PPAR Agonists Protect Mesangial Cells from Interleukin 1β-Induced Intracellular Lipid Accumulation by Activating the ABCA1 Cholesterol Efflux Pathway

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Abstract. Previous studies have demonstrated that inflammatory cytokines such as interleukin-1β (IL-1β) promote lipid accumulation in human mesangial cells (HMC) by dysregulating the expression of lipoprotein receptors. Intracellular lipid accumulation is governed by both influx and efflux; therefore, the effect of IL-1β on the efflux of lipid from HMC was investigated. IL-1β was shown to inhibit ^{3}H-cholesterol efflux from HMC and increase total intracellular cholesterol concentration, probably as a result of reduced expression of the adenosine triphosphate (ATP) binding cassette A1 (ABCA1), a transporter protein involved in apolipoprotein-A1 (apo-A1)–mediated lipid efflux. To ascertain the molecular mechanisms involved, expression of peroxisome proliferator-activated receptors (PPAR) and liver X receptorα (LXRα) were examined. IL-1β (5 ng/ml) reduced PPARα, PPARγ, and LXRα mRNA expression. Activation of PPARγ with the agonist prostaglandin J2 (10 μM) and of PPARα with either bezafibrate (100 μM) or Wy14643 (100 μM) both increased LXRα and ABCA1 gene expression also and enhanced apoA1-mediated cholesterol efflux from lipid-loaded cells, even in the presence of IL-1β. A natural ligand of LXRα, 25-hydroxycholesterol (25-OHC), had similar effects; when used together with PPAR agonists, an additive effect was observed, indicating cooperation between PPAR and LXRα in regulating ABCA1 gene expression. This was supported by the observation that overexpression of either PPARα or PPARγ by transfection enhanced LXRα and ABCA1 gene induction by PPAR agonists. Taken together with previous data, it appears that, in addition to increasing lipid uptake, inflammatory cytokines promote intracellular lipid accumulation by inhibiting cholesterol efflux through the PPAR-LXRα-ABCA1 pathway. These results suggest potential mechanisms whereby inflammation may exacerbate lipid-mediated cellular injury in the glomerulus and in other tissues and indicate that PPAR agonists may have a protective effect.

Clinical studies indicate that correction of dyslipidemia associated with kidney disease may slow progression of chronic renal failure (1). Inflammation, lipid accumulation, and foam cell formation are recognized features of glomerular and tubulointerstitial injury, and it is plausible that renal injury and atherosclerosis share common pathogenic mechanisms (2,3). In most cells, intracellular cholesterol accumulation is prevented by tight regulation of influx and efflux pathways (4). It is generally accepted that intracellular cholesterol accumulation only occurs in cells, such as macrophages, that are able to take up modified lipoproteins via a scavenger-type receptor (5). We have previously demonstrated that cytokines induce scavenger receptor expression by mesangial cells and promote foam cell formation (6). However, we also observed that under inflammatory conditions, intracellular lipid accumulation occurred even when scavenger receptors were blocked with polyisonosinic acid, suggesting that additional pathways were involved. Further studies indicated that inflammatory cytokines caused dysregulation of LDL receptor expression, which is usually tightly controlled by intracellular cholesterol concentration (7), thereby allowing unregulated uptake of cholesterol.

Intracellular lipid content is governed by both influx and efflux; we therefore set out to examine the impact of inflammatory cytokines on cholesterol efflux, a process mediated by the protein adenosine triphosphate (ATP) binding cassette A1 (ABCA1). ABCA1, a member of the ATP binding cassette (ABC) transporter super family, is a cholesterol transporter containing 12 putative transmembrane domains and two ABC (8,9). ABCA1 deficiency causes both Tangier disease and familial HDL deficiency, characterized by accumulation of cholesterol esters in various tissues such as tonsils, liver, spleen, and intestinal mucosa. Individuals with these disorders have very low levels of plasma HDL cholesterol (10,11). ABCA1 is dramatically induced in lipid-loaded macrophages and facilitates the efflux of cellular cholesterol to extracellular apoA1 or HDL. ABCA1 activation seems to be the first step of

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the reverse cholesterol transport pathway and is therefore important in the control of plasma level of HDL, which is an important factor in the development of atherosclerosis (12,13).

It has been recently demonstrated that ABCA1 gene expression in macrophages can be induced by PPARα, PPARγ, and LXRα agonists (14–16). PPAR are ligand-activated nuclear receptors, which upon heterodimerization with the retinoid X receptors (RXR) induce a number of genes controlling lipid and glucose metabolism, cellular differentiation, and inflammation (17–19). The LXR were originally cloned as orphan receptors based on their homology to RXR. LXRα, which is known to regulate the metabolic conversion of cholesterol to bile acids, has also been shown to regulate the expression of ABCA1 in macrophages and fibroblasts (15,16). A PPAR response element has been identified in the LXR gene promoter; it therefore seems likely that PPAR regulate ABCA1 gene expression through the LXR pathway.

