Letters to Nature Medicine

Spontaneous glomerular deposition of immunoglobulins for ACE (Angiotensin Converting Enzyme) induces spontaneous nephropathy in diabetogenic rats

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We first discovered autoantibodies to ACE (angiotensin converting enzyme) in patients with type 2 diabetes mellitus[1]. The antibodies were positive for 64.5% of the patients with diabetes and were positive in 83.3% in the early stage of clinical diabetic nephropathy. In addition, in genetically diabetogenic OLETF (Otsuka Long-Evans Tokushima Fatty) rats[2], one of the characteristics of which strain is spontaneous nephropathy resembling those of human type 2 diabetes, and in control LETO rats[2], immunization with rabbit lung ACE developed glomerulopathy similar to that seen in diabetics[3]. Also, in normal New Zealand white rabbits, immunization with the rabbit lung ACE induced glomerular changes similar to those seen in diabetic nephropathy[3]. In this study, renal tissues identical to those examined in research of diabetic nephropathy by PAS staining and electron microscope in preceding study[3], were examined by immunostaining methods, only to prove that the diabetic glomerular changes may occur by immunization with ACE, not by non-specific responses to ACE, in non-diabetogenic rats and rabbits.

Studies on OLETF rats[3](referred to the former study). To be brief, LETO(Long-Evans Tokushima Otsuka)rats, a control strain of OLETF rat, and OLETF rats were monthly immunized with rabbit lung ACE. The immunization was terminated at the 15th injection when the kidneys were extirpated for histological examinations. The kidneys were fixed by 10% formaldehyde solution and PAS stained for light microscopic examination and were fixed by 2.5% glutaraldehyde in 0.1 M PBS, pH 7.4 (1st fixation) and osmium (VIII) oxide solution (2nd fixation) for electronmicroscopic examination. Immunoperoxidase staining of the kidney was performed in rats at Special Reference Laboratories (Tokyo, Japan) as reported previously[4]. Cryosections were permeabilized with absolute ethanol, and then exposed to microwave irradiation for 10 min in 10 mM citricbuffer, pH 6.0, at 98℃, or treated with 0.05% protease (Sigma, type XXVII), at 37℃, for 30 min for activation of the antigen. Next, sections were treated for 5 min with hydrogen peroxidase and blocked with 10 % porcine serum. After washing, sections were incubated with

goat anti-rat IgG (Bethyl Lab) or goat anti-rat IgM (Bethyl Lab), 1 : 100, 4℃ overnight. Anti-goat IgG (SBA Inc.) conjugated to horseradish peroxidase (DAKO) was used as secondary antibody. For staining, incubation for 7 min in 3, 3'-diaminobenzidine containing H_2O_2 was used. Nuclear staining was done with haematoxylin. ELISA for anti-rabbit ACE antibodies was done as reported[5]. Studies on rabbits as a control study of the OLETF rat[3]. NZ white female rabbits were immunized with the rabbit lung ACE by monthly injections. The immunization was terminated at the 15th injection when the kidneys were extirpated for histological examinations. Then, immunoperoxidase staining of the kidney was performed as in OLETF rats.

The mean body weight of the rats was 280g for LETO rats and 350g for OLETF rats at 10 weeks old, but after the 14th immunization the same weight of 560g was shown for both groups. In immunized LETO and OLETF rats, circulating antibodies to ACE were detected from the 4th immunization on, and titers of the antibodies reached a plateau after the 6th immunization. In OLETF rats glomerular changes were observed from 10 months on. Also in LETO rats glomerular changes were demonstrated after immunization. It is reprted, by the institute which established the strain, that OLETF rats were demonstrating proliferation of the mesangial matrix and thickening of the basement membrane of glomerulus after 40 weeks of age and at 70 weeks of age a PAS-positive nodular lesion was observed in almost every glomerulus[2]. The immunization with ACE did not induce changes in early morning plasma glucose and HbA_{1c} levels in rats and rabbits[3].

