Macrophage impairment produced by Fc receptor gamma deficiency plays a principal role in the development of lipoprotein glomerulopathy in concert with apoE abnormalities

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Abstract

Background. To obtain a clear understanding of the pathogenesis of lipoprotein glomerulopathy (LPG), we studied the role of the deficiency of Fc receptor gamma chain (FcRγ) for the development of LPG in concert with apolipoprotein E (apoE) abnormalities.

Methods. We generated apoE and FcRγ double-knockout (FcRγ/apoE-KO) mice, and subsequently introduced several kinds of human recombinant apoE genes. At 21 days after infection, the mice were sacrificed and histologically examined. Peritoneal macrophages were evaluated for their response to modified lipids.

Results. In the FcRγ/apoE-KO mice, the human apoE3-injected mice showed the most drastic LPG-like changes, as well as prominent hypertriglyceridemia. Meanwhile, relative to the human apoE3-injected mice, the FcRγ/apoE-KO mice showed greater lipoprotein deposition and less macrophage infiltration into the mesangial area. Moreover, the peritoneal macrophages in the apoE/FcRγ-KO mice were impaired in lipid uptake and secretion of the cytokines monocyte chemotactic protein-1 and regulated upon activation, normal T-cell expressed and secreted, after the uptake of oxidized low-density lipoprotein.

Conclusions. These results suggest that the impairment of macrophage function resulting from FcRγ deficiency plays a principal role in the development of LPG in the presence of apoE abnormalities.

Keywords: apolipoprotein E; Fc receptor gamma chain; lipoprotein glomerulopathy; macrophage

Introduction

Lipoprotein glomerulopathy (LPG) is a recently identified renal disease [1] that was originally described in 1989 by Saito et al. and mainly affects people of Japanese and Chinese origin. LPG patients commonly show severe proteinuria and progression to renal failure. A histological characteristic of LPG is the deposition of a thrombus-like substance in markedly dilated glomerular capillaries; these substances positively stain for Sudan IV and oil red O. Moreover, patients with LPG have elevated concentrations of intermediate-density lipoprotein (IDL) and plasma apolipoprotein E (apoE) levels similar to those seen in type III dyslipidemia [2].

In 1997, a novel apoE variant, apoE-Sendai (Arg145Pro), was identified in Japanese patients with LPG [3]. Since the identification of apoE-Sendai, various other apoE mutations have subsequently been discovered [4–12]. In murine studies, Ishigaki et al. [13] and our group [14] reported that the apoE-knockout (KO) mice that received virus-mediated transduction of apoE-Sendai developed the murine counterpart of LPG. Therefore, the dyslipidemia that resulted from the apoE variants was
considered an important factor in LPG development. However, non-responders of renal lesions in apoE-Sendai-transfected mice [13], healthy carriers with apoE variants in families of patients with LPG [2] and LPG patients with normal lipid profiles [15] suggest the involvement of other factors in the development of LPG.

In another murine experimental study published by Kanamuru et al. [16], renal lesions specific to LPG were shown to develop in Fc receptor (Fcr) gamma chain (Fcrγ)-KO mice in which chronic graft-versus-host disease (cGVHD) had been induced. Fcrs are expressed in the hematopoietic cell lineage and mediate phagocytosis and antibody-dependent cell cytotoxicity in the immune and resident cells of many tissues [17]. Two general immunoglobulin G (IgG) Fc receptor (FcγR) classes are known: the activating receptors and the inhibitory receptors, which transmit their signals via immunoreceptor tyrosine-based activation motif and immunoreceptor tyrosine-based inhibitory motif, respectively [18]. The activating FcγRs require an FcγR homodimer for surface expression and signal transduction in the mouse system. In Fcγ KO mice, the activating FcγRs (FcγRI and FcγRIII) are not expressed, but the inhibitory FcγR (FcγRIIb) is expressed and remains functional [19]. Kanamuru et al. [16] suggested that the absence of an activating inflammatory signal in cGVHD-induced Fcγ-KO mice might affect the development of LPG in chronic inflammatory kidneys through FcRs. Further, Fcγ deficiency was suggested to be another potential factor for LPG development. However, these researchers were unable to examine how Fcγ deficiency in their murine model played a role in lipid accumulation in LPG lesions in the combination with an apoE abnormality, since Fcγ-KO mice have no evident impairment on lipid metabolism.

