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An electron microscopic study of glomerular lesions in hereditary nephrotic mice (ICGN strain)

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Summary. Glomerular lesions in hereditary nephrotic mice (ICGN strain) were investigated by electron microscopy. The glomeruli of unaffected animals, which appeared normal by light microscopy, had developed an ultrastructural change in the glomerular capillary basement membrane (GCBM). There was a partial thickening of the GCBM with bilaminar splitting of the lamina densa and an electron-dense fibrillar material exhibiting cross-striations. In affected animals, light microscopy revealed a marked thickening of GCBM and an increase of mesangial matrix without cellular proliferaton. By electron microscopy, multilaminar splitting of the lamina densa in the thickened GCBMs and fusion of the epithelial foot processes were observed. In some severely affected animals, immune complex deposition was found in GCBM, but little if any was observed in other animals. In the end, the glomeruli were globally sclerosed. Our findings suggest that initial structural abnormalities in GCBM may play an important role in the onset and development of the disease, though subsequent events such as immune complex deposition would modify the disease.

Key words: Mouse – Nephrotic syndrome – Hereditary nephritis – Electron microscopy

Introduction

In our previous papers (Ogura et al. 1989a, b), we reported some clinical and pathological characteristics of the hereditary nephrotic mice (ICGN strain, inbred) maintained at the National Institute of Health (Japan), and presented their availability as a model for human idiopathic nephrotic syndrome. The affected mice of this strain consistently show the clinical signs of nephrotic syndrome, developing various degrees of glomerular lesions. The onset of the disease is usually observed before 150 days of age. Affected animals die from chronic renal failure within a few months after onset. The progress of the disease can roughly be divided into the early, middle, and terminal stages according to changes in the macroscopic and microscopic appearances of the kidneys, which are closely correlated with the results of the clinical examinations. Light microscopy shows that glomerular lesions usually consist of thickening of the glomerular capillary basement membrane (GCBM) and mesangial expansion without noticeable cellular proliferation. Noteworthy in this disease is the absence of abnormalities in other organs or tissues.

The present investigation was undertaken to make a more exact evaluation of the glomerular lesions in ICGN mice by electron microscopy. It demonstrated characteristic ultrstructural development of the glomerular lesions including splitting of the lamina densa in the GCBM.

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Materials and methods

A total of 31 inbred ICGN mice (15 males, 16 females; weight 14.0–39.0 g; age 21–234 days) from a specific-pathogen-free colony in our laboratory were used in this study. They were housed in autoclaved metal cages and provided free access to laboratory diet and water. Four female ICR mice obtained from SLC (Shizuoka, Japan) were housed under the same conditions and served as controls on 40, 70, 100 and 130 days after birth, respectively. The animals were killed by an overdose of ether, and the specimens were taken for light microscopic [haematoxylin and eosin (H & E), periodic acid-Schiff (PAS) and phosphotungstic acid haematoxylin stains], immunofluorescent (anti-mouse IgG, IgA, IgM, and C3), and electron microscopic observations. For light and immunofluorescence microscopy, these materials were processed by the methods previously reported (Ogura et al. 1989a). For electron microscopy, blocks of renal cortex 1–2 mm square and 0.5 mm thick were fixed in 3.1% glutaraldehyde in cacodylate buffer (0.1 M, pH 7.3) for 3–12 h, rinsed in cacodylate buffer containing 5% (w/v) sucrose, and then post-fixed in 1% osmium tetroxide. After dehydration through ascending grades of ethanol, they were embedded in a mixture of Epon and Araldite. Ultra-thin sections (60–80 nm thick)

were made on an LKB III microtome, mounted on copper grids, and stained with aqueous uranyl acetate and Reynolds' lead citrate. They were observed with a Hitachi H-7000 electron microscope at 75 keV. In each animal, between two and five glomeruli were examined.

Results

For clarity, the electron microscopic findings as well as the light microscopic and immunofluorescent observations are presented in order. By light microscopy, 12 of 31 animals appeared unaffected. Of the 19 affected aniamls, 4, 4 and 11 animals were evaluated as early, middle, and terminal stages, respectively. All affected animals showed proteinuria, hypoproteinaemia, hyperlipidaemia, and either systemic oedema or emaciation.

