IGF-1 induces rat glomerular mesangial cells to accumulate triglyceride

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MESANGIAL CELLS (MC) ARE SPECIALIZED glomerular pericytes that share many properties with vascular smooth muscle cells (VSMC) (77) and macrophages (81). Like VSMC, MC provide structural support through production of extracellular matrix (2) and regulate glomerular blood flow and intracapillary pressure via contraction (39). MC properties typical of macrophages include phagocytosis and production of reactive oxygen species (7). Recent studies of progression of chronic kidney disease indicate that, like atherosclerosis, lipid accumulation can contribute to glomerular injury (53). In some cases, lipid accumulates in MC, and they take on the appearance of foam cells. These observations are relevant to understanding vascular disease and progressive renal diseases that are accelerated by abnormalities in lipid metabolism.

chronic kidney disease; cholesterol; peroxisome proliferator-activated receptor; foam cells

IGF-1 has been implicated as a cytokine that contributes to the development of glomerulosclerosis (56). Mice that overexpress IGF-1 develop glomerulosclerosis (28), and MC from diabetic mice that develop nephropathy secrete increased amounts of IGF-1 (28). MC express IGF-1 receptors (3) and synthesize IGF-1 in vitro (23). Exposure to IGF-1 induces an increase in the synthesis of extracellular matrix, indicating that IGF-1 might contribute to mesangial matrix accumulation in glomerular disease (6). IGF-1 is thought to contribute to VSMC proliferation in vessel walls and participate in lesion progression in atherosclerosis (Ref. 76 and references therein).

In prior studies, we observed that rat MC exposed to IGF-1 for several days become lipid laden, developing a foam cell appearance (11). We demonstrated that lipid uptake was primarily through increased endocytosis and that lipid accumulation could be increased by supplementing the medium with cholesterol ester (12). As lipid accumulated, the actin cytoskeleton became disordered, and the membrane could no longer reorganize sufficiently to engulf Escherichia coli particles (11). Furthermore, lipid-laden MC were unable to migrate in response to insulin-like growth factor binding protein-5 (IGFBP-5) or to contract in response to angiotensin II (Berfield AK, Andress DL, and Abrass CK. Kidney Int 62: 1229–1237, 2002); however, their migratory response to IGF binding protein-5 is unaffected. This differs from cholesterol loading, which impairs both phagocytosis and migration. These findings have important implications for understanding the mechanisms that contribute to lipid accumulation in MC and the functional consequences of different forms of foam cells. These observations are relevant to understanding vascular disease and progressive renal diseases that are accelerated by abnormalities in lipid metabolism.

MATERIALS AND METHODS

Materials. The following reagents were purchased: human recombinant IGF-1 (Collaborative Research, Waltham, MA); IGFBP-5 peptide (AA201–218, RKGFYKRRQCKPSRGRKR, Fred Hutchinson Cancer Research Center, Seattle, WA); Lucifer yellow (Sigma, St. Louis, MO);125I (DuPont-New England Nuclear, Boston, MA); and RPMI 1640 tissue culture medium (GIBCO, Grand Island, NY).

Experimental design. MC were cultured in routine medium (20% FCS-RPMI 1640) with or without IGF-1 (100 nM) for 1–7 days. The lipid content of this medium was (in mg/dl) 10 cholesterol, 2 high-

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density lipoprotein (HDL), 5 LDL, 15 TG, and 8 phospholipids. Medium was changed every 2–3 days. After designated times, MC were assessed for cholesterol and TG content; LDL and acetylated LDL (AcLDL) binding and degradation; and levels of protein expression of sterol-regulatory element binding protein-1c (SREBP-1c), the peroxisome proliferator-activated receptors (PPAR), PPARα, PPARγ, and PPARδ, scavenger receptor (SR) SR-B1, CD36, ATP-binding cassette A-1 (ABCA-1), and 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase. Macropinocytosis was visualized with Lucifer yellow staining and electron microscopic examination. To measure the effects of lipid accumulation on IGFBP-5-mediated migration, MC propagated with and without IGF-1 (100 nM) were incubated in standard medium or loaded with cholesteryl ester (2.5 μg/ml) or linoleic and oleic acids conjugated to albumin (9.3 μg/ml, Sigma) for 7 days. Migration was measured as described elsewhere (12).