In this study, we generated apoE and Fcγ double-knockout (Fcγ-γKO) mice by crossing apoE-KO mice with Fcγ-KO mice and subsequently introduced several kinds of human recombinant apoE genes using virus-mediated gene transfer. We then evaluated their renal histology, lipid profiles and macrophage function and elucidated the role of Fcγ in the pathogenesis of LPG in the presence of various expressed human apoE proteins.

Materials and methods

Experimental animals

ApoE-KO mice in the C57BL/6 background were obtained from Dr Okawa, Nippon Medical School (Tokyo, Japan); Fcγ-KO mice in the C57BL/6 background were provided by Dr Takai, Institute of Development, Aging and Cancer, Tohoku University (Sendai, Japan). Male apoE-KO mice were crossed with female Fcγ-KO mice, and the progeny were repeatedly bred with one another to obtain a Fcγ-apoE-KO lineage. The apoE and Fcγ genotypes were determined by genomic PCR using specific primers [48–50]. Mice were bred and maintained at the Fukuoka University Animal Center under SPF conditions and protocols in accordance with the institutional guidelines for animal experiments at Fukuoka University. The mice had free access to a standard diet (CRF-1®, containing normal 5.7% fat and 0% cholesterol; Japan Chares River Ltd., Yokohama, Japan) and water.

Propagation, purification and administration of RAs

RAs containing the entire coding regions of the human apoE-Sendai, and the human apoE3 and LacZ gene were provided by Dr Ishigaki, Tohoku University School of Medicine (Sendai, Japan), and had been generated as described previously [13]. The RAs were propagated in 293 cells, purified by CsCl ultracentrifugation and stored in phosphate-buf- fered saline containing 10% (w/w) glycerol at −80°C. Each RA was injected into age-matched male mice (5 months of age) via the tail vein (2 × 10⁹ plaque-forming units/mouse).

Evaluation of urine and serum samples

Spot urine was collected at each time point, and urinary protein concentrations and Cr levels were assessed. Urinary protein excretion was estimated from the urine protein/Cr ratio. Blood was collected from the retro- or orbital venous plexus after 4 h of fasting at each time point. Serum TC, TG and UN levels were determined in individual mice at each time point by enzymatic assay kits (Wako Pure Chemical Co., Osaka, Japan). Serum human apoE levels were determined using an enzyme-linked immunodiffusion assay kit (Sekisui Medical Co., Tokyo, Japan). Furthermore, plasma lipoprotein profiles were analyzed by an online dual enzymatic method for simultaneous quantification of cholesterol and TGs by HPLC as previously described [51].

Histological analysis

Kidneys were dissected 21 days after viral administration and examined by light microscopy, electron microscopy and immunohistochemistry. For light microscopy, the removed kidneys were fixed in Carnoy’s solution, and paraffin-embedded sections were stained with PAS and AM. In addition, lipid staining was performed by a modified method described by Mochizuki et al. [20] as follows: the specimens were initially fixed in formalin and then post-fixed in a mixed solution of 2% OsO₄ and 10% potassium dichromate for lipid fixation; the samples were then dehydrated through an alcohol series and embedded in paraffin; the sections were then stained with oil red O by a standard procedure. This method allows a detailed observation of lipoprotein thrombi in glomerular capillaries, since the OsO₄-fixed lipids remain in the specimens after alcohol dehydration. For electron microscopy, small blocks of kidney tissue were fixed with 2.5% glutaraldehyde and 2% paraformaldehyde, post-fixed with 1% OsO₄ and embedded in Epon 812 by conventional methods. The sections were double-stained with uranyl acetate and lead citrate. For immunohistochemistry, 95% alcohol-fixed and paraffin-embedded sections were incubated with anti-mouse CD68 antibody (Mac1Biotech Inc., Uden, The Netherlands) at a concentration of 1 μg/ml/mL in PBS with 1% bovine serum albumin. Staining was performed using anti-mouse IgG horseradish peroxidase-labeled polymer (DakoCytomation Inc., Carpinteria, USA). The numbers of anti-CD68 Ab-positive cells were calculated in 50 randomly selected glomeruli in each mouse.

