Inflammatory stress exacerbates lipid-mediated renal injury in ApoE/CD36/SRA triple knockout mice

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Submitted 11 June 2010; accepted in final form 24 July 2011


In 1982, MOORHEAD AND COLLEAGUES (22) suggested that lipoprotein-induced nephron damage could result in progressive renal disease. In recent years, a number of studies have shown that the histological features of glomerulosclerosis are similar to the changes observed in atherosclerosis, and the term “glomerular atherosclerosis” has been proposed (21). It has long been established that cholesterol supplementation of the diets of several animal species leads to focal segmental glomerulosclerosis (FSGS). Studies using the puromycin amino nucleoside nephrotic rat model have also shown that cholesterol feeding increases the severity of proteinuria and FSGS (9, 14). Apolipoprotein B (ApoB) and ApoE were deposited in increased amounts in the mesangium and colocalized with oil red O-positive lipid deposits (33). However, the pathways of lipid accumulation and damage in kidney remain unclear.

Multiple pathways may be involved in renal lipid accumulation. Initially, uptake of modified LDL (oxidized or glycosylated) via the scavenger receptors was thought to be the major pathway for lipid accumulation. We have demonstrated that inflammatory cytokines cause lipid accumulation in human mesangial cells (HMCs) by inducing scavenger receptor type A (SRA) expression (27). However, evidence of involvement of macrophage scavenger receptors on foam cell formation is conflicting. Incubation of human monocyte-derived macrophages with oxidized LDL produces little macrophage cholesterol accumulation (27). Also, oxidized LDL is poorly metabolized within macrophages and reduces cholesterol esterification (8), the hallmark of foam cell formation. Boyanovsky (1) reported that phospholipase A2-modified LDL promotes foam cell formation by an SRA- and CD36 (scavenger receptor type B)-independent process. Kruth and colleagues (13) have recently demonstrated that macrophages can accumulate substantial quantities of lipids via macropinocytosis of LDL particles. In vivo, macrophage-specific expression of SRA in LDL receptor (LDLr) knockout (KO) mice decreases atherosclerosis (35). Moore et al. (20) recently reported that deprivation of either SRA- or CD36-mediated lipid uptake does not prevent atherosclerotic lesion progression, which suggests that lipid uptake by other receptors may be involved in intracellular lipid accumulation.

LDLr is the major lipoprotein receptor in maintaining whole body cholesterol homeostasis and is highly expressed in renal mesangial cells (27, 28, 29). Cholesterol uptake via LDLr and cholesterol synthesis is tightly regulated by feedback to intracellular cholesterol. Sterol-regulatory element-binding proteins (SREBPs) and its chaperone, SREBP-cleavage activating protein (SCAP), are two regulatory factors for LDLr transcription (7). LDLr gene activation is mediated by translocation of SREBP precursor from the endoplasmic reticulum to the Golgi apparatus for cleavage, following which the N terminal of SREBP2 enters the nucleus (5, 24). We have previously demonstrated that inflammatory stress can disrupt LDLr feedback regulation by increasing SCAP/SREBP expression and cause LDL cholesterol accumulation in HMCs, vascular smooth muscle cells, and liver cells (4, 19, 27, 28).

It seems that the main lipoprotein receptor in both HMCs and tubular cells is LDLr. We hypothesize that LDLr upregulation under inflammatory stress results in cholesterol redistribution under inflammatory stress results in cholesterol redistribution.
Table 1. Primers of genes in ApoE/CD36/SRA KO mice used for real-time PCR

