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

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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<sup>-/-</sup>) mice were reconstituted with bone marrow from PPARα<sup>-/-</sup> or PPARα<sup>+/+</sup> 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<sup>-/-</sup>→LDLR<sup>-/-</sup> mice was significantly increased (44% and 46%, respectively) compared with controls. Male PPARα<sup>-/-</sup>→LDLR<sup>-/-</sup> mice also had larger (44%) atherosclerotic lesions in the proximal aorta than male PPARα<sup>+/+</sup>→LDLR<sup>-/-</sup> mice. Peritoneal macrophages from PPARα<sup>-/-</sup> mice had increased uptake of oxidized LDL and decreased cholesterol efflux. PPARα<sup>-/-</sup> 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<sup>-/-</sup>→LDLR<sup>-/-</sup> mice compared with the lesions of control PPARα<sup>+/+</sup>→LDLR<sup>-/-</sup> 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 derivates, have widespread clinical application in the treatment of dyslipidemia. 3 They have been shown to reduce the progression of coronary disease in angiographic trials 4-5 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 al 11 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<sup>+/+</sup>/apoE<sup>-/-</sup> litter mates, which suggests that systemic PPARα deficiency has complex effects on atherogenesis. In one study, treatment of apoE<sup>-/-</sup> mice with PPARα agonists severely aggravates their hypercholesterolemia and accelerated atherosclerotic development. 12 In contrast, several reports have demonstrated that treatment with PPARα agonists...
improved lipoprotein metabolism and reduced atherosclerosis in low-density lipoprotein receptor (LDL−/-)–deficient (LDL−−)–deficient (apoE−−) mice, and human apoE2–expressing (instead of mouse apoE–expressing) mice.13–15 In addition, PPARα agonists have been reported to reduce cholesterol esterification in macrophages.16 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,17 smooth muscle cells,18 and monocyte/macrophages.19 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 gene20 were crossed with LDLR−/− mice21 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 (Teklad, Madison, Wis). Animal care and experimental procedures were performed according to the regulations of Vanderbilt University’s Animal Care Committee.

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.22

Serum Lipids and Lipoprotein Distribution

Mice were fasted for 4 hours, and then serum total cholesterol and triglyceride levels were determined.23 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.24 Sections of the proximal aorta were prepared25 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-1-3,3,3,3′-tetramethylindocarbocyanine perchlorate (DiI)–labeled acetylated or oxidized LDL (Intracel Corp, Rockville, Md) for 4 hours, and examined by flow cytometry as described previously.26 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 µg/mL [3H]-cholesterol and 70 µg/mL human acetylated LDL for 36 hours. 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.27

Quantitative Real-Time Polymerase Chain Reaction

Peritoneal macrophages were treated with RPMI media containing 5% fetal lipoprotein-deficient serum (Intracel) and 2 PPARα agonists, WY14643 (Biomol 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) and then blotted onto nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ). Cells were probed 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 horseradish peroxidase–conjugated goat anti-rabbit antibodies (Upstate Cell Signaling, Lake Placid, NY) and quantified by densitometry.

Western Blotting

Proteins were extracted from macrophages in the presence of protease inhibitors (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). Western blots were probed 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 horseradish 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.29 Serial sections were stained with specific anti-macrophage antibody, MOMA-2 (Serotec, Raleigh, NC). Macrophage-rich atherosclerotic lesions were captured (PicoCell 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 cells were used for 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 performed.
were conducted. First, 7-week-old female LDLR<sup>−/−</sup> mice were lethally irradiated and reconstituted with marrow from female PPAR<sub>α</sub><sup>+/−</sup>/LDLR<sup>−/−</sup> (n=12, experimental group) or PPAR<sub>α</sub><sup>+/−</sup>/LDLR<sup>−/−</sup> (n=12, control group) mice. Second, 7-week-old male LDLR<sup>−/−</sup> mice were lethally irradiated and reconstituted with marrow from male PPAR<sub>α</sub><sup>+/−</sup>/LDLR<sup>−/−</sup> (n=15) or PPAR<sub>α</sub><sup>+/−</sup>/LDLR<sup>−/−</sup> (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 PPAR<sub>α</sub><sup>+/−</sup>/LDLR<sup>−/−</sup> mice gained more body weight than control PPAR<sub>α</sub><sup>+/−</sup>/LDLR<sup>−/−</sup> 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 triglycerides (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 PPAR<sub>α</sub><sup>+/−</sup>/LDLR<sup>−/−</sup> 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, PPAR<sub>α</sub> 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 PPAR<sub>α</sub><sup>+/−</sup>/LDLR<sup>−/−</sup> mice, the extent of atherosclerotic lesions in the proximal aorta was significantly higher (44%) than in PPAR<sub>α</sub><sup>+/−</sup>/LDLR<sup>−/−</sup> mice (90±127±9690 versus 62 648±7424 µm<sup>2</sup>, P=0.048; Figure 2D). A similar increase in atherosclerotic lesions was found in en face aorta preparations of male PPAR<sub>α</sub><sup>+/−</sup>/LDLR<sup>−/−</sup> mice compared with PPAR<sub>α</sub><sup>+/−</sup>/LDLR<sup>−/−</sup> 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 PPAR<sub>α</sub><sup>+/−</sup>/LDLR<sup>−/−</sup> and control PPAR<sub>α</sub><sup>+/−</sup>/LDLR<sup>−/−</sup> recipients (15 3763±19 932 versus 14 8036±20 770 µm<sup>2</sup>, 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 PPAR<sub>α</sub><sup>+/−</sup>/LDLR<sup>−/−</sup> mice compared with PPAR<sub>α</sub><sup>+/−</sup>/LDLR<sup>−/−</sup> mice (0.41±0.06% versus 0.28±0.02%, respectively, P<0.043; Figure 2B). Thus, PPAR<sub>α</sub> deficiency in marrow-derived cells significantly increased atherosclerosis in both female and male LDLR<sup>−/−</sup> mice.

