Immunopathology of Renal Extracellular Membranes in Kidneys Transplanted into Patients with Diabetes Mellitus

S. Michael Mauer, M.D., Kenneth Miller, M.D., Frederick C. Goetz, M.D., Jose Barbosa, M.D., Richard L. Simmons, M.D., John S. Najarian, M.D., and Alfred F. Michael, M.D., Minneapolis

SUMMARY

Kidneys of patients with severe diabetic nephropathy demonstrate marked linear immunofluorescent staining of extracellular membranes, including the tubular and glomerular basement membranes (TBM and GBM) and Bowman's capsule. Immunofluorescent studies were carried out on kidney tissue obtained from 12 diabetic and 17 nondiabetic patients from two to 12 years following renal transplantation. The frequency and intensity of IgG and albumin staining of these membranes were significantly greater in the diabetic than in the nondiabetic patients ($P < 0.0005$). TBM, GBM, and Bowman's capsule staining did not occur in any of the seven kidneys studied at the time of their transplantation into diabetic recipients.

Thus, the abnormalities leading to the deposition or trapping of proteins in renal extracellular membranes occur early after the placement of normal kidneys into the abnormal metabolic environment of the diabetic transplant recipient. The present study supports the concept that basement membrane alterations in diabetes are a consequence of the biochemical perturbations of diabetes rather than a separately inherited genetically linked disorder.
DIABETES *25*:709-12, August, 1976.

Controversy still exists as to whether the lesions underlying the secondary complications of diabetes mellitus are due to a separately inherited but closely linked genetic disorder[1] or to the metabolic derangements inherent in the disease.[2] However, certain recently established lines of evidence appear to support the latter hypothesis. Animals with diabetes induced by pancreatectomy[3] or by the administration of pancreatic beta-cell toxins[4] develop lesions that resemble those seen in human diabetes. The diabetic glomerulopathy seen in kidneys of rats with long-standing chemically induced diabetes can be reversed by transplantation of the diabetic kidney into a normal host[5] or by cure of the diabetic state with successful pancreatic islet transplantation.[6] Further, it has been shown that normal kidneys transplanted into diabetic humans develop, within two to three years, characteristic vascular lesions indistinguishable from those seen in the natural historical course of diabetic nephropathy.[7] Herein we report that immunopathologic findings previously described in renal extracellular membranes in diabetes[8] develop in normal kidneys transplanted into diabetic patients.

From the Departments of Pediatrics, Medicine, and Surgery, University of Minnesota Medical School, Minneapolis, Minnesota 55455.

Address reprint requests to S. Michael Mauer, M.D., Department of Pediatric Nephrology, Box 491—Mayo, University of Minnesota, Minneapolis, Minnesota 55455.

Accepted for publication April 21, 1976.

MATERIALS AND METHODS

Patients

The technics of renal transplantation, maintenance immunosuppression and transplantation rejection treatment standard to this institution have been reported.[9] Transplant renal tissues from 12 diabetic and 17 nondiabetic patients with renal grafts in place for at least two years were studied. Nine of the 12 diabetics received related donor grafts. One of the diabetic patients was transplanted because of chronic glomerulonephritis and developed diabetes following transplantation, presumably secondary to steroid therapy. Her biopsy was performed 12 years following transplantation. Eleven of the diabetic patients had renal biopsies done as part of a prospective study. Several months after biopsy, two of these patients died and one required transplant nephrectomy for chronic rejection, and, thus, further tissue became available

for study. In one patient only autopsy tissue was examined. The mean time following transplantation at which tissue was obtained from the diabetic patients was 39 months (range 24-112 months). In addition, seven renal biopsies obtained from the related donor kidneys at the time of their transplantation into diabetic recipients were studied.

The original renal diseases in the 17 nondiabetic transplant recipients are listed in table 1. Fourteen of these patients received related donor grafts. Renal tissue was obtained by percutaneous biopsy in 10 and transplant nephrectomy in seven instances. The biopsies were performed for the diagnosis of renal malfunction and transplant nephrectomies for severe chronic rejection. The mean time following transplantation at which tissue was obtained from the nondiabetic patients was 61 months (range 24-128 months).

