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Case Report

Spontaneous hyaline glomerulopathy in a young Slc:ICR mouse

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Abstract: Hyaline glomerulopathy is a type of glomerular lesion that occurs in aging mice. Spontaneous hyaline glomerulopathy is rare in young mice. Here, we report spontaneous hyaline glomerulopathy in a young adult (15-week-old) ICR mouse. Necropsy revealed discoloration and roughness of the kidney surface. Microscopically, diffuse glomerular lesions were prominent. Amorphous, eosinophilic materials were deposited globally in the glomeruli. The mesangial region was expanded; however, the mesangial cells showed no proliferation. Thickening of the Bowman's capsule with proliferation of parietal epithelial cells was observed. Glomerular deposits were strongly positive for anti-IgM, anti-IgG, and periodic acid-Schiff stain and were stained red by Masson's trichrome stain. The deposits were negative for anti C3 and stained negatively with Congo red stain. Periodic acid methenamine silver and electron microscopy revealed glomerular deposits limited to intraglomerular capillaries. Based on the histological features, we diagnosed this lesion as hyaline glomerulopathy. This case could improve our understanding of spontaneous lesions in toxicological and pharmacological studies. (DOI: 10.1293/tox.2020-0027; J Toxicol Pathol 2020; 33: 303–307)

Key words: hyaline glomerulopathy, glomerulopathy, ICR mouse, spontaneous lesion, immunoglobulin

Slc:ICR mice are widely used in toxicological and pharmacological studies. Background data on ICR mice are useful in drug development to determine whether the encountered lesions are related to chemical toxicity or are spontaneous. With regard to glomerular lesions, spontaneous lesions in aging ICR mice have been extensively studied[1, 2]. Mutant strains derived from outbred ICR mice, termed ICGN mice, develop glomerular lesions characterized by thickened basement membranes of the capillary loops with irregular spike-like protrusions and enlargement of the mesangial region unaccompanied by cellular proliferation at an early age[3]. However, few reports have described glomerular lesions in young ICR mice[4–6].

Hyaline glomerulopathy is a type of glomerular lesion. The term hyaline glomerulopathy is used to describe a specific glomerular lesion, defined as the accumulation of non-amyloid, eosinophilic material in the glomeruli[7]; however, this term does not indicate composition of the accumulated material. Spontaneous hyaline glomerulopathy occurs in aging mice and rats[8, 9]; however, spontaneous hyaline glomerulopathy in young mice is rare. In this report, we describe a case of spontaneous hyaline glomerulopathy in a young ICR mouse.

A female Slc:ICR mouse was purchased from Japan SLC Inc. (Shizuoka, Japan) at 5 weeks of age. It was housed under controlled conditions (12-h light/dark cycle, temperature of 23 ± 3°C, relative humidity of 50 ± 20%) for microbial monitoring and was provided free access to food (autoclaved CRF-1; Oriental Yeast Co., Ltd., Tokyo, Japan) and tap water. The mouse showed emaciation and continuous loss of body weight from 14 weeks of age. For the humane endpoint, after blood collection, the mouse was euthanized by exsanguination via the abdominal aorta and post cava under deep isoflurane anesthesia at 15 weeks of age. Then, necropsy and blood chemical examination were performed.

For histopathological examination, the kidneys were collected and fixed in 10% neutral buffered formalin. Next, cross-sections of the kidney were dehydrated and embedded in paraffin. The paraffin-embedded sections (4 μm thick) were stained with hematoxylin and eosin (H&E). The kidneys were further examined by periodic acid-Schiff (PAS); periodic acid methenamine silver (PAM); Congo red; Masson's trichrome (MT); and immunohistochemical staining for IgG, IgM, and C3 and electron microscopy. For immunohistochemical staining, deparaffinized sections were incubated with 3% H_2O_2 in distilled water for 10 min and using an appropriate blocking reagent for 30 min at room temperature. Block Ace (DS Pharma Biomedical Co., Ltd., Osaka, Japan) for C3 and Avidin/Biotin Blocking Kit (Vector Laboratories Inc., Burlingame, CA, USA) for IgG and IgM

