and it increased moderately with time. Pancreas transplants of both types prevented abnormal enlargement of the total glomerular area. Measurements in groups PDT and DLPT were not significantly different from each other or from those in group NC.

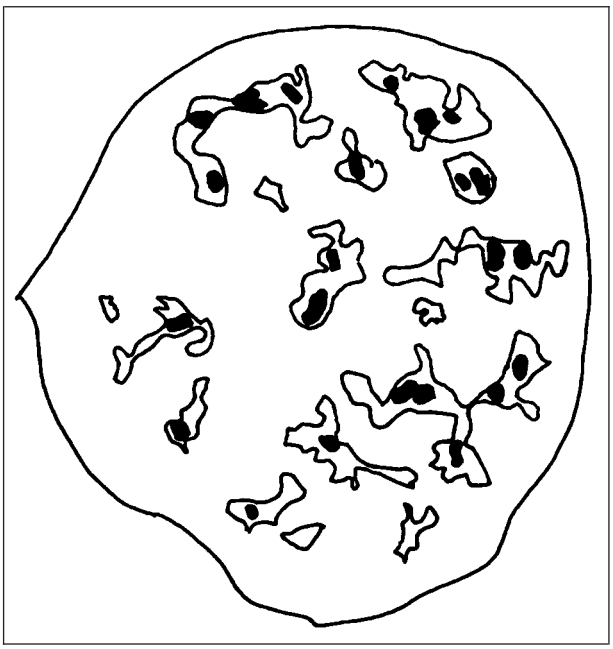

Figure 2. *Camera lucida tracing of a kidney glomerulus (original magnification, ×1250). The mesangium appears as islands containing solid areas, which are the mesangial nuclei. The inner surface of Bowman capsule is the circle surrounding the islands.*

Table 1. Plasma Glucose Concentrations in Nondiabetic Control Rats, Diabetic Control Rats, Diabetic Rats That Received Pancreaticoduodenal Transplants, and Diabetic Rats That Received Duct-Ligated Pancreas Transplants*

	Time After							
	1-3 mo		4-6 mo		7-9 mo		10-12 mo	
Group	No.	Glucose, mmol/L (mg/dL)	No.	Glucose, mmol/L (mg/dL)	No.	Glucose, mmol/L (mg/dL)	No.	Glucose, mmol/L (mg/dL)
Nondiabetic controls	116	4.8 ± 0.09 (87 ± 1.6)	105	6.6 ± 0.12 (119 ± 2.2)	98	4.8 ± 0.06 (87 ± 1.1)	113	4.6 ± 0.04 (82 ± 0.7)
Diabetic controls†	272	15.9 ± 0.3 (288 ± 6.0)	245	16.9 ± 0.33 (305 ± 5.9)	198	16.5 ± 0.26 (297 ± 4.6)	156	16.8 ± 0.28 (303 ± 5.1)
Pancreaticoduodenal transplant	171	4.77 ± 0.06 (86 ± 1.1)	154	5.11 ± 0.09 (92 ± 1.6)	129	4.9 ± 0.07 (89 ± 1.3)	106	4.9 ± 0.07 (89 ± 1.3)
Duct-ligated pancreas transplant	166	4.7 ± 0.07 (85 ± 1.3)	127	5.11 ± 0.11 (92 ± 1.9)	100	4.8 ± 0.1 (87 ± 1.8)	93	4.8 ± 0.08 (87 ± 1.5)

*Values are mean ± SEM.
†P<.001 compared with nondiabetic controls.

Table 2. Total Glomerular Area in Nondiabetic Control Rats, Diabetic Control Rats, Diabetic Rats That Received Pancreaticoduodenal Transplants, and Diabetic Rats That Received Duct-Ligated Pancreas Transplants*

	Total Glomerular Area by Time			
Group	1-3 mo	4-6 mo	7-9 mo	10-12 mo
Nondiabetic controls	9140 ± 433 (6)	9924 ± 510 (8)	10 770 ± 872 (5)	10 710 ± 1015 (5)
Diabetic controls	13 241 ± 1650 (4)†	11 730 ± 355 (4)†	12 126 ± 756 (9)†	14 225 ± 405 (11)†
Pancreaticoduodenal transplant	9250 ± 522 (13)	9780 ± 514 (12)	10 564 ± 723 (15)	10 811 ± 814 (15)
Duct-ligated pancreas transplant	9112 ± 487 (15)	9823 ± 493 (15)	10 497 ± 688 (20)	11 040 ± 734 (16)

*Values are mean ± SEM (number of rats).
†P<.01 compared with nondiabetic controls.

