A Protective Role for Kidney Apolipoprotein E
REGULATION OF MESANGIAL CELL PROLIFERATION AND MATRIX EXPANSION*

Guangping Chen‡, Latha Paka‡, Yuko Kako§, Pravin Singhal¶, Wenlan Duan‡, and Sivaram Pillarisetti‡‡

Received for publication, May 29, 2001, and in revised form, September 27, 2001
Published, JBC Papers in Press, September 28, 2001, DOI 10.1074/jbc.M104879200

Mesangial expansion is a key feature in the pathogenesis of numerous renal diseases involving the glomerulus. Studies indicate that mutations in apolipoprotein E (apoE) might independently contribute to kidney dysfunction. Although the role of apoE as an atheroprotective molecule is well established, its role in kidney is unclear. In this study, we sought to explore whether apoE has a protective function in kidney. Northern blotting and reverse transcriptase-polymerase chain reaction showed apoE expression in kidney, and mesangial cell is a major source of apoE in kidney. In the kidneys of 14–16-month-old apoE-null mice, hematoxylin-eosin (HE) staining revealed increased mesangial cell proliferation and matrix formation compared with wild type mice or apoE-overexpressing mice, which have elevated plasma cholesterol and triglycerides. These data suggest that lack of apoE, rather than hyperlipidemia, contributes to increased mesangial expansion. We isolated mesangial cells from mouse kidney and determined the effect of apoE on cell growth. ApoE (E3, 10 μg/ml) completely inhibited serum, platelet-derived growth factor (10 ng/ml), as well as low density lipoprotein-induced mesangial cell proliferation. Among the three isoforms, E3 was found to be most effective in inhibiting mesangial cell proliferation. ApoE did not show any cytotoxic effect, and moreover, inhibited mesangial cell apoptosis induced by oxidized low density lipoprotein. These data suggest that apoE regulates growth as well as survival of mesangial cells. We previously showed that apoE induces matrix heparan sulfate proteoglycan (HSPG) in vascular cells, which has an antiproliferative effect. Similarly, apoE induced the mesangial matrix HSPG. Perlecan is the major HSPG of mesangial matrix and subendothelial space, and consistent with this, blockade of perlecan reversed the antiproliferative effect of apoE. Immunohistochemistry revealed reduced staining of perlecan in kidney from apoE-null mice. Because the loss of anionic HSPG in the basement membrane and mesangial matrix is associated with disruption of filtration barrier, these data suggest a novel role for kidney apoE in preserving the filtration barrier. In summary, apoE has a protective function in kidney as an autocrine regulator of mesangial expansion and kidney function.

Many forms of renal disease that progress to renal failure are characterized by mesangial cell proliferation and more prominently accumulation of mesangial matrix (1–3). Factors that control mesangial cell function include cytokines and growth mediators, matrix components such as heparan sulfate proteoglycans (HSPG),¹ and interactions with other cells such as the endothelial and epithelial cells (2). Besides mesangial expansion, a prominent feature of glomerulosclerosis and nephropathy is decreased matrix HSPG (4–7). Because HSPG is a key regulator of mesangial growth (8), it is conceivable that the decreased HSPG in part contributes to increased mesangial proliferation. Understanding the regulation of mesangial proliferation is important for the design of therapeutic strategies to alleviate or arrest proliferative glomerular disease.

ApoE is a major protein component of plasma lipoproteins and plays a key role in lipoprotein clearance (9, 10). A lack of apoE results in hyperlipidemia and in the development of atherosclerosis (11, 12). Several studies now show that apoE can be atheroprotective even in a hyperlipidemia setting (13–15). This atheroprotective effect of apoE could be because of any of the recently identified novel functions, prominent among these, their ability to inhibit smooth muscle cell proliferation and increase vascular HSPG (16–18). Atherosclerosis and glomerulosclerosis have several common features including loss of HSPG, endothelial dysfunction, and unregulated cell proliferation. Thus, kidney apoE could be protective against the development of glomerulosclerosis. Although apoE expression was shown in kidney (19, 20), its role in the kidney is not known. In the current study, we explored the contribution of apoE to kidney function. Our studies show that apoE regulates mesangial expansion and HSPG levels, and a lack of apoE contributes to increased mesangial proliferation and matrix accumulation.

