PPARα and PPARγ activators suppress the monocyte-macrophage apoB-48 receptor[1]

Go Haraguchi,* Yasushi Kobayashi,* Matthew L. Brown,† Akira Tanaka,§ Mitsuaki Isobe,* Sandra H. Gianturco,[2,†] and William A. Bradley[2,†]

Tokyo Medical and Dental University,* Department of Cardiovascular Medicine, Tokyo 113-8519, Japan; University of Alabama at Birmingham,† Department of Medicine, Division of Gerontology and Geriatric Medicine, Birmingham, AL 35294-0012; and Kanto Gakuin University,§ Department of Health and Nutrition, College of Human and Environmental Studies, Yokohama 236-8501, Japan

Abstract Certain triglyceride-rich lipoproteins (TRLs), specifically chylomicrons, dyslipemic VLDLs, and their remnants, are atherogenic and can induce monocyte-macrophage foam cell formation in vitro via the apolipoprotein B-48 receptor (apoB-48R). Human atherosclerotic lesion foam cells express the apoB-48R, as determined immunohistochemically, suggesting it can play a role in the conversion of macrophages into foam cells in vivo. The regulation of the apoB-48R in monocyte-macrophages is not fully understood, albeit previous studies indicated that cellular sterol levels and state of differentiation do not affect apoB-48R expression. Since peroxisome proliferator-activated receptors (PPARs) regulate some aspects of cellular lipid metabolism and may be protective in atherogenesis by up-regulation of liver X-activated receptor α and ATP-binding cassette transporter A1, we examined the regulation of apoB-48R by PPAR ligands in human monocyte-macrophages. Using real-time PCR, Northern, Western, and functional cellular lipid accumulation assays, we show that PPARα and PPARγ activators significantly suppress the expression of apoB-48R mRNA in human THP-1 and blood-borne monocyte-macrophages. Moreover, PPAR activators inhibit the expression of the apoB-48R protein and, notably, the apoB-48R-mediated lipid accumulation of TRL by THP-1 monocytes in vitro. If PPAR activators also suppress the apoB-48R pathway in vivo, diminished apoB-48R-mediated monocyte-macrophage lipid accumulation may be yet another anti-atherogenic effect of the action of PPAR ligands.—Haraguchi, G., Y. Kobayashi, M. L. Brown, A. Tanaka, M. Isobe, S. H. Gianturco, and W. A. Bradley. **PPARα and PPARγ activators suppress the monocyte-macrophage apoB-48 receptor. *J. Lipid Res.* 2003.** 44: **1224–1231.**

Supplementary key words lipoproteins • triglyceride • atherosclerosis • apolipoprotein B • foam cells • peroxisome proliferator-activated receptor

Elevated levels of plasma triglycerides (TGs) and VLDL, persistent dietary chylomicrons (CMs), and their remnants are risk factors for cardiovascular disease, a major cause of death worldwide (1). Atherogenesis involves both endothelial cell dysfunction and the appearance of lipid-filled foam cells, primarily of macrophage origin, in the arterial intima throughout the atherosclerotic process. The TG-rich lipoproteins (TRLs), hypertriglyceridemic (HTG) VLDL, CMs, and their remnants are the only native, nonmodified lipoproteins that cause rapid receptor-mediated macrophage lipid engorgement in vitro; normal VLDL and LDL do not (2, 3). Indeed, THP-1 monocyte-macrophages and native blood-borne macrophages show massive lipid accumulation (3- to 10-fold increase in TG mass) in ~4 h when exposed to physiological levels of these TRLs in vitro via the apolipoprotein B-48 receptor (apoB-48R) described below (4). Similar lipid accumulation occurs in vivo: humans with persistently elevated CMs have foam cells in their bone marrow, spleen, liver, and skin (5). As these TRLs also cause endothelial cell cholesterol uptake (6, 7) and fibrinolytic dysfunction in vitro (8), TRLs may be atherothrombogenic in subjects with elevated fasting and postprandial plasma TG.

TRLs interact with cells by many described mechanisms. HTG-VLDL and CM remnants bind to the LDL receptor and gene family members via apoE (9–12). Diet-derived TRLs lack the C-terminal domain of the apoB-100 that binds to the LDL receptor (13) and therefore cannot bind to the LDL receptor via apoB-48, the major apoB species formed in the intestine. Although the majority of CM is lipolyzed into remnants that are cleared by the liver via apoE (14, 15), a small but significant fraction of CM seemed to be cleared directly by reticuloendothelial cells,

Manuscript received 12 February 2003 and in revised form 21 March 2003.

Published, JLR Papers in Press, April 16, 2003.
DOI 10.1194/jlr.M300077-JLR200

[1] Portions of this work were presented in abstract form in: Haraguchi G., Y. Kobayashi, A. Tanaka, M. Isobe, M. L. Brown, W. A. Bradley, and S. H. Gianturco. 2001. Peroxisome proliferator-activated receptor-α and γ ligands inhibit expression of the monocyte-macrophage apolipoprotein B-48 receptor in human monocytes and THP-1 cells. *Circulation.* **104(17):** II-45.

[2] To whom correspondence should be addressed.
e-mail: wbradley@uab.edu, shg@ucb.edu

such as accessible macrophages in bone marrow and spleen, independent of apoE (16–18).

The apoB-48R is an apoE-independent receptor in human and murine monocyte-macrophages (19, 20). Transfection of the apoB-48R minigene into Chinese hamster ovary cells in vitro confers all the known properties of the apoB-48R characterized in human monocytes and macrophages, including converting these cells in vitro into a foam cell phenotype upon challenge with TRL, with identical kinetic and saturation characteristics as seen in macrophages (4). The apoB-48R binds apoB-48 of dietary TRL to a like domain of apoB-100 in HTG-VLDL (21) and may account, in part, for the observed direct reticuloendothelial uptake of CM in vivo (16–18), and for foam cell formation seen in humans with elevated TRL (5). Moreover, the apoB-48R likely participates in the foam cell formation in atherosclerosis-susceptible apoE-null mice, which have elevated apoB-48-containing lipoproteins (22–24).

