Nephropathy in Dogs Induced by Treatment with Antiserum against Renal Basement Membrane

Kinji SHIROTA* and Kōsaku FUJIWARA

Department of Veterinary Pathology, Faculty of Agriculture,
University of Tokyo, Bunkyo, Tokyo 113

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ABSTRACT. The dogs having received intravenously or intraperitoneally rabbit antiserum to renal tubular basement membrane (TBM) or glomerular basement membrane (GBM) developed hematuria and proteinuria. Pathologically observed were focal and/or diffuse interstitial mononuclear infiltration with tubular degenerative changes in the renal cortex and generalized proliferative glomerulonephritis. Interstitial and glomerular lesions were not consistently correlated in intensity. By electron microscopy, mononuclear cells were occasionally in close contact with TBM, and some of them invaded the space between the tubular epithelial cells in the cortex. A continuous linear pattern of rabbit IgG was seen on GBM in all cases treated with both antisera, whereas a discontinuous linear pattern was observed on cortical TBM of a single case by immunofluorescence. In the dogs immunized with canine TBM, neither renal lesions nor autoantibodies against homologous renal tissues were produced.

Anti-tubular basement membrane (TBM)-mediated interstitial nephritis (IN) has extensively been studied during the past decade. In human beings, tubular and interstitial renal lesions associated with anti-TBM antibody deposit were found in cases of Goodpasture's syndrome [14,20,28], immune complex glomerulonephritis [27], primary IN [2], methicillin-associated IN [4], and renal transplantation [9].

Experimental studies on anti-TBM-mediated IN have been made mostly in the guinea-pigs [1,12,25] and rats [13,21,26] immunized with the homologous kidney homogenate or renal basement membrane. Only a few reports, however, dealt nephrotoxic glomerulonephritis of dogs without any detailed description of interstitial lesions [19,29].

This paper deals with the histopathological and immunopathological observations of renal lesions in the dogs treated with either anti-TBM or anti-glomerular basement membrane (GBM) rabbit serum.

MATERIALS AND METHODS

TBM and GBM samples: Both TBM and GBM samples were prepared from the perfused kidneys of adult mongrel dogs by a modification of the method described by Krisko et al. [11]. The renal cortex was minced and forced to pass through 600, 250, 177 and 125-μm pore-sized stainless-steel sieves by adding a large amount of chilled physiological saline (PS). The material passing through the 125-μm pore-sized mesh was once again filtrated through the sieve of the same size. The filtrate, being rich in tubular fragments, was sonicated for 10 min to give a TBM sample. The material retained on the 125-μm pore-sized sieve was subjected to sonication for 9 min, and then it was allowed to pass through a 74-μm pore-sized mesh. The sample retained on this sieve was found to be rich in glomeruli, and it was subjected to sonication

* Present address: Department of Veterinary Pathology, Azabu University Veterinary School, 1-17-71 Fuchinobe, Sagamihara, Kanagawa 229
for 30 min to isolate GBM.

**Antiserum to TBM and GBM**: Male albino rabbits weighing about 3 kg were each injected into the footpads with 4 ml of a PS-suspension containing 100 mg wet weight of the canine TBM or GBM sample mixed with an equal volume of Freund's complete adjuvant (FCA). A month later, a booster injection was given intracutaneously with 0.5 ml of a suspension containing 25 mg of each sample and the animals were bled 14 days later. The anti-TBM and anti-GBM antisera as well as normal rabbit serum (NRS) were absorbed three times with canine red blood cells and heated at 56°C for 30 min, and they were let pass through 0.8-μm and 0.45-μm pore-sized filters (Millipore Corporation, Bedford, Mass., U.S.A.). As shown in Fig. 1, both anti-TBM and anti-GBM rabbit sera were shown to react with cortical TBM and GBM of the normal dog kidney in immunofluorescence. Both the antisera reacted also with Bowman's capsule.

**Serum treatment**: Three of four mongrel dogs of the same litter aged 13 weeks weighing 3 to 4 kg received intravenous injection with anti-TBM serum (Cases A, B and C) and the remaining one with NRS (Case D) (2 ml/kg body weight). Two mongrels of another litter aged 20 weeks weighing 5 to 6 kg received intraperitoneal injection with 50 ml of anti-TBM serum (Case E) or NRS (Case F). In addition, four mongrel littersmates aged 18 weeks weighing 3.5 to 4.8 kg were also treated intravenously with anti-GBM serum (Cases G, H and I) or NRS (Case J) (2 ml/kg body weight). Animals were sacrificed at different times after treatment, as shown in Table 1 and 2. Urine samples were checked for proteinuria and hematuria by reagent strips (Hema-Combi-stix-II, Miles-Sankyo Co., Tokyo). Urinary sediments of the dogs treated with anti-TBM serum were subjected to light microscopy.