Table 3. Nuclear Area in Nondiabetic Control Rats, Diabetic Control Rats, Diabetic Rats That Received Pancreaticoduodenal Transplants, and Diabetic Rats That Received Duct-Ligated Pancreas Transplants*

	Nuclear Area by Time After Entry				
Group	1-3 mo	4-6 mo	7-9 mo	10-12 mo	13-15 mo
Nondiabetic controls	144 ± 14 (6)	183 ± 37 (8)	195 ± 24 (5)	196 ± 40 (5)	290 ± 34 (6)
Diabetic controls	268 ± 44 (4)†	229 ± 25 (4)	205 ± 18 (9)	294 ± 37 (11)†	368 ± 51 (5)†
Pancreaticoduodenal transplant	142 ± 16 (13)	165 ± 22 (12)	180 ± 18 (15)	189 ± 21 (15)	204 ± 27 (14)
Duct-ligated pancreas transplant	146 ± 15 (15)	169 ± 19 (15)	182 ± 15 (20)	190 ± 22 (16)	199 ± 18 (14)

*Values are mean ± SEM (number of rats).
†P<.01 compared with nondiabetic controls.

Table 4. Total Mesangial Area in Nondiabetic Controls Rats, Diabetic Control Rats, Diabetic Rats That Received Pancreaticoduodenal Transplants, and Diabetic Rats That Received Duct-Ligated Pancreas Transplants*

	Total Mesangial Area by Time After Entry					
Group	1-3 mo	4-6 mo	7-9 mo	10-12 mo	13-15 mo	16-18 mo
Nondiabetic controls	952 ± 102 (6)	1046 ± 185 (8)	1262 ± 75 (5)	1155 ± 189 (5)	1471 ± 109 (6)	1082 ± 126 (7)
Diabetic controls	1716 ± 151 (4)†	1470 ± 226 (4)†	1554 ± 197 (9)†	2027 ± 127 (11)†	2544 ± 359 (5)†	2109 ± 81 (7)†
Pancreaticoduodenal transplant	936 ± 91 (13)	1031 ± 89 (12)	1148 ± 82 (15)	1201 ± 108 (15)	1284 ± 143 (14)	1292 ± 117 (12)
Duct-ligated pancreas transplant	941 ± 84 (15)	998 ± 100 (15)	1184 ± 90 (20)	1211 ± 119 (16)	1263 ± 127 (14)	1302 ± 142 (14)

*Values are mean ± SEM (number of rats).
†P<.01 compared with nondiabetic controls.

Entry Into Study								
13-15 mo		16-18 mo		19-21 mo		22-24 mo	
No.	Glucose, mmol/L (mg/dL)	No.	Glucose, mmol/L (mg/dL)	No.	Glucose, mmol/L (mg/dL)	No.	Glucose, mmol/L (mg/dL)
106	4.7 ± 0.07 (85 ± 1.3)	91	4.61 ± 0.06 (83 ± 1.1)	76	4.7 ± 0.07 (85 ± 1.2)	42	2.3 ± 0.09 (98 ± 1.6)
134	16.4 ± 0.29 (296 ± 5.3)	84	14.9 ± 0.48 (270 ± 8.6)	43	16.6 ± 0.83 (299 ± 14.9)	28	14.7 ± 0.35 (264 ± 6.3)
81	4.9 ± 0.12 (89 ± 2.1)	41	4.9 ± 0.14 (89 ± 2.6)	12	5.8 ± 0.69 (105 ± 12.4)	9	5.11 ± 0.33 (92 ± 6.0)
68	4.7 ± 0.11 (85 ± 2.0)	37	4.61 ± 0.16 (83 ± 2.9)	20	6.3 ± 0.43 (113 ± 7.7)	8	5.3 ± 0.5 (96 ± 9.0)