Preparation of peritoneal macrophages

Mice were killed and exsanguinated 21 days after viral administration. The peritoneal cavities were rinsed with 5 mL of cold Hank’s solution (HS; Sigma-Aldrich Inc., St. Louis, USA). Cells suspended in HS were washed by centrifugation and resuspended in RPMI1640 (HyClone Laboratories Inc., South Logan, USA) containing 20% fetal bovine serum (FBS). These collected peritoneal macrophages were cultured at a concentration of 2 × 10⁶ cells/well and used for the ox-LDL uptake analysis and ELISA assay.

Ox-LDL uptake and ELISA assay of cultured macrophages

Ox-LDL was purchased from Biomedical Technologies Inc. (Stoughton, USA). After overnight incubation at 37°C, macrophages were cultured for 24 h at 37°C in the presence of ox-LDL (10 μg/mL) in RPMI1640–20% FBS. Cultured macrophages were stained with oil red O by a standard procedure. Afterwards, foam-cell formation was observed by light microscopy, and the numbers of cells changing into foam cells were determined and divided by the total number of glomeruli in five randomly selected sections in each mouse. Meanwhile, the supernatants were collected at each time point, and the concentrations of murine MCP-1 and RANTES were measured with commercial ELISA kits (R&D Systems Europe Ltd., Abingdon, UK).
Real-time quantitative PCR

Preparation of total RNA from kidney specimens, quantitative cDNA amplification, the reverse transcription reaction, TaqMan PCR and the detection of sequence-specific amplification were performed as previously described [41]. The transcription levels of the LDL-R gene (Ldbr) and SR genes (Scara3, Cd63, Cd68, Lox1, Scarf2 and Cxcl16) were assessed. Oligonucleotide primers and probes were designed using the Primer Express program (Applied Biosystems Japan) and synthesized; Ldbr (Mm00440169_m1), Scara3 (Mm00553769_m1), Cd63 (Mm 01135198_m1), Cd68 (Mm000839636_g1), Lox1 (Mm00454586_m1), Scarf2 (Mm00464144_m1), Cxcl16 (Mm00469712_m1) and Actb (Mm00607839_s1). The transcription levels in all the samples were normalized to the amount of β-actin (Actb).

Statistical analysis

All data are presented as the mean ± SE. Statistical comparisons between two groups of parametric data were made using an unpaired t-test, while those between two groups of non-parametric data were determined using the Mann–Whitney U-test. For multiple comparisons, analysis of variance was performed with subsequent post hoc analysis according to Bonferroni. Kendall’s rank correlation was used to assess the relationships between the number of CD68-positive cells/glomeruli and the percentage of lipoprotein thrombi-positive glomeruli. All statistical tests were done using SPSS version 18.0. A value of P < 0.05 was regarded as statistically significant.

