Atherogenic scavenger receptor modulation in the tubulointerstitium in response to chronic renal injury

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Okamura DM, López-Guisa JM, Koelsch K, Collins S, Eddy AA. Atherogenic scavenger receptor modulation in the tubulointerstitium in response to chronic renal injury. Am J Physiol Renal Physiol 293: F575–F585, 2007. First published May 30, 2007; doi:10.1152/ajprenal.00063.2007.—Oxidized low-density lipoproteins (oxLDL) and their scavenger receptor (SR) binding partners play a central role in atherosclerosis and by analogy may play a role in chronic kidney disease pathogenesis. The present study was designed to investigate in C57BL/6 mice the effects of hypercholesterolemia on renal injury severity and oxLDL generation after unilateral ureteral obstruction (UUO). The expression profiles of CD36, SR class AI/II (SR-A), lectin-like receptor for oxidized low-density lipoprotein-1 (Lox-1), and SR that binds phosphatidylserine and oxLDL (SR-PSOX/CXCL16) were examined. Four experimental groups were studied: sham and UUO male mice on either a high-fat Western diet or a control diet. Significantly more oxLDL accumulated in the tubulointerstitium of hypercholesterolemic mice compared with normocholesterolemic mice on day 14 (P < 0.01). Total kidney collagen was significantly higher in the obstructed kidneys of hypercholesterolemic mice compared with normocholesterolemic mice on day 14 (P < 0.01). After 14 days of obstruction, the number of interstitial F4/80+ macrophages and NF-κB activation increased in hypercholesterolemic mice compared with normocholesterolemic mice (P < 0.01). In normal kidneys, CD36, SR-A, Lox-1, and CXCL16 were primarily localized to renal tubular epithelia. After ureteral obstruction, CD36 increased at day 7; SR-A and Lox-1 progressively decreased in a time-dependent manner; and CXCL16 increased significantly with the onset of obstruction (P < 0.01). Strong tubular expression suggests that in addition to inflammatory interstitial cells, renal tubular scavenger receptors may help to orchestrate the inflammatory and fibrogenic pathways that are activated by oxLDL.

interstitial fibrosis; CD36; SR-A; Lox-1; CXCL16; SR-PSOX; hypercholesterolemia; oxidized lipoprotein

THE NUMBER OF PATIENTS WITH chronic and end-stage renal disease (ESRD) is increasing at an alarming rate (12). Several studies have demonstrated that chronic kidney disease increases the risk for cardiovascular morbidity and mortality primarily by accelerating atherosclerotic disease (12, 29). Many parallels have been drawn between atherogenesis and the pathogenetic mechanisms that cause progressive kidney destruction by fibrosis. Can the same pathological mechanisms that accelerate atherosclerotic disease in chronic kidney disease amplify fibrogenic pathways in the kidney?

Accumulation of oxidized low-density lipoproteins (oxLDL) has been reported in the circulation and renal interstitium in both experimental models and patients with chronic kidney disease and ESRD (7, 40). In vitro and in vivo studies suggest that oxLDL may exert biologically relevant responses in situ in the kidney. In vitro, LDL and oxLDL stimulate mesangial cells to secrete chemoattractant chemokines that may trigger an influx of monocytes (41). Several animal studies have further suggested that chronic exposure to oxLDL promotes collagen synthesis and activates proinflammatory pathways (9, 40). Few studies of progressive renal fibrosis have examined the relationship between renal oxidized lipoproteins and their primary binding partner, members of the scavenger receptor superfamily. Scavenger receptors mediate the endocytosis and degradation of oxLDL in vivo, but they may also activate intracellular signaling pathways that contribute to the proinflammatory milieu that initiates and propagates atherosclerosis (37). However, little is known about the modulation and function of scavenger receptors in kidneys during normal and hypercholesterolemic states and how scavenger receptors are regulated in response to acute and chronic injury.

