Selective Binding of IgG_4 and Other Negatively Charged Plasma Proteins in Normal and Diabetic Human Kidneys

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Renal tissue from 9 patients with diabetes mellitus (4 with mild and 5 with end-stage disease) and 3 with anti-glomerular basement membrane (GBM) nephritis, as well as 5 normal human kidneys, were examined by immunofluorescence microscopy for the presence of plasma proteins of varying isoelectric point (pI). In normal and diabetic kidneys, IgG deposition in basement membranes was restricted to IgG_4 (pI 5.5–6.0), the subclass present in lowest concentration in human plasma. IgG_1, IgG_2, and IgG_3 (pI 7.0–9.5) were not detected. In contrast, in anti-GBM nephritis, all four subclasses were present in a linear pattern in GBM. Other plasma proteins of low isoelectric point were detected in basement membranes: albumin (pI 4.9), α-1-acid glycoprotein (pI 2.7), amyloid P (pI 3.9–4.8), and α-1-antitrypsin (pI 4.5). These studies are consistent with the hypothesis that circulating anionic plasma proteins are electrostatically bound *in vivo* to positively charged moieties in normal and especially diabetic basement membranes. (Am J Pathol 1984, 115:443–446)

LINEAR DEPOSITION of albumin and IgG in renal basement membranes (BM) has been observed in normal human kidneys and in increased amounts in diabetes mellitus.[1-3] In the latter disease, increased immunofluorescence for albumin also has been detected in vascular basement membranes of muscle and skin, as well as in sarcolemmal and thyroidal basement membranes.[4,5]

The possibility that the binding of plasma proteins to basement membranes is charge-dependent is suggested by the fact that both albumin and one subclass of IgG, IgG_4, are anionic under physiologic conditions, ie, have low isoelectric points, 4.7[6] and 5.5–6.0,[7] respectively. To investigate the possibility that this binding may be charge-related, we examined renal tissue from normal and diabetic patients by immunofluorescence techniques for the presence of plasma proteins of varying isoelectric points. These studies demonstrate that proteins of low isoelectric point are present in the glomerular BM (GBM) and tubular basement membrane (TBM) of normal and diabetic kidneys and that plasma proteins with high isoelectric points (>7)—eg, IgM, properdin—are not detected.

Materials and Methods

Tissue

Kidney tissue was obtained from patients undergoing treatment at the University of Minnesota Hospitals between 1975 and 1982. The diagnosis of diabetes mellitus had been confirmed by conventional clinical and laboratory features. Kidney biopsies were obtained from 4 patients with diabetes mellitus who were undergoing evaluation for pancreatic transplantation (kindly provided by Drs. S. Michael Mauer and David Sutherland, University of Minnesota) because of progressive retinopathy or insulin resistance. The glomerular filtration rate and urinary protein excretion were normal in 3; the fourth patient had proteinuria with a creatinine clearance of 75/ml/min/1.73 m^2. Kidney tissues were also obtained from 5 patients with end-stage diabetes. Kidney biopsies from 3 patients with anti-GBM nephritis were obtained for clinical indications. Tissue from 5 normal kidneys was obtained at the time of surgery for other clinical conditions, selected from a total of 15 on the basis of bright linear immunofluorescence for IgG in the GBM. These kidneys were histologically normal by light and immunofluorescence microscopy. All studies were approved by the Committee on

Supported in part by grants from the NIH (AM-07087, AI10704, AM26149, AM25518) and the Viking Childrens Fund.

Accepted for publication January 25, 1984.

