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Interstitial inflammation and fibrosis in rats with diet-induced hypercholesterolemia

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Interstitial inflammation and fibrosis in rats with diet-induced hypercholesterolemia. Abnormalities in lipid metabolism appear to play a pathogenic role in progressive renal disease. To elucidate the cellular and molecular basis of renal interstitial fibrosis in uninephrectomized rats with diet-induced hypercholesterolemia, we fed experimental rats with standard rat chow supplemented with 4% cholesterol and 1% cholic acid. Control rats were fed an isocaloric diet. Groups of 7 control and 7 experimental rats were killed after 4, 8, and 12 weeks. Hypercholesterolemic rats developed albuminuria; serum creatinine was elevated at 12 weeks. By 12 weeks numerous oil red O-positive cells were present throughout the interstitium and to a lesser extent in tubules. Total renal lipid-peroxidation products were significantly increased (172 ± 15, 198 ± 28, and 197 ± 13 mmol malondialdehyde/kidney at 4, 8, and 12 weeks vs. 123 ± 17, 144 ± 6, and 125 ± 10 mmol in controls). Immunostaining revealed oxidatively modified lipoproteins within tubular and interstitial cells. The interstitial disease was characterized by an interstitial infiltrate of monocytes. Significant increases were detected in renal cortical mRNA levels for monocyte chemoattractant protein-1 (MCP-1), osteopontin, and vascular cell adhesion molecule-1 (VCAM-1), associated with changes in the pattern of immunostaining for each encoded proteins. Total kidney collagen was significantly increased at 12 weeks (9.8 ± 0.9 mg/kidney vs. 7.8 ± 0.9 mg in controls). At 12 weeks there was a significant increase in interstitial immunostaining for collagen I, collagen III, collagen IV, fibronectin and tenascin. A significant threefold increase in renal cortical mRNA levels for transforming growth factor beta-1 (TGF-β1) at 4 and 12 weeks was associated with the appearance of TGF-β1-positive interstitial cells. Renal matrix protein mRNA levels were measured at 4, 8, and 12 weeks. The only statistically significant elevations were procollagen α1(I) and procollagen α1(III) at weeks 8 and 12. In contrast, renal cortical mRNA levels for the tissue inhibitor of metalloproteinases-1 (TIMP-1) were significantly increased at 4, 8 and 12 weeks (1.4 ± 0.5, 2.7 ± 0.9 and 2.7 ± 1.4 arbitrary densitometric units, respectively, vs. 1.0 ± 0.4, 1.0 ± 0.5 and 1.0 ± 0.4 units for controls), and urokinase-type plasminogen activator (μPA) mRNA levels were significantly decreased at 4, 8, and 12 weeks (0.4 ± 0.1 arbitrary densitometric units for all three experimental groups vs. 1.0 ± 0.4, 1.0 ± 0.3, and 1.0 ± 0.4 units for the control groups). In summary, rats with diet-induced hypercholesterolemia develop renal interstitial fibrosis over several weeks. Following the accumulation of lipids within tubulointerstitial cells, interstitial nephritis develops. The fibrotic phase is characterized by modest changes in matrix protein mRNA levels, up-regulated TIMP-1, and down-regulated μPA levels, suggesting that altered matrix degradation plays a role in the interstitial fibrogenesis in this model.

Abnormalities in lipid metabolism appear to play a pathogenic role in progressive renal disease. Supportive evidence dates all the way back to Virchow's 1860 report of fatty degeneration in the kidneys of patients with Bright's disease [1]. However, progress in this field was relatively slow until 1982, when Moorhead and colleagues suggested that lipoproteins produced by the liver in response to proteinuria might contribute to progressive renal disease [2]. Since that time, several studies based on animal models have provided evidence that a pathogenetic relationship exists between elevated plasma lipid levels and glomerular injury [3–7]. For example, manipulations that alter serum lipids influence glomerular injury in rats with puromycin aminonucleoside nephrosis [8, 9], ureteral obstruction [10], and 5/6 nephrectomy [11, 12], and they influence the glomerular disease that develops spontaneously in obese Zucker rats [13]. Furthermore, feeding high-cholesterol diets to normal rats [14, 15], guinea pigs [16], and rabbits [17] causes renal disease that is not present in animals fed standard laboratory chow [14–17].

To date very little attention has been paid to the potential role of hyperlipidemia as a cause of the tubulointerstitial damage that typifies progressive renal injury, despite speculation by Moorhead et al that lipiduria may be an independent progression factor [2]. Wellmann and Volk [17] reported interstitial foam cells and foci of interstitial fibrosis in rabbits with diet-induced hypercholesterolemia. Peric-Golia and Peric-Golia [18] described interstitial fibrosis in rats fed a high-cholesterol diet for 80 weeks. In both normal and uninephrectomized rats, Kasiske et al reported significant tubulointerstitial damage in the rats fed a diet high in cholesterol for 19 weeks compared to rats fed standard chow [15]. This group also reported the presence of significant tubulointerstitial injury in obese Zucker rats with endogenous hypercholesterolemia 32 weeks after uninephrectomy [19]. In both of these rat studies, the investigators found a significant positive correlation between the content of esterified cholesterol in the renal cortex and the severity of the tubulointerstitial damage. Modi et al reported an attenuation of tubulointerstitial disease associated with a significant reduction in serum cholesterol levels in rats that were treated with probucol after subtotal nephrectomy [12]. ApoB, the major human low-density lipoprotein (LDL) apoprotein, has been shown to accumulate in the interstitium and in tubular cells in patients with nephrotic syndrome [20].

The pathogenetic basis of the relationship between hyperlipidemia and renal injury remains unclear, although certain morphologic parallels with atherosclerosis suggest that common mechanisms of tissue injury may exist. In particular, oxidized lipoproteins (oxLDL) and cells of the monocyte-macrophage lineage appear to play a critical role [21–23]. Oxidized LDL has

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monocyte chemotactic activity [24]. Exposure of endothelial cells to oxidized LDL may also enhance monocyte-endothelial interactions by upregulating the expression of adhesion molecules such as the $\beta 2$ integrins [25, 26], intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1) [27, 28], and by increasing the synthesis of monocyte chemotactic factors [29]. Macrophages express scavenger receptors that process oxidized [30] but not native LDL. Although tubular cells can internalize oxidized LDL [31, 32], the specific receptors involved have not yet been identified. Two scavenger receptors for oxidized LDL have recently been cloned from several animal species [33–36], and they appear to be products of a single gene [37]. In the current model of atherogenesis, it has been proposed that the uptake of oxidized LDL by macrophage scavenger receptors stimulates the synthesis of growth factors, cytokines, and other mediators of matrix-protein synthesis [38, 39].

The present study was designed to elucidate the cellular and molecular basis of renal interstitial fibrosis in uninephrectomized rats with diet-induced hypercholesterolemia. Our results show that tubulointerstitial disease develops slowly. The earliest morphologic change is the detection of lipid deposits within interstitial cells. At 4 weeks renal cortical osteopontin mRNA levels are significantly increased, and remain elevated at 8 and 12 weeks. By 8 weeks there is a significant increase in renal monocyte chemoattractant protein-1 mRNA levels and the number of interstitial macrophages. At 12 weeks the kidney collagen content and interstitial immunostaining for several matrix proteins are increased. The results of a serial evaluation of renal mRNA levels suggest that increased expression of transforming growth factor beta-1 (TGF-$\beta 1$) and the tissue inhibitor of metalloproteinases-1 (TIMP-1) and decreased expression of urokinase-type plasminogen activator (μPA) may contribute to interstitial fibrosis in this model.

Methods

Experimental design

Forty-two female Sprague-Dawley rats weighing 100 to 120 g were purchased from Charles River Breeding Laboratories (Wilmington, MA, USA). We performed a left nephrectomy on each animal five days before the study began. Half of the animals were fed standard rat chow (Purina Rodent Laboratory Chow 5001; Purina Mills Inc., Richmond, IN, USA) supplemented with 4% cholesterol and 1% cholic acid (Purina Test Diets). The control group was fed the standard chow containing 270 mg cholesterol per kilogram that was prepared by the same manufacturer. Groups of 7 control rats and 7 rats on the high-cholesterol diet were killed at 4, 8, and 12 weeks.

Each rat was housed individually in a metabolic cage at 4, 8, and 12 weeks, and the spontaneously voided urine was collected over a 24-hour period. Animals were killed by exsanguination under general anaesthesia by inhalation of nitrous oxide, oxygen, and enflurane. At the time of death, a blood sample was taken, and the remaining kidney was decapsulated and carefully weighed (wet weight). The kidney was divided in half longitudinally. The renal cortex was carefully dissected from one-half of the first piece and snap-frozen in liquid nitrogen for extraction of total RNA. The remainder of the first half was divided into smaller pieces of cortex that were snap-frozen in isopentane pre-cooled in liquid nitrogen for subsequent immunostaining. All tissues were stored at −70°C.

The second half was divided into two parts, and both were carefully weighed. The first piece was frozen in liquid nitrogen for total collagen measurement and the second for measurement of lipid peroxidation products. Renal peroxidation products were assessed using the thiobarbituric-acid assay to assess the renal malondialdehyde content according to the methods of Ohkawa, Ohishi and Yagi [40].

Biochemical studies

Urinary albumin concentrations were measured by radial immunodiffusion [41]. Plasma creatinine and total cholesterol levels were measured using the Kodak Ektachem Clinical Chemistry slide technique on individual samples of heparinized blood obtained when the rats were exsanguinated.

Total kidney collagen was calculated as previously described [42], based on measurements of the hydroxyproline in kidney homogenates according to the technique of Kivirikko, Laitinen and Prockop [43]. Collagen was assumed to contain 12.7% hydroxyproline by weight. Final results, based on the wet wt of the kidney, are expressed as milligrams of collagen per kidney.

Renal immunofluorescence studies

Interstitial and intraglomerular monocytes/macrophages expressing the cytoplasmic marker ED-1 (Serotec, Oxford, UK) were counted on acetone-fixed kidney cryosections 3 μm thick, using the dual fluorochrome labeling and enumeration technique previously described [41, 44].

