Peroxisome Proliferator–Activated Receptor Activators
Target Human Endothelial Cells to Inhibit
Leukocyte–Endothelial Cell Interaction

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Abstract—An early event in acute and chronic inflammation and associated diseases such as atherosclerosis and rheumatoid arthritis is the induced expression of specific adhesion molecules on the surface of endothelial cells (ECs), which subsequently bind leukocytes. Peroxisome proliferator–activated receptors (PPARs), members of the nuclear receptor superfamily of transcription factors, are activated by fatty acid metabolites, peroxisome proliferators, and thiazolidinediones and are now recognized as important mediators in the inflammatory response. Whether PPAR activators influence the inflammatory responses of ECs is unknown. We show that the PPAR activators 15-deoxy-
\(\Delta^{12,14}\)-prostaglandin J2 (15d-PGJ2), Wyeth 14643, ciglitazone, and troglitazone, but not BRL 49653, partially inhibit the induced expression of vascular cell adhesion molecule-1 (VCAM-1), as measured by ELISA, and monocyte binding to human aortic endothelial cells (HAECs) activated by phorbol 12-myristate 13-acetate (PMA) or lipopolysaccharide. The “natural” PPAR activator 15d-PGJ2 had the greatest potency and was the only tested molecule capable of partially inhibiting the induced expression of E-selectin and neutrophil-like HL60 cell binding to PMA-activated HAECs. Intracellular adhesion molecule-1 induction by PMA was unaffected by any of the molecules tested. Both PPAR-\(\alpha\) and PPAR-\(\gamma\) mRNAs were detected in HAECs by using reverse transcription–polymerase chain reaction and a ribonuclease protection assay; however, we have yet to determine which, if any, of the PPARs are mediating this process. These results suggest that certain PPAR activators may help limit chronic inflammation mediated by VCAM-1 and monocytes without affecting acute inflammation mediated by E-selectin and neutrophil binding. (Arterioscler Thromb Vasc Biol. 1999;19:2094-2104.)

Key Words: peroxisome proliferator–activated receptors ■ endothelial cells ■ adhesion molecules ■ inflammation

A key step in the inflammatory response is adhesion of leukocytes to endothelial cells (ECs) lining the blood vessels, with subsequent extravasation of the leukocytes into the involved tissue.1 ECs are stimulated by inflammatory agents to express selectins, such as endothelial-leukocyte adhesion molecule-1 (E-selectin), which interact with carbohydrate ligands on leukocytes, and to express immunoglobulin superfamily proteins, such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1), which interact with \(\alpha_4\beta_1\) integrin receptors and \(\beta_2\) integrin receptors on leukocytes, respectively.1

Acute inflammation is the short-term response to bacterial infections and injuries, whereas chronic inflammation is the longer-term response to sustained infections, chronic injury, or foreign bodies.2 When uncontrolled, chronic inflammation may lead to a variety of common, serious ailments such as atherosclerosis, arthritis, inflammatory bowel disease, and fibrotic lung disease. However, acute inflammation is essential for survival against bacterial infections. Development of new therapeutic agents that preserve the beneficial effects of acute inflammation while controlling the devastating effects of chronic inflammation would have major clinical value. Nonsteroidal anti-inflammatory agents and glucocorticoids achieve this goal to some degree, but with significant side effects. Although both acute and chronic forms of inflammation involve the endothelial-leukocyte interactions described above, E-selectin and neutrophils are associated with acute inflammation, and VCAM-1 and monocytes are associated more closely with chronic inflammation.3

Peroxisome proliferator–activated receptors (PPARs), members of the nuclear receptor superfamily,4 have a newly recognized role in inflammation.5,6 All 3 subtypes, \(\alpha, \delta,\) and \(\gamma\), are ligand-activated transcription factors. PPAR-\(\alpha\) and PPAR-\(\gamma\) are known to regulate the expression of genes