As shown in Figs. 1 and 2, both the immunized and unimmunized LETO rats and OLETF rats demonstrated glomerular deposition of immunoglobulins for ACE. LETO rat showed spontaneously deposited glomerular immunoglobulins for ACE, though glomerulus did not show nephropathy[3]. However, when immunized with ACE, glomerular changes similar to those seen in diabetic nephropathy were induced in LETO rat, resulting in a decrease in deposition of the immunoglobulins to a similar extent observed in OLETF rat or immunized

OLETF rat or even immunized rabbit. OLETF rat demonstrated both of spontaneous glomerular immunodeposit and spontaneous nephropathy similar to those of immunized LETO rat, but when immunized with ACE, glomerulus showed more remarkable nephropathy than unimmunized OLETF rat[3]. A normal NZ white rabbit showed glomerular PAS-positive nodular lesions after immunization with ACE[3] and also demonstrated glomerular deposition of immunoglobulins equivalent to that of OLETF rats after immunization(Fig. 3), though no deposition was seen in an unimmunized rabbit.

Thus, the immunoglobulins for ACE deposited in glomorulus decreased after immunization with ACE in unimmunized LETO rats, but not in unimmunized OLETF rats. Concerning the decrease in glomerular deposition of immunoglobulins, Mauer et al[6]. demonstrated complete disappearance of intensely stained immunoglobulin G from mesangium 2 months after transplantation of the kidney of a diabetic rat into a normal rat. Mauer et al[6]. also have demonstrated progressive localization of immunoglobulins G and M, and complement C3 within the mesangium in rats made diabetic by alloxan or streptozotocin, and they stated the glomerulopathy of diabetes in rats is secondary to the abnormal metablolic environment in which the kiney resides or a consequence of the diabetic state rather than genetic disorder. In our former study[1], autoantibodies to ACE, which were recognized most frequently in the pre-nephropathy and incipient-nephropathy stages, gradually decreased in frequency towards the final stage. This may be due to the loss of autoantibody in urine, and this loss was most prominent in the IgG of small molecule. From the results obtained in the studies for LETO and OLETF rats, though control strain, LETO rats appear to share some of diabetogenic genes with OLETF rats[7], it is suggested that spontaneous, “silent” deposit of immunoglobulins for ACE seen in LETO rats begins to decrease when additional deposition by immunization with ACE was added only to create the abnormal metabolic environment. On the other hand, as glomerular function becomes impaired by immunization with ACE, this leads to further glomerular pathology

and as nephropathy progresses the immunoglobulins gradually disappear from glomerus. However, if diabetic state exists, nephropathy can occur only by spontaneous deposition of the immunoglobulins, without additional deposit, as in OLETF rats. Also, it is very interesting that, though diabetic state does not exist, nephropathy similar to that of diabetics occurs in normal rabbit by immunization with ACE. A previous paper[8] is suggesting for OLETF rats that since disturbance of renal function exists already at 6 week-old age, before diabetes being found, specific genes independent of diabetogenic genes must be working separately for process of nephropathy. In respect of immunology, we are easily accessible to reports showing glomerular deposition of immunoglobulin or complement for unidentified antigenic substance in rat nephropathy[9,10].

From all the results combined[1,3], it is suggested that the immunoglobulin for ACE plays an important role for the occurrence of diabetic nephropathy.

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Legends for Figures

Fig. 1. Glomerular deposition of immunoglobulins M(a) and G(b) for ACE in a 68 week-old LETO rat. Also, Ig M(c) and IgG(d) in a 68 week-old LETO rat immunized with ACE. Original magnification × 100. Glomeruli ; demonstrating diffusely intense deposition of IgM and IgG in capillary endothelial cell and in mesangial region.

Fig. 2. Glomerular deposition of immunoglobulins M(a) and G(b) for ACE in a 68 week-old OLETF rat. Also, Ig M(c) and IgG(d) in a 68 week-old OLETF rat immunized with ACE. Original magnification × 100. Glomeruli ; demonstrating diffuse deposition of IgM and IgG in capillary endothelial cell and in mesangial region.