We assessed the accumulation of extracellular matrix proteins within the cortical interstitium semiquantitatively with fluorescence microscopy, using the technique previously described [45]. The primary antibodies used were: sheep anti-human collagen I; goat anti-human collagen III; goat anti-human collagen IV (Southern Biotechnology Associates, Birmingham, AL, USA); rabbit anti-human tenascin (a gift from Dr. H. Erickson, Duke University, NC, USA); and a murine monoclonal antibody to human extracellular domain A (EDA+) fibronectin, an attenuatively spliced form of fibronectin (a gift from Dr. L. Zardi, Instituto Nazionale perla Rererca sul Cancro, Italy).

Cryostat sections were stained with oil red O to evaluate the renal accumulation of neutral lipids. Evidence of recent tubular injury was evaluated by immunostaining (with murine monoclonal anti-vimentin antibody; ESBE Laboratory Supplies, Markham, ON, Canada) for vimentin, an intermediate filament that is transiently expressed by regenerating tubules. Tissue sections from each animal group were evaluated by indirect immunofluorescence staining for the pattern of expression of alpha smooth-muscle actin (anti-rat smooth-muscle actin; Sigma Chemical Company, St. Louis, MO, USA); arachidonic acid-modified and linoleic acid-modified LDL (murine monoclonal antibodies; a gift from Dr. U. Steinbrecher, University of British Columbia, Vancouver, BC, Canada) [46]; MCP-1 (rabbit anti-rat MCP-1 antiserum; a gift from Dr. J.S. Warren, University of Michigan Medical Center, Ann Arbor, MI, USA); osteopontin (goat anti-rat smooth-muscle osteopontin; a gift from Dr. C.M. Giachelli, University of Washington, Seattle, WA, USA); VCAM-1 (anti-rat VCAM-1 monoclonal antibody; a gift from Dr. P. Chisholm, Biogen, Cambridge, MA, USA); ICAM-1 (anti-rat ICAM-1 monoclonal antibody; Cedarlane Laboratories Ltd., Hornby, ON,

Table 1. Renal functional parameters

Parameter	Group	Week 4	Week 8	Week 12
Body weight *g*	C	250 ± 11	292 ± 18	318 ± 21
	H	238 ± 18	259 ± 11[a]	286 ± 23[a]
Urinary albumin *mg/100 g body wt/day*	C	0.1 ± 0.1	0.1 ± 0.1	0.3 ± 0.2
	H	8.1 ± 8.6[a]	19.4 ± 8.1[a]	57.2 ± 32.0[a]
Serum creatinine *μmol/liter*	C	51 ± 5	49 ± 4	46 ± 6
	H	44 ± 3[a]	46 ± 6	52 ± 4[a]
Interstitial macrophages *per 1000 tubulointerstitial cells*	C	10 ± 6	4 ± 2	7 ± 3
	H	13 ± 7	12 ± 6[a]	44 ± 11[a]
Glomerular cells *per glomerular cross-section*	C	0.7 ± 0.4	0.5 ± 0.5	1.3 ± 1.0
	H	2.8 ± 1.1[a]	2.7 ± 1.3[a]	10.5 ± 3.0[a]
Kidney weight *mg*	C	1184 ± 30	1273 ± 67	1246 ± 54
	H	1307 ± 121	1434 ± 103[a]	1786 ± 184[a]

Results are means ± 1 SD; N = 7/group. Abbreviations are: C, control group; H, hypercholesterolemic group.

[a] $P < 0.05$ compared to the corresponding control group, by Mann-Whitney U test

Table 2. Lipid abnormalities

Parameter	Group	Week 4	Week 8	Week 12
Plasma cholesterol *mmol/liter*[a]	C	1.3 ± 0.1	1.4 ± 0.1	1.3 ± 0
	H	5.1 ± 1.5	4.3 ± 1.1	3.1 ± 1.0
Renal peroxidation products *mmol malondialdehye per kidney*	C	123 ± 17	144 ± 6	125 ± 10
	H	172 ± 15	198 ± 28	197 ± 13

Results are means ± 1 SD; N = 7/group. Results for all experimental groups are significantly increased over those of the corresponding control group ($P < 0.05$ by the Mann-Whitney U test). Abbreviations are: C, control group; H, hypercholesterolemic group.

[a] Results less then 1.3 mmol/liter (the lowest detectable level) were entered as 1.3 mmol/liter for statistical analysis

Canada); TGF-β1 [rabbit anti-TGF-β1 (anti-LC) antibody; a gift from Dr. K. Flanders, National Institute of Health, Bethesda, MD, USA]; TIMP (rabbit anti-bovine TIMP; a gift from Dr. Y.A. De Clerck, Children's Hospital of Los Angeles, Los Angeles, CA, USA); and μPA (rabbit anti-human μPA; American Diagnostica Inc., Greenwich, CT, USA). Unreferenced antibodies are cited in our previous publications [42, 45, 47, 48]. The secondary antisera for detecting polyclonal antibodies were fluorescein isothiocyanate (FITC)-conjugated goat anti-rabbit IgG and FITC-conjugated rabbit anti-goat IgG antisera (Chemicon International Inc., Temecula, CA, USA). The secondary antiserum for detecting murine monoclonal antibodies was FITC-conjugated goat anti-mouse IgG (Zymed Laboratories Inc., San Francisco, CA, USA). The FITC-conjugated antisera were absorbed with normal rat plasma; lack of staining showed them to be nonreactive with control kidney sections. Code numbers were used to blind the investigator to the animal group as each investigation was performed.

Gene expression

We isolated total renal cortical RNA according to the guanidinium isothiocyanate-cesium chloride method of Chirgwin et al [49], separated it by electrophoresis in a 1% agarose-formaldehyde gel, transferred it to a nylon membrane, and probed it with ^{32}P dCTP-labeled complementary DNA probes, as described previously [47]. We obtained autoradiographs and quantified the bands by laser densitometry. The density reading of each band on the autoradioaudiograph was adjusted for any RNA-loading inequality as previously described [47].

The cDNA probes used were: rat MCP-1 (a gift from Dr. T. Yoshimura, National Cancer Institute, Bethesda, MD, USA); rat osteopontin (a gift from Dr. C.M. Giachelli, University of Washington, Seattle, WA, USA); rat VCAM-1 (a gift from Dr. T. Collins, Harvard Medical School, Boston, MA, USA); rat TGF-β1 (a gift from Dr. S.W. Qian, National Cancer Institute); rat macrophage colony-stimulating factor (M-CSF, American Tissue Culture Collection no. 63,057); rat tumor-necrosis factor alpha (TNF-α, a gift from Dr. W. Fiers, Roche Research, Gent, Belgium); rat interleukin-1 beta (IL-1β, a gift from Dr. T. Nishida, Otsuka Pharmaceutical Company Ltd., Japan) [50]; rat platelet-derived growth factor A (PDGF-A, a gift from Dr. D. Mercola, University of California, San Diego, CA, USA) [51]; rat α1(I) procollagen (supplied by Dr. S. Thorgeirsson, National Cancer Institute, Bethesda, MD, USA); murine α1(III) procollagen (supplied by Dr. Y. Yamada, National Institutes of Health, Bethesda, MD, USA); murine α2(IV) procollagen (supplied by Dr. M. Kurkinen, University of New Jersey, Rutgers Medical School, Piscataway, NJ, USA); fibronectin (supplied by Dr. R. Hynes, Center for Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, USA); mouse tenascin (supplied by Dr. P. Ekblom, Uppsala University, Sweden); murine TIMP-1 (supplied by Dr. R. Khokha, London Regional Cancer Centre, London, ON, Canada); rat plasminogen-activator inhibitor-1 (rat PAI-1; supplied by Dr. Z.R. Gelehrter, University of Michigan, Ann Arbor, MI, USA); and rat μPA (supplied by Dr. J. Degan, Children's Hospital Research Foundation, Cincinnati, OH, USA). Unreferenced probes have been cited in earlier publications [42, 45, 47, 48].

Statistical analysis

Results for experimental animals at weeks 4, 8, and 12 were compared with corresponding results for control animals using the Mann-Whitney U-test. A P value under 0.05 was considered statistically significant.

Results

Renal function

Excretion rates for urinary albumin among hypercholesterolemic rats were significantly elevated at 4, 8, and 12 weeks; serum creatinine levels were elevated at 12 weeks (Table 1).

Lipid abnormalities

Rats fed the high-cholesterol diet had significantly increased serum cholesterol levels and renal peroxidation products at weeks 4, 8, and 12 (Table 2). Oil red O staining demonstrated significant intraglomerular lipid deposits by week 4; these deposits continued to enlarge at 8 and 12 weeks (Fig. 1A). At weeks 4 and 8, a few interstitial cells contained lipid deposits, while positive-staining tubules were very infrequent. By 12 weeks, numerous oil red O-positive cells were seen throughout the interstitium. The tubules showed positivity, but to a lesser extent (Figs. 1 B, C). Immunostaining using a monoclonal antibody to arachidonic acid-modified lipoproteins showed an increase in tubular staining and reactivity with tubular droplets of protein (Fig. 2A). Staining in control kidney sections showed reactivity with vessel walls and glomerular endothelial cells, and weak reactivity with some tubules. Immunostaining using a monoclonal antibody to linoleic

