

RENAL TRANSPLANTATION IN DIABETES MELLITUS IN RATS

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The deposition of immunoglobulins and complement in the glomerular mesangium is a striking feature of the progressive glomerulosclerosis which accompanies chronic experimental diabetes mellitus in the rat (1). The importance of this deposition in the pathogenesis of the diabetic glomerular lesions in these animals is uncertain. It is known that one function of the mesangium is the uptake and processing of a variety of circulating macromolecules (2). It has been theorized that the immunohistochemical alterations noted above represent a significant alteration in this mesangial activity (1). In previous studies it was demonstrated that achievement of long-lasting normoglycemia in diabetic rats by pancreatic tissue transplantation results in almost complete disappearance of the glomerular immunohistopathology and arrest or reversal of the mesangial sclerosis seen by light microscopy.¹ These experiments suggested that cure of the diabetic state allows for recovery of the mesangial lesions.

The studies reported herein confirm and amplify these findings. We demonstrated that diabetic glomerular changes developed in normal kidneys transplanted into diabetic rats. Conversely, we established that diabetic glomerular changes are reversible upon transplantation of kidneys from diabetic rats into normal recipients.

Materials and Methods

These studies were carried out in highly inbred Lewis rats (Microbiological Associates, Inc., Bethesda, Md.) weighing approximately 100 g at the start of the experiment. Experimental animals were made diabetic, following an 18 h fast, by the injection of 65 mg/kg of streptozotocin, intravenously, and 2 ml of a 30% glucose and water solution, intraperitoneally. Nondiabetic animals were age-matched litter mates. Induction of the diabetic state was confirmed by the development of persistent heavy glycosuria by Tes-Tape (Eli Lilly & Co., Indianapolis, Ind.). Diabetic and nondiabetic animals were maintained on ad libitum water and Purina rat chow (Ralston Purina Co., St. Louis, Mo.).

Approximately 6 mo after the induction of diabetes the following experiments were per-

¹ Mauer, S. M., D. E. R. Sutherland, M. W. Steffes, R. J. Leonard, J. S. Najarian, A. F. Michael, and D. M. Brown. 1973. Pancreatic transplantation: effects on the glomerular lesions of experimental diabetes in the rat. Manuscript submitted for publication.

formed: Kidneys were removed from diabetic rats and transplanted into nondiabetic recipients; kidneys were removed from nondiabetic animals and transplanted into diabetic recipients; kidneys were removed from nondiabetic animals and transplanted into nondiabetic recipients.

Renal transplantation was performed under ether anesthesia using microsurgical techniques similar to those previously described (3, 4). Briefly, following in situ perfusion of the donor kidney with cold Ringer's lactate solution containing 100 U heparin/ml, the donor kidney and ureter were removed complete with aortic, vena cava, and bladder cuffs. In the recipient rat one kidney was removed. The donor kidney was transplanted with aortic and caval end to side anastomoses using 8-0 nylon and 7-0 silk suture materials, respectively. Ischemia time was approximately 20-30 min. The donor bladder cuff was sutured to the recipient's bladder with 6-0 silk.

Renal biopsies were obtained at the time of transplantation from the donor's nontransplanted kidney and from the recipient's removed kidney. Further, biopsies of both the donor kidney and the recipient's remaining kidney were performed at 2 and 4 mo after the transplantation procedure. Part of each renal biopsy was placed in formalin for paraffin embedding and subsequent staining with hematoxylin and eosin and periodic acid-Schiff (PAS). The rest of the biopsy was frozen in isopentane, precooled in liquid nitrogen, sectioned at 4 μ m in a Lipshaw cryostat (Lipshaw Mfg. Co., Greenville, S. C.) and processed for immunofluorescent microscopy with appropriate controls using fluorescein isothiocyanate-labeled rabbit antisera directed, respectively, against rat IgG, IgM, and β_1 C. These antisera were prepared as previously described (reference 1 and footnote 1). The amount of immunofluorescence was arbitrarily graded as negative, trace, 1+, 2+, and 3+.

RESULTS

In 11 of 21 attempts successful renal transplantation of kidneys from diabetic donors into normal recipients was achieved with follow-up biopsies available from donor and recipient kidneys at 2 and usually 4 mo after transplantation. However, diabetic animals withstood major surgical procedures not as well as normal rats. Only 8 of 41 attempts were successful in the transplantation of normal kidneys into diabetic recipients. Six successful transplants of normal kidneys into normal rats were performed.