In the unaffected animals, no morphological changes were detectable in the glomeruli by light microscopy. Faint to moderate fluorescence of IgA, IgG and IgM was observed only in the mesangial area. Electron microscopy occasionally revealed a mild thickening of the GCBM, which showed bilaminar splitting of the lamina densa, with the subepithelial portion protruding toward the urinary space (Fig. 1a). Small hump-like structures of GCBM protruding into the epithelial cells were often observed (Fig. 1b). These protrusions were usually filled with an electron-dense mass continuous with the lamina densa of the neighbouring areas. In some of the thickened GCBMs, there was unique fibrillary electron-dense

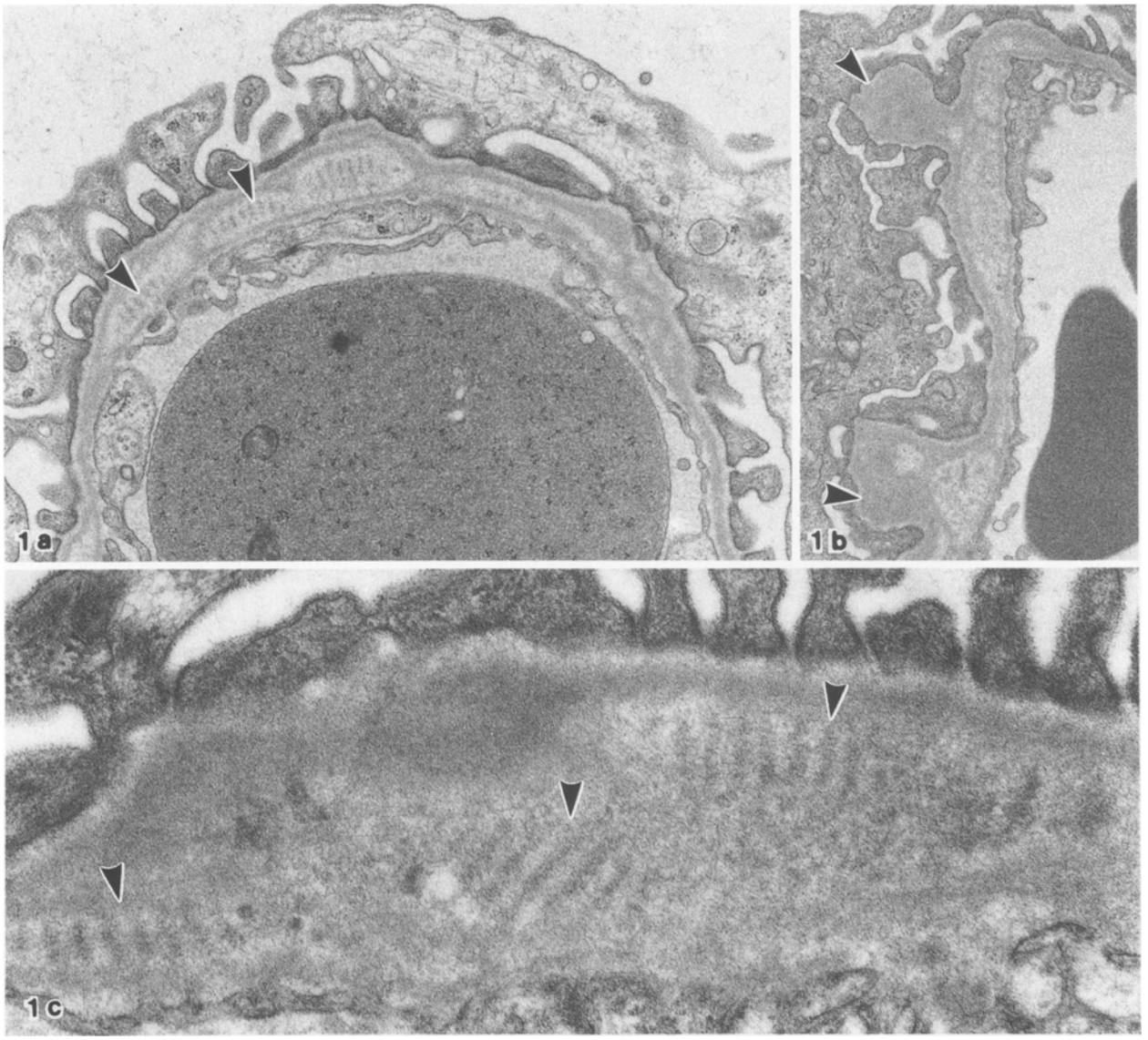