Scavenger receptors are transmembrane receptors that bind several ligands in addition to oxidized lipoproteins. They are expressed by many cell lineages but are best characterized in macrophages and the microvascular endothelium. Epithelial cell expression of several members of the scavenger receptor family has been reported, but in general much less is known about their biological effects after ligand binding by this cell type. Nine classes of scavenger receptors have been identified based on structural similarities: class A (e.g., SR-A); SR-A-like scavenger receptors; class B (e.g., CD36); class D (e.g., macroselectin); class E [lectin-like oxLDL receptor (Lox)]; class F [SRs expressed by endothelial cells (SREC)]; class G [scavenger receptor for phosphatidylserine and oxidized lipoprotein (SR-PSOX)]; fasciclin epidermal growth factor (EGF)-like, laminin-type EGF-like, and link domain (FEEL); and CD163. However, SR-A, CD36, Lox-1, and CXCL16 appear to be the primary receptors for oxLDL based on atherogenesis data. Each receptor has been shown to promote or attenuate atherogenic effects in vivo through the endocytosis of oxLDL and activation of proinflammatory cascades following ligand binding (4, 18, 52, 54). Many of the other scavenger receptors function primarily as receptors in the immune response system (3, 6).

In murine models of atherosclerosis, both SR-A and CD36 have clearly been shown to play important functional roles in oxLDL processing and in disease progression. SR-A and CD36 account for up to 90% of the uptake of oxLDL by macrophages in vitro (28). Both are multiligand transmembrane receptors expressed by macrophages. In addition, CD36 is expressed by...
several other cell types, including microvascular endothelium, platelets, and epithelial cells. Recent evidence suggests that SR-A and CD36 may also serve as signal transduction receptors that modulate the inflammation associated with atherosclerosis (14, 26, 28, 35).

Lox-1 differs from other scavenger receptors in that it is primarily expressed on microvascular endothelial cells, with low-level expression on macrophages, platelets, and smooth muscle cells (8). Recent studies suggest that Lox-1 may also mediate endothelial dysfunction by modulating the expression of endothelial constitutive nitric oxide synthase (eNOS) and by upregulating adhesion molecules (34).

SR-PSOX/CXCL16 is one of the few scavenger receptors that is found in two distinct forms: membrane bound and soluble. Membrane-bound CXCL16 binds and internalizes ox-LDL and promotes adhesion of cells expressing its cognate receptor CXCR6 (45, 46). Proteolytic cleavage of membrane-bound CXCL16 releases soluble CXCL16 (1, 20), which is a chemoattractant for CXCR6+ cells such as polarized T helper cells (27). CXCL16 is expressed on endothelial cells, macrophages, and smooth muscle cells (22, 45).

To gain further insight into the role of renal scavenger receptors in response to intrarenal oxLDL generation, the present study was designed to investigate the expression pattern of the four atherogenic scavenger receptors (CD36, SR-A, Lox-1, and CXCL16) in kidneys chronically damaged by unilateral ureteral obstruction, in both a normal and a hypercholesterolemic environment. It is known from previous studies that oxidant stress and oxLDL production occur within kidneys that are damaged by chronic obstruction. This study was also designed to investigate the hypothesis that hypercholesterolemia is associated with higher renal oxLDL levels during chronic injury, an effect that leads to worse fibrosis.

METHODS

Experimental Design

Studies were performed using male C57BL/6 mice purchased from Harlan Laboratories (Kent, WA). Male mice were fed either a high-fat Western diet (15.8% total fat with 0.5% sodium cholate, Harlan Teklad, Madison, WI) (39) or a control diet of standard chow supplemented with 0.5% sodium cholate beginning after weaning at 3–4 wk of age. After a run-in period of 8 wk on the experimental diets, animals were randomly assigned to one of four experimental groups: sham surgery or unilateral ureteral obstruction (UUO) and either a high-fat diet or a control diet. Groups of mice (n = 4–10 each) were killed at 3, 7, and 14 days after surgery. These time points were selected as representative of the early phase when cellular recruitment is just beginning (day 3), a midpoint when growth factor-dependent matrix accumulation has begun (day 7), and a more advanced phase when structural kidney damage is becoming evident (day 14). All surgeries were performed under general anesthesia with isoflurane.