Peroxisome proliferator activated receptors (PPARs) have been implicated in macrophage biology, lipid homeostasis, and atherogenesis. PPARs are ligand-activated transcription factors which, upon heterodimerization with the 9-*cis*-retinoic acid receptor, bind to specific peroxisome proliferator response elements (PPREs), thus regulating the expression of target genes involved in intra- and extracellular lipid metabolism. The naturally occurring prostaglandin 15-deoxy Δ12, 14-prostaglandin J2 (15-d-PGJ2), and the synthetic antidiabetic glitazones are ligands for PPARγ, while hypolipidemic fibrates are synthetic ligands for PPARα (25). PPARα is expressed in human monocytes and in fully differentiated macrophages (26), while PPARγ is expressed in cells undergoing differentiation into macrophages (27–30). In addition, both PPARα and PPARγ are detected in macrophage-rich regions of human atherosclerotic lesions (27, 31, 32). In macrophages, PPARs inhibit inflammatory cytokine-induced activation (33), promote apoptosis, and control lipid homeostasis through their effects on the expression of several key genes, including scavenger family members class A macrophage receptor (SR-A), class B scavenger receptor CD36, scavenger receptor class B type I, and cholesterol transporter ATP binding cassette transporter A1 (ABCA1) (28–30, 32). The relative contributions of these PPAR-regulated genes in atherosclerotic processes remain unclear, although on balance PPAR ligands appear to inhibit atherogenesis in animal models.

Since the apoB-48 receptor is involved in lipid metabolism in monocyte-macrophages, we hypothesized that PPAR might regulate its expression in macrophages. Here we report that PPAR ligands, somewhat surprisingly, suppress, rather than activate, expression of the macrophage apoB-48R mRNA and protein. Furthermore, our results indicate that the down-regulation of apoB-48R expression by PPAR activators in human monocyte-macrophages is accompanied by a parallel diminished cellular uptake of TRL, independent of apoE. If similar phenomena occur in vivo, the suppression of the apoB-48R pathway may contribute to the beneficial effects of PPAR agonists on atherogenesis by diminishing macrophage lipid accumulation via this pathway, particularly in hypertriglyceridemia.

METHODS

Reagents

Pioglitazone was kindly provided by Takeda Pharmaceutical Co., Osaka, Japan, and troglitazone was from Sankyo Pharmaceutical Co., Osaka, Japan. Wy14643 was purchased from Sigma (St. Louis, MO). 15-d-PGJ2 was from Cayman Chemical (Ann Arbor, MI).

Cells

THP-1 cells were maintained in RPMI-1640 containing 10% FBS, L-glutamine, penicillin (100 U/ml), and streptomycin (10 μg/ml). THP-1 monocytes were differentiated to macrophages by the addition of phorbol ester as previously described (20). Human peripheral blood-borne monocytes were isolated using Ficoll-Paque (Pharmacia Biotech, Uppsala, Sweden) (34) and then followed with the DYNAL monocyte-negative isolation kit (DYNAL A.S., Oslo, Norway) using the enriched monocyte fraction per the manufacturer's protocol.

Northern blot analyses

Total RNA (20 μg per sample) was separated by agarose gel electrophoresis, transferred to nylon membranes (4), and detected with human apoB-48R-, CD36-, and GAPDH-radiolabeled probes generated using RediPrime (Amersham Bioscience, Uppsala, Sweden) according to the manufacturer's instructions.

RNA isolation and real-time quantitative RT-PCR

Total RNA was isolated from the monocytes using RNAzol B (Tel-Test, Friendswood, TX). Genomic DNA was removed by treatment with RNase-free DNase. The method of RT-PCR is briefly outlined here. One microgram of total RNA was reverse-transcribed in 1× PCR buffer using 10 U/μl reverse transcriptase (Life Technologies, Grand Island, NY) in the presence of 5 mM $MgCl_2$, 1 mM dNTPs, 1 U/μl RNase inhibitor, and 2.5 mM random hexanucleotide primers. The RT reaction was carried out at 42°C for 50 min, followed by denaturation at 99°C for 5 min and cooling at 4°C for 5 min. The primers used in these studies are illustrated in **Table 1**. Quantitative real-time PCR was

TABLE 1. Primers for RT-PCR

Human apoB-48R	
Sense	GGC-CTT-AGA-GGC-AGC-CAA-AA
Antisense	TTC-CCA-GCT-TCT-CAG-CCT-CT
Human β-actin	
Sense	TCA-CCC-ACA-CTG-TGC-CCA-TCT-ACG-A
Antisense	CAG-CGG-AAC-CGC-TCA-TTG-CCA-ATG-G
Human ATP-binding cassette transporter A1	
Sense	TGT-CCA-GTC-CAG-TAA-TGG-TTC-TGT-GT
Antisense	GCG-AGA-TAT-GGT-CCG-GAT-TG

performed in a 20 μl reaction mixture (PCR kit from Roche Diagnostics) that contained 3 μl of reverse-transcribed cDNA, 5 μM forward primers, and 5 μM reverse primers. For each sample, triplicate analyses were performed. The resulting relative increase in reporter fluorescent dye emission, SYBR Green I, was monitored by the LightCycler system (Roche) using a protocol to reduce primer-dimer background interference (35). The level of the apoB-48R and ABCA1 mRNA, relative to human β-actin, was calculated. The data are expressed as the ratio of target mRNA (relative to internal control) obtained from cells pretreated with PPAR ligands relative to mRNA obtained from cells treated with vehicle only.

Western blot analysis of human apoB-48R

Monocytes (~10^6 cells) were disrupted in the lysis buffer containing 50 mM Tris-HCl (pH 8.0), 150 mM NaCl, 0.1 mM EDTA, 1% Triton X-114, and incubated with rotation for 30 min. After centrifugation of the sample to remove insoluble debris, the supernatant was used as total protein cell lysate. Twenty-five micrograms of protein were applied to SDS-PAGE gels and immunoblotted as described (36). Rabbit polyclonal antibody against human apoB-48R was used as previously described (4). Mouse anti-actin monoclonal antibody was from Chemicon International, Temecula, CA. Visualization of specific proteins was detected using enhanced chemiluminescence (Amersham Pharmacia Biotech, Newark, NJ).

