Glucosylated and Normal Human or Rat Albumin Do Not Bind to Renal Basement Membranes of Diabetic and Control Rats

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SUMMARY

Hyperglucosylated and normal human and rat albumin were injected intravenously into control and streptozotocin-induced diabetic rats. Binding of injected human albumin to renal basement membranes was not observed by immunofluorescence microscopy in the diabetic or control rats irrespective of the form of injected albumin. However, human albumin was found as tubular droplets in both the control and diabetic rats injected with either form of albumin. Spontaneous binding of endogenous rat albumin was observed in a linear pattern on diabetic rat glomerular basement membranes (GBM), but not in the GBM of control rats. No appreciable differences in the intensity of staining for rat albumin was observed in diabetic rats injected with either glucosylated or normal rat albumin. Similarly, no binding of rat albumin to the GBM was observed in control rats irrespective of the type of albumin injected. These studies demonstrate that binding of albumin to renal basement membranes is not dependent on glucosylation. DIABETES 32:380–382, April 1983.

The increased proportions of nonenzymatic glucosylation of hemoglobin,[1] albumin,[2,3] erythrocyte membrane proteins,[4] lipoproteins,[5] lens crystallin proteins,[6,7] aortic collagen,[8,9] and glomerular basement membrane (GBM) collagen[10] in diabetes mellitus have been postulated to contribute to the pathogenesis of the secondary complications of diabetes.[11] Furthermore, repeated injections of glycosylated serum proteins in mice were associated with thickening of the GBM, a characteristic feature of diabetes.[12] However, in these mice binding of serum proteins along renal basement membranes was not demonstrated. We have demonstrated binding of albumin and IgG to basement membranes of the kidney,[13] muscle,[14] skin capillaries,[15] and thyroid[16] of diabetic patients and linear binding of albumin and IgG to the renal basement membranes of spontaneously diabetic dogs.[17] The affinity of albumin for basement membranes including GBM and tubular basement membrane (TBM) in diabetic animals and man is unexplained,[18] and the effect of its presence on the basement membrane is unknown.

To test the hypothesis that the binding of albumin to the GBM and TBM in diabetes is due either to increased glucosylation of albumin or of GBM, we injected hyperglucosylated human and rat albumin and normal human and rat albumin into diabetic and age-matched nondiabetic rats. We examined the renal tissue for deposition of human albumin at various time intervals by immunofluorescence microscopy.

MATERIALS AND METHODS

Diabetes was induced in 3-mo-old male Sprague-Dawley rats (Holtzman, Madison, Wisconsin) by intravenous injection of streptozotocin (citrate buffer, 0.3 M, pH 4.5) 55 mg/kg, and this was followed immediately by the intraperitoneal administration of 20 ml of 30% dextrose. Diabetes was confirmed by the presence of hyperglycemia, which was checked 4 days after administration of streptozotocin and then once a month for 3 mo at which time the experiments were performed.

One gram of albumin (normal human or rat serum albumin, Sigma, St. Louis, Missouri) was added to 500 ml of D-glucose in 0.05 M sodium phosphate buffer with 0.15 M sodium chloride, pH 7.4. Sodium azide (0.01%) and 2 drops of toluene were added to the mixture, which was incubated at 37°C for 3 wk. Glucosylation of albumin was evaluated by the modified method of Fluckiger and Winterhaller.[19] Ten-milligram samples of glucosylated human or rat albumin and standard were dissolved in 1.5 ml of 1.0 M oxalic acid and 5-hydroxymethylfurfural (range 10^{-6} to 10^{-4} M). The mixtures were heated to 100°C with venting for 5 h and then were allowed to cool to room temperature, precipitated with 0.5 ml of 40%

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trichloracetic acid and centrifuged at 1000 × *g* for 10 min. One-half milliliter of 0.05% W/2-thiobarbituric (wt/vol = 0.05%) acid was added to 1.5 ml of the supernatant and the mixture was incubated for 30 min at 37°C. The absorbance of the samples was determined on a Zeiss spectrophotometer at 443 nm. Standard plots were made of the log of absorbance at 443 nm versus the log concentration of 5-hydroxymethylfurfural.

The isoelectric points of glucosylated and normal human or rat albumin, determined by isoelectric focusing (LKB) on agarose with ampholines producing a gradient of isoelectric points from 3.5 to 10.0, were similar (4.9–5.3).

Six diabetic rats of 3 mo duration and six age-matched control rats were each injected intravenously with 40 mg (1.0 ml) of glucosylated or normal human albumin. Also, two diabetic rats of 2 mo duration and two of 3 mo duration and four age-matched control rats were each injected intravenously with 40 mg (1.0 ml) of hyperglucosylated or normal rat albumin. Renal biopsies were performed at 20 min and at 24 h, and the rats were killed 48 h after administration of glucosylated or normal human or rat albumin.

Kidney biopsy samples were frozen immediately in isopentane precooled in liquid nitrogen, sectioned at 4 μm in a Lipshaw cryostat, and processed for immunofluorescent microscopy.[8] Sections from rats injected with glucosylated or normal human albumin were stained for human albumin, rat albumin, and rat IgG, while sections from rats injected with glucosylated or normal rat albumin were stained for rat albumin and rat IgG. Specificity of the antisera was determined by immunodiffusion. All tissue sections were coded and examined independently without prior knowledge of the condition of the rat or the protein injected. The intensity of fluorescence was scored as follows: 0, staining background; 0.5, staining slightly higher than background; 1–3, indicating relative increased intensity of staining. Variations in observer scores did not exceed 0.5. The scores were averaged and the code then broken for tabulation of data.