Light Microscopy.—Glomeruli in the renal biopsies from animals diabetic for approximately 6 mo demonstrated slight widening of the mesangium with PAS-positive mesangial matrix material (Fig. 1 A). This mesangial thickening extended to surround the intraglomerular portion of the arterioles resulting in mild thickening of the walls and narrowing of the lumina of these vessels at the hilus of the glomerulus. In most biopsies, nodular PAS-positive deposits were seen. These hyaline, glassy masses of variable size and shape were found in the mesangium, around capillary loops, or along the parietal layer of Bowman's membrane. Cortical tubular dilatation and cyst formation were common, and focal areas of tubular atrophy associated with minimal interstitial fibrosis and infiltration with round cells were occasionally noted. In all these biopsies vacuolated tubular epithelial cells containing PAS-positive cytoplasmic granules were noted. Biopsies from kidneys of nondiabetic animals were normal by light microscopy at this time.

The following observations were made in kidneys transplanted from diabetic rats into normal recipients when biopsied 2 mo later: In six kidneys glomeruli were unchanged from the pretransplantation biopsy. However, in five kidneys

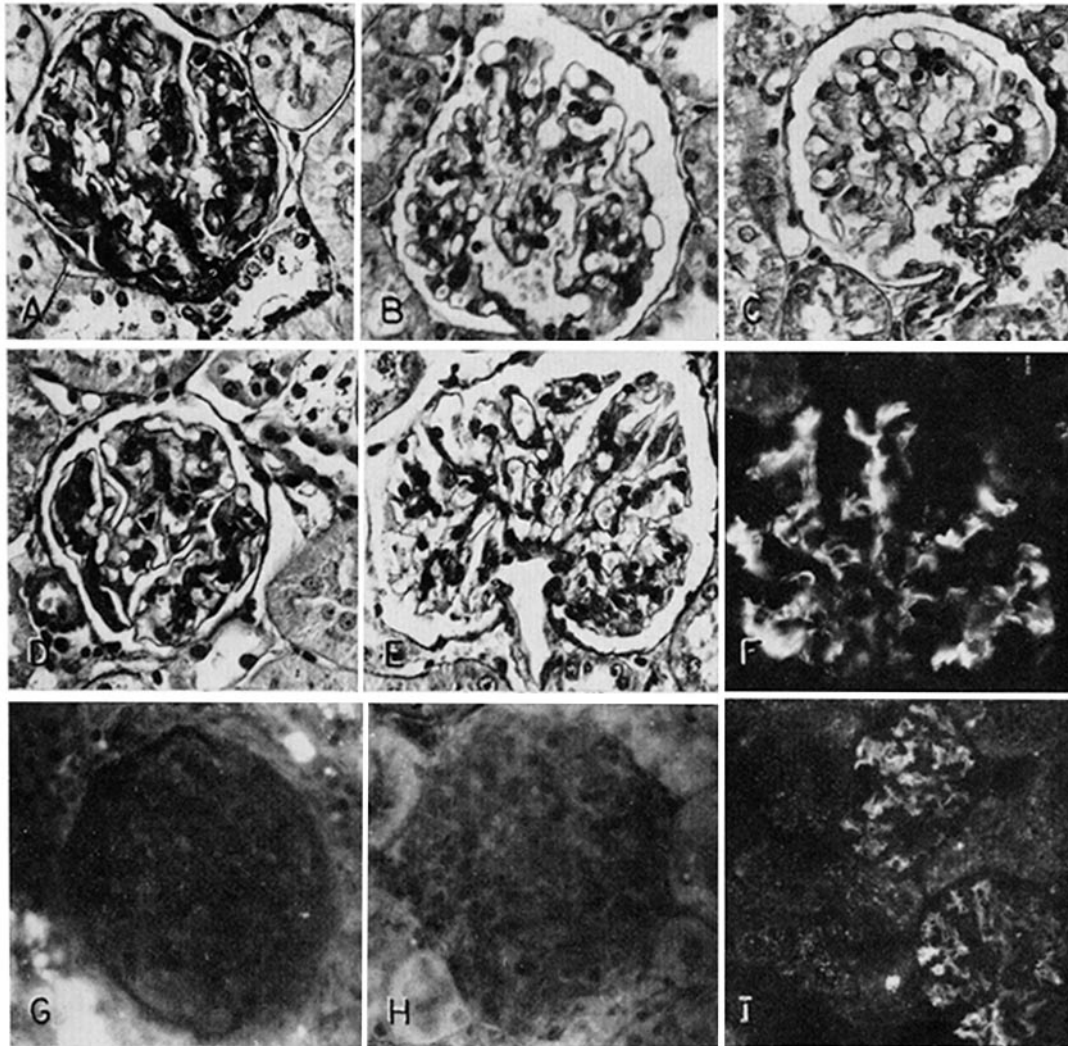


FIG. 1. (A) Glomerulus from a rat diabetic for 6 mo before transplantation into a normal recipient, demonstrating increased mesangial matrix material (PAS). (B) Glomerulus from kidney of the same rat as in Fig. 1 A 2 mo after transplantation demonstrating a decrease in mesangial matrix material (PAS). (C) Glomerulus from a normal rat before transplantation of kidney into a diabetic recipient (PAS). (D) Glomerulus from kidney of the same rat as in Fig. 1 C 2 mo after transplantation, demonstrating increased mesangial matrix material (PAS). (E) Glomerulus from a kidney of a normal rat 2 mo after transplantation into a normal recipient (PAS). (F) Glomerulus from a rat diabetic for 6 mo before transplantation into a normal recipient, demonstrating intense (3+) mesangial staining for rat IgG. (G) Glomerulus from kidney of the same rat as in Fig. 1 F, demonstrating complete disappearance of rat IgG from the mesangium. (H) Glomerulus from a normal rat before transplantation of kidney into a diabetic recipient stained for rat IgG. (I) Glomeruli from kidney of same rat as in Fig. 1 H 2 mo after transplantation, demonstrating rat IgG (2+) in the mesangium.