**Cell culture.** Rat glomerular MC were prepared by modification (4, 5) of routine methods (47). In brief, minced rat kidney cortex was sieved. Isolated glomeruli were plated in medium containing a 1:1 mix of 20% FCS-RPMI 1640 and previously collected glomerular conditioned medium for an additional week, after which the conditioned medium was sieved. Isolated glomeruli were plated in medium containing a 1:1 mix of 20% FCS-RPMI 1640 and previously collected glomerular conditioned medium. Insulin routinely added to supplement MC cultures was omitted. MC outgrowths were harvested and passed in this medium for an additional week, after which the conditioned medium was omitted. MC were cloned and studied at passages 8–12.

**Cholesterol and TG analysis.** MC were plated (5 × 10^4 cells/35-mm dish) and grown for 7 days with and without IGF-1 (100 nM). Lipids were extracted in hexane/isopropyl alcohol (3:2) or chloroform-methanol and isopropanol (13). Cholesterol and cholesterol ester were measured by mass spectrometer (PerkinElmer) as previously described (9). TG were assayed with a G HPO trinder kit (Sigma) using the manufacturer’s instructions and a modification of McGowan (61). Protein was measured by the method of Lowry (55). Cell counts were performed using a Coulter counter; n = 3–4/condition and experiments were repeated on four separate occasions.

**LDL and AcLDL binding and degradation.** LDL was prepared from human plasma as described previously (9). LDL was acetylated by repeated additions of acetic anhydride to LDL (10 mg/ml) diluted with saturated ammonium acetate. LDL and AcLDL were radiolabeled by the iodine monochloride method as modified for lipoproteins as described (43). MC (3 × 10^5 cells/75-cm² flask) were cultured for 1, 3, or 7 days with and without IGF-1 (100 nM). To measure LDL and AcLDL binding, cells were washed in warm RPMI 1640 tissue culture medium and 0.5 mg/ml fatty acid-free albumin, chilled, and incubated on ice for 3 h with 125I-LDL or 125I-AcLDL [250 cpm (counts/min/ng) in the presence and absence of unlabeled lipoproteins. The cells were washed with PBS, dissolved in 0.1 N NaOH, and bound counts were determined. For measurements of lipoprotein degradation, cultures were prepared as described. After 125I-LDL and 125I-AcLDL binding, replicate samples were washed and warmed to 37°C. After incubation for 4 h, the supernatants were collected, precipitated with trichloroacetic acid, and measured in a gamma counter. Cell-free degradation, which is <5% of total radioactivity, is subtracted from total degradation (43). Protein was measured by the method of Lowry (55).

**Western blot analysis.** MC (5 × 10^6 cells/75-cm² flask) were extracted using Triton-glycerol lysate buffer (1% Triton X-100, 10% glycerol, 20 mM HEPES, 100 mM NaCl) containing protease inhibitors (Complete Protease Inhibitor Cocktail tablets, Roche, Indianapolis, IN). The concentrated samples were assayed for total protein by BCA (Pierce, Rockford, IL). Samples (10–20 μg) were subjected to electrophoresis using 10% (HMG-CoA), 12% (SR-B1), or 4–12% (ABCA-1) SDS precast gels (Bio-Rad, Richmond, CA). PPARs were immunoprecipitated from cell lysates with respective antibodies and subjected to electrophoresis on 7% SDS gel. Proteins were transferred to nitrocellulose and incubated with the following antibodies: monoclonal mouse anti-mouse CD36 (MAB1258, Chemicon International, Temecula, CA); rabbit anti-SR-B1, mouse anti-SREBP1c (Santa Cruz Biotechnology, Santa Cruz, CA); rabbit anti-ABCA-1 (Novus Biologicals, Littleton, CO); rabbit anti-HMG-CoA reductase (gift from P. A. Edwards, UCLA, Los Angeles, CA); or rabbit PPARα, PPARγ, and PPARδ (Santa Cruz Biotechnology) followed by horseradish peroxidase-labeled donkey anti-rabbit or mouse IgG (Amersham Pharmacia, Piscataway, NJ) (41). Staining was enhanced with an ECL chemiluminescence kit (Amersham Pharmacia). Negative controls included the secondary antibody alone. Semiquantitative analysis was by optical density scanning of the relevant bands (Kodak).