**Oxidized LDL Uptake and Cholesterol Efflux by PPARα<sup>−/−</sup> Macrophages**

To study the impact of PPAR<sub>α</sub> on the uptake of modified LDL, macrophages from PPAR<sub>α</sub><sup>−/−</sup> and PPAR<sub>α</sub><sup>+/−</sup> mice were incubated with Dil-labeled AcLDL or oxidized LDL (OxLDL). The uptake of Dil-AcLDL was similar in PPAR<sub>α</sub><sup>−/−</sup> and PPAR<sub>α</sub><sup>+/−</sup> macrophages with the exception of the highest concentration of AcLDL (Figure 3A). In contrast, PPAR<sub>α</sub><sup>−/−</sup> macrophages had increased uptake of Dil-OxLDL (by 46.7%, 64.1%, and 93.3% at doses of 5, 10, and 20 µg/mL, respectively) compared with PPAR<sub>α</sub><sup>+/−</sup> macrophages (Figure 3B). To examine whether macrophage PPARα deficiency had an impact on cholesterol efflux, we used 2 alternative strategies.
Inflammatory Response Genes

PPAR levels (Figure 4B) and SR-BI protein content (19%) in WY14643 treatment increased the SR-BI gene expression. Analysis demonstrated that the agonist upregulates CD36 in a PPAR-dependent manner (Figure 4C). 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.

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 PPAR agonist WY14643 (2 µmol/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

**Figure 2.** Atherosclerotic lesion area in female (A and B) and male (C and D) LDLR−/− mice in the proximal (A and C) and distal (B and D) aorta after reconstitution with PPARα−/− (solid bars) and PPARα−/− (open bars) marrow. Data are average (mean±SEM) lesion area for female (n=12 and n=12) and male (n=15 and n=13) recipients.
controls. Thus, local macrophage PPAR expression in these mice occurred in the absence of significant differ-
ences in serum lipids or lipoprotein profiles compared with controls. Thus, local macrophage PPAR expression in the artery wall has protective antiatherogenic effects 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.

Figure 3. Uptake of Dil-AcLDLR (A), Dil-OxLDLR (B), HDL-mediated cholesterol efflux (C), and total cholesterol content (D) in peritoneal macrophages isolated from PPARα+/− (solid bars) and PPARα−/− (open bars) mice. Cells were cultured with Dil-AcLDLR or Dil-AcLDLR for 4 hours alone or with HDL for 48 hours. For cholesterol mass analysis (D), groups were treated with human LDLR ligand WY14643 (WY; 2 μmol/L) for 24 hours alone or with LPS for 5 hours. Gene expression levels and protein content were measured by real-time PCR or Western blot. The experiment was repeated twice with the same number of mice (n=4 per group; P<0.05).

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 oil red O–staining area in the proximal aorta of female recipients 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 descending aortas. In contrast, Tordjman et al11 reported that systemic PPARα deficiency in apoE−/− mice decreased insulin resistance, blood pressure, and atherosclerosis compared with apoE−/− mice. Recently, Bernal-Mizrachi et al15 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 PPAR agonists induce cholesterol removal from human macrophage-derived foam cells through stimulation of the ABCA-1 pathway. In our studies, we present evidence that PPAR-null macrophages have increased CD36 gene expression levels and uptake of OxLDL. Although the CD36 receptor promoter does not have PPAR-response elements, PPAR 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 PPAR-deficient mice may have proatherogenic effects.

The present results indicate that PPAR deficiency suppresses cholesterol efflux in macrophages. Although the impact of PPARs 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 PPAR agonist has been reported to reduce ABCA1 protein levels. Although PPAR 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 PPAR-/- cells, consistent with an important role for PPAR 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 PPAR on macrophage cellular cholesterol homeostasis. Interestingly, PPAR activation stimulated SR-BI gene and protein expression, and ABCG4 mRNA levels in wild-type but not in PPAR-/- 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.
SR-BI mediates the efflux of cholesterol from macrophages to HDL and facilitates the selective uptake of cholesterol esters from lipoproteins into liver. Ji and coworkers demonstrated that macrophage-derived foam cells in atherosclerotic lesions of apoE−/− mice express abundant SR-BI. PPARα agonists upregulated SR-BI protein levels in human and murine macrophages, and SR-BI protein level is significantly increased in atherosclerotic lesions of apoE-null mice treated with a PPARα ligand. In contrast, mice lacking SR-BI exclusively in bone marrow—derived cells developed accelerated atherosclerosis compared with wild-type mice. 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 wild-type macrophages than in mice transplanted with PPARα−/− 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 stimuli and acquire a state of redox imbalance. PPARα agonists stimulate reactive oxygen species production and lipolysis with the generation of endogenous PPARα ligands that can further activate PPARα and subsequently limit inflammation. 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. 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 COX-2, 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.
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

CLINICAL 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 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−/− mice developed significantly larger atherosclerotic lesions than control mice with wild-type marrow. Macrophage PPARα deficiency was accompanied with decreased expression of scavenger receptor B type I, reduced expression 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−/− mice compared with the lesions of control PPARα+/− 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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