Results

Plasma expression of apoE

Recombinant adeno viruses (RA) containing human apoE-Sendai and apoE3 were injected into Fcγ/a reinforced mice (designated Fcγ(−)/apoE−;E-Sendai) and Fcγ(−)/apoE−;E3 mice, respectively), and the plasma levels of human apoE at multiple time points were determined. A control RA containing a modified chicken β-galactosidase gene (referred to as LacZ) was also injected into Fcγ/a apoE-KO and Fcγ-KO mice. These control mice were designated Fcγ(−)/apoE−;LacZ and Fcγ(−)/apoE+(−;LacZ) mice, respectively. In the mice carrying the human apoE genes, the levels of plasma human apoE rapidly reached maximal levels of about 6–9 ng/μL at 4 days after infection and then declined rapidly to near baseline at 10–14 days after injection. No significant differences were observed in apoE levels between the Fcγ(−)/apoE−;E-Sendai and the Fcγ(−)/apoE−;E3 mice. Meanwhile, serum apoE was not detected in the Fcγ(−)/apoE−;LacZ mice (Figure 1A). Furthermore, no serum apoE was detected in the
Fcγ(−)/apoE(+;LacZ) mice, because of differences between human and murine apoE.

**Injected apoE variants differentially affect the serum lipid profiles of Fcγ/apoE-KO mice**

Body weight, food intake, urinary protein/creatinine (Cr) ratios and serum levels of urea nitrogen (UN), total cholesterol (TC) and triglyceride (TG) were assessed at multiple time points. The urinary protein/Cr ratios and serum UN levels showed no significant difference among all the groups during the experiment (data not shown). On the other hand, serum TC levels in the Fcγ(−)/apoE(−;E3) mice were initially 7-fold higher than the baseline levels observed in the Fcγ-KO mice (Figure 1B). Serum TC levels in the Fcγ(−)/apoE(−;E-Sendai) mice rapidly decreased to a normal range after RA injection, and this marked decrease was sustained throughout the experimental period. The Fcγ(−)/apoE(−;LacZ) and Fcγ(−)/apoE(+;LacZ) mice remained stable during the experiment. In contrast to the pattern observed for the TC values, serum TG levels in the Fcγ(−)/apoE(−;E-Sendai) and the Fcγ(−)/apoE(−;E3) mice transiently increased after RA injection and then declined toward the baseline 2 weeks after injection (Figure 1C). The two other groups injected with LacZ showed no apparent changes in TG levels. Because body weight and food intake remained unchanged throughout the experimental period (data not shown), these alterations in serum TC and TG levels were dependent on the expression of apoE.

High-performance liquid chromatography (HPLC) analysis was carried out to determine in greater detail the effects of the expression of each injected apoE. Consistent with the reduction in TC levels, chylomicron (CM), very low-density lipoprotein (VLDL), IDL and LDL cholesterol levels were almost normalized in the Fcγ(−)/apoE(−;E3) mice (Figure 1D), with the reduction much less substantial in the Fcγ(−)/apoE(−;E-Sendai) mice. In terms of serum TG, extremely high levels of VLDL- and IDL-TG were seen in the Fcγ(−)/apoE(−;E3) and the Fcγ(−)/apoE(−;E-Sendai) mice after RA injection (Figure 1E), with the increase significantly steeper for the Fcγ(−)/apoE(−;E3) mice. These differences were especially prominent in the fractions containing the smaller VLDLs and the larger LDLs. The Fcγ(−)/apoE(−;LacZ) and Fcγ(−)/apoE(+;LacZ) mice showed similar lipoprotein profiles before and after RA injection.

**Fcγ(−)/apoE(−;E3) mice displayed the most drastic LPG-like changes**

Kidney samples taken from mice 3 weeks after RA injection were examined histologically. Lipoprotein-containing,
thrombus-like substances in dilated vascular lumens, a feature mimicking human LPG, were observed under light microscope in FcR\(\gamma^{-}\)/apoE\(+;\text{LacZ}\), FcR\(\gamma^{-}\)/apoE\(\neg;\text{E-Sendai}\) and FcR\(\gamma^{-}\)/apoE\(\neg;\text{E3}\) samples visualized with periodic acid Schiff (PAS) (data not shown) and Azan-Mallory (AM) stain (Figure 2A, a–c). These substances also stained with oil red O in osmium tetroxide (OsO\(_4\))-post-fixed specimens (Figure 2A, e–g). Moreover, as revealed by electron microscopy, the bodies containing the substances possessed lamellar-like structures of various electron densities, as well as numerous, small lipid vacuoles (Figure 2A, i–k). These were compatible with lipoprotein thrombi in patients with LPG. In contrast, these abnormalities were not detected in the FcR\(\gamma^{-}\)/apoE\(\neg;\text{LacZ}\) mice (Figure 2A, panels d and h).