The ability of ABCA1 to facilitate cholesterol efflux suggests that it may be a useful target for intervention in human diseases involving lipid accumulation. The present study examines the effects of the inflammatory cytokine IL-1β on cholesterol efflux in human mesangial cells (HMC), investigates the underlying molecular mechanisms and explores the potential protective influence of PPAR and LXRα agonists on lipid-mediated injury.

Materials and Methods

Cell Culture

An established stable line of HMC that had been immortalized by transfection with T-SV40 and H-ras oncogenes was used in all experiments (kindly donated by Dr. J. D. Sraer, Hôpital Tenon, Paris). These cells have been fully characterized and retain most of the morphologic and physiologic features of nontransfected mesangial cells (20,21). These cells were cultured in growth medium comprising RPMI supplemented with 5% fetal calf serum (FCS), 2 mmol/L glutamine, 100 unit/ml penicillin, 100 μg/ml streptomycin, 2.5 μg/ml amphotericin, 5 μg/ml insulin, 5 μg/ml human transferrin, and 5 ng/ml sodium selenite. Experiments were carried out in serum-free RPMI medium containing 0.2% bovine serum albumin (BSA; Sigma, Poole, Dorset, UK). All reagents for cell culture were obtained from Life Technologies BRL (Paisley, UK). Recombinant interleukin-1 (IL-1β, 1.0 to 3.3 $\times$ 10^8 U/mg) was obtained from R&D Systems (Europe Ltd, Abingdon, UK).

Transient Expression of PPAR

HMC were cultured and transfected with supercoiled PPARα or PPARγ expressive vectors (pSG5hPPARα and pSG5hPPARγ, kindly provided by Professor B. Staels, Lille, France) by electroporation at 340 V and 125 uF with a Gene Pulser (Bio-Rad, Hemel Hempstead, Herefordshire, UK). After electroporation, the cells were placed in six-well plates with growth medium. Approximately 24 h after replating, cells were washed with PBS and the medium was replaced by fresh serum-free RPMI medium in the presence or absence of bezafibrate (100 μM) or PPARγ agonist PGJ2 (10 μM) for 24 h. The cells were then collected and RNA was analyzed.

Reverse Transcriptase–PCR

Total RNA (500 ng) was used as a template for RT-PCR using an RNA PCR kit from ABI (Applied Biosystems, Warrington, Cheshire, UK). The RT reaction was set up in a 20 μl mixture containing 50 mmol/L KCl, 10 mmol/L Tris/HCl, 5 mmol/L $MgCl_2$, 1 mmol/L of each dNTP, 2.5 μmol/L random hexamers, 20 U RNAsin, and 50 U of M-MLV reverse transcriptase. Incubations were performed in a DNA Thermal Cycler (Perkin-Elmer 9700) for 10 min at room temperature, followed by 30 min at 42°C and 5 min at 99°C. After cDNA synthesis by RT, the incubation mixture was split into two 10-μl aliquots for separate amplification of ABCA1, PPARα, PPARγ, LXRα, and GAPDH cDNA using specific primers. The final concentrations of the PCR reaction mixture were 50 mmol/L KCl, 10 mmol/L Tris/HCl, 2 mmol/L $MgCl_2$, 200 μmol/L dNTP, 0.125 μmol/L of primers, 1.25 U *Taq* DNA polymerase. The following primers were used for PCR. ABCA1 upper primer (nucleotide positions 4503–4523) 5'-TTAAACGCCCTCACCAAAGAC-3', and lower primer (4873–4896) 5'-AAAAGCCGCCATACCTAAACTCAT-3', PPARα upper primer (733–757) 5'-GACGAATGCCAAGATCTGAGAAAGC-3' and lower primer (1660–1683) 5'-CGTCTCCTTTGTAGTGCTGTCAGC-3', PPARγ upper primer (493–522) 5'-GGCAATTGAATGTCGTGTCTGTGGAGATAA-3' and lower primer (1402–1432) 5'-AGCTCCAGGGCTTGTAGCAGGTTGTCTTGA-3', LXRα upper primer (379–399) 5'-GCGAGGGCTGCAAGGGATTCT-3' and lower primer (735–754) 5'-ATGGGCCAAGGCGTGACTCG-3', GAPDH upper primer (nucleotide positions 73–92) 5'-TCATAGACAAGATGGTGAAG-3', and lower primer (303–327) 5'-TGACGGGATCTCGCTCCTGGAAGAT-3' (22). Twenty microliters of each PCR reaction were subjected to electrophoresis in a 2% agarose gel.