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primers</th>
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</thead>
<tbody>
<tr>
<td>LDLr</td>
<td>F:5'-AGCCATGGGCCGCACTTGA-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-CCCTGGGGGCTGTTGATGCT-3'</td>
</tr>
<tr>
<td>SREBP2</td>
<td>F:5'-GATTGATTGCAATGGGAGG-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-CTCTGGAGTTGCTGTCCT-3'</td>
</tr>
<tr>
<td>SCAP</td>
<td>F:5'-GTCCTGGGGTCCACTACATT-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-GTCTTGGCCTGGAGCTT-3'</td>
</tr>
<tr>
<td>18S rRNA</td>
<td>F:5'-GGTTCAAGACCTTATAGTATGG-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-GGGTAGGCACACCGTCAA-3'</td>
</tr>
<tr>
<td>a-SMA</td>
<td>F:5'-GGGTCCTTGGAGGAGCTT-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-GTCCTGGGGTCCACTACATT-3'</td>
</tr>
<tr>
<td>Collagen type I</td>
<td>F:5'-GGGATGCATGGACACCTGATT-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-GGGTAGGCACACCGTCAA-3'</td>
</tr>
<tr>
<td>Collagen, type IV</td>
<td>F:5'-GGGATGCATGGACACCTGATT-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-GGGTAGGCACACCGTCAA-3'</td>
</tr>
<tr>
<td>Fibronectin</td>
<td>F:5'-GGGATGCATGGACACCTGATT-3'</td>
</tr>
<tr>
<td></td>
<td>R:5'-GGGTAGGCACACCGTCAA-3'</td>
</tr>
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ApoE, apolipoprotein E; KO, knockout; LDLr, LDL receptor; SREBP2, sterol-responsive element-binding protein 2; SCAP, SREBP cleavage-activating protein; TGF, transforming growth factor; SMA, smooth muscle actin.

bution from the circulation to the tissues, causing renal injury and lowering blood cholesterol levels. ApoE-deficient mice (ApoE KO) 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 (34). These data suggest that ApoE/CD36/SRA triple KO mice could be the best model for studying the roles of LDLr pathways in lipid accumulation. To further explore these phenomena, this study examined the effects of inflammatory stress and hypercholesterolemia on renal injury in ApoE KO mice with targeted deletions of both SRA and CD36, thus making the LDLr the dominant receptor in this model.

MATERIALS AND METHODS

Animal model and genotyping. Male C57BL/6J mice, ApoE KO mice, and ApoE/CD36/SRA triple KO mice in C57BL/6J genetic background (donated by Dr. Maria Febbraio, Lerner Research Institute) were used for this study. Three types of mice at 8 wk old (n = 6/group), fed a Western diet containing 21% fat and 0.15% cholesterol, were randomly assigned to subcutaneous injections of 0.5 ml 10% casein every other day or PBS as a control for 14 wk. At termination, plasma was collected for assays of total cholesterol, LDL cholesterol (LDLc), HDL cholesterol (HDLc; Rongsheng, Shanghai, China), amyloid A (SAA; R&D), and IL-6 levels (R&D). The kidneys were collected for histological assessment and examination of gene and protein expression. Animal care and experimental procedures were performed with approval from the Animal Care Committees of Chongqing Medical University.

Perfusion fixation of kidneys. Mice were anesthetized and perfused with 3% paraformaldehyde and 0.05% picric acid in a 6:4 mixture of cacodylate buffer (pH 7.4) and 10% hydroxyethyl starch. After 5 min of fixation, the mice were perfused for an additional 5 min with cacodylate buffer.

Measurements of blood urea nitrogen, creatinine, urine protein, and creatinine. Blood urea nitrogen (BUN) and creatinine concentrations were determined by urease/glutamate dehydrogenase coupled-enzyme reaction and Jaffe’s reaction using commercial kits (Dade Behring, Newark, NJ). Urine was collected in a metabolic cage at 14 wk for protein and creatinine assays. Urine protein concentration was determined by Lowry’s assay and normalized by urine creatinine.

Lipid accumulation in the kidney. Lipid accumulation in frozen sections from kidneys of the three types of mice was evaluated by oil red O staining. Briefly, samples were fixed with 5% formalin solution and then stained with oil red O for 30 min at room temperature. After washing, the samples were counterstained with hematoxylin for 5 min and examined by light microscopy (Olympus).

Quantitative measurement of tissue cholesterol. Quantitative measurements of total and free cholesterol in kidneys were performed using the method described by Gamble et al. (6). In brief, samples were collected and lipids were extracted by addition of 1 ml chloroform/methanol (2:1). The lipid phase was collected, vacuum dried, then dissolved in 2-propanol containing 10% Triton X-100. The concentrations of total and free cholesterol were analyzed using a standard curve and normalized to total sample protein. The concentrations of cholesterol ester were calculated using total cholesterol (LDL) and HDL cholesterol (HDL; Rongsheng, Shanghai, China), amyloid A (SAA; R&D), and IL-6 levels (R&D). The kidneys were collected for histological assessment and examination of gene and protein expression. Animal care and experimental procedures were performed with approval from the Animal Care Committees of Chongqing Medical University.

