Activation of Peroxisome Proliferator-activated Receptor γ Inhibits Interleukin-1β-induced Membrane-associated Prostaglandin E₂ Synthase-1 Expression in Human Synovial Fibroblasts by Interfering with Egr-1*

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Membrane-associated prostaglandin (PG) E₂ synthase-1 (mPGES-1) catalyzes the conversion of PGH₂ to PGE₂, which contributes to many biological processes. Peroxisome proliferator-activated receptor γ (PPARγ) is a ligand-activated transcription factor and plays an important role in growth, differentiation, and inflammation in different tissues. Here, we examined the effect of PPARγ ligands on interleukin-1β (IL-1β)-induced mPGES-1 expression in human synovial fibroblasts. PPARγ ligands 15-deoxy-Δ12,14-prostaglandin J₂ (15d-PGJ₂) and the thiazolidinedione troglitazone (TRO), but not PPARα ligand Wy14643, dose-dependently suppressed IL-1β-induced PGE₂ production, as well as mPGES-1 protein and mRNA expression. 15d-PGJ₂ and TRO suppressed IL-1β-induced activation of the mPGES-1 promoter. Overexpression of wild-type PPARγ further enhanced, whereas overexpression of a dominant negative PPARγ alleviated, the suppressive effect of both PPARγ ligands. Furthermore, pretreatment with an antagonist of PPARγ, GW9662, relieves the suppressive effect of PPARγ ligands on mPGES-1 protein expression, suggesting that the inhibition of mPGES-1 expression is mediated by PPARγ. We demonstrated that PPARγ ligands suppressed Egr-1-mediated induction of the activities of the mPGES-1 promoter and of a synthetic reporter construct containing three tandem repeats of an Egr-1 binding site. The suppressive effect of PPARγ ligands was enhanced in the presence of a PPARγ expression plasmid. Electrophoretic mobility shift and supershift assays for Egr-1 binding sites in the mPGES-1 promoter showed that both 15d-PGJ₂ and TRO suppressed IL-1β-induced DNA-binding activity of Egr-1. These data define mPGES-1 and Egr-1 as novel targets of PPARγ and suggest that inhibition of mPGES-1 gene transcription may be one of the mechanisms by which PPARγ regulates inflammatory responses.

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‡‡‡ The abbreviations used are: PG, prostaglandin; 15d-PGJ₂, 15-deoxy-Δ12,14-prostaglandin J₂; COX, cyclooxygenase; cPGES, cytosolic prostaglandin E synthase; EMISA, electrophoretic mobility shift assay; HSF, human synovial fibroblast; IL, interleukin; iNOS, inducible nitric-oxide synthase; MMP, metalloproteinase; mPGES, membrane-associated prostaglandin E synthase; PPAR, peroxisome proliferator-activated receptor; TRO, troglitazone; TNF, tumor necrosis factor; OA, osteoarthritis; NF-κB, nuclear factor-κB; PMSF, phenylmethylsulfonyl fluoride; DMEM, Dulbecco's modified Eagle's medium; FCS, fetal calf serum; DTT, dithiothreitol; DN, dominant negative; PPRE, PPAR-responsive element.

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zyme immunoassay reagents for PGE2 assays were purchased from Cayman Chemical. The detection limit and sensitivity was 9 pg/ml. All assays were performed in duplicate.

Western Blot Analysis—Cells were lysed in ice-cold lysis buffer (50 mM HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA, 1 mM PMSF, 10 μg/ml each of aprotinin, leupeptin, and pepstatin, 1% Nonidet P-40, 1 mM sodium orthovanadate (Na3VO4), and 1 mM NaF). Lysates were sonicated on ice and centrifuged at 12,000 rpm for 15 min. The protein concentration of the supernatant was determined using the bicinchoninic acid method (Pierce). 20 μg of total cell lysate or nuclear extracts was subjected to SDS-polyacrylamide gel electrophoresis and electrotransferred to a nitrocellulose membrane (Bio-Rad). After blocking in 20 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, 0.1% Tween 20, and 5% (w/v) nonfat dry milk, blots were incubated overnight at 4 °C with primary antibodies and washed with wash buffer (Tris-buffered saline, pH 7.5, with 0.1% Tween 20). The blots were then incubated with horseradish peroxidase–conjugated secondary antibody (Pierce), washed again, incubated with SuperSignal Ultra Chemiluminescent reagent (Pierce), and finally exposed to X-Omat film (Eastman Kodak Co., Rochester, NY).