Fig. 3. Glomerular deposition of immunoglobulins M(a) and G(b) for ACE in a NZ white rabbit after 15th monthly immunization with ACE. Original magnification × 100. Glomeruli ; demonstrating diffuse deposition of IgM and IgG in capillary endothelial cell and in mesangial region.

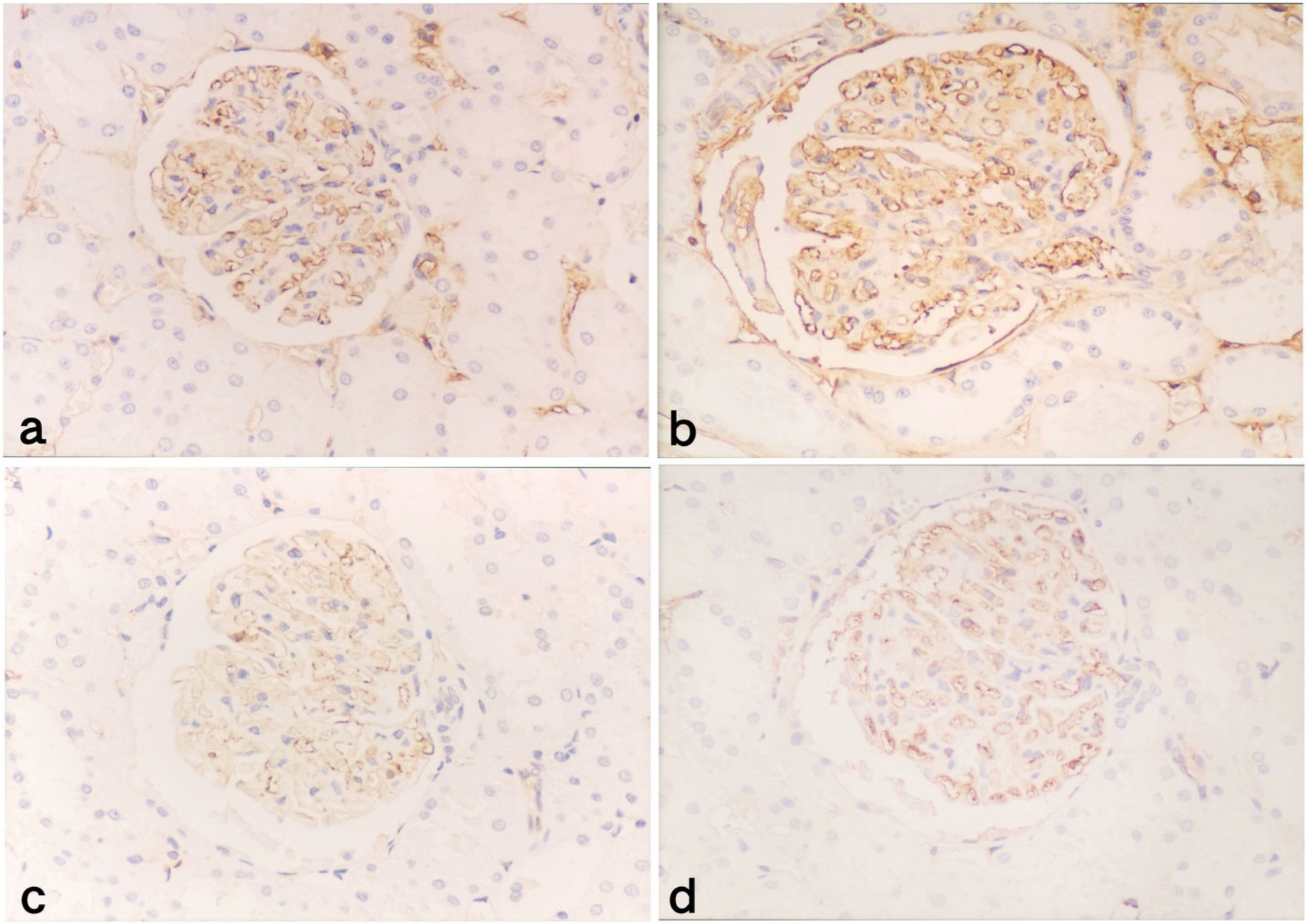
Addendum

Figures from Reference 3 as a reference.

