Effect of interleukin-6 receptor blockage on renal injury in apolipoprotein E-deficient mice

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Tomiyama-Hanayama M, Rakugi H, Kohara M, Mima T, Adachi Y, Ohishi M, Katsuya T, Hoshida Y, Aozasa K, Ogihara T, Nishimoto N. Effect of interleukin-6 receptor blockage on renal injury in apolipoprotein E-deficient mice. Am J Physiol Renal Physiol 297: F679–F684, 2009. First published July 1, 2009; doi:10.1152/ajprenal.90680.2008.—Hyperlipidemia has been demonstrated to be associated with renal disease, yet the mechanism of renal injury is still poorly understood. Inflammation that occurs with the hyperlipidemia has been considered to play an important role in development of glomerular injury. In the present study, we investigated the role of interleukin-6 (IL-6), a key inflammatory molecule, on renal injury in apolipoprotein E-deficient (ApoE−/−) mice with severe hypercholesterolemia. The 6-wk-old mice were fed a high-fat diet and administered weekly rat anti-IL-6 receptor monoclonal antibody (MR16-1), control rat IgG, or saline for a total of 4 wk. We examined histopathological changes in the kidney and urinary excretion of protein and albumin. Saline- and IgG-treated mice showed remarkable proteinuria at 10 wk of age, whereas MR16-1-treated mice exhibited significantly lower levels. Renal histopathology of saline- and IgG-treated mice revealed striking lipid deposits and foam cells in the glomerular tuft, juxtaglomerular area, and arteriolar wall along with range of mesangial cell proliferation and matrix expansion. Notably, the severity of lipid deposits and mesangial cell proliferation were significantly reduced in MR16-1-treated mice. Immunohistochemistry demonstrated that mesangial IL-6 expression was dramatically reduced in MR16-1-treated mice compared with IgG-treated mice. Blocking the IL-6 receptor prevented progression of proteinuria and renal lipid deposit, as well as the mesangial cell proliferation associated with severe hyperlipoproteinemia. These results clearly demonstrate that IL-6 plays an essential role in the pathogenesis of hyperlipidemia-induced glomerular injury in ApoE−/− mice and suggests the usefulness of anti-IL-6 receptor antibody in treatments for hyperlipidemia-induced organ damage.

mesangial cells; macrophages; anti-mouse interleukin-6 receptor antibody MR16-1

HYPERLIPIDEMIA IS A WELL-KNOWN RISK FACTOR for cardiovascular diseases and is thought to accelerate the progression of renal diseases. Recent experimental and clinical investigations have suggested a correlation between the progression of renal disease and dyslipidemia, including an altered apolipoprotein profile and elevated plasma cholesterol and triglyceride levels (2, 3, 28). Apolipoprotein E-deficient (ApoE−/−) mice are considered a well-accepted model of severe hyperlipidemia; ApoE−/− mice on high-fat diets exhibit a dramatic increase in both blood cholesterol and triglyceride levels, as well as accelerated atherosclerosis (19, 34). Recently, ApoE−/− mice were reported to present not only atherosclerosis but also renal disease with remarkable pathological alterations, including glomerular infiltration with foam cells, lipid deposits at glomerular capillaries, and expanded mesangium (33). The underlying pathophysiological mechanisms for the relationship between lipid levels and progression of renal disease, however, are not yet fully understood.

Studies in the hypercholesterolemic animal model revealed an association between renal injury, as well as atherosclerosis, and inflammation. Rats with diet-induced hypercholesterolemia exhibited interstitial inflammation and fibrosis in the kidney (6). Another study in pigs showed a high-cholesterol diet induced endothelial dysfunction, accompanied by increased intrarenal oxidative stress and inflammation (5).