TRL uptake analysis in THP-1 cells

THP-1 monocytes were subcultured into 6-well plates and grown in the presence of phorbol ester to induce adherence as previously described (20). After 2 days, cells were washed and incubated with fresh medium containing buffer (control), DMSO (vehicle control), or the PPAR ligands PGJ2 at 10 μM or Wy14643 at 100 μM for 24 h at 37°C. At the end of this incubation period, cells were washed and the test lipoproteins, trypsinized VLDL Sf 100-400, were added at the levels indicated and further incubated for 4 h at 37°C, as previously published (20). Cells were then washed thoroughly with cold PBS to remove lipoproteins and the wells analyzed for TG and protein content as previously described (20). Duplicate analyses, corrected for no-cell controls, for each lipoprotein concentration were determined. Values from each well differed by <10% and three independent experiments were performed.

RESULTS

PPARγ activators suppress apoB-48R expression in THP-1 monocytes

To determine whether PPARγ activators regulate apoB-48R expression in human monocytes, we evaluated apoB-48R expression at the mRNA level and protein level in the human monocytic leukemic cell line, THP-1, previously used to characterize, purify, and clone the receptor (4). THP-1s were incubated with several PPAR activators for up to 24 h prior to analyses. Real-time RT-PCR analysis (**Fig. 1**) showed that treatment of cells with the PPARγ ligand 15-d-PGJ2 at 10 μM resulted in an ~95% decrease in mRNA levels of apoB-48R (Fig. 1A). At 20 μM of 15-d-PGJ2, the expression of apoB-48R was almost abolished. At lower 15-d-PGJ2 concentrations, 0.1 and 1.0 μM, the levels of apoB-

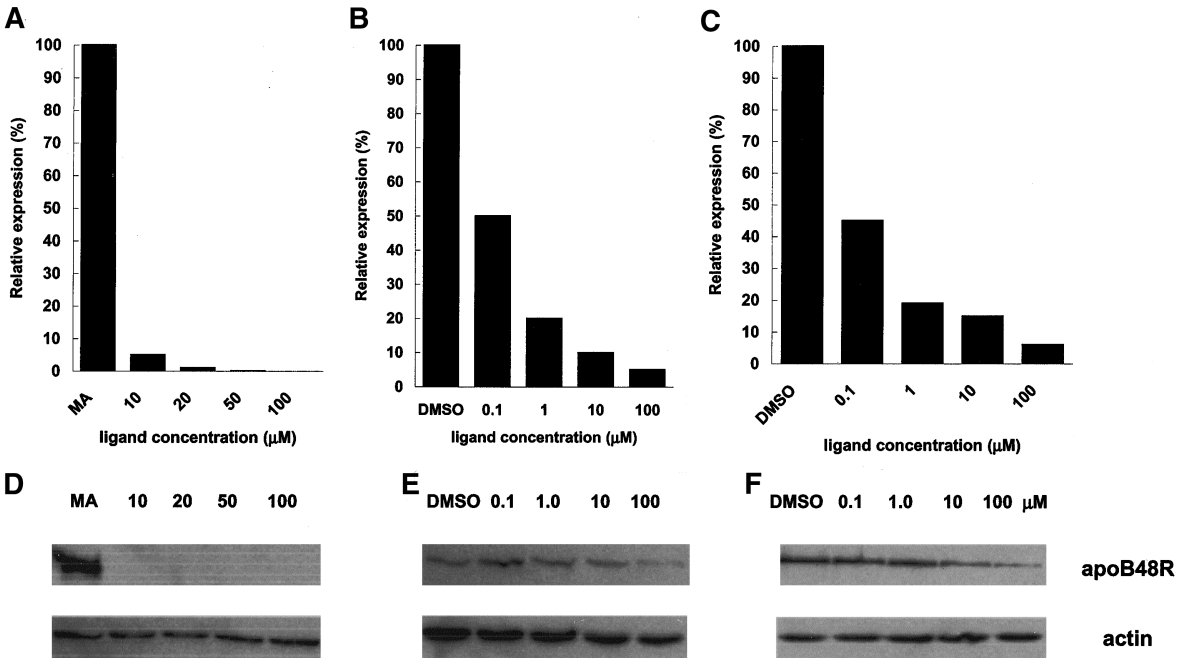

Fig. 1. Peroxisome proliferator activated receptor γ (PPARγ) activators suppress apolipoprotein B-48 receptor (apoB-48R) mRNA and protein expression in THP-1 monocyte-macrophages. THP-1 monocyte-macrophages were grown as described in Methods and treated with vehicles methyl acetate (MA; control), DMSO (control), or 15-deoxy Δ12, 14-prostaglandin J2 (15-d-PGJ2) (in MA), troglitazone, or pioglitazone (in DMSO) at levels indicated for 24 h. Total RNA was isolated and real-time RT-PCR, using primers shown in Table 1 as described in Methods, quantified mRNA expression of human apoB-48R. A: 15-d-PGJ2. B: Troglitazone. C: Pioglitazone. Values represent expression of apoB-48R mRNA relative to β-actin mRNA at each level of PPAR ligand, with 100% representing the control/vehicle only. Total protein extracts (25 μg) were used for Western blot analysis and visualized by enhanced chemiluminescence as described in Methods. D: 15-d-PGJ2. E: Troglitazone. F: Pioglitazone. Expression of actin (lower panel) was used as a control.

48R mRNA expression were 60% and 40%, respectively (unpublished observations). The PPARγ activators, troglitazone and pioglitazone, also suppressed the level of apoB-48R mRNA in a dose-dependent manner with ~50% reduction at 0.1 μM (Fig. 1B, C). Northern blot analysis corroborated the PCR analysis, indicating similar suppression of apoB-48R mRNA at these PPAR activator concentrations (unpublished observations). The time course of mRNA suppression was determined at 100 μM PPAR activators by densitometric analysis of Northern blots and indicated that 15-d-PGJ2 was the most effective agonist, with a reduction in mRNA to 67% at 3 h, 6% at 6 h, and not detectable at 24 h relative to control (100%; relative to GAPDH). Likewise, but later and less effective, troglitazone and pioglitazone showed no lowering of mRNA levels until 6 h with ⩾50% and >90% mRNA reductions at 24 h, respectively. Western blot analysis also showed that 15-d-PGJ2 at a low concentration (10 μM) almost completely abolished apoB-48R protein expression in the same time frame (Fig. 1D). Furthermore, and consistent with the mRNA data, addition of the synthetic PPARγ ligands troglitazone and pioglitazone at 100 μM, suppressed apoB-48R protein expression by >50% (Fig. 1E, F).