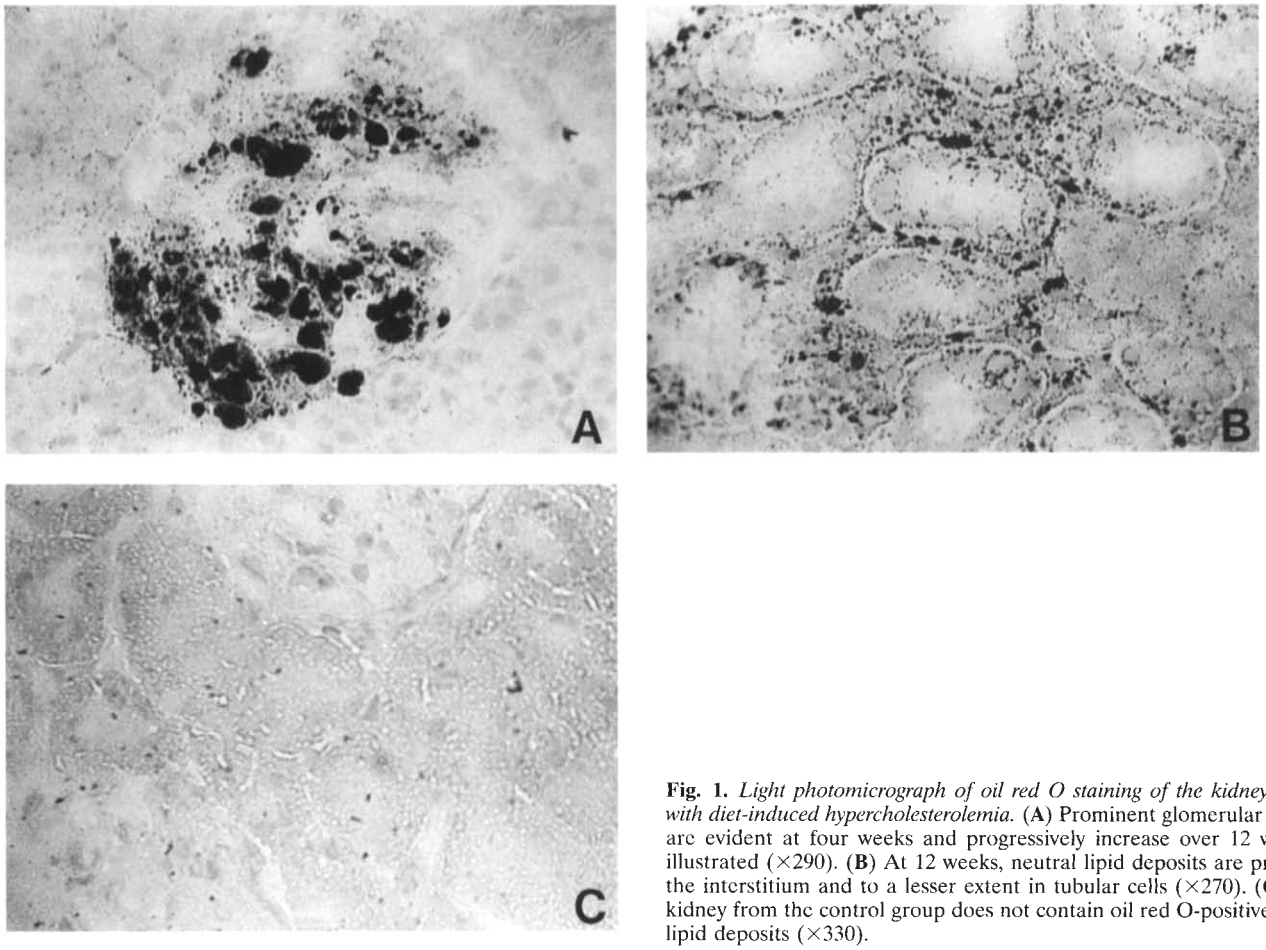

Fig. 1. *Light photomicrograph of oil red O staining of the kidneys of rats with diet-induced hypercholesterolemia.* (**A**) Prominent glomerular deposits are evident at four weeks and progressively increase over 12 weeks as illustrated (×290). (**B**) At 12 weeks, neutral lipid deposits are present in the interstitium and to a lesser extent in tubular cells (×270). (**C**) A rat kidney from the control group does not contain oil red O-positive neutral lipid deposits (×330).

acid-modified lipoproteins demonstrated reactivity with discrete interstitial cells and tubular protein droplets (Fig. 2B), whereas sections of control kidneys showed negative results. Using vimentin expression as a marker for regenerating tubules, tubular damage did not appear to be a major feature of the tubulointerstitial disease associated with diet-induced hypercholesterolemia. No vimentin-positive tubules were seen at 4 or 8 weeks; cortical tubules only very rarely expressed vimentin reactivity at 12 weeks.

Renal expression of monocyte chemoattractants, adhesion molecules, and α smooth-muscle actin

The number of interstitial macrophages was significantly increased in the experimental groups in those killed at weeks 8 and 12 (Table 1). Renal MCP-1 mRNA levels were significantly increased at week 8 (Table 3). Although not seen in control kidneys, occasional MCP-1-positive cells were present within the interstitium after 8 and 12 weeks of hypercholesterolemia. Renal cortical osteopontin mRNA levels were significantly elevated in all three groups of experimental animals (Table 3). Osteopontin was not expressed in the immunostained renal cortex of normal rats. Several cortical tubules expressed osteopontin, primarily along the apical membrane, in rats on the high-cholesterol diet. VCAM-1 mRNA levels were significantly elevated in rats of the high-cholesterol group examined at 12 weeks (Table 3). In normal rats, VCAM-1 protein was found in peritubular interstitial cells; in the high-cholesterol group there was an increase in the number of VCAM-1-positive interstitial cells at 4, 8, and 12 weeks (Fig. 3 A, B). In kidneys from control rats, cortical arterioles showed strong α smooth-muscle actin staining (Fig. 3D). Occasional foci of cells positive for α smooth-muscle actin were present in the interstitium after 12 weeks of hypercholesterolemia (Fig. 3C).

Renal cytokine and growth factor expression

Renal TGF-β1 mRNA levels were significantly increased at 4 and 12 weeks (Fig. 4). In control animals TGF-β1 immunostaining revealed positive cells in vessel walls and glomeruli and occasional positive tubules. At 4 and 8 weeks there was an increase in the number of TGF-β1 positive intraglomerular cells and positive tubules. At 12 weeks foci of positive interstitial cells were also present (Fig. 5). Renal cortical M-CSF mRNA levels were significantly elevated in the hypercholesterolemic rats at 4 weeks (1.4 ± 0.2 vs. 1.0 ± 0.3 arbitrary units) and IL-1β mRNA levels were significantly elevated in the hypercholesterolemic rats at 12 weeks (2.9 ± 1.6 vs. 1.0 ± 0.3 arbitrary units). Renal mRNA levels were not significantly increased for TNF-α or for PDGF-A at 4, 8, or 12 weeks in the hypercholesterolemic rat groups. In fact renal mRNA levels were significantly decreased at four weeks for TNFα (0.5 ± 0.3 vs. 1.0 ± 0.4 arbitrary units, respectively) and for MCSF at 12 weeks (0.6 ± 0.4 vs. 1.0 ± 0.3 arbitrary units).

Renal interstitial matrix proteins

The total kidney collagen content was significantly increased after 12 weeks of hypercholesterolemia (9.8 ± 0.9 mg/kidney vs.

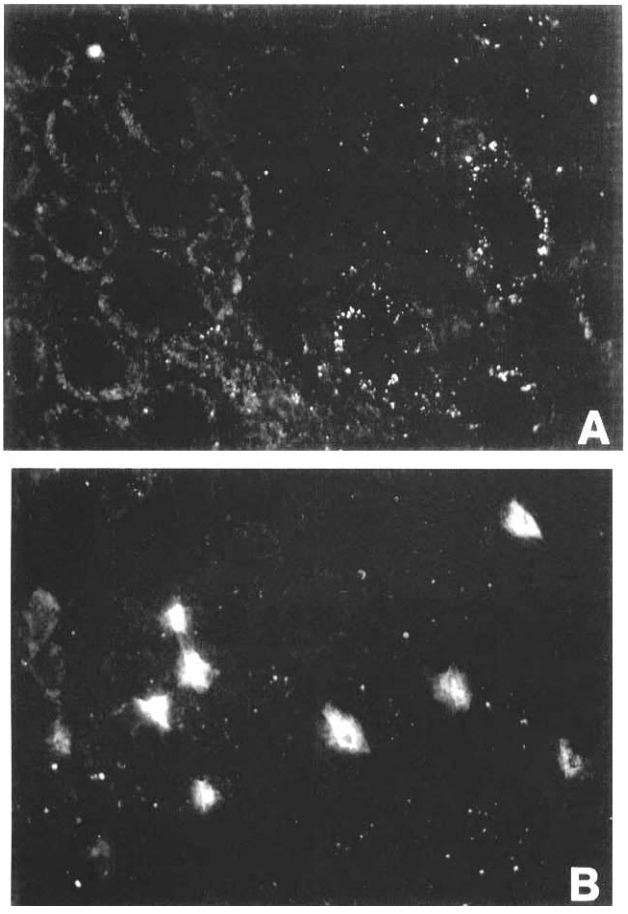

Fig. 2. *Immunostaining for oxidatively modified lipoproteins in hypercholesterolemic rats.* (**A**) Arachidonic acid-modified lipoproteins within tubular cells and droplets (×290). (**B**) Linoleic acid-modified lipoproteins within interstitial cells (×250).

7.8 ± 0.9 mg in controls; Fig. 6). At this time, a significant number of interstitial fields had increased immunostaining for collagen I, collagen III, collagen IV, fibronectin and tenascin (19 ± 27%, 11 ± 13%, 9 ± 11%, 26 ± 26% and 16 ± 21% of interstitial fields, respectively, showed increased interstitial immunostaining compared to control animals; Fig. 7). Interstitial immunostaining for collagen III did not show a statistically significant difference at 4 or 8 weeks in the hypercholesterolemic rats (0 ± 0% and 2 ± 3% of interstitial fields with increased immunostaining, respectively).

Renal matrix protein mRNA levels at weeks 4, 8, and 12 are summarized in Table 4. The only statistically significant elevations were in procollagen α1(I) at 12 weeks and procollagen α1(III) levels at 8 and 12 weeks (Fig. 8).

Renal protease/protease inhibitor mRNA levels

Renal TIMP-1 mRNA levels were significantly elevated at 4, 8 and 12 weeks in the hypercholesterolemic rats (Table 5 and Fig. 9). By immunostaining, TIMP-1 protein appeared *de novo* in the interstitium of the hypercholesterolemic rats, beginning at four weeks (Fig. 10A). Renal PAI-1 mRNA levels were increased at four weeks, but were similar in control and experimental groups at 8 and 12 weeks. Renal μPA mRNA levels were significantly decreased in all experimental groups (Table 5 and Fig. 11).