TABLE 1

Diagnosis of the primary renal diseases in the nondiabetic patients

Chronic glomerulonephritis (type unknown)	5
Obstructive uropathy with chronic pyelonephritis	4
Hypoplastic-dysplastic kidneys	2
Familial nephrotic syndrome	1
Medullary cystic disease	1
Anaphalactoid purpura nephritis	1
Nail-patella syndrome	1
Familial interstitial nephritis	1
Steroid-resistant nephrotic syndrome with segmental sclerosis	1

Immunofluorescent Studies

The renal tissue was snap-frozen in isopentane precooled in liquid nitrogen, sectioned at 4 μ in a Lipshaw cryostat, and stained with fluorescein isothiocyanate (FITC)-conjugated antisera to human albumin, IgG, and C3 by previously described methods.[8] Specificity of these antisera was demonstrated by established procedures.[8]

Examination of Tissues

The tissue sections were coded and examined without knowledge of the diagnosis or source by two investigators using a Zeiss transmission fluorescent microscope with an FITC interference filter. Following tabulation of the data, the code was broken. Intensity of membrane staining was arbitrarily scored as follows: 0, no significant difference from background; 0.5, fluorescence slightly above background; and increasing degrees of positivity up to 2.0.

RESULTS

The renal transplant tissue obtained from diabetic patients more than two years following transplantation frequently demonstrated intense linear tubular basement membrane (TBM), glomerular basement membrane (GBM), and Bowman's capsule staining for IgG and albumin (figure 1). In most instances the TBM and Bowman's capsule staining was focal and involved 30 to 80 per cent of these membranes present on the biopsy specimens (figure 2). However, the linear GBM staining was usually diffuse and involved all of the GBM in all glomeruli (figure 2). The differences between the intensity of TBM, Bowman's capsule, and GBM staining on comparing diabetic and nondiabetic tissues for both IgG and albumin were in all instances highly statistically significant with all P values being less than 0.0005 (Student's t test). In fact, there was very little overlap in the comparative results except for linear IgG staining of the GBM (figure 3). Moreover, when membrane staining occur-

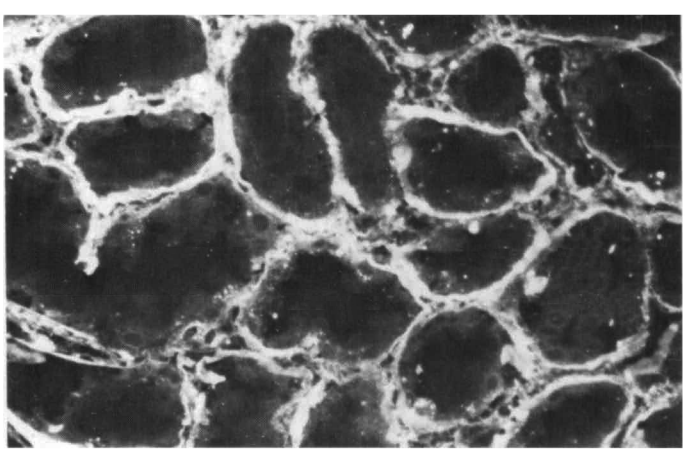

FIGURE 1

Linear TBM staining for albumin of 2.0 intensity in a diabetic transplant recipient three years following transplantation (X 200).

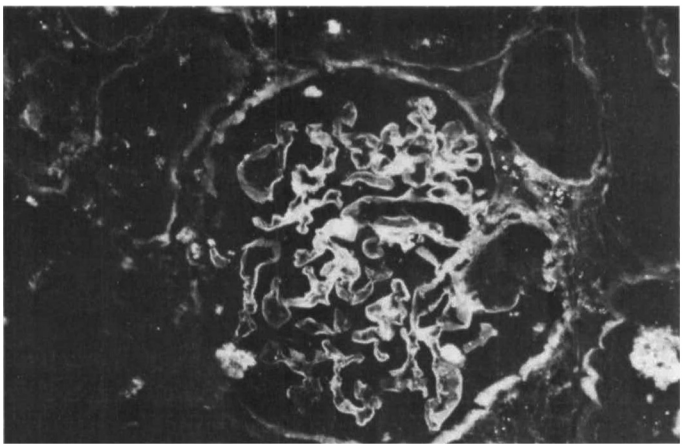

FIGURE 2

Diffuse linear GBM staining (1.5 intensity) and focal TBM and Bowman's capsule staining for IgG in a diabetic transplant recipient two years following transplantation (X 180).

red in the nondiabetic patients it was much more focal in distribution than in the diabetic patients.