After Entry Into Study, μm²				
13-15 mo	16-18 mo	19-21 mo	22-24 mo	25-28 mo
13 389 ± 927 (6)	11 689 ± 664 (7)	13 497 ± 663 (9)	12 090 ± 804 (3)	11 603 ± 1216 (6)
16 373 ± 1665 (5)†	12 878 ± 781 (7)†	13 725 ± 777 (7)†	14 573 ± 571 (5)†	16 610 ± 853 (5)†
12 889 ± 822 (14)	11 914 ± 763 (12)	12 994 ± 704 (5)	12 447 ± 639 (5)	12 082 ± 821 (6)
13 011 ± 693 (14)	12 119 ± 973 (14)	13 166 ± 817 (12)	12 280 ± 777 (12)	11 841 ± 884 (8)

Into Study, μm²			
16-18 mo	19-21 mo	22-24 mo	25-28 mo
175 ± 23 (7)	250 ± 41 (9)	190 ± 38 (3)	250 ± 53 (6)
304 ± 40 (7)†	375 ± 28 (7)†	346 ± 87 (5)†	518 ± 78 (5)†
203 ± 17 (12)	217 ± 23 (5)	208 ± 20 (5)	216 ± 31 (6)
195 ± 19 (14)	211 ± 15 (12)	210 ± 17 (12)	219 ± 29 (8)

Into Study, μm²		
19-21 mo	22-24 mo	25-28 mo
1559 ± 157 (9)	1248 ± 257 (3)	1585 ± 305 (6)
2621 ± 299 (7)	2300 ± 299 (5)†	3114 ± 303 (5)†
1376 ± 128 (5)	1393 ± 119 (5)	1489 ± 137 (6)
1394 ± 152 (12)	1407 ± 134 (12)	1485 ± 141 (8)

Nuclear Area

Measurements of the nuclear area of the mesangium in the 4 groups of rats over a period of 28 months are shown in **Table 3**. Group DC had a significantly larger nuclear area than the other 3 groups at almost all intervals ($P<.01$), and the nuclear area increased moderately with duration of DM. The transplanted rats were not significantly different from group NC. Results following the 2 types of transplantations were similar.

Total Mesangial Area

Table 4 shows the total mesangial area, containing the mesangial matrix and cell cytoplasm as well as the mesangial nuclei, in the 4 groups of rats during the 28 months after entry into the study. Total mesangial area more than doubled during the course of the study in group DC rats and at all intervals was significantly larger than in group NC ($P<.01$). Both types of pancreas transplants prevented the abnormal increase of total mesangial area. Measurements in groups PDT and DLPT were similar to each other and to those in group NC.

Nuclear-Free Mesangial Area (Absolute Mesangial Area)

The most meaningful indices of ME are the nuclear-free mesangial area, also known as the absolute mesangial area,

Table 5. Nuclear-Free Mesangial Area (Absolute Mesangial Area) in Nondiabetic Control Rats, Diabetic Control Rats, Diabetic Rats That Received Pancreaticoduodenal Transplants, and Diabetic Rats That Received Duct-Ligated Pancreas Transplants*

	Nuclear-Free Mesangial Area by Time After Entry					
Group	1-3 mo	4-6 mo	7-9 mo	10-12 mo	13-15 mo	16-18 mo
Nondiabetic controls	809 ± 91 (6)	863 ± 152 (8)	1067 ± 63 (5)	959 ± 149 (5)	1182 ± 87 (6)	907 ± 104 (7)
Diabetic controls†	1448 ± 151 (4)	1470 ± 226 (4)	1350 ± 183 (9)	1733 ± 101 (11)	2176 ± 323 (5)	1806 ± 151 (7)
Pancreaticoduodenal transplant	794 ± 81 (13)	866 ± 71 (12)	968 ± 67 (15)	1012 ± 88 (15)	1081 ± 121 (14)	1089 ± 103 (12)
Duct-ligated pancreas transplant	795 ± 79 (15)	829 ± 83 (15)	1002 ± 76 (20)	1021 ± 100 (16)	1065 ± 110 (14)	1107 ± 123 (14)

*Values are mean ± SEM (number of rats).
†P<.01 compared with nondiabetic controls.