Interleukin-6 (IL-6) is a well-known critical mediator of inflammatory changes (1). Studies in IL-6 transgenic mice suggested proliferative glomerulonephritis developed in the presence of high concentrations of IL-6 (23, 25). The glomerulonephritis was inhibited by treatment with MR16-1 (anti-IL-6 receptor antibody), whose specificity to block IL-6 signaling was well-confirmed in the previous studies (12, 26, 27). However, the function of IL-6 in hypercholesterolemia-induced renal injury and associated inflammation is still unclear. Hypercholesterolemia is usually associated with metabolic changes in adipose tissue, which produces and releases a variety of proinflammatory and anti-inflammatory factors, including leptin, adiponectin, and resistin, as well as cytokines, such as tumor necrosis factor (TNF-α). Accordingly, IL-6 is expressed along with its receptor in adipose tissue and, to a large extent, is secreted from adipose tissue during noninflammatory conditions (16). Proinflammatory molecules produced by adipose tissue are accelerated in obesity. The presence of systemic inflammation has been linked to an increased risk of developing of cardiovascular diseases in obesity (31) and may consequently associate with renal injury. These evidences suggest IL-6, systemically produced from adipose tissue and locally produced in the kidney, may play a role in renal injury induced in the hyperlipidemia ApoE−/− mouse model.

In the present study, we investigated the role of IL-6 in hyperlipidemia-induced renal injury. We assessed the effect of IL-6 inhibition by its receptor antibody treatment on kidney structure and function in ApoE−/− mice fed a high-fat diet.
MATERIALS AND METHODS

Animals and experimental protocol. Male ApoE−/− mice, 5 wk of age, of C57BL/6 backgrounds were purchased from Taconic Farms (Germantown, NY). The mice were maintained under specific pathogen-free conditions and monitored weekly. At 6 wk of age, the mice were randomly divided into three groups (20 mice/group): the saline group, the rat IgG group (class-matched control, catalog no. 14131; Sigma-Aldrich, St. Louis, MO), or the MR16-1 group. Mice in the MR16-1 group were administered rat anti-mouse IL-6 receptor (mIgL-6R) monoclonal antibody (MR16-1, isotype IgG1k). The specificity and blocking ability of this monoclonal antibody was well-confirmed in previous reports (12, 26, 27).

Mice were first injected intraperitoneally with saline, 2 mg of MR16-1, or control rat IgG antibody at 6 wk of age to induce tolerance against rat IgG (12). Subsequent injections were with saline or 0.5 mg of antibody weekly from 7 to 9 wk of age (12). Mice were fed a high-fat diet (15% cacao butter, 1.25% cholesterol, and 0.5% sodium cholate, F2HFD1; Oriental Yeast, Tokyo, Japan) from 6 to 10 wk of age (24).

Blood serum and urine were collected at 10 wk of age and stored at −80°C. Kidneys were removed and either fixed with 10% neutral buffered formalin for histological examination or frozen for immunofluorescence staining. This study was approved by the Institutional Laboratory Animal Care and Use Committee of the Osaka University.

Plasma and urinary data analysis. Total cholesterol, triglyceride, free fatty acid, and glucose levels were measured with enzymatic kits (Wako, Osaka, Japan), and blood urea nitrogen levels were measured via an enzymatic method (SRL, Tokyo, Japan). Proteinuria was measured semiquantitatively at the age of 10 wk using the urinary reagent strip method (Uropinas S; Kyowa, Tokyo, Japan). Protein concentration in urine was categorized into five grades and defined as follows: +/-, 15 mg/dl; 1 +, 30 mg/dl; 2 +, 100 mg/dl; 3 +, 250 mg/dl; and 4 +, 1,000 mg/dl (according to the “Urinary Reagent Strip Method” guidelines set by the Japanese Committee for Clinical Laboratory Standards). The urinary albumin concentration was measured by ELISA (Shibayagi, Gunma, Japan), and the urinary creatinine concentration was measured spectrophotometrically using a creatinine assay kit (Exocell, Philadelphia, PA). The urinary albumin levels were calculated, normalized with creatinine levels, and determined as the index of excreted urinary albumin.