Renal histology. Sequential paraffin-embedded tissue sections from the renal cortex to the renal medulla were cut. Cross sections (3 μm) were placed on gelatin-coated slides and stained with periodic acid-Schiff (PAS) and Masson’s trichrome. Indices of glomerulosclerosis and tubulointerstitial fibrosis were determined using Image-Pro plus software (Media Cybernetics, Silver Spring, MD) (n = 6). Twenty

Table 2. Levels of inflammatory cytokines and cholesterol in serum of C57BL/6J, ApoE KO, and ApoE/CD36/SRA triple KO mice

<table>
<thead>
<tr>
<th>Type of Mice</th>
<th>Control (n = 6)</th>
<th>Casein (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAA, ng/ml</td>
<td>C57BL/6J</td>
<td>12.01 ± 2.10</td>
</tr>
<tr>
<td></td>
<td>ApoE KO</td>
<td>17.19 ± 4.94</td>
</tr>
<tr>
<td></td>
<td>ApoE/CD36/SRA triple KO</td>
<td>16.46 ± 3.33</td>
</tr>
<tr>
<td>IL-6, pg/ml</td>
<td>C57BL/6J</td>
<td>20.77 ± 5.10</td>
</tr>
<tr>
<td></td>
<td>ApoE KO</td>
<td>25.21 ± 5.34</td>
</tr>
<tr>
<td></td>
<td>ApoE/CD36/SRA triple KO</td>
<td>20.94 ± 6.01</td>
</tr>
<tr>
<td>TC, mmol/l</td>
<td>C57BL/6J</td>
<td>3.17 ± 0.40</td>
</tr>
<tr>
<td></td>
<td>ApoE KO</td>
<td>86.32 ± 5.60</td>
</tr>
<tr>
<td></td>
<td>ApoE/CD36/SRA triple KO</td>
<td>51.96 ± 2.1</td>
</tr>
<tr>
<td>LDL, mmol/l</td>
<td>C57BL/6J</td>
<td>2.10 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>ApoE KO</td>
<td>56.38 ± 4.80</td>
</tr>
<tr>
<td></td>
<td>ApoE/CD36/SRA triple KO</td>
<td>16.80 ± 1.73</td>
</tr>
<tr>
<td>HDL, mmol/l</td>
<td>C57BL/6J</td>
<td>0.90 ± 0.14</td>
</tr>
<tr>
<td></td>
<td>ApoE KO</td>
<td>10.30 ± 1.47</td>
</tr>
<tr>
<td></td>
<td>ApoE/CD36/SRA triple KO</td>
<td>9.07 ± 1.18</td>
</tr>
</tbody>
</table>

Values are means ± SD. SAA, serum amyloid A; TC, total cholesterol; LDL and HDL, LDL and HDL cholesterol, respectively. *P < 0.05 vs. control.
fields per section from 10 sections/mouse were examined. The formula used for determining the ratio of the positive areas to each glomerulus or tubulointerstitial total area was (positive areas/total area selected) × 100%. The positive areas where the intensity was beyond a threshold were calculated by the software as previously described (11).

Immunohistochemistry. All immunohistochemical studies were performed on sections of paraformaldehyde-fixed and paraffin-embedded kidney tissues. Endogenous peroxidase activity was blocked with 0.3% hydrogen peroxide in PBS for 15 min. After blocking nonspecific binding sites with 10% normal horse serum in PBS for 30 min, slides were incubated with primary antibodies for 1 h at room temperature. Antibody reactivity was detected using horseradish peroxidase-conjugated biotin-avidin complexes and developed with diaminobenzidine tetrahydrochloride (Zhongshan, Beijing, China) as a substrate. Primary antibodies included the following: rabbit anti-mouse transforming growth factor (TGF)-β1 polyclonal antibody (1:200, Santa Cruz Biotechnology, Santa Cruz, CA), rabbit anti-mouse collagen type I polyclonal antibody (1:100, Bioss, Beijing, China), rabbit anti-mouse collagen type IV polyclonal antibody (1:100, Bioss), rabbit anti-mouse α-smooth muscle actin (α-SMA) polyclonal antibody (1:100, Bioss), and rabbit anti-mouse fibronectin polyclonal antibody (1:100, Bioss). Results were examined by light microscopy, and the expression of the target protein was quantified using the Image Pro Plus software on 20 high-power fields/section from 10 renal sections/mouse (n = 6).

