Kidney International, Vol. 55, Suppl. 71 (1999), pp. S-37–S-41

Lipoprotein glomerulopathy: Significance of lipoprotein and ultrastructural features

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Lipoprotein glomerulopathy: Significance of lipoprotein and ultrastructural features.

Background. Lipoprotein glomerulopathy (LPG) is a unique disease characterized by intraglomerular lipoprotein thrombi and type III hyperlipoproteinemia. Recently, we have demonstrated that LPG is associated with inherited apolipoprotein E (apoE) variants including apoE Sendai. On the other hand, electron microscopy shows that intraglomerular lipoprotein thrombi consist of lipid granules of various sizes. To elucidate the relationship between the peculiar histology and abnormal lipid metabolism related to apoE Sendai, we studied lipoprotein profiles and ultrastructural features.

Methods. The subjects were 11 patients with LPG. Four patients were nephrotic, and two others became nephrotic within six months following the biopsy. Eight patients underwent apoE gene analysis and showed apoE Sendai. The other three were presumed to have apoE Sendai because this mutation was demonstrated in their kindreds. Under electron microscopy, diameters of more than 1000 lipid granules were measured in several glomeruli, and a mean value was calculated in each case. Lipoprotein profiles were analyzed by the ultracentrifugation methods.

Results. The mean diameter of intraglomerular lipid granules correlated inversely with the levels of plasma triglyceride (TG; $r_s = -0.73$, $P < 0.05$), TG ($r_s = -0.77$, $P < 0.01$) and cholesterol (Chol; $r_s = -0.75$, $P < 0.05$) in very low-density lipoprotein (VLDL) fraction and TG in high-density lipoprotein (HDL) fraction ($r_s = -0.75$, $P < 0.05$). The inverse correlation was also seen between the mean lipid diameter and TG/Chol ratios in whole plasma ($r_s = -0.80$, $P < 0.01$) and in HDL ($r_s = -0.80$, $P < 0.01$). In addition, the cases showing smaller lipid granules and higher TG/Chol ratios in plasma and in HDL were nephrotic or became nephrotic within six months.

Conclusion. These results suggest that the size of lipid granules in LPG may become smaller under the influence of hypertriglyceridemia and particularly elevated plasma VLDL and HDL-TG, which may lead to heavy proteinuria.

Lipoprotein glomerulopathy (LPG) is a unique disease characterized by intraglomerular lipoprotein thrombi and type III hyperlipoproteinemia [1–3]. Differed from other hyperlipoproteinemia, however, the lesions appear to be limited in the kidney because hypertension, liver dysfunctions, and any other findings specific to previously known lipid abnormalities are not usually recognized [4]. Recently, we have demonstrated that three cases of LPG have a novel apolipoprotein E (apoE) variant (arginine 145→proline) named apoE Sendai [5]. Moreover, different mutations of apoE have been recognized in other cases of LPG. These studies suggest that some variants in apoE molecule may contribute to the pathogenesis of LPG, as reported in familial type III hyperlipoproteinemia (FIIIHLP) and delayed type Alzheimer's disease [6].

Electron microscopy shows that intraglomerular lipoprotein thrombi consist of variously sized lipid granules and form "fingerprint" appearance [4]. In addition, there are some specific findings deeply related to the pathogenesis and clinical symptoms of this disease [4, 7, 8]. To clarify the disease specificities of LPG, we describe the relationship between the ultrastructural features and the clinical and laboratory profiles. Particularly, we newly measure the size of lipid granules in the intraglomerular lipoprotein thrombi and investigate the relationship between the lipid size and plasma lipid and lipoprotein levels in detail.

METHODS

Subjects

For the histological study, the subjects were three males and three females. LPG was diagnosed by the histological findings in renal biopsy specimens. For the histometrical study of lipid granules, electron micrographs were obtained from four additional males and one female in collaborating institutions. In these 11 cases, mean values of age, urinary protein excretion (U_{Prot}), and creatinine clearance were 30 (7 to 57) years old, 4.1 (0.5 to 10) g/day, and 91 (60 to 134) ml/min, respectively.

Key words: lipoprotein thrombi, apolipoprotein E, lipid granules, type III hyperlipoproteinemia, nephrotic syndrome.

Four patients were nephrotic, and two others became nephrotic within six months of their biopsy.