Southern Blot Analyses and Quantitative Evaluation

Nucleic acids were transferred to a nylon membrane (Boehringer Mannheim, Lewes, East Sussex, UK) using the Southern analysis method. The nylon membranes were probed with [γ-^{32}P]ATP labeled oligonucleotides (3000 Ci/mmol; Amersham, Little Chalfont, Buckinghamshire, UK). The following probes were used: ABCA1 probe (nucleotide position 4552–4575) 5'-AGGGCGTGTCTGGGATTGGGTTTC-3', PPARα probe (1456–1479) 5'-AGAGAAAGATATCGTCCGGGTGGT-3', PPARγ probe (878 to 898): 5'-TGTCTTTCCTGTCAAGATCGC-3'), LXRα probe (410–433) 5'-ATGTAGTGCGCTCCCTTGATGACG-3', and GAPDH probe (170–191) 5'-AATGAAGGGGTCGTTGATGGCA-3' (22). The DNA probes were labeled using a 5'-end labeling system (Promega, Southampton, Hampshire, UK). Membranes were irradiated by UV stratalinker for 3 min, prehybridized in 50% formamide, 5 $\times$ Denhardt solution, 5 $\times$ SSPE, 100 μg/ml hs DNA, and 50 mmol/L sodium phosphate (pH 6.8) at 42°C for 4 h, and hybridized overnight at 42°C with at least 1 $\times$ 10^6 cpm/ml of the labeled probe in the same solution used for prehybridization but without Denhardt reagents. The membranes were washed twice for 15 min at room temperature with 5 $\times$ SSC, and then washed for 10 min at 42°C with 5 $\times$ SSC. The blots were exposed to x-ray films (Eastman Kodak, Rochester, NY) for 4 h.

Cholesterol Loading and Cholesterol Efflux Assay

Oxidized LDL (Ox-LDL) was prepared from human LDL as described previously (23). HMC were incubated with medium A, which contains RPMI 1640, 1% CPSR (low lipid serum replacement; Sigma, Poole, Dorset, UK), 1 μl of [^{3}H] cholesterol (1 μCi/μl; Amersham, Little Chalfont, Buck, UK), and 50 μg/ml of Ox-LDL. After 72 h, the fresh serum-free medium containing PPARα agonists Bezafibrate (100 μM) or Wy14643 (100 μM), or PPARγ agonist PGJ2 (10 μM) were added in the presence or absence of the inflammatory cytokine IL-1β (5 ng/ml) for a further 24 h. The oxysterol 25-OHC (1 μg/ml) was also used as an agonist of LXRα. After this incubation period,

cells were washed twice in PBS, and apoA1-mediated cholesterol efflux studies were immediately performed by adding fresh serum-free RPMI 1640 medium with or without 50 μg/ml of ApoA1 for 24 h. At the end of this incubation, the supernatant was collected and centrifuged at 13,000 rpm for 10 min. Cellular lipids was extracted using isopropanol. The radioactivity in both the supernatant and cellular lipid was measured by scintillation counting. ApoA1-induced [^{3}H]-cholesterol efflux was calculated as by subtracting the radioactivity in supernatants without apoA from the counts in supernatants containing apo A. The data were normalized by determining total [^{3}H]-cholesterol radioactivity (including radioactivity in supernatant and cell) and were expressed as a percentage of control.

Total Cholesterol Measurement

HMC were cultured in six-well plates and loaded with cholesterol as described above. After 72 h, cells were cultured in serum-free medium without (control) or with 5 ng/ml of IL-1β for 24 h. Cells were then washed twice in PBS and incubated with 50 μg/ml of ApoA1 for a further 24 h. At the end of this second incubation period, intracellular lipids were extracted in isopropanol and dried under vacuum, and the total cholesterol content was measured by enzymatic assay (Dade Behring, Milton Keynes, UK). Cellular proteins were collected by digestion in NaOH and measured by the Bradford assay (BioRad, Hertfordshire, UK). The results were normalized for total cellular protein.

Statistical Analyses

In all experiments, data were evaluated for significance by one-way ANOVA using Minitab software. Data were considered significant at $P \leq 0.05$.

Results

We examined the effects of the inflammatory cytokine IL-1β on cholesterol efflux. At a concentration of 5 ng/ml, IL-1β reduced Apo-A1-mediated cholesterol efflux from lipid-loaded HMC (Figure 1A) and increased intracellular total cholesterol content (Figure 1B). To investigate the potential mechanisms by which this occurred, the effect of IL-1β on expression of the ABCA1 gene was studied. IL-1β reduced ABCA1 gene expression in a time-dependent manner in both non-lipid–loaded (Figure 2A) and lipid-loaded HMC (Figure 2B). Interestingly, IL-1β also inhibited both PPAR and LXRα mRNA expression with a similar temporal response pattern (Figure 3, A through C). These results suggest that both PPAR and LXRα may regulate the ABCA1 pathway and thereby mediate the reduction in cholesterol efflux induced by IL-1β.

The PPARα agonists, bezafibrate (100 μM) and Wy14643 (100 μM), and the PPARγ agonist, PGJ2 (10 μM), increased Apo-A1–dependent cholesterol efflux from HMC and were able to override IL-1β-induced suppression (Figure 4). Essentially similar results were observed in the presence of the LXRα agonist 25-OHC, and an additive effect was observed when this oxysterol was used in conjunction with PPAR agonists, suggesting that PPAR and LXRα cooperatively induce cholesterol efflux (Figure 5).