Fig. 1a–c. Electron micrograph of glomerular capillaries from unaffected mice. **a** Bilaminar splitting of the lamina densa. Note electron-dense material with cross-striations (*arrowheads*). ×17500. **b** Small hump-like lesions protruding into the epithelial cells (*arrowheads*). They are filled with electron-dense material which seems to be homologous with the lamina densa. ×12500. **c** Higher magnification of the electron-dense material in the glomerular capillary basement membrane (GCBM) (*arrowheads*), showing that the material consists of a number of fibrils. The cross-striations are repeated at about 100 nm intervals. ×43000

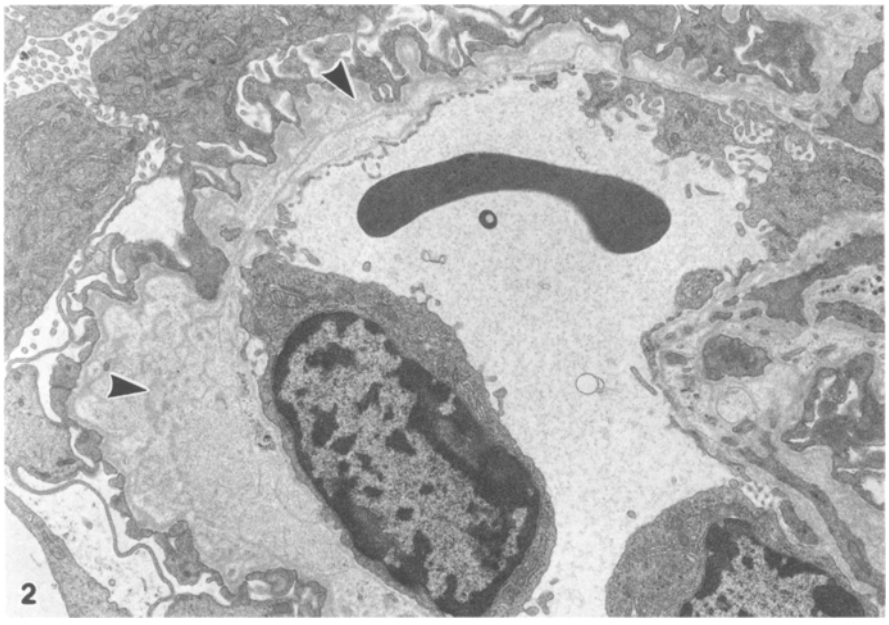
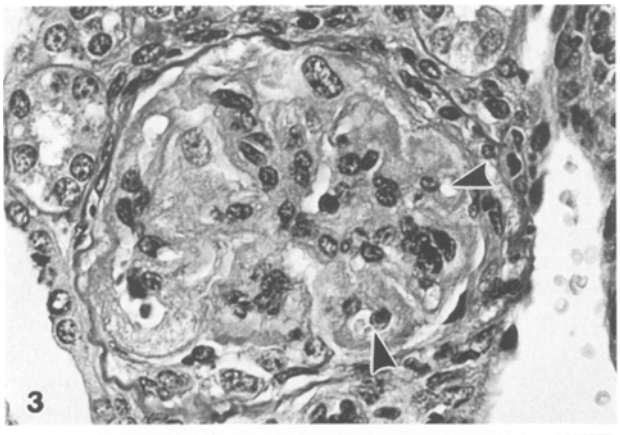


Fig. 2. Electron micrograph of irregularly thickened GCBM in the early stage. Multilaminar splitting of the lamina densa (*arrowheads*) is evident. × 7000



