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PPAR-γ dependent and independent effects on macrophage-gene expression in lipid metabolism and inflammation

AJAY CHAWLA[1,2], YAACOV BARAK[1], LASZLO NAGY[4], DEBBIE LIAO[1], PETER TONTONOZ[3] & RONALD M. EVANS[1]

[1]*The Salk Institute for Biological Studies, Howard Hughes Medical Institute, La Jolla, California 90237, USA*
[2]*Division of Endocrinology and Metabolism, University of California, San Diego, California 92093, USA*
[3]*Department of Pathology and Laboratory Medicine, Howard Hughes Medical Institute, University of California, Los Angeles, California 90095, USA*
[4]*Department of Biochemistry and Molecular Biology, University of Debrecen, Medical and Health Science Center, Nagyerdei krt. 98, Debrecen H-4012, Hungary*
Correspondence should be addressed to R.M.E.; e-mail: evans@salk.edu

Peroxisome proliferator-activated receptor-γ (PPAR-γ) is highly expressed in lipid-accumulating macrophages of the coronary artery. In light of this, the wide-spread clinical use of thiazolidinediones (TZDs) in the treatment of type II diabetes raises concerns about the role of PPAR-γ in macrophage function and disease progression. To define the role of PPAR-γ in macrophage biology, we used homologous recombination to create embryonic stem cells that were homozygous for a null mutation in the PPAR-γ gene. We demonstrate here that PPAR-γ is neither essential for nor substantially affects the development of the macrophage lineage both *in vitro* and *in vivo*. In contrast, we show it is an important regulator of the scavenger receptor CD36, which has been genetically linked to lipid accumulation in macrophages. Both 15-deoxy-$\Delta^{12,14}$prostaglandin J_2 and thiazolidinediones have anti-inflammatory effects that are independent of PPAR-γ. We show that PPAR-γ is required for positive effects of its ligands in modulating macrophage lipid metabolism, but that inhibitory effects on cytokine production and inflammation may be receptor independent.

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Peroxisome proliferator-activated receptor-γ (PPAR-γ) is a ligand-dependent nuclear receptor that has a critical role in adipogenesis, glucose metabolism and placental function[1–3]. This receptor is the molecular target of the thiazolidinedione (TZD) class of antidiabetic drugs, which include rosiglitazone and pioglitazone[4,5]. PPAR-γ is prominently expressed in activated monocytes and tissue macrophages *in vivo*, including the foam cells of atherosclerotic lesions[6,7]. Multiple functions have been proposed for PPAR-γ in macrophages, but the true role of this transcription factor in macrophage physiology remains unclear. How the PPAR-γ signaling pathway may affect the atherosclerotic process is an important issue because more than one million type II diabetics, who are already highly susceptible to atherosclerotic disease, are currently being treated with TZDs (ref. 8).

Activation of PPAR-γ in THP-1 cells by either 15-deoxy-$\Delta^{12,14}$prostaglandin J_2 (15d-PGJ_2) or TZD promotes changes in surface marker expression characteristic of monocytic differentiation, including induction of the class B scavenger receptor CD36 (ref. 6). As a consequence of CD36 induction, PPAR-γ–activated THP-1 cells have an increased capacity to take up oxidized low-density lipoproteins (OxLDL)[9]. These observations indicate that PPAR-γ may have an important role in macrophage differentiation as well as in macrophage lipid metabolism. However, the requirement of PPAR-γ for monocytic differentiation and the relative contribution of the PPAR-γ–CD36 pathway to lipid uptake in differentiated macrophages have not been tested.

Several studies have reported potentially anti-inflammatory effects of PPAR-γ ligands in monocytes and macrophages. Treatment of monocytes and macrophages with high concentrations of PPAR-γ agonists reduced secretion of inflammatory cytokines and inhibited macrophage activation[10]. For example, treatment of monocytes with either 15d-PGJ_2 or TZD reduced release of inflammatory cytokines, such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 (ref. 11). Similarly, treatment of elicited macrophages with PPAR-γ ligands induced a resting phenotype and suppressed inducible nitric oxide synthase (iNOS), gelatinase B and scavenger receptor A (SRA)[12]. These observations indicated that PPAR-γ might represent a target for anti-inflammatory therapy. At present, however, no definitive studies support the premise that PPAR-γ expression is required for anti-inflammatory effects in macrophages.