To examine the interrelationship between PPAR, LXRα-and ABCA1 in mediating cholesterol efflux, the effects of PPAR agonists on LXRα and ABCA1 gene expression were investigated. Bezafibrate and Wy14643 induced PPARα mRNA expression, and PGJ2 induced PPARγ mRNA expression as expected (Figure 6, A and B). Both PPARα and PPARγ agonists also enhanced LXRα expression in a dose- and time-dependent manner in both non-lipid–loaded (Figure 7, A through C) and lipid-loaded HMC (Figure 8A), even in the presence of IL-1β (Figure 8B). To further clarify the relationship between PPAR and LXRα, both PPAR were overexpressed in HMC by transfection. Overexpression of PPARα and PPARγ increased LXRα gene expression induced by bezafibrate or PGJ2, respectively (Figure 8A). Furthermore, both

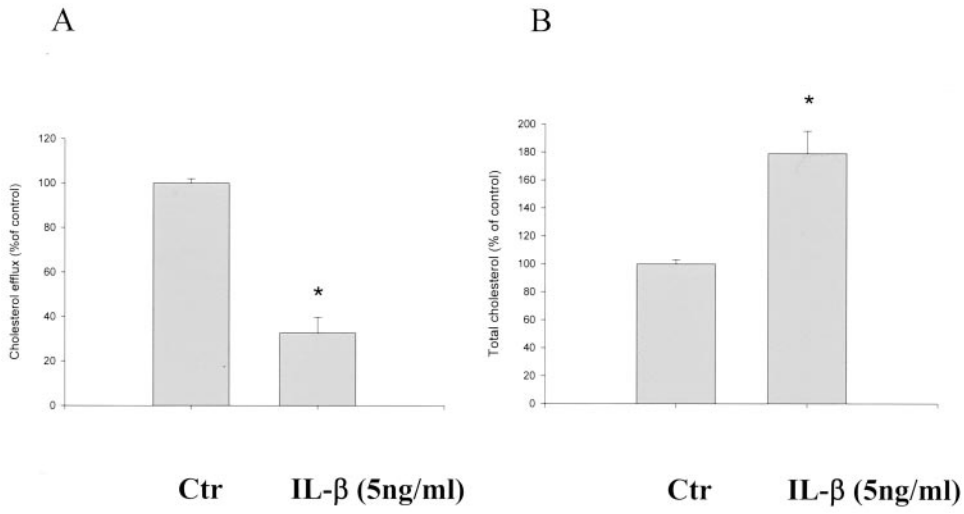

Figure 1. IL-1β reduced cholesterol efflux in human mesangial cells (HMC). Lipid-loaded HMC were treated with serum-free medium without (control) or with IL-1β (5 ng/ml) for 24 h. The cells were subsequently incubated in serum-free RPMI 1640 medium with or without 50 μg/ml of ApoA for 24 h. ApoA1-mediated [^{3}H] cholesterol efflux (A) and total intracellular cholesterol (B) were measured as described in the Materials and Methods section. Results are expressed as percentage of the untreated control. * $P < 0.001$ *versus* control.

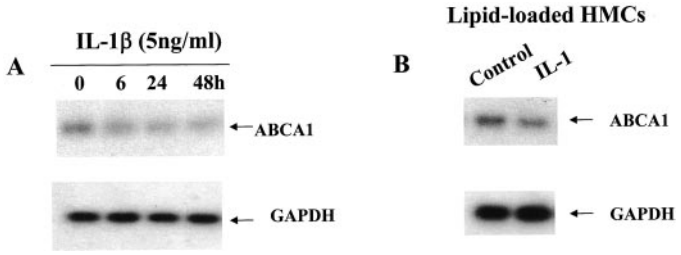

Figure 2. The effects of IL-1β on ABCA1 gene expression. HMC were incubated in serum-free medium for 24 h, then fresh medium containing IL-1β (5 ng/ml) was added and cells were cultured for 0 (control), 6, 24, and 48 h (A). In addition, lipid-loaded HMC were treated with IL-1β (5 ng/ml) for 24 h (B). The expression of ABCA1 mRNA, was examined using RT-PCR followed by Southern blotting as described in Materials and Methods.

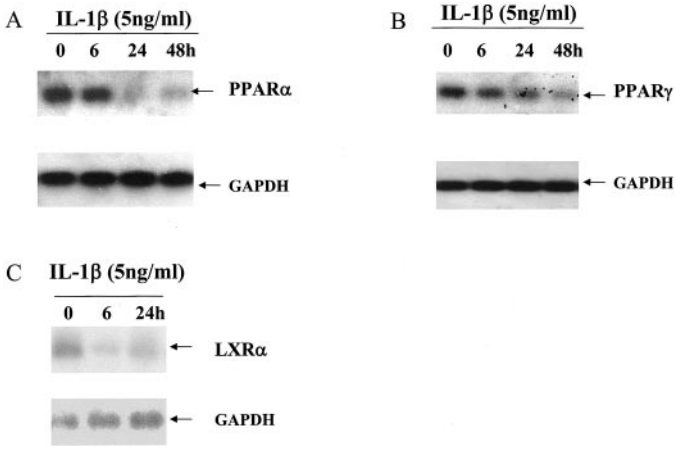

Figure 3. The effects of the inflammatory cytokine IL-1β on peroxisome proliferator-activated receptors (PPAR) and liver X receptorα (LXRα) gene expression. HMC were incubated in serum-free medium for 24 h, then the medium was changed to a fresh medium containing IL-1β (5 ng/ml) and cells were cultured for 0 (control), 6, 24, and 48 h (A, B, C). Both PPAR and LXRα mRNA were examined using RT-PCR followed by Southern blotting as described in Materials and Methods.