Fig. 1. A 68 week-old, unimmunized LETO rat weighing 590 g demonstrates normal glomerulus in PAS –staining (a, ×400) and a 68 week-old LETO rat weighing 500 g, treated with ACE demonstrates expansion of PAS-positive mesangial matrix and thickening of lumen wall (b, ×400). A 68 week-old, unimmunized diabetic OLETF rat weighing 530 g demonstrates the increase in mesangial matrix and the deposit of PAS-positive materials in it, which perform segmental sclerosis (c, ×400). A 68 week-old diabetic OLETF rat weighing 575 g, immunized with ACE demonstrates a chronic thickening of lumen wall and expansion of the mesangial matrix where

cell infiltration and, partly, nodular lesions are seen(d, ×400).

Fig.2. Photos a and b of a control rabbit demonstrate normal glomerulus and tubulointerstitium in PAS staining(a, ×100) and electron microscopic examination (b, ×3000). PAS staining of immunized rabbits (×100); rabbit 1 (c) demonstrates PAS-positive materials mainly at the mesangium region. Rabbit 2 (e) demonstrates PAS-positive materials mainly at the mesangium region. The expansion of mesangial region and a decrease in mesangial cells together form a nodular lesion similar to that in diabetic nephropathy. Electron microscopic examination(d and f, ×3000); rabbit 2 (f) shows an increase in mesangial matrix and low electron-dense deposits in the mesangial region.