Blood pressure and heart rate measurements. Systolic and diastolic blood pressure and heart rate were measured twice at 6 and 10 wk of age. Blood pressure and heart rate measurements were conducted in stable position, using an appropriate-size tail cuff and a mercury sphygmomanometer; measurements were calculated as the average of three separate readings.

Histology and immunohistochemistry. Mice were euthanized and perfused at physiological pressure. Then, the kidneys were dissected. The samples were fixed in 10% formalin and routinely processed for paraffin embedding. Histological sections cut at 4 μm were stained with hematoxylin/eosin and periodic acid-Schiff (PAS) staining. A part of the samples was embedded in OCT compound, snap frozen in liquid nitrogen, and stored at −80°C before use. Frozen materials were cut at 6 μm with a cryotome and used for immunohistochemistry and oil-red O staining. At immunohistochemistry, fixed tissue sections were incubated with 0.3% H2O2 in 0.1 M sodium azide to inhibit endogenous peroxidase activity, and then incubated with 20% goat serum for 60 min at room temperature (RT), followed by incubation with a monoclonal antibody against mouse IL-6 (Innogenetics, Ghent, Belgium) or against mouse CD68 (AbD Serotec, Oxford, UK), and further processed with universal immunoenzyme polymer, anti-rat (Histofine Simplestain Max PO; Nichirei, Tokyo, Japan) for 30 min at RT (9). Immunoreactivity was visualized with 3,3′-diaminobenzidine (DAB; Nichirei, Tokyo, Japan). CD68 antibody was used to distinguish macrophages from mesangial cells in the kidney. Sections were counterstained with hematoxylin and, finally, double-stained with oil-red O.

Histopathological evaluation of the kidney. All histopathological evaluations were performed by pathologists blinded to the specific treatment of each sample. The resulting indexes in each animal are expressed as a mean of all scores obtained. On the PAS-stained sections, lipid deposit could be identified as the translucent areas in the glomerular tuft; the percentage of lipid deposit was determined in 100 randomly selected glomeruli per animal (4). Each glomerulus was graded for lipid deposits as follows: none, no changes; mild, <40% lesions observed; moderate, 40–70% lesions; and severe, >70% of the field contained lesions. As indexes for renal damage of the extent of glomerulosclerosis, the increase in mesangial cells and matrices in the mesangial region were analyzed in 100 randomly selected glomeruli per kidney and graded as follows: grade 0, no changes; grade 1, focal and segmental lesions detected; grade 2, segmental and diffuse lesions; and grade 3, global lesions and diffuse within the field.

Statistical analysis. Statistical analysis was performed using StatView software (version 5; Abacus Concepts, Berkeley, CA), and values are means ± SD. An unpaired Student’s t-test was used for comparison between the two groups. The significance of differences in severity of lipid deposit and mesangial cell proliferation among the three groups was evaluated using the Kruskal-Wallis test. Lipid deposit and mesangial cell proliferation were compared among the grades categorized by histopathological outcome with the Kruskal-Wallis test. A value of P < 0.05 was accepted as statistically significant.

RESULTS

The metabolic and hemodynamic characteristics of ApoE−/− mice after 4 wk of treatment are summarized in Table 1. The high-fat diet remarkably increased total cholesterol, triglyceride, and fatty acid levels in each of the ApoE−/− mice treatment groups. Glucose levels and hemodynamic indexes did not differ among the three groups. The weights of whole body, heart, kidney, and fat did not differ among the study groups, but the spleen weight was significantly greater in the saline and rat IgG groups than in the MR16-1 group (data not shown).

The mortality rate varied among the study groups; eight mice in the saline group (n = 20), three in the IgG group (n = 20), and three in the MR16-1 group (n = 20) died during the 4-wk treatment period. The cause of death is unclear. Two of the seven mice in the saline group, which subsequently died, were examined for proteinuria before death, and urinary protein levels were >1,000 mg/dl.