MATERIALS AND METHODS

Morphological Study—Kidneys used in these studies were obtained from 14–16-month-old mice (Jackson Laboratories) of the following strains: C57BL/6J mice (n = 4), apoE-null mice on C57BL/6 background (n = 6), and human apoB transgenic (HuBTg) mice (n = 6). HuBTg mice had a mixed genetic background of predominantly C57BL/6J (>75%) and FVB/N strains (21) and were fed on a western-type diet. The cholesterol and triglyceride levels of these mice are as follows: cholesterol (mg/dl) = 70 ± 23 (WT), 315 ± 45 (apoE-null), and 260 ± 27 (HuBTg) and triglycerides (mg/dl) = 64 ± 29 (WT), 91 ± 30 (apoE-null), and 140 ± 38 (HuBTg). Kidney tissues were cut into small pieces, fixed in 10% formalin, embedded in paraffin, and 4-μm sections were stained.

¹ The abbreviations used are: HSPG, heparan sulfate proteoglycan; HuBTg, human apoB transgenic mice; WT, wild type; LDL, low density lipoprotein; FCS, fetal calf serum; PBS, phosphate-buffered saline; Ox-LDL, oxidized LDL; TUNEL, terminal deoxynucleotidyl transferase end-labeling; RT-PCR, reverse transcriptase-polymerase chain reaction; PDGF, platelet-derived growth factor; LpL, lipoprotein lipase.
with HE and examined under microscope. At least 20 glomeruli for each tissue were evaluated for cell count. The extracellular matrix expansion was analyzed and quantitated as described previously (22).

**Cell Culture**—Mesangial cells from C57BL/6 mice were isolated and cultured as described previously (23). The cells were grown in RPMI 1640 medium (Life Technologies, Inc.) supplemented with insulin-transferrin-selenium selenite media (Sigma) and 10% fetal calf serum (FCS). Cells used in experiments were from passages 5–10. Glomerular epithelial cells were maintained in 10% FCS-RPMI 1640 medium. Tubular epithelial cell lines were obtained from American Type Culture Collection (Madin-Darby canine kidney) and grown in Dulbecco's modified Eagle's medium containing 10% FCS.

**Lipoproteins and Apolipoproteins**—LDL (d < 1.063) was isolated from fresh human plasma in the presence of EDTA by ultracentrifugation and dialyzed against phosphate-buffered saline (PBS) containing 0.5 mM EDTA. Oxidized LDL (OxLDL) was prepared by dialyzing the LDL with 10 μM copper sulfate for 24 h at room temperature as described previously (24). Apolipoprotein E isoforms, E2, E3, and E4 were obtained from Calbiochem. Unless indicated otherwise, apoE3 was used in all experiments.

**Cell Proliferation**—Cell proliferation was assessed by [3H]thymidine incorporation as described previously (18). Mesangial cells were seeded at a density of 2 × 10^4/well in a 48-well plate, and experiments were performed the following day (40–50% confluence). Cells were treated with serum-free medium for 24 h followed by serum medium with or without various agents for 24 h. The cells were then labeled with [3H]thymidine for 6 h, and the radioactivity incorporated into DNA was determined by a scintillation counter.

**Northern Blot Analysis**—Total RNA was extracted from kidneys of C57BL/6 and apoE-null mice and used for Northern analysis. Plasmid (pJS381) containing apoE clone was kindly provided by Dr. Jonathan Smith (Rockefeller University, NY). A 1053-bp apoE fragment was subjected to RT-PCR using the following primers: forward 5′—GCCATCAGCGGACAAGAT-3′ and reverse 5′—CTTCTGCACCTGCTCAGAC-3′. The predicted polymerase chain reaction product using these primers is a 261-bp fragment.