Here, we report that PPAR-γ is not essential for the development of the macrophage lineage *in vitro* and *in vivo*, but is an important regulator of macrophage gene expression. The scavenger receptor CD36 is shown to be a target gene for PPAR-γ. Retroviral expression of PPAR-γ in NIH-3T3 cells or J774 macrophages facilitates induction of CD36 in response to PPAR-γ ligands. Moreover, TZDs induce CD36 expression in wild-type but not in PPAR-γ–deficient macrophages. We further show that the influence of PPAR-γ expression on OxLDL uptake is cell-type– and context-dependent. Expression of PPAR-γ in NIH-3T3 cells, which lack scavenger receptor expression, confers the ability to take up OxLDL. Finally, we show that PPAR-γ expression is not essential for PPAR-γ ligands to exert anti-inflammatory effects in macrophages and thus the receptor may be an inappropriate tar-

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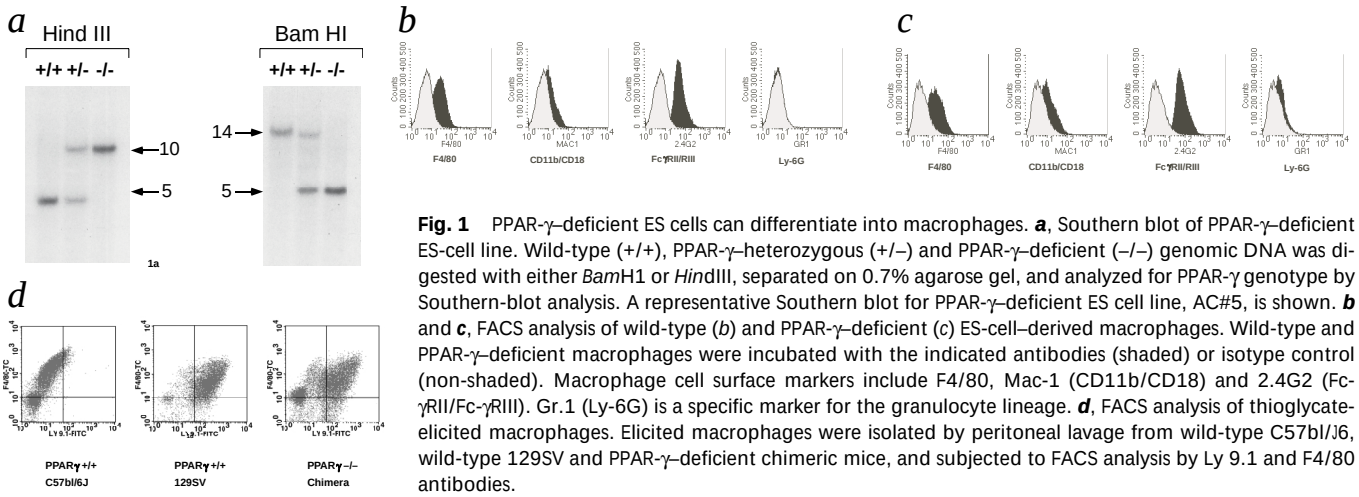

Fig. 1 PPAR-γ–deficient ES cells can differentiate into macrophages. ***a***, Southern blot of PPAR-γ–deficient ES-cell line. Wild-type (+/+), PPAR-γ–heterozygous (+/–) and PPAR-γ–deficient (–/–) genomic DNA was digested with either *Bam*H1 or *Hin*dIII, separated on 0.7% agarose gel, and analyzed for PPAR-γ genotype by Southern-blot analysis. A representative Southern blot for PPAR-γ–deficient ES cell line, AC#5, is shown. ***b*** and ***c***, FACS analysis of wild-type (*b*) and PPAR-γ–deficient (*c*) ES-cell–derived macrophages. Wild-type and PPAR-γ–deficient macrophages were incubated with the indicated antibodies (shaded) or isotype control (non-shaded). Macrophage cell surface markers include F4/80, Mac-1 (CD11b/CD18) and 2.4G2 (Fc-γRII/Fc-γRIII). Gr.1 (Ly-6G) is a specific marker for the granulocyte lineage. ***d***, FACS analysis of thioglycate-elicited macrophages. Elicited macrophages were isolated by peritoneal lavage from wild-type C57bl/J6, wild-type 129SV and PPAR-γ–deficient chimeric mice, and subjected to FACS analysis by Ly 9.1 and F4/80 antibodies.

get for the development of anti-inflammatory drugs.