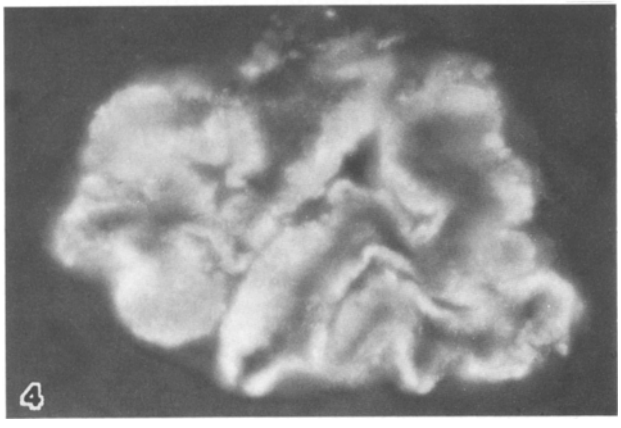


Fig. 3. Light micrograph of the glomerulus of a mouse in the middle stage. Note accumulation of PAS-positive material in the capillary wall and the mesangium, which causes reduction of the capillary lumina (*arrowheads*). PAS, × 440

Fig. 4. Immunofluorescent staining for IgG. The middle stage. Granular deposition along the capillary loops is noted. × 440

material with cross-striations repeated at about 100 nm intervals (Fig. 1a, c). Fusions of the epithelial foot processes or swelling of the endothelial cells were rarely found.

In the early stage, light microscopy revealed a moderate thickening of the GCBM and a slight, segmental enlargement of the mesangial area. Cellular proliferation was not usually observed in the glomeruli. Immunofluorescence for IgA, IgG and IgM revealed a fine granular pattern along the capillary loops. Electron microscopy showed extensive multilaminar splitting of the lamina densa (Fig. 2). Swelling of the epithelial cells and fusion of their foot processes were constantly observed. The endothelial cells often showed slight swelling.

In the middle stage, light microscopy showed severe thickening of the GCBM and moderate to great mesangial expansion, both of which were responsible for reduction of the patency of the capillary lumina (Fig. 3). Immunofluorescent examination demonstrated a heavy deposition of IgA, IgG and IgM along the capillary loops (Fig. 4). In contrast, in the mesangium, the intensity of immunofluorescence was significantly less than that of the previous stages. Electron microscopy showed varying degrees of splitting of the lamina densa, which was often frayed and poorly defined (Fig. 5a). In 2 of 4 animals, electron-dense deposits were occasionally observed in the thickened GCBM (Fig. 5b). These deposits showed a great variety in size and lay apart from the epithelial cells and the endothelial cells. The endothelial pores had usually disappeared and the epithelial foot processes were fused extensively. Collagen fibrils were identified within the markedly expanded mesangial matrix and in the thickened GCBM. Partial mesangial interposition in the glomerular capillary wall was often observed (Fig. 5a).