**Endocytic assay.** MC were grown for 7 days with or without IGF-1 in chamber slides, then incubated in media containing Lucifer yellow dipotassium salt (1 μg/ml) for 1 h at 37°C and other slides at 4°C. The 4°C control was included to determine the degree of nonspecific surface binding of antigen to the cells. Following incubation, cells were washed twice with PBS to remove excess Lucifer yellow. Slides were examined by fluorescence microscopy.

**Electron microscopy.** MC were plated and treated as above, then subsequently fixed in 2% glutaraldehyde for 2 h. Cells were treated with 1% osmium tetroxide before sequentially dehydrated to Medcast. Thin sections were examined with a JEOL S100 electron microscope.

**MC migration.** MC migration was measured with and without IGF-1. 500–218 (30 μg/ml) in a wounding assay as described (12). In brief, MC were plated in 60-mm tissue culture dishes, grown to near confluence, growth arrested in 2% FCS-RPMI, and scraped with a sterile razor blade to create a linear wound across the dish. IGFBP-500–218 peptide was added to the cultures, and migration was examined 48 h later. Controls were maintained in 2% FCS-RPMI medium. Cultures were stained with toluidine blue. Migrating cells along a 1-mm length of the wound edge were counted in 0.1-mm increments. Five areas were examined per sample. Experiments were repeated three times. Data were analyzed as the total number of migrating cells and were expressed as a percentage of control.

**Calculations and statistics.** Unless otherwise stated, results were calculated on triplicate experiments, done on two to four separate occasions. For variables, means and SE were computed. An unpaired Student’s t-test was used to compare means between two experimental groups, whereas for multiple comparisons ANOVA was used, followed by Tukey’s test. Statistical significance was defined as P < 0.05.

**RESULTS**

Previously, we showed that MC cultured with IGF-1 for >3 days develop morphological changes characteristic of foam cells (11). Compared with controls, IGF-1-treated MC show increased accumulation of intracellular neutral lipids as evidenced by Nile red staining. The presence of neutral lipid droplets indicates accumulation of either cholesterol or TG. As we previously showed that cholesterol uptake was enhanced in IGF-1-treated MC when the medium was supplemented with cholesterol, we expected that the foam cells that developed after chronic exposure to IGF-1 in standard medium would be cholesterol rich. Furthermore, because oxidized LDL accumulation is thought to be an important contributor to foam cell formation, we postulated that IGF-1 might enhance uptake of modified lipids. To address these possibilities, we systematically evaluated the potential contribution of cholesterol synthesis and uptake of LDL and oxidized LDL to IGF-1-induced foam cell formation.

**IGF-1 has modest effects on receptor-mediated cholesterol uptake.** Most cholesterol and some TG uptake occur via LDL receptor-mediated endocytosis (38, 74). As shown in Fig. 1, there were no differences in the specific binding of LDL in the first day in untreated compared with IGF-1-treated MC. After 3 and 7 days of exposure to IGF-1, there was a minimal reduction (25%) in LDL binding. This suggests that IGF-1 had no direct effect on LDL receptor expression and that the modest fall in LDL binding at days 3 and 7 may have occurred.
as a result of downregulation by the intracellular accumulation of lipid. As shown in Fig. 1B, 1 day of exposure to IGF-1 was associated with a very modest increase in the amount of LDL internalized and degraded by MC. This increase was not sustained with longer exposure to IGF-1; thus changes in the rates of uptake and metabolism of LDL do not account for our findings.