Real-time PCR. Half of the kidney tissue (100 mg) was homogenized in TRIzol reagent, and total RNA was extracted according to the TRIzol protocol (Takara Life Technologies). Total RNA (2 μg) was subjected to DNase digestion followed by cDNA synthesis using a kit (Bio-Rad, Richmond, CA). Real-time PCR was performed on Opticon-2 using Power SYBR Green PCR master mix (Bio-Rad). 18S rRNA served as an internal standard for data normalization. The amount of mRNA was calculated by the comparative CT method (4). Primers designed by Primer Express Software V2.0 (Applied Biosystems) are listed in Table 1.

Protein extraction and Western blotting. Kidneys were homogenized and extracted using a protein extraction kit (Keygen, Nanjing, China). Identical amounts of total protein were denatured and subjected to electrophoresis on a 5% stacking and 8% separating SDS polyacrylamide gel. The protein was transferred to a nitrocellulose membrane. The membrane was then blocked with 5% blocker (Amersham Bioscience, Bucks, UK) for 1 h at room temperature followed by two 5-min washes in Tris-buffered saline/0.1% Tween 20 (TBST). The membrane was incubated with goat anti-mouse LDLr polyclonal antibody (1:1,000, Santa Cruz Biotechnology), goat anti-mouse SREBP polyclonal antibody (1:800, Santa Cruz Biotechnology), and rabbit anti-human SCAP polyclonal antibody (1:500, Centre for Neurology, UCL, UK) for 1 h. A goat anti-rabbit or a donkey anti-goat horseradish peroxidase-labeled antibody (1:2,500, Santa Cruz Biotechnology) was added to the membrane for 1 h. Finally, detection procedures were performed using an ECL Advance Western blotting Detection kit (Amersham Biosciences).

Data analysis. In all experiments, data were evaluated for statistical significance using a two-sided paired t-test by SPSS 13.0 Software. Values are reported as means ± SD with six mice/group unless otherwise indicated. A difference was considered significant if the P value was <0.05.

RESULTS

Chronic inflammatory stress lowered serum lipid levels in mice. Serum levels of SAA and IL-6 were significantly increased in the casein-injected C57BL/6J, ApoE KO, and ApoE/CD36/SRA triple KO mice compared with respective controls (Table 2), suggesting that chronic inflammatory stress was successfully induced. There were no significant differences
INFLAMMATORY STRESS INDUCES LIPID-MEDIATED RENAL FIBROSIS

A

C57BL/6J (Glomerulus)

C57BL/6J (Tubulointerstitium)

Tubulointerstitium

Tubulointerstitium

Control

Casein

B

C57BL/6J (Glomerulus)

C57BL/6J (Tubulointerstitium)

Tubulointerstitium

Tubulointerstitium

Control

Casein

C

Glomerular sclerosis index

Tubulointerstitial fibrosis

Control

Casein

Control

Casein

Control

Casein

Control

Casein

C57BL/6J mice

Triple KO mice

C57BL/6J mice

Triple KO mice
between different types of mice. Serum levels of total cholesterol, LDL, and HDL were lower in all casein-treated animals than the levels in respective controls (Table 2).