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involved in lipid metabolism. PPARs combine with the retinoid X receptor-α (RXR-α) to form a functional heterodimer bound to specific DNA sequences within the promoters of target genes. Activating ligands for PPARs are semi-selective for the subtypes, and selectivity is ligand-concentration and cell-type dependent. 7 The so-called “natural or endogenous ligand” prostaglandin 15-deoxy-DΔ12,14-prostaglandin J2 (15d-PGJ2), is γ-selective at low concentrations but activates α at higher levels. Many of the eicosanoids, certain nonsteroidal anti-inflammatory drugs, and the long-chain fatty acids examined are activators of all PPAR subtypes, whereas peroxisome proliferators (such as Wyeth 14643) are considered α-selective at low concentrations. The most selective γ-ligands examined are the insulin-sensitizing drugs, the thiazolidinediones (such as ciglitazone, troglitazone, and BRL 49653). Ligands for RXR-α, such as 9-cis-retinoic acid, can induce similar responses to PPAR ligands by activating the PPAR/RXR-α heterodimer.8

Although PPAR-α is predominantly localized to the liver where it orchestrates β-oxidation of long-chain fatty acids and PPAR-γ is expressed predominantly in adipose tissue where it induces differentiation and triglyceride synthesis,4 PPARs are also now known to be expressed in both the vasculature and in leukocytes.9–16 Indeed, Ricote et al9 and Jiang et al10 recently demonstrated that activation of PPAR-γ inhibited inflammatory cytokine production by stimulated monocytes/macrophages. Importantly, Marx et al11 found that PPAR-γ activation in monocytes/macrophages prevented induction of matrix metalloproteinases, which are associated with atherosclerotic plaque rupture. Staels et al12 detected PPAR-α in human aortic smooth muscle cells and found that an α-selective activator (Wyeth 14643) inhibited an inflammatory response in these cells. These authors further showed that patients receiving fenofibrate (also an α-activator) for lipid lowering had lower plasma C-reactive protein, fibrinogen, and interleukin-6 concentrations, all of which are markers of inflammation.13 Though not related specifically to the vasculature, Devchand et al14 showed that the inflammatory mediator leukotriene B4 is a ligand for PPAR-α and that its activation of PPAR-α leads to catabolism of leukotriene B4, which may ultimately limit the inflammatory response. These important findings are consistent with a protective effect of PPAR activators against complications of atherosclerosis, such as myocardial infarction caused by plaque rupture.

However, Nagy et al14 and Tontonoz et al15 showed that PPAR-γ activators promote monocyte differentiation into macrophages and promote uptake of lipids by the scavenger receptor, thus leading to increased foam cell formation. These results suggest the converse, that PPAR-γ activators would promote development and growth of atherosclerotic plaque.

There is preliminary evidence suggesting that ECs express PPARs.16 However, it is not known what effect PPAR activators have on ECs and whether the former can affect even earlier stages of atherogenesis and other chronic inflammatory diseases, specifically leukocyte-EC interaction. This information would be critical in predicting the effects of PPAR activators on chronic inflammatory diseases. In this study, we show that PPAR activators directly affect leukocyte interaction with ECs, partly through preventing upregulation of adhesion molecules by ECs in response to inflammatory stimuli. Most synthetic PPAR activators tested inhibited only the chronic inflammatory mediators—monocyte binding and VCAM-1 expression—and not the acute inflammatory mediators—neutrophil binding and E-selectin expression. These results suggest that PPAR activators may be beneficial in ameliorating chronic inflammatory disease such as atherosclerosis by reducing extravasation of monocytes to the involved tissue without limiting the response to acute infection.