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were used as the blocking reagents. Subsequently, sections were incubated at 4°C for 16 h with primary antibodies. Details of the primary antibodies are summarized in Table 1. Following incubation with Histofine Simple Stain Mouse MAX-PO (Nichirei Bioscience Inc., Tokyo, Japan) for C3 or streptavidin/horseradish peroxidase (Agilent Technology Inc., Santa Clara, CA, USA) for IgG and IgM for 30 min at room temperature, positive reactions were visualized with a Peroxidase Stain DAB Kit (Nacalai Tesque Inc., Kyoto, Japan). All immunostained slides were counterstained with hematoxylin. For electron microscopic examination, small pieces of the kidney were fixed in 2.5% glutaraldehyde, postfixed in 1% osmium tetroxide, and embedded in epoxy resin using standard methods. Ultrathin sections were prepared, contrasted with EM stainer (Nisshin EM Co., Ltd., Tokyo, Japan) and lead citrate, and examined using a transmission electron microscope HT7700 (Hitachi High-Tech Corp., Tokyo, Japan).

All procedures were approved by the Institutional Animal Care and Use Committee of Mitsubishi Tanabe Pharma Corporation.

Necropsy revealed bilateral discoloration and roughness of the kidney surface. Edema of the cervical subcutis and atrophy of the thymus were observed. Increased serum levels of blood urea nitrogen (BUN; 649 mg/dL) and creatinine (1.11 mg/dL) were detected. Histopathological examination of the kidney revealed prominent diffuse glomerular lesions (Fig. 1A). Amorphous, eosinophilic materials were deposited globally in the glomeruli (Fig. 1B). The mesangial region was expanded; however, the mesangial cells did not proliferate. Thickening of the Bowman's capsule was seen with proliferation of the parietal epithelial cells (Fig. 1C). In

Table 1. Primary Antibodies Used for Immunohistochemistry

Antibody	Host species	Clonality	Clone	Dilution	Antigen retrieval	Supplier
IgG	Goat	Polyclonal	-	1:15,000	Incubation with protease at 37°C for 10 min	Vector Laboratories, Burlingame, CA, USA
IgM	Goat	Polyclonal	-	1:100	Incubation with protease at 37°C for 10 min	Vector Laboratories, Burlingame, CA, USA
C3	Rabbit	Monoclonal	EPR19394	1:2,000	Heated with Tris/EDTA buffer (pH 9.0) for 10 min	Abcam, Cambridge, UK