The differences noted above were not as striking when C3 stainings of kidneys of diabetic and nondiabetic patients were compared. Nonetheless seven of 12 tissues from diabetics had TBM staining of 1.0 intensity or more while only two of 17 nondiabetic kidneys had similar staining. Further, in diabetics, the GBM staining in four of 12 instances and Bowman's capsule staining in two of 12 instances was 1.0 or greater, while in nondiabetics in only two of 15 and none of 15 cases, respectively, was GBM and Bowman's capsule staining of this intensity seen. Abnormal staining of renal extracellular membranes occurred in none of the seven related donor kidney biopsies obtained at the time of transplantation to diabetic patients.

Three of the diabetic patients each had several glomerular arterioles exhibiting striking homogeneous circumferential vessel wall staining for IgG, albumin, and C3. Two of these patients also had intense homogeneous glomerular nodular staining for these proteins. Periodic-acid Schiff (PAS) staining of these slides demonstrated that these immunopathologic findings corresponded to PAS positive hyaline arteriole degenerative changes and hyaline glomerular nodules. These findings were not present in the biopsies of nondiabetic patients.

DISCUSSION

Glomerular[2] and muscle capillary basement membranes[1,10] have been intensively studied in diabetes mellitus, and it is clear that thickening of these structures is a highly uniform occurrence in this disease. Although there is still no general agreement as to whether muscle capillary basement membrane thickening precedes the onset of overt diabetes[11] it is known that thickening progresses with time, especially in patients with juvenile-onset disease.[1] GBM is of normal thickness at the onset of juvenile diabetes but is measurably thickened one and one-half to two and one-half years later.[2] Westberg and Michael demonstrated linear GBM staining for IgG and other proteins in two children with minimal light microscopic glomerular abnormalities two and four years after onset of diabetes.[12] Further, within two years of onset, exercise-induced albuminuria can be documented in most juvenile-type diabetics.[13] Thus,

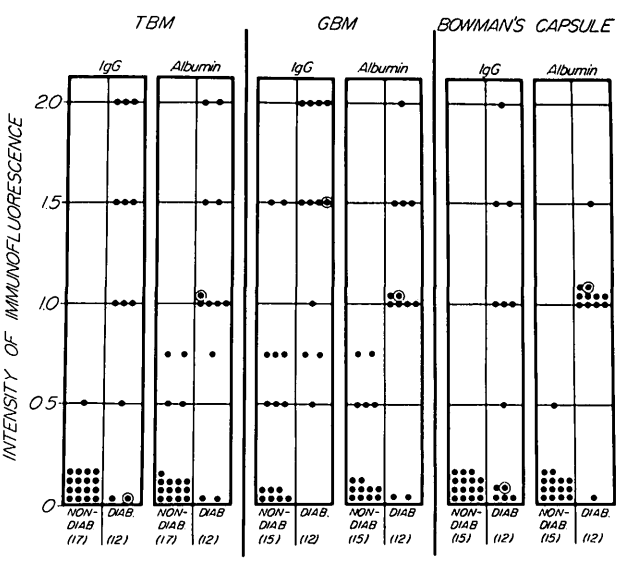

FIG. 3. Intensity of immunofluorescence for IgG and albumin in the TBM, GBM, and Bowman's capsule of kidneys transplanted into diabetic and nondiabetic patients. The numbers in parentheses are totals of patients studied. ⊙ represents the patient with posttransplantation steroid-induced diabetes.

structural and functional alterations in glomerular capillaries occur very early in the natural history of juvenile diabetes. Until recently little attention was paid to the renal TBM and Bowman's capsule in diabetes. Careful morphometric studies of the thickness of these membranes are lacking; however, Miller and Michael in a companion report[8] have shown that these membranes stain intensely for IgG and albumin in patients with advanced diabetic nephropathy. However, immunopathologic studies of renal TBM and Bowman's capsule early after onset of juvenile diabetes have not been done.

The present study clearly demonstrates increased TBM, Bowman's capsule, and GBM staining for IgG and albumin two or more years following the transplantation of normal kidneys into diabetic patients. Thus, the abnormalities leading to the deposition or trapping of proteins in renal extracellular membranes in diabetes may occur early in the natural history of the disease and may correspond to the early morphologic and functional glomerular capillary changes mentioned above. The immunopathologic changes noted herein appear to result from the placement of a normal kidney into the abnormal metabolic environment of the diabetic recipient in that none of the donor biopsies performed at the time of transplantation had similar changes. Further, the changes did not result from transplantation per se or from transplantation rejection. In fact, the nondiabetic patients had little significant membrane staining despite, as a group, having much more severe chronic rejection than the diabetic patients. Thus, the present study may be taken as further evidence in support of the theory that basement membrane abnormalities of diabetes are a consequence of the abnormal metabolic environment rather than a separately inherited genetically linked disorder.

