Macrophage Expression of Peroxisome Proliferator–Activated Receptor–α Reduces Atherosclerosis in Low-Density Lipoprotein Receptor–Deficient Mice

Vladimir R. Babaev, PhD; Hiroyuki Ishiguro, MD; Lei Ding, BA; Patricia G. Yancey, PhD; Dwayne E. Dove, MD, PhD; William J. Kovacs, MD; Clay F. Semenkovich, MD; Sergio Fazio, MD, PhD; MacRae F. Linton, MD

Background—The peroxisome proliferator–activated receptor-α (PPARα) plays important roles in lipid metabolism, inflammation, and atherosclerosis. PPARα ligands have been shown to reduce cardiovascular events in high-risk subjects. PPARα expression by arterial cells, including macrophages, may exert local antiatherogenic effects independent of plasma lipid changes.

Methods and Results—To examine the contribution of PPARα expression by bone marrow–derived cells in atherosclerosis, male and female low-density lipoprotein receptor–deficient (LDLR–/–) mice were reconstituted with bone marrow from PPARα–/– or PPARα+/- mice and challenged with a high-fat diet. Although serum lipids and lipoprotein profiles did not differ between the groups, the size of atherosclerotic lesions in the distal aorta of male and female PPARα–/–→LDLR–/– mice was significantly increased (44% and 46%, respectively) compared with controls. Male PPARα–/–→LDLR–/– mice also had larger (44%) atherosclerotic lesions in the proximal aorta than male PPARα+/-→LDLR–/– mice. Peritoneal macrophages from PPARα–/– mice had increased uptake of oxidized LDL and decreased cholesterol efflux. PPARα–/– macrophages had lower levels of scavenger receptor B type I and ABCA1 protein expression and an accelerated response of nuclear factor-κB–regulated inflammatory genes. A laser capture microdissection analysis verified suppressed scavenger receptor B type I and increased nuclear factor-κB gene expression levels in vivo in atherosclerotic lesions of PPARα–/–→LDLR–/– mice compared with the lesions of control PPARα+/-→LDLR–/– mice.

Conclusions—These data demonstrate that PPARα expression by macrophages has antiatherogenic effects via modulation of cell cholesterol trafficking and inflammatory activity. (Circulation. 2007;116:1404-1412.)

Key Words: atherosclerosis ■ macrophages ■ proteins ■ receptors

Peroxisome proliferator–activated receptor-alpha (PPARα) is a ligand-activated transcription factor that plays an important role in lipid metabolism and inflammation. PPARα regulates a number of genes involved in inflammation and the metabolism of cellular lipids, plasma lipoproteins, and glucose.1,2

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PPARα ligands, such as fibric acid derivatives, have widespread clinical application in the treatment of dyslipidemia.3 They have been shown to reduce the progression of coronary disease in angiographic trials4,5 and to reduce the incidence of cardiovascular events in outcome studies.6–8 The protective effects of PPARα agonists on atherosclerosis may be due to their impact on both inflammation and cholesterol homeostasis in the artery wall.9 It was recently shown that a PPARα gene polymorphism significantly affects progression of atherosclerosis and ischemic heart disease without causing changes in plasma cholesterol levels.10

Studies of the role of PPARα in atherosclerosis in murine models using PPARα agonists and knockout mice have yielded conflicting results. Tordjman et al11 showed that mice with double deletion of the PPARα and apolipoprotein E (apoE) genes had higher levels of atherogenic lipoproteins but less aortic atherosclerosis, improved insulin sensitivity, and significantly lower levels of blood pressure than PPARα+/−/apoE−/− litter mates, which suggests that systemic PPARα deficiency has complex effects on atherogenesis. In one study, treatment of apoE−/− mice with PPARα agonists severely aggravated their hypercholesterolemia and accelerated atherosclerotic development.12 In contrast, several reports have demonstrated that treatment with PPARα agonists

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improved lipoprotein metabolism and reduced atherosclerosis in low-density lipoprotein receptor (LDLR)–deficient (LDLR−/−), apolipoprotein E–deficient (apoE−/−), and human apoE2–expressing (instead of mouse apoE–expressing) mice. In addition, PPARα agonists have been reported to reduce cholesterol esterification in macrophages. Taken together, these data demonstrate that the effects of PPARα on atherogenesis are complex and may vary in different conditions.