Table 6. Percentage of Total Glomerular Area Occupied by Nuclear-Free Mesangial Area (Relative Mesangial Area) in Nondiabetic Control Rats, Diabetic Control Rats, Diabetic Rats That Received Pancreaticoduodenal Transplants, and Diabetic Rats That Received Duct-Ligated Pancreas Transplants*

	Relative Mesangial Area by Time After Entry					
Group	1-3 mo	4-6 mo	7-9 mo	10-12 mo	13-15 mo	16-18 mo
Nondiabetic controls	8.6 ± 0.7 (6)	8.5 ± 1.3 (8)	10.0 ± 0.7 (5)	9.3 ± 1.2 (5)	8.9 ± 1.2 (6)	7.9 ± 1.0 (7)
Diabetic controls	10.9 ± 1.3 (4)	10.5 ± 1.6 (4)†	10.6 ± 0.9 (9)†	12.0 ± 0.7 (11)†	13.1 ± 2.1 (5)†	14.0 ± 0.8 (7)†
Pancreaticoduodenal transplant	8.5 ± 0.6 (13)	8.4 ± 0.9 (12)	9.3 ± 0.9 (15)	9.4 ± 1.0 (15)	8.5 ± 0.8 (14)	8.8 ± 0.7 (12)
Duct-ligated pancreas transplant	8.7 ± 0.7 (15)	8.4 ± 1.0 (15)	9.4 ± 0.8 (20)	9.2 ± 0.9 (16)	8.3 ± 0.6 (14)	9.0 ± 0.9 (14)

*Values are mean ± SEM (number of rats).
†P<.01 compared with nondiabetic controls.

and the relative mesangial area, both of which reflect deposition of mesangial matrix and cell cytoplasm. **Table 5** shows the absolute mesangial area over the 28-month period in the 4 groups of rats. In group DC, there was more than a 200% increase in absolute mesangial area during 28 months, and the mesangium was significantly larger at every interval than in group NC ($P<.01$). Absolute mesangial area increased only about 65% over 28 months in group NC. Whole pancreas transplantation prevented the accelerated ME seen in group DC rats and resulted in an absolute mesangial area similar to that found in group NC. Measurements in groups PDT and DLPT were about the same.

Relative Mesangial Area

The percentage of total glomerular area occupied by the nuclear-free mesangial area, also known as the relative mesangial area, in the 4 groups of rats is shown in **Table 6** and **Figure 3**. Over the course of 28 months, the mesangium enlarged from 8.6% to 10.7% of the glomerulus in group NC but to almost 16% of the glomerulus in group DC rats. At every interval except one, the relative mesangial area was significantly larger in group DC than in group NC ($P<.01$). The 2 types of pancreas transplants were equally effective in preventing mesangial expansion and produced measurements that were similar to those in group NC rats.

Figure 4 shows the rate of ME determined by regression analysis. It is obvious that group DC had a markedly accelerated rate of mesangial growth compared with the other 3 groups ($P<.001$) and that whole pancreas transplantation of both types normalized the rate of ME.

COMMENT

Mesangial enlargement is one of the most important lesions of diabetic nephropathy. Diabetic glomerulosclerosis is characterized by proliferation of basement membrane–like material within the mesangial matrix, a process that has been shown to begin during the first 2 years of clinical DM.[4,32] As DM progresses, the area of the glomerulus occupied by the mesangium increases,[31-34] and the mesangium insinuates itself between the endothelium and epithelium of the peripheral portions of the glomerular tuft.[35] It is this enlarging mesangium that is responsible for the histologic characteristics of both diffuse and nodular glomerulosclerosis associated with established diabetic nephropathy. Mesangial enlargement and glomerular capillary basement membrane thickening are the hallmark lesions of kidney disease in IDDM.