PPAR agonists also induced the expression of ABCA1 mRNA in non-lipid–loaded (Figure 9, A and B) and lipid-loaded HMC (Figure 10A), even in the presence of IL-1β (Figure 10B). Overexpression of PPARα or PPARγ also significantly increased ABCA1 gene expression induced by bezafibrate or PGJ2 (Figure 10A). These results suggest that PPAR induce ABCA1 gene expression through the LXRα pathway. Finally, we examined the effect of simultaneous activation of PPAR and LXRα on ABCA1 gene expression. 25-OHC alone induced ABCA1 gene expression in keeping with its effect on cholesterol efflux (Figure 10C). When activators of both PPAR and LXRα were used together, an additive effect on ABCA1 gene expression was observed (Figure 10C), suggesting that PPAR and LXRα may act jointly to control ABCA1 gene expression.

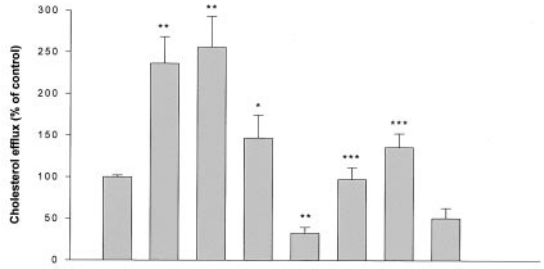

Figure 4. PPAR agonists increased apoA1-mediated cholesterol efflux from lipid-loaded HMC. Lipid-loaded HMC were treated with vehicle (control), bezafibrate (100 μM), Wy14643 (100 μM), or PGJ2 (10 μM) in the presence or absence of IL-1β (5 ng/ml) for 24 h. The cells were subsequently incubated in serum-free RPMI 1640 medium with or without 50 μg/ml of ApoA1 for 24 h. ApoA1-mediated [^{3}H] cholesterol efflux was measure as described in Materials and Methods. Results are expressed as a percentage of the untreated control. *$P < 0.05$ *versus* control, **$P < 0.001$ *versus* control, ***$P < 0.05$ *versus* IL-1 alone.

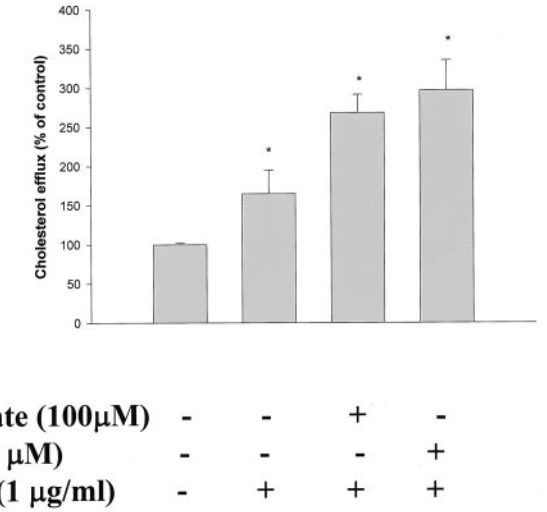

Figure 5. The effect of 25-OHC on ABCA1-mediated cholesterol efflux. Lipid-loaded HMC were treated with vehicle (control) or 25-OHC (1 μg/ml) in the presence or absence of bezafibrate (100 μM) or PGJ2 (10 μM) for 24 h. The cells were subsequently incubated in serum-free RPMI 1640 medium with or without 50 μg/ml of ApoA1 for 24 h. ApoA1-mediated [^{3}H] cholesterol efflux was measured. *$P < 0.001$ *versus* control.