In a quantitative examination of renal sections stained for lipids, an average of 30.1 ± 9.3% of the glomeruli in the FcR\(\gamma^{-}\)/apoE\(\neg;\text{E3}\) mice showed lipoprotein thrombi, whereas only 8.8 ± 4.4 and 2.4 ± 2.4% were positive in the FcR\(\gamma^{-}\)/apoE\(\neg;\text{E-Sendai}\) and FcR\(\gamma^{-}\)/apoE\(\neg;\text{LacZ}\) mice, respectively (Figure 2B).

The lack of FcR\(\gamma\) is related to the development of LPG in concert with expressed human apoEs

To confirm the influence of FcR\(\gamma\) deficiency in the development of LPG, we injected RAs containing the human apoE3 gene into the age-matched apoE-KO [referred to as FcR\(\gamma\)(+)/apoE\(\neg;\text{E3}\)] mice and compared the results with those for the FcR\(\gamma^{-}\)/apoE\(\neg;\text{E3}\) mice. As a control, the FcR\(\gamma\)-KO mice with murine wild-type apoE were also infected with RAs containing the human apoE3 gene; these mice were designated as FcR\(\gamma^{-}\)/apoE\(+;\text{E3}\) mice. In all the groups, the body weight and food intake were comparable throughout the experimental period (data not shown). Moreover, as seen for the FcR\(\gamma^{-}\)/apoE\(\neg;\text{E3}\) mice, the FcR\(\gamma\)(+)/apoE\(\neg;\text{E3}\) mice also showed a rapid decrease in serum TC levels and a transient increase in serum TG levels after RA injection (data not shown). Thus, serum lipid profiles were not affected by the lack of FcR\(\gamma\). In a histological examination, enlarged capillary lumina with lipoprotein thrombi were seen in FcR\(\gamma\)(+)/apoE\(\neg;\text{E3}\) samples, but the frequency at which glomeruli with lipoprotein thrombi were observed was only 3.8 ± 2.7%.

![Fig. 3. Glomerular finding and macrophage infiltration into glomeruli in the FcR\(\gamma^{-}\)/apoE\(\neg;\text{E3}\) and the FcR\(\gamma\)(+)/apoE\(\neg;\text{E3}\) mice.](image-url)

(A) The percentage of glomeruli with lipoprotein thrombi in the FcR\(\gamma^{-}\)/apoE\(\neg;\text{E3}\) and the FcR\(\gamma\)(+)/apoE\(\neg;\text{E3}\) mice. The data are shown as the mean (SE), and statistical differences are shown as P < 0.01(**). (B) Representative findings of immunohistologically examined glomeruli in the FcR\(\gamma^{-}\)/apoE\(\neg;\text{E3}\) and FcR\(\gamma\)(+)/apoE\(\neg;\text{E3}\) mice with anti-mouse CD68 Ab. (C) The percentages of anti-CD68 Ab-positive cells per glomerulus for the FcR\(\gamma^{-}\)/apoE\(\neg;\text{E3}\) \((n = 10)\) and FcR\(\gamma\)(+)/apoE\(\neg;\text{E3}\) \((n = 10)\) mice. The numbers of anti-CD68 Ab-positive cells were calculated in 50 randomly selected glomeruli in each mouse. The data are shown as the mean (SE). Statistical differences are shown as P < 0.01(**).
(Figure 3A). Meanwhile, no LPG lesions were present in the FcRγ(−)/apoE(+;E3) mice.