For mice in the UUO group, the left ureter was exposed through a midabdominal incision and ligated using 4-0 silk. Sham and UUO kidneys were harvested and processed for RNA and protein extraction and histological studies as previously described (31). Frozen tissue samples were stored at −80°C. All procedures were performed in accordance with the guidelines established by National Research Council Guide for the Care and Use of Laboratory Animals and approval of our Institutional Animal Care and Use Committee. Serum cholesterol levels were measured in blood samples that were obtained at death using the Total Cholesterol Kit (WAKO Chemicals USA, Richmond, VA).

Collagen Content

Hydroxyproline content of kidney tissue (μg hydroxyproline/mg wet wt kidney section) was performed by acid hydrolysis of the tissue section using procedures established in our laboratory (31).

Histological Examination

Immunohistochemical staining was performed on sections of paraffin-embedded tissue or cryosections of snap-frozen tissue using procedures established in our laboratory with Vectastain Elite ABC Kits (Vector Laboratories, Burlingame, CA) and a AEC Substrate Chromogen K3464 (Dako, Carpinteria, CA) as the peroxidase substrate. Sections were blocked with an Avidin/Biotin blocking kit (Vector Laboratories). For CXCL16 immunostaining, the Tyramide Signal Amplification Biotin System was used (PerkinElmer, Boston, MA). Primary antibodies used were reactive with F4/80 (rat antimouse F4/80 monoclonal antibody, AbD Serotec, Raleigh, NC); CD36 (rabbit anti-human CD36 polyclonal antibody, Cayman Chemical, Ann Arbor, MI); Lox-1 (goat anti-mouse oxLDL receptor-1 polyclonal antibody, Santa Cruz Biotechnology, Santa Cruz, CA); scavenger receptor A (goat anti-mouse macrophage class A scavenger receptor, Santa Cruz Biotechnology); IxB-α and phosphorylated IxB-α (rabbit anti-human IxBα and rabbit anti-human phosphorylated IxB-α, Cell Signaling Technology, Danvers, MA); and oxLDL (rabbit anti-human hypochlorous acid (HOCl)-modified LDL polyclonal antibody, Chemicon International, Temecula, CA). Secondary antibodies were shown to be nonreactive with tissue sections stained without primary antibody. Semiquantitative computer-assisted image analysis of tubulointerstitial proteins was performed on six randomly selected х1000-magnified images of slides from individual animals with Image-Pro Plus software (MediaCybernetics). The glomerular area and space not occupied by tissue were subtracted in the analysis. Final results were expressed as the mean positive tubulointerstitial area. The investigator was blinded to the experimental groups at the time of analysis.

Northern Blotting

Total kidney RNA was extracted using the TRizol single-step reagent (GIBCO BRL Life Technologies, Grand Island, NY). Total kidney RNA (20 μg) from each individual animal was separated by electrophoresis in 1.2% agarose formaldehyde gel, transferred to a nylon membrane (Hybond-C, Schleicher & Schuell, Keene, NH) by capillary blotting, and fixed by ultraviolet cross-linking. Northern blotting was performed as previously described (31). After being washed, membranes were scanned with a Typhoon 9410 PhosphorImager (GE Healthcare, Pittsburgh, PA). The results were adjusted for RNA loading inequality based on the density of the 18S ribosomal RNA band. The cDNA probe used was mouse SR-PSOX/CXCL16 [EST GenBank AI019535 (33), ATCC, Manassas, VA].

Western Blotting

Pieces of frozen kidney were homogenized in buffer (1% SDS in 50 mM Tris, pH 7.6), frozen and thawed, and centrifuged at 13,000 rpm in a microfuge at 4°C. The supernatant was removed, and protein concentration was determined using a BCA protein assay (Pierce). Protein bands were visualized using the enhanced chemiluminescence detection system (Pierce Biotechnology, Rockford, IL). Protein bands were visualized using the enhanced chemiluminescence detection system (Pierce Biotechnology, Rockford, IL). Band intensities were measured using Image-Pro Plus software and normalized for protein loading equality as determined by staining membranes.
with Ponceau red. The major band for each scavenger receptor that was detected by Western blotting was analyzed by densitometry.