**Immunohistochemical Detection of Perlecan**—Rat monoclonal anti-perlecan antibody was purchased from Neo Markers Inc., and conjugated with fluorescein by QuickTag FITC conjugation kit (Roche Molecular Biochemicals) according to the recommended procedures. Kidneys from WT and apoE-null mice were embedded in OCT and snap frozen in liquid nitrogen. 6 μm of frozen sections were fixed in cold acetone, rinsed in PBS, and incubated in PBS containing 1% bovine serum albumin for 20 min. The sections were then incubated with FITC-conjugated perlecan antibody (1:100 dilution) followed by detection by fluorescence microscope. For negative control, slides were preincubated with 5× non-FITC-labeled perlecan antibody.

**RESULTS**

**Lack of ApoE Results in Mesangial Expansion**—ApoE is known to be antiproliferative in smooth muscle cells (17, 18). Because the mesangial cell is phenotypically similar to the smooth muscle cell, we first investigated whether a lack of apoE results in altered mesangial morphology in kidney. ApoE-null mice have both high plasma cholesterol and triglycerides, and in order to distinguish the effects of apoE from those of hyperlipidemia, we also examined HuBtg mice. These mice are transgenic for human apoB and are a distinct model for hyperlipidemia. Kidney sections from wild type mice (WT, C57BL/6), apoE-null mice, and HuBtg mice were examined by HE staining (Fig. 1). Significantly increased mesangial proliferation was seen in kidneys from apoE-null mice compared with kidneys from WT mice (p < 0.01) or HuBtg mice (p < 0.05). The most striking change in apoE-null mice was the matrix hyperproduction in comparison with WT and apoB mice (p < 0.01). Interestingly, the gross kidney of HuBtg mouse is much bigger than that of WT and apoE-null mice, and enlarged glomerulus was observed with mild cell proliferation, which was significantly different from that of WT mouse. However, there was no prominent matrix expansion in HuBtg mice (Fig. 1, Table I). These data suggest that a lack of apoE contributes to mesangial cell proliferation and matrix expansion in the kidney.
extracellular matrix expression as described under ‘Materials and Methods.’ At least 20 glomeruli for each tissue were evaluated for cell count and were stained with hematoxylin-eosin and examined under microscope.

LDL (25 μg/ml) and LDL (50 μg/ml) com-pletely inhibited serum-stimulated cell growth (Fig. 3B). The addition of apoE completely blocked this effect. These data show that the antiproliferative effect of apoE is specific for mesangial cells.

**ApoE Inhibits Platelet-derived Growth Factor (PDGF) and LDL-stimulated Cell Proliferation**—Although the trigger for mesangial proliferation is not clear, studies have identified several possible candidates including PDGF, interleukin-6, and LDL (25–27). We tested whether apoE can suppress the growth-promoting effects of these agents. Incubation of mesangial cells with PDGF (10 ng/ml) resulted in a 2–2.5-fold increase in [3H]thymidine incorporation into DNA (Fig. 4A). Similarly, LDL (20 μg/ml) increased [3H]thymidine incorporation into DNA by ~2-fold (Fig. 4B). The addition of apoE completely reversed this effect. These data show that the antiproliferative effects of apoE extend to a variety of mesangial growth inducers.

We next tested whether the antiproliferative effects of apoE are isoform-specific. At similar concentrations, apoE3 was found to be most effective (~50%) in inhibiting mesangial cell proliferation (Fig. 4C). ApoE4 also showed significant inhibition (~30%, p < 0.05). ApoE2 showed moderate effect (~19%) on mesangial proliferation.

**ApoE Inhibit OxLDL-induced Apoptosis**—Several antiproliferative agents can also induce cell apoptosis. To rule out the possibility that the antiproliferative effect of apoE is because of apoptosis, we determined the effect of apoE on OxLDL-induced apoptosis. Confluent mesangial cells were incubated with Ox-LDL (50 μg/ml) and LDL (50 μg/ml) in the presence or absence of apoE for 48 h. Under these conditions only Ox-LDL but not LDL induced mesangial cell apoptosis as determined by TUNEL staining. Ox-LDL-induced cell apoptosis was completely blocked by apoE, and virtually no apoptotic cells were observed (Fig. 5). These data show that apoE plays a key role in both the regulation of proliferation as well as the survival of mesangial cells.