glomeruli demonstrated a significant decrease in mesangial matrix thickening when compared with biopsies from the same kidney obtained before transplantation (Fig. 1 B). In several of these transplanted kidneys areas of interstitial fibrosis, round cell infiltration, and tubular atrophy were prominent. However, in areas where these changes were absent the tubules appeared completely normal. Biopsies of the recipient rats' own kidneys were normal at this time.

The following observations were made in kidneys transplanted from normal animals into diabetic recipients when biopsied 2 mo later: In five of eight instances glomeruli demonstrated a slight but definite increase in mesangial matrix and hilar expansion with thickening of the walls of arterioles (Figs. 1 C and D). In three animals glomeruli of the donor kidney were unchanged from pretransplantation biopsies. Areas of interstitial fibrosis, round cell infiltration, and tubular atrophy were present in four biopsies. However, in areas uninvolved with interstitial changes in these biopsies and in the four biopsies lacking interstitial changes, tubular epithelial cell vacuolization, PAS-positive cytoplasmic droplets, and tubular dilatation were present. Glomeruli of the diabetic recipients' own kidneys showed progressive glomerulosclerosis at the 2-mo time period. Compared with the biopsies obtained at the time of transplantation there was increased mesangial thickening, focal glomerular tuft sclerosis, and more prominent thickening of afferent arterioles. Tubular and interstitial findings were unchanged.

Glomeruli of kidneys transplanted from normal animals into normal recipients were normal by light microscopy in biopsies obtained 2 mo later (Fig. 1 E). In four of six donor kidneys interstitial changes as described above were present. The recipients' own kidneys remained normal.

Immunofluorescent Microscopy—Glomeruli in the renal biopsies of animals diabetic for 6 mo demonstrated intense (3+ in most instances) staining for rat IgG in a mesangial distribution (Fig. 1 F). Rat β_1C was present in an identical location but in a more granular pattern and in lesser intensity (1-2+) when compared with IgG. Mesangial IgM was present in some animals, but the intensity of staining was variable. Glomeruli from biopsies of kidneys of non-diabetic rats were either negative or had only trace quantities of rat immunoglobulins and complement at this time.