Altered foam cell function has traditionally been attributed to intracellular accumulation of cholesterol and oxidized LDL by heightened scavenger receptor action. Scavenger receptor expression was assessed by measuring receptor binding, internalization, and degradation of AcLDL (9). As shown in Fig. 1C, no significant differences in AcLDL binding were detected over a period of 7 days. Similarly, in Fig. 1D, although a slight decrease in degradation occurred the first day, this was not sustained. This is in agreement with our previous data in which we found no change in the uptake of fluorescent-labeled AcLDL (11). These data indicate that IGF-1 did not significantly influence uptake of lipids by scavenger receptors, but it does not exclude the possibility that IGF-1 modulates other scavenger receptors, including SR-B1 and CD36 (67).

IGF-1 has little effect on intracellular synthesis, transfer, or efflux of cholesterol. MC express HMG-CoA reductase, which regulates intracellular synthesis of cholesterol (1). In most cells, intracellular accumulation of cholesterol is followed by a decrease in expression of HMG-CoA reductase (74), which permits cholesterol levels to return to baseline. As shown in Fig. 2, HMG-CoA reductase protein content was unchanged in MC after 7 days of exposure to IGF-1; thus there is no evidence for enhanced cholesterol synthesis.

SR-B1, the HDL receptor, serves as both a scavenger receptor for uptake of oxidized LDL, uptake of cholesterol and cholesterol ester from HDL, and as a passive cholesterol efflux mechanism by transferring cholesterol to HDL (22, 69). There were no differences in expression of SR-B1 in IGF-1-treated MC to account for the lipid accumulation in these cells (Fig. 2). This correlates with the results of the scavenger receptor and suggests that there is little lateral transfer of cholesterol to HDL.

A second scavenger receptor, CD36, has received considerable attention because of its role in uptake of oxidized LDL and long-chain fatty acids, its regulation in inflammatory states, and demonstration that null mutations in this receptor protect against the development of atherosclerosis (62). As shown in Fig. 2, chronic IGF-1 was associated with a marked decrease in CD36 content in MC. Such a prominent decrease in CD36 expression would be expected to reduce lipid accumulation and foam cell formation. Given the development of foam cells in the face of a decrease in CD36 expression, it argues that IGF-1

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**Fig. 1. LDL or acetylated LDL (AcLDL) binding and degradation.** For the binding assay, I125-labeled LDL (A) and AcLDL (C) bound to untreated control and IGF-1-treated mesangial cells (MC) were determined. For the degradation assay, cultures were incubated for 4 h, and the released isotope in the supernatant was counted LDL (B) and AcLDL (D). Values are means ± SE expressed as ng/μg cell protein. *P < 0.05, ANOVA.
The primary lipid that accumulates in IGF-1-treated MC is TG. As shown in Table 1, IGF-1 treatment was associated with a modest increase in the total cholesterol measured per sample; however, this difference disappears when the cholesterol measurement for cellular protein or cell number is factored in. In contrast to the measurement of cholesterol, TG content was significantly increased even when the measurements were expressed per cell. These results indicate that in routine medium (in mg/dl: 10 cholesterol, 2 HDL, 5 LDL, 15 TG, 8 phospholipids), IGF-1-treated MC preferentially accumulate TG. This is a novel and unexpected finding.

For many years, foam cell formation has been attributed to intracellular accumulation of cholesterol and oxidized LDL (33). Recent studies have implicated TG accumulation in foam cell formation and implicated TG in some of the aberrant functions of foam cells (8, 34); yet, little is known about the factors that lead to TG accumulation, the subsequent change in lipid metabolism, or the mechanisms that mediate changes for cell function.

TG uptake is enhanced by macrophagocytosis. Fluid and small, soluble antigens can be taken up by micro (up to ~0.1 μm)- and macrocytosis (from 0.5 to 3 μm). We previously showed that IGF-1 induces an increase in fluid-phase endocytosis (12). By uptake of low-molecular-weight FITC-dextran and electron microscopy, we revealed increased internalization by macrophagocytosis via caveoli and coated pits. Accumulation of Lucifer yellow has been shown to be a marker of macrophagocytosis (66), and now in Fig. 3A we show that IGF-1 also increases lipid uptake by macrophagocytosis. This is verified by the electron micrograph in Fig. 3B showing not only caveoli and endosomes of microphagocytosis but also, more specifically, many ruffles of macrophagosomes. The circular ruffles of macrophagosomes are heterogeneous in size, closed by purse-string movement, nonselectively enclosing bulk-fluid and macromolecules. These distinctive features of IGF-1-treated MC have not been previously described. These data indicate that IGF-1 induces lipid uptake by both micro- and macrophagocytosis.