Occasionally weak and focal TBM and Bowman's capsule staining for IgG and albumin may be seen in normals and in patients with nondiabetic renal diseases.[8] However, as in our studies, immunofluorescent staining of these membranes discriminated diabetic from nondiabetic kidneys more clearly than did GBM staining. In the present study, as compared with the previous work,[8] a slightly higher incidence of weak, focal albumin and IgG staining of TBM and Bowman's capsule was found in nondiabetic patients. This may reflect the diabetogenic effects of corticosteroid therapy in these patients. In fact, significant staining was seen in the single patient with steroid-induced diabetes.

ACKNOWLEDGMENTS

We thank Justine Wilmert, R.N., and Lois Recker, R.N., for their major contributions to the care of these patients.

This research was aided by grants from the National Institutes of Health (AM17697-02, AI-10704, and HL-06314), the General Clinical Research Centers Program of the Division of Research Resources, N.I.H. (RR-400), the Bush Foundation of St. Paul, Minnesota, and U.S.P.H.S. grant A.M. 13083.

REFERENCES

[1]Siperstein, M.D., Unger, R.H., and Madison, L.L.: Studies of muscle capillary basement membranes in normal subjects, diabetic and pre-diabetic patients. J. Clin. Invest. *47*:1973, 1968.

[2]Østerby, R.: Early phases in the development of diabetic glomerulopathy. Acta Med. Scand. (Suppl.) *574*:1, 1975.

[3]Foglia, V.G., Mancini, R.E., and Cardeza, A.F.: Glomerular lesions in the diabetic rat. Arch. Pathol. *50*:75, 1950.

[4]Mauer, S.M., Michael, A.F., Fish, A.J., and Brown, D.M.: Spontaneous immunoglobin and complement deposition in glomeruli of diabetic rats. Lab. Invest. *27*:488, 1972.

[5]Lee, C.S., Mauer, S.M., Brown, D.M., Sutherland, D.E.R., Michael, A.F., and Najarian, J.S.: Renal transplantation in diabetes mellitus in rats. J. Exp. Med. *139*:793, 1974.

[6]Mauer, S.M., Sutherland, D.E.R., Steffes, M.W., Leonard, R.J., Najarian, J.S., Michael, A.F., and Brown, D.M.: Pancreatic islet transplantation. Effects on the glomerular lesions of experimental diabetes in the rat. Diabetes *23*:748, 1974.

[7]Mauer, S.M., Barbosa, J., Vernier, R.L., Kjellstrand, C.M., Buselmeier, T.J., Simmons, R.L., Najarian, J.S., and Goetz, F.C.: Diabetic vascular lesions develop in normal kidneys transplanted into patients with diabetes mellitus. N. Engl. J. Med. In press.

[8]Miller, K., and Michael, A.F.: Immunopathology of renal extracellular membranes in diabetes mellitus: Specificity of tubular basement membrane immunofluorescence. Diabetes *25*:701-08, 1976.

[9]Simmons, R.L., Kjellstrand, C.M., and Najarian, J.S.: Kidney transplantation. *In* Transplantation. Najarian, J.S., and Simmons, R.L., Eds. Philadelphia, Lea and Febiger, 1972, p. 445.

[10]Williamson, J.R., Vogler, B.S., and Kilo, C.: Microvascular disease in diabetes. Med. Clin. N. Amer. *55*:847, 1971.

[11]Raskin, P., Marks, J.F., Burns, H., Jr., Plumer, M.E., and Siperstein, M.D.: Capillary basement membrane width in diabetic children. Am. J. Med. *58*:365, 1975.

[12]Westberg, N.G., and Michael, A.F.: Immunohistopathology of diabetic glomerulosclerosis. Diabetes *21*:163, 1972.

[13]Mogensen, C.E., and Vittinghus, E.: Urinary albumin excretion during exercise in juvenile diabetes. Scand. J. Clin. Lab. Invest. *35*:295, 1975.