Table 3. Renal adhesion and chemoattractant molecule mRNA levels

Parameter	Group	Week 4	Week 8	Week 12
MCP-1	C	1.0 ± 0.4	1.0 ± 0.2	1.0 ± 0.8
	H	0.7 ± 0.2	2.2 ± 1.1[a]	2.4 ± 1.9
Osteopontin	C	1.0 ± 0.4	1.0 ± 0.6	1.0 ± 0.7
	H	2.9 ± 0.4[a]	2.4 ± 0.8[a]	2.1 ± 0.7[a]
VCAM-1	C	1.0 ± 0.4	1.0 ± 0.6	1.0 ± 0.4
	H	1.1 ± 0.4	1.0 ± 0.5	2.1 ± 0.5[a]

Results are means ± 1 SD (N = 7/group), expressed in arbitrary densitometric units. Abbreviations are: C, control group; H, hypercholesterolemic group; MCP-1, monocyte chemoattractant protein-1; VCAM-1, vascular cell adhesion molecule-1.

[a] $P < 0.05$ compared to the corresponding control group, by the Mann-Whitney U test

Several cortical tubules reacted with the anti-human μPA antiserum; however, no significant difference in the intensity of the staining could be discerned by immunofluorescence microscopy (Fig. 10B).

Discussion

Oxidized low-density lipoproteins play a pivotal role in the pathogenesis of atherosclerosis [23]. Several lines of evidence also suggest that lipoproteins are among the list of factors that cause glomerulosclerosis, perhaps by mimicking some of the atherosclerotic mechanistic pathways [5, 6, 14, 15, 52–56]. The results of the present study in uninephrectomized rats with diet-induced hypercholesterolemia suggest that lipoproteins may also contribute to the interstitial inflammation and fibrosis that typifies chronic progressive renal disease. In this model, glomerular changes occur early, while convincing tubulointerstitial pathology is delayed until after eight weeks of hypercholesterolemia.

Interstitial macrophages and renal tubular cells are able to internalize lipoproteins *in vitro* [31, 57]. After four weeks of hypercholesterolemia and prior to the onset of interstitial inflammation, oil red O staining identified lipid deposits within a few interstitial cells. By 12 weeks, numerous oil red O-positive interstitial cells were seen, likely within both resident and infiltrating interstitial macrophages. Not evident at four weeks, by 12 weeks lipid deposits were also detected in many tubules. Additional evidence suggests that a proportion of these tubulointerstitial lipid deposits are oxidized lipoproteins. Based on measurement of renal malondialydehyde levels, renal lipid peroxidation products were significantly increased. Immunostaining using monoclonal antibodies that recognize oxidation-specific epitopes characteristic of oxidized LDL confirmed the intracellular uptake of arachidonic acid-modified lipoproteins by tubular cells and linoleic acid-modified lipoproteins by interstitial cells. In addition, both of these antibodies reacted with tubular protein droplets.

Oxidized LDL is taken up by scavenger receptors but not by native LDL receptors. The molecular basis of *in vivo* LDL oxidation remains unclear, although the release of reactive oxygen metabolites by perivascular cells and interstitial macrophages likely plays a role [31]. It is possible that oxidation occurs mainly in the extravascular interstitial space, where the balance between endogenous oxidants and antioxidants favors oxidation [7]. After oxidation, LDL has an affinity for type I collagen [58] that may lead to entrapment in the interstitial microenvironment. Macrophages are an important source of scavenger receptors; studies

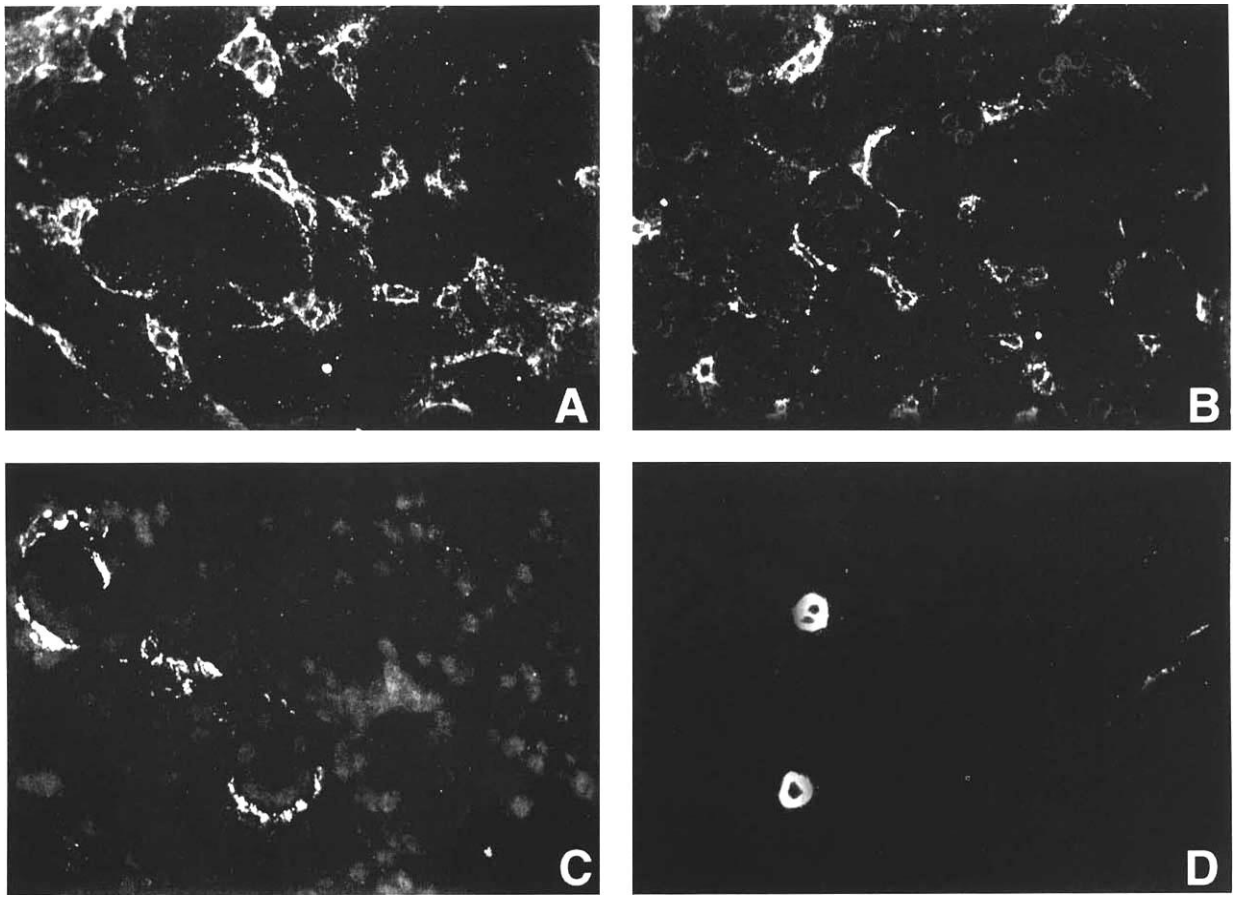

Fig. 3. *Immunofluorescence photomicrographs illustrating* (**A**) *increased numbers of interstitial cells expressing VCAM-1 (×240) compared to* (**B**) *control animals (×270), and* (**C**) *foci of interstitial cells expressing α smooth-muscle actin in the kidneys of hypercholesterolemic rats (×300).* (**D**) In control rats, α smooth-muscle actin is only found in the walls of arterioles (×310).

have not yet been done to confirm the expression of these receptors on renal tubular cells.

In the present study, tubular expression of the intermediate filament vimentin was used as a marker for recent tubular injury. The results of this study suggest that tubular uptake of lipoproteins does not cause significant tubular damage *in vivo*. This finding contrasts with *in vitro* studies documenting that significant tubular injury can be induced by oxLDL [31, 32]. This observation led us to speculate that the accumulation of oxidized lipoproteins within tubular and interstitial cells might trigger intrarenal proinflammatory and profibrogenic events. Scavenger receptors may activate numerous cellular pathways. *In vitro* studies have reported increased production of granulocyte and macrophage colony-stimulating factors, basic fibroblast growth factor [59], and platelet-derived growth factor [60] by endothelial cells exposed to oxLDL.

In this study we attempted to determine whether hypercholesterolemia altered the renal cortical expression of cytokines that have been implicated in fibrogenesis. At the mRNA level, TGF-β1 expression was biphasic, with significantly increased levels at 4 and 12 weeks. By 12 weeks, several TGF-β1-positive cells were present in the glomeruli and the interstitium, and the

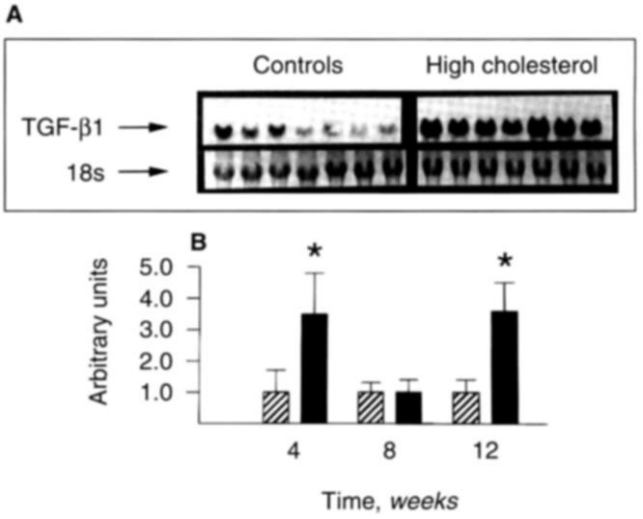

Fig. 4. *TGF-β1.* (**A**) Northern blot of renal cortical RNA from individual control rats and rats after 12 weeks of hypercholesterolemia. (**B**) The results of the TGF-β1 densitometric analysis, expressed in arbitrary units and shown as means ± 1 SD. Symbols are: (▨) control; (■) high cholesterol; $^*P < 0.05$, Mann-Whitney *U*-test.