Results

To better define the role of PPAR-γ in macrophage differentiation, we used embryonic stem (ES) cells in which both alleles of the gene expressing PPAR-γ were deleted. PPAR-γ–deficient ES cells were established *de novo* from the inner cell mass of blastocysts that were isolated from PPAR-γ–heterozygous matings[13]. We established 11 ES cell lines in this manner, 2 of which were homozygous for PPAR-γ–deficient alleles (Fig. 1*a*). To determine whether PPAR-γ–deficient ES cells give rise to macrophages, we used a two-step *in vitro* differentiation assay[14]. First, wild-type and PPAR-γ–deficient ES cells were cultured in methylcellulose differentiation medium to generate embryoid bodies. These embryoid bodies, which contained hematopoetic precursors, were then isolated and further cultured in medium containing IL-3 and M-CSF-1, cytokines that support the development of monocytes and macrophages, respectively[15]. Under these conditions, both wild-type and PPAR-γ–deficient ES cells were capable of differentiating into macrophages, as judged by morphology and expression of cell surface markers, such as F4/80, CR3 (CD11bl/cD18) and Fc-γRII/Fc-γRIII (Fig. 1*b* and *c*)[16]. Lack of significant expression of the granulocyte-specific marker, Gr-1, verified that differentiation occurred primarily along the macrophage lineage (Fig. 1*b* and *c*). To verify that PPAR-γ is dispensable for generation of macrophage lineage *in vivo*, we analyzed thioglycolate-elicited macrophages from PPAR-γ–deficient chimeric mice. Elicited macrophages from the C57b/JG strain do not express the Ly9.1 antigen, whereas those from the 129SV strain are positive for Ly9.1 expression (Fig. 1*d*). Because the PPAR-γ–deficient ES cells were derived from 129SV mice and injected into C57bl/6J blastocysts to generate the chimeric mice, we used the Ly9.1 and F4/80 antibodies to analyze the elicited macrophages in these chimeric mice. Consistent with our *in vitro* results, PPAR-γ–deficient cells were also able to contribute to the macrophage lineage *in vivo* (Fig. 1*d*). Thus PPAR-γ is not required for macrophage differentiation either *in vitro* or *in vivo*.

Next, we tested the role of PPAR-γ as a regulator of gene expression in differentiated macrophages. Treatment of undifferentiated THP-1 cells with PPAR-γ ligands promotes expression of the class B scavenger receptor CD36 and uptake of OxLDL (refs. 6,9). To determine whether PPAR-γ directly regulates CD36 expression, we used a retroviral vector to express PPAR-γ in J774 macrophages and NIH-3T3 fibroblasts (J774-PPAR-γ and NIH-PPAR-γ, respectively). These cell lines were chosen because neither expresses significant levels of PPAR-γ (Fig. 2; ref. 17). Treatment of control cells (J774-vector, NIH-vector) with the TZD troglitazone or the RXR-specific ligand LG268 had no effect on the expression of CD36 mRNA (Fig. 2*a* and *b*). Treatment of J774-vector cells but not NIH-3T3 cells with 15d-PGJ_2 led to a small induction of CD36 and endogenous PPAR-γ mRNA expression. In contrast, treatment of NIH-PPAR-γ and J774-PPAR-γ with