Statistical Analysis

All data are presented as the mean and SD. A nested ANOVA was utilized for all semiquantitative computer-assisted image analysis. For image analysis data, the arithmetic mean of six randomly selected images of slides for each animal was used to calculate the reported mean of the group and the SD. All other results were analyzed by an unpaired Student’s t-test. A P value <0.05 was considered statistically significant.

RESULTS

Normal Renal Scavenger Receptor Expression and Effects of Diet-Induced Hypercholesterolemia

Tubular expression of renal scavenger receptors. To elucidate the intrarenal scavenger receptor expression pattern in normal kidneys, protein and/or mRNA expression levels were analyzed in sham-operated kidneys. By immunostaining, CD36, SR-A, Lox-1, and CXCL16 were detected primarily on renal tubular epithelial cells in the sham-operated kidneys of normocholesterolemic and hypercholesterolemic mice (Fig. 1, A–D). In normal kidneys, CD36 was detected predominantly in distal renal tubular epithelial cells and peritubular cells, likely microvascular endothelium; CD36 immunolocalized to the basolateral side of distal tubular epithelial cells (Fig. 1A). SR-A and CXCL16 were also expressed in low levels on normal renal tubular epithelium (Fig. 1, C and D).

By Western blotting the CD36 (88-kDa glycosylated), SR-A (70 kDa), and Lox-1 (84-kDa dimer) protein bands were detectable to varying degrees in sham kidneys from normal and hypercholesterolemic mice (Fig. 1E). Two protein bands were detected for Lox-1 and CD36, suggesting either differential glycosylation or alternative splicing (2, 38). Basal SR-A protein densities was relatively low and variable between individual animals compared with the expression levels of the other scavenger receptors. Due to low expression, a specific CXCL16 band was not detected by Western blotting. By Northern blotting, low CXCL16 steady-state mRNA levels were detected in normal sham-operated kidneys (Fig. 1G).

Modulation of normal renal scavenger receptor expression with a high-fat diet. To determine whether levels of renal scavenger receptor expression are modulated in response to hypercholesterolemia, as has been reported for macrophages, protein or mRNA levels were compared between sham-operated kidneys from mice fed a control diet and mice fed a high-fat diet. Fourteen days after sham operation, renal protein or mRNA levels for each of the four scavenger receptors were not significantly different between sham kidneys of normocholesterolemic and hypercholesterolemic mice (Fig. 1, F and H). Western blotting for peroxisome proliferator-activated receptor-γ in sham-operated kidneys was also not significantly different between normocholesterolemic and hypercholesterolemic mice [control vs. high-fat diet, n = 4–6/group: 22 (SD 6) vs. 22 (SD 5), P = 0.97].

Renal SR Expression in Response to UUO and Effects of Diet-Induced Hypercholesterolemia

Renal fibrosis. To investigate whether the severity of renal fibrosis induced by UUO was accentuated by hypercholesterolemia, total collagen levels were measured as hydroxyproline content per wet weight of kidney tissue. Fibrosis was signifi-

Fig. 1. Scavenger receptor expression in sham-operated kidneys: effect of hypercholesterolemia. Representative photomicrographs of CD36 (A), scavenger receptor SR-A (B), Lox-1 (C), and CXCL16 (D) tubular immunostaining in sham-operated kidneys are shown. E: representative Western blots of day 14 sham-operated kidneys from mice on a control and high-fat Western diet with protein loading measured by Ponceau S staining shown below each immunoblot. Lox-1 protein was expressed in the kidney predominantly as a homodimer at ∼84 kDa. G: representative Northern blot of day 14 sham-operated kidneys. RNA loading for CXCL16 Northern blot is indicated by the 18s band. F and H: graphs summarizing the results of Western blot or Northern blot major band density measurements. Differences in renal scavenger receptor expression from sham-operated kidneys were not significant between normocholesterolemic and hypercholesterolemic mice. All results are expressed as means (SD); n = 4–6.
Significantly worse in obstructed kidneys from hypercholesterolemic mice compared with normocholesterolemic mice at day 3 and day 14 [control vs. high-fat diet, n = 8–10/group; day 3: 3.9 μg/mg (SD 0.4) vs. 4.7 μg/mg (SD 0.3), P = 0.002; day 7: 6.7 μg/mg (SD 0.9) vs. 6.6 μg/mg (SD 0.8), P = 0.91; day 14: 8.2 μg/mg (SD 0.9) vs. 10.0 μg/mg (SD 1.0), P = 0.001] (Fig. 2).