Renal function. Table 2 summarizes renal function at 10 wk of age as assessed by blood urea nitrogen and urinary excretion of protein and albumin. Blood urea nitrogen levels did not differ among the groups, and serum creatinine levels were not

| Table 1. Metabolic and hemodynamic characteristics at 10 wk |
|-----------------|-----------------|-----------------|
|                 | Saline          | Rat IgG         | MR16-1         |
| n               | 12              | 17              | 17             |
| Total cholesterol, mg/dl | 1,660±284        | 1,588±255       | 1,740±377      |
| Triglycerides, mg/dl | 193±46          | 199±64          | 224±68         |
| Free fatty acid, mEq/l | 3.8±0.8         | 3.6±0.8         | 4.3±0.9        |
| Glucose, mg/dl | 195±34          | 213±30          | 203±30         |
| Body weight, g | 24±1            | 25±2            | 23±2           |
| Kidney weight, mg | 123±12          | 123±16          | 122±13         |
| Systolic blood pressure, mmHg | 105±15          | 105±14          | 105±11         |
| Diastolic blood pressure, mmHg | 50±10           | 55±11           | 51±10          |
| Heart rate, beats/min | 622±64          | 663±66          | 600±72         |

Values are means ± SD; n = no. of apolipoprotein E-deficient mice treated with saline, rat IgG, or rat anti-IL-6 receptor antibody (MR16-1).
measured due to severe chylemia in all mice. The saline- and rat IgG-treated mice showed remarkable proteinuria, whereas the MR16-1-treated mice showed only low levels. Urinary albumin excretion, normalized by creatinine, was also remarkably elevated in saline- and IgG-treated mice but not in MR16-1-treated mice.

Histopathological indices of renal damage and glomerulus lipid deposit. A striking alteration, observed in renal structure, was the presence of foam cells and lipid deposits in the glomerular tuft, juxtaglomerular area, and arteriolar wall in the saline (Fig. 1a) and IgG-treated mice (Fig. 1c). The severity of lipid deposits was significantly suppressed in the MR16-1 group (Fig. 1e). Semiquantitative analysis of lipid deposition showed significant reduction of lipid deposits by treatment with MR16-1 (Table 3). There was no significant difference in lipid deposits between the saline and rat IgG groups.

Various degrees of mesangial cell proliferation and matrix expansion as revealed by PAS-positive area in the mesangial region were present with form cells and/or lipid deposits, contributing to the enlargement of glomerulus (Fig. 1, a–d). Only a few CD68-positive macrophages were detected in the mesangial region, and there was no obvious increase of infiltrating lymphocytes, indicating that these proliferating cells were mesangial cells (see Fig. 3). The proportion of mesangial cell proliferation and lipid deposition differed among the glomeruli. In several severe cases, glomerular lobulation or sclerosis was observed (Fig. 1, b and d) and capillary lumens were occasionally occluded by thrombuslike structures. The mesangial cell proliferation and matrix expansion were significantly milder in the MR16-1 group compared with the saline and rat IgG groups (Table 3). There was no significant difference in the grade of severity between the saline and rat IgG groups (Table 3).

Table 2. Renal function and urinary protein

<table>
<thead>
<tr>
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<th>Saline</th>
<th>Rat IgG</th>
<th>MR16-1</th>
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<tbody>
<tr>
<td>n</td>
<td>12</td>
<td>17</td>
<td>17</td>
</tr>
<tr>
<td>BUN, mg/dl</td>
<td>23.1±3.3</td>
<td>21.7±5.6</td>
<td>22.3±4.4</td>
</tr>
<tr>
<td>Proteinuria, mg/dl</td>
<td>187.5±74.0</td>
<td>72.7±58.0*</td>
<td>18.8±6.7†</td>
</tr>
<tr>
<td>Urinary albumin/creatinine, mg/g Cre</td>
<td>55.2±54.2</td>
<td>60.3±78.1*</td>
<td>10.6±3.7†</td>
</tr>
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Values are means ± SD. BUN, blood urea nitrogen. *P < 0.05 vs. saline group. †P < 0.05 vs. IgG group.