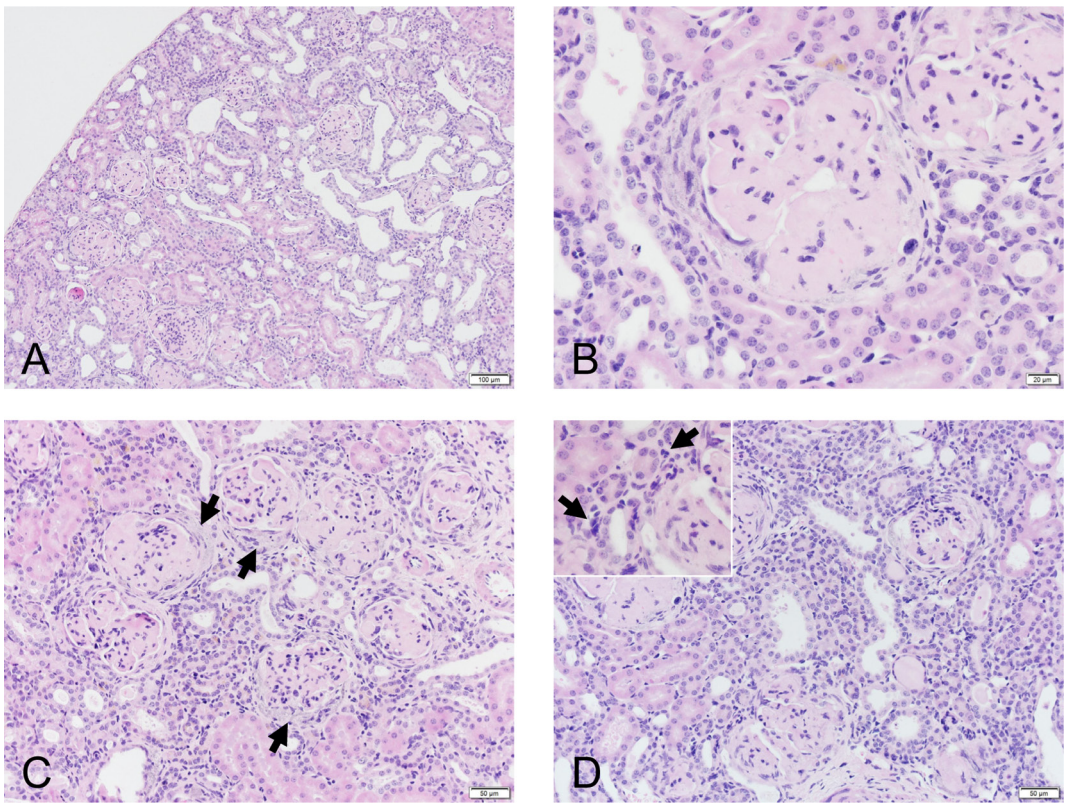

Fig. 1. Histopathological findings in the kidney. Hematoxylin and eosin (H&E) staining. (A) The glomeruli are diffusely enlarged. The renal tubules are dilated, regenerated, and atrophic. Bar = 100 µm. (B) High magnification of the glomeruli. Amorphous eosinophilic materials are deposited globally in the glomeruli. The mesangial region is expanded; however, the mesangial cells show no proliferation. Bar = 20 µm. (C) Thickened basement membrane of the Bowman's capsule is prominent (arrows). Bar = 50 µm. (D) Mononuclear cells infiltrate the interstitium (insert: arrows). Bar = 50 µm.

other parts of the kidney, dilatation, regeneration, atrophy of renal tubules, and interstitial mononuclear cell infiltration were observed (Fig. 1D). The glomerular deposits were positively stained with PAS staining (Fig. 2A) and stained red with MT (Fig. 2B). PAM staining revealed that the glomerular deposits were limited to the intraglomerular capillaries, and the basement membrane did not present any abnormal findings, such as thickened or double contouring (Fig. 2C). The glomerular deposits were negatively stained with Congo red under polarized light (Fig. 2D). Immunohistochemically, the glomerular deposits were strongly positive for IgG (Fig. 3A) and IgM (Fig. 3B) but were negative for C3 (Fig. 3C). Ultrastructurally, the glomerular deposits were present in the mesangial area underneath the capillary basement membrane and were identified as high electron-dense deposits (Fig. 4A). At higher magnification, these electron-dense deposits were composed of accumulations of curvilinear and fibrillary structures, the diameter of which was approximately 10 nm (Fig. 4B). In other organs, calcification was observed in the liver, lung, and heart.

Based on the above histological findings, the kidney lesion was diagnosed as hyaline glomerulopathy. Calcification in other organs was a secondary change that was thought to be related to decreased renal function. The lesions in hyaline glomerulopathy closely resembled those in renal amyloidosis on H&E-stained tissues[9]; amorphous, eosinophilic, hyaline extracellular material was present within the glomeruli in both lesions[10]. Hyaline glomerulopathy was identified by positive staining of the glomerular deposits with PAS staining and MT staining as well as by immunohistochemical positivity for IgM, IgG, and IgA but negativity or weak positivity for C3[7–9, 11]. The ultrastructure of glomerular deposits in hyaline glomerulopathy exhibited a well-circumscribed accumulation of closely packed tubules, which were nonbranching, curvilinear, and fibrillary and were arranged in parallel rows or double layers[9, 12, 13]. Amyloid in renal amyloidosis was characterized by amorphous eosinophilic deposits in the glomeruli and interstitium[10, 13]. The features of the present case were similar to those of hyaline glomerulopathy described above; in particular, the glomerular deposits were characterized as immunoglobulin, and not amyloid. Furthermore, a few reports have described glomerular lesions in young ICR mice. Two reports described glomerular lesions: membranoproliferative glomerulonephritis, characterized by thickening and double contour of the basement membrane and mesangial cell proliferation, has been reported in young ICR mice[5, 6]. However, these characteristics were not seen in the present case. Therefore, the present case can be distinguished from membranoproliferative glomerulonephritis. Another glo-