Three patients were already reported to have apoE Sendai [5]. Another five (two in our hospital and three in others) newly underwent apoE gene analysis with the methods described previously [5], and all showed apoE Sendai. Three patients who did not undergo gene analysis were also presumed to have apoE Sendai because the same apoE variant was demonstrated in their kindreds.

Histological analyses

The tissue obtained from the renal biopsy was processed using routine methods for light microscopy, immunohistological technique, and electron microscopy. For light microscopy, paraffin-embedded sections were cut at 2 to 3 micron and were stained with hematoxylin and eosin, periodic acid-Schiff (PAS), periodic acid-methenamine silver (PAM), Masson's trichrome-elastica, and azan-Mallory. For immunohistology, sections were stained using antisera monospecific to IgG, IgA, IgM, C3, and fibrinogen.

In snap-frozen sections, lipids were studied with oil-red O stain, while apoA, apoB, and apoE were studied with the indirect immunofluorescence technique using antisera monospecific to each.

For electron microscopy, small blocks of a specimen were fixed in 2.5% glutaraldehyde and 2% paraformaldehyde followed by postfixation in 1% osmium tetroxide and were embedded in Epon 812 according to the conventional methods. The samples were double stained with uranyl acetate and lead citrate.

Measurement of lipid granule sizes

Under electron microscopy at 10,000 magnification, diameters of more than 1000 lipid granules were measured in several glomeruli. A mean value was then calculated in each case. For the convenience of measurement, all granules were rounded off to the nearest size on electron micrographs, which did not substantially influence the final results. Granules under 200 nm were cut off because of the difficulty of measurement.

Lipids and lipoproteins analyses

After overnight fasting, blood was drawn from the antecubital vein into tubes containing 0.1% of ethylenediaminetetraacetic acid (EDTA)-2Na. Plasma was prepared by centrifugation (3000 r.p.m. for 15 min) for measurements of total cholesterol (TC) and triglyceride (TG). Very low-density lipoprotein (VLDL), intermediate-density lipoprotein (IDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) were isolated from plasma by sequential preparative ultracentrifugation as previously described by Hatch and Lees [9]. TC and TG levels of each lipoprotein were measured similar to the method described for the plasma.

Statistical analyses

Correlations between mean diameters of lipid granules and plasma lipid and lipoprotein levels were evaluated by nonparametric test using Spearman's rank correlation coefficient because it was not probable that these variables were normally distributed. A P of less than 0.05 was considered significant.

RESULTS

Routine histology

In the tissues obtained from the six examined cases, most glomeruli showed extreme hypertrophy and enlarged capillary lumina with pale-stained, mesh-like substances, which we called "lipoprotein thrombi" (Fig. 1). Mesangial proliferation was moderate and sometimes associated with membrane reduplication in capillary walls. Advanced glomerular lesions with segmental sclerosis and periglomerular fibrosis were obvious in one case. In these cases, nonspecific tubulointerstitial changes were dependent on the degree of glomerular lesions. There were no vascular changes characteristic of abnormal lipid metabolism, although lipoprotein thrombi were seen in a part of venules in one case.

Immunoglobulins, C3, and fibrinogen were mildly deposited along the capillary walls, but did not appear to have any pathological significance. Meanwhile, one case showed moderate deposits of IgA and fibrinogen in the mesangial area, indicating IgA nephropathy.

Histology specific for lipids and apolipoproteins

Oil red-O staining showed lipid droplets in the capillary lumina. ApoB and apoE were demonstrated intensively in the capillary lumina associated with fine granular staining in the mesangial area and capillary walls.

Electron microscopy

In all cases, the capillary lumina were filled by granules and vacuoles of various sizes, which formed striae resembling a fingerprint (Fig. 2). By these structures, erythrocytes and endothelial cells were squeezed on the capillary walls. Mesangial cells were sometimes detached from capillary walls. In most cases, curious osmiophilic deposits were observed (Fig. 3). In one case, huge electron-dense substances occupied the mesangial area, and the fragments were fluxed in the capillary lumina.