In atherosclerotic lesions, PPARα is expressed by endothelial cells, smooth muscle cells, and monocyte/macrophages. To dissect the role of macrophage PPARα expression in early atherosclerosis, we generated female and male LDLR−/− mice chimeric for PPARα−/− macrophages. When fed the Western diet, recipient mice reconstituted with PPARα−/− bone marrow–derived cells developed significantly larger atherosclerotic lesions than control mice with wild-type marrow. Peritoneal macrophages isolated from PPARα−/− mice had slightly decreased cholesterol efflux, increased uptake of oxidized LDL, and accelerated response of proinflammatory genes. These data together demonstrate that PPARα expression by macrophages has antiatherogenic effects via modulation of cell cholesterol trafficking and inflammatory activity.

Methods

Animal Procedures

Mice with targeted disruption of the PPARα gene were crossed with LDLR−/− mice onto the C57BL/6 background (fifth backcross). Recipient LDLR−/− mice on the C57BL/6 background were purchased from Jackson Laboratories Inc (Bar Harbor, Me). All mice were maintained on a rodent chow diet (PMI No. 5010, St. Louis, Mo) or the Western-type diet consisting of 21% fat and 0.15% cholesterol and may vary in different conditions.

Quantitative Real-Time Polymerase Chain Reaction

Peritoneal macrophages were treated with RPMI media containing 5% fetal lipid-deficient serum (Intracel) and 2 PPARα agonists, WY14643 (Bioul Res Lab Inc, Plymouth Meeting, Pa) (2 μmol/L) or GW7647 (Calbiochem, San Diego, Calif) (600 nmol/L), for 24 hours. In addition, some cells were treated with lipopolysaccharide (LPS; 50 ng/mL; Sigma, St. Louis, Mo) for 5 hours. Total RNA was isolated from peritoneal macrophages with Trizol reagent (Invitrogen, Carlsbad, Calif), purified by RNasey kit (Qiagen, Valencia, Calif). Relative quantitation of the target mRNA was performed on the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, Calif) and normalized with 18S RNA as an endogenous control as described previously. All probes were purchased from Applied Biosystems.

Western Blotting

Proteins were extracted from macrophages in the presence of protease inhibitor (Sigma) and resolved (100 μg/well) in NuPAGE 4% to 12% Bis-Tris gel (Invitrogen) and then blotted onto nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ). Proteins were detected with rabbit antibodies to scavenger receptor B type I (SR-BI), ABCA1 (both from Novus Biologicals, Littleton, Colo), and β-actin (Abcam, Inc, Cambridge, Mass). Immunoreactive bands were visualized with horse-radish peroxidase–conjugated goat anti-rabbit antibodies (Upstate Cell Signaling, Lake Placid, NY) and quantified by densitometry.

Laser Capture Microdissection

Cryosections (7 μm) from the proximal aorta were used for laser capture microdissection (LCM) as reported previously. Serial sections were stained with specific anti-macrophage antibody, MOMA-2 (Serotec, Raleigh, NC). Macrophage-rich atherosclerotic lesions were captured (PixCell II LCM system; Arcturus, Mountain View, Calif) from the proximal aorta of mice reconstituted with wild-type and PPARα−/− marrow (n=5 in each group). Approximately 500 shots were performed with a 15-μm diameter infrared laser beam to obtain approximately 200 to 1500 cells of interest (CapSure LCM caps; Arcturus Biosciences). Total mRNA concentration was measured by a NanoDrop ND-1000 spectrophotometer (Wilmington, Del), and its purity was analyzed by an Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, Calif) with the DNA markers. RNA was isolated from captured tissues and amplified (PicoPure RNA isolation and amplification kits, Arcturus).

Statistical Analysis

The statistical differences in mean serum lipids and aortic lesion areas between the groups were determined with SigmaStat version 2 software (SPSS Inc, Chicago, Ill) by Student t test and the Mann-Whitney rank sum test, respectively.

The authors had full access to and take full responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Macrophage PPARα Deficiency and Serum Lipids

To study the role of PPARα expression by bone marrow–derived cells in atherosclerosis, 2 main sets of experiments were studied.