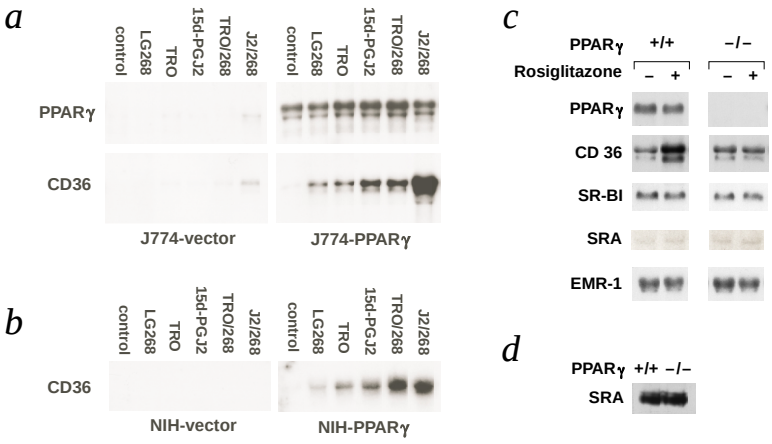

Fig. 2 CD36 is a PPAR-γ target gene. ***a***, J774-vector and J774-PPAR-γ cells were treated for 48 h with troglitazone (5 μM), 15d-PGJ2 (3 μM) and/or LG268 (50 nM) as indicated. ***b***, NIH-vector and NIH-PPAR-γ cells were treated as in (*a*). ***c***, ES-derived macrophages were treated with either rosiglitazone (1 μM) or vehicle for 24 h. Total RNA (5 μg) was analyzed by northern blot using [^{32}P]-labeled cDNA probes. An equivalent amount of intact RNA was run in each lane as indicated by hybridization to a 36B4 cDNA probe (data not shown).

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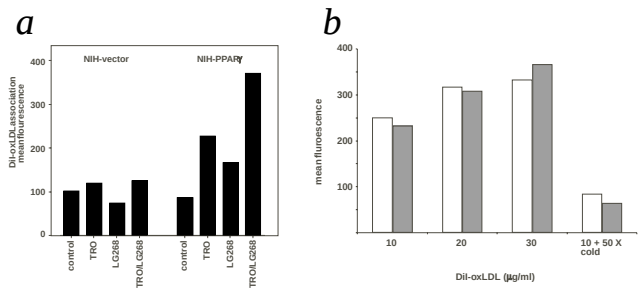

Fig. 3 PPAR-γ is required for OxLDL uptake in NIH-3T3 cells, but not in differentiated macrophages. ***a***, NIH-vector and NIH-PPAR-γ cells were treated for 48 h with troglitazone (10 μM), LG268 (50 μM) or both, and incubated in the presence of 20 μg protein/ml DiI-OxLDL. ***b***, ES-derived macrophages were pretreated with rosiglitazone (1 μM) for 48 h and then incubated with indicated concentrations of DiI-OxLDL. □, PPARγ +/+; ■, PPARγ –/–. Cell associated fluorescence was quantified by flow cytometry. Mean fluorescence of the counted populations is presented. We added 50-fold excess of unlabeled OxLDL as a competitor to show specific binding.

TZDs, 15d-PGJ_2 or LG268, led to an induction of CD36 expression. These results demonstrate that expression of PPAR-γ can promote ligand inducibility on the gene expressing CD36.

To evaluate whether PPAR-γ is required for the regulation of the gene expressing CD36 in differentiated macrophages, we studied gene expression in ES-derived macrophages that had been treated with rosiglitazone for 24 hours. PPAR-γ is abundantly expressed in macrophages derived from wild-type cells and absent from those derived from PPAR-γ–deficient cells (Fig. 2*c*). Treatment of wild-type macrophages with rosiglitazone resulted in a robust increase in CD36 expression. In contrast, we observed no change in CD36 expression in PPAR-γ–deficient macrophages with rosiglitazone treatment. Upregulation of CD36 by rosiglitazone was specific, as the expression of SR-BI, another class B scavenger receptor, was unchanged in either wild-type or PPAR-γ–deficient macrophages. Equal loading and comparable differentiation among the various samples were assessed by hybridization to EMR1, a cDNA that encodes the macrophage-specific F4/80 antigen[18]. In addition, expression of SRA protein was equivalent in both wild-type and PPAR-γ–deficient macrophages (Fig. 2*d*), and its mRNA was not induced by PPAR-γ ligands in both wild-type and PPAR-γ–deficient macrophages (Fig. 2*c*). Similar results were obtained with the other PPAR-γ–deficient ES-cell line (data not shown). These results show that although PPAR-γ is not essential for the development of the macrophage lineage, it is required for the regulation of the CD36 gene in response to PPAR-γ ligands.

