7. Rat Glomerulonephritis Induced by Soluble Antigens from Bovine Glomerular Basement Membrane

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(Communicated by Shigeo TAKAHARA, M. J. A., Feb. 12, 1983)

Experimental glomerulonephritis can be induced in animals by injecting the fraction of glomerular basement membrane (GBM) with Freund's complete adjuvant (FCA).^{1),9)-12)} The glomeruli of the injected animals are damaged by their own antibodies raised against the injected antigens.³⁾ The antigens normally used were those from different species as well as the same ones; and were used as the insoluble GBM fractions lacking biochemical details.

In 1971 Shibata *et al.*⁶⁾ were able to solubilize the nephritogenic antigen from rat GBM. This was the first step to make the analysis of the antigen possible. However, there seemed to be difficulty in obtaining a large quantity of the antigen enough for the chemical analysis as far as rats are used. We recently have obtained the soluble fraction of bovine GBM (GBM-ST) by the method of Shibata *et al.*,⁴⁾ 500 mg from 1 kg bovine renal cortex. By injecting GBM-ST into rats the autoimmune glomerulonephritis was induced, resulting heavy and long-lasting proteinuria accompanied by hematuria initiating at about 3 weeks after the injection. The proteinuria lasted until sacrifice 15 weeks after a single antigen injection and kidneys showed typical glomerulonephritis. In this paper we demonstrated the nephritic symptoms with the severe histologic changes specific to glomerulonephritis caused in the rats injected with GBM-ST.

Materials and methods. Fresh bovine kidneys were used. From cortical fragments, GBM was isolated⁸⁾ and solubilized with trypsin.⁴⁾ The solubilized GBM was dialyzed against distilled water and then lyophilized. Before use, the whitish GBM-ST lyophil, kept in refrigeration, was dissolved in the phosphate buffered isotonic saline.

For the study, female Wistar rats weighing 150–180 g were used being divided into 3 groups. The animals in the first group received a single injection of 1 to 8 mg GBM-ST dissolved in 0.1 ml saline and emulsified with 0.1 ml of FCA (Difco Lab.) into hind footpads. The second group received the phosphate buffered saline with FCA alone and the third group was left with no treatment as controls.

After the injection 15-hour urine (from 6:00 P.M. to 9:00 A.M.)

was collected from each rat every week for 10 to 15 weeks. Urinary protein contents were measured by 3% sulfosalicylic acid precipitation,²⁾ followed by determination of the optical density at 450 nm. Urinary hemoglobin was checked with test paper (Hemastix, Miles-Sankyo) and hematuria by light microscopy.

Ten to 15 weeks later the animals were sacrificed by decapitation, the kidneys and some tissues were fixed in Dubosq-Brazil fixative for light microscopy and others were frozen-sectioned for immunofluorescence study. The fixed tissues were embedded, sectioned then stained with Hematoxylin-eosin and periodic acid-Schiff. The frozen-sections were stained with FITC-labeled anti-rat IgG (Hyland), anti-rat IgM (Fuji Zoki), and anti-rat C3 (Cappel Lab.).

Results. Ten out of 14 rats receiving GBM-ST excreted proteinuria and hematuria beginning 3 to 9 weeks after the injection and lasted for at least 12 weeks (Fig. 1). Protein contents were 10 to 360 mg/15 hr. The proteinuria was always with microscopic hematuria. The animals in the second and third groups excreted some protein, 2 mg or less per 15 hr throughout the study.

Histologically, almost all the glomeruli exhibited an accentuated lobulation with segmental lesions of endocapillary hypercellularity of mononuclear cells. Epithelial crescent formation which was usually restricted to the area of capsular adhesion, and protein droplets in the epithelial cells were seen (Fig. 2a). The hyaline thickening of the GBM was hardly seen. No glomerular infiltration of polymorphonuclear leukocytes was found. In severe cases some tubules were dilated and some were atrophied, and hyaline casts within the lumina

Fig. 1. Time course of proteinuria of rats injected with GBM-ST.

Fig. 2. a: Histological changes of the kidney from a rat injected with 1 mg of GBM-ST at 10 weeks. (PAS staining, original magnification, $\times 150$.) b: Linear deposition of rat IgG along the GBM of a rat injected with GBM-ST. (Stained with FITC-labeled anti-rat IgG, original magnification, $\times 200$.)

of tubules were prominent. Immunofluorescence study revealed the intense uninterrupted linear deposits of rat IgG along the GBM (Fig. 2b). No granular deposition of IgG was observed along the capillary wall. The IgG fluorescence intensity was nearly the same among the rats injected with GBM-ST, and it did not correlate with the severity of proteinuria nor with that of histologic changes. No IgG deposition in glomeruli was seen in the control. Neither IgM nor C3 deposition was detected whatsoever.

Discussion. As demonstrated, the rats receiving GBM-ST developed typical glomerulonephritis. The sign of the glomerular damage appeared about 3 weeks after the injection. The latent period suggests the time required for the antibody formation enough to induce the disease. Immunofluorescence study revealed the linear deposition of rat IgG along the GBM. The proteinuria indicating the damage of glomeruli lasted more than 12 weeks after the onset of nephritic symptoms which were induced by only one injection of GBM-ST.

Although Shibata *et al.*'s antigen-solubilization method was applied in this study, the symptoms induced seems to be different from those of their model. Heavy proteinuria was found 3 to 4 months after the injection in their cases,⁷⁾ whereas it appeared only 3 weeks after the injection in ours. The immunofluorescence pattern of IgG in theirs was mainly mesangial or granular,^{5),7)} but was linear in ours.

The mechanism of development of glomerulonephritis induced by GBM-ST should be the same as that of development of glomerulonephritis inducible by the injection of insoluble GBM fractions from the same and different species, e.g. Steblay's model in sheep.^{9),10)}

GBM-ST is soluble and makes the purification of the nephritogenic antigen possible. Therefore, we believe that GBM-ST from bovine kidney gives a more adequate tool for the study of autoimmune glomerulonephritis.

Acknowledgements. The authors are very grateful to Dr. Haruo Takamiya for valuable advice and Mr. Katsuhiro Hongo for his excellent assistance.

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