Fig. 1. Histological features of the kidneys in apolipoprotein E-deficient (ApoE<sup>−/−</sup>) mice. The glomerulus in ApoE<sup>−/−</sup> mice was treated with saline (a and b), rat IgG (c and d), or rat anti-IL-6 receptor monoclonal antibody MR16-1 (e). Wild-type control is shown in f. The ApoE<sup>−/−</sup> mice treated with saline and rat IgG showed lipid deposit (a and c; arrows) and mesangial cell proliferation (b and d). There was a diffuse mesangial cell proliferation accompanied with deposition of red-purple mesangial matrix (b and d). In contrast, only minor mesangial cell proliferation and lipid deposit were observed in the ApoE<sup>−/−</sup> mice treated with MR16-1 (e). All magnification, ×600; staining is periodic acid-Schiff.
F682 ROLE OF IL-6 IN RENAL INJURY

Table 3. Histopathology of the kidney

<table>
<thead>
<tr>
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<th>Saline</th>
<th>Rat IgG</th>
<th>MR16-1</th>
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<tr>
<td><strong>Lipid deposits, % of 100 glomeruli</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None or mild</td>
<td>55±1*</td>
<td>58±14</td>
<td>79±14†</td>
</tr>
<tr>
<td>Moderate</td>
<td>44±17</td>
<td>40±14</td>
<td>21±14</td>
</tr>
<tr>
<td>Severe</td>
<td>1.2±1.6</td>
<td>0.9±2.1</td>
<td>0*+</td>
</tr>
<tr>
<td><strong>Mesangial cell proliferation, no. (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grade 0</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>1 (5.9)</td>
</tr>
<tr>
<td>Grade 1</td>
<td>1 (8.3)</td>
<td>1 (5.9)</td>
<td>12 (70.6)</td>
</tr>
<tr>
<td>Grade 2</td>
<td>4 (23.5)</td>
<td>4 (23.5)</td>
<td></td>
</tr>
<tr>
<td>Grade 3</td>
<td>10 (83.3)</td>
<td>12 (70.6)</td>
<td>0 (0)§§</td>
</tr>
</tbody>
</table>

Values are means ± SD. The percentage of lipid deposit was determined in 100 randomly selected glomeruli per animal; each glomerulus was graded for lipid deposits as follows: none, no changes; mild, <40% lesions observed; moderate, 40–70% lesions; and severe, >70% of the field contained lesions. Mesangial cell proliferation was analyzed in 100 randomly selected glomeruli per kidney and graded as follows: grade 0, no changes; grade 1, focal and segmental lesions detected; grade 2, segmental and diffuse lesions; and grade 3, global lesions and diffuse within the field (percentages are shown in parentheses). Statistical analyses using the Kruskal-Wallis test show significant difference among the 3 groups in the severity of lipid deposit (P = 0.001) and mesangial cell proliferation (P = 0.00001). Post hoc analyses to compare 2 groups were performed using the nonparametric Newman-Keuls multiple comparison test. *P = 0.002, MR16-1 vs. IgG group. †P = 0.001, MR16-1 vs. saline group. §P = 0.0001, MR16-1 vs. IgG group. §§P = 0.0001, MR16-1 vs. saline group.

Immunohistochemistry. Using immunohistochemistry, we compared the level of IL-6 in the glomeruli between the rat IgG and MR16-1 groups. IL-6 was expressed in the mesangial cells of glomerulus from ApoE<sup>−/−</sup> mice treated with control rat IgG (Fig. 2b). In addition, double-staining with oil-red O demonstrated lipid deposition was also found in the glomerulus, which expresses IL-6 (Fig. 2f). In the MR16-1 treatment group, levels of both IL-6 expression and lipid deposition in the glomerulus had been dramatically reduced compared with the control IgG treatment group (Fig. 2d).