Methods
Cell culture materials were purchased from Fischer Scientific and FBS was from Hyclone Laboratories. Lipopolysaccharide (LPS) and phorbol 12-myristate 13-acetate (PMA) were obtained from Sigma Chemical Co. Molecular biology reagents were purchased from Stratagene, and oligonucleotides were synthesized by Gibco BRL. Anti–adhesion molecule monoclonal antibodies were obtained from Biosource. Radiolabel was obtained from Amersham. PPAR activators were purchased from Biomol and Cayman Chemical Co except for BRL 49653 (a kind gift from SmithKline Beecham, King of Prussia, Pa) and troglitazone (kindly provided by Parke Davis, Ann Arbor, Mich).

Cell Culture
Human aortic ECs (HAECs) at passages 4 to 7 were cultured as previously described.18 Primary human monocytes were obtained by a modification of the Recalde method.19 The neutrophil-like cell line, human promyelocytic leukemic cells (HL60), were obtained from the American Type Culture Collection (Manassas, Va) and cultured in RPMI medium with 10% FBS.

Leukocyte Adhesion Assay
Leukocytes were added to control or activated HAECs, and after a 10-minute incubation at 37°C, unbonded leukocytes were removed by washing, the cells were fixed with 4% paraformaldehyde, and the number of bound leukocytes was determined by light microscopy as previously described.20 HAEC activation was achieved by incubation with 5 to 50 nmol/L PMA (Sigma) or 1 ng/mL LPS (Sigma) for 4 hours. PPAR activators were added 18 hours before HAEC activation and/or during the 4-hour activation period. The activators were initially prepared as 1000× stock solutions in dimethyl sulfoxide and then added to an appropriate volume of medium at 37°C. The samples were mixed and incubated for 30 minutes at 37°C, after which an appropriate aliquot was placed on the cells in each 48-well plate. Extensive washing before addition of leukocytes removed both PPAR activators and the EC activator (PMA or LPS), thus precluding interaction of these molecules with the leukocytes.

As a validation, we directly treated monocytes with the PPAR activators over a range of concentrations and found no effect on binding to activated ECs for the 10-minute adhesion-time period used in these experiments (data not shown).

Adhesion Molecule Expression
The expression of VCAM-1, E-selectin, and ICAM-1 on control and on PMA- or LPS-activated HAECs was quantified by ELISA as previously described.21 In brief, at 95% confluence in 96-well dishes, PPAR activators were added to HAECs 18 hours before activation and/or during the 4-hour activation period. The cells were washed and incubated with anti–VCAM-1 (1:100), anti–E-selectin (1:500), or anti–ICAM-1 (1:500) antibody for 2 hours on ice. Subsequently, the cells were fixed with 4% paraformaldehyde and incubated for 1 hour with secondary antibody conjugated to alkaline phosphatase at room temperature (for VCAM-1, 1:2000; E-selectin, 1:2000; and ICAM-1, 1:5000 dilution) and developed with p-nitrophenyl phosphate substrate (Sigma). The colorimetric assay was quantified using a Molecular Devices plate reader. Because the cells were not permeabilized, this ELISA detected cell surface–expressed protein.

RNA Analysis
Total RNA was isolated from confluent monolayers of HAECs with Trizol reagent (Gibco BRL) according to the manufacturer’s direc-
tions. RNA (2 µg) was reverse-transcribed (RT) as previously described.22 Polymerase chain reaction (PCR) was performed using 3.5 µL of cDNA, 100 ng of each primer (synthesized by Gibco BRL), 14 µmM dNTPs, and 1.75 U of fFru DNA polymerase (Stratagene) in a 50-µL reaction mix with a Perkin-Elmer Gene Amp PCR System 2400. The sense (5’-CCATTTGGCCCAACCAAC-3’) and antisense (5’-CTGAAAACGCCAGTACTG-3’) primers were capable of amplifying both human PPAR-γ1 and -γ2 isoforms,23 thereby producing a product of 479 bp. The sense (5’-CATCAGGAGCAACGGCTTTCACC-3’) and antisense (5’-GTTCCTCAAGTGGCCTGTAG-3’) primers were used for amplification of human PPAR-α24 producing a product of 450 bp. Each cycle consisted of 45 seconds at 96°C, 45 seconds at 62°C, and 2 minutes at 72°C for 35 cycles, terminating with 5 minutes at 72°C. A 15-µL aliquot was electrophoresed on a 1.5% agarose gel and visualized by an ethidium bromide stain. The size of PCR products was confirmed by comparison with a 1-kb DNA ladder (Stratagene). PPAR band intensities were normalized to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) levels, amplified as previously described.23