The cells were incubated with human HDL (100 μg/mL) for 24 hours, then radioactivity was measured in media, and lipids were extracted with hexane-isopropanol. Cholesterol efflux was expressed as the percentage of the counts in the cellular lysate. In a separate set of experiments, the cells were incubated in DMEM containing 0.25% FBS that contained human acetylated LDL (AcLDL; 70 μg/mL) for 48 hours. The cells were equilibrated in DMEM containing 1% BSA for 18 hours and then treated with DMEM alone or with human HDL (100 μg/mL; Intracel, Frederick, Md) for 24 and 48 hours. Cell lipids were extracted by overnight incubation at room temperature in isopropanol containing cholesteryl methyl ether (2.5 μg/well) as an internal standard. Total cholesterol of lipid extracts was measured by gas-liquid chromatography after the procedure.

Bone Marrow Transplantation

Recipient mice were lethally irradiated (9 Gy) from a cesium gamma source, and 5×10⁶ bone marrow cells were injected as described previously.

Serum Lipids and Lipoprotein Distribution

Mice were fasted for 4 hours, and then serum total cholesterol and triglyceride levels were determined. Fast-performance liquid chromatography was performed with a Superose 6 column (Pharmacia, Piscataway, NJ) on a high-performance liquid chromatography (HPLC) system model 600 (Waters, Milford, Mass). High-density lipoprotein (HDL) cholesterol was measured on an automated ACE analyzer with the direct HDL test (Schiapparelli Biosystems, Inc, Fairfield, NJ).

Analysis of Aortic Lesions

The entire aorta was flushed and dissected for en face preparation. Sections of the proximal aorta were prepared and analyzed with an imaging system KS 300 (Carl Zeiss Inc, Thornwood, NY).

Modified LDL Uptake and Cholesterol Efflux Measurements

Thioglycolate-elicited peritoneal macrophages were cultured for 2 days, incubated with human 1-dioctadecyl-1,3,3',3'-tetramethylinodocarboxylic perchlorate (DiI)–labeled acetylated or oxidized LDL (Intracel Corp, Rockville, Md) for 4 hours, and examined by flow cytometry as described previously. For cholesterol loading, macrophages were cultured in Dulbecco’s modified Eagle’s medium (DMEM) with 2% fetal calf serum containing 1.5 to 2.0 μCi/mL [3H]-cholesterol and 70 μg/mL human acetylated LDL for 36 hours.
Total Serum Cholesterol and Triglyceride Levels in Female and Male LDLR−/− Mice Transplanted With PPARα+/−/LDLR−/− and PPARα−/−/LDLR−/− Bone Marrow

<table>
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<th>Group of Animals</th>
<th>Serum Lipid</th>
<th>Baseline</th>
<th>6 Weeks of Chow Diet</th>
<th>4 Weeks of Western Diet</th>
<th>10 Weeks of Western Diet</th>
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<tr>
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<td>Cholesterol</td>
<td>207±6</td>
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<td>698±15</td>
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<tr>
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<td>198±6</td>
<td>708±21</td>
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<td>86±4</td>
<td>179±14</td>
<td>311±11</td>
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<tr>
<td><strong>Males</strong></td>
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<tr>
<td>PPARα+/− (n=15)</td>
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<td>313±27</td>
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</table>

Values are in mg/dL (mean±SEM). Differences between groups were not significant at any time point.

were conducted. First, 7-week-old female LDLR−/− mice were lethally irradiated and reconstituted with marrow from female PPARα+/−/LDLR−/− (n=12, experimental group) or PPARα+/−/LDLR−/− (n=12, control group) mice. Second, 7-week-old male LDLR−/− mice were lethally irradiated and reconstituted with marrow from male PPARα−/−/LDLR−/− (n=15) or PPARα−/−/LDLR−/− (n=13) mice. After 6 weeks on chow diet, the recipient mice were challenged with the Western diet for 10 and 8 weeks, respectively. Female but not male PPARα−/−→LDLR−/− mice gained more body weight than control PPARα−/−→LDLR−/− mice (30.8±1.0 versus 27.1±0.6 g, mean±SEM; P<0.043). Female and male recipients had no changes in systolic blood pressure or plasma postheparin lipoprotein lipase activity (data not shown).