Having established CD36 as a PPAR-γ target gene, we examined the functional consequence of PPAR-γ–mediated CD36 induction in fibroblasts and macrophages. First, we investigated whether retroviral expression of PPAR-γ in fibroblasts altered the ability of these cells to internalize OxLDL. We chose NIH-3T3 cells for these studies because they do not express scavenger receptors and have almost no ability to uptake OxLDL. Consistent with their ability to induce CD36 mRNA expression, troglitazone and LG268 promoted OxLDL uptake in NIH-PPAR-γ cells but not in NIH-vector cells (Fig. 3*a*). The combination of troglitazone and LG268 had an additive effect. These results show that expression of PPAR-γ in cells that express few scavenger receptors confers the ability to take up OxLDL.

Next, we examined the contribution of the PPAR-γ–CD36 pathway to OxLDL uptake in differentiated macrophages that express multiple scavenger receptors. We measured OxLDL uptake in wild-type and PPAR-γ–deficient macrophages that had been treated with rosiglitazone. Although rosiglitazone treatment resulted in an increase in DiI-OxLDL binding to wild-type macrophages (data not shown), there was no significant difference in net uptake of DiI-OxLDL between wild-type and PPAR-γ–deficient macrophages (Fig. 3*b*). This indicates that other scavenger receptors expressed in ES-derived macrophages can compensate for reduced CD36 expression in PPAR-γ–deficient macrophages.

PPAR-γ ligands exert potent anti-inflammatory effects on macrophages, but because the concentration of PPAR-γ ligands required to produce inhibition is much higher than the dissociation constants of these ligands, a role for the receptor is not assured. To test whether expression of PPAR-γ is required to inhibit the inflammatory response, we stimulated wild-type and PPAR-γ–deficient macrophages with lipopolysaccharide (LPS) and measured the inhibition of cytokine secretion in response to PPAR-γ ligands. Stimulation with LPS resulted in an equivalent increase in the secretion of pro-inflammatory cytokines, TNF-α and IL-6, in both wild-type and PPAR-γ–deficient macrophages (data not shown). 15d-PGJ_2 inhibited the secretion of TNF-α and IL-6 in wild-type macrophages; however, it was equally effective in PPAR-γ–deficient macrophages (Fig. 4*a*). Treatment of wild-type

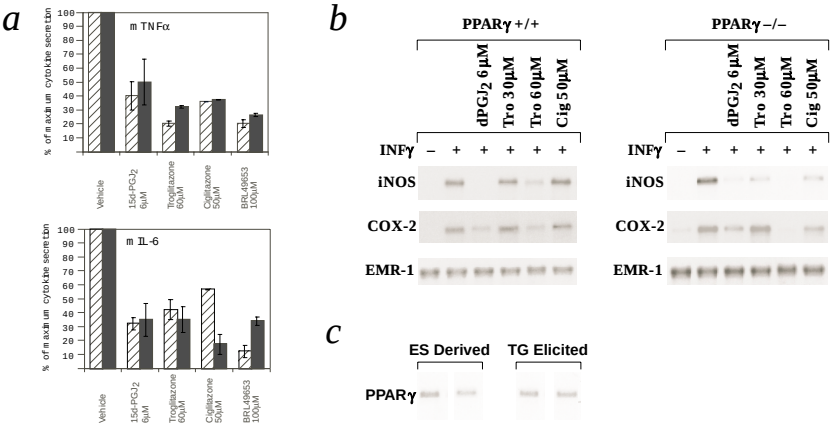

Fig. 4 PPAR-γ ligands can down regulate inflammatory response independent of PPAR-γ. ***a***, Wild-type and PPAR-γ–deficient macrophages were treated with LPS (0.1 μg/ml) and various PPAR-γ ligands. ELISA assays were used to measure TNF-α and IL-6 released into the media. Results expressed are the mean of 3 or 4 determinations and are shown as a percentage of maximum cytokine concentration measured. ***b***, Wild-type and PPAR-γ–deficient macrophages were stimulated with mINF-γ and various PPAR-γ ligands. Gene expression analysis was carried out by northern blots for iNOS, COX-2 and EMR-1. ***c***, Expression of PPAR-γ mRNA in wild-type ES-derived and thioglycolate-elicited macrophages. Two representative experiments with each macrophage population are shown. Equal loading of RNA was confirmed by staining of ribosomal RNA (data not shown). ▨, PPARγ +/+; ■, PPARγ –/–