Discussion

Our previous studies have shown that inflammatory cytokines promote lipid accumulation in human mesangial cells, both by inducing expression of type A scavenger receptor (6) and by overriding the feedback mechanisms by which intra-

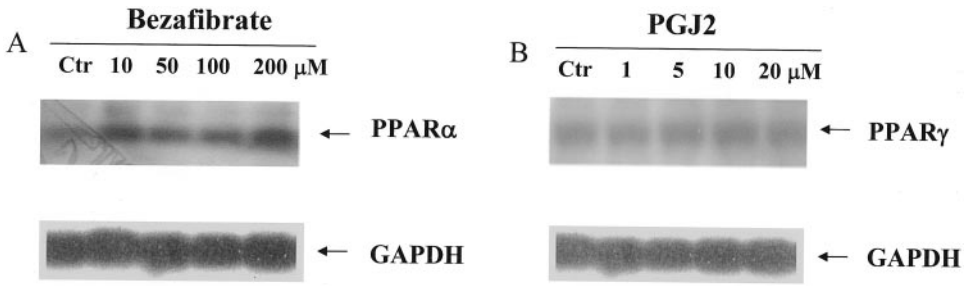

Figure 6. The effect of PPAR agonists on PPAR gene expression. HMC were incubated in serum-free medium with vehicle (control) or serum-free medium containing various concentrations of bezafibrate (10, 50, 100, 200 μM) or PGJ2 (1, 5, 10, 20 μM) for 24 h. The expression of both PPARα and PPARγ mRNA was examined respectively.

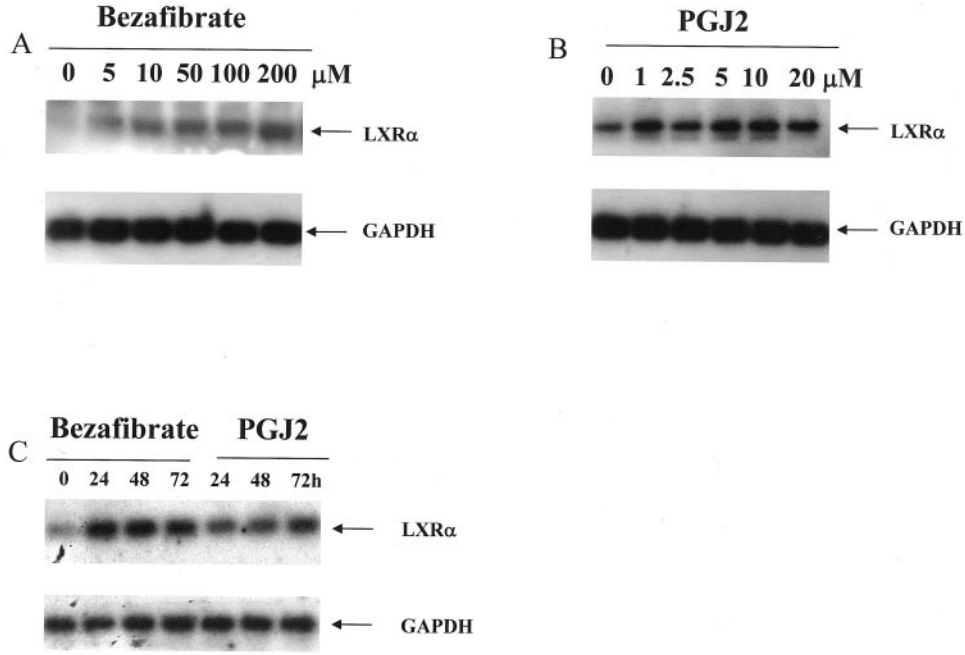

Figure 7. The effect of PPAR agonists on LXRα gene expression. HMC were incubated with vehicle (control), various concentration of bezafibrate (5, 10, 50, 100, 200 μM) or PGJ2 (1, 2.5, 5, 10, 20 μM) for 24 h (A and B), or the cells were incubated with 100 μM of bezafibrate or 10 μM of PGJ2 for different times (C). The expression of LXRα mRNA was examined.

cellular cholesterol concentration regulates LDL receptor expression (7). We have previously observed that exposure of mesangial cells to oxidized LDL (Ox-LDL), a natural ligand of the type A scavenger receptor (ScrA), induces both ScrA and CD36 (unpublished observations). Unlike the LDL receptor, expression of the scavenger receptor is not regulated by intracellular cholesterol concentration (5). Thus incubation of mesangial cells with Ox-LDL for 72 h achieves lipid loading. Using this method to pre-load HMC, we demonstrated that the inflammatory cytokine IL-1β also increased intracellular cholesterol by reducing cholesterol efflux.

Furthermore, we investigated the molecular mechanisms that may contribute to cytokine-induced dysregulation of cholesterol efflux. Several studies have confirmed that ABCA1 expression is induced by lipid-loading of macrophages, whereas studies using antisense oligonucleotides have supported the role of this protein in facilitating the efflux of cholesterol to extracellular apo A1 or to HDL. Moreover, fibroblasts derived from patients with Tangier disease (24) or from ABCA1 null mice (25) exhibit a 60% reduction in the rate of efflux of cholesterol and phospholipids to extracellular acceptors. Taken together, these observations indicate that ABCA1 activation is the first step in the reverse cholesterol transport pathway and regulates plasma levels of HDL, a lipoprotein that is recognized to protect against atherosclerosis (12,13). Defective efflux of cellular cholesterol is also thought to account for the low levels of plasma HDL observed in ABCA1 null mice and in familial HDL deficiency, an inherited condition in which an ABCA1 gene mutation has been described (11,13). Our results demonstrating that IL-1β inhibits ABCA1 mRNA expression provide a plausible mechanism by which this inflammatory cytokine might impair cellular cholesterol efflux. Inhibition of the ABCA1 pathway by inflammatory cytokines might also explain the clinical observation