**Macrophages rarely infiltrated into the glomeruli of FcRγ(−)/apoE(−;E3) mice compared with FcRγ(+)/apoE(−;E3) mice**

Macrophage infiltration of glomeruli was examined by immunohistological examination using anti-mouse CD68 Ab. Cells positive for CD68 had markedly infiltrated the glomeruli in the FcRγ(+)/apoE(−;E3) mice, and often showed foam-cell formation. In contrast, the macrophage infiltration of glomeruli and foam-cell formation were rarely observed in the FcRγ(−)/apoE(−;E3) mice (Figure 3B). A significant difference in the numbers of cells positive for anti-CD68 Ab per glomerulus was observed between the FcRγ(−)/apoE(−;E3) and FcRγ(+)/apoE(−;E3) samples. The numbers were as follows: FcRγ(−)/apoE(−;E3), 2.29 ± 0.29 cells/glomeruli and FcRγ(+)/apoE(−;E3), 4.71 ± 0.50 cells/glomeruli (Figure 3C). Furthermore, slight correlations were found between the number of CD68-positive cells/glomeruli and the percentage of lipoprotein thrombi-positive glomeruli ($r = 0.301$, $P < 0.05$).

The macrophage response to modified LDL was suppressed in FcRγ(−)/apoE(−;E3) mice

We then examined the macrophage response to oxidized LDL (ox-LDL), which is a representative modified LDL. Number-matched peritoneal macrophages collected from the FcRγ(−)/apoE(−;E3) and FcRγ(+)/apoE(−;E3) mice were cultured with ox-LDL (10 μg/mL) for 24 h. Afterwards, the macrophages were stained with oil red O. Ox-LDL that had been taken up was observed as oil red O-positive substances in the macrophages. The ratios of cells positive for oil red O were 28.9 ± 5.2% for the FcRγ(−)/apoE(−;E3) samples and 79.3 ± 1.6% for the FcRγ(+)/apoE(−;E3) samples. The uptake of ox-LDL by the peritoneal macrophages was significantly reduced for FcRγ(−)/apoE(−;E3) relative to that for FcRγ(+)/apoE(−;E3) (Figure 4A and B). In the supernatant with the peritoneal macrophages, the expression of monocyte chemotactic protein-1 (MCP-1) and regulated upon
activation, normal T-cell expressed and secreted (RANTES) was evaluated by enzyme-linked immunosorbent assay (ELISA) at each time point. The concentrations of MCP-1 and RANTES in the FcγR(−)/apoE(−;E3) samples were significantly reduced after administration of ox-LDL compared with those in the FcγR(+)/apoE(−;E3) samples (Figure 4C and D).

The transcript levels of the LDL receptor gene (Ldlr) in the kidney were reduced in FcγR(−)/apoE(−;E3) mice relative to those in FcγR(+)/apoE(−;E3) mice

To obtain a clear understanding of the mechanism for the reduced lipid uptake in the macrophages, mRNA transcripts of the LDL receptor (LDL-R), i.e. Ldlr, and major scavenger receptors (SRs), i.e. Scarf2, Cxcl16, were evaluated by quantitative real-time polymerase chain reaction (PCR) in the kidney. No significant differences in the levels of mRNA transcripts for these SRs (data not shown) were observed. However, Ldlr transcripts were significantly reduced in the FcγR(−)/apoE(−;E3) mice compared with those in the FcγR(+)/apoE(−;E3) mice (Figure 4E).

Discussion

LPG is a disease characterized by the deposition of lipoprotein thrombi in markedly dilated glomerular capillaries. Moreover, apoE-Sendai and several other apoE mutations have often been found in patients with LPG [4–12], so that apoE variants are considered to be an important factor in LPG development. However, healthy carriers of apoE variants in families of patients with LPG have not been rare [2]. Further, Ishigaki et al. [13] described a murine LPG model in which mutated human apoE had induced LPG in apoE-KO mice and found that only some of the mice were responders of renal lesions.

