Abnormal Distribution of Anionic Sites in the Glomerular Basement Membrane in Glomerulonephritis of Dogs Infected with *Dirofilaria immitis*

Junichi KAMIIE, Kinji SHIROTA, Munetaka YAMAKI, Hitoshi KITAGAWA, Masahiko WASAKI and Hong-Keun OOI

1Laboratory of Veterinary Pathology and 2Research Institute of Biosciences, Azabu University, 1–17–71 Fuchinobe, Sagamihara, Kanagawa 229–8501, 3Laboratory of Internal Medicine, Division of Veterinary Medicine, Faculty of Agriculture, Gifu University, Yanagido 1–1, Gifu 501–1193, 4Mitsubishi Tokyo Pharmaceutical Co., Ltd, 1000 Kamoshida, Midori-ku, Yokohama 227–0033, Japan and 5Department of Veterinary Medicine, National Chung Hsing University, 250 Ku Kuan Road, Taichung 40227, Taiwan

(Received 11 April 2000/Accepted 11 July 2000)

**Abstract.** Ultrastructural alteration of anionic sites (ASs) in the glomerular basement membrane (GBM) was studied in glomerulonephritis characterized by linear capillary IgG deposition in four dogs infected with *Dirofilaria immitis* and two normal control dogs using polyethyleneimine. ASs were identified as small dense particles distributed regularly in the lamina rara externa (LRE), but there were no ASs in the lamina densa (LD) of the GBM of the control dogs. In the glomeruli of the infected dogs, ASs were distributed regularly or irregularly in the thickened LD. ASs were in addition localized over the characteristic continuous bands of subendothelial dense deposits. The number of ASs of the LRE increased in all four infected cases as compared to the controls (p<0.01).

**Keywords:** anionic site, canine dirofilariasis, glomerulonephritis.

Glomerulonephritis accompanied by proteinuria is common in dogs naturally infected with *Dirofilaria immitis* (*D. immitis*), and histologically the condition is characterized by thickening of the glomerular basement membrane (GBM) with electron-dense deposits and mesangial expansion [2, 4]. Ultrastructural studies using cationic probes such as polyethyleneimine (PEI) have shown a reduction in the number of ASs in the GBM in humans [3, 8] and experimental glomerulonephropathies [9, 11]. These studies have shown that the reduction of ASs in the GBM might be associated with development of proteinuria. However, the charge properties of the GBM have not been studied in dirofilarial glomerulopathy. The purpose of this study is to demonstrate an alteration of ASs of the GBM in the glomerulonephritis in canine dirofilariasis.

Four *D. immitis*-infected dogs (Nos. 3–6) with proliferative glomerulonephritis (Fig. 1) showing mild or severe proteinuria checked by reagent strips were examined. Two normal beagle dogs, Nos. 1 and 2, were used as a control.

The kidney samples were fixed in 10% neutral-buffered formalin and embedded in paraffin. Histologic sections were stained with hematoxylin-eosin (H.E), periodic acid-Schiff (PAS). For immunofluorescence microscopy, dewaxed sections were digested with 0.2% trypsin and incubated overnight at 4°C with fluorescein isothiocyanate-conjugated goat anti-dog IgG (Organ Teknika Corp., Durham, NC). After washing the sections in phosphate-buffered saline (PBS) for 15 min, we examined them using a fluorescence microscope.

Ultrastructural study for ASs with PEI (molecular weight =1,800; Polysciences, Warrington, PA) was performed as described previously [10,11]. For the quantitative analysis of ASs in peripheral portions of the GBM, the PEI particles within the lamina rara externa (LRE) were counted. A total of 76 to 128 portions of 1,000 nm-length of GBM in two or three glomeruli were observed in each dog. The PEI particles were counted only in clearly cross-sectioned regions that afforded a clear view of epithelial cell membrane. Statistical comparison of the number of ASs per 1,000 nm of GBM in each dog infected with *D. immitis* and that in the two control beagle dogs was performed by Mann-Whitney's U-test.

Histopathologically, moderate to marked and diffuse global mesangial expansion and thickening of the capillary wall with frequent duplication of the GBM was found in the glomeruli of all infected dogs.

Electron microscopy showed moderate to marked thickening of the GBM and a continuous dense band composed of fine granular deposits in the subendothelial regions in all infected dogs. The mesangial interposition into the subendothelial space resulting in the formation of a new GBM-like matrix was frequently observed. The effacement of the epithelial foot processes was found over a wide area.

Immunofluorescence microscopy revealed a characteristic diffuse global distribution of intense linear IgG deposition over the glomerular capillary walls in all infected dogs (Fig. 2). No IgG deposition was seen in the renal glomeruli of the two control beagles.