Serum cholesterol and triglycerides did not differ between the control and experimental groups in the course of the diet (Table). Lipoprotein profiles (Figure 1A and 1B) and HDL cholesterol levels in female and male PPARα−/−→LDLR−/− mice were similar to those of control recipients (208±12 versus 196±11 mg/dL, P=0.34, and 164±16 versus 164±13 mg/dL, P=0.99, respectively). Thus, PPARα deficiency in bone marrow-derived cells has no impact on serum lipids and lipoprotein profiles.

Macrophage PPARα Deficiency and Atherosclerosis

After 10 and 8 weeks on the diet, aortic lesions were detected in all female and male recipients. These lesions consisted mainly of macrophage-derived foam cells, as verified by analysis of the sections stained for macrophages (data not shown). Atherosclerotic lesions in female recipients often contained a fibrous cap and had a lower ratio of macrophage area to oil red O staining area (0.50 versus 0.53, respectively) than male recipients (0.68 versus 0.69, respectively). In male PPARα−/−→LDLR−/− mice, the extent of atherosclerotic lesions in the proximal aorta was significantly higher (44%) than in PPARα+/−→LDLR−/− mice (90 127±9690 versus 62 648±7424 μm², P=0.048; Figure 2D). A similar increase in atherosclerotic lesions was found in en face aorta preparations of male PPARα−/−→LDLR−/− mice compared with PPARα+/−→LDLR−/− male recipients (0.13±0.015% versus 0.09±0.01%, P=0.044; Figure 2C). In contrast, no difference in lesion size existed in the proximal aorta of female PPARα−/−→LDLR−/− and control PPARα+/−→LDLR−/− recipients (15 3763±19 932 versus 14 8036±20 770 μm², P=0.85; Figure 2A).

However, a significant 46% increase in lesion area was noted by en face analysis of the distal aorta of female PPARα−/−→LDLR−/− mice compared with PPARα+/−→LDLR−/− mice (0.41±0.06% versus 0.28±0.02%, respectively, P<0.043; Figure 2B). Thus, PPARα deficiency in marrow-derived cells significantly increased atherosclerosis in both female and male LDLR−/− mice.

Oxidized LDL Uptake and Cholesterol Efflux by PPARα−/− Macrophages

To study the impact of PPARα on the uptake of modified LDL, macrophages from PPARα−/− and PPARα+/− mice were incubated with DiI-labeled AcLDL or oxidized LDL (OxLDL). The uptake of DiI-AcLDL was similar in PPARα−/− and PPARα+/− macrophages with the exception of the highest concentration of AcLDL (Figure 3A). In contrast, PPARα−/− macrophages had increased uptake of DiI-OxLDL (by 46.7%, 64.1%, and 93.3% at doses of 5, 10, and 20 μg/mL, respectively) compared with PPARα+/− macrophages (Figure 3B).

To examine whether macrophage PPARα deficiency had an impact on cholesterol efflux, we used 2 alternative

Figure 1. Lipoprotein distribution in female (A) and male (B) LDLR−/− mice transplanted with PPARα+/− (●) and PPARα−/− (○) bone marrow after 10 and 8 weeks on the Western diet, respectively. Data are represented as the average (n=3) of the percent of total cholesterol for each fraction. Fractions 14 to 17 contain very low-density lipoprotein (VLDL); fractions 18 to 24 are intermediate-density lipoprotein/LDL; and fractions 25 to 30 contain HDL.
approaches: efflux of radiolabeled cholesterol and bulk changes in cellular cholesterol content. PPARα−/− macrophages had slightly (12%, \(P<0.05\)) lower levels of HDL-mediated cholesterol efflux than PPARα+/- macrophages (Figure 3C). Similarly, wild-type macrophages treated with WY14643 and HDL for 24 and 48 hours had a significantly greater decrease (18.2% and 14.7%, respectively) in total cholesterol mass than cells treated with HDL only (Figure 3D). Although there appeared to be a trend for a decrease (12.6% and 9.9%) in cholesterol mass in PPARα−/− macrophages, this difference was not statistically significant (Figure 3D). Thus, PPARα-deficient macrophages had increased OxLDL uptake and decreased levels of cholesterol efflux compared with wild-type cells.