DISCUSSION

Our study demonstrated, for the first time, that blocking the IL-6 receptor prevents the progression of proteinuria, mesangial cell proliferation, and renal lipid deposit in ApoE<sup>−/−</sup> mice. In our study, the ApoE knockout mice were fed a high-fat diet containing 15% cacao butter, 1.25% cholesterol, and 0.5% sodium cholate, which caused an earlier onset of renal phenotype than in those with regular chow diet (18). Hyperlipidemia has been discussed to accelerate the induction and progression of renal injury leading to glomerulosclerosis and tubulointerstitial lesions (17). Lipid-lowering treatments are known to reduce renal lesions and preserve renal function (14). However, the mechanism by which hyperlipidemia contributes to renal injury is less well studied. Hyperlipidemia is thought to induce a classic proinflammatory response within the kidney glomerulus through production of well-described macrophage chemotactic and adhesion molecules, which results in the recruitment of macrophages and the development of glomerular injury (10). Supporting this idea, macrophage depletion was shown to inhibit hyperlipidemia-induced renal injury (13).

The effects to induce kidney injury are often associated with severe hyperlipidemia, as seen in ApoE knockout mice, but are relatively uncommon with “common trait” hyperlipidemia. ApoE deficiency causes a severe renal lipodisosis, which resembles a unique and rare human disease, lipoprotein glomerulopathy (22). Although we are looking at rare pathological changes caused by severe hyperlipidemia in ApoE<sup>−/−</sup> mice, defining mechanisms of injury in this model may identify pathways of renal injury in less severe hypercholesterolemia. Our experimental results showed that IL-6 was pathologically involved in the renal injury related to the severe hyperlipidemia in ApoE<sup>−/−</sup> mice. We assume that IL-6 might also be involved in the development of the renal disease caused by human hyperlipidemia.

The present study provides novel information as to the role of IL-6 in hyperlipidemia-induced renal injury that occurs in ApoE<sup>−/−</sup> mice. There are several possible mechanisms for the renal protection observed upon anti-IL-6 receptor antibody (MR16-1) treatment in ApoE<sup>−/−</sup> mice. Although severe hyperlipidemia has been shown to be the cause of renal injury in ApoE<sup>−/−</sup> mice, the protective effect of MR16-1 was not related to improvement in lipid metabolism, because MR16-1
treatment does not affect the development of hyperlipidemia. Many extraglomerular factors, such as arterial hypertension and diabetes mellitus, are known to initiate glomerulosclerosis (32). The present study, however, showed no differences in blood pressure and plasma glucose levels among the three groups. This suggests systemic circulating IL-6 and/or locally generated IL-6 in the kidney might be involved in the development of the renal injury.

Recently, ApoE was shown to bind lipid antigens and deliver them into endosomal compartments that contain CD1 molecules in antigen-presenting cells (APC). The lipid antigens are presented at the surface of APC to lipid antigen-reactive T cells, and a deficiency of ApoE causes the inactivation of the T cells to produce γ-interferon (29). This finding raises a possibility that MR16-1 treatment might modify the immune response that is characteristic in ApoE-deficient mice. According to this model, however, IL-6 could be reduced in ApoE knockout mice, because IL-6 is also secreted by activated APC and T cells. On the other hand, we showed a marked increase of IL-6 expression in the mesangial cells of glomerulus from ApoE knockout mice by immunohistochemistry. Furthermore, we did not find an obvious increase of infiltrating macrophages or T cells in the glomerulus of ApoE knockout mice (Fig. 3). Together, our results indicate that IL-6 is secreted by mesangial cells and may affect the mesangial cells in an autocrine manner and that anti-IL-6 therapy mainly inhibits this process.