PPARα activator suppresses apoB-48R expression in THP-1 monocytes

We then examined whether a PPARα activator also regulated apoB-48R expression in THP-1 monocytes at both the mRNA level and the protein level (**Fig. 2**). Real-time RT-PCR analysis showed that treatment of cells with the PPARα ligand Wy14643 at 25 μM resulted in an ~40% reduction of apoB-48R mRNA levels and a ~90% reduction at 50 μM (Fig. 2A). Northern blot analysis showed similar suppression of apoB-48R mRNA by Wy14643 (unpublished observations). Western blot analysis (Fig. 2B), on the other hand, revealed that Wy14643 (50 μM) reduced the expression of apoB-48R protein in THP-1 monocytes by only ~50% relative to actin, as determined by densitometry, suggesting that the mRNA pool was turning over more rapidly than the apoB-48R protein.

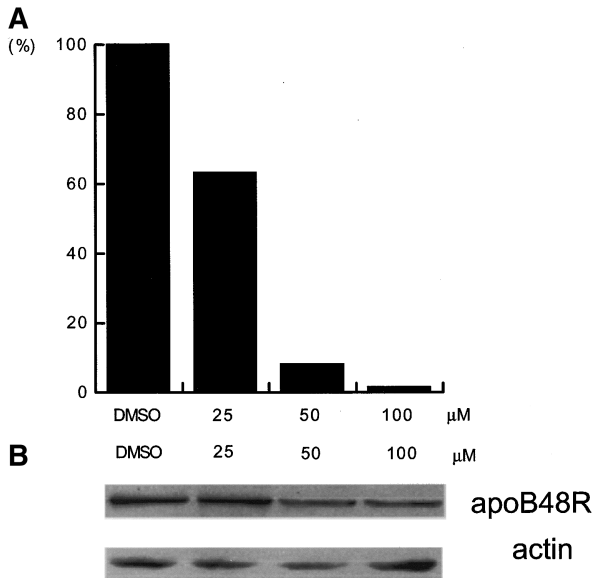

Fig. 2. The PPARα activator Wy14643 suppresses apoB-48R expression in THP-1 monocytes. A: THP-1 cells were treated with DMSO (control) and Wy14643 at the levels for 24 h, and total RNA was isolated from the cells. Expression of human apoB-48R mRNA was quantified by real-time RT-PCR. Expression of β-actin was determined as an internal control. Values represent expression of apoB-48R mRNA relative to β-actin mRNA at each level of PPAR ligand, with 100% representing the control/vehicle only. B: For Western blotting, total cell protein extracts (25 μg) were analyzed for apoB-48R protein as described in the legend of Fig. 1. Expression of actin was determined as an internal control.

PPARγ and PPARα activators suppress apoB-48R protein expression in human peripheral blood-borne monocytes

To demonstrate that the regulation of apoB-48R by PPARα and PPARγ activators seen in the human leukemic monocytic cell line THP-1 was more universal and potentially valid in vivo, we also determined and observed similar regulation of apoB-48R protein expression in human native peripheral blood-borne monocytes (**Fig. 3**). Western blot analysis showed that 15-d-PGJ2 (10 μM) treatment for 24 h almost completely abolished the apoB-48R protein expression in the monocyte-macrophages (Fig. 3A), similar to effects in THP-1 monocyte-macrophages. Troglitazone (50 μM) and pioglitazone (50 μM) suppressed apoB-48R protein expression to a lesser extent, by ~60% and 40%, respectively, relative to actin controls, as determined by densitometry (Fig. 3B, C). The PPARα activator Wy14643 (100 μM) also suppressed apoB-48R protein expression (Fig. 3D).

PPARγ activators up-regulate ABCA1 and CD36 mRNA expression in human monocyte-macrophages

To demonstrate that the PPAR activators were working properly in our hands, since apoB-48R expression was inhibited by the PPAR activators rather than enhanced, we monitored the previously documented enhanced expres-

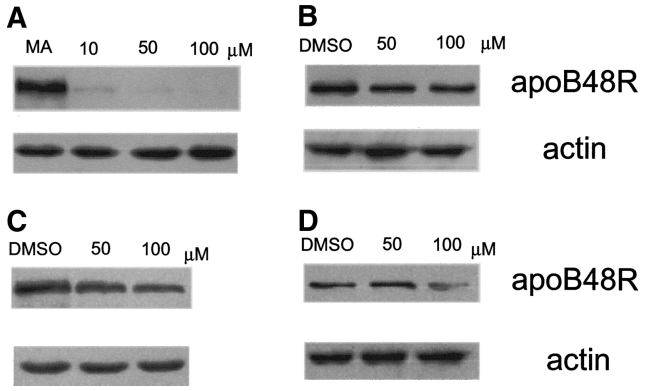

Fig. 3. PPARγ and -α activators suppress apoB-48R protein expression in human blood-borne monocyte-macrophages. Cells were isolated as described in Methods and incubated with MA, DMSO (control), and the indicated concentrations of 15d-PGJ2, troglitazone, pioglitazone, and Wy14643 for 24 h. Total protein extracts (25 μg) were used for Western blot analysis as described in Methods. A: 15-d-PGJ2. B: Troglitazone. C: Pioglitazone. D: Wy14643. Expression of actin was determined as a control.

sion of CD36/FAT and ABCA1 in the human THP-1 monocytes (30, 37, 38). Under the conditions shown above that inhibit apoB-48R expression in THP-1 monocytes, mRNA analyses demonstrated that the PPARγ ligands 15-d-PGJ2, troglitazone, and pioglitazone increased CD36 and ABCA1 mRNA expression levels, as expected, in a dose-dependent fashion. **Figure 4A** represents the densitometric analyses of the CD36 Northern blots relative to a GAPDH internal control: at ⩾10 μM, 15-d-PGJ2 caused a 2-fold increase in CD36 mRNA levels, while troglitazone increased mRNA levels ~4-fold at the maximal level of drug tested (100 μM), and pioglitazone increased CD36 mRNA levels ~3.2-fold at both 10 μM and 100 μM. Moreover, as seen in Fig. 4B, using an alternate technique for measuring mRNA levels, real-time RT-PCR treatment of THP-1 monocytes with PPARγ ligands also increased ABCA1 mRNA levels in a dose-dependent manner, as reported earlier (30, 37, 38). Here, the ABCA1 mRNA levels were increased maximally ~5-fold upon treatment with 50 μM and 100 μM 15-d-PGJ2, and ~4-fold and 6-fold with 100 μM troglitazone and pioglitazone, respectively. As with CD36, ABCA1 mRNA levels were increased by lower levels of pioglitazone than troglitazone, with significant increases at 0.1 μM and 10 μM. These data indicate that the PPARγ ligands were indeed functioning as previously described and that the inhibition of apoB-48R expression was not due to nonspecific mechanisms.