On the other hand, Kanamaru et al. [16] indicated that FcγR deficiency was involved in the development of LPG in mice with cGVHD. However, this group could not explain in particular the apoE abnormalities observed in human LPG because FcγR-KO mice have normal lipid profiles. In the present study, to examine the mechanism by which the combination of an apoE mutation and FcγR deficiency affects the development of LPG, we generated FcγR(−)/apoE-KO mice and subsequently introduced several kinds of human recombinant apoE genes using virus-mediated gene transfer.

In particular, we performed lipid fixation by OsO4 [20] for the kidney specimens. Lipoprotein thrombi are seen as vacuolar or empty spaces by light microscopy. This manifestation has hindered the detailed observation of the glomerular features, and evaluations using traditional paraffin-embedded sections pose the risk of minor lesions with lipoprotein thrombi being overlooked with light microscopy. This method allowed us to observe lipid deposition in the murine glomeruli in detail.

In the current study, we observed that FcγR(−)/apoE (+;LZ) mice, which expressed the murine wild-type apoE, displayed a few LPG-like lipoprotein thrombi (Figure 2), indicating that LPG barely develops under conditions of FcγR deficiency when wild-type apoE is present. On the other hand, LPG lesions were present under conditions with human recombinant apoE(S, as shown with the FcγR(−)/apoE(−;E-Sendai) and FcγR(+)/apoE(−;E3) mice (Figure 2). Against our expectations, FcγR(−)/apoE(−;E3) mice, which carried the wild-type human apoE, showed more drastic LPG-like changes than FcγR(−)/apoE(−;E-Sendai) mice, although apoE-Sendai has been reported to be one of most important apoE variants identified in patients with LPG (Figure 2B) [2, 13, 21].

The structural differences between human and murine wild-type apoE have been reported [22]. In addition to human apoE-Sendai, human apoE3 may also act as an abnormal apoE in mice and lead to LPG in a murine model, although apoE3 is the wild-type version of the human apoE. In addition, an alteration in the lipoprotein composition may be related to it. Xenogeneic apoE injection induced severe hypertriglyceridemia [13], though the mechanism was unknown. In this study, the serum TG levels were elevated after apoE injection and were substantially higher in the FcγR(−)/apoE(−;E3) mice than in the FcγR(−)/apoE(−;E-Sendai) mice. These results suggest that the overloading of TG-rich lipoproteins may play a more important role in the development of LPG than hypercholesterolemia. This interpretation is consistent with previous reports that abnormal TG metabolism may have a pathogenic role in the peculiar glomerular lesions of human LPG [23], and that TG-lowering therapies, such as the use of fribates, are more effective than cholesterol-lowering therapies, such as the use of human menopausal gonadotrophin-CoA inhibitors in patients with LPG [24–26]. Furthermore, the increased TGs in the FcγR(−)/apoE(−;E3) and FcγR(+)/apoE(−;E-Sendai) mice were primarily distributed within the smaller VLDLs and larger LDLs (Figure 1E). These fractions are elevated in patients with type III hyperlipoproteinemia [27], and the levels in these groups are comparable with those seen in LPG patients [21].

In this study, we also observed that the frequency of lipoprotein thrombi in the FcγR(−)/apoE(−;E3) mice was significantly greater than that in the FcγR(+)/apoE(−;E3) mice (Figure 3A), despite the similar lipid profiles. These results clearly indicate that FcγR deficiency leads to worsened LPG under conditions of human apoE3 expression. Moreover, we noticed fewer infiltrated macrophages and foam-cell changes in the glomeruli of the FcγR(−)/apoE(−;E3) mice than in those of the FcγR(+)/apoE(−;E3) mice (Figure 3B and C). These findings were consistent with previous reports that foam cells are rarely observed in the glomeruli of patients with LPG, despite their hyperlipidemia [2, 21]. In contrast, renal lipodosis, which is induced by type III hyperlipidemia, is characterized by severe glomerulosclerosis with a large number of foam cells [28, 29]. Analogous to the lesions for atherosclerosis [30–33], the importance of macrophages with SRs has been suggested to be an aggravating factor [34, 35]. These findings, which distinguished LPG from renal lipodosis, suggest that the lack of macrophage infiltration into