Intracellular mechanisms of IGF-1-treated MC show that TG accumulation occurs not by increased biosynthesis but by decreased efflux. SREBP-1 plays an important role in TG biosynthesis, whereas SREBP-2 mediates cholesterol synthesis. As shown in Fig. 4, there was no change in the expression of SREBP-1 in IGF-1-treated MC to account for TG accumulation in these cells, reinforcing the concept that external lipids are endocytosed.

PPAR are nuclear hormone ligand-activated transcription factors, which play important roles in cholesterol and TG efflux through regulation of ABCA1 activity and other less well-defined transporters (50). PPARα is primarily found in the liver and promotes fatty acid oxidation to generate energy.

### Table 1. Cholesterol and triglyceride content of IGF-1-treated mesangial cells

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<th>Cholesterol Content</th>
<th>TG Content</th>
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<td></td>
<td>μg/sample*</td>
<td>μg/mg protein</td>
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<td></td>
<td>2.9±1.8</td>
<td>14.8±1.6</td>
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<tr>
<td>Control</td>
<td>8.4±1.6</td>
<td>22.4±4.9</td>
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<tr>
<td>IGF-1</td>
<td>12.7±2.5*</td>
<td>21.3±3.9</td>
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Values are means ± SD. MC, mesangial cells; TG, triglyceride. TG cholesterol and levels were assessed by mass spectrometry and a triglyceride kit. *P < 0.05, ANOVA.
for peripheral tissues (36). PPARγ potentiates adipocyte differentiation and modulates lipid storage and glucose homeostasis (36, 50). PPARδ activates TG uptake and efflux, has recently shown to regulate and mediate VLDL signaling in macrophages (19, 50), and exerts both pro- and anti-inflammatory activity (49). As shown in Fig. 5, MC express protein for all three PPAR isoforms. By density analysis, we found that although exposure of MC to IGF-1 for 7 days had no affect on expression of PPARα or γ, expression of PPARδ was markedly reduced. These findings have been repeated in triplicate on three separate occasions, with similar reductions in PPARδ expression. Given that PPARδ is the major determinant of long-chain fatty acid and TG efflux, it is likely that this change is a significant contributor to TG accumulation. This is a new and novel observation.

Differences in intracellular lipid composition influence MC function. In prior studies, we showed that MC migratory response to IGFBP-5 is affected by treatment with IGF-1, particularly following the addition of cholesteryl esters to the culture medium. At that time, we presumed that the absence of an effect without supplementation of the medium reflected a dose effect of cholesterol. In light of our findings described above showing that the lipid that accumulates in IGF-1-treated MC grown in standard medium is TG, we wondered what the effects of additional TG loading would be. In Table 2, we confirmed prior studies showing that chronic exposure to IGF-1 alone has no effect on IGFBP-5-mediated MC migration and that supplementation with cholesteryl esters blunts the migratory response. Consistent with our present data showing that MC exposed to IGF-1 in standard medium accumulate TG and have a normal migratory response, MC that were TG loaded by the addition of TG to the medium also had a normal migratory response. Phagocytosis and endocytosis, which were impaired by chronic IGF-1 treatment, were still impaired with supplemental TG. These data indicate that the nature of the lipid that accumulates determines the consequences to specific MC functions. In this case, cholesterol, but not TG, accumulation interferes with IGFBP-5-mediated migration. The data in Table 2 summarize the different functional modification of supplemental lipids in growth media and reaffirm that IGF-1-treated MC are TG loaded.