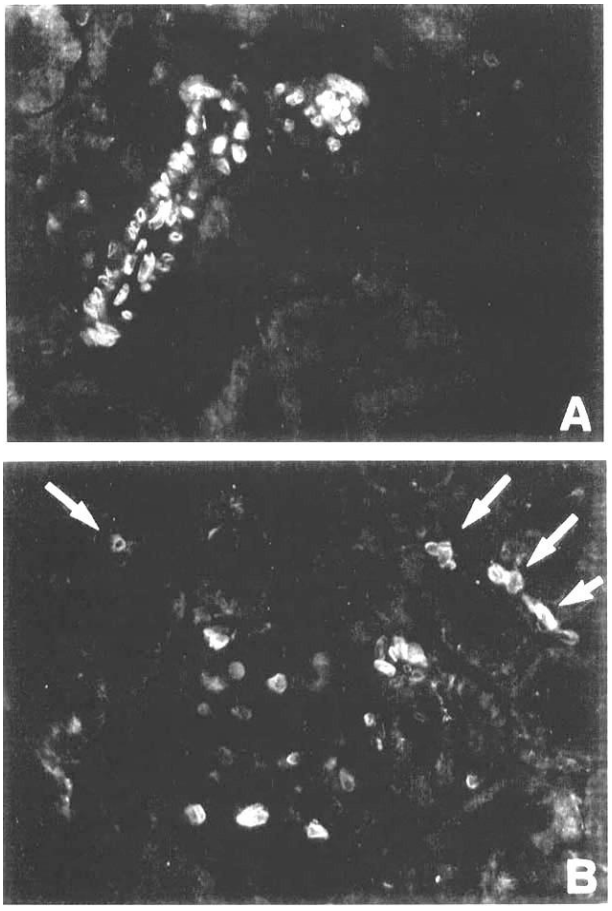

Fig. 5. *TGF-β1 immunofluorescence photomicrographs.* (**A**) In normal rats, TGF-β1-positive cells are present within vessel walls (×340). (**B**) After 12 weeks of diet-induced hypercholesterolemia, TGF-β1-positive cells appear within glomeruli and the interstitium (arrows) (×350).

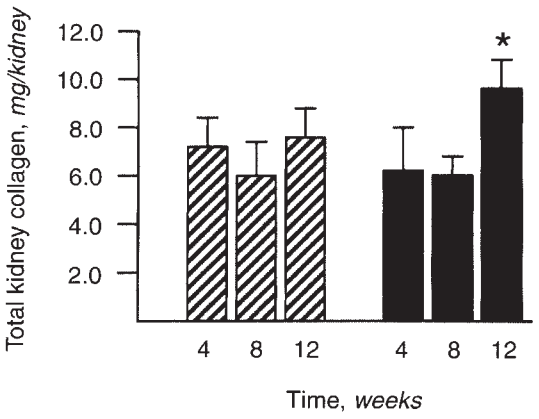

Fig. 6. *Total kidney collagen in hypercholesterolemic rats.* Results are means ± 1 SD. Symbols are: (▨) controls; (■) high cholesterol; $^*P < 0.05$, Mann-Whitney *U*-test.

intensity of tubular staining was increased. Compared to those in control kidneys, mRNA levels in the kidneys from rats with hypercholesterolemia were significantly increased for M-CSF at 4 weeks and IL-1β at 12 weeks. Effects of oxLDL on TGF-β1 expression have not been extensively studied. Ding and Diamond [61] have reported enhanced expression of TGF-β1 by cultured human glomerular epithelial cells in the presence of oxLDL. Native LDL augmented TGF-β1 bioactivity in supernatants of cultured rat mesangial cells [62].

Interstitial macrophages were significantly increased in the hypercholesterolemic group. Based on the results of immunostaining, interstitial macrophages appear to be an important source of TGF-β1 production. Although the molecular basis of interstitial monocyte recruitment in this model remains unclear, MCP-1, VCAM-1, and osteopontin appear to be candidates. Renal MCP-1 mRNA levels were significantly increased at 8 weeks. However, *de novo* renal expression of MCP-1 protein was not observed via immunostaining. Only the occasional MCP-1-positive interstitial cell was identified in the hypercholesterolemic rats. VCAM-1 mRNA levels were significantly increased at 12 weeks in the hypercholesterolemic rats. Again, immunostaining suggested that this was due to an influx of VCAM-1-expressing interstitial inflammatory cells rather than an increase in VCAM-1 expression by intrinsic renal cells. It is possible that oxidized lipoproteins up-regulated VCAM-1 expression on the interstitial macrophages [27, 28], although we observed a similar population of VCAM-1-positive interstitial cells in rats with protein-overload proteinuria [47] in the absence of hypercholesterolemia [63]. Osteopontin is a glycoprotein with monocyte chemotactic activity [64]. *De novo* expression of osteopontin by renal cortical tubules co-localizes with interstitial monocyte accumulation in several experimental models of renal disease [47, 65–67].

The appearance of interstitial myofibroblasts identified by their expression of α smooth-muscle actin is characteristic of renal diseases with progressive interstitial fibrosis. The origin and specific function of these myofibroblasts remains unclear. They may derive from resident interstitial fibroblasts, or they may be perivascular cells. It is interesting that the interstitial disease that developed in rats with hypercholesterolemia was characterized by the appearance of relatively few interstitial myofibroblasts, compared to other tubulointerstitial diseases such as obstructive uropathy [68]. It is likely that the 12-week timepoint represented the initiation of myofibroblast transformation/migration and that these cells may continue to increase with more advanced interstitial fibrosis. However, it is worth noting that during the first 12 weeks of hypercholesterolemia, renal mRNA levels for genes encoding matrix proteins were normal or only modestly increased (see below), suggesting a link between myofibroblast transformation and up-regulated expression of matrix-encoding genes.

By 12 weeks, hypercholesterolemic rats developed significant renal interstitial fibrosis, associated with a small but significant elevation in the mean serum creatinine level. Semiquantitative immunostaining confirmed a significant increase in collagen I, collagen III, collagen IV, EDA^+ fibronectin and tenascin. The molecular basis of renal interstitial fibrosis in hypercholesterolemic rats is not yet clear, although the results of this study suggest some possibilities. Studies in several other animal models of renal interstitial fibrosis [42, 45, 47, 48, 69–74] have suggested that both increased matrix-protein synthesis and decreased matrix turnover participate. What is particularly intriguing in the present study is that renal matrix protein mRNA levels did not show a striking difference between control and experimental animals. The elevations in procollagen α1(I) and procollagen α1(III) mRNA levels at 12 weeks were only 30% and 70% above control levels,

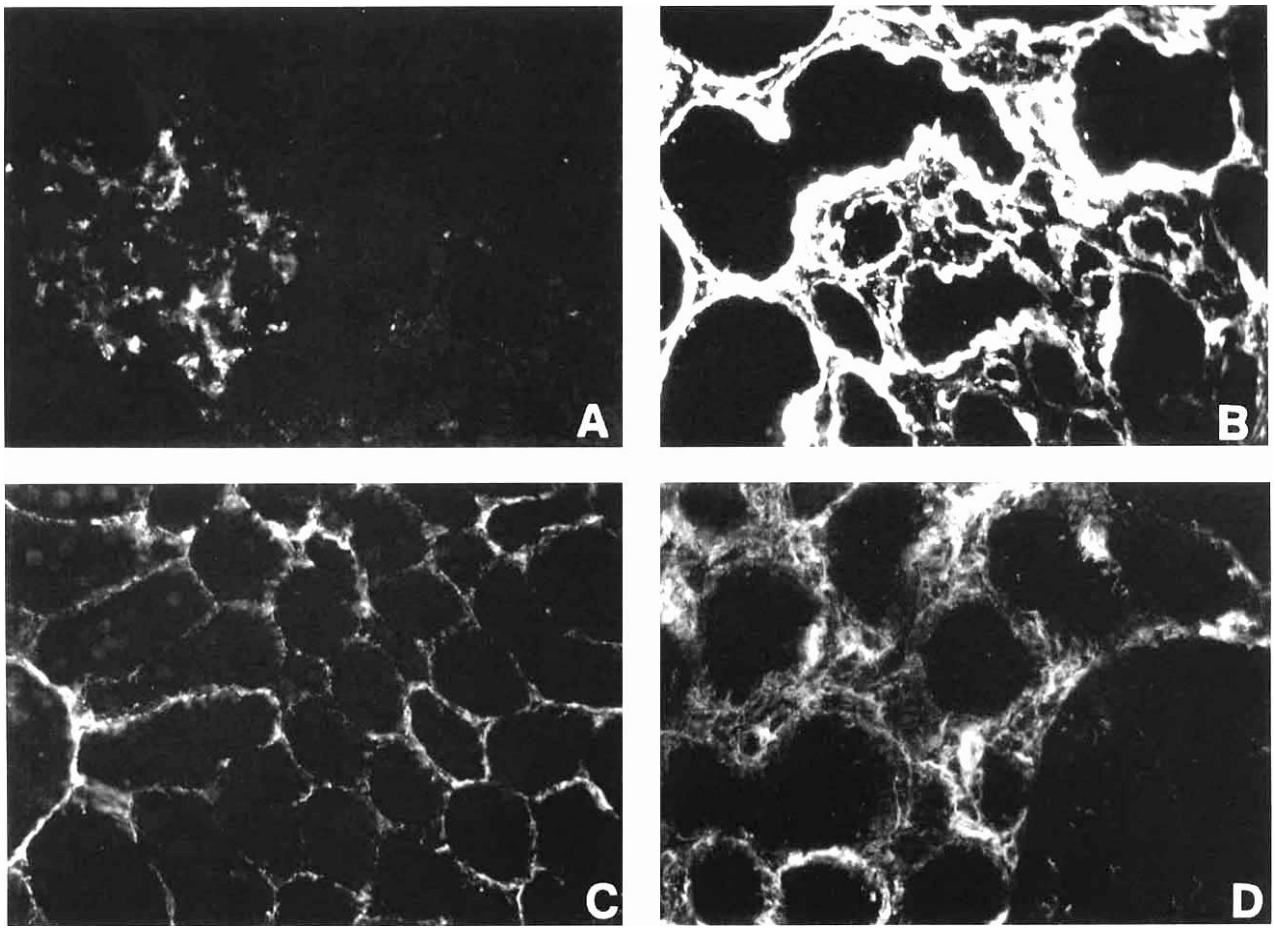

Fig. 7. *Immunofluorescence photomicrographs illustrating the renal interstitial accumulation of matrix proteins in hypercholesterolemic rats.* (**A**) control EDA^+ fibronectin; (**B**) 12-week experimental EDA^+ fibronectin; (**C**) control collagen I; and (**D**) 12-week experimental collagen I (all ×310).