PPARα Deficiency Affects CD36, SR-BI, and Inflammatory Response Genes

To evaluate the impact of macrophage PPARα on genes implicated in cholesterol homeostasis, peritoneal macrophages from wild-type and PPARα−/− mice were treated with the PPARα agonist WY14643 (2 \(\mu\)mol/L) for 24 hours. Quantitative real-time polymerase chain reaction (PCR) analysis demonstrated that the agonist upregulates CD36 in a PPARα-independent manner (Figure 4A). Interestingly, WY14643 treatment increased the SR-BI gene expression levels (Figure 4B) and SR-BI protein content (19%) in PPARα+/- macrophages but not in PPARα−/− macrophages (Figure 4C and 4D). In contrast, LPS treatment significantly decreased SR-BI gene expression (data not shown) and protein levels in both types of cells (41% and 31%) compared with untreated cells (Figure 4C and 4D). Similar effects on macrophage SR-BI expression were seen with another PPARα agonist, GW7647 (Figure 5A). Wild-type macrophages treated with this ligand had a trend for an increase in ABCA1 and ABCG1 gene expression (Figure 5B and 5C), but only ABCG4 gene expression was statistically significant compared with PPARα−/− macrophages (Figure 5D). In addition, WY14643 treatment did not dramatically change ABCA1 protein expression in either type of macrophage, but wild-type cells had higher levels of ABCA1 protein content than PPARα−/− macrophages (Figure 5E and 5F).

Next, to examine whether macrophage PPARα has an impact on the inflammatory response, wild-type and PPARα−/− peritoneal macrophages were stimulated with LPS (50 ng/mL) for 5 hours, and the expression pattern of inflammatory genes was analyzed by real-time PCR. Compared with wild-type cells, PPARα−/− macrophages had significantly increased mRNA expression levels of inflammatory genes including COX-2, IL1β, IL6, Gro1, MCP-1, and p65 (Figure 6A through 6F). Thus, macrophage PPARα deficiency eliminates PPARα-mediated SR-BI activation, suppresses ABCA1 protein production, and accelerates LPS-induced inflammatory responses.

SR-BI and Nuclear Factor-κB Expression Levels in Atherosclerotic Lesions

Finally, to examine whether the expression of SR-BI and nuclear factor (NF)-κB genes are compromised in vivo in atherosclerotic lesions, we used an LCM approach as reported by Trogan et al.\(^{29}\) Using serial sections of the proximal aorta stained with a specific anti-macrophage antibody (Figure 7A) as a guide, macrophage-rich atherosclerotic lesions were collected by LCM (Figure 7B...
through 7D). Real-time PCR analysis of amplified RNA samples indicated that PPARα+/− cells in atherosclerotic lesions had no changes in CD36, SR-A, or ABCA1 gene expression levels (data not shown) but had diminished expression of SR-BI (Figure 7E) and increased expression of p65 (Figure 7F) genes compared with control lesions with wild-type macrophages. Thus, mice reconstituted with PPARα+/− macrophages had lower levels of SR-BI and elevated levels of NF-κB mRNA expression in atherosclerotic lesions in vivo.

**Discussion**

In the present study, the role of macrophage PPARα expression in atherogenesis was examined in male and female LDLR−/− mice reconstituted with PPARα+/− or PPARα+/+ macrophages. Both male and female PPARα−/−→LDLR−/− mice had significantly increased (44% and 46%, respectively) atherosclerotic lesions in the distal aorta compared with controls. Male PPARα−/−→LDLR−/− mice also had larger (44%) atherosclerotic lesions in the proximal aorta than male PPARα+/+→LDLR−/− mice. The increase in atherosclerosis in these mice occurred in the absence of significant differences in serum lipids or lipoprotein profiles compared with controls. Thus, local macrophage PPARα expression in the artery wall has protective antiatherogenic effects in vivo.

The lack of a significant difference in the proximal aortas of the female mice may be the result of a sexual dimorphism specific for PPARα−/−. Female PPARα−/− mice are prone to develop obesity and have increased plasma triglycerides, whereas PPARα-null males have hepatic accumulation of triglycerides and higher serum cholesterol and apoB levels. Therefore, gender differences in atherosclerosis susceptibility may have contributed to the lack of difference in atherosclerosis in the proximal aortas of the females, although the mechanism for this sexual dimorphism remains unclear. Alternatively, accelerated formation of complicated lesions in the females with a low ratio of macrophage area to lesion area may have obscured the effect of macrophage PPARα expression on lesion area.