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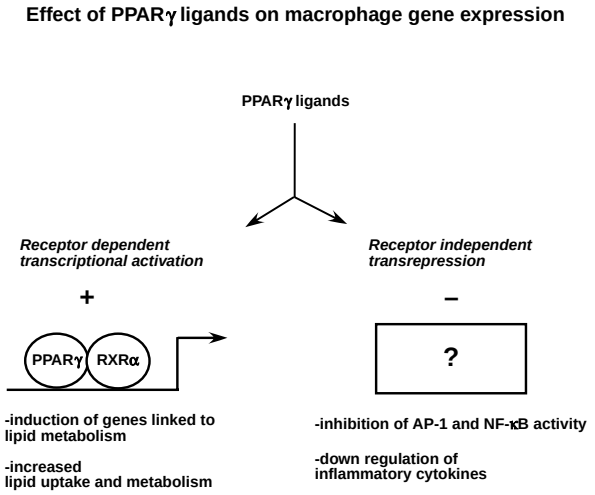

Fig. 5 Model for gene regulation by PPAR-γ and its ligands. Target gene activation by PPAR-γ ligands requires the receptor, whereas transrepression by PPAR-γ ligands can occur independently of the receptor.

macrophages with various TZDs also resulted in inhibition of secretion of TNF-α and IL-6 by 60–80% (Fig. 4*a*). However, similar effects of TZDs were observed in PPAR-γ–deficient macrophages (Fig. 4*a*), indicating that PPAR-γ is not required for mediating the anti-inflammatory effects of 15d-PGJ_2 or TZDs in macrophages.

To further verify that PPAR-γ ligands can independently inhibit the inflammatory response of the macrophages, we stimulated wild-type and PPAR-γ–deficient macrophages with interferon (INF)-γ, and evaluated gene expression for iNOS and cycloxygenase (COX)-2. Stimulation of either wild-type or PPAR-γ–deficient macrophages with INF-γ resulted in quantitatively similar induction of iNOS and COX-2 mRNAs (Fig. 4*b*). The expression of these pro-inflammatory genes was also inhibited by whether natural and synthetic PPAR-γ ligands in both the wild-type and PPAR-γ–deficient macrophages (Fig. 4*b*). Finally, to determine results obtained with the ES-cell–derived macrophages extend to other macrophage populations, we examined the expression level of PPAR-γ in ES-cell–derived macrophages and thioglycolate-elicited macrophages. The expression level of PPAR-γ mRNA in both macrophage populations was equivalent (Fig. 4*c*), indicating that the anti-inflammatory effects of PPAR-γ ligands in macrophages are independent of their ability to activate PPAR-γ.

Discussion

A variety of functions have been attributed to PPAR-γ in different cell types[19]. Activation of PPAR-γ in immortalized cell lines, such as THP-1 and HL-60, promotes differentiation along the macrophage lineage, as shown by changes in gene expression and uptake of OxLDL by CD36 (ref. 6). This led to the identification of PPAR-γ in peripheral human monocytes and lipid-accumulating macrophages of coronary artery lesions of both mice and humans[6,7]. PPAR-γ ligands were independently shown to inhibit macrophage activation, thus acting as typical anti-inflammatory agents[11,12]. Our goal was to use homologous recombination to assess the role of PPAR-γ in mediating these three critical functions. We show that macrophage differentiation can occur *in vitro* in PPAR-γ–deficient ES cells and *in vivo* in mice chimeric for PPAR-γ–deficient alleles. Although PPAR-γ is essential for the regulated expression of CD36, it is not required for the anti-inflammatory effects of 15d-PGJ_2 and TZDs.