Macrophage infiltration. Since fibrosis severity was increased in hypercholesterolemic mice, the degree of inflammation was compared in obstructed kidneys from normocholesterolemic and hypercholesterolemic mice. There was significantly a larger F4/80+ interstitial area in obstructed kidneys from hypercholesterolemic mice at day 14 and a nonsignificant increase at day 3 after UUO [control vs. high fat, n = 4–7/group; day 3: 0.8% (SD 0.5) vs. 1.6% (SD 1.3), P = 0.60; day 14: 5.2% (SD 2.8) vs. 8.2% (SD 4.0), P = 0.011 by nested ANOVA] (Fig. 3).

Renal oxLDL levels. To determine whether intrarenal ox-LDL generation was modulated by diet in normal kidneys, renal oxLDL levels in sham-operated kidneys were first compared between normocholesterolemic and hypercholesterolemic mice. C57BL/6 mice were consistently hypercholesterolemic after 8 wk on a high-fat Western diet (Table 1) without a significant change in body weight between the diet groups (Table 2). By immunostaining, HOCl-modified LDL localized predominantly to renal tubules in sham-operated kidneys (Fig. 4, A and C). Semiquantitative analysis of kidney sections stained for HOCl-modified LDL showed a nonsignificant increase in oxLDL levels in sham-operated kidneys of hypercholesterolemic mice compared with normocholesterolemic mice [percent tubulointerstitial area, control vs. high-fat diet, n = 4: 4.9% (SD 2.5) vs. 9.8% (SD 4.1), P = 0.09].

Since fibrosis was worse when kidneys were obstructed in hypercholesterolemic mice, studies were performed to determine whether intrarenal levels of oxidized lipoproteins were modified by serum cholesterol levels. Semiquantitative analysis of kidney sections immunostained for HOCl-modified LDL identified significantly higher levels in kidneys from hypercholesterolemic mice compared with obstructed kidneys from normocholesterolemic mice at each time point [percent tubulointerstitial area, control vs. high-fat diet, n = 6–7/group; day 3: 9% (SD 5) vs. 20% (SD 6), P = 0.0002; day 7: 7% (SD 5) vs. 19% (SD 3), P = 0.0001; day 14: 5% (SD 4) vs. 24% (SD 8), P = 0.0002 by nested ANOVA] (Fig. 4E). By immunostaining, HOCl-modified LDL appeared de novo after the onset of obstruction with the intensity of oxLDL staining increased in both tubular cells and the interstitium of obstructed kidneys (Fig. 4, B and D).

NF-κB activation. To investigate whether proinflammatory pathways that are activated in obstructed kidneys were influenced by hypercholesterolemia, activation of the NF-κB pathway was examined on days 3 and 14. NF-κB heterodimers are inactive in the cytoplasm due to IκB-α binding. Phosphorylation of IκB-α results in release and translocation of NF-κB heterodimers into the nucleus to initiate transcription of several proinflammatory genes. Phosphorylated IκB-α is then ubiquinated and degraded. Nuclear translocation of NF-κB was evaluated indirectly by Western blotting measurements of phosphorylated IκB-α and IκB-α. Phosphorylated