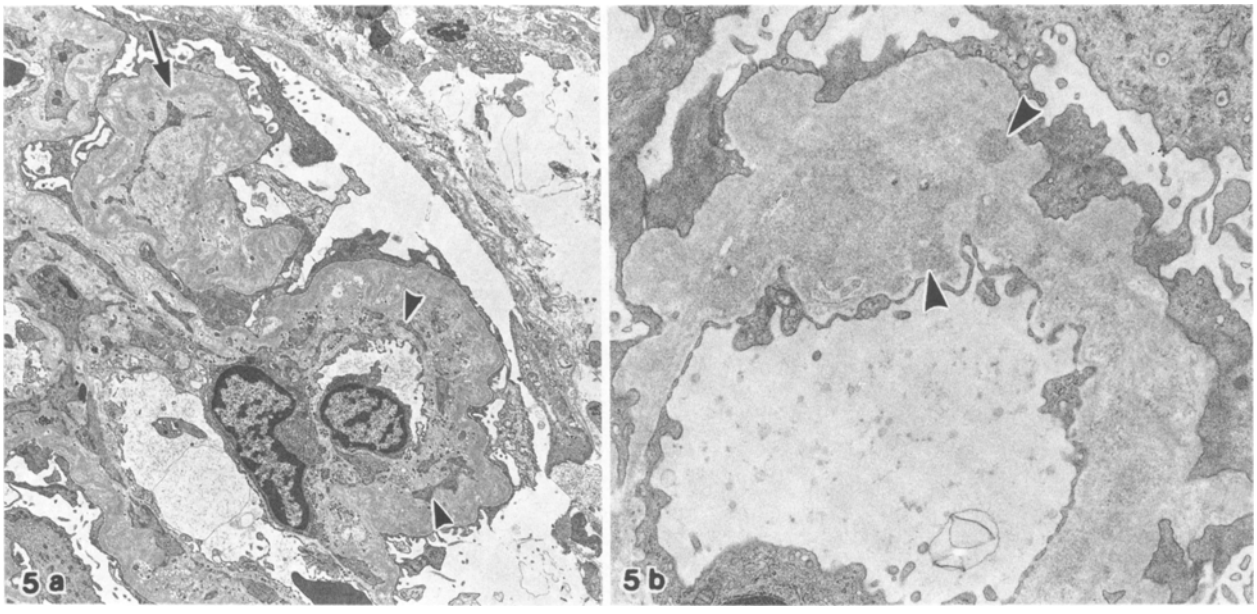


Fig. 5a, b. Electron micrographs of glomerular capillaries of mice in the middle stage. **a** Capillary loops showing severe thickening of GCBM. The lamina densa is often frayed, developing irregular, multilaminar splitting (*arrow*). Mesangial interposition is visible (*arrowheads*). ×3100. **b** Electron-dense deposits in GCBM (*arrowheads*). ×10500

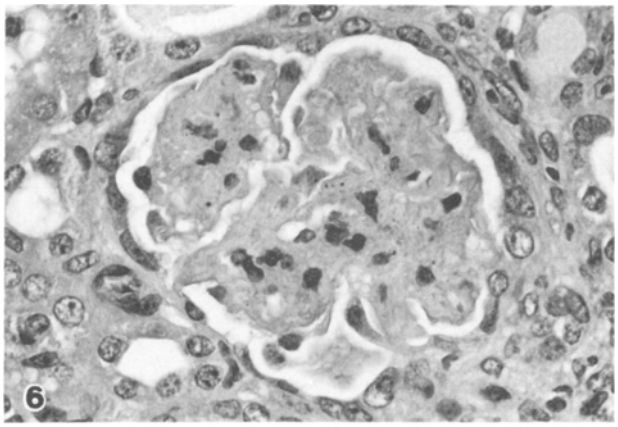
In the terminal stage, examination light microscopy showed apparent sclerotic mesangial expansion with loss of structure and almost complete occlusion of the glomerular capillaries (Fig. 6). Finally, the glomeruli were composed of a large amount of PAS-positive material and a few cellular components. Immunofluorescence demonstrated that deposits of IgA, IgG and IgM formed lumpy and globular lesions of the occluded capillary loops. The cells of the glomeruli on electron microscopy were highly transformed, appearing as polygonal fragments (Fig. 7). In 7 of 11 animals, large amounts of electron-dense deposits were found in the GCBM. There were membrane-bound spherical structures of variable sizes which were interpreted as cell debris. In most GCBMs, the lamina densa was poorly defined. Fibrin-like material was often observed in the urinary space. Glomerular crescent-like structures appeared in 30–50% of the glomeruli in this stage.

The positive staining for mouse C3, which was no more intensive than that for immunoglobulins, was often present in the capillary loops from the middle stage. Immunofluorescence for C3 tended to be relatively intensive in the animals with a large amount of electron-dense deposits. Sections stained with phosphotungstic acid haematoxylin did not indicate intravascular deposition of fibrin. Inflammatory cells were rarely observed in the glomeruli. Retrovirus-like particles, which resembled type-C particles, sometimes appeared in the mesangial matrices and less frequently in GCBMs, but there was no correlation between the appearance of these particles and the severity of the disease. No significant differences were noted between sexes in the morphology of the glomerular lesions.