Table 4. Renal matrix protein mRNA levels

Parameter	Group	Week 4	Week 8	Week 12
Procollagen αI(I)	C	1.0 ± 0.2	1.0 ± 0.2	1.0 ± 0.1
	H	0.9 ± 0.1	1.0 ± 0.3	1.3 ± 0.3[a]
Procollagen αI(III)	C	1.0 ± 0.2	1.0 ± 0.3	1.0 ± 0.2
	H	1.2 ± 0.2	1.4 ± 0.2[a]	1.7 ± 0.3[a]
Procollagen αI(IV)	C	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.3
	H	1.2 ± 0.4	0.6 ± 0.1[a]	1.0 ± 0.2
Fibronectin	C	1.0 ± 0.5	1.0 ± 0.4	1.0 ± 0.4
	H	1.0 ± 0.4	0.6 ± 0.2[a]	1.1 ± 0.2
Tenascin	C	1.0 ± 0.5	1.0 ± 0.3	1.0 ± 0.3
	H	0.9 ± 0.4	1.7 ± 0.9	0.7 ± 0.3

Results are means ± 1 SD (N = 7/group), expressed in arbitrary densitometric units. Abbreviations are: C, control group; H, hypercholesterolemic group.

[a] $P < 0.05$ compared to the corresponding control group, by the Mann-Whitney U test.

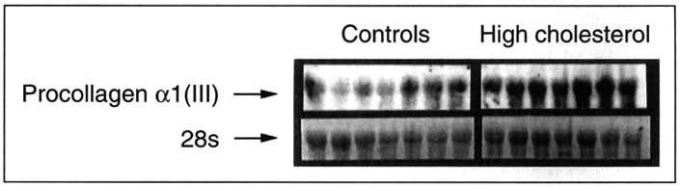

Fig. 8. *Northern blot of renal cortical procollagen α1(III) mRNA levels in uninephrectomized control rats and rats after 12 weeks of diet-induced hypercholesterolemia.* The mean densitometric scores expressed in arbitrary units were 1.0 ± 0.2 for the control group and 1.7 ± 0.3 for the hypercholesterolemic group.

respectively. Although we did not measure actual matrix protein synthesis rates in this study, transcriptional regulation of matrix-encoding genes is an important and often rate-limiting step. A recent study by Tamaki et al [75] showed a good correlation between renal fibronectin mRNA levels and rates of fibronectin synthesis in rats with adriamycin-induced nephrosis.

The modest changes in renal matrix protein mRNA levels suggested the possibility that decreased rates of matrix turnover played a role in the pathogenesis of interstitial fibrosis in the hypercholesterolemic rats. Two proteolytic cascades are thought to participate in degradation of renal interstitial matrix proteins: the metalloproteinases and the serine proteases. The metalloproteinase family is a large family consisting of three enzyme groups and three enzyme inhibitors. TIMP-1 is a glycoprotein that is elaborated by virtually all mesenchymal tissues, including intrinsic glomerular cells, inflammatory cells such as macrophages, and fibroblasts [76]. TIMP-1 inhibits all active as well as many latent matrix metalloproteinases.

TIMP-1 mRNA levels were significantly increased in the hypercholesterolemic rats at 4, 8 and 12 weeks. TIMP-1 mRNA levels are elevated in several experimental models of renal fibrosis,

Table 5. Renal protease and protease-inhibitor mRNA levels

Paramcter	Group	Week 4	Week 8	Week 12
TIMP-1	C	1.0 ± 0.4	1.0 ± 0.5	1.0 ± 0.4
	H	1.4 ± 0.5[a]	2.7 ± 0.9[a]	2.7 ± 1.4[a]
PAI-1	C	1.0 ± 0.4	1.0 ± 0.3	1.0 ± 0.5
	H	1.3 ± 0.3[a]	0.9 ± 0.3	1.1 ± 0.3
μPA	C	1.0 ± 0.4	1.0 ± 0.3	1.0 ± 0.4
	H	0.4 ± 0.1[a]	0.4 ± 0.1[a]	0.4 ± 0.1[a]

Results are means ± 1 SD (N = 7/group), expressed in arbitrary densitometric units. Abbreviations are: C, control group; H, hypercholesterolemic group; μPA, urokinase-type plasminogen activator; PAI-1, plasminogen-activator inhibitor-1; TIMP-1, tissue inhibitor of metalloproteinases-1.

[a] $P < 0.05$ compared to the corresponding control group, by the Mann-Whitney U test

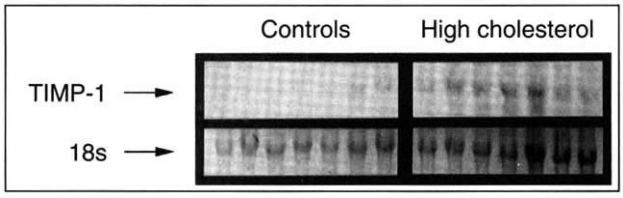

Fig. 9. *Northern blot of renal cortical TIMP-1 mRNA levels for uninephrectomized control rats and rats fed a high-cholesterol diet for eight weeks.* The mean densitometric scores were 1.0 ± 0.4 arbitrary units for the control group and 2.7 ± 0.9 units for the hypercholesterolemic group.

including puromycin aminonucleoside nephrosis [42, 45, 48], protein-overload proteinuria [47], passive Heymann nephritis [77], anti-tubular basement membrane nephritis [71], obstructive uropathy [69, 70], and diabetic nephropathy [78]. Absent in control kidney sections, TIMP-1 protein appeared *de novo* as a extracellular interstitial protein in rats with hypercholesterolemia, a pattern that has been observed in other animal models. Preliminary *in situ* hybridization studies (A.A. Eddy, unpublished observations) in rats with puromycin aminonucleoside nephrosis have indicated that both tubular and interstitial cells are the site of increased TIMP-1 expression during interstitial fibrosis. We have never found increased renal mRNA levels for matrix protein-degrading metalloproteinases in any of the models of renal interstitial fibrosis that we have studied. These results predict a reduction in metalloproteinase activity in the kidneys of hypercholesterolemic rats.

The second important pathway that has been implicated in renal matrix remodeling is the plasmin-dependent pathway. Perhaps most important is the ability of plasmin to activate latent procollagenases, although plasmin itself may degrade certain matrix proteins such as fibronectin. Biologically active plasmin is released from latent plasminogen by the proteolytic activity of the plasminogen activators. Within the kidney, urokinase-type plasminogen activator (μPA) is thought to be the most abundant enzyme and is produced by proximal, distal, and collecting tubules [79, 80]. In rats with hypercholesterolemia, renal μPA mRNA levels were significantly reduced at 4, 8, and 12 weeks, compared to control kidneys. The activity of μPA is inhibited by plasminogen activator inhibitor-1. In the hypercholesterolemic rats, renal PAI-1 mRNA levels were transiently increased at four weeks but were similar to control rats at 8 and 12 weeks. These results suggest that the intrarenal activity of the plasmin-dependent pathway is also depressed in hypercholesterolemic rats.

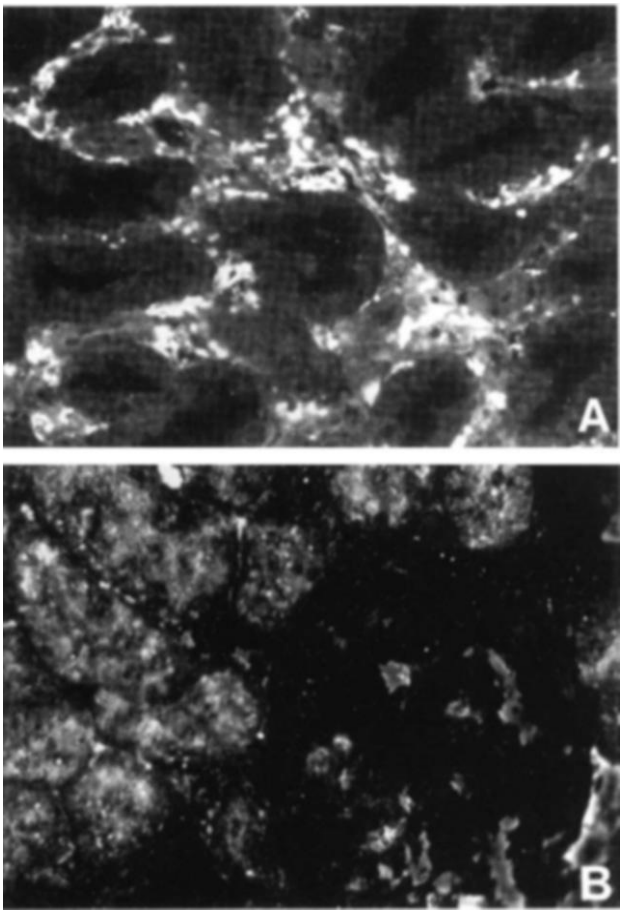

Fig. 10. *Immunofluorescence photomicrographs illustrating:* (**A**) *the de novo expression of TIMP-1 within the interstitium of rats after 12 weeks of hypercholesterolemia (×280); and* (**B**) *the tubular expression of μPA, a pattern that was similar in control and hypercholesterolemic rats (×280).*

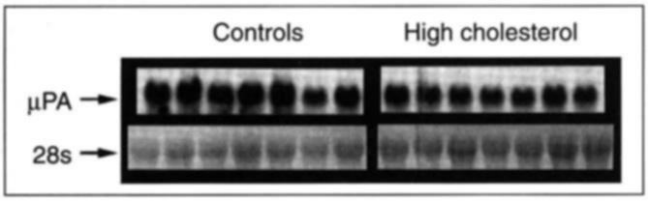

Fig. 11. *Renal cortical μPA mRNA levels for uninephrectomized control rats and rats fed a high-cholesterol diet for 12 weeks.* The mean densitometric scores were 1.0 ± 0.4 arbitrary units for the control group and 0.4 ± 0.1 units for the hypercholesterolemic group.