Northern blot analysis was performed for the detection of VCAM-1 mRNA expression by using a VCAM-1 cDNA probe (a kind gift of Dr Myron Cybulsky, Department of Pathology, Brigham and Women’s Hospital, Boston, Mass). In brief, total RNA (12 µg) isolated from confluent HAEC monolayers, left untreated or treated with LPS (100 µg/mL) in the presence or absence of Wyeth 14463 (100 µmol/L), was run on a formaldehyde/agarose gel. Fractionated RNA was transferred to a Duralon-UV membrane (Stratagene) and subsequently hybridized with a random primed labeled (Prime-It II, Stratagene) cDNA probe and Rapid-Hyb buffer (Amersham) according to the manufacturers’ recommendations at 65°C for 2.5 hours. The blot was subsequently washed once at room temperature in 2× SSC (1× SSC is 0.3 mol/L NaCl, 0.03 mol/L sodium citrate), 0.1% SDS; once at 65°C in 0.5× SSC, 0.1% SDS; and once at 65°C in 0.1× SSC, 0.1% SDS, with each wash requiring 15 minutes. After exposure overnight on film (Hyperfilm MP, Amersham), band intensity was determined by densitometry and normalized to the level of mRNA of the human acidic ribosomal phosphoprotein PO (36B4).25

The solution-hybridization RNase protection assay was performed to determine PPAR-γ and RXR-α expression in HAECs and human primary monocytes as previously described.26 In brief, antisense RNA probes were prepared from pGEM cloning vectors containing partial cDNA probes for PPAR-γ (kindly provided by Dr Jeffrey Flter, Harvard University, Cambridge, Mass)26,27 or RXR-α (kindly provided by Dr Sunil Naggal, Allergen Inc, Irvine, Calif). Labeled probes were incubated with RNA (10 µg) for 12 hours at 50°C and then digested with RNase A (90 µg/mL) and RNase T1 (600 U/mL) for 1 hour at 45°C. Samples were electrophoresed on 6% nondenaturing polyacrylamide gels. Protected bands of 348 (PPAR-γ), 285 (PPAR-γ), or 101 (RXR-α) bp were detectable. RNA from control or PMA-activated (5 µmol/L, 4 hours) human monocytes was used as a positive control for PPAR-γ expression.2,3,13,16

Statistical Analysis
Computer-assisted statistical analyses were performed using the ANOVA program, and probability values were calculated using Fisher’s protected least significant difference test. A value of P<0.05 was considered significant.

Results
PPAR Activators Inhibit Monocyte Binding and VCAM-1 Expression
We chose PMA and LPS to activate HAECs, and under our experimental conditions, PMA (1 and 5 µmol/L) and LPS (1 ng/mL) induced a 3- to 4-fold increase in the binding of monocytes to HAECs. This induction was partially blocked by the synthetic PPAR activators ciglitazone, troglitazone, Wyeth 14643, and the natural PPAR activator 15d-PGJ2 (Figures 1A and 2A). The synthetic PPAR activator BRL 49653 had no effect on monocyte binding (Figure 2A). These findings were mirrored by the effects on expression of VCAM-1 protein, an adhesion molecule implicated in EC binding of monocytes.1,3 The induction of VCAM-1 expression on activated HAECs was partially blocked by the PPAR activators with the exception of BRL 49653 (Figures 1B and 2B). The approximate relative effectiveness was similar to that for monocyte binding, such that 15d-PGJ2 (10 µmol/L) > ciglitazone (60 µmol/L) > Wyeth 14643 (100 µmol/L) > troglitazone (15 µmol/L) = ciglitazone (30 µmol/L). The PPAR activators alone did not significantly increase or decrease the protein expression levels of the adhesion molecules relative to control untreated cells (data not shown). To examine whether PPAR activation could affect VCAM-1 mRNA levels, we performed Northern blot analysis, which showed that the induction in VCAM-1 mRNA levels on activation by LPS could be partially inhibited by treatment with 100 µmol/L Wyeth 14643 (39.4±4.9% inhibition versus LPS alone, Figure 3). This inhibition was comparable to the reduced induction in VCAM-1 protein expression after LPS activation in the presence of Wyeth 14643 (53.3±11.5% inhibition versus LPS alone, Figure 2B). Thus, the PPAR activators tested were capable of reducing monocyte binding to activated HAECs by partially inhibiting the induction of VCAM-1 expression.