**ApoE Induces HSPG in Mesangial Cells**—The mechanism by which apoE inhibits mesangial cell proliferation is not clear. Heparan sulfate and HSPG are potent inhibitors of mesangial proliferation, and we previously showed that apoE induced HSPG in smooth muscle cells (18). We next tested whether apoE inhibits mesangial cell proliferation by increasing HSPG. Subconfluent monolayers of mesangial cells were incubated with apoE, and cellular HSPG were expressed as a ratio to cell number. ApoE3 increased HSPG to cell ratio by ~2-fold (Fig. 6A). E2 and E4 were less effective, and this may in part explain their effects on mesangial cell proliferation (Fig. 4C). The kidney, like other tissues, contain different HSPGs including syndecan and glypican (cell surface) and perlecan (extracellular

### Table I

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Morphological Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>WT (n = 4)</td>
<td>35.1 ± 0.1</td>
</tr>
<tr>
<td>ApoE-null (n = 6)</td>
<td>59.8 ± 14.2^a,b</td>
</tr>
<tr>
<td>ApoB-Tg (n = 4)</td>
<td>42.9 ± 2.5^c</td>
</tr>
</tbody>
</table>

^a p < 0.01 versus WT.  
^b p < 0.05 versus ApoB-Tg.  
^c p < 0.01 versus WT.  
^d p < 0.05 versus ApoB-Tg.  
^e p < 0.05 versus WT.  
^f p < 0.01 versus ApoB-Tg.

Mesangial Cell Is a Source of ApoE in Kidney—To correlate apoE expression to mesangial expansion, we determined apoE expression in kidney and kidney cell types. Northern blot analysis of total kidney RNA showed apoE expression in kidneys of WT but not of apoE-null mice (Fig. 2A). RT-PCR of total RNA isolated from different kidney cell types revealed a strong expression of apoE in mesangial cells (Fig. 2B). A weak band was also seen in glomerular epithelial cells. Both tubular epithelial cells and endothelial cells were negative for apoE expression. The results suggest that mesangial cell is a major contributor of kidney apoE.

**ApoE Inhibits Mesangial Cell Proliferation**—To determine the effects of apoE on cell proliferation, we isolated mesangial cells from wild type mouse kidney and tested whether apoE inhibits mesangial cell proliferation. ApoE (10 μg/ml) completely inhibited serum-stimulated cell growth (Fig. 3A). At a similar dose, apoE did not affect glomerular epithelial cell proliferation (Fig. 3B). We previously reported that apoE does not inhibit endothelial proliferation (18). Thus, the antiproliferative effect of apoE is specific for mesangial cells.

**ApoE Inhibit OxLDL-induced Apoptosis**—Several antiproliferative agents can also induce cell apoptosis. To rule out the possibility that the antiproliferative effect of apoE is because of apoptosis, we determined the effect of apoE on OxLDL-induced apoptosis. Confluent mesangial cells were incubated with Ox-LDL (50 μg/ml) and LDL (50 μg/ml) in the presence or absence of apoE for 48 h. Under these conditions only Ox-LDL but not LDL induced mesangial cell apoptosis as determined by TUNEL staining. Ox-LDL-induced cell apoptosis was completely blocked by apoE, and virtually no apoptotic cells were observed (Fig. 5). These data show that apoE plays a key role in both the regulation of proliferation as well as the survival of mesangial cells.