PPARγ and PPARα activators suppress TGRLP uptake of monocytes through apoB-48R

In the present study, we found PPARα and PPARγ activators significantly suppressed apoB-48R mRNA and protein expression in monocyte-macrophages in vitro. Therefore, we next determined whether these PPARγ and PPARα activators also regulate the functional endpoint of the receptor pathway, the cellular TG accumulation induced by the apoE-independent uptake of TRL (4, 20). THP-1 macrophages were preincubated for 24 h with either 15-d-PGJ2 (10 μM) or Wy14643 (100 μM), and then tested for lipid accumulation with the model ligand, trypsinized-VLDL Sf 100–400, at physiological concentrations previously shown to cause rapid and saturable lipid (TG) accumulation in THP-1 cells (20) and in apoB-48R-transfected CHOs (4, 24). The data suggest PPARγ and

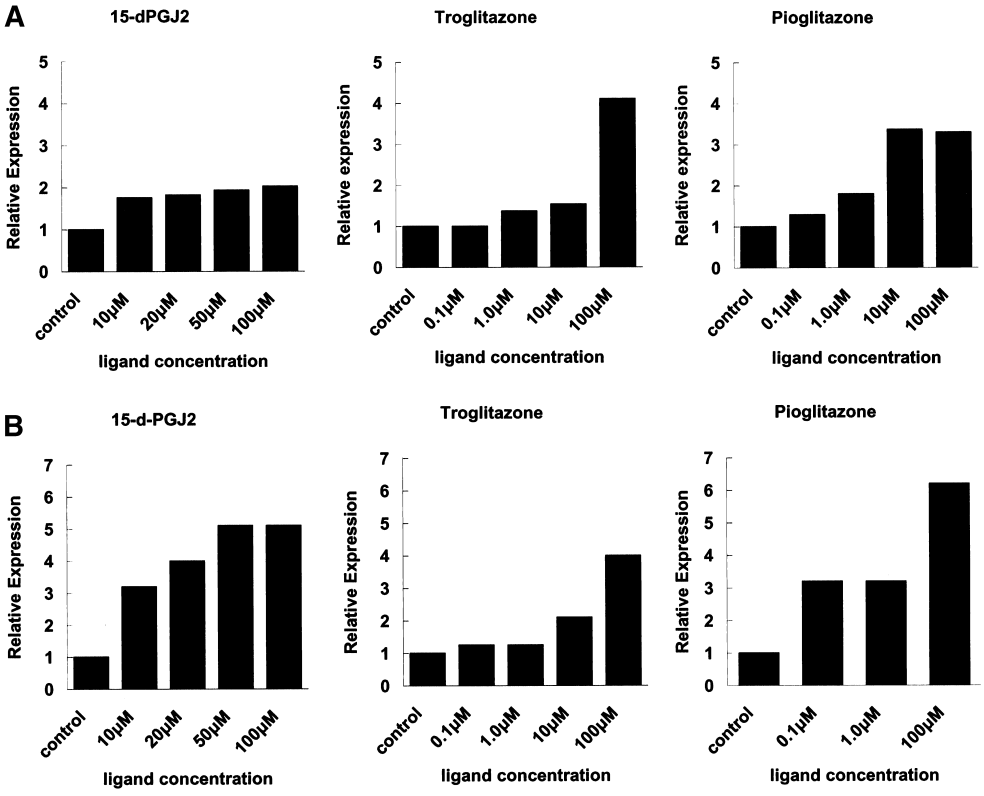

Fig. 4. As a positive control, under conditions that decrease expression of apoB-48R, PPARγ activators increased class B scavenger receptor CD36 and ATP binding cassette transporter A1 (ABCA1) mRNA expression in THP-1 monocyte-macrophages. THP-1 cells were incubated at levels of 15-d-PGJ2, troglitazone, and pioglitazone. A: Total RNA was isolated and Northern blot and densitometric analyses carried out as described in Methods. Expression of GAPDH was determined as an internal control. Values represent expression of CD36 mRNA relative to GAPDH mRNA at each level of PPAR ligand with 1.0 representing the control/vehicle only. B: Total RNA was isolated as described in Methods and expression of human ABCA1 mRNA was quantified by real-time RT-PCR as described in the legend of Fig. 1 and Methods. Expression of β-actin was used as an internal control. Values represent expression of ABCA1 mRNA relative to β-actin mRNA at each level of PPAR ligand with 1.0 representing the control/vehicle only.

PPARα activators have a key role in TG uptake in monocyte-macrophages via apoB-48R regulation. As seen in **Fig. 5**, cells not pretreated (open circles) or pretreated with vehicle only (closed circles) showed significant, rapid, saturable accumulation of TG mass per milligram of cell protein in the short 4 h incubation period over the indicated concentration range of the apoE-free surrogate ligand. Strikingly, and consistent with the effect of PPARs seen on mRNA and protein levels of apoB-48R in these cells, the TG accumulation was reduced by more than 50% by both 15-d-PGJ2 (Fig. 5, closed triangles) and Wy14643 (Fig. 5, closed squares), demonstrating that functionally the PPAR ligands also serve to reduce lipid accumulation through the apoB-48R pathway.

DISCUSSION

We had previously shown that the apoB-48R is not regulated by sterol content or state of differentiation of human and murine monocyte-macrophages. The new findings reported here showing that apoB-48R is suppressed by PPARγ, and PPARα activators represent the first report of the down-regulation of this unique lipoprotein receptor. In Fig. 1, we show that the native PPARγ activator, 15-d-PGJ2, lowers by >95% the level of apoB-48R mRNA in THP-1 monocytes, which is reflected by an almost complete loss of immunochemically detectable apoB-48R protein at this level (10 μM) of activator (Fig. 1D). Importantly, the insulin-sensitizing PPARγ activators thiazolidinediones, troglitazone, and pioglitazone also significantly reduce the mRNA expression levels of apoB-48R in a dose- (Fig. 1B, C) and time-dependent manner (data stated in Results) with an ~50% reduction of apoB-48R mRNA at the lowest levels of drug tested (0.1 μM). The thiazolidinediones did not appear to be as effective as the prostaglandins in lowering apoB-48R protein levels, which might reflect either an inability to efficiently enter the cells under our conditions or an inherent difference in the potency of the PPARγ agonists.