**TBM immunization**: Four dogs, two mongrels and two beagles, aged 33 weeks weighing 8 to 10.5 kg, each received an intradermal injection at multiple sites of 1.3 ml of a suspension containing 60 ml of a canine TBM sample mixed with an equal volume of FCA. Beside the injection sites, a pertussis vaccine containing 2×10^8 organisms was injected. As controls, each of one 33-week-old mongrel and beagle dogs weighing 8 and 10 kg, respectively, received 1.3 ml of PS mixed with an equal volume of FCA and the pertussis vaccine. Forty-two and 91 days later, two TBM-immunized, one each of of beagle and mongrel, and one control beagle were sacrificed. The remaining cases received additional four injections in the same way every other week and sacrificed 28 and 35 days after the last injection.

**Histopathology**: Kidney tissues were fixed in 10% neutral buffer-formalin, embedded in paraffin, and 4-μm sections were made and stained with hematoxylin-eosin (HE) and periodic acid-Schiff (PAS). For electron microscopy, small tissue pieces were fixed overnight in 2.5% glutaraldehyde, post-fixed in 1% osmium tetroxide for 90 min, and embedded in Epon 812. Ultra-thin sections were made with an LKB ultramicrotome and stained with uranyl acetate and lead nitrate. Observation was made under a JEM 100S electron microscope at 80 kV.

**Immunofluorescence**: Small samples of the kidney tissues from dogs of different groups were frozen in hexane-dry ice acetone. Cryostat sections were made and treated overnight at 4°C with fluorescein isothiocyanate (FITC)-labelled rabbit anti-dog gamma globulin. Sections were stained overnight also with FITC-labelled anti-rabbit IgG goat serum at 4°C. The specificities of anti-TBM and anti-GBM rabbit sera were confirmed by treating normal dog kidney sections with each antiserum at 37°C for 60 min followed by treatment with FITC-labelled anti-rabbit IgG goat serum. The serum samples from actively immunized dogs were checked for the anti-renal basement membrane activity by indirect immunofluorescence with a normal canine kidney substrate.

**RESULTS**

**Changes after treating with anti-TBM rabbit serum**

All cases treated with the anti-TBM rabbit serum developed hematuria and proteinuria. From 3 days postinjection, urinary casts, red blood cells and epithelial cells were seen in urinary sediments.

At autopsy, the kidneys of the treated dogs were slightly swollen, and some of them showed several red pin-point foci on the surface.

As presented in Table 1, interstitial infiltration of mononuclear cells and a few neutrophils were detected in the cortical area of all animals examined of this group. On day 7 (Case C), interstitial infiltration of mononuclear cells was the severest with some mitotic figures. A few spindle-shaped cells were present in the lesion. Some
renal tubules showing degenerative and necrotizing changes were surrounded by migrating cells, and sometimes the normal structure disappeared with wrinkling, thickening and partial disappearance of TBM (Figs. 2 and 3). Mitotic figures of the cortical tubular epithelial cells were seen and some tubules contained proteinaceous casts, cell debris and erythrocytes. Edema and cellular infiltration resulted in distention of the interstitium.

In the glomeruli, seen were necrosis of the tufts with some mitotic figures and hypercellularity. Proliferation of parietal epithelial cells, crescent formation and capsular adhesion were observed (Fig. 4) and there were fibrinous materials in the Bowman’s spaces.

Electron microscopy revealed a large number of lymphoid cells and macrophages as well as some neutrophils and plasma cells having been accumulated in the interstitium. Some cortical tubular cells were degenerated showing irregular thickening, wrinkling and duplication of TBM. Frequently, the migrating cells were in contact with TBM by their microvilli. Some mononuclear cells or neutrophils invaded either the cytoplasm of tubular cells or the spaces between the epithelial cells. There were no TBM gaps in the adjacent sites (Fig. 5).

Within the glomerular capillary lumens, many mononuclear cells and some neutrophils were accumulated, and some of them were in direct contact with GBM (Fig. 6). The epithelial foot processes were frequently fused each other with irregular dilatation of the lamina rara interna including some flocculent materials.

As shown in Table 1, a linear-pattern deposition of rabbit IgG was detected along GBM in all anti-TBM-treated cases (Fig. 7). In Case E treated intraperitoneally with the antiserum, however, some TBM were stained with rabbit IgG in a discontinuous linear fashion (Fig. 8). Canine gamma-globulin was also found as a linear fluorescence on GBM in Cases B and C.

There were no changes in the kidneys of NRS-injected controls.

### Changes after treating with anti-GBM rabbit serum

Various intensities of proteinuria and hematuria were revealed in all cases treated with anti-GBM serum. Gross renal lesions were similar to those of the cases treated with anti-TBM serum.

As shown in Table 2, interstitial cellular infiltration was seen in all cases having
received anti-GBM serum. In Case H, there were severe tubular and interstitial changes with diffuse or focal mononuclear infiltration around the cortical vessels and tubules (Fig. 9). A few multinucleated giant cells were seen. Glomerular changes were similar to those in the case treated with anti-TBM serum.

By immunofluorescence, rabbit IgG was clearly demonstrable on GBM in a linear pattern as shown in Table 2. Additionally in Case I, deposition of canine gamma globulin was detected in a linear fashion on GBM as well as TBM. Active immunization with canine TBM samples

None of those immunized with canine TBM developed renal lesions, and direct immunofluorescence revealed no canine gamma globulin in the kidneys of the TBM-immunized cases or controls. The serum from these animals failed to react with TBM and GBM on the normal dog kidney sections.