Table 1. Mean cholesterol levels on control and high-fat diet

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Surgery</th>
<th>Control, mg/dl (n = 4–6)</th>
<th>High Fat, mg/dl (n = 6–10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 14</td>
<td>Sham</td>
<td>102 (SD 2)</td>
<td>167 (SD 18)</td>
</tr>
<tr>
<td>Day 3</td>
<td>UUO</td>
<td>103 (SD 9)</td>
<td>140 (SD 16)</td>
</tr>
<tr>
<td>Day 7</td>
<td>UUO</td>
<td>80 (SD 4)</td>
<td>193 (SD 71)</td>
</tr>
<tr>
<td>Day 14</td>
<td>UUO</td>
<td>86 (SD 11)</td>
<td>191 (SD 39)</td>
</tr>
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Values are means and SD, n. No. of mice; sham, sham-operated mice; UUO, unilateral ureteral obstruction.
IkB-α-to-total IkB-α ratios were significantly higher in obstructed kidneys of hypercholesterolemic mice at both time points [control vs. high fat, \( n = 4-6/\text{group} \): sham: 0.6 (SD 0.1) vs. 0.6 (SD 0.1); day 3: 0.9 (SD 0.2) vs. 1.5 (SD 0.4), \( P = 0.01 \); day 14: 0.2 (SD 0.1) vs. 0.6 (SD 0.1), \( P = 0.0007 \) (Fig. 5).

Renal scavenger receptor expression. Since CD36, SR-A, Lox-1, and CXCL16 are the major oxLDL scavenger receptors, their expression was examined in obstructed kidneys harvested from the hypercholesterolemic mice. Changes in SR expression were measured by comparing sham-operated and UUO kidneys on the same Western or Northern blot for each time point and analyzed by an unpaired Student’s \( t \)-test. By Western blotting, total kidney CD36 protein levels significantly decreased 3 and 14 days after UUO relative to sham kidneys by 70 and 90%, respectively [mean sham group = 1.0, \( n = 4-9/\text{group} \): day 3: 0.3 (SD 0.2), \( P = 0.01 \); day 14: 0.1 (SD 0.1), \( P = 0.00001 \) (Fig. 6A). However, 7 days after ureteral obstruction, a time point representing a midpoint between inflammation and fibrosis, CD36 total protein levels returned to baseline levels relative to sham kidneys [mean sham group = 1.0, \( n = 4-10/\text{group} \): 1.7 (SD 0.9), \( P = 0.18 \)]. By immunostaining, tubular CD36 expression initially decreased on day 3 after obstruction, then intensified on day 7, but by day 14 only a few tubules were positive compared with sham-operated

### Table 2. Percent weight increase on control and high-fat diet

<table>
<thead>
<tr>
<th>Time Point</th>
<th>Surgery</th>
<th>Control, % (( n = 4-6 ))</th>
<th>High Fat, % (( n = 6-10 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 14</td>
<td>Sham</td>
<td>80% (SD 30)</td>
<td>87% (SD 26)</td>
</tr>
<tr>
<td>Day 3</td>
<td>UUO</td>
<td>80% (SD 30)</td>
<td>87% (SD 26)</td>
</tr>
<tr>
<td>Day 7</td>
<td>UUO</td>
<td>66% (SD 14)</td>
<td>61% (SD 31)</td>
</tr>
<tr>
<td>Day 14</td>
<td>UUO</td>
<td>67% (SD 17)</td>
<td>66% (SD 16)</td>
</tr>
</tbody>
</table>

Values are means and SD. \( n \), No. of mice.
epithelial cells in normal kidneys, adding to the growing list of cellular lineages that may express this family of receptors. Second, when levels of oxLDL, the major ligands for these scavenger receptors, are enhanced within the kidney during chronic damage by systemic hypercholesterolemia, renal fibrosis is exacerbated. Third, the kinetic expression profiles of the scavenger receptors are altered in a receptor- and cell-specific manner during chronic kidney injury.

Despite extensive information on the role of scavenger receptors in endothelial and macrophage biological responses, information on epithelial scavenger receptor function is still in its infancy. In fact, previously published studies have reported Lox-1 by endothelial cells and macrophages but not in epithelia. The present findings demonstrate that the dimeric form of Lox-1 (38) is expressed by renal tubular epithelial cells. CD36 has also been identified on epithelial cells in the intestine and retina, where it may mediate fatty acid transport and photoreceptor phagocytosis, respectively (15, 42). SR-A and CXCL16 have recently been reported to have low levels of expression in epithelial cells, but their function at these sites still remains unclear (21, 25). Several studies have reported that scavenger receptors expressed by macrophages or endothelial cells are upregulated by oxLDL (8, 19, 32). However, in the present study relatively short-term hypercholesterolemia did not enhance tubular scavenger receptor expression in vivo in normal (sham-operated) kidneys. A recent study demonstrated that the transcriptional control of CD36 is very complex, with at least five alternative transcripts and expression patterns that are cell type dependent (2). Cell type-specific regulation may infer differences in biological function. In atherosclerosis, macrophage scavenger receptors are primarily responsible for oxLDL clearance by endocytosis, but these receptors are also known to trigger intracellular signaling pathways that may lead to the production of chemokines and cytokines (11, 24). Whether renal tubular CD36, SR-A, Lox-1, and CXCL16 are involved in oxLDL endocytosis and/or intracellular signaling is still unclear and requires further investigation.