**Inflammation stress increased renal lipid accumulation.** There was more lipid droplet accumulation in glomeruli and tubules of kidneys in hyperlipidemia ApoE KO (Fig. 1AIII) and ApoE/CD36/SRA triple KO mice (Fig. 1AV) compared with C57BL/6J (Fig. 1AI). CD36/SRA KO in ApoE KO mice did not significantly reduce lipid accumulation in kidneys compared with ApoE KO mice. Interestingly, casein injection significantly increased lipid accumulation in kidneys in C57BL/6J (Fig. 1AII) and to a great extent in ApoE KO (Fig. 1AIV) and ApoE/CD36/SRA triple KO mice (Fig. 1AV), suggesting that hypercholesterolemia and inflammatory stress have a synergistic effect in lipid accumulation in kidneys. Quantitative analysis of kidney cholesterol confirmed the results of oil red O staining (Fig. 1B). This result suggests that inflammatory stress increased cholesterol accumulation in kidney while lowering serum cholesterol levels, and knocking out scavenger receptors was not able to block cholesterol accumulation induced by inflammation in kidneys.

**Inflammatory stress exacerbated renal injury.** Next, we examined the effects of chronic inflammatory stress on renal pathological changes and renal function in ApoE/CD36/SRA triple KO mice and C57BL/6J mice. PAS staining demonstrated massive structural changes in kidneys from casein-injection mice, to a great extent in ApoE/CD36/SRA triple KO mice (Fig. 2A, VI and VIII, vs. Fig. 2A, II and IV), including PAS-positive glomerular deposits, glomerulosclerosis, mesangial matrix expansion with reduction of Bowman’s space, attenuation of glomerular capillaries, cellular degeneration of tubules, glomerular epithelial proliferation, and architectural disorganization (Fig. 2A, II, IV and VI, VIII) compared with age-matched controls (Fig. 2A, I, III, and V, VII). Masson’s trichrome staining clearly revealed that casein injection enhanced glomerulosclerosis with an increase in trichrome-positive deposits in glomeruli of C57BL/6J (Fig. 2BII) and triple KO mice (Fig. 2BVI) and tubulointerstitial fibrosis indicated by tubular atrophy, basement membrane thickening in tubulointerstium of C57BL/6J (Fig. 2BIV) and triple KO mice (Fig. 2BVIII) compared with respective controls (Fig. 2B, I, III, and V, VII). Quantitative analyses of these changes showed that inflammation was associated with an increase in the index of glomerular sclerosis and tubulointerstitial fibrosis (Fig. 2C). The changes in indices of glomerular sclerosis and tubulointerstitial fibrosis were in parallel with urinary protein excretion. Blood BUN, creatinine levels, and urine protein excretion were higher in casein-injected mice than those in controls in triple KO mice (Table 3). These results suggest that hypercholesterolemia and inflammatory stress synergistically induced renal injury and renal dysfunction.

**Inflammation-induced lipid accumulation increased renal fibrosis.** Next, we focused on mechanisms by which casein-induced inflammation increased renal fibrosis. Inflammation increased mRNA expression of TGF-β, α-SMA, collagen type I, collagen type IV, and fibronectin in the kidney of ApoE/CD36/SRA triple KO mice compared with noninflamed controls (Fig. 3A). Inflammation also increased the intensity of immunostaining for TGF-β in both glomeruli and tubulointerstitium (Fig. 3B, III vs. II), α-SMA in epithelial cells and tubulointerstitium (Fig. 3B, VI vs. V), and collagen type I (Fig. 3B, IX vs. VIII), collagen type IV (Fig. 3B, XII vs. XI), and fibronectin (Fig. 3B, XV vs. XIV) in tubulointerstitium compared with noninflamed controls. Quantitative analyses of these changes showed that inflammation increased relative density of TGF-β, α-SMA, collagen type I, collagen type IV, and fibronectin in kidneys of the triple KO mice (Fig. 3C).

**Inflammatory stress increased SREBP2 and LDLr expression.** Gene (Fig. 4A) and protein (Fig. 4, B and C) expression of LDLr was upregulated in inflamed mouse kidneys compared with controls. Inflammation increased SCAP and SREBP mRNA expression (Fig. 4A). Abundant SCAP, total SREBP2, and N-terminal SREBP2 proteins were observed in inflamed kidneys compared with controls (Fig. 4, B and 4C).