After 2 mo glomeruli of diabetic kidneys transplanted into normal rats demonstrated complete disappearance or a marked reduction in the intensity of mesangial staining for rat IgG, IgM, and β_1C (Fig. 1 G and Fig. 2). Glomeruli of the recipients' own kidneys were unchanged from the earlier observations.

Significant (1+ or greater) mesangial IgG deposition occurred in glomeruli in six of eight kidneys transplanted from normal rats into diabetic recipients (Fig. 1 H and I, and Fig. 3). In five biopsies significant mesangial β_1C staining was seen and in three biopsies significant IgM deposits occurred. In the diabetic rats' own kidneys quantities of mesangial immunoglobulins and complement were generally increased, and in no instance were they reduced, when compared with the earlier biopsies. The glomeruli in biopsies of the donor and recipients'

own kidneys in normal control transplant animals were unchanged when compared with pretransplant biopsies.

Results of light and immunofluorescent microscopy studies on renal biopsies performed 4 mo after transplantation in all three groups of animals confirmed

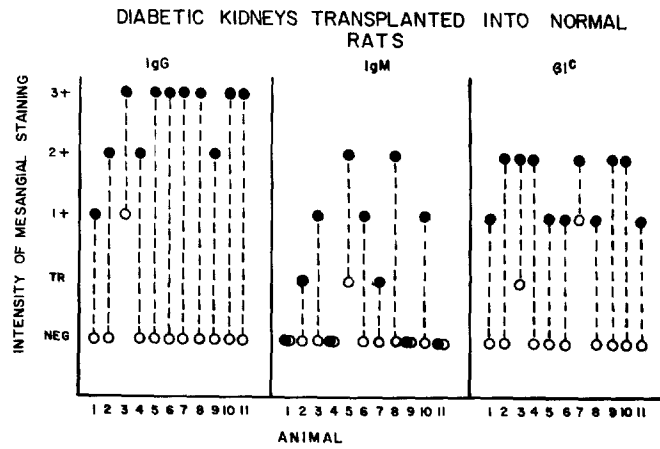


FIG. 2. Results of immunofluorescent microscopy studies of kidneys transplanted from diabetic rats into normal recipients; ●, biopsies before transplantation; ○, biopsies 2 mo after transplantation.

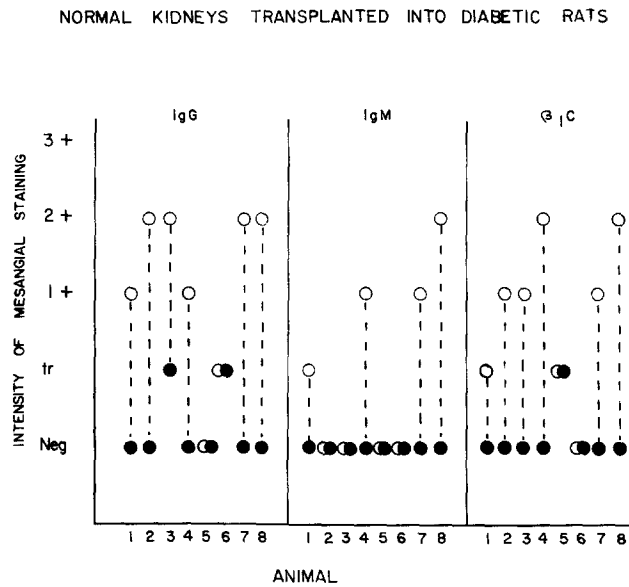


FIG. 3. Results of immunofluorescent microscopy studies of kidneys transplanted from normal rats into diabetic recipients; ●, biopsies before transplantation; ○, biopsies 2 mo after transplantation.

the observations made at the 2-mo time period. It should be pointed out that the light microscopic glomerular changes described above were generally focal in distribution, however, the immunofluorescent findings were uniform in all glomeruli in a given biopsy specimen.

DISCUSSION

Expansion of the volume of the glomerular mesangial matrix without a corresponding increase in the number of mesangial cells is a prominent histological feature in human diabetic glomerulosclerosis (5). In rats with long-standing diabetes induced by beta cell toxins or by pancreatectomy expansion of the mesangial matrix is a uniform finding (1, 6, 7). In the present studies, renal transplantation was carried out in a highly inbred strain of rats in order to obviate morphological changes associated with graft rejection. It was shown that transplantation per se was not associated with detectable glomerular light or immunohistochemical alterations.