In the present study, hypercholesterolemia significantly increased renal oxLDL tubulointerstitial deposition in chronically damaged kidneys. After 14 days of UUO, renal oxLDL levels were significantly increased >450% in hypercholesterolemic mice compared with normocholeserolemic mice. OxLDL may be generated within the kidney by one or more pathways that include copper oxidation, myeloperoxidase (MPO)-generated reactive nitrogen species, and HOCl-mediated oxidation (10). Data from the current study provide further evidence that, similar to atherogenesis, oxLDL generated in the kidney are pathological and promote renal fibrogenesis. Previous studies from our own and other laboratories demonstrated that long-term hypercholesterolemia (12 wk) alone may increase intrarenal oxidative stress and promote fibrosis in otherwise normal kidneys (9, 40). The present study suggests that hypercholesterolemia, and more specifically oxidized lipoproteins, leads to a progressive increase in interstitial macrophage numbers after the onset of obstruction and results in more severe tissue injury. After 14 days of obstruction, interstitial macrophages increased ~160% in hypercholesterolemic mice compared with normocholeserolemic mice. These changes were associated with greater NF-κB activation that may have primed the kidney for a more severe injury and fibrosis when it encountered a second insult such as ureteral obstruction.
Antioxidant therapy has been shown to decrease oxLDL generation and to reduce renal injury (8, 16, 43). Perhaps limiting the downstream inflammatory and profibrotic effects of oxLDL by blocking their binding to scavenger receptors will prove to be an effective strategy for reducing renal fibrosis.

In the present study, both oxLDL and its scavenger receptors CD36, SR-A, Lox-1, and CXCL16 immunolocalized to the tubulointerstitial compartment after chronic obstruction, suggesting that they may be important in activating the profibrotic pathways activated by oxLDL. It has previously been reported that oxLDL can interact with CD36-, CXCL16-, and Lox-1-dependent NF-κB activation in both macrophages and endothelial cells (13, 30). The present study demonstrated that increased NF-κB activation correlated with time points of increased fibrosis severity in hypercholesterolemic mice. After 3 and 14 days of obstruction, NF-κB activation levels increased 160 and 240%, respectively, in hypercholesterolemic mice compared with normocholesterolemic mice. Based on the findings in the present study, oxLDL-scavenger receptor interactions leading to NF-κB activation is one potentially relevant pathophysiological mechanism in progressive renal fibrosis.

The present study also demonstrates that the renal expression profile of each scavenger receptor examined was altered in a receptor-specific pattern during chronic injury. After UUO, two convergent events appear to influence overall scavenger receptor expression: infiltration of scavenger receptor-bearing
macrophages and changes in renal tubular epithelial cell expression with progressive renal injury. Lox-1 and SR-A protein levels decreased in a time-dependent manner after UUO despite an increase in levels of oxLDL, likely as a consequence of direct tubular injury. By contrast, Lox-1 expression has been reported to increase after hypertensive and vascular models of renal endothelial cell injury in rats (34, 50). Hypoxic injury is recognized as an integral component of progressive renal injury.
damage after ureteral obstruction. Despite the progressive loss of tubular Lox-1 after UUO, increased Lox-1 expression was noted in the interstitium likely due to increased expression by peritubular capillaries (see arrows, Fig. 7H) (47).