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glomeruli is a key observation elucidating the pathogenesis of LPG. Therefore, we hypothesized that the macrophage impairment resulting from FcγR deficiency prompts lipoprotein thrombi to be deposited in the glomerular capillaries, in the current murine model.

To examine this hypothesis, we collected peritoneal macrophages from FcγRγ(+/−)/apoE(−;E3) and FcγRγ(−/−)/apoE(−;E3) mice and examined lipid uptake and chemokine secretion after culturing the macrophages with excess ox-LDL, a representative modified LDL. The uptake of ox-LDL in FcγRγ(−/−)/apoE(−;E3) macrophages was impaired relative to that in FcγRγ(+/−)/apoE(−;E3) macrophages. Moreover, the secretion of MCP-1 and RANTES in the FcγRγ(−/−)/apoE(−;E3) macrophages was also significantly reduced (Figure 4C and D). These results indicate that the lack of FcγR causes an impairment in the macrophage response to modified lipids.

Macrophages possess several SRs [36, 37], which are involved in phagocytosis and are defined by their ability to bind modified forms of LDL [38]. Moreover, FcγR has been implicated in the up-regulation of SRs [39, 40]. Our group previously described a drastic decline in the mRNA expression of Cd36 in cGVHD-induced FcγRγ-KO mice that had developed LPG [41]. However, an analysis of the mRNA expression of major SRs showed no significant differences between FcγRγ(−/−)/apoE(−;E3) and FcγRγ(+/−)/apoE(−;E3) mice. On the other hand, LDL-R has been implicated in the formation of foam cells and in the uptake of LDL-containing immune complexes through FcγR [36, 42, 43]. In this study, the Ldlr were reduced in the kidneys of FcγRγ(−/−)/apoE(−;E3) mice, and the down-regulation of LDL-R through FcγR may be one of the mechanisms in the impairment of macrophage function in the uptake of modified lipids. Moreover, FcγR activation triggers the production of cytokines, proteolytic enzymes and reactive oxygen intermediates [40, 42, 44], and FcγR is also known to be one of the receptors on macrophages involved in lipid metabolism [45–47]. In particular, FcγRI and FcγRII are involved in the uptake of modified LDL [45, 47]. Accordingly, the deficiency of FcγRI may contribute directly to the impairment of lipid uptake into macrophages in the current model.

These macrophage impairments derived from FcγR deficiency are insufficient for the development of LPG, since lipoprotein thrombi are rarely observed in FcγRγ(+/−)/apoE(+;LZ) and FcγRγ(−/−)/apoE(+;E3) mice, which express normal murine apoE. However, under conditions with xenogeneic apoE, especially human apoE3, the FcγRγ(−/−)/apoE(−;E3) mice developed severe LPG. These results indicate that impaired macrophages are unable to clear abnormal lipoproteins containing substantial amounts of TGs, causing excessive lipid deposits in tissues and thereby resulting in lipoprotein thrombi. Therefore, macrophage dysfunction may cause the development of LPG, though an increased function in macrophages has been regarded as an aggravating factor for renal lipodosis and atherosclerosis [34, 35].

In conclusion, our results suggested that the impairment of macrophage function resulting from FcγR deficiency plays a principal role in the development of LPG in concert with apoE abnormalities. Unfortunately, macrophage functions have not been examined in previously reported cases of LPG. An accumulation of more cases of LPG and research into macrophage function in these cases may facilitate the elucidation of its pathogenesis, as well as the evidence-based development of new therapies for LPG.

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