DISCUSSION

IGF-1-treated MC become lipid-laden foam cells, which are unable to phagocytose or contract in response to physiological stimuli (11, 12). Lipid uptake occurs through IGF-1-mediated enhancement of endocytosis and is not associated with in-
creases in surface concentration of lipid transporters. The type of lipid that accumulates can be influenced by the concentration of specific lipid moieties in the medium; however, TG preferentially accumulate in IGF-1-treated MC grown in standard serum-containing medium. We noted a 40% reduction in expression of PPARδ; thus TG may accumulate as a result of failure of PPARδ-dependent downregulation of the VLDL receptor and/or reduced TG efflux (19, 49). The role of TG in foam cell formation, the mechanisms of TG accumulation, the influence of TG on cellular lipid metabolism, and the functional consequences of TG to foam cells are just beginning to be examined (70). Data presented in this study complement our previous studies and show that the composition of the lipid that accumulates determines the effect on MC function (Table 2). Cholesterol accumulation interferes with phagocytosis, contraction, and IGFBP-5-mediated migration, whereas TG accumulation alters contraction but has no affect on migration.

Considerable data show that intracellular cholesterol content is tightly regulated and under normal circumstances synthesis and uptake are appropriately modulated to maintain stable cholesterol content. Current concepts of the pathogenesis of atherosclerosis (30) and progressive renal disease (74) indicate that inflammatory cytokines disrupt the feedback control mechanisms that maintain normal intracellular cholesterol levels, thereby allowing for intracellular lipid accumulation and foam cell formation. Oxidized lipoproteins have a propensity to accumulate in vessel walls through binding to matricular proteins (14, 18). Uptake of oxidized lipids stimulates macrophages (25, 59) and MC (15, 60) to release inflammatory cytokines, which perpetuate progression of the vascular disease and extracellular matrix accumulation (51). Until recently, TG were thought to play little role in this process; however, there is growing evidence that TG also contribute to the pathogenesis of vascular and renal diseases (34, 57). Additional studies are needed to define the mechanisms whereby TG accumulation interferes with MC function.

Little is known about changes that occur in lipid metabolism in the presence of TG accumulation. In macrophages, VSMC, and MC, cytokines such as TNF-α and IL-1β dysregulate expression of LDL and scavenger receptors, which contribute to lipid accumulation. In the present studies, we found no significant differences in receptor binding or degradation of LDL or AcLDL in MC chronically exposed to IGF-1. These observations are consistent with the unchanged levels of cholesterol and indicate that neither IGF-1 nor intracellular TG levels directly influence expression of these receptors. As we previously showed that IGF-1 enhances endocytosis and that cholesteryl esters can be taken up by these mechanisms and accumulate when the medium is supplemented with cholesteryl ester, it is possible that cholesterol metabolism is abnormal in that setting. Additional studies would be required to determine the role of IGF-1 in dysregulation of cholesterol homeostasis in the cholesterol-loaded MC.

In the present studies, we show that HMG-CoA reductase levels were equal in untreated and IGF-1-treated MC. Not only does this correspond to the normal levels of cholesterol in the cell, but it also indicates that neither IGF-1 nor TG modulate HMG-CoA reductase levels in MC. CD36 and SR-B1/CLA-1 are members of a family of receptors that contribute to cholesterol transport. SR-B1 receptors contribute to VLDL uptake; however, their major role is in cholesterol efflux, which is influenced by the external concentration of HDL (40). As prior studies had shown that IGF-1 downregulates expression of this

| Table 2. Functions of TG- and cholesterol-loaded MC |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Phagocytosis                    | Normal                          | Impaired                        | Impaired                        |
| Migration                       | Normal                          | Normal                          | Normal                          |
| Cholesterol content            | Normal                          | Elevated                        | Normal                          |
| TG content                      | Normal                          | Elevated                        | Elevated                        |