Previous investigations of the role of PPARα in atherosclerosis in apoE−/− mice that used PPARα agonists and gene knockouts have yielded conflicting results. Mice treated with PPARα agonists had severe hypercholesterolemia and accelerated atherosclerosis but reduced cholesterol content in the arterial wall. In contrast, Tordjian et al reported that systemic PPARα deficiency in apoE−/− mice decreased insulin resistance, blood pressure, and atherosclerosis compared with apoE−/− mice. Recently, Bernal-Mizrachi et al identified PPARα expression in the liver as a key mediator of insulin resistance and hypertension in mice. Collectively, these results indicate that the systemic absence of PPARα does not produce the opposite phenotype of fibrate-mediated activation of PPARα and that the role of PPARα in atherosclerosis is complex.

Macrophage transformation into foam cells can be viewed as an imbalance between cholesterol influx attributed mainly to scavenger receptors type A and CD36 and to lipid efflux...
mediated by membrane transporters ABCA1, ABCG1, and SR-BI. Chinetti and coworkers have demonstrated that PPARα agonists induce cholesterol removal from human macrophage-derived foam cells through stimulation of the ABCA-1 pathway. In our studies, we present evidence that PPARα-null macrophages have increased CD36 gene expression levels and uptake of OxLDL. Although the CD36 receptor promoter does not have PPARα-response elements, PPARα can regulate the CD36 receptor gene via induction of other transcriptional factors such as Sp-1. Febbraio and coworkers demonstrated that CD36 expression by macrophages significantly accelerates atherosclerosis. Thus, activation of scavenger receptor CD36 expression in PPARα-deficient mice may have proatherogenic effects.

The present results indicate that PPARα deficiency suppresses cholesterol efflux in macrophages. Although the impact of PPARα on cholesterol efflux was relatively low (12% to 18%), this difference was statistically significant and consistently reproducible. Therefore, we believe that over time, differences of this magnitude may significantly impact macrophage cholesterol homeostasis. Treatment of macrophages cholesterol loaded in vivo with a PPARα agonist has been reported to reduce ABCA1 protein levels. Although PPARα activation in the present study did not change ABCA1 gene and protein expression levels in the absence of cholesterol loading, wild-type macrophages had significantly higher levels of ABCA1 protein content than PPARα-deficient cells, consistent with an important role for PPARα in modulating ABCA1 posttranscriptionally. Given that Neufeld and coworkers have shown a role for ABCA1 in mobilizing lysosomal cholesterol, this may represent an important effect of PPARα on macrophage cellular cholesterol homeostasis. Interestingly, PPARα activation stimulated SR-BI gene and protein expression, and ABCG4 mRNA levels in wild-type but not in PPARα-deficient macrophages. The impact of increased ABCG4 expression on cholesterol efflux is uncertain, because studies have shown that increased expression of ABCG4 promotes efflux, but elimination of ABCG4 gene expression does not appear to impact cholesterol efflux. The present data on SR-BI are consistent with the results of recent studies indicating that PPARα specifically induces gene activi-
SR-BI mediates the efflux of cholesterol from macrophages to HDL and facilitates the selective uptake of cholesterol esters from lipoproteins into liver.43 Ji and coworkers44 demonstrated that macrophage-derived foam cells in atherosclerotic lesions of apoE-null mice express abundant SR-BI. PPARα agonists upregulated SR-BI protein levels in human and murine macrophages,19,45 and SR-BI protein level is significantly increased in atherosclerotic lesions of apoE-null mice treated with a PPARα ligand.19,45 In contrast, mice lacking SR-BI exclusively in bone marrow–derived cells developed accelerated atherosclerosis compared with wild-type mice.19,45,46,47 In addition, we used the LCM approach to demonstrate directly that the SR-BI gene is expressed at higher levels in atherosclerotic lesions of mice reconstituted with PPARα-null marrow. Taken together, these results indicate that PPARα-mediated SR-BI activation may play an important protective role in atherosclerosis.