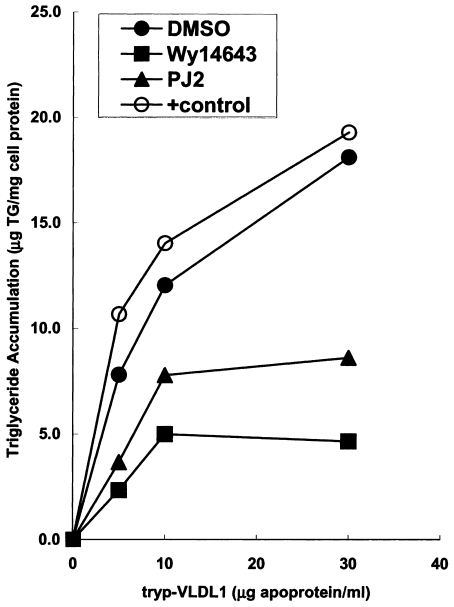

Fig. 5. PPARγ and PPARα activators suppress the apoB-48R pathway: the functional endpoint of triglyceride-rich lipoproteins-induced lipid accumulation is inhibited in THP-1 macrophages. THP-1 macrophages were preincubated with PPARγ activator, 15-d-PGJ2 (10 μM), PPARα activator Wy14643 (100 μM), DMSO, or buffer controls for 24 h. Cells were washed and incubated with the model ligand (apoE-negative), tryp-VLDL (S_f 100–400), at the concentrations indicated for 4 h at 37°C. The cells were harvested and TG mass and protein quantified as described in Methods. Open circles indicate no treatment; closed circles, DMSO vehicle; closed triangles, 10 μM 15-d-PGJ2; and closed squares, 100 μM Wy14643. Values represent the micrograms of TG per mg cell protein (the average of duplicate dishes, which differ by <10%). The experiment shown is representative of three independent experiments.

We next tested whether the PPARα activator Wy14643, a fibric acid, also regulates apoB-48R expression. In the current study, Wy14643 is shown to suppress the expression of mRNA (Fig. 2A) and protein levels (Fig. 2B) of apoB-48R in human THP-1 monocytes in vitro, and to significantly diminish lipid accumulation in the macrophages (Fig. 5). As stated in Results, the level of mRNA is suppressed by ~90% at 50 μM and by >98% at 100 μM Wy14643, whereas the protein is decreased to ~50% at both concentrations of agonist. The reason for this difference is not known; however, the ability of the THP-1s to accumulate TG under these conditions was also ~50% of control. A possible explanation might be a metabolic pool of apoB-48R protein that turns over more slowly than does the apoB-48R mRNA.

We also tested whether the PPAR activator effects, seen in the human monocytic cell lines, are evident in native, human blood-borne monocyte-macrophages. Using protein expression as the endpoint, PPARγ activators 15-d-PGJ2, troglitazone, and pioglitazone all caused a decreased expression of the apoB-48R in these primary cell cultures. ApoB-48R protein expression decreased by ~93% at 10 μM 15-d-PGJ2; troglitazone and pioglitazone demonstrated ~40% and 60% decreased expression, respectively, at 50 μM. We interpret these data to suggest that this mechanism potentially occurs in vivo.

We further compared apoB-48R expression levels relative to other reported macrophage proteins putatively involved in the atherosclerotic process. The known up-regulation of ABCA1 with PPAR activation is considered beneficial since the macrophage uses this route to efflux cholesterol from the cell in the first step of the reverse cholesterol transport pathway (39). Indeed, under the conditions where we found significantly decreased expression of apoB-48R, there was an opposite 2- to 4-fold increase in ABCA1 mRNA levels, suggesting opposing but beneficial effects of the PPAR activators on these two regulators of macrophage cellular lipid content.

It is widely believed that macrophage scavenger receptors SR-A, CD36, and CD 68 are major routes of uptake for lipid accumulation in arterial macrophages (40–42). However, for lipid recruitment to occur, these receptors require that the lipoproteins they bind first be modified in some fashion and/or the receptor be induced by cytokines

or by transcriptional ligands for PPARs or liver X-activated receptors (43). The apoB-48R receptor, however, is expressed in both monocytes and differentiated macrophages (4, 20) and would be available in the initial stages for receptor-mediated uptake and accumulation of lipid from dietary lipoproteins or from elevated levels of VLDLs in certain dyslipemic individuals. Even when the cells accumulate extensive cholesterol, the levels of the apoB-48R do not diminish, thereby allowing continued uptake of these deleterious lipoproteins by this pathway (20). It is also anticipated that if oxidized lipid is essential for the atherosclerotic process, the apoB-48R may provide yet another pathway for these lipids to enter the cell by delivering exogenous or endogenous oxidized lipid carried by TRL.

Finally, our in vitro data indicate that the functional endpoint of the apoB-48R pathway, i.e., the rapid, saturable lipid accumulation by macrophages induced by TRL ligands of this receptor, is substantially diminished upon treatment with either PPARγ or PPARα activators (Fig. 5). Thus, the finding that the PPAR activators lower the expression of apoB-48R in monocyte-macrophages and thereby diminish macrophage lipid accumulation may represent yet another beneficial effect of PPAR activator therapy for atherosclerosis.

The authors thank Prof. Fujio Numano of Tokyo Medical and Dental University for continued encouragement, and Ruiling Song of the University of Alabama at Birmingham for excellent technical assistance. This study was supported in part by a grant-in-aid (No. 10877108) from the Ministry of Education, Science, Sports, and Culture of Japan (Y.K.); a grant from Pfizer (M.L.B.); and National Institutes of Health Grants HL-44480 (S.H.G.) and HL-64156 (W.A.B).