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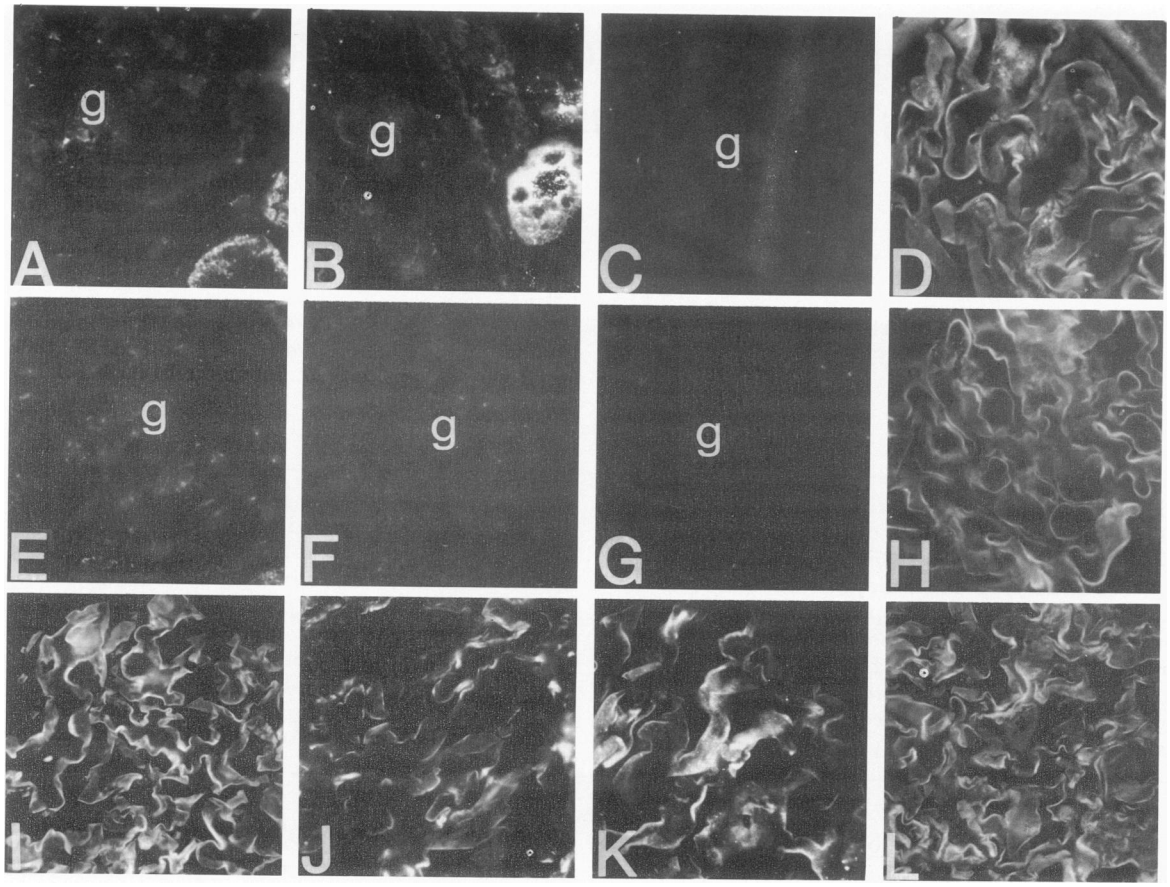

Figure 1 — Immunofluorescence micrograph of sections of diabetic kidney (**A–D**), normal kidney (**E–H**), and kidney with anti-GBM nephritis (**I–L**). The tissue was stained by indirect immunofluorescence for IgG_1 (**A, E, I**), IgG_2 (**B, F, J**), IgG_3 (**C, G, K**), and IgG_4 (**D, H, L**). Note the positive immunofluorescence for IgG_4 in the GBM of diabetic (**D**) and normal (**H**) glomeruli and the absence of IgG_1 (**A, E**), IgG_2 (**B, F**), and IgG_3 (**C, G**). Negative glomeruli are indicated by *g*. Some tubules in diabetic kidney contain IgG_1 (**A**) and IgG_2 (**B**). In contrast, the GBM in kidney from a patient with anti-GBM nephritis contains all four subclasses of IgG (**I–L**). (All except **K**, ×300; **K**, ×384)

Use of Human Subjects in Research of the University of Minnesota.

Immunohistochemical Techniques

Goat anti-human IgG (γ chain) and anti-human IgM (μ chain) conjugated with fluorescein isothiocyanate (FITC) were obtained from Cappel Laboratories, Cochranville, Pennsylvania. Murine monoclonal antibodies to the four IgG subclasses were obtained as ascites from the following investigators and commercial laboratories: Dr. Mary Conley (Philadelphia, Pa)—anti-γ chain, anti IgG_3; Dr. J. Donald Capra (Dallas, Tex)—antibodies to IgG_1, IgG_2, and IgG_4; Seward Laboratories (London, England)—antibodies to IgG_1 IgG_2, IgG_3, and IgG_4; and Bethesda Research Laboratories (Bethesda, MD)—antibodies to IgG_3 and IgG_4. FITC-labeled goat antisera to mouse IgG (Cappel Laboratories) and to rabbit IgG (Kallestad Laboratories, Austin, Tex) were absorbed with human plasma prior to use. Rabbit antisera to α-1-acid glycoprotein and α-1-antitrypsin were obtained from Behring Diagnostics (Sommerville, NJ), and rabbit anti-human amyloid P was obtained from Dr. Merrill D. Benson, Indianapolis, Ind. Rabbit anti-properdin was used as previously reported.[8] Immunofluorescence was carried out as previously described.[9] Each tissue was examined by three observers without knowledge of the particular antibody, and intensity was graded on the following scale: no immunofluorescence in basement membrane, 0; mild to moderate immunofluorescence, 1+; and intense immunofluorescence, 2+.

Results

In kidneys from 2 groups of patients with diabetes mellitus, 5 with end-stage and 4 with early disease, IgG (γ chain) was detected in a linear pattern in GBM and TBM with an intensity range of 1-2+ using polyclonal and monoclonal antibodies. The reactivity of three different anti-IgG_4 reagents were concordant,

Table 1—Presence of Plasma Proteins in Renal Basement Membranes of 9 Patients With Diabetes Mellitus as Detected by Immunofluorescence Microscopy