In the histometrically examined 11 cases, the mean diameter of intraglomerular lipid granules ranged widely from 281 to 722 nm. The mean diameters showed significantly inverse correlation with plasma TG levels ($r_s = -0.73$, $P < 0.05$), but not with plasma TC levels. There was also a significant correlation between the diameters and VLDL-TG ($r_s = -0.77$, $P < 0.01$), VLDL-Chol ($r_s = -0.75$, $P < 0.05$), and HDL-TG ($r_s = -0.75$, $P <$

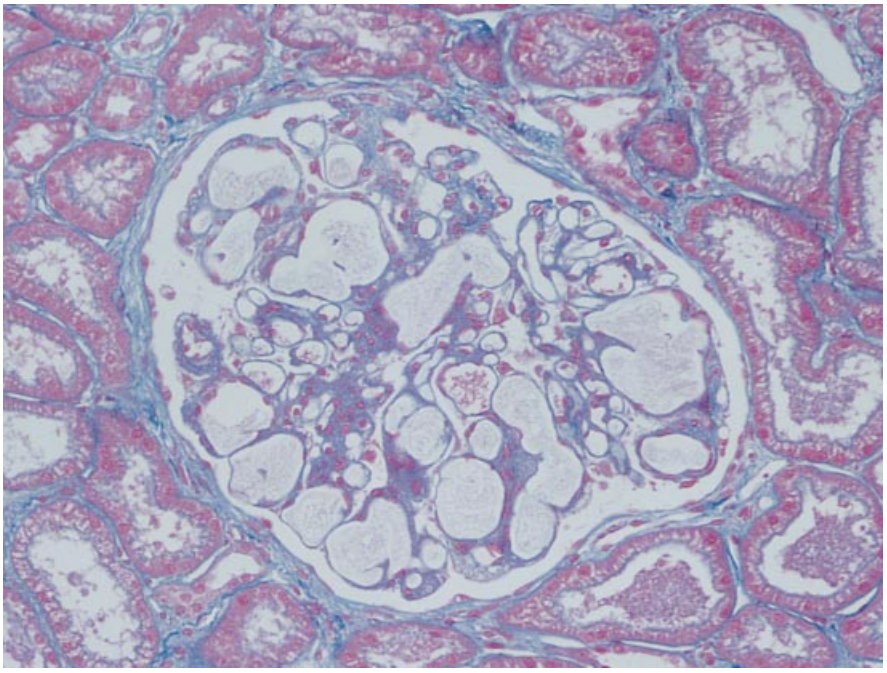

Fig. 1. Glomerulus with lipoprotein thrombi. Capillary lumina are enlarged with pale-stained and mesh-like substances (Azan-Mallory stain, ×300).

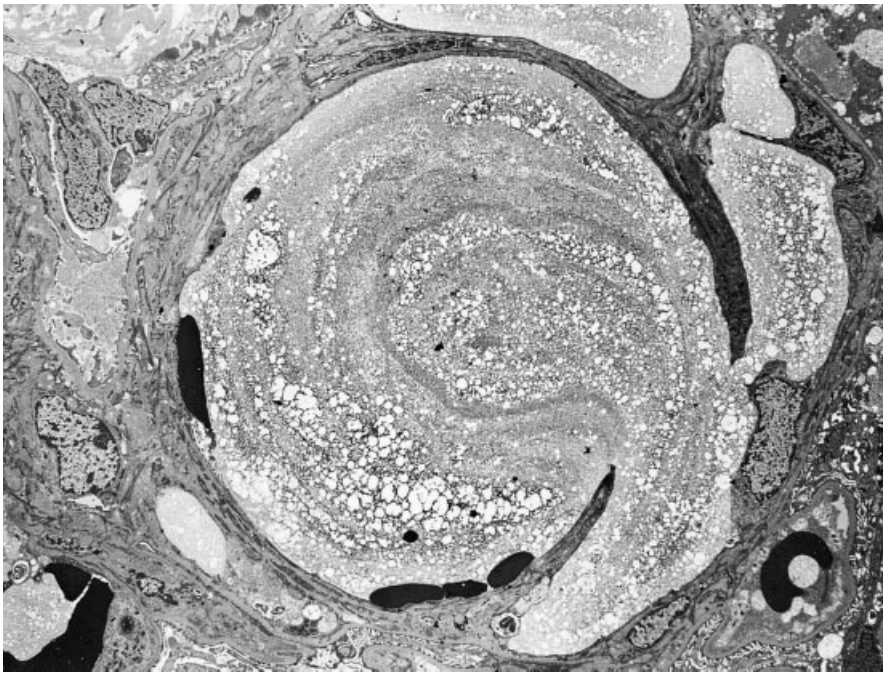

Fig. 2. Electron micrograph of a glomerulus. A layered structure resembling a fingerprint is composed of granules and vacuoles in the capillary lumen (×3000).