REFERENCES

1. Hokanson, J. E., and M. A. Austin. 1996. Plasma triglyceride level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. *J. Cardiovasc. Risk.* **3:** 213–219.
2. Brown, M. S., and J. L. Goldstein. 1983. Lipoprotein metabolism in the macrophage: implications for cholesterol deposition in atherosclerosis. *Annu. Rev. Biochem.* **52:** 223–261.
3. Gianturco, S. H., W. A. Bradley, A. M. Gotto, J. D. Morrisett, and D. L. Peavy. 1982. Hypertriglyceridemic very low density lipoproteins induce triglyceride synthesis and accumulation in mouse peritoneal macrophages. *J. Clin. Invest.* **70:** 168–178.
4. Brown, M. L., M. P. Ramprasad, P. K. Umeda, A. Tanaka, Y. Kobayashi, T. Watanabe, H. Shimoyamada, W. L. Kuo, R. Li, R. Song, W. A. Bradley, and S. H. Gianturco. 2000. A macrophage receptor for apolipoprotein B48: cloning, expression, and atherosclerosis. *Proc. Natl. Acad. Sci. USA.* **97:** 7488–7493.
5. Fredrickson, D. S., J. L. Goldstein, and M. S. Brown. 1978. The familial hyperlipoproteinemias. *In* The Metabolic Basis of Inherited Diseases. J. G. Stanbury, M. F. Wyngaarden, and D. S. Fredrickson, editors. McGraw-Hill, New York. 604–655.
6. Gianturco, S. H., S. G. Eskin, L. T. Navarro, C. J. Lahart, L. C. Smith, and A. M. Gotto. 1980. Abnormal effects of hypertriacylglycerolemic very low-density lipoproteins on 3-hydroxy-3-methylglutaryl-CoA reductase activity and viability of cultured bovine aortic endothelial cells. *Biochim. Biophys. Acta.* **618:** 143–152.
7. Fielding, C. J., I. Vlodavsky, P. E. Fielding, and D. Gospodarowicz. 1979. Characteristics of chylomicron binding and lipid uptake by endothelial cells in culture. *J. Biol. Chem.* **254:** 8861–8868.
8. Li, X-N., J. C. Kroon, R. L. Benza, J. M. Parks, V. K. Varma, W. A. Bradley, S. H. Gianturco, K. B. Taylor, J. R. Grammer, E. M. Tabengwa, and F. M. Booyse. 1996. Hypertriglyceridemic VLDL decreases plasminogen binding to endothelial cells and surface-localized fibrinolysis. *Biochemistry.* **35(19):** 6080–6088.
9. Gianturco, S. H., A. M. Gotto, S. L. Hwang, J. B. Karlin, A. H. Lin, S. C. Prasad, and W. A. Bradley. 1983. Apolipoprotein E mediates uptake of Sf 100–400 hypertriglyceridemic very low density lipoproteins by the low density lipoprotein receptor pathway in normal human fibroblasts. *J. Biol. Chem.* **258:** 4526–4533.
10. Bradley, W. A., S. L. Hwang, J. B. Karlin, A. H. Lin, S. C. Prasad, A. M. Gotto, and S. H. Gianturco. 1984. Low-density lipoprotein receptor binding determinants switch from apolipoprotein E to apolipoprotein B during conversion of hypertriglyceridemic very-low-density lipoprotein to low-density lipoproteins. *J. Biol. Chem.* **259:** 14728–14735.
11. Beisiegel, U., W. Weber, G. Ihrke, J. Herz, and K. K. Stanley. 1989. The LDL-receptor-related protein, LRP, is an apolipoprotein E-binding protein. *Nature.* **341:** 162–164.
12. Kowal, R. C., J. Herz, J. L. Goldstein, V. Esser, and M. S. Brown. 1989. Low density lipoprotein receptor-related protein mediates uptake of cholesteryl esters derived from apoprotein E-enriched lipoproteins. *Proc. Natl. Acad. Sci. USA.* **86:** 5810–5814.
13. Yang, C. Y., S. H. Chen, S. H. Gianturco, W. A. Bradley, J. T. Sparrow, M. Tanimura, W. H. Li, D. A. Sparrow, H. DeLoof, M. Rosseneu, F. S. Lee, Z. W. Gu, A. M. Gotto, and L. Chan. 1986. Sequence, structure, receptor-binding domains and internal repeats of human apolipoprotein B-100. *Nature.* **323:** 738–742.
14. Havel, R. J. 1985. George Lyman Duff memorial lecture. Role of the liver in atherosclerosis. *Arteriosclerosis.* **5:** 569–580.
15. Brown, M. S., and J. L. Goldstein. 1986. A receptor-mediated pathway for cholesterol homeostasis. *Science.* **232:** 34–47.
16. Nagata, Y., and D. B. Zilversmit. 1987. Blockade of intestinal lipoprotein clearance in rabbits injected with Triton WR 1339-ethyl oleate. *J. Lipid Res.* **28:** 684–692.
17. Hussain, M. M., R. W. Mahley, J. K. Boyles, M. Fainaru, W. J. Brecht, and P. A. Lindquist. 1989. Chylomicron-chylomicron remnant clearance by liver and bone marrow in rabbits: factors that modify tissue-specific uptake. *J. Biol. Chem.* **264:** 9571–9582.
18. Hussain, M. M., R. W. Mahley, J. K. Boyles, P. A. Lindquist, J. W. Brecht, and T. L. Innerarity. 1989. Chylomicron metabolism. Chylomicron uptake by bone marrow in different animal species. *J. Biol. Chem.* **264:** 17931–17938.
19. Gianturco, S. H., A. H. Lin, S. L. Hwang, J. Young, S. A. Brown, D. P. Via, and W. A. Bradley. 1988. Distinct murine macrophage receptor pathway for human triglyceride-rich lipoproteins. *J. Clin. Invest.* **82:** 1633–1643.
20. Gianturco, S. H., M. P. Ramprasad, A. H. Lin, R. Song, and W. A. Bradley. 1994. Cellular binding site and membrane binding proteins for triglyceride-rich lipoproteins in human monocyte-macrophages and THP-1 monocytic cells. *J. Lipid Res.* **35:** 1674–1687.
21. Gianturco, S. H., M. P. Ramprasad, R. Song, R. Li, M. L. Brown, and W. A. Bradley. 1998. Apolipoprotein B-48 or its apolipoprotein B-100 equivalent mediates the binding of triglyceride-rich lipoproteins to their unique human monocyte-macrophage receptor. *Arterioscler. Thromb. Vasc. Biol.* **18:** 968–976.
22. Plump, A. S., J. D. Smith, T. Hayek, K. Aalto-Setala, A. Walsh, J. G. Verstuyft, E. M. Rubin, and J. L. Breslow. 1992. Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell.* **71:** 343–353.
23. Zhang, S. H., R. L. Reddick, J. A. Piedrahita, and N. Maeda. 1992. Spontaneous hypercholesterolemia and arterial lesions in mice lacking apolipoprotein E. *Science.* **258:** 468–471.
24. Brown, M. L., K. Yui, J. D. Smith, R. C. LeBoeuf, W. Weng, P. K. Umeda, R. Li, R. Song, S. H. Gianturco, and W. A. Bradley. 2002. The murine macrophage apoB-48 receptor gene (Apob-48r): homology to the human receptor. *J. Lipid Res.* **43:** 1181–1191.
25. Barbier, O., I. P. Torra, Y. Duguay, C. Blanquart, J. C. Fruchart, C. Glineur, and B. Staels. 2002. Pleiotropic actions of peroxisome proliferator-activated receptors in lipid metabolism and atherosclerosis. *Arterioscler. Thromb. Vasc. Biol.* **22:** 717–726.
26. Wahli, W., O. Braissant, and B. Desvergne. 1995. Peroxisome proliferator activated receptors: transcriptional regulators of adipogenesis, lipid metabolism and more. *Chem. Biol.* **2:** 261–266.
27. Ricote, M., A. C. Li, T. M. Willson, C. J. Kelly, and C. K. Glass. 1998. The peroxisome proliferator-activated receptor-gamma is a negative regulator of macrophage activation. *Nature.* **391:** 79–82.