The development of glomerular mesangial light and immunohistochemical pathology in normal donor kidneys 2 mo after their placement in diabetic recipients was surprising; in our previous studies in rats, lesions were not seen in the glomerulus until at least 4 mo after diabetes was induced (1). This rapidity of the development of lesions may be related to some added effects of renal transplantation or to the fact that the kidney donors were 6 mo older than the rats employed in earlier studies. Alternately, this phenomenon may reflect abnormalities in the diabetic environment which takes several months to develop but which, once present, lead rapidly to renal injury. This accelerated progression of glomerular damage is unlikely to be due to hyperglycemia per se as this factor was approximately constant when blood sugar values from the previous and the present studies were compared. If pancreatic islet cell insufficiency results in the development of metabolic sequelae, other than hyperglycemia, which accelerate glomerular damage, this could relate to the occasional finding, in humans, of advanced diabetic glomerulosclerosis in the presence of minimal manifestations of glucose intolerance. Patients with end-stage diabetic nephropathy have undergone renal transplantation. Biopsies performed 1 yr later did not demonstrate unequivocal evidence of recurrence of diabetic glomerulosclerosis (8). However, kidney tissue from two patients obtained 3 and 4 yr after transplantation did show definite evidence of the recurrence of diabetic vascular and glomerular lesions (personal observations). The development of diabetic lesions upon placing a normal kidney in a diabetic environment 6 mo after the administration of streptozotocin to the recipient rat does emphasize the primacy of the abnormal metabolic environment as opposed to the drug itself in the etiology of these lesions.

Diabetic kidneys transplanted into normal animals and studied 2 mo later have arrest or reversal of light microscopic glomerular lesions and complete reversal of immunohistopathological findings. These studies confirm our pre-

vious observations of the reversibility of early glomerular lesions in diabetic rats treated by pancreatic tissue transplantation.¹ The effects of placing kidneys from diabetic into normal animals is more striking when these kidneys are compared to those remaining in diabetic hosts; over the 2-mo time period of the study, glomeruli of the diabetic recipient's own kidney underwent significant morphological deterioration.

The present studies suggest that the mesangial changes in diabetic rats may relate to alterations in the dynamics of mesangial function in the uptake and processing of circulating macromolecules (2), disturbance in this function leading to the accumulation of immunoglobulins, complement, and, possibly, other potentially noxious substances in the mesangium. Preliminary studies in diabetic rats² have demonstrated a relative inability of the diabetic mesangium to clear macromolecules which had been made to localize therein. Reversal of the diabetic metabolic milieu of the kidney presumably restores the balance between the mesangial uptake and the mesangial processing of macromolecules. This could explain the appearance and striking disappearance of immunoglobulins and complement from the mesangium in our experiments.

Tubular atrophy, dilatation, cyst formation, and tubular epithelial cell vacuolization and glycogen deposition are present in rats with alloxan- (9) or streptozotocin-induced diabetes. It is known that alloxan causes acute tubular injury in the first few days after injection (10). In dogs given alloxan into one renal artery, tubular and interstitial changes developed although these animals did not become diabetic (11). Thus, it had been thought that tubular changes in animals with long-standing chemically induced diabetes were at least in part secondary to direct toxicity of the inducing agent. Our studies with renal transplantation and pancreatic transplantation demonstrated complete reversibility of the chronic tubular pathology thus indicating that these alterations are due to the diabetic state and not to streptozotocin.

SUMMARY

Immunoglobulins and complement are deposited in the glomerular mesangium of rats with progressive glomerulosclerosis secondary to chemically induced diabetes mellitus. Isotransplantation of a kidney from a rat diabetic for 6 mo into a normal recipient results within 2 mo in the disappearance of IgG, IgM, and β_1C from the mesangium and arrest or reversal of the light microscopic glomerular lesions. Kidneys isotransplanted from normal donors into diabetic rats developed mesangial matrix thickening and deposition of IgG, IgM, and β_1C in the mesangium. No glomerular changes occur upon transplantation of a normal kidney into a normal rat. These findings indicate that diabetic glomerular changes in the rat are reversible and are secondary to the diabetic state rather than to the inducing agent.

² Mauer, S. M., A. F. Michael, and D. M. Brown. Manuscript in preparation.

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