The table provides a summary of data obtained from various experiments provided herein, as well as those published previously (12). CE, cholesteryl esters. These studies indicate that cellular migration is most impaired by changes in intracellular cholesterol content, whereas the contractile response to angiotensin II is affected by TG accumulation.
receptor in fibroblasts (65) and HepG2 cells (16), we expected it to be reduced. No changes in protein content of this receptor were observed; however, we examined it after 7 days, and studies in fibroblasts and hepatic cells were done after short-term exposure to IGF-1. Additional studies would be needed to determine the appropriate expression of this receptor in TG-loaded cells; however, our results provide no evidence for increased expression of these receptors as a mechanism for TG accumulation in IGF-1-treated MC. We found that IGF-1 markedly decreased the protein content of CD36 in MC, a finding that has not been reported previously. CD36 was originally identified as a platelet membrane glycoprotein and as a receptor for thrombospondin 1, and it is the primary receptor that mediates uptake of oxidized LDL and long-chain fatty acids (reviewed in Refs. 22 and 62). Uptake of oxidized LDL by CD36 leads to induction of CD36 and further lipid uptake, thereby aggravating macrophage foam cell formation. Null mutations in CD36 actually protect against the development of atherosclerosis; thus it is of note that we found such a marked decrease in expression of this receptor in IGF-1-treated MC. Although additional studies are needed to understand the importance of this observation in the net effects on lipid metabolism in vivo, they exclude increased CD36 activity as a contributor to lipid accumulation in our studies.

In our previous studies and the ones described herein, we found that IGF-1 increased both micro- and macropinocytosis (11, 12), processes that have been implicated in foam cell formation (48). Micropinocytosis can occur in clathrin-associated, caveolin-associated, or other vesicles, which involve lipid-raft domains of the plasma membrane, whereas macropinocytosis is an actin-dependent process that involves larger regions of the cell membrane (48). Caveolin-1-rich caveolae can influence lipid uptake by fluid-phase endocytosis/transcytosis and receptor-mediated endocytosis, as in the case of CD36 (31). Lipid rafts lacking caveolin-1 also participate in long-chain fatty acid uptake, as many members of the fatty acid translocases, including SR-B1 and CD36, can attach to the rafts directly (68). Although caveolin-1 enhances CD36 function, the influence of caveolin-1 on SR-B1 function in endothelial cells and macrophages is less clear as studies have produced variable results (31). The role of caveolae in lipid handling by smooth muscle cells has been less well studied, but caveolin-1 appears to play little or no role in lipid uptake in these cells (31). Similar to vascular smooth muscle cells, caveolin-1 suppresses MC proliferation (32), but no studies have evaluated the role of caveolin-1 in MC handling of lipids or the effects of IGF-1 on caveolar-dependent lipid uptake. Understanding the pinocytic uptake of lipids is complicated, as different lipid moieties are processed differently by the rafts, with variable contributions by caveolin-1, and there are differences in different cell types and in the response to different stimuli and with different translocases. Additional studies are needed to specifically define the role of caveolin-1 and lipid rafts in mediating IGF-1-induced changes in cholesterol and TG uptake in MC.

PPARs are fatty acid receptors that play important roles in fatty acid catabolism, lipid storage, adipocyte differentiation, inflammation, and glucose homeostasis (50). PPARα stimulates hepatic fatty acid oxidation and ketogenesis. PPARγ responds to oxidized LDL and promotes cholesterol efflux through activation of the ABCA-1 transporter (20). We found no evidence through measurements of PPARα, PPARγ, or ABCA-1 that they played a role in the TG accumulation in IGF-1-treated MC. As ABCA-1 activity is very low in MC, other transporters may be more important to lipid efflux in MC. In a recent report, Davies et al. (24) demonstrated that adipogenic conditions induce VSMC to enhance TG synthesis, leading to lipid accumulation in intracellular vacuoles as a result of liver X receptor (LXR)/SREBP1c activation. As VSMC in atherosclerotic plaques lack a scavenger receptor phenotype but express adipocyte markers (e.g., fatty acid synthase, SREBP-1, LXRα, and adipin), this would suggest that they become foam cells by mechanisms that differ from those of macrophage foam cells. Similar to their reports, we found no evidence that IGF-1-treated MC primarily developed lipid or cholesterol accumulation via enhanced expression and activity of LDL or scavenger receptors. Although we did not examine LXRα function directly, we did not find an increase in the expression of SREBP1c or ABCA1 as evidence of LXRα activity (24, 82); thus IGF-1 might mediate foam cell formation by yet another mechanism. Although the effects of PPARα and PPARγ involve LXRα in the development of macrophage foam cells, PPARδ does not (54). The reduced expression of PPARδ may contribute to TG accumulation because of reduced fatty acid oxidation, as occurs in cardiac muscle of animals with targeted deletion of PPARδ (21). Furthermore, IGF-1 increases gene expression for fatty acid synthase in adipose tissue (78). Effects in smooth muscle cells have not been examined.