Rats with DM induced by destruction of beta cells with alloxan or streptozotocin develop ME and other renal lesions similar to those that occur in human diabetic nephropathy.[11-13,17,18] Biochemical studies have demonstrated increased synthesis of glomerular basement membrane material in experimental DM.[36-38] The increase in mesangial matrix is slowly progressive and ultimately leads to obliteration of the glomerular capillary spaces. Aging normal rats develop some of these same renal abnormalities, but we have found that they are much more severe in age-matched diabetic rats.[39]

Into Study, μm²		
19-21 mo	22-24 mo	25-28 mo
1309 ± 131 (9)	1059 ± 244 (3)	1335 ± 253 (6)
2246 ± 277 (7)	1954 ± 217 (5)	2796 ± 227 (5)
1159 ± 109 (5)	1185 ± 103 (5)	1273 ± 110 (6)
1183 ± 140 (12)	1197 ± 121 (12)	1266 ± 114 (8)

Into Study, %		
19-21 mo	22-24 mo	25-28 mo
9.6 ± 1.0 (9)	8.9 ± 1.7 (3)	10.7 ± 1.4 (6)
15.8 ± 1.2 (7)†	13.3 ± 1.5 (5)†	15.2 ± 1.1 (5)†
9.0 ± 0.8 (5)	9.5 ± 1.1 (5)	10.4 ± 1.0 (6)
9.1 ± 0.7 (12)	9.6 ± 1.0 (12)	10.7 ± 0.9 (8)

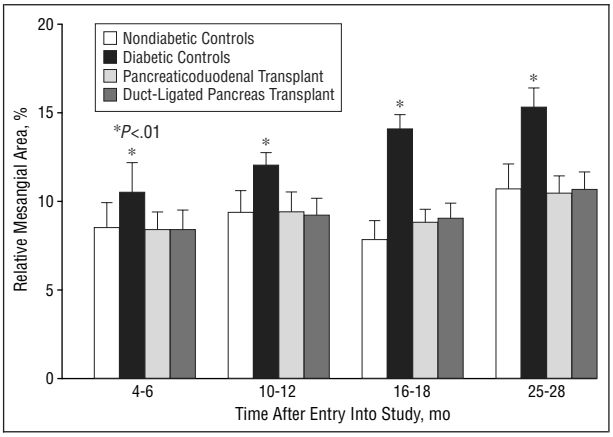

Figure 3. *Relative mesangial areas in the 4 groups of rats at several intervals after the start of the study. T-shaped bars indicate SEs.* P *values are for comparison with other groups.*

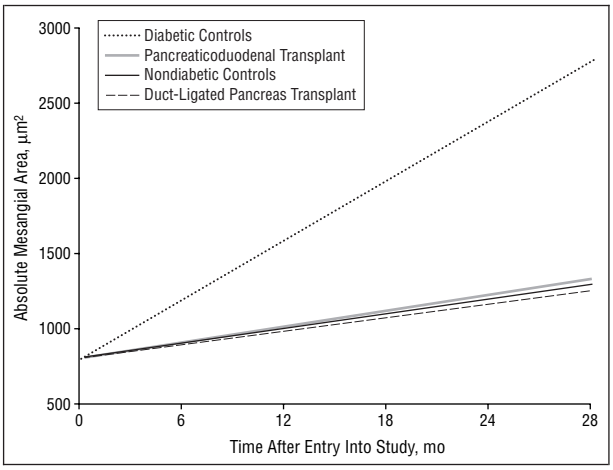

Figure 4. *Regression analysis showing the rate of increase of absolute mesangial area by time after entry into study in the 4 groups of rats. The slope for the diabetic controls is significantly different from the slopes in the other 3 groups (*P*<.001).*

The importance of ME in human diabetic nephropathy has been demonstrated in studies of structural-functional relationships in patients with IDDM.[6-9] Morphometric studies of renal biopsy specimens have shown a significant correlation between mesangial expansion and the clinical manifestations of diabetic nephropathy, such as albuminuria, hypertension, and decreased glomerular filtration rate. On the other hand, glomerular basement membrane thickening, which some authors believe is the most important lesion in diabetic renal disease,[40] has related poorly or not at all to the clinical manifestations of nephropathy.