PPAR Activators Fail to Inhibit Neutrophil Binding and E-Selectin Expression
To determine whether the synthetic PPAR activators also affect neutrophil binding, we performed the same experiments as above after replacing the monocytes with the neutrophil-like cell line HL60. In contrast to their effects on monocyte binding, the synthetic PPAR activators failed to inhibit, and in some cases further increased, the induced binding of HL60 cells to PMA-activated HAECs (ciglitazone 30 µmol/L, 45.8±15.4% increased binding; ciglitazone 60 µmol/L, 58.7±14.1% increased binding over PMA alone; Wyeth 14643, 34.6±16.3% increase; and troglitazone, 11.4±13.2% decreased binding versus PMA alone; Figure 4A). A similar trend was observed for protein levels of E-selectin (Figure 4B), an adhesion molecule implicated in the binding of neutrophils by ECs.1,3 The synthetic PPAR activators tested therefore block the induction in monocyte binding and VCAM-1 expression but not neutrophil binding and E-selectin expression.

Greater Potency of 15d-PGJ2
The prostaglandin-like PPAR ligand 15d-PGJ2, a metabolite of PGD2, blocked the induction of E-selectin protein expression (56.6±2.0% inhibition versus PMA alone), unlike the synthetic PPAR activators tested (Figure 4B). To investigate the activity of 15d-PGJ2 further, we applied more stringent assay conditions. Instead of preincubating HAECs with PPAR activators, cells were exposed to PPAR activators only during the 4-hour PMA activation period. Moreover, a higher dose of PMA (50 nmol/L) was utilized. Under these conditions, 15d-PGJ2 partially blocked the induction of monocyte binding (75.2±7.7% inhibition versus PMA alone, Figure 5A) and VCAM-1 protein expression (72.7±2.2% inhibition versus PMA alone, Figure 5B), whereas ciglitazone, Wyeth 14643, troglitazone, and BRL 49653 were ineffective. Fur-
thermore, 15d-PGJ₂ partially blocked the induction of HL60 binding (84.2±5.1% inhibition versus PMA alone, Figure 6A) and E-selectin protein expression (46.1±6.7% inhibition versus PMA alone, Figure 6B), whereas ciglitazone, Wyeth 14643, troglitazone, and BRL 49653 were ineffective. Therefore, 15d-PGJ₂ appears to have a much greater potency than any of the other molecules tested.

PPAR Activators Fail to Inhibit ICAM-1 Expression

The level of expression of ICAM-1 protein on unactivated control HAECs was greater than that for the other adhesion molecules examined, but the levels were induced 2- to 3-fold by PMA activation (data not shown). The synthetic PPAR activators ciglitazone (30 µmol/L) and troglitazone (15 µmol/L) and the natural activator 15d-PGJ₂ (5 µmol/L) were incapable of blocking the induction in ICAM-1 protein expression in HAECs activated by 5 to 50 nmol/L PMA, even under preincubation conditions (data not shown). The PPAR activator effects were therefore rather specific among adhesion molecules.