Protein	Immunofluorescence with antisera to specific proteins*		Physicochemical parameters†		
	GBM	TBM	Molecular weight (daltons)	Isoelectric point	Serum concentration (mg/dl)
α-1-Acid glycoprotein	+	+	44,000	2.7	75–100
Amyloid P	+	+	235,000	3.9–4.8	3.9–71.4
α-1-Antitrypsin‡	+	+	58,000	4.5	210–500
Albumin	+	+	68,000	4.9	3500–4500
IgG_4	+	+	146,000	5.5–6.0	40§
IgM	–	–	900,000	5.8–8.5	37–261
IgG_1	–	–	146,000	7.0–9.5	640§
IgG_2	–	–	146,000	7.0–9.5	250§
IgG_3	–	–	170,000	8–9	70§
Properdin	–	–	146,000–153,000	>10‖	1.6–3.6

* Positive immunofluorescence of the basement membrane, +; negative immunofluorescence, –.
† Data taken from references 6, 7, 10, 11, 12, 13, 14, 15.
‡ Weak linear staining of GBM, predominantly TBM.
§ Based on idealized total IgG concentration of 1000 mg/dl.
‖ Personal communication, Dr. Robert Schreiber, La Jolla, California.

staining GBM and TBM with an intensity of 1+ (Figure 1). No reactivity in BM was observed with the use of two different monoclonal antibodies to IgG_1, two antibodies to IgG_2, and three antibodies to IgG_3. In contrast, linear staining of the GBM was evident with all monoclonal antibodies to IgG_1, IgG_2, IgG_3, and IgG_4 in 2 patients with anti-GBM nephritis; in the third IgG_2, IgG_3, and IgG_4 were present.

Monoclonal antibodies to IgG_1, IgG_2, and IgG_3 stained non-BM regions of diabetic kidneys: IgG_1 was uniformly present as granular deposits in tubular epithelium; IgG_2 and IgG_3 also were detected in a focal homogeneous pattern in some tubules.

In addition, α-1-acid glycoprotein (pI 2.7),[6] α-1-antitrypsin (pI 4.5),[6] albumin (pI 4.9), and amyloid P (pI 3.9–4.8)[10] were detected with an intensity of 1–2+ in a linear pattern in GBM and TBM of three out of three early diabetic kidneys.

Goat and monoclonal antibodies to IgG (γ chain) reacted with the GBM in all five normal kidneys studied and the TBM in two of five. IgG_4, but not the other subclasses, was present in the GBM of four kidneys; in the fifth, no subclass was detected. The anionic proteins (α-1 acid glycoprotein, α-1-antitrypsin, amyloid P, albumin) were detected in the GBM of all five normal human kidneys in an intensity of 1+. Only α-1-antitrypsin was detected in normal TBM.

No linear staining of GBM or TBM was detected with antisera to IgM or properdin, in any diabetic or normal kidney. These findings are summarized in Table 1.[11–15]

Discussion

The IgG present in renal basement membranes of normal and diabetic patients is restricted to the IgG_4 subclass—a unique finding in view of the fact that its serum concentration is lower than those of IgG_1, IgG_2, and IgG_3.[11] The relatively low isoelectric point of IgG_4 (5.5–6.0) separates it from the other subclasses (Table 1). In addition, four other anionic plasma proteins with pIs in the range of 2.7–4.9 were observed in a linear pattern on GBM and TBM. None of 5 plasma proteins with higher isoelectric points were detected. The findings cannot be explained on the basis of plasma protein charge alterations in patients with diabetes, for two reasons. First, the proteins are localized in similar fashion in normal human kidneys; and second, it has been demonstrated that there is no measurable change in charge of the major anionic plasma protein, albumin, in patients with diabetes (A. F. Michael, unpublished observations). The findings in this study are most consistent with the view that anionic plasma proteins are bound electrostatically to renal basement membranes.

However, a number of prior studies have shown the presence of negatively charged sites within the glomerular capillary and have suggested that these provide a barrier to the filtration of anionic plasma proteins. Two moieties have been recognized—a proteoglycan containing heparan sulfate and a sialoprotein, the glomerular polyanion.[16,17] *In vivo* studies using charged probes have shown that only the cationized species binds to sites within the GBM, whereas anionic probes do not appear to localize within the capillary wall.[18]

These experimental studies, which appear to be in conflict with the present studies, may be reconciled by the hypothesis that positively charged sites within BM are abrogated by endogenous circulating anionic plasma proteins (eg, albumin, IgG_4). Our conclusions are consistent with the observations of Izui et al,[19] who

demonstrated an affinity of anionic DNA for BM and bovine skin collagen. Both Type IV and Type V collagen, shown previously to be present in isolated human renal BM,[20] have pIs in the range of 8.3–9.0 and may be potential ligands for circulating anionic proteins.[21] The mechanism for increased binding of anionic plasma proteins to diabetic basement membranes is unknown. However, recent evidence suggests that BM-fixed negative-charged sites (ie, heparan sulfate) are decreased in diabetic mice.[22] If a similar phenomenon is true in humans, it could result in a net increase in available cationic sites to which the anionic proteins bind.

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Acknowledgments

The authors wish to thank Ms. Kathy Divine, Ms. Crystal Blocher, and Ms. Kim Pinkham for technical assistance, Mr. Marshall Hoff for the preparation of figures, and Ms. Cindy Dawis for typing the manuscript.