PPARδ is ubiquitously expressed but is most abundant in muscles, which rely heavily on fatty acids for energy (50). PPARδ functions primarily as a sensor for TG in the VLDL particle and to promote fatty acid oxidation (19, 49). TG participates in macrophage differentiation by PPARδ-dependent induction of transcription of adipocyte differentiation-related protein (19). In PPARδ null cells, the VLDL receptor gene is induced, indicating that activation of PPARδ reduces TG uptake by this receptor. Furthermore, TG loading down-regulates the VLDL receptor in a PPARδ-dependent fashion (19). Thus our findings that PPARδ expression is significantly decreased in IGF-1-treated MC indicate that TG accumulation may have occurred as a result of impaired suppression of VLDL receptor expression and reduced TG efflux. These alterations in PPARδ expression are particularly interesting given the known increased expression of IGF-1 in MC from animals with type 2 diabetes (17, 28), the elevated levels of TG that are typical of dyslipidemia in diabetes, and recent studies showing the importance of this receptor in mediating insulin resistance (80). Furthermore, two fatty acids common to TG, oleate and linolate, enhance the growth-promoting effects of IGF-1 in SMC (8), which have been implicated in vascular (76) and renal disease progression (27, 57). Additional studies are needed to define the mechanisms responsible for IGF-1-mediated alterations in TG metabolism and PPARδ-mediated effects.

Considerable evidence implicates lipids in the progression of renal diseases, including diabetic nephropathy (reviewed in Refs. 44 and 1). Hypercholesterolemia accelerates the rate of progression of kidney disease and leads to macrophage infiltration and foam cell formation in rats (37). Genetic abnormal-
ities in VLDL handling and apoE lead to MC foam cell formation and progressive glomerulosclerosis (75). Obese Zucker rats have significant glomerulosclerosis, which is ameliorated by correction of hypertriglyceridemia (42). In nephrotic syndrome, reduced VLDL clearance enhances interstitial injury (64). The potential role of IGF-1 in glomerulosclerosis and progression of diabetic nephropathy is well established (17, 26). Given the increased expression of IGF-1 by MC in diabetes mellitus (28), and the TG-rich plasma typical of uncontrolled diabetes mellitus, our findings have important implications for understanding the pathogenesis of diabetic nephropathy. Definition of the mechanisms of lipid accumulation in MC may lead to interventions that could modify the outcome of diseases such as diabetic nephropathy and focal and segmental glomerulosclerosis in which mesangial foam cells are prominent (58, 63).

In summary, IGF-1 leads to an increase in MC lipid accumulation primarily as a result of increased endocytosis (11, 12). The lipid moiety that accumulates, in part, is dependent on the concentration of lipids in the medium. When the medium is not supplemented with specific lipids, IGF-1-treated MC preferentially accumulate TG. Our findings that these MC have reduced expression of PPARÎ¸ suggest that chronic exposure to IGF-1 might limit the normal TG-mediated downregulation of the VLDL receptor and reduce TG efflux, both of which would favor TG accumulation. The specificity of these effects was further supported by the absence of alterations in HMG-CoA reductase, LDLR, or scavenger receptors that might have increased lipid accumulation. The abnormal function of mesangial foam cells is dependent on the lipid moiety that accumulates as cholesterol-loaded cells fail to contract, phagocytose, or migrate normally, whereas TG-loaded cells migrate normally in response to IGFFBP-5 but are unable to contract in response to angiotensin II. Additional studies are needed to further define the specific effects of IGF-1 on fatty acid synthase, VLDL receptors, and PPARÎ¸ expression.

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