Previously reported studies showed that whole pancreas transplantation performed shortly after the induction of diabetes with alloxan in rats produced good health, normal growth and weight gain, and lifelong normalization of the metabolic abnormalities of DM.[27] Severe DM invariably recurred when the pancreas graft was removed. Furthermore, in an electron microscopic study using a morphometric technique of measurement, we found that whole pancreas transplants prevented abnormal glomerular basement membrane thickening for the full natural life span of the rats.[28] The current study was undertaken to determine, by an accurate, quantitative method of morphometry, if whole pancreas transplantation is also capable of preventing ME, a lesion that some believe is the main cause of diabetic renal failure.

The results of the present study demonstrate convincingly that whole pancreas transplantation performed early in the course of DM was capable of preventing ME for life. Induction of DM with alloxan produced a progressive increase in the size of the glomerulus and progressive enlargement of all elements of the mesangium over a period of 28 months. The indices of mesangial matrix deposition showed the greatest increases. Total mesangial area and nuclear-free mesangial area more than doubled and the proportion of the glomerular area occupied by the mesangium (relative mesangial area) almost doubled in group DC. Whole pancreas transplantation maintained the size of all of the mesangial elements at the levels of group NC throughout life. In groups PDT and DLPT, transplants were equally effective in preventing abnormal ME. Both types of whole pancreas transplants maintained normal plasma levels of glucose and insulin throughout the 28 months of study, so that prevention of ME took place against a background of very tight metabolic control. Although there are other techniques of measuring ME,[4-9] the method used in this study is well established in our laboratory and has proved to be highly reproducible.

Many experimental studies directed at determining whether endocrine pancreas replacement therapy can prevent diabetic nephropathy have been reported.[41-48] Most

of these studies used isolated islet transplants, which often do not eliminate the metabolic disorders of diabetes and therefore may not provide a valid test of the capabilities of pancreas transplantation.[25] Moreover, many of the studies did not use quantitative morphologic methods or conduct a morphometric assessment of ME. In a recent well-conducted study, Leow et al[48] reported that transplantation of 3000 pancreatic islets in diabetic rats prevented glomerular basement membrane thickening for life, confirming our previous results with whole pancreas transplants. Leow et al did not study ME.

Although more than 7000 pancreas transplantations have been done in patients with diabetic nephropathy, insufficient information has been obtained to answer the critical questions regarding prevention and reversal of ME in the native kidney or in a kidney transplant.[49] Bilous et al[50] showed that pancreas transplantation in patients with IDDM prevented or slowed the progression of ME in kidneys transplanted 1.0 to 7.2 years previously. Similarly, Wilczek et al,[51] comparing simultaneous pancreas and kidney transplantations with kidney transplants alone in patients with IDDM, observed that ME and glomerular basement membrane thickening were prevented or reduced by pancreas transplantation. On the other hand, Fioretto et al[52] and Esmatjes et al[53] reported that pancreas transplantation failed to reverse established glomerular lesions in the native kidneys of patients with IDDM. The findings of Fioretto et al have been contested by Ferguson et al.[54]

Many studies have shown that conventional treatment of IDDM with injections of insulin, diet, and medications often has not prevented the development or progression of nephropathy.[23-25] Transplantation of a normal kidney into patients with end-stage diabetic renal disease caused by IDDM is often followed by recurrence of nephropathy in the transplanted kidney, despite attempts at metabolic control with conventional diabetes therapy.[55-59] If pancreas transplants are capable of preventing diabetic nephropathy, they should be helpful to patients with DM who require simultaneous kidney transplantation, a circumstance in which most pancreas transplantations are performed today. Furthermore, pancreas transplantation might prove useful clinically as a preventive measure on a wider scale if it becomes possible to predict the development of severe renal disease early in its course in patients with IDDM. Our previous demonstration of lifelong prevention of abnormal glomerular capillary basement membrane thickening and our current finding of lifelong prevention of ME suggest that whole pancreas transplantation is likely to prevent diabetic nephropathy in humans with IDDM.

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