**DISCUSSION**

Both TBM and GBM antisera used in this study were shown to react in vitro with not only cortical TBM and GBM but also Bowman’s capsule of the canine kidney. Such cross reactions may have probably been due to either the presence of a common antigen shared by GBM and TBM [6, 12] or the impurity of both base-
ous linear deposition of immunoglobulin on TBM as well as GBM, but they had no correlation with glomerular lesions in intensity. The tubular damage might be attributed to the antibody binding to TBM. Electron microscopy demonstrated that mononuclear cells were in close contact with TBM sometimes penetrating the epithelial side as already described in the rat [26] and guinea-pig [1] models of immune-mediated IN, suggesting the importance of mononuclear cells in development of tubular damages.

In nephrototoxic glomerulonephritis, the fixation of antibody on GBM is said to be an initial step triggering the activation of the complement system followed by neutrophil infiltration and glomerular injury [5, 7]. Recently, it was suggested that mononuclear cells may also be involved in pathogenesis of anti-GBM glomerulonephritis [3, 8, 10]. In the guinea-pig model of anti-TBM-mediated IN, Andres et al. [1] suggested that the TBM damage followed by tubular epithelial changes would result from the antibody-mediated toxicity of lymphocytes. In the rat model of anti-TBM-mediated IN, it was shown that the lesions were initiated by infiltration of polymorphonuclear leukocytes followed soon by that of mononuclear cells [13, 21, 26]. In this study, however, the presence of an early leukocyte-rich phase was not evidenced in the renal interstitial lesions.

In man, interstitial mononuclear infiltration in association with tubular damage has been observed in various diseases including different types of glomerulonephritis. These lesions have erroneously been attributed to superimposed pyelonephritis or to a certain preceding glomerular changes [17, 26]. In dogs, we reported that interstitial mononuclear infiltration with or without glomerular lesions was still high incidence accompanying frequently glomerular lesions and without evidences of leptospirosis [23]. From the results of this and previous studies [24], it is assumed that some of naturally occurring IN in dogs might be attributed to autoimmunological reactions involving renal tubular components.

As a trigger for the production of anti-renal basement membrane antibody, chemical substances or infective agents were proposed [4, 22, 28]. In addition, the presence of GBM-cross-reactive materials was demonstrated in the urine of normal human [18] and of the dogs infected with adenovirus [30]. The basement membrane in the urine was shown to have some autoantigenic potential to induce anti-GBM glomerulonephritis [16].

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REFERENCES


[29] Wright, N. G., Thompson, H., and Cornwell,


**EXPLANATION OF FIGURES**

Figs. 2 to 6 are from Case C on day 7 post-injection with anti-TBM rabbit serum.

Fig. 1. Indirect immunofluorescence on TBM, GBM and Bowman's capsule of a normal dog kidney treated with anti-TBM rabbit serum. ×280.

Fig. 2. Prominent mononuclear infiltration in the cortical interstitium. HE stain. ×320.

Fig. 3. Destruction of TBM and tubular structure. PAS stain. ×360.

Fig. 4. Crescent formation in a glomerulus. PAS stain. ×370.

Fig. 5. Mononuclear cell infiltration in the cytoplasm of tubular epithelium. Asterisk=TBMs. Bar=1 μm.

Fig. 6. Mononuclear cell accumulation in a glomerular capillary lumen (CL) in direct contact with GBM (arrow). Asterisk=GBM. BS=Bowman's space. Bar=1 μm.

Fig. 7. A linear intense rabbit IgG-specific fluorescence along GBM. Case A on day 3 post-injection with anti-TBM rabbit serum. ×175.

Fig. 8. A discontinuous linear deposition of rabbit IgG on cortical TBM. Case E on day 5 post-injection with anti-TBM rabbit serum. ×190.

Fig. 9. Mononuclear infiltration around the cortical vessels and tubules. Case H on day 5 post-injection with anti-GBM rabbit serum. HE stain. ×165.

**要　約**

抗腎基底膜抗体による犬の腎 病 変：代田欣人・藤原公策（東京大学農学部家畜病理学教室）——抗腎尿細管基底膜（TBM）、抗系球体基底膜（GBM）ウサギ血清をイヌの静脈内あるいは腔内に投与すると、全例に血尿・タンパク尿が認められた。病理学的には皮質間質における尿細管の変性を伴う尿細管の高電流状及び細胞内浮遊状の変性が見られた。腎実質変化は、腎質においてしばしば TBM に密着した単核細胞が見られ、尿細管上皮細胞内及び細胞内へ侵入する像も認められた。免疫蛻光法では、両抗血清投与のいずれの GBM にウサギ IgG の線状沈着を認めたが、皮質 TBM では不連続線状の沈着が 1 例のみに見られた。イヌ TBM で免疫されたイヌでは腎病理の発現、同種腎組織に対する自己抗体の産生は認められなかった。