0.05). On the other hand, the diameters were significantly correlated with TG/Chol ratios in whole plasma ($r_s = -0.80$, $P < 0.01$) and in HDL fraction ($r_s = -0.80$, $P < 0.01$; Fig. 4) but not in VLDL or LDL. In addition, the cases showing smaller lipid granules and higher TG/Chol ratios in plasma and in HDL were nephrotic or became nephrotic within six months (Fig. 4).

DISCUSSION

It is reported that FIIIHLP occasionally has renal lesions [10–12]. In these cases, however, glomerulosclerosis with massive foam cells is mainly observed, different from intraglomerular lipoprotein thrombi in LPG. Moreover, the nephropathy of FIIIHLP is associated with systemic organ damage, for example, arteriosclerosis and

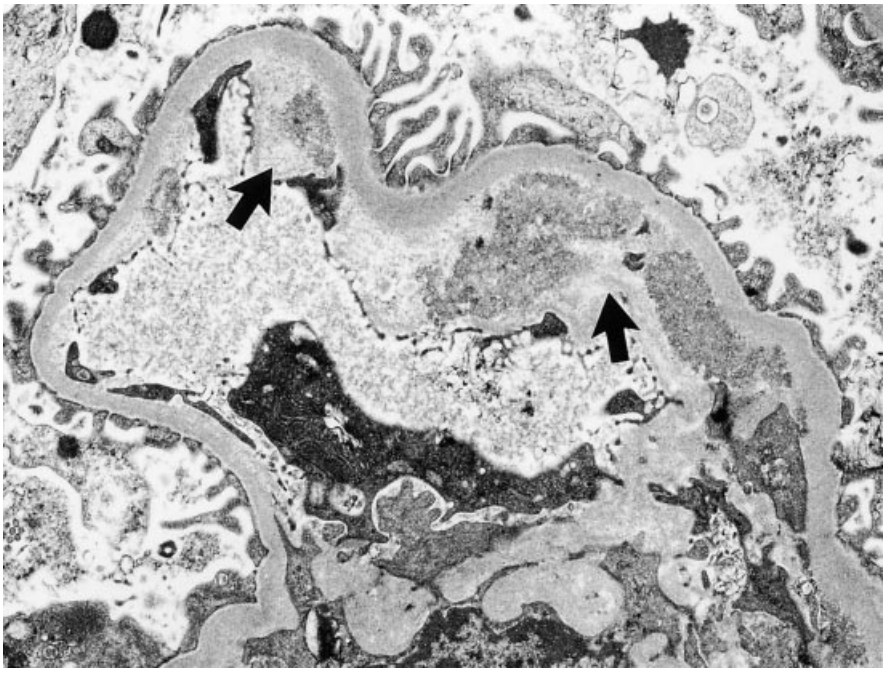

Fig. 3. Electron micrograph of a glomerulus. Osmiophilic substances (arrows) are deposited in dilated subendothelial space (×7000).

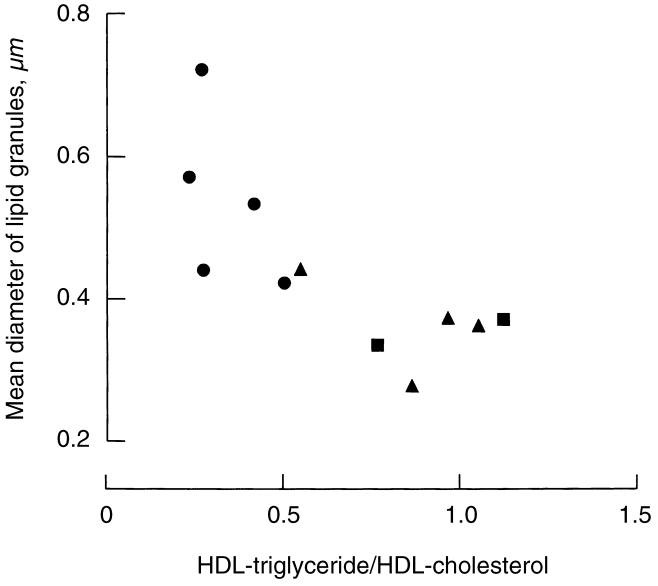

Fig. 4. Correlation between high density lipoprotein (HDL) triglyceride/HDL cholesterol ratio and mean diameters of intraglomerular lipid granules in nephrotic cases (●), non-nephrotic cases (▲), and cases developing nephrotic within six months (■). $r_s = -0.80$, $P < 0.01$.

diabetes mellitus, which is caused by hyperlipidemia. The contrast between LPG and FIIIHLP may be related to the abnormal lipoproteins degenerated by different apoE variants.