Previous studies demonstrated that PPARα null mice display a prolonged response to inflammatory stimuli48 and acquire a state of redox imbalance.49 PPARα agonists stimulate reactive oxygen species production50 and lipolysis with the generation of endogenous PPARα ligands51 that can further activate PPARα and subsequently limit inflammation.52 The agonists also inhibited the expression of LPS-mediated inflammatory cytokines (interleukin [IL]-1β, IL6, IL8, and tumor necrosis factor-α) by antagonizing the constitutively active NF-κB.49,53 Here, we used a genetic approach to demonstrate that peritoneal macrophages execute these antiinflammatory effects. Deficiency of the PPARα gene in macrophages significantly accelerated LPS-mediated activation of the inflammatory response genes (such as COX2, IL-1β, IL-6, MCP-1, and p65). Moreover, macrophage-derived foam cells in atherosclerotic lesions of mice reconstituted with PPARα-null macrophages have increased expression levels of the NF-κB transcription factor subunit p65 than control lesions reconstituted with wild-type cells. Although not definitive in the absence of protein data, these results are consistent with increased expression of NF-κB–regulated genes. The results also suggest that macrophage PPARα deficiency promotes a proinflammatory phenotype, which has increased sensitivity to oxidative stress. Thus, macrophage PPARα expression suppresses the NF-κB pathway, inducing protective and antiatherogenic effects in vivo within atherosclerotic lesions.

Figure 6. Inflammatory response gene (COX2, IL1β, IL6, Gro1, MCP1, and p65) expression in macrophages isolated from PPARα+/− (solid bars) and PPARα−/− (open bars) mice. Cells were treated with LPS (50 ng/mL) for 5 hours, and gene expression levels were measured by real-time PCR. The experiment was repeated 2 times with the same number of mice (n=3 per group; *P<0.05).

Figure 7. Serial sections from the proximal aorta were stained for macrophages (A) or used for LCM (B through D). B and C, Representative sections of the aortic sinus before (B) and after (C) LCM, plus a sample of captured tissue (D) from atherosclerotic lesions (×10). SR-BI (E) and NF-κB (F) mRNA expression levels in atherosclerotic lesions isolated from LDLR−/− mice reconstituted with PPARα+/− (solid bars) or PPARα−/− (open bars) marrow (*P<0.05).
In summary, we demonstrate here that macrophage PPARα expression plays an important protective role in atherosclerotic lesion formation in vivo. Macrophage PPARα deficiency significantly increases uptake of oxidized LDL, eliminates the PPARα-mediated SR-BI activation, diminishes ABCA1 protein content, and accelerates the response to inflammatory stimuli. These results support the concept that local PPARα expression in the artery wall coordinates macrophage cholesterol homeostasis and inflammatory activity, thereby mediating protective antiatherogenic effects.

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Disclosures

None.

References

CHEMICAL PERSPECTIVE

Peroxisome proliferator-activated receptor-α (PPARα) is a nuclear receptor or ligand-activated transcription factor with important roles in lipid metabolism and inflammation. Fibric acid derivatives are PPARα ligands used for treatment of dyslipidemia that have been shown to reduce cardiovascular event rates in clinical trials. Although PPARα activation may also impact atherosclerosis directly, the role of PPARα expression by vascular cells in atherogenesis remains to be defined in vivo. To dissect the role of macrophage PPARα expression in early atherosclerosis, female and male low-density lipoprotein–receptor–null (LDLR−/−) mice were lethally irradiated and transplanted with bone marrow from wild-type or PPARα−/−null mice. When fed the Western diet, recipient mice reconstituted with PPARα−/− bone marrow–derived cells (PPARα−/−→LDLR−/−) developed significantly larger atherosclerotic lesions than control mice with wild-type marrow (PPARα+/+→LDLR−/−). To further investigate the mechanisms responsible for the increase of atherosclerosis, we examined cholesterol homeostasis and inflammatory gene responses in peritoneal macrophages. PPARα−/− macrophages had increased uptake of oxidized low-density lipoprotein and decreased cholesterol efflux compared with wild-type cells. Macrophage PPARα deficiency was accompanied with decreased expression of scavenger receptor B type I, reduced amounts of ABCA1 protein, and accelerated response to inflammatory stimuli. A laser capture microdissection analysis verified suppressed scavenger receptor B type I and increased nuclear factor-κB gene expression levels in vivo in atherosclerotic lesions of PPARα−/−→LDLR−/− mice compared with the lesions of control PPARα+/+→LDLR−/− mice. Thus, these results support the concept that local PPARα expression in the artery wall induces protective antiatherogenic effects in macrophages by regulating cholesterol homeostasis and inflammatory activity.
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