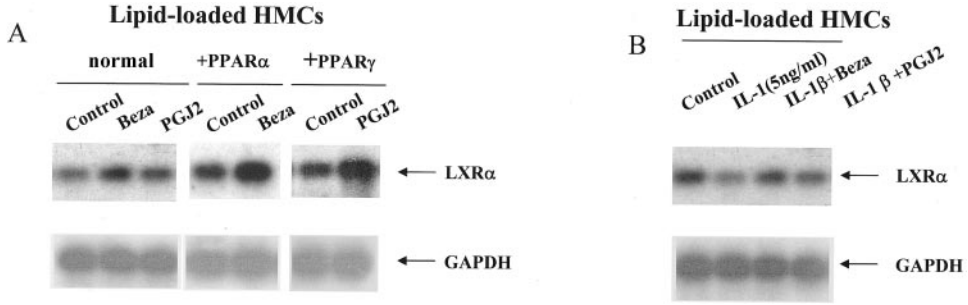

Figure 8. The lipid-loaded cells were treated with vehicle (control), bezafibrate (100 μM), or PGJ2 (10 μM) for 24 h (A). In addition, lipid-loaded HMC were also treated with IL-1β (5 ng/ml) in the absence or presence of bezafibrate (100 μM) or PGJ2 (10 μM) for 24 h (B). HMC were also transfected by PPARα or PPARγ expressive vectors. The transfected cells were loaded with lipid and treated with vehicle (control) or bezafibrate (100 μM) or PGJ2 (10 μM) for 24 h (A). The expression of LXRα mRNA was examined.

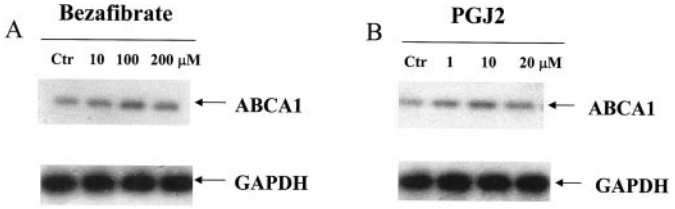

Figure 9. Both PPARγ and PPARα agonists induced ABCA1 mRNA expression in HMC. HMC were incubated in serum-free medium with vehicle (control) or in serum-free medium containing various concentrations of bezafibrate (10, 100, 200 μM) or PGJ2 (1, 10, 20 μM) for 24 h (A and B). The expression of ABCA1 mRNA was examined.

that patients with an activated inflammatory response have low circulating levels of apo A1 (26).

We next describe how IL-1β inhibits ABCA1 gene expression. PPAR control the expression of a number of genes involved with lipid metabolism, including ABCA1 (14); we therefore examined whether these ligand-activated nuclear receptors might be involved in cytokine-induced downregulation of the cholesterol efflux protein. There are three types of PPAR, termed α, δ (β), and γ, all of which belong to the nuclear receptor super family. PPARα is highly expressed in liver, heart, muscle, kidney,and cells of the arterial wall (such as endothelial cells, smooth muscle cell, and monocyte-derived macrophages) and can be activated by fatty acids, eicosanoids, and lipid-lowering drugs of the fibrate class; PPARγ has a much more restricted expression,and its ligands are prostaglandin J2, oxidized fatty acids, and thiazolidinedione (glitazone)-type antidiabetic agents (27,28). After binding to respective ligands in the nucleus, PPAR change conformation and interact with the peroxisome proliferator response element (PPRE), thereby promoting activation of target genes (18). We observed that IL-1β caused suppression of both PPARα and PPARγ mRNA expression over a time scale that would be consistent with their role in regulating ABCA1 gene transcription. This observation suggests that PPAR may mediate the inhibition of ABCA1 gene induced by IL-1β.

Importantly, we demonstrated that agonists of PPARα and PPARγ increased ABCA1 gene expression and cholesterol efflux, even in the presence of IL-1β, suggesting that PPAR activation may have a protective role in preventing lipid accumulation by overriding the reduction in cholesterol efflux induced by inflammatory cytokines. There is no recognized PPAR regulatory element in the ABCA1 gene promoter region; we therefore examined the role of other potential mediators. One obvious candidate was LXRα, which has been identified as an ABCA1 gene regulator (16) and has a PPAR response element (PPRE) in its gene promoter region (29). The involvement of the PPAR-LXRα-ABCA1 pathway in mediating cholesterol efflux has previously been reported (30). We therefore, investigated the role of the interaction of PPAR and LXRα in the regulation of ABCA1 gene expression. Our results show that both PPARα and PPARγ activation induced LXRα genes expression, even in the presence of the inflammatory cytokine IL-1β. 25-OHC, an LXRα agonist, also increased ABCA1 gene expression and cholesterol efflux in HMC. The induction of LXRα and ABCA1 genes was further increased when both PPARα and PPARγ gene were overexpressed in HMC. This is probably due to direct induction of LXRα gene transcription as a result of PPAR interacting with PPRE in the LXRα promoter (29). Finally, we examined the effect of the combination of PPAR and LXRα on ABCA1 gene expression. The combination of both PPAR and LXRα agonists had an additive effect in increased ABCA1 gene expression and cholesterol efflux. This would suggest that the effects of PPAR agonists on ABCA1 gene expression might be mediated by their stimulatory action on LXRα expression and activity. Thus PPAR and LXRα appear to act jointly to control ABCA1-mediated cholesterol efflux in HMC. To further clarify the relationship between PPAR and LXRα or PPAR and ABCA1 genes, PPAR or LXRα null cells will be required.