**ApoE Induces HSPG in Mesangial Cells**—The mechanism by which apoE inhibits mesangial cell proliferation is not clear. Heparan sulfate and HSPG are potent inhibitors of mesangial proliferation, and we previously showed that apoE induced HSPG in smooth muscle cells (18). We next tested whether apoE inhibits mesangial cell proliferation by increasing HSPG. Subconfluent monolayers of mesangial cells were incubated with apoE, and cellular HSPG were expressed as a ratio to cell number. ApoE3 increased HSPG to cell ratio by ~2-fold (Fig. 6A). E2 and E4 were less effective, and this may in part explain their effects on mesangial cell proliferation (Fig. 4C). The kidney, like other tissues, contain different HSPGs including syndecan and glypican (cell surface) and perlecan (extracellular
matrix). ApoE treatment resulted in the induction of HSPG in all pools (Fig. 6B). Perlecan is the major HSPG in the glomerular basement membrane as well as mesangial matrix and may contribute to the regulation of mesangial cell proliferation (28, 29). We next tested whether the induction of perlecan mediates the antiproliferative effect of apoE (Fig. 6C). Perlecan antibody but not mouse IgG reversed the antiproliferative effect of apoE, suggesting that perlecan contributes to the antiproliferative effect of apoE.

**ApoE-null Mice Have Reduced Perlecan in Kidney**—We next determined whether the absence of apoE results in altered perlecan expression in kidney. Immunohistochemical analysis of perlecan in WT and apoE-null mice revealed significantly decreased staining in apoE-null mice (Fig. 7). These data suggest that the morphological changes seen in apoE-null kidney may in part be due to decreased perlecan.

**DISCUSSION**

Our data for the first time identify a protective role for apoE in preventing a proliferative phenotype in kidney. Mice that are deficient in apoE have both increased proliferation as well as matrix overproduction, a hallmark of kidney pathogenesis. Several human studies identified an association between apoE polymorphism and nephropathy (30–33). A recent large case-controlled study with 223 subjects showed a 3.1-fold increase in the risk of diabetic nephropathy in subjects carrying E2 allele of apoE (34). Although the molecular mechanisms underlying this increased risk are unclear, it is often thought to be because of dyslipidemia, in particular, to increased triglycerides. However, our data on mesangial matrix accumulation in HuBTg mice suggest lipid-independent effects on mesangial expansion.
in apoE-null mice(51,121),(970,935). HuBTg mice, despite having hypercholesterolemia and hypertriglyceridemia, clearly did not show changes in mesangial morphology that are seen in apoE-null mice. ApoE2, as we previously showed, has no significant antiproliferative effect on smooth muscle cells (18). Thus, it is conceivable that the increased risk of nephropathy in E2 subjects is in part attributed to the inability of apoE2 to regulate mesangial expansion.

Apart from liver, studies showed that kidney is a major source of apoE (19, 20). Our Northern data confirmed this finding and showed an intense band of apoE in WT mice but not in apoE-null mice. Although kidney expression of apoE has been clearly demonstrated, the source of apoE in kidney is not known. Early studies showed relatively greater amounts of apoE synthesis in the cortex compared with medulla (20). RT-PCR analysis of different kidney cell types in the current study showed that mesangial cells are a major source of apoE expression.

Mesangial cell proliferation and matrix overproduction are the predominant pathological features of many forms of glomerulonephritis, such as IgA nephropathy, lupus nephropathy, and diabetic nephropathy and frequently precedes the increase of extracellular matrix in the mesangium and the development of glomerulosclerosis (2, 3). When exposed to injurious stimuli such as hyperglycemia, glycated proteins, or oxidants, the mesangial cell responds by cellular proliferation and matrix synthesis. A key example of this is the pro-sclerotic cytokine transforming growth factor-β, which is induced by many diabetic stimuli (35, 36). The up-regulation of transforming growth factor-β may be a necessary event in the repair process and repair of damaged matrix. However, a loss of proper regulation of this repair process may ultimately progress to the development of glomerulosclerosis. Expression of apoE in mesangial cells may serve as an autocrine regulator of such uncontrolled mesangial expansion.