**DISCUSSION**

LDL is the major carrier of cholesterol in humans, and LDLr plays an important role in regulating blood LDL concentrations. In the kidney, scavenger receptor expression in HMCs is very limited although it can be induced (29). Both HMCs and tubular cells express LDLr. Brown and Goldstein (2–4) observed that LDLr activity is under tight metabolic control via a feedback system that depends on intracellular cholesterol concentration. This mechanism maintains a constant level of cholesterol in both the cells and circulation by controlling both the rates of cholesterol uptake of LDL and cellular cholesterol synthesis. We have previously demonstrated that inflammation can increase LDLr-mediated cholesterol uptake by disrupting its feedback regulation in vitro (19, 26, 28). This study was specifically designed to explore the role of LDLr in lipid accumulation induced by chronic inflammatory stress in vivo and avoid the involvement of scavenger receptors SRA and CD36.

LDLr-related protein (LRP) may be expressed in kidney, but its expression is much lower than levels of LDLr in our...
It has been demonstrated that LRP is important in the clearance of ApoE-enriched particles, such as H9252-VLDL and chylomicron remnants, and native LDL is not normally recognized by LRP (32, 15). Therefore, LRP is unlikely to be involved in native LDL accumulation in kidney with deletion of ApoE in this study.

Fig. 3. Effects of inflammation on mRNA and protein expression of transforming growth factor (TGF)-β1, α-smooth muscle actin (SMA), collagen type IV (Col IV), collagen type I (Col I), and fibronectin (FN) in the kidney of ApoE/CD36/SRA triple KO mice. A: mRNA expression of TGF-β1, α-SMA, Col IV, Col I, and FN was determined by real-time PCR as described in MATERIALS AND METHODS. 18S rRNA served as the housekeeping gene. Results are means ± SD (n = 6). *P < 0.05 vs. control. B: protein levels of TGF-β1, α-SMA, Col I, Col IV and FN in kidney sections from casein-injected group (III, VI, IX, XII, and XV), control (II, V, VIII, XI, and XIV), and negative control (I, IV, VII, X, and XIII) were determined by immunohistochemical staining (×400). C: histogram represents means ± SD (n = 6) for the quantification of immunostaining and is expressed as a fold of control. *P < 0.05 vs. control.
ApoE/CD36/SRA KO mice model allowed us to exclude the involvement of scavenger receptors and LRPs and to investigate the roles of the LDLr pathway in cholesterol accumulation under inflammatory stress.

We have previously induced a low-grade long-term chronic systemic inflammation characterized by increased serum SAA in ApoE KO mice by injecting 10% casein subcutaneously. Casein injection increased lipid accumulation in the liver (19) and aorta in ApoE KO mice. In this study, we used ApoE/CD36/SRA triple KO, ApoE KO, and C57BL/6J mice fed a Western diet and subcutaneously injected with casein to induce a predictable low-grade systemic inflammation and to test whether this inflammatory stress results in cholesterol redistribution from the circulation to the tissues via LDLr, causing renal injury and lowering blood cholesterol levels. Plasma levels of SAA, IL-6, and kidney TGF-β showed that the casein-injected mice had a more proinflammatory phenotype than controls, suggesting that systemic and local renal inflammatory stress was successfully induced. This model provides an approach to studying the role of inflammatory stress independent of other metabolic disorders caused by chronic renal dysfunction. We demonstrated that inflammation increased lipid accumulation in glomeruli and tubules of kidneys in inflamed ApoE KO compared with C57BL/6J mice. Similar lipid accumulation was found in ApoE/CD36/SRA triple KO mice, suggesting that LDLr may be one of players in inflammation-mediated lipid accumulation in the kidney.