In regard to scavenger receptor expression, we show that retroviral expression of PPAR-γ in NIH-3T3 cells or J774 macrophages facilitates the induction of CD36 in response to PPAR-γ ligands. Consistent with this gain-of-function data, TZDs induced CD36 expression in wild-type but not in PPAR-γ–deficient macrophages. The unaltered basal expression of CD36 in PPAR-γ–deficient macrophages indicates that PPAR-γ is unnecessary for macrophage-specific expression of CD36, but is important in conferring lipid inducibility to the promoter of this gene. We have also shown that the influence of PPAR-γ expression on OxLDL uptake is cell-type– and context-dependent. Expression of PPAR-γ in NIH-3T3 cells, which lack scavenger receptor expression, endows these cells with the ability to take up OxLDL. In differentiated ES-cell–derived macrophages, however, which express several other scavenger receptors, loss of PPAR-γ does not substantially compromise OxLDL net uptake *in vitro*.

In macrophages, PPAR-γ has been implicated in both activation and repression of transcription (Fig. 5). However, PPAR-γ is not known to function as a direct transcriptional repressor and this inhibitory effect requires concentrations of PPAR-γ ligands that are orders of magnitude higher than their dissociation constants. PPAR-γ has also been reported to interfere with AP-1 and NF-κB activity in transient transfection assays, although it is not clear that this 'transrepression' mechanism is relevant *in vivo*. The possibility that certain PPAR-γ ligands may have biological activities that are independent of PPAR-γ has not been tested. Using ES-cell–derived macrophages that were stimulated with LPS and INF-α, we showed that PPAR-γ ligands have anti-inflammatory effects that are independent of PPAR-γ. Although the natural PPAR-γ ligand, 15d-PGJ_2, is a potent inhibitor of TNF-α and IL-6 secretion in LPS stimulated macrophages, this activity is not diminished in PPAR-γ–deficient macrophages, indicating that alternative signaling pathways are involved. These observations are consistent with the recent findings that 15d-PGJ_2 directly inhibits NF-κB activity[20,21]. Our results emphasize that caution needs to be exercised in ascribing functions to PPAR-γ on the basis of the action of its ligands, especially when such ligands are used at concentrations far exceeding those required to bind the receptor. On the basis of this data, PPAR-γ seems to be a poor choice as a target for the development of anti-inflammatory drugs.

The role of PPAR-γ in various macrophage functions *in vivo* remains to be determined. Given the widespread clinical use of TZDs, the influence of these particular PPAR-γ ligands on atherosclerosis must be considered. Early studies indicate that TZDs improve atherosclerosis in patients with type II diabetes[22]. The relative contribution of macrophage PPAR-γ signaling pathways to these effects, however, is difficult to assess as TZDs have multiple effects on systemic lipid and glucose metabolism in addition to their effects in vascular cells[23]. Thus, the consequence of PPAR-γ activation may be atherogenic in some contexts and protective in others. For example, *in vivo*, PPAR-γ ligands induce CD36 expression in adipose tissue, skeletal muscle and cardiac muscle as well as in macrophages (H.S. Ahuja *et al.*, submitted). In these tissues, CD36 is thought to function primarily as a fatty acid transporter, and, therefore, its regulation by PPAR-γ may contribute to the control of blood lipid levels. Moreover, loss of CD36 expression by homologous recombination in mice causes increased serum triglycerides and impairment of lipid transport

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in macrophages[24]. Thus, inability to regulate CD36 in PPAR-γ–deficient cells is likely to have implications for lipid homeostasis *in vivo*. Further genetic studies, including tissue-specific disruption of the gene encoding PPAR-γ in myeloid cells, will be required to precisely define the role of this nuclear receptor and its ligands in macrophage biology and vascular disease.