In summary, uninephrectomized rats fed a high cholesterol diet develop hypercholesterolemia and progressive renal disease. The renal interstitial disease develops slowly over several weeks. Following the accumulation of lipids within tubulointerstitial cells, interstitial nephritis develops, characterized by an infiltrate of macrophages and the appearance of occasional myofibroblasts. Interstitial fibrosis and renal functional impairment are established by 12 weeks. The fibrosis is characterized by increased intrarenal expression of TGF-β1 and TIMP-1 and decreased expression of μPA. We speculate that oxidized LDL-mediated

scavenger receptor activation plays a role in the fibrogenic cascade, a hypothesis that is currently under investigation using antioxidant drugs.

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Appendix

Abbreviations are: μPA, urokinase-type plasminogen activator; EDA, extracellular domain A; ICAM-1, intercellular adhesion molecule-1; IL-1β, interleukin-1 beta; LDL, low-density lipoprotein; MCP-1, monocyte chemoattractant protein-1; M-CSF, macrophage colony-stimulating factor; oxLDL, oxidized low-density lipoprotein; PAI-1, plasminogen-activator inhibitor-1; PDGF, platelet-derived growth factor; TGF-β1, transforming growth factor beta-1; TIMP-1, tissue inhibitor of metalloproteinases-1; TNF-α, tumor-necrosis factor alpha; μPA, urokinase-type plasminogen activator; VCAM-1, vascular cell adhesion molecule-1.

References

1. VIRCHOW R: A more precise account of fatty metamorphosis, in *Cellular Pathology* (2nd edition), edited by CHANCE FG, England, 1860, pp 324–366
2. MOORHEAD JF, CHAN MK, EL-NAHAS M, VARGHESE Z: Lipid nephrotoxicity in chronic progressive glomerular and tubulointerstitial disease. *Lancet* 2:1309–1311, 1982
3. KLAHR S, HARRIS K: Role of dietary lipids and renal eicosanoids on the progression of renal disease. *Kidney Int* 36(Suppl 27):S27–S31, 1989
4. MOORHEAD JF: Lipids and progressive kidney disease. *Kidney Int* 39(Suppl 31):S35–S40, 1991
5. KEANE WF, MULCAHY WS, KASISKE BL, KIM Y, O'DONNELL MP: Hyperlipidemia and progressive renal disease. *Kidney Int* 39(Suppl 31):S41–S48, 1991
6. DIAMOND JR: Effect of lipid abnormalities on the progression of renal damage: Analogous pathobiologic mechanisms in glomerulosclerosis and atherosclerosis. *Kidney Int* 39(Suppl 31):S29–S34, 1991
7. SCHLONDORFF D: Cellular mechanisms of lipid injury in the glomerulus. *Am J Kidney Dis* 22:72–82, 1993
8. DIAMOND JR, KARNOVSKY MJ: Exacerbation of chronic aminonucleoside nephrosis by dietary cholesterol supplementation. *Kidney Int* 32:671–677, 1987
9. DIAMOND JR, HANCHAK NA, MCCARTER MD, KARNOVSKY MJ: Cholestyramine resin ameliorates chronic aminonucleoside nephrosis. *Am J Clin Nutr* 51:606–611, 1990
10. MODI KS, MORRISSEY J, SHAH SV, SCHREINER GF, KLAHR S: Effects of probucol on renal function in rats with bilateral ureteral obstruction. *Kidney Int* 38:843–850, 1990
11. KASISKE BL, O'DONNELL MP, GARVIS WJ, KEANE WF: Pharmacologic treatment of hyperlipidemia reduces glomerular injury in rat 5/6 nephrectomy model of chronic renal failure. *Circ Res* 62:367–374, 1988
12. MODI KS, SCHREINER GF, PURKERSON ML, KLAHR S: Effects of probucol in renal function and structure in rats with subtotal kidney ablation. *J Lab Clin Med* 120:310–317, 1992
13. KASISKE BL, O'DONNELL MP, CLEARY MP, KEANE WF: Treatment of hyperlipidemia reduces glomerular injury in obese Zucker rats. *Kidney Int* 33:667–672, 1988
14. GRÖNE H-J, WALLI A, GRÖNE E, NIEDMANN P, THIERY J, SEIDEL D, HELMCHEN U: Induction of glomerulosclerosis by dietary lipids: A functional and morphologic study in the rat. *Lab Invest* 60:433–446, 1989
15. KASISKE BL, O'DONNELL MP, SCHMITZ PG, KIM Y, KEANE WF: Renal injury of diet-induced hypercholesterolemia in rats. *Kidney Int* 37:880–891, 1990
16. AL-SHEBEB T, FROHLICH J, MAGIL AB: Glomerular disease in hypercholesterolemic guinea pigs: A pathogenetic study. *Kidney Int* 33:498–507, 1988
17. WELLMANN KF, VOLK BW: Renal changes in experimental hypercholesterolemia in normal and in subdiabetic rabbits: II. Long-term studies. *Lab Invest* 24:144–155, 1971
18. PERIC-GOLIA L, PERIC-GOLIA M: Aortic and renal lesions in hypercholesterolemic adult, male, virgin Sprague-Dawley rats. *Atherosclerosis* 46:57–65, 1983
19. KASISKE BL, O'DONNELL MP, CLEARY MP, KEANE WF, PHILLIPS F: Effects of reduced renal mass on tissue lipids and renal injury in hyperlipidemic rats. *Kidney Int* 35:40–47, 1989
20. KASHYAP ML, OOI BS, HYND RA, GLUECK CJ, POLLAK VE, ROBINSON K: Sequestration and excretion of high-density and low-density lipoproteins by the kidney in human nephrotic syndrome. *Artery* 6:108–121, 1979
21. FROSTEGŚRD J, NILSSON J, HAEGERSTRAND A, HAMSTEN A, WIGZELL H, GIDLUND M: Oxidized low-density lipoprotein induces differentiation and adhesion of human monocytes and the monocytic cell line U937. *Proc Natl Acad Sci USA* 87:904–908, 1990
22. ROSENFELD ME, KHOO JC, MILLER E, PARTHASARATHY S, PALINSKI W, WITZTUM JL: Macrophage-derived foam cells freshly isolated from rabbit atherosclerotic lesions degrade modified lipoproteins, promote oxidation of low-density lipoproteins, and contain oxidation-specific lipid-protein adducts. *J Clin Invest* 87:90–99, 1991
23. WITZTUM JL, STEINBERG D: Role of oxidized low-density lipoprotein in atherogenesis. *J Clin Invest* 88:1785–1792, 1991
24. QUINN MT, PARTHASARATHY S, FONG LG, STEINBERG D: Oxidatively modified low-density lipoproteins: A potential role in recruitment and retention of monocyte/macrophages during atherogenesis. *Proc Natl Acad Sci USA* 84:2995–2998, 1987
25. LEHR H-A, KRÖBER M, HÜBNER C, VAJKOCZY P, MENGER MD, NOLTE D, KOHLSCHÜTTER A, MESSMER K: Stimulation of leukocyte/endothelium interaction by oxidized low-density lipoprotein in hairless mice: Involvement of CD11b/CD18 adhesion receptor complex. *Lab Invest* 68:388–395, 1993
26. LEHR H-A, KROMBACH F, MÜNZING S, BODLAJ R, GLAUBITT SI, SEIFFGE D, HÜBNER C, VON ANDRIAN UH, MESSMER K: *In vitro* effects of oxidized low-density lipoprotein on CD11b/CD18 and L-selectin presentation on neutrophils and monocytes with relevance for the *in vivo* situation. *Am J Pathol* 146:218–227, 1995
27. KUME N, CYBULSKY MI, GIMBRONE MAJ: Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. *J Clin Invest* 90:1138–1144, 1992
28. KHAN BV, PARTHASARAPTHY SS, ALEXANDER RW, MEDFORD RM: Modified low-density lipoprotein and its constituents augment cytokine-activated vascular cell adhesion molecule-1 gene expression in human vascular endothelial cells. *J Clin Invest* 95:1262–1270, 1995
29. YLÄ-HERTTUALA S, LIPTON BA, ROSENFELD ME, SARKIOJA T, YOSHIMURA T, LEONARD EJ, WITZTUM JL, STEINBERG D: Expression of monocyte chemoattractant protein 1 in macrophage-rich areas of human and rabbit atherosclerotic lesions. *Proc Natl Acad Sci USA* 88:5252–5256, 1991
30. GOLDSTEIN JL, HO YK, BASU SK, BROWN MS: Binding site on macrophages that mediates uptake and degradation of acetylated low density lipoprotein, producing massive cholesterol deposition. *Proc Natl Acad Sci USA* 76:333–337, 1979
31. ONG ACM, MOORHEAD JF: Tubular lipidosis: Epiphenomenon or pathogenetic lesion in human renal disease? *Kidney Int* 45:753–762, 1994
32. AGARWAL A, BALLA J, CROATT A, NATH K: Oxidized LDL injuries renal tubular epithelial cells: Implications for tubulointerstitial disease. (abstract) *J Am Soc Nephrol* 5:935, 1994
33. KODAMA T, FREEMAN M, ROHRER L, ZABRECKY J, MATSUDAIRA P, KRIEGER M: Type 1 macrophage scavenger receptor contains alpha-helical and collagen-like coiled coils. *Nature* 343:531–535, 1990
34. ROHRER L, FREEMAN M, KODAMA T, PENMAN M, KRIEGER M:

Coiled-coil fibrous domains mediate ligand binding by macrophage scavenger receptor type II. *Nature* 343:570–572, 1990
35. Matsumoto A, Naito M, Itakura H, Ikemoto S, Asaoka H, Hayakawa I, Kanamori H, Aburatani H, Takaku F, Suzuki H, Kobari Y, Miyal T, Takahashi K, Cohen EH, Wydro R, Housman DE, Kodama T: Human macrophage scavenger receptors: Primary structure, expression, and localization in atherosclerotic lesions. *Proc Natl Acad Sci USA* 87:9133–9137, 1990
36. Bickel PE, Freeman MW: Rabbit aortic smooth muscle cells express inducible macrophage scavenger receptor messenger RNA that is absent from endothelial cells. *J Clin Invest* 90:1450–1457, 1992
37. Freeman M, Ashkenas J, Rees DJG, Kingsley DM, Copeland NG, Jenkins NA, Krieger M: An ancient, highly conserved family of cysteine-rich protein domains revealed by cloning type I and type II murine macrophage scavenger receptors. *Proc Natl Acad Sci USA* 87:8810–8814, 1990
38. Clinton SK, Libby P: Cytokines and growth factors in atherogenesis. *Arch Pathol Lab Med* 116:1292–1300, 1992
39. Liptay MJ, Parks WC, Mecham RP, Roby J, Kaiser LR, Cooper JD, Botney MD: Neointimal macrophages colocalize with extracellular matrix gene expression in human atherosclerotic pulmonary arteries. *J Clin Invest* 91:588–594, 1993
40. Ohkawa H, Ohishi N, Yagi K: Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem* 95:351–358, 1979
41. Eddy AA, Michael AF: Acute tubulointerstitial nephritis associated with aminonucleoside nephrosis. *Kidney Int* 33:14–23, 1988
42. Eddy A: Protein restriction reduces transforming growth factor-β and interstitial fibrosis in nephrotic syndrome. *Am J Physiol* 35:F884–F893, 1994
43. Kivirikko KI, Laitinen O, Prockop DJ: Modifications of a specific assay for hydroxyproline in urine. *Anal Biochem* 19:249–255, 1967
44. Eddy AA, Crary GS, Michael AF: Identification of lymphohemopoietic cells in the kidneys of normal rats. *Am J Pathol* 124:335–342, 1986
45. Jones CL, Buch S, Post M, McCulloch L, Liu E, Eddy AA: The pathogenesis of interstitial fibrosis in chronic purine aminonucleoside nephrosis. *Kidney Int* 40:1020–1031, 1991
46. Magil AB, Frohlich JJ, Innis SM, Steinbrecher UP: Oxidized low-density lipoprotein in experimental focal glomerulosclerosis. *Kidney Int* 43:1243–1250, 1993
47. Eddy AA, Giachelli CM: Renal expression of genes that promote interstitial inflammation and fibrosis in rats with protein-overload proteinuria. *Kidney Int* 47:1546–1557, 1995
48. Jones CL, Buch S, Post M, McCulloch L, Liu E, Eddy AA: Renal extracellular matrix accumulation in acute purine aminonucleoside nephrosis in rats. *Am J Pathol* 141:1381–1396, 1992
49. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ: Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. *Biochem* 18:5294–5299, 1979
50. Nishida T, Hirato T, Nishino N, Mizuno K, Sekiguchi Y, Takano M, Kawai K, Nakai S, Hirai Y: Cloning of the cDNAs for rat interleukin-1α and β: Monokines and other non-lymphocytic cytokines, in *Progress in Leukocyte Biology* (vol 8), edited by Powanda MC, Oppenheim JJ, Kluger MJ, Dinarello CA, New York, Alan R. Liss Inc., 1988, pp 73–78
51. Mercola M, Wang CY, Kelly J, Brownlee C, Jackson-Grusby L, Stiles C, Bowen-Pope D: Selective expression of PDGF A and its receptor during mouse embryogenesis. *Dev Biol* 138:114–122, 1990
52. Magil AB, Cohen AH: Monocytes and focal glomerulosclerosis. *Lab Invest* 61:404–409, 1989
53. Kasiske BL, O'Donnell MP, Cowardin W, Keane WF: Lipids and the kidney. *Hypertension* 15:443–450, 1990
54. Schönholzer KW, Waldron M, Magil AB: Intraglomerular foam cells and human focal glomerulosclerosis. *Nephron* 62:130–136, 1992
55. Keane WF: Lipids and the kidney. *Kidney Int* 46:910–920, 1994
56. Gröne EF, Walli AK, Gröne H-J, Miller B, Seidel D: The role of lipids in nephrosclerosis and glomerulosclerosis. *Atherosclerosis* 107: 1–13, 1994
57. Roh DD, Ha H, Pai R, Irwin FL Jr, Lim H, Kim S, Kamanna VS, Kirschenbaum MD: Effect of atherogenic lipoproteins on extracellular matrix protein expression by murine renal tubular epithelial cells. (abstract) *J Am Soc Nephrol* 6:908, 1995
58. Hoover GA, McCormick S, Kalant N: Interactions of native and cell-modified low-density lipoprotein with collagen gel. *Arteriosclerosis* 8:525–534, 1988
59. Kraemer R, Pomerantz KB, Joseph-Silverstein J, Hajjar DP: Induction of basic fibroblast growth factor mRNA and protein synthesis in smooth muscle cells by cholesteryl ester enrichment and 25-hydroxycholesterol. *J Biol Chem* 268:8040–8045, 1993
60. Kume N, Gimbrone MJ: Lysophosphatidylcholine transcriptionally induces growth factor gene expression in cultured human endothelial cells. *J Clin Invest* 93:907–911, 1994
61. Ding G, Diamond J: Cholesterol-supplemented diet (CSD) rapidly upregulates glomerular transforming growth factor-β1 (TGF-β1), fibronectin (FN), and type I collagen (Col I) gene expression (GE) in normal rats. (abstract) *J Am Soc Nephrol* 3:467, 1992
62. Studer RK, Craven PA, DeRubertis FR: Low-density lipoprotein stimulation of mesangial cell fibronectin synthesis: Role of protein kinase C and transforming growth factor-β. *J Lab Clin Med* 125:86–95, 1995
63. Eddy AA: Interstitial nephritis induced by heterologous protein-overload proteinuria. *Am J Pathol* 135:719–733, 1989
64. Singh RP, Patarca R, Schwartz J, Singh P, Cantor H: Definition of a specific interaction between the early T lymphocyte activation 1 (Eta-1) protein and murine macrophages *in vitro* and its effect upon macrophages *in vivo*. *J Exp Med* 171:1931–1942, 1990
65. Pichler R, Giachelli CM, Lombardi D, Pippin J, Gordon K, Alpers CE, Schwartz SM, Johnson RJ: Tubulointerstitial disease in glomerulonephritis: Potential role of osteopontin (uropontin). *Am J Pathol* 144:915–926, 1994
66. Giachelli CM, Pichler R, Lombardi D, Denhardt DT, Alpers C, Schwartz S, Johnson RJ: Osteopontin expression in angiotensin II-induced tubulointerstitial nephritis. *Kidney Int* 45:515–524, 1994
67. Pichler RH, Franceschini N, Young BA, Hugo C, Andoh TF, Burdmann EA, Shankland SJ, Alpers CE, Bennett WM, Couser WG, Johnson RJ: Pathogenesis of cyclosporine nephropathy: Roles of angiotensin II and osteopontin. *J Am Soc Nephrol* 6:1186–1196, 1995
68. Diamond JR, van Goor H, Ding G, Engelmyer E: Myofibroblasts in experimental hydronephrosis. *Am J Pathol* 146:121–129, 1995
69. Sharma AK, Mauer SM, Kim Y, Michael AF: Interstitial fibrosis in obstructive nephropathy. *Kidney Int* 44:774–788, 1993
70. Engelmyer E, van Goor H, Edwards DR, Diamond JR: Differential mRNA expression of renal cortical tissue inhibitor of metalloproteinase-1, -2 and -3 in experimental hydronephrosis. *J Am Soc Nephrol* 5:1675–1683, 1995
71. Tang WW, Feng L, Xia Y, Wilson CB: Extracellular matrix accumulation in immune-mediated tubulointerstitial injury. *Kidney Int* 45:1077–1084, 1994
72. Yamamoto T, Noble NA, Miller DE, Border WA: Sustained expression of TGF-β1 underlies development of progressive kidney fibrosis. *Kidney Int* 45:916–927, 1994
73. Nakamura T, Ebihara I, Shirato I, Tomino Y, Koide H: Increased steady-state levels of mRNA coding for extracellular matrix components in kidneys of NZB/W F1 mice. *Am J Pathol* 139:437–450, 1991
74. Haralambous-Gasser A, Chan D, Walker RG, Powell HR, Becker GJ, Jones CL: Collagen studies in newborn rat kidneys with incomplete ureteric obstruction. *Kidney Int* 44:593–605, 1993
75. Tamaki K, Okuda S, Ando T, Iwamoto T, Nakayama M, Fujishima M: TGF-β1 in glomerulosclerosis and interstitial fibrosis of adriamycin nephropathy. *Kidney Int* 45:525–536, 1994
76. Woessner JF Jr: Matrix metalloproteinases and their inhibitors in connective tissue remodeling. *FASEB J* 5:2145–2154, 1991
77. Eddy A, Li Z, Liu E, McCulloch L: Macrophages may mediate interstitial fibrosis via the release of transforming growth factor β1 in passive Heymann nephritis. (abstract) *J Am Soc Nephrol* 2:573, 1991
78. Wu K, Setty S, Staskus K, Mauer M, Rozen S, Tsilibary EC: Altered matrix genes expression in experimental diabetes. (abstract) *J Am Soc Nephrol* 6:915, 1995
79. Kristensen P, Eriksen J, Dano K: Localization of urokinase-type plasminogen activator messenger RNA in the normal mouse by in situ hybridization. *J Histochem Cytochem* 39:341–349, 1991
80. Sappino A-P, Huarle J, Vassalli J-D, Belin D: Sites of synthesis of urokinase and tissue-type plasminogen activators in the murine kidney. *J Clin Invest* 87:962–970, 1991