In ICR mice, light microscopy showed no abnormalities in the kidneys. Immunofluorescence was observed only in the mesangial area. Electron microscopy revealed no particular glomerular lesions except for occasional small hump-like structures in the GCBM.

Discussion

The present study illustrates the development of glomerular lesions in nephrotic ICGN mice with electron microscopy. The results support the main findings of light microscopy (Ogura et al. 1989a); thickening of GCBM, a significant increase of the mesangial matrix without noticeable cellular proliferation, and resultant occlusion of the glomerular capillaries. The most prominent feature of the GCBM structure observed with electron microscopy was splitting of the lamina densa. It was also found in unaffected animals and extensively developed as the disease advanced. Multilaminar splitting of the lamina densa is the most important manifestation of hereditary nephritis in human and Samoyed dogs, in which nephrotic syndrome develops (Atkin et al. 1988; Jansen et al. 1984). In these hereditary diseases, the structural disorder of the GCBM is known to be closely related to an absence of Goodpasture antigen, which is contained in the collagenase-resistant sequences of type IV collagen (Wieslander et al. 1984; Thorner et al. 1987). Splitting of the lamina densa was also reported in experimentally produced GCBM lesions, which were accompanied by an excess accumulation of normal GCBM components (Thorning and Vracko 1977; Morel-Maroger et al. 1978; Moss et al. 1988). These lesions were not followed by severe structural changes in the glomeruli and did not lead to nephrotic syndrome. Therefore, it is reasonable to assume that the GCBM lesions in nephrotic ICGN mice are attributed to struc-



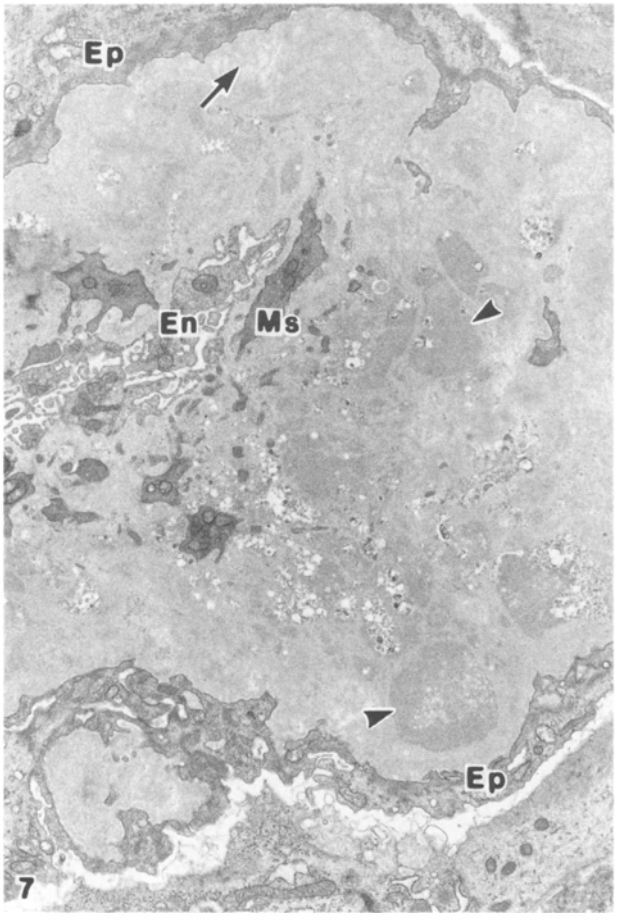


Fig. 6. Light micrograph of a glomerulus from a mouse in the terminal stage. It is globally sclerosed. H & E, ×440

Fig. 7. Electron micrograph of a capillary loop in the terminal stage. Large electron deposits are noted within the matrix (*arrowheads*). The component cells are highly transformed and the lamina densa is poorly defined (*arrow*). *En*, Endothelium; *Ep*, epithelium; *Ms*, mesangial cells. ×6700

tural abnormalities of collagenous proteins and/or other GCBM components which affect size and charge selectivity in filtration of macromolecules. The presence of regularly oriented fibrillar electron-dense material with cross-striations also suggests a structural disorder in the GCBM. Immunohistochemical studies using antibodies against GCBM components are now in progress in ICGN mice.