Methods

ES-cell derivation. PPAR-γ–deficient ES cells were derived from the inner cell mass (ICM) of blastocysts collected from PPAR-γ–heterozygous matings[13]. PPAR-γ–heterozygous females were super-ovulated with gonadotrophins and mated with PPAR-γ–heterozygous males. Blastocysts were collected on d 4 from the uteri of pregnant females. Blastocysts were washed in M2 medium (Specialty Media, Lavallette, New Jersey) and cultured for 2 d on feeders in the presence of recombinant LIF (Gibco, Grand Island, New York). On d 6, blastocysts that had hatched were disaggregated with trypsin and replated on feeder cells in DMEM (Gibco) supplemented with LIF. ES-cell colonies that developed after 7–10 d of disaggregation were expanded, genotyped by Southern blot and used in the experiments outlined below. For control cells, we used the parental ESJ1 cell line in which the initial gene targeting was undertaken.

ES-cell differentiation. Before differentiation, ES cells were trypsinized and depleted of feeder cells by culturing in IMDM (Gibco) supplemented with 5% FCS for 3–4 h (ref. 15). Non-adherent cells were seeded in 0.9% methylcellulose in IMDM supplemented with 15% FCS, transferrin (300 μg/ml, Roche), PFHM-II (5%, Gibco), ascorbic acid (50 μg/ml) and monothioglycerol (4×10^{-4} M). Embryoid bodies were collected on d 6, trypsinized and replated in IMDM supplemented with 15% L929 conditioned medium (CM) and mIL-3 (1 ng/ml). Two days later, macrophage precursors that developed as non-adherent cells were collected and cultured in IMDM supplemented with 15% L929 CM. Adherent macrophages developed over the next 4 to 5 d and were used for experiments starting on d 5.

Stable cell lines. The pBabe-puro–PPAR-γ expression vector has been described[17]. Control and PPAR-γ expression vectors were packaged into retrovirus by transient transfection of Phoenix E cells as described[17]. NIH-3T3 cells or J774 macrophages were infected at 50% confluence with equal titres of recombinant retrovirus. Stable cell lines were selected with puromycin (2 μg/ml).

RNA and protein analysis. Total RNA was isolated using Trizol reagent (Gibco). Blots were probed with [^{32}P]-labeled cDNA probes for mCD36, mPPAR-γ, mSR-BI and mEMR1 as described[6]. For western blot analysis, cells were lysed in 0.5% TNET buffer and equivalent amount of protein was run on 8% SDS–PAGE gels. Proteins were transferred to PVDF membranes, blocked in TBS-T buffer containing 5% non-fat milk, and probed for SRA with 2F8 antibody (1:100) followed by HRP-conjugated rat antibody (1:10,000). Immunoreactive proteins were visualized with ECL (Promega Madison, Wisconsin).

Flow cytometry. Macrophages were cultured in petri dishes and then detached for FACS analysis with ice cold PBS containing 5 mM EDTA. All samples, except those for 2.4G2 staining, were blocked with mouse IgG for 15 min and then incubated for 15 min at 4 ºC with FITC-conjugated antibodies F4/80 (Serotec, Raleigh, North Carolina), Mac-1, Gr1 and 2.4G2 (Pharmingen, San Diego, Califonia). Samples were analyzed on the Becton-Dickinson FACScan using CellQuest software, and propidium iodide for live cell gating.

Cytokine assay. Macrophages were treated with LPS (0.1 μg/ml) and PPAR-γ ligands simultaneously, and the medium was collected for analysis 18–20 h later. Concentrations of mTNF-α and mIL-6 in the supernatants were measured using enzyme-linked immunosorbent assay (ELISA) per manufacturer's protocols (Pharmingen). Samples were diluted 1:10 before analysis, and all samples were run in triplicate. For gene expression analysis, macrophages were treated with mINF-γ (20 ng/ml, Sigma) and PPAR-γ ligands simultaneously for 20 h, and processed for northern-blot analysis.

DiI-OxLDL uptake. All lipoproteins were purchased from Intracel DiI-OxLDL binding and uptake experiments were performed as described earlier with the following changes. For OxLDL-binding experiments, macrophages were pretreated with PPAR-γ ligands for 48 h and then incubated with indicated concentration of DiI-OxLDL at 4 ºC for 4 h. For OxLDL uptake experiments, cells were treated with PPAR-γ ligands for 48 h and then incubated with DiI-OxLDL for 2–4 h at 37º C. Cells were then washed with PBS, and analyzed by flow cytometry for DiI-OxLDL binding and uptake.

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