Electron-dense particles of PEI interpreted as ASs were distributed in a single layer in the LRE and lamina rara interna (LRI) in the normal GBM of the two control dogs (Fig. 3). Particularly in the LRE, they were arranged regularly. In the LRI, ASs were observed to be irregular in pattern and fewer in number. There were no ASs in the lamina densa (LD) of the GBM of the control dogs. In the thickened GBM of the infected dogs, the ASs were distributed regularly.
in the LRE and were arranged regularly in several layers or irregularly in the others in the thickened LD (Fig. 4). The size of PEI particles in the thickened LD were smaller than that in the LRE. PEI particles of various sizes were localized over the subendothelial dense deposits (Fig. 5). In the markedly thickened capillary walls associated with mesangial interposition, PEI particles of various sizes were distributed irregularly over an increased GBM-like matrix.

The number of ASs per 1,000 nm-length of the LRE in infected dogs was significantly higher than that in control dogs (P<0.01) (Table 1). This study showed alteration of the number of ASs in the GBM in four dogs infected with *D. immitis*. ASs in the LRE of the normal GBM were regularly distributed, however, those in the LRI distributed irregularly and were much fewer than those in LRE as described previously [10]. We thought that it was not easy to evaluate the charge property of the LRI. Therefore, we quantitatively assessed ASs in the LRE of the GBM in this study.

The reduction in the number of ASs in the LRE has been reported in cases of human [3, 8] and experimental [9, 11] glomerulonephritis. The present four dogs infected with *D. immitis* had histopathologically membranoproliferative glomerulonephritis-like nephropathy, which is common in this parasitic disease [2, 4]. Glomerular ASs in the LRE were
Table 1. Glomerular lesions and anionic sites in the kidneys of dogs infected with *Dirofilaria immitis*

<table>
<thead>
<tr>
<th>Dog No.</th>
<th>Double contour of glomerular capillary wall</th>
<th>Proliferation of mesangial cells</th>
<th>Distribution of IgG</th>
<th>Thickening of GBM</th>
<th>Sebenothelial dense deposit</th>
<th>Mesangial interposition</th>
<th>No. of anionic site/1000 nm-length of LRE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>17.1 ± 2.1</td>
</tr>
<tr>
<td>2</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>18.4 ± 2.1</td>
</tr>
<tr>
<td>3</td>
<td>++</td>
<td>+++</td>
<td>D-G</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>18.8 ± 2.6**</td>
</tr>
<tr>
<td>4</td>
<td>++</td>
<td>+++</td>
<td>D-G</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>21.1 ± 2.7**</td>
</tr>
<tr>
<td>5</td>
<td>++</td>
<td>+++</td>
<td>D-G</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>19.4 ± 2.5**</td>
</tr>
<tr>
<td>6</td>
<td>++</td>
<td>+++</td>
<td>D-G</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>20.0 ± 2.1**</td>
</tr>
</tbody>
</table>

* Abbreviations: LM=Light microscopy; IF=Immunofluorescence microscopy; EM=Electron microscopy; GBM=Glomerular basement membrane; LRE=Lamina rara externa

** Significantly different from control dogs (Nos. 1 and 2), Mann-Whitney U-test, p<0.01.

increased in number irrespective of the presence of thickened GBM or mesangial interposition in the infected dogs. There were no ASs in the LD of normal GBM in control dogs; however, ASs were regularly arranged in several layers or distributed irregularly in the thickened LD of infected dogs.

It has been reported that ASs of the GBM are mainly composed of heparan sulphate proteoglycan (HSPG), which is one of the major constituents of the GBM [6]. We performed immunostaining of HSPG in the four cases examined in this study, but we could not obtain usable results for the evaluation of staining property (data not shown). Nonetheless, it is possible that the altered distribution of ASs in thickened GBM might be due to irregular distribution of HSPG.

PEI particles did not bind to the immune deposits in the glomeruli from human patients with glomerulonephritis [7] and pigs with mesangial proliferative glomerulonephritis [10]. In this study, we detected continuous bands of subendothelial dense deposits that were consistent with linear continuous IgG deposition along the capillary walls in infected dogs by immunofluorescence as described previously [1]. Experimental studies suggest that *in situ* immune complex formation is a part of glomerular damage in canine dirofilariasis [5]. In the present study, we found that the characteristic subendothelial dense deposits were associated with various sized PEI particles. In the theory of *in situ* immune complex formation in dirofilarial glomerulonephritis, the parasitic antigens bind to GBM followed by reaction of antibodies against these antigens resulting in formation of immune deposits in GBM [5]. Therefore, the components of subendothelial dense deposits in dirofilariasis might be different from those of the other types of glomerulonephritis and might include some GBM components or parasitic antigens engulped into dense deposits.

In conclusion, the alteration of ASs in dirofilarial glomerulonephritis is mainly a matter of their distribution in the GBM, which might be associated with a disturbance of the charge-selective barrier. However, the clinical significance and the specificity of abnormal distribution of ASs in the GBM in dirofilarial glomerulonephritis were not clarified in this study.

ACKNOWLEDGEMENTS. The authors thank Drs. Yasuo Nomura and Yumi Une, Laboratory of Veterinary Pathology, Azabu University for encouragement of this study. This study was partially supported by Kitayama Labes Co., Ltd.

REFERENCES