Expression of PPARs in ECs

The ribonuclease protection assay detected low levels of PPAR-γ₁ mRNA but no PPAR-γ₂ mRNA in HAECs (Figure 7A). Human monocytes expressed relatively high levels of PPAR-γ₁ mRNA and detectable PPAR-γ₂ mRNA after stimulation by PMA (Figure 7A), in agreement with previous studies. The level of RXR-α was approximately equivalent in HAECs, monocytes, and PMA-activated monocytes (Figure 7A). The mRNA level of PPAR-γ (both γ₁ and γ₂ isoforms together) was compared with PPAR-α in HAECs by semiquantitative RT-PCR. The expression of PPAR-α was greater than that of PPAR-γ in HAECs (Figure 7B), and PPAR-γ expression was lower in HAECs than in human monocytes (as shown by the ribonuclease protection assay). GAPDH expression was approximately equivalent in HAECs and monocytes (Figure 7B). No PCR product was detected
when cDNA derived from mouse 3T3-L1 adipocytes was used as a template for the human PPAR-γ and PPAR-α primers, thus demonstrating the specificity of this system (Figure 7B).

**Discussion**

These results indicate that some synthetic PPAR activators (structures shown in Figure 8), such as a peroxisome proliferator and certain thiazolidinediones, inhibit monocyte binding by activated HAECs and their expression of VCAM-1, an adhesion molecule relatively specific for monocyte and lymphocyte adhesion and chronic inflammation. These activators showed no effect on neutrophil binding by activated HAECs or their expression of E-selectin, an adhesion molecule associated with acute inflammation. A natural PPAR activator, 15d-PGJ₂, a prostaglandin metabolite of PGD₂, inhibited both monocyte and neutrophil binding concomitant with the inhibition of both induced VCAM-1 and E-selectin expres-
These results further fuel the discussion on the positive versus negative aspects of PPAR activation on atherosclerosis.

Induction of adhesion molecules by stimuli such as LPS and various cytokines is mediated by signal transduction pathways that subsequently regulate the activity of transcription factors. There are various binding sites for oxidation, proliferation, and inflammation-related transcription factors in the promoter elements of the adhesion molecule genes. The transcription factor nuclear factor-κB (NF-κB) is critical for the induction of adhesion molecules; however, it works in concert with other transcription factors that are specific for each adhesion molecule. Ricote et al. postulated that PPAR-γ activation inhibited the activity of transcription factors such as NF-κB, activating protein-1, and STAT, subsequently inhibiting the inducible form of NO synthase, gelatinase B, and scavenger receptor A expression in activated monocytes. Such a molecular mechanism may apply here; however, NF-κB is required for induction of all of the adhesion molecules examined in this study. So why do we observe the specific inhibition of VCAM-1? We can only speculate that because NF-κB is a dimeric complex that can comprise a range of distinct subunits, such as p50 (NF-κB1), p52 (NF-κB2), p65 (Rel A), c-Rel, and Rel B, the mechanism for the specificity of the synthetic PPAR activators may be due to an inhibitory interaction with a specifically composed NF-κB complex or specific auxiliary transcription factors on the VCAM-1 promoter. A similar specificity of inhibition of adhesion molecule expression was previously observed after EC treatment with all-trans-retinoic acid or certain antioxidant molecules. Troglitazone contains a vitamin E moiety that endows it with some antioxidant properties, perhaps partially accounting for its activity in this study. The other PPAR activators used in our study have no reported antioxidant activity. In addition, using an assay that detects lipid hydroperoxide formation (in LDL, in this