Taken together with our previous studies of mesangial cell lipid uptake, our results suggest that there may be several mechanisms that cause intracellular lipid accumulation in the presence of an inflammatory response. To avoid intracellular accumulation of lipid, there must be a functional balance between influx via lipoprotein receptors and efflux via the ABCA1 or other pathways. Inflammatory cytokines interfere with this balance, not only by promoting increased lipoprotein uptake by LDL and scavenger receptors (6,7), but also by

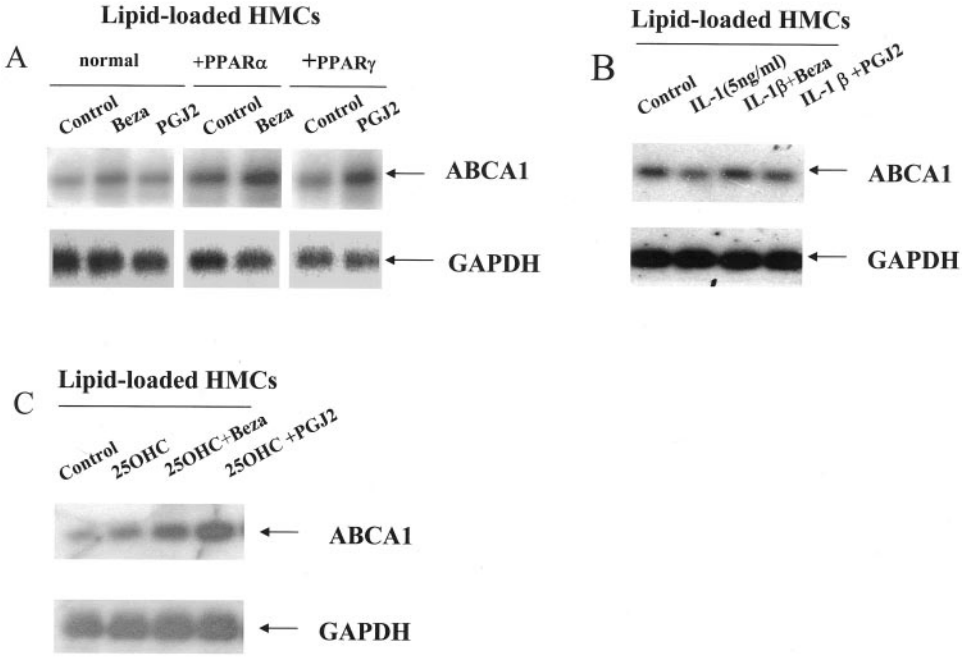

Figure 10. The lipid-loaded cells were treated with vehicle (control), bezafibrate (100 μM), or PGJ2 (10 μM) for 24 h (A). In addition, the lipid-loaded cells also were treated with vehicle (control) or IL-1β (5 ng/ml) in the absence (control) or presence of 100 μM of bezafibrate or 10 μM of PGJ2 for 24 h. The expression of ABCA1 mRNA was examined (B). HMC were also transfected by PPARα or PPARγ expressive vectors. The transfected cells were loaded with lipid. The cells then were treated with bezafibrate (100 μM) or PGJ2 (10 μM) for 24 h (A). The expression of ABCA1 mRNA was examined. Lipid-loaded HMC were also incubated with vehicle (control) or 25-OHC (1 μg/ml) in the absence or presence of 100 μM of bezafibrate or 10 μM of PGJ2 for 24 h (C), the ABCA1 gene expression was examined as described in Materials and Methods.

inhibiting ABCA1-mediated cholesterol efflux to HDL. Both PPARα and PPARγ activation can reset the level of intracellular cholesterol by increasing ABCA1 gene expression, even in the presence of inflammatory cytokines, thereby potentially inhibiting foam cell formation. Such anti-atherogenic actions of PPAR activation are in keeping with studies of cholesterol accumulation in macrophages (14,31) and in LDL receptor knockout mice (32). These data would imply that pharmacologic agents such as fibrates and thiazolidinediones, which function as PPAR agonists, may protect against intracellular lipid accumulation. This may help to explain the protective role of such agents in animal models of lipid-induced renal injury (33,34). Such findings may also be relevant to other disease processes characterized by intracellular lipid deposition such as atherosclerosis.

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