28. Li, A. C., K. K. Brown, M. J. Silvestre, T. M. Willson, W. Palinski, and C. K. Glass. 2000. Peroxisome proliferator-activated receptor gamma ligands inhibit development of atherosclerosis in LDL receptor-deficient mice. *J. Clin. Invest.* **106:** 523–531.
29. Tontonoz, P., L. Nagy, J. G. Alvarez, V. A. Thomazy, and R. M. Evans. 1998. PPARgamma promotes monocyte/macrophage differentiation and uptake of oxidized LDL. *Cell.* **93:** 241–252.
30. Chinetti, G., S. Lestavel, V. Bocher, A. T. Remaley, B. Neve, I. P. Torra, E. Teissier, A. Minnich, M. Jaye, N. Duverger, H. B. Brewer, J. C. Fruchart, V. Clavey, and B. Staels. 2001. PPAR-alpha and PPAR-gamma activators induce cholesterol removal from human macrophage foam cells through stimulation of the ABCA1 pathway. *Nat. Med.* **7:** 53–58.
31. Marx, N., G. Sukhova, C. Murphy, P. Libby, and J. Plutzky. 1998. Macrophages in human atheroma contain PPARgamma: differentiation-dependent peroxisomal proliferator-activated receptor gamma(PPARgamma) expression and reduction of MMP-9 activity through PPARgamma activation in mononuclear phagocytes in vitro. *Am. J. Pathol.* **153:** 17–23.
32. Chinetti, G., F. G. Gbaguidi, S. Griglio, Z. Mallat, M. Antonucci, P. Poulain, J. Chapman, J. C. Fruchart, A. Tedgui, J. Najib-Fruchart, and B. Staels. 2000. CLA-1/SR-BI is expressed in atherosclerotic lesion macrophages and regulated by activators of peroxisome proliferator-activated receptors. [In Process Citation] *Circulation.* **101:** 2411–2417.
33. Jiang, C., A. T. Ting, and B. Seed. 1998. PPAR-gamma agonists inhibit production of monocyte inflammatory cytokines. *Nature.* **391:** 82–86.
34. Peper, R. J., W. Z. Tina, and M. M. Mickelson. 1968. Purification of lymphocytes and platelets by gradient centrifugation. *J. Lab. Clin. Med.* **72:** 842–848.
35. Chou, Q., M. Russell, D. E. Birch, J. Raymond, and W. Bloch. 1992. Prevention of pre-PCR mis-priming and primer dimerization improves low-copy-number amplifications. *Nucleic Acids Res.* **20:** 1717–1723.
36. Ramprasad, M. P., R. Li, W. A. Bradley, and S. H. Gianturco. 1995. Human THP-1 monocyte-macrophage membrane binding proteins: distinct receptor(s) for triglyceride-rich lipoproteins. *Biochemistry.* **34:** 9126–9135.
37. Moore, K. J., E. D. Rosen, M. L. Fitzgerald, F. Randow, L. P. Andersson, D. Altshuler, D. S. Milstone, R. M. Mortensen, B. M. Spiegelman, and M. W. Freeman. 2001. The role of PPAR-gamma in macrophage differentiation and cholesterol uptake. *Nat. Med.* **7:** 41–47.
38. Chawla, A., Y. Barak, L. Nagy, D. Liao, P. Tontonoz, and R. M. Evans. 2001. PPAR-gamma dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation. *Nat. Med.* **7:** 48–52.
39. Chen, W., D. L. Silver, J. D. Smith, and A. R. Tall. 2000. Scavenger receptor-BI inhibits ATP-binding cassette transporter 1-mediated cholesterol efflux in macrophages. *J. Biol. Chem.* **275:** 30794–30800.
40. Kodama, T., P. Reddy, C. Kishimoto, and M. Krieger. 1988. Purification and characterization of a bovine acetyl low density lipoprotein receptor. *Proc. Natl. Acad. Sci. USA.* **85:** 9238–9242.
41. Endemann, G., L. W. Stanton, K. S. Madden, C. M. Bryant, R. T. White, and A. A. Protter. 1993. CD36 is a receptor for oxidized low density lipoprotein. *J. Biol. Chem.* **268:** 11811–11816.
42. Boullier, A., D. A. Bird, M. K. Chang, E. A. Dennis, P. Friedman, K. Gillotre-Taylor, S. Horkko, W. Palinski, O. Quehenberger, P. Shaw, D. Steinberg, V. Terpstra, and J. L. Witztum. 2001. Scavenger receptors, oxidized LDL, and atherosclerosis. *Ann. N. Y. Acad. Sci.* **947:** 214–222.
43. Li, A. C., and C. K. Glass. 2002. The macrophage foam cell as a target for therapeutic intervention. *Nat. Med.* **8:** 1235–1242.