**Figure 4.** Effect of synthetic PPAR activators on neutrophil binding and E-selectin expression of HAECs activated by PMA. Confluent HAECs were preincubated for 18 hours with PPAR activators (PPAR-A) ciglitazone (30 or 60 μmol/L; Cig30, Cig60), troglitazone (15 μmol/L; Tro15), Wyeth 14643 (100 μmol/L; Wy100), BRL 49653 (10 μmol/L; BRL10), or 15d-PGJ2 (10 μmol/L; PGJ10) and then incubated along with PMA (1 or 5 nmol/L) during a 4-hour activation period. Neutrophil binding was assessed using HL60 cells (A), and ELISA for E-selectin was performed (B) using the conditions as described in the legend to Figure 1. A, *P<0.001 vs PMA alone; B, *P<0.001 vs 5 nmol/L PMA or #1 nmol/L PMA alone. HPF indicates high-power field; O.D., optical density.
case), we found that although troglitazone prevented lipid hydroperoxide formation in LDL, the activators Wyeth 14643, ciglitazone, and 15d-PGJ2 at the concentrations used in the adhesion experiments did not (data not shown). This result suggests that an antioxidant effect is not a major factor accounting for the observed effects of PPAR activators. The mechanism by which certain PPAR activators induce E-selectin expression and HL60 binding to ECs in our study may also pertain to specific interactions with the transcription factor complexes on the promoter or with the promoter itself. Further studies are required to define the mechanism(s) involved.

Downregulation of adhesion molecule expression for therapeutic purposes has followed a number of strategies. Monoclonal antibodies to adhesion molecules were shown to significantly reduce leukocyte binding,39 and oligonucleo-

Figure 5. Effect of the natural PPAR activator 15d-PGJ2 on monocyte binding and VCAM-1 expression of HAECs activated by PMA. A, Confluent HAECs were cotreated during a 4-hour activation period with high-dose PMA (50 nmol/L) with PPAR activators (PPAR-A) ciglitazone (30 μmol/L; Cig30), Wyeth 14643 (10 μmol/L; Wy10), or 15d-PGJ2 (5 μmol/L; PGJ5). HAECs were washed and incubated with 4×10^5 human monocytes for 10 minutes. Cells were washed and fixed with glutaraldehyde, and the number of adherent monocytes per high-power field (HPF) was determined. Data are mean±SD of 12 HPFs from 1 experiment performed in quadruplicate wells and are representative of 3 other experiments. *P<0.001 vs PMA alone. B, ELISA was performed with anti–VCAM-1 antibody as described in Methods after cotreatment of HAECs with PMA (50 nmol/L) and PPAR activators (PPAR-A) Wyeth 14643 (100 μmol/L; Wy100), troglitazone (15 μmol/L; Tro15), BRL 49653 (10 μmol/L; BRL10), or 15d-PGJ2 (5 μmol/L; PGJ5) for 4 hours. Control cells received no treatment. Results are representative of 2 experiments and are reported as mean±SD, n=8 wells. *P<0.001 vs PMA alone. O.D. indicates optical density.

Figure 6. Effect of the natural PPAR activator 15d-PGJ2 on neutrophil binding and E-selectin expression of HAECs activated by PMA. A, Confluent HAECs were cotreated during a 4-hour activation period with high-dose PMA (50 nmol/L) with PPAR activators (PPAR-A) ciglitazone (30 μmol/L; Cig30), Wyeth 14643 (10 μmol/L; Wy10), or 15d-PGJ2 (5 μmol/L; PGJ5). HAECs were washed and incubated with 4×10^5 cells of the human neutrophil-like cell line HL60 for 10 minutes. Cells were washed and fixed with glutaraldehyde, and the number of adherent HL60 cells per high-power field (HPF) was determined. Data are mean±SD of 12 HPFs from 1 experiment performed in quadruplicate wells and are representative of 2 experiments. *P<0.001 versus PMA alone. B, ELISA was performed with anti–E-selectin antibody as described in Methods after cotreatment of HAECs with PMA (50 nmol/L) and PPAR activators (PPAR-A) ciglitazone (30 μmol/L; Cig30), Wyeth 14643 (10 μmol/L; Wy10), or 15d-PGJ2 (5 μmol/L; PGJ5). HAECs were washed and incubated with 4×10^5 human neutrophils for 10 minutes. Cells were washed and fixed with glutaraldehyde, and the number of adherent neutrophils was determined. Data are mean±SD of 12 HPFs from 1 experiment performed in quadruplicate wells and are representative of 3 other experiments. *P<0.001 vs PMA alone. O.D. indicates optical density.
tides were utilized as transcription factor decoys. Most pertinent to the present study, De Caterina et al showed that certain lipids could block the induction of VCAM-1 expression on activated ECs. These authors suggested that this may partially explain the purported protective effect of various unsaturated dietary lipids in atherogenesis. Such lipids, for instance, docosahexaenoic acid, are now understood to be activators of PPARs.