The decline in tubular SR-A expression and the early appearance of small numbers of SR-A+ interstitial cells after UUO suggest that SR-A may play a role in the inflammatory segment of renal fibrogenesis. SR-A+ interstitial cells were detected on day 3 (data not shown) and day 7 but not on day 14 when fibrosis is well established. Other studies report that SR-A expression is upregulated during macrophage activation, suggesting that it may play a role in acute inflammation (36). In addition, Beamer et al. (5) demonstrated that following bleomycin administration, SR-A null mice developed more extensive inflammation but less collagen deposition in the lung, suggesting that SR-A may be an important switch in the complex process of inflammatory fibrosis. A recent study further suggests that SR-A may promote macrophage infiltration in a model of diabetic nephropathy (51). However, in the present study the overall levels of SR-A protein expression levels were low relative to the other scavenger receptors studied, perhaps suggesting that its role in the progression of renal fibrosis is limited.

In contrast to declining SR-A and Lox-1 levels after UUO, CD36-positive cells infiltrated the interstitium during chronic obstruction and tubular CD36 expression intensified, leading to a relative increase in total kidney CD36 protein levels at day 7 after UUO. In agreement with the lack of studies reporting CD36 expression by fibroblasts, dual staining for F4/80 and CD36 indicated that most of the CD36+ renal interstitial cells in the present study were macrophages (data not shown). As monocytes differentiate into macrophages in vitro, CD36 expression has been reported to increase (23). Phorbol esters (PMA), macrophage colony-stimulating factor (M-CSF), and IL-4 have also been shown to increase monocyte/macrophage CD36 expression (53). Therefore, the results of the present study suggest that CD36+ macrophages infiltrating the interstitium in response to persistent kidney damage may promote renal fibrogenesis, and in particular they may mediate profibrotic effects of oxLDL. Consistent with this hypothesis, a recent study in humans with pulmonary fibrosis reported increased levels of oxidized phosphatidylcholine colocalizing with CD36+ macrophages in fibrotic areas (54). Oxidized phosphatidylcholine is a specific binding moiety for CD36 (28). Because phosphatidylcholine is a primary component of plasma membranes, it is possible that the part of the oxidized lipoproteins generated during chronic injury represent apoptotic cells, which are known CD36 ligands. A recent study suggests that in diabetic nephropathy, CD36 may induce proximal tubular epithelial cell apoptosis through src kinase activation of caspase 3 (48). In addition to oxLDL, other CD36 ligands that have been implicated in the pathogenesis of chronic kidney disease are thrombospondin and advanced glycosylated end-products (17). Together, these data suggest that both CD36+ macrophages and CD36+ renal tubular cells may play important roles in the progression of renal fibrosis.

The CXCL16 expression pattern was unique among the oxLDL-binding scavenger receptors examined in that its basal expression level was low and increased by 400% with the onset of chronic kidney injury caused by obstruction. In intestinal epithelial cells, membrane-bound CXCL16 may recruit activated T cells (21) and it may play a similar role during chronic injury in the kidney by directing lymphocyte chemotaxis toward injured tubules. However, unlike the protective effects of genetic CD36 or SR-A deficiency in mouse models of atherosclerosis (18, 44, 49), CXCL16-deficient mice develop worse disease (4). The role of CXCL16 as a possible modulator of progressive renal fibrosis deserves further investigation.

Conclusions

Systemic hypercholesterolemia was confirmed to enhance intrarenal oxLDL generation and worsen fibrosis severity during chronic kidney injury by obstruction. The results of the present study begin to elucidate the cellular pathways that may be involved. Normal renal tubular epithelial cells were shown to express CD36, SR-A, Lox-1, and CXCL16 in vivo, thus adding renal tubular epithelial cells to the growing list of cells that express scavenger receptors. As receptors for oxLDL, any of these receptors may serve as the bridge between hyperlipidemia, oxidative stress, and more aggressive renal fibrosis. However, based on the observed changes in expression after UUO-induced renal damage, CD36 and CXCL16 appear to be the best candidates and both renal tubules and inflammatory interstitial cells may participate. Further studies should determine their functional role and potential as therapeutic targets in chronic kidney disease.

GRANTS

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generally exhibit scavenger receptor activity through their receptor-bind- 