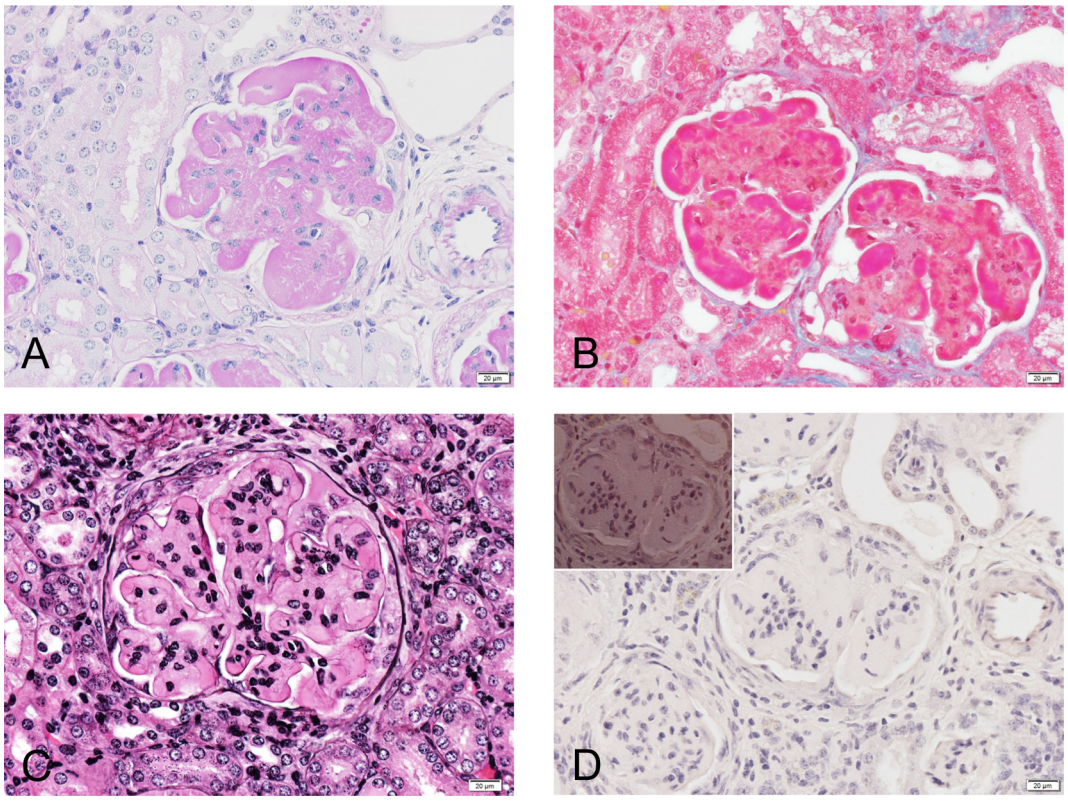

Fig. 2. Special staining of the glomeruli. (A) The glomerular deposits are seen using the periodic acid-Schiff stain. (B) The glomerular deposits stain red with Masson's trichrome stain. (C) The basement membrane is stained black with periodic acid methenamine silver staining; it shows no thickening or double contouring. The glomerular deposits are limited to intraglomerular capillaries. (D) The glomerular deposits are negatively stained with Congo red stain (insert: under polarized light). Bars = 20 µm.