RNA Extraction and cdNA Synthesis—Total RNA was isolated from HSFs using the TRIzol reagent (Invitrogen) and dissolved in 20 μl of diethylpyrocarbonate-treated H2O. 1 μg of total RNA was treated with RNase-free DNase and reverse-transcribed using Moloney Murine Leukemia Virus reverse transcriptase (Fermentas, Burlington, Ontario, Canada) as detailed in the manufacturer’s guidelines. One-fifth of the reverse transcription reaction was analyzed by real-time PCR as described below. The following primers were used for mPGES-1, sense 5′-TGCTTGG-3′ and antisense 5′-GGAAAGCAGCAAGTGCTAC-3′; pPGES, sense 5′-GCAAAGTGTTAGCTGAAGG-3′ and antisense 5′-TGCTCCGTTCCTTTATGTTGCTGG-3′; and gliclazine-dehydrogenase, sense 5′-CAGAACATCATCCTGCCTCCT-3′ and antisense 5′-GCTGGAGAAAAAGTCGTTGAG-3′.

Real-time Quantitative PCR—Quantitative PCR analysis was performed in a total volume of 50 μl containing cdNA template, 200 nM of sense and antisense primers, and 25 μl of SYBR® Green master mix (Qiagen). Incorporation of SYBR® Green dye into PCR products was monitored in real time using a Gene Amp 5700 sequence detector (Applied Biosystems) allowing determination of the threshold cycle (Ct) value for the exponential amplification of PCR products. The amplification was performed at 95 °C for 10 min to activate the AmpliTaq Gold enzyme, the mixtures were subjected to 40 amplification cycles (15 s at 95 °C for denaturation and 1 min for annealing and extension at 60 °C). After PCR, dissociation curves were generated with one peak, indicating the specificity of the amplification. A threshold cycle (Ct) value was obtained from each amplification curve using the software provided by the manufacturer (Applied Biosystems). Data were expressed as -fold changes relative to control conditions (unstimulated cells) using the ΔΔCt method as detailed in the manufacturer’s guidelines (Applied Biosystems). The ΔΔCt value was first calculated by subtracting the Ct value for the housekeeping gene glyceraldehyde-3-phosphate dehydrogenase (GAPDH) for each sample. A ΔCt value was then calculated by subtracting the ΔCt value of the control from the ΔCt value of each treatment. The -fold changes compared with the control (unstimulated cells) were then determined by raising 2 to the ΔΔCt power. Each PCR reaction generated only the expected specific amplification as shown by the melting-temperature profiles of the final product and no gel electrophoresis. Each PCR reaction was performed in triplicate on two separate occasions from at least three independent experiments.

**Materials**—Human recombinant IL-1β was obtained from R&D Systems Inc. 15d-PGJ2, troglitazone (TRO), Wy14643, GF120918, GW9226, and enalapril were purchased from Cayman Chemical. The detection limit and sensitivity was 9 pg/ml. All assays were performed in duplicate.

**Western Blot Analysis**—Cells were lysed in ice-cold lysis buffer (50 mM HCl, pH 7.4, 150 mM NaCl, 2 mM EDTA, 1 mM PMSF, 10 μg/ml each of aprotinin, leupeptin, and pepstatin, 1% Nonidet P-40, 1 mM sodium orthovanadate (Na3VO4), and 1 mM NaF). Lysates were sonicated on ice and centrifuged at 12,000 rpm for 15 min. The protein concentration of the supernatant was determined using the bicinchoninic acid method (Pierce). 20 μg of total cell lysate or nuclear extracts was subjected to SDS-polyacrylamide gel electrophoresis and electrotransferred to a nitrocellulose membrane (Bio-Rad). After blocking in 20 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, 0.1% Tween 20, and 5% (w/v) nonfat dry milk, blots were incubated overnight at 4 °C with primary antibodies and washed with wash buffer (Tris-buffered saline, pH 7.5, with 0.1% Tween 20). The blots were then incubated with horseradish peroxidase–conjugated secondary antibody (Pierce), washed again, incubated with SuperSignal Ultra Chemiluminescent reagent (Pierce), and finally exposed to X-Omat film (Eastman Kodak Co., Rochester, NY).

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**Plasmids and Transient Transfection**—The human mPGES-1 promoter construct (~550–28) was kindly provided by Dr. Terry J. Smith (University of California, Los Angeles) (13). The human expression vector pEGF120918 (Roche Applied Science) for h IL-1β was transfected into the cells by the procedure described below. The human Egr-1 expression vector and the pEgr-1Mutx3-TK-Luc reporter construct were generously provided by Dr. Yuqing E. Chen (Morehouse School of Medicine, Atlanta, GA) (31). A β-galacto-
sidase reporter vector under the control of SV40 promoter (pSV40-β-galactosidase) was from Promega.

Transient transfection experiments were performed using FuGENE 6 (1 μg of DNA/μl of FuGENE 6) (Roche Applied Science) according to the manufacturer's recommended protocol. Briefly, HSFs were seeded and grown to 50–60% confluence. The cells were transfected with 1 μg of the reporter construct and 0.5 μg of the internal control pSV40-β-galactosidase. In cotransfection experiments the amount of transfected DNA was kept constant by using a corresponding empty vector. Six hours later, the medium was replaced with DMEM containing 1% FCS. The next day, the cells were treated for another 14 h with or without IL-1β in the absence or presence of 15d-PGJ2 or TRO. After harvesting, luciferase activity was determined and normalized to β-galactosidase activity (27).