RESULTS

Binding of normal or glucosylated human albumin to the GBM, TBM, or mesangium was not demonstrated by immunofluorescence in kidneys of diabetic or control rats at any time period (Figure 1). However, human albumin was found as tubular droplets in both the diabetic and the control rat injected with either glucosylated or normal human albumin.

Deposition of rat IgG and albumin in the mesangium or along the GBM and TBM were not observed in the control rats. Linear immunofluorescence of endogenous rat albumin along the GBM and TBM was observed in the diabetic rats. However, appreciable differences in the intensity of staining for rat albumin was not observed between diabetic rats injected with glucosylated or normal rat albumin. However, in all diabetic rats significant endogenous deposition of rat albumin and rat IgG were observed in the mesangium (Figure 2) as previously described.[8] Further linear fluorescence of rat IgG was observed along the GBM of diabetic rats (Figure 2). The proximal tubular cells of diabetic rats but not of control rats contained droplets of rat albumin.

DISCUSSION

Administration of exogenous human or rat glucosylated albumin or normal albumin to the diabetic rats did not result in binding of appreciable amounts of albumin to the renal basement membranes of diabetic rats. Our observations are in agreement with those of McVerry et al.,[12] who did not observe binding of serum proteins to renal basement membrane of mice after weekly administration of glucosylated serum proteins.

The lack of binding of glucosylated human or rat albumin to the renal basement membranes of diabetic as well as nondiabetic rats suggests that glucosylation of albumin does not alter its binding to renal basement membranes. Another possibility for these findings is that the dose of glucosylated albumin administered and the time periods at which we ex-

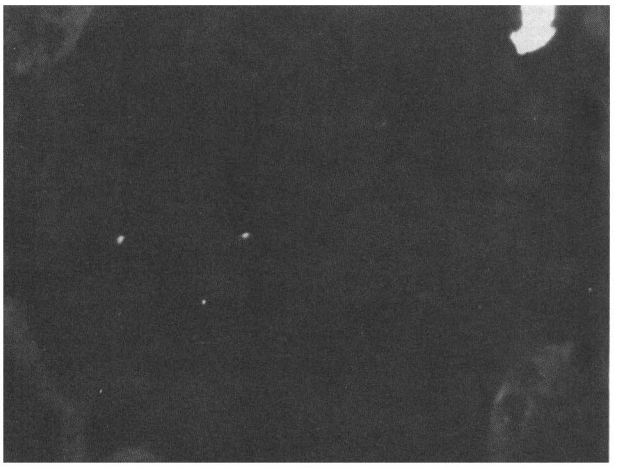

FIGURE 1. Direct immunofluorescence of a kidney of a diabetic rat injected with glucosylated albumin. The section was stained with rabbit anti-human IgG. No reaction was observed, indicating no binding of glucosylated albumin to the renal basement membranes of the diabetic rat. (× 550.)

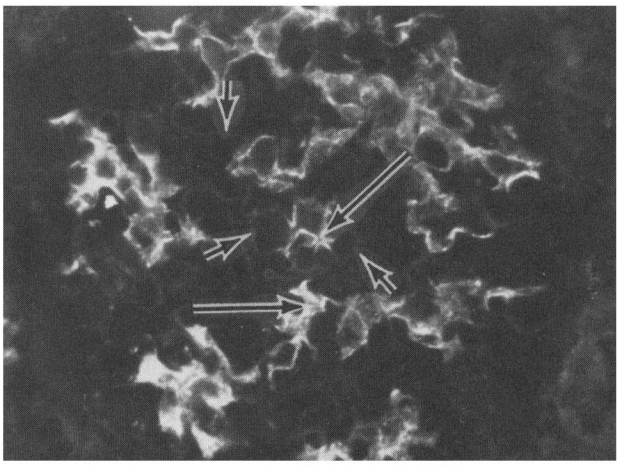

FIGURE 2. Direct immunofluorescence of a kidney of a diabetic rat injected with glucosylated human albumin. The section was obtained with rabbit anti-rat albumin. Deposition of rat albumin was noted in the mesangium (long arrow) and in a linear pattern along the glomerular basement membrane (short arrow). (× 550.)

amined the renal tissues were inappropriate. This is unlikely since the dose of albumin used in these studies was similar to that used by Purtell et al.,[20] who demonstrated binding of cationized (isoelectric point 7.2–8.2) albumin to renal basement membranes after 2 h of renal perfusion, but failed to demonstrate binding of normal albumin (isoelectric point 4.5–5.5).

Another possibility is that 3 mo duration of diabetes in these rats was insufficient for basement membrane glucosylation, an explanation that is unlikely since Cohen et al.,[10] have demonstrated glucosylation of GBM in rats with diabetes of 3–4 wk duration.

The presence of endogenous IgG and albumin binding to diabetic basement membranes in our studies suggests that the mechanism(s) accounting for this phenomenon may have prevented the binding of exogenously administered human or rat albumin to renal basement membranes.

The immunofluorescence technique used in these experiments is exquisitely sensitive and would have been expected to detect meaningful increases in binding of exogenously delivered albumin. The ability to detect small quantities of albumin by immunofluorescence is verified by the relatively small amounts of albumin recovered from GBM and TBM as measured by radioimmunoassay.[16]

Thus, in vitro glucosylation of albumin does not appear to increase its affinity for renal basement membranes. Similarly, glucosylation of renal basement membranes does not appear to attract exogenous glucosylated albumin. However, in the latter situation it is possible that the binding sites of the renal basement membranes may have been saturated by endogenous glucosylated albumin.

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