In contrast to the synthetic PPAR activators tested, the natural PPAR activator 15d-PGJ blocked both E-selectin as well as VCAM-1 expression and acted without the need for pretreatment of the cells. This difference may be related to the prostaglandin nature of 15d-PGJ, which, by analogy with other prostaglandins such as PGE, may interact with a cell surface receptor to generate an increase in intracellular cAMP levels. Such an increase in cAMP in this manner has been shown previously to block induction of VCAM-1 and E-selectin but not ICAM-1 expression on activated ECs. However, no known specific cell surface receptor for 15d-PGJ has been identified. It is also possible that 15d-PGJ has other pharmacological properties, such as conjugation with glutathione, as occurs with prostaglandin metabolites containing an α,β-unsaturated carbonyl group resulting in varied biological activity. Alternatively, one might argue that these effects are solely mediated by PPAR and that 15d-PGJ may be a very effective PPAR activator in ECs. Even though 15d-PGJ2 is less effective than most thiazolidinediones at inducing adipocyte differentiation or transactivation of a PPAR-γ promoter-reporter construct in fibroblast-type cells, it has often been shown to have the greatest efficacy in other processes in various cell types, such as inhibiting cytokine production by activated monocytes, inducing the differentiation of monocytes into macrophages, and inhibiting growth of breast cancer cells. The reason for this paradox is currently unknown.

We are unable to state, as yet, which PPAR subtype might be mediating this process in ECs. The ability of the peroxisome proliferator Wyeth 14643 to have an effect suggests that PPAR-α is involved, whereas the effects of the thiazolidinediones indicate that PPAR-γ is involved. The potency of 15d-PGJ2 also predicts a role for PPAR-γ in this process; however, the ineffectiveness of BRL 49653, the most potent γ-activator in most systems, is puzzling. Analysis of the mRNA levels indicates that PPAR-α is more abundant and perhaps more active than PPAR-γ in ECs; however, this is not definitive, since we have as yet to determine the functional protein levels of the PPARs in ECs. Given that there is no truly specific activator of a particular PPAR, we can only conclude that the process described in this study may be mediated by any of the PPARs expressed in ECs.

Our results do not exclude a role for the effects of PPAR activators on the expression of other factors involved in leukocyte-EC interaction, such as inhibition of endothelium-derived chemokines and other adhesion molecules. Nor do
they rule out the possibility that the molecules tested may act through unrelated mechanisms in addition to PPAR activation. Regardless of the exact mechanism(s), it is hoped that such molecules will have the same effects in vivo.

Because inflammatory lipid factors such as prostaglandins, eicosanoids, and leukotrienes are well-recognized activators of PPARs, these transcription factors are poised to mediate inflammatory responses in general and in specific diseases. The present results suggest that, even though certain PPAR activators may promote foam cell formation in the atherosclerotic lesion, they may also block the initial entry of monocytes into the artery wall, thus preventing the onset of atherosclerotic lesion formation but aggravating preexisting lesions. These findings carry enormous clinical significance, as increasing numbers of diabetic patients are receiving PPAR agonists as routine therapy for insulin resistance.

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