In ICGN mice, immunofluorescence revealed various degrees of granular deposition of immunoglobulins either in the mesangium or in the capillary walls, depending on the severity of the disease. By electron microscopy, however, electron-dense deposits were either absent in the glomeruli or, if present, less than expected from immunofluorescence. Therefore, most immunoglobulins detected by the immunofluorescent method may only be trapped, not involved in the formation of large molecules of immune complexes. Such incompatibility between the findings of immunofluorescence and electron microscopy has already been reported in normal mice and rats (Shimizu et al. 1977; Goode et al. 1988). Although electron-dense deposits observed in the middle and terminal stages probably represent trapped immune complexes, they never appeared in the earlier stages. Therefore, immune complex deposition may not play a primary causative role in this disease, though it would modify the disease.

Electron microscopy often revealed hump-like structures in the GCBM. They were filled with basement membrane material, not with immune complexes. Thus, the spike-like protrusions observed with light microscopy (Ogura et al. 1989a) may partly represent well-developed hump-like structures of GCBM. However, this feature is not specific for this disease, because ICR mice also showed the same small protrusions.

Previous studies have shown that nephrotic ICGN mice may serve as model for human nephrotic syndrome (Ogura et al. 1989a, b). The present study suggests that the disease in ICGN mice is caused by initial structural abnormalities in the GCBM rather than by immunopathological mechanisms. The ICGN strain of mice is unique in this respect. Although the exact nature of the GCBM lesions in ICGN mice remains to be determined, detailed investigation of the mice may provide new information on the pathology of the GCBM, especially that related to the onset of nephrotic syndrome.

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References

Atkin CL, Gregory MC, Border WA (1988) Alport syndrome. In: Schrier RW, Gottschalk CW (eds) Diseases of the kidney. Little Brown, Boston, pp 617–641

Goode NP, Davison AM, Gowland G, Shires M (1988) Spontaneous glomerular immunoglobulin deposition in young Sprague-Dawley rats. Lab Anim 22:287–292

Jansen B, Thorner PS, Singh A, Patterson JM, Lumsden JH, Valli VE, Baumal R, Basrur PK (1984) Hereditary nephritis in Samoyed dogs. Am J Pathol 116:175–178

Morel-Maroger L, Sloper JC, Vinter J, Biol MI, Woodrow D,

Gresser I (1978) An ultrastructural study of the development of nephritis in mice treated with interferon in the neonatal period. Lab Invest 39:513–522

Moss J, Shore I, Woodrow D, Gresser I (1988) Interferon-induced glomerular basement membrane and endothelial cell lesions in mice. Am J Pathol 133:557–563

Ogura A, Asano T, Matsuda J, Takano K, Nakagawa M, Fukui M (1989a) Characteristics of mutant mice (ICGN) with spontaneous renal lesions: a new model for human nephrotic syndrome. Lab Anim 23:169–174

Ogura A, Asano T, Matsuda J, Noguchi Y, Yamamoto Y, Takano K, Nakagawa M (1989b) Development of nephrotic ICGN mice. The origin, reproductive ability, and incidence of glomerulonephritis. Exp Anim 38:349–352

Shimizu F, Abe F, Ito K, Kawamura S (1977) On the age-associated presence of immunoglobulin and complement in the renal glomeruli of mice. Contrib Nephrol 6:79–93

Thorner P, Jansen B, Baumal R, Valli VE, Goldberger A (1987) Samoyed hereditary glomerulopathy: immunohistochemical staining of basement membranes of kidney for laminin, collagen type IV, fibronectin, and Goodpasture antigen, and correlation with electron microscopy of glomerular capillar basement membranes. Lab Invest 56:435–443

Thorning D, Vracko R (1977) Renal glomerular basal lamina scaffold. Lab Invest 37:105–119

Wieslander J, Barr JF, Butkowski RJ, Edwards SJ, Bygren P, Heinegard D, Hudson BG (1984) Goodpasture antigen of the glomerular basement membrane: localization to noncollagenous regions of type IV collagen. Proc Natl Acad Sci USA 81:3838–3842