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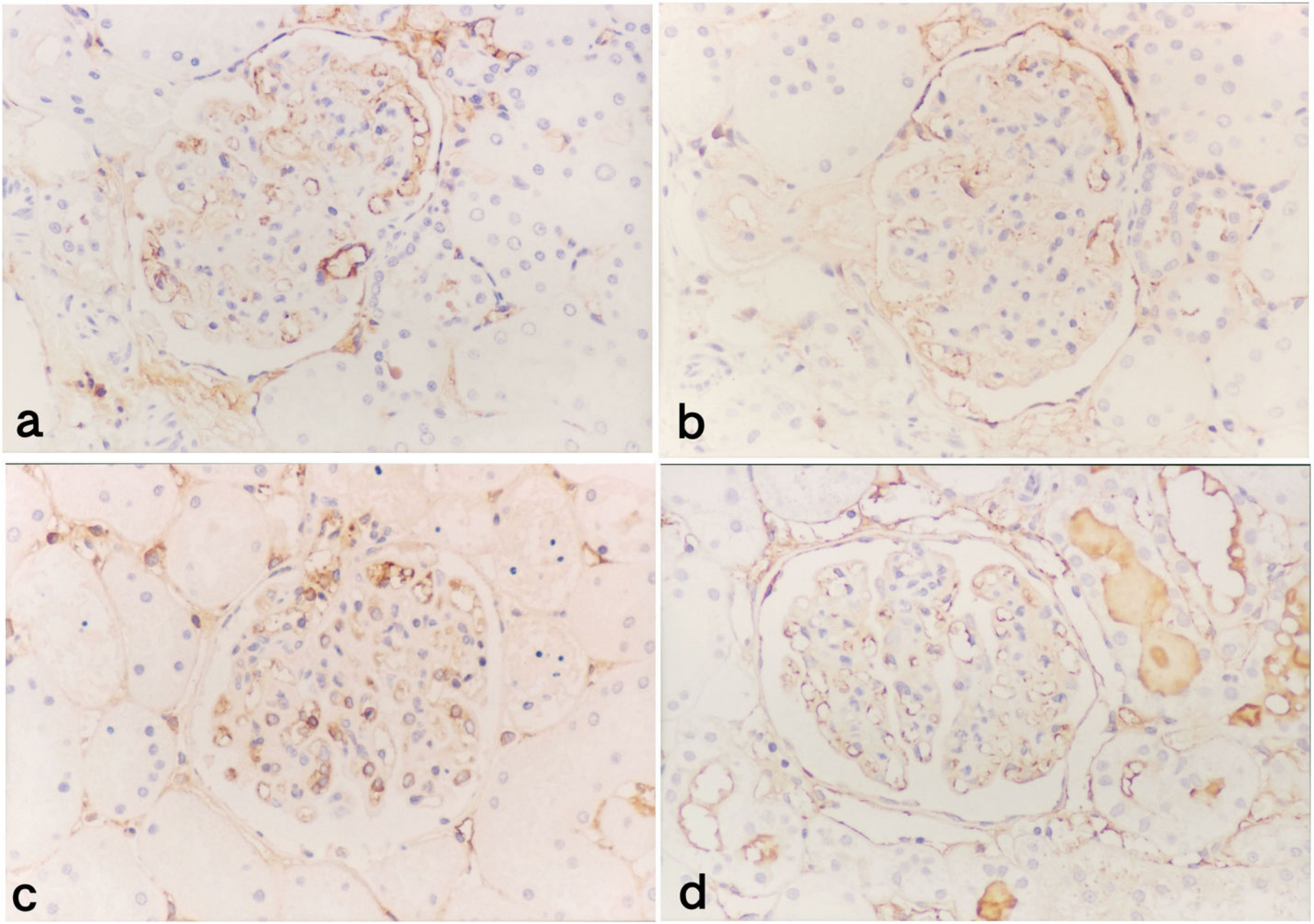
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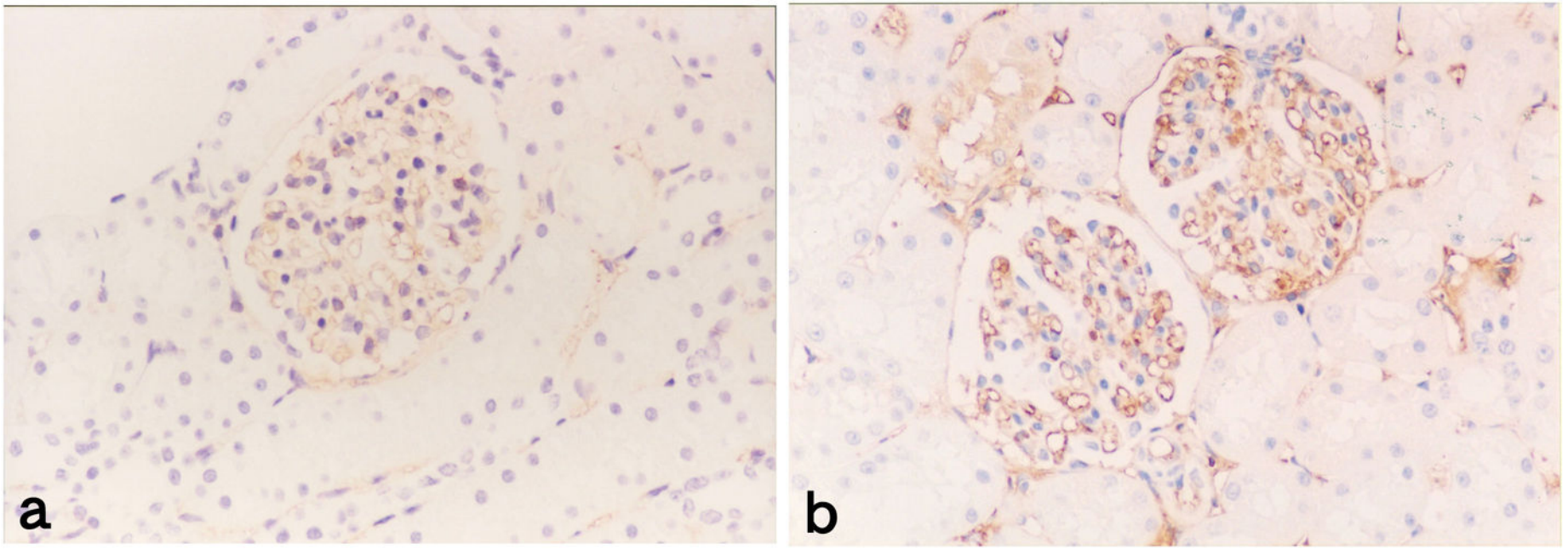
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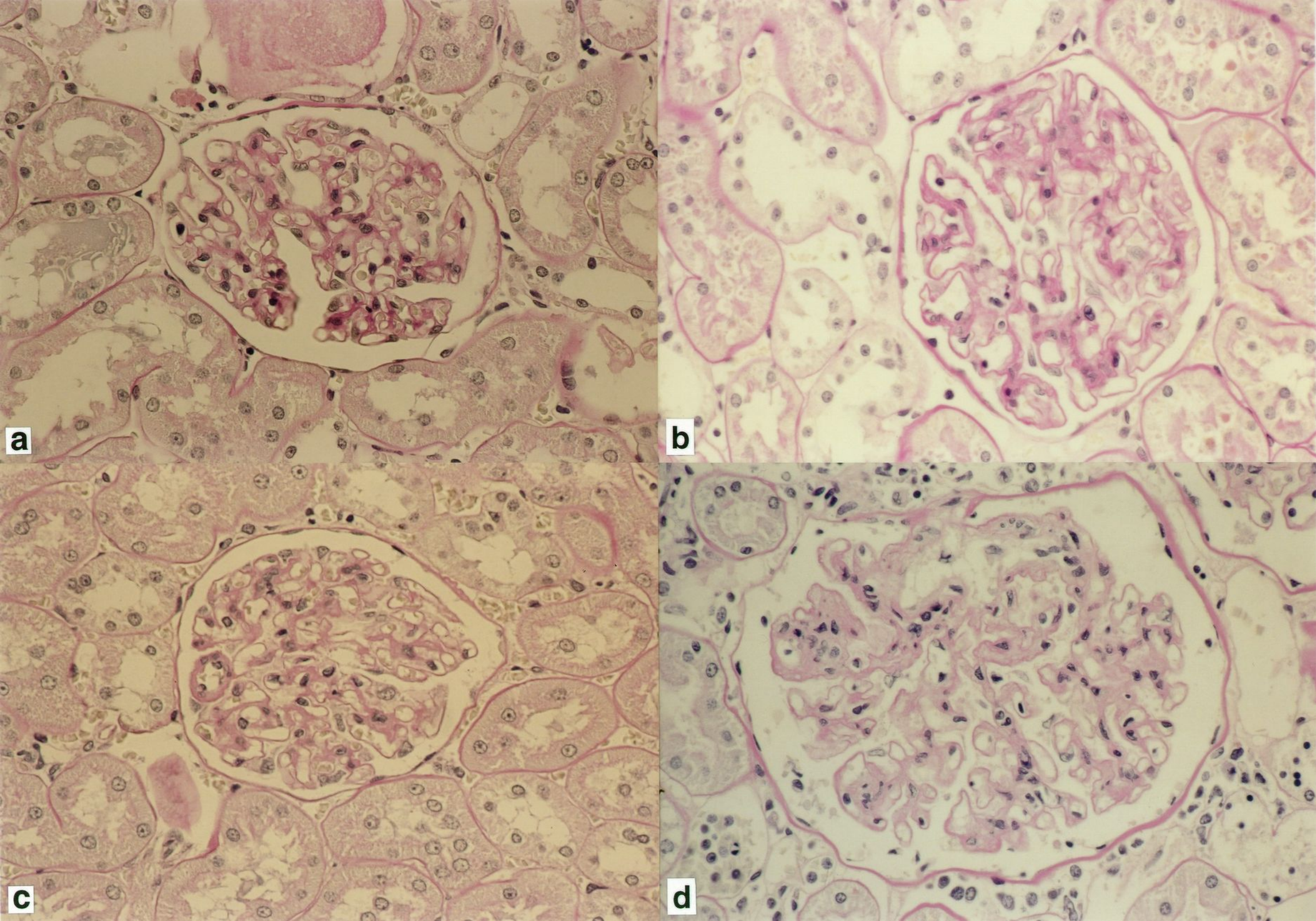
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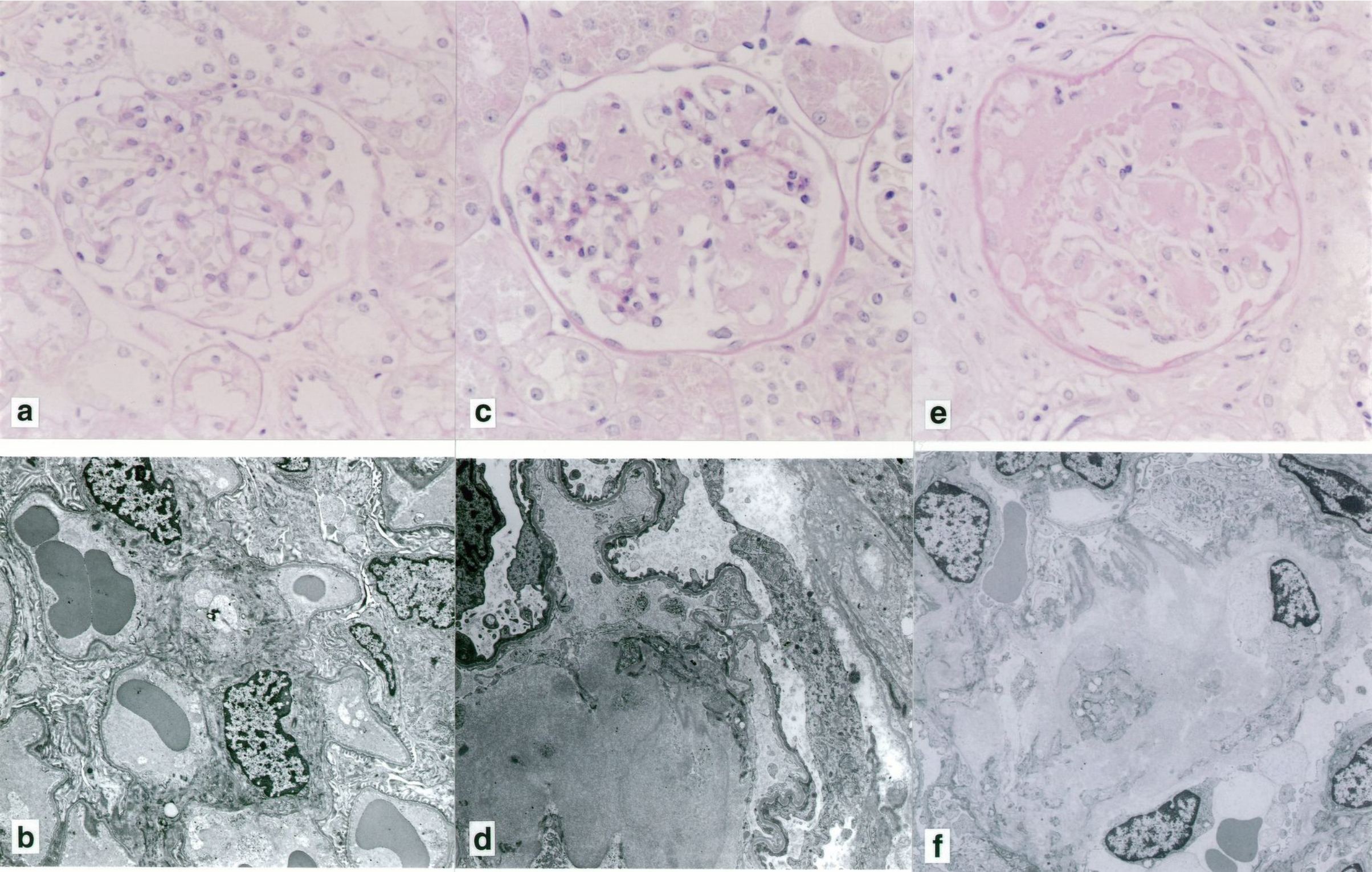
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