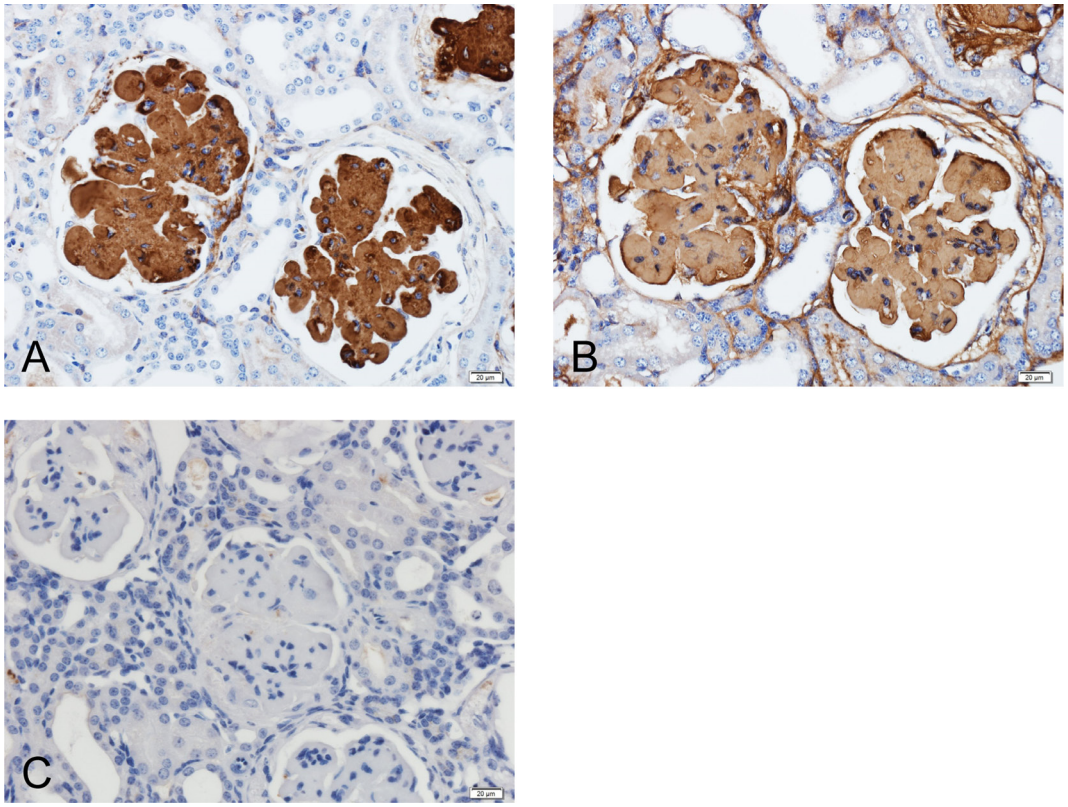

Fig. 3. Immunohistochemical staining of the glomeruli. (A) The glomerular deposits are strongly positive for IgG. (B) The glomerular deposits are strongly positive for IgM. (C) The glomerular deposits are negative for C3. Bars = 20 µm.

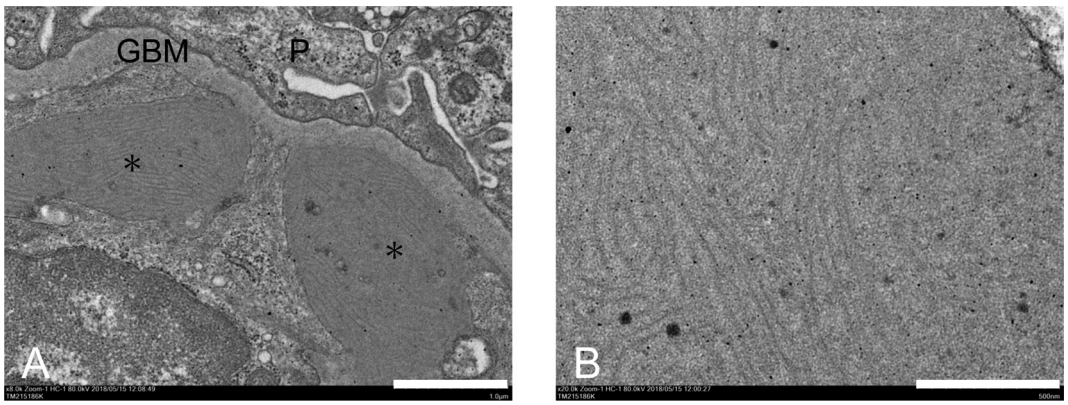

Fig. 4. Electron microscopy of the glomeruli. (A) The glomerular deposits (asterisk) are present in the mesangial area underneath the capillary basement membrane and exhibit high electron density. GBM: glomerular basement membrane. P: podocyte. Bar = 1.0 µm. (B) High magnification of the glomerular deposits. Electron-dense deposits composed of accumulations of curvilinear and fibrillary structures, with diameters of approximately 10 nm. Bar = 500 nm.

merular lesion has been reported in a 33-day-old mouse; it was diagnosed as idiopathic glomerulopathy[4]. The morphological characteristics in this lesion, such as deposition of hyaline materials without mesangial cell proliferation, were similar to those in hyaline glomerulopathy. However, the deposits in the glomeruli were not composed of immunoglobulins, contrary to the present case and hyaline glomerulopathy. Although the etiology of the glomerular lesions remains undetermined, immunological effects are strongly suggested[8, 14–16]. Glomerular deposits are related to immunoglobulins in many strains of mice[17, 18]. Immunoglobulin may exhibit exudation and/or entrapment in serum components because this animal showed high serum levels of BUN and creatinine[7]. Additionally, the observed immunoglobulin

deposition suggested that the present case of hyaline glomerulopathy was associated with immunological effects, supporting the hypothesis that the immune system affected the development of glomerular lesions.

In conclusion, in this report, we described a case of spontaneous severe hyaline glomerulopathy in a young (15-week-old) ICR mouse. Hyaline glomerulopathy in young mice is rarely observed; to the best of our knowledge, there is only one previous report of hyaline glomerulopathy diagnosed in young ddY mice[11]. Therefore, the present report is important to understand spontaneous lesions in ICR mice.

Disclosure of Potential Conflicts of Interest: The authors declare that there are no conflicts of interest.

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