Upregulation of circulating IL-6 is widely observed in atherosclerotic and inflammatory human diseases (15, 20). ApoE−/− mice fed a high-fat diet presented marked atherosclerosis, suggesting circulating IL-6 may be upregulated. We could not detect circulating IL-6 in ApoE−/− mice (8). This is consistent with the findings from Elhage et al. (8), although the failure to detect serum IL-6 could be due to the lower sensitivity of assaying mouse IL-6 compared with human IL-6.

In contrast, we clearly demonstrated increased IL-6 expression in the kidney of ApoE−/− mice using immunohistochemistry. IL-6 was expressed and might be secreted by mesangial cells, but not by leukocytes, and its expression was dramatically reduced in ApoE−/− mice treated with MR16-1 compared with rat IgG. These findings suggest IL-6 promotes positive feedback upregulation of IL-6 expression in mesangial cells.

In general, mesangial cell proliferation is the predominant pathological feature of many types of glomerulonephritis; it frequently precedes the increase of extracellular matrix in the mesangium and the development of glomerulosclerosis. A previous study showed IL-6 is an autocrine growth factor for mesangial cells, although this remains controversial (7, 21). Our study demonstrated IL-6 is a possible autocrine growth factor for mesangial cells and could accelerate mesangial proliferative glomerulonephritis.

Studies of mesangial proliferative glomerulonephritis, such as IgA nephropathy and lupus nephritis, showed IL-6 mRNA expression in the glomeruli of renal biopsy specimens using RT-PCR methods (11). In our renal injury model, MR16-1 treatment suppressed mesangial proliferation in ApoE−/− mice, confirming IL-6 to be responsible for the proliferation.

Glomerular lipid deposits were also reduced in ApoE−/− mice treated with MR16-1 compared with control groups. This finding suggests that increased IL-6 could accelerate lipid deposition in the kidney. Foam cells with lipid deposition in kidney are known to originate from macrophages and smooth muscle cells (33). The numbers of glomerular macrophages and foam cells have been reported to be increased in hyperlipidemic rats (30). Since IL-6 is known to accelerate transformation of monocytes to macrophages (1), IL-6 may accelerate lipid deposition through macrophage activation. However, only a few macrophages were detected in the glomeruli of ApoE−/− mice in this study. Furthermore, blockade of IL-6 using MR16-1 did not affect the number of the glomerular macrophages. Therefore, our findings suggest the existence of a distinct mechanism as to how IL-6 mediates lipid deposit in the kidney, and MR16-1 can inhibit this process. Further study is required to elucidate this mechanism.

Fig. 3. CD68 staining of the kidney from ApoE−/− mice. Immunohistochemical staining of kidney sections from ApoE−/− mice treated with saline (a), rat IgG (b), or MR16-1 (c) with anti-CD68 antibody is shown. There is no obvious difference in the number of CD68-positive macrophages (brown) among the 3 groups. d, positive control staining of spleen section. All magnification, ×400.

AJP-Renal Physiol • VOL 297 • SEPTEMBER 2009 • www.ajprenal.org
In summary, the present study clearly demonstrated IL-6 plays an essential role in the pathogenesis of severe hyperlipidemia-induced renal injury associated with mesangial cell proliferation and lipid deposit. Our report further suggests anti-IL-6 receptor antibody may provide a new model to study treatments for lipid-induced organ damage. The inhibition of IL-6 signal transduction may lead to the development of a new therapeutic strategy for glomerulonephritis accompanied with severe hyperlipidemia.

GRANTS

This work was financially supported by a grant from Chugai Pharmaceutical Co., Ltd., Tokyo, Japan, the manufacturer of MR16-1 (rat anti-mouse IL-6 receptor antibody) and tocilizumab (humanized anti-IL-6 receptor antibody).

DISCLOSURES

N. Nishimoto and T. Mima have served as consultants to and received honoraria from Chugai Pharmaceutical. The other authors have no conflicting interests.

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