Although some apoE variants, including apoE Sendai, have been identified in LPG as one of pathogenetic factors, the mechanisms binding these abnormalities to characteristic and pathological conditions in the kidney are still unknown. In FIIIHLP, the representative disease associated with apoE abnormality, structural deformity of the receptor-binding domain based on apoE2 amino acid substitution may decrease the binding activity between the apoE molecule and its receptor, resulting in the abnormal lipoprotein metabolism and hyperlipidemia [13]. ApoE Sendai also has the mutation in the receptor-binding domain and may play a causative role in LPG [5]. However, hyperlipidemia is not marked in LPG when the patient does not develop nephrotic syndrome [4, 14]. Accordingly, it is considered that the glomerular lesions with lipoprotein thrombi are directly induced by a novel apoE variant but not through hyperlipidemia.

Observations on the intraglomerular lipoprotein thrombi using electron microscopy give very important information regarding the pathogenesis of LPG. These findings are summarized as follows. Lipoprotein thrombi in the capillary lumina are essentially composed of sand to stone-like round-shaped granules and space-occupying matrices. It is clarified by oil-red O staining in frozen-sections that the round-shaped granules are lipids [4, 7]. Moreover, Zhang et al have demonstrated using immunoelectron microscopy that apoE molecules are included in the matrices bound to lipid granules [7].

It is also of interest that osmiophilic substances similar to lipoprotein thrombi occasionally appears in the subendothelial spaces and around mesangial cells even if lipoprotein thrombi are not seen in the capillary lumina [4, 7, 8]. However, these materials seem to be immature

because they are irregularly formed different from "fingerprint" appearance such as in lipoprotein thrombi. Accordingly, it is postulated that these substances are initially deposited as the precursors of lipoprotein thrombi in the subendothelial and mesangial areas and that the fragments are fluxed into capillary lumina when deposits are excessively accumulated. Finally, lipoprotein thrombi may develop with a "fingerprint" appearance. As described earlier in this article, deposition of osmiophilic substances in the glomeruli may be related to the affinity of an apoE variant for the capillary wall or mesangium.

This study shows that elevated plasma VLDL and HDL-TG with hypertriglyceridemia as well as VLDL-TG/LDL-Chol reported previously [8] influence the lipid size of lipoprotein thrombi to be smaller, which may lead to heavy proteinuria. This evidence suggests that VLDL and HDL including apoE variants may contribute to the development of nephrotoxic lipoprotein thrombi in the glomerulus. In addition, because we have preliminary findings in the immunocolumn study showing that apoE Sendai is mostly included in HDL fraction, we paid attention to the significance of HDL-TG/HDL-Chol.

It is well-known that hyperlipidemia associated with the increase of remnant is usually induced by abnormal LPL activity and has atherogenic action. Some investigators have reported that hypertriglyceridemia and the decrease of HDL-Chol are deleterious factors in cardiovascular disease [15]. On the other hand, it has been discussed that hyperlipidemia is implicated in the pathogenesis of accelerated glomerulosclerosis, which is analogous to atherosclerosis [12, 16]. In LPG, however, LPL activity is within normal range [14], and lipoproteins in the glomerulus appear not to have atherogenic action but to have some specific affinity for the capillary walls and mesangium as shown in this study. Actually, the abnormal triglyceride metabolism through VLDL and HDL may be induced by novel apoE variants such as apoE Sendai and may have a pathogenetic role in the peculiar glomerular lesions different from the analogue of atherosclerosis.

ACKNOWLEDGMENTS

This work was supported by Grant for the "Progressive Renal Disease" Research Project from the Ministry of Health and Welfare, Japan, and Grant-in-Aid 10670982 from the Ministry of Education, Science and Culture, Japan. We are grateful to the late Professor Hiroshi Sakaguchi for his valuable advice. We also thank Dr. Kenichi Kudo, Dr. Yasushi Koitabashi, Dr. Tetsuya Mitarai, Dr. Toshikatsu Shibata, Dr. Nobuaki Yamanaka, Dr. Takeshi Matsuyama, and Dr. Toshisada Morita for providing materials.

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