**Fig. 3.—Continued**
Fig. 4. Effects of inflammation on mRNA and protein expression of LDL receptor (LDLr), sterol-responsive element-binding protein (SREBP) cleavage-activating protein (SCAP), and SREBP2 in the kidneys of C57BL/6J mice and ApoE/CD36/SRA triple KO mice. A: mRNA expression of LDLr, SCAP, SREBP2 in mice was determined by real-time PCR. 18S rRNA served as a housekeeping gene. B: protein levels of LDLr, SCAP, and SREBP2 were examined by Western blotting. C: histogram represents means ± SD of densitometric scans for the bands from 6 experiments expressed as a fold of control. *P < 0.05 vs. control.
Furthermore, this study demonstrated that systemic inflammatory stress increased lipid accumulation by disrupting SCAP-SREBP-mediated feedback regulation of LDLr, thereby exacerbating lipid accumulation. Oil red O, PAS, and Mason’s trichrome staining demonstrated massive lipid accumulation in the kidney of casein-injected mice characterized by the appearance of numerous fat vacuoles, glomerular expansion, glomerulosclerosis, degeneration of the renal tubules, tubular atrophy, the presence of tubular casts, and overexpression of ECM proteins including collagen (type I and IV), fibronectin, and α-SMA observed in inflamed kidneys compared with controls. These results suggest inflammatory stress exacerbates lipid-mediated glomerular and interstitial injury. Interstitial fibrosis was even more pronounced than glomerulosclerosis in this study by unknown mechanisms. This confirms our previous finding from in vitro study (25, 28) and also provides a plausible explanation for the observation that abnormal kidney deposition of cholesterol is found in various inflammatory renal diseases (37).

Next, we examined the expression of SCAP and SREBP2, which are two important molecules in regulating LDLr expression. Our data show that inflammatory stress upregulated both mRNA and protein expression of LDLr, SREBP2, and SCAP in the mice fed a Western diet, suggesting that inflammatory stress disrupts LDLr normal feedback regulation mediated by SCAP and SREBP2, inappropriately increasing LDL uptake with transformation of kidney cells into foam cells. Upregulation of LDLr in the kidney and liver (19, 28) may cause lipid redistribution from the circulation to the tissues. This may explain why inflammatory stress lowers LDL cholesterol levels in this model.

Our data are also consistent with some clinical studies (10, 12, 16, 31) which showed that blood LDL levels were not increased in CKD or hemodialysis patients. These data may explain why in hemodialysis patients, the higher risk of death from cardiovascular disease is counterintuitively associated with low blood cholesterol (“reverse epidemiology”) (17, 18, 35) and also the data from a retrospective study of patients with nephritic progressive renal disease showing that severe proteinuria and hypercholesterolemia accompanied renal disease progression, but plasma cholesterol gradually fell to normal levels as patients approached end-stage renal disease (23, 36, 38).

It is important to mention that cholesterol homeostasis is governed by multipathways including intracellular cholesterol uptake, synthesis, and efflux (26, 28). The increased cholesterol synthesis and impaired cholesterol efflux via the ABCA1 pathway under inflammatory stress may also account for renal lipid accumulation (30) in addition to the LDLr-mediated cholesterol uptake. Loss of CD36 could adversely affect reverse cholesterol transport, which may account for the lipid accumulation in the kidney as observed in this study. Furthermore, the experimental model in this study does not include a manipulation of LDLr, and thus a causal relationship cannot be fully confirmed and needs to be investigated in the future.

In conclusion, inflammatory stress in chronic kidney disease may fundamentally modify cholesterol homeostasis by disrupting SCAP-mediated LDLr feedback regulation. This induces at least partially lipid accumulation in the kidney by diverting lipid from the plasma to the kidney via the SCAP-SREBP2-LDLr pathway to cause renal injury and fibrosis while at the same time lowering plasma cholesterol. These data suggest that low blood cholesterol levels may be associated with a high risk for chronic renal injury under inflammatory stress and that plasma LDL cholesterol in patients with chronic kidney disease might be unhelpful or even misleading in risk assessment of lipid-mediated renal injury.

ACKNOWLEDGMENTS

We thank Dr. Maria Febbraio (Lerner Research Institute) for providing the ApoE/CD36/scavenger receptors class A triple knockout mice.

GRANTS

This study was supported by the Moorhead Trust, Royal Free Hospital Special Trustees Grant-115 through Dr. Zac Varghese, the National Natural Science Foundation of China (30871159, 81070631, 81070317, and the Key Program, No. 81030008), National Basic Research Program of China (973 Program, 2006CB503907), National Natural Science Foundation Project of CQ CSTC (2008BA5016), and Kidney Research UK (RP37/2008).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

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AJP-Renal Physiol • VOL 301 • OCTOBER 2011 • www.ajprenal.org