Our data clearly establish apoE as an antiproliferative molecule for mesangial cells. It inhibited mesangial proliferation induced by different stimuli including growth factors and lipids. This finding is consistent with the observed apoE effects on smooth muscle cells in which the proliferative effects of serum, PDGF, OxLDL, and lyssolecithin were inhibited by apoE (18). The antiproliferative effects of apoE, however, were not attributed to the induction of apoptosis. Instead, apoE was found to be antiproliferative and prevented oxidant-induced apoptosis. Proliferation as well as apoptosis of mesangial cells has been shown in glomerular diseases (37, 38). Both processes may regulate the cellular content of the mesangium by closely influencing each other. Thus, apoE may offer a dual protection against proliferation and apoptosis.

Increasing matrix HSPG (perlecan) is a probable mechanism by which apoE can be antiproliferative to mesangial cells. Consistent with previous studies with smooth muscle cells (18), perlecan antibody significantly blocked the antiproliferative effect of apoE on mesangial cells. Obunike et al. (39) previously showed that lipoprotein lipase (LpL), like apoE, can induce proteoglycans in other cell types. However, it is not known whether this increase is in HSPG or in perlecan. Recent studies (40, 41) from two different laboratories showed that LpL, in contrast to apoE, stimulates proliferation of smooth muscle and mesangial cells. The antiproliferative effects of apoE on smooth muscle cells appear to require LpL activity. It is conceivable that residual serum lipids (lipoprotein-derived) or cellular lipids are hydrolyzed by LpL leading to the generation of fatty acids or lysolipids, both of which can activate protein kinase C, a mitogenic signal. Not surprisingly, these authors found that protein kinase C inhibitors completely blocked LpL-induced proliferation.

The ability of ApoE to increase mesangial (current study) and endothelial HSPG (16) may have other implications. Apart from being antiproliferative to mesangial cells, HSPG plays an important role in barrier function (4–6). Increasing evidence supports the hypothesis that a loss of heparan sulfate may play a pathophysiological role in the development of diabetic vascular complications. Our data show decreased staining for perlecan in apoE-null mice. Perlecan, the major HSPG of the basement membrane and mesangial matrix, plays an important role in the assembly and structure of the basement membrane and regulation of basement membrane permeability (42). In diabetic patients with mesangial cell expansion and clinical nephropathy, a negative correlation was observed among the number of anionic sites representing HSPG in the glomerular basement membrane, and urinary albumin secretion was also observed (6). Heparin, which increases HSPG, has been shown to reduce albuminuria in patients with incipient diabetic nephropathy (43). Thus, apoE by virtue of its ability to induce perlecan may help preserve barrier function and inhibit the hyperpermeability associated with kidney dysfunction.

To determine whether morphological changes seen in apoE-null mice correlate with permeability changes, we determined urine albumin in wild type and apoE-null mice. Serum creatinine levels were similar in wild type and apoE-null mice (27.27 ± 6.53 μmol/liter versus 28.32 ± 5.01 μmol/liter). Although it did not reach statistical significance, urinary albumin was elevated in apoE-null mice (11.7 ± 0.24 mg/mmol creatinine in apoE-null mice compared with 1.38 ± 0.1 mg/mmol creatinine in wild type mice, p = 0.09, n = 4). Further studies requiring large number of animals are needed to conclusively show kidney dysfunction in apoE-null mice.

Several non-traditional and novel functions of apoE are only beginning to be realized (42). Data presented here add to the growing list of protective effects that apoE possesses. In addition to its antiproliferative and HSPG-inducing effects, apoE
can also be an antioxidant (44). Because oxidant stress is a major player in nephropathy, it will be of interest to see whether overexpression of apoE offers protection against the development of nephropathy and kidney dysfunction.

Acknowledgments—We thank Drs. Rick Timmer and Uday Saxena for comments on the manuscript.

REFERENCES

A Protective Role for Kidney Apolipoprotein E: REGULATION OF MESANGIAL CELL PROLIFERATION AND MATRIX EXPANSION
Guangping Chen, Latha Paka, Yuko Kako, Pravin Singhal, Wenlan Duan and Sivaram Pillarisetti

doi: 10.1074/jbc.M104879200 originally published online September 28, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M104879200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 44 references, 15 of which can be accessed free at http://www.jbc.org/content/276/52/49142.full.html#ref-list-1