Nuclear Extract Preparation and Electrophoretic Mobility Shift Assay—Nuclear extracts were prepared as previously described (28). Briefly, HSFs were washed in ice-cold phosphate-buffered saline and gently scrapped in ice-cold hypotonic buffer containing 10 mM HEPES-KOH, pH 7.9, 10 mM KCl, 1.5 mM MgCl2, 0.5 mM DTT, 0.2 mM EDTA, 1 mM MgCl2, 4% glycerol, 0.5 mM PMSF, 1 mM Na2VO3, and 10 μg/ml each of aprotinin, leupeptin, and pepstatin. The cells were allowed to swell on ice, and the nuclei were recovered by brief centrifugation. The pellets were resuspended in high salt buffer containing 20 mM HEPES, pH 7.9, 420 mM NaCl, 1.2 mM MgCl2, 0.5 mM DTT, 0.2 mM EDTA, 25% glycerol, 0.5 mM PMSF, 1 mM Na2VO3, and 10 μg/ml each of aprotinin, leupeptin, and pepstatin, followed by incubation on ice for 20 min. The nuclear extracts were recovered by centrifugation, and protein concentration was determined by the method of Bradford (Bio-Rad).

A synthetic double-stranded oligonucleotide, corresponding to the Egr-1 motifs in the human mPGES-1 promoter (5'-TGGGGGTCCCTGGGCCTGGTCT-3'), was end-labeled by T4 polynucleotide kinase in the presence of [γ-32P]ATP. The mutant competitor oligonucleotide had the following sequence with a 4-bp substitution (underlined): 5'-TGGGGGTCCCTGTTCCCTGGTCT-3'. The binding buffer consisted of 10 mM Tris-HCl, pH 7.5, 50 mM NaCl, 0.5 mM DTT, 0.5 mM EDTA, 1 mM MgCl2, 4% glycerol, and 2.5 μg of poly(dI-dC). Binding reactions were conducted with 5 μg of nuclear extract and 100,000 cpm of end-labeled oligonucleotide probe at 22°C for 20 min in a final volume of 10 μl. In supershift assays, the antibody to Egr-1 (1 μg/reaction) was incubated with the reaction mixture for 1 h at 4°C before the addition of 32P-labeled oligonucleotide. In cold competition assays, 100-fold molar excess of cold wild-type or mutant oligonucleotide was used. Binding complexes were resolved on non-denaturing 6% polyacrylamide gel electrophoresis in a Tris borate buffer system, after which the gels were fixed, dried, and subjected to autoradiography.

Statistical Analysis—All results were calculated as the mean ± S.E. of independent experiments. Statistics were analyzed using Student's 2-tailed t test. p values of <0.05 were considered significant.

RESULTS

Effect of PPAR Ligands on IL-1β-induced PGE2 Production and mPGES-1 Protein Expression in HSF—We initially examined the effect of three distinct classes of PPAR ligands on IL-1β-induced PGE2 production in HSF: 15d-PGJ2 and TRO, natural and synthetic PPARγ activators, respectively, and Wy14643, a selective PPARα agonist. Quiescent HSF were stimulated with IL-1β (100 pg/ml) in the absence or presence of increasing concentrations of 15d-PGJ2 (5, 10, and 20 μM), TRO, or Wy14643 (10, 25, and 50 μM), and PGE2 production was determined. Under control cell culture conditions, HSFs released low levels of PGE2, and stimulation with IL-1β led to severalfold increase in PGE2 production (Fig. 1). Pretreatment with increasing concentrations of the PPARγ ligands 15d-PGJ2 and TRO suppressed IL-1β-induced PGE2 production in a dose-dependent manner. Conversely, the selective PPARα agonist had no effect on IL-1β-induced PGE2 production (Fig. 1).

To determine whether these changes in PGE2 release were related to differences in amounts of mPGES-1, Western blotting of cell lysate protein was carried out. As expected, treatment with IL-1β resulted in a strong induction of mPGES-1 protein expression (16 kDa) (Fig. 2, A–C). Interestingly, both PPARγ ligands, 15d-PGJ2 and TRO, suppressed IL-1β-induced mPGES-1 protein expression in a dose-dependent manner.
The possibility that PPARγ is involved in the repression of mPGES-1 is further tested using the PPARγ antagonist. HSFs were preincubated with increasing concentrations of GW9662 (1, 5, and 10 μM) for 30 min prior to the addition of 15d-PGJ₂ (20 μM) or TRO (50 μM) (Fig. 6B), suggesting that the inhibition of IL-1β-induced mPGES-1 expression by 15d-PGJ₂ and TRO is mediated by PPARγ.

To confirm the involvement of PPARγ in the suppressive effects of 15d-PGJ₂ and TRO on IL-1β-induced mPGES-1 expression, we examined the action of GW9662, a selective and irreversible PPARγ antagonist. HSFs were preincubated with increasing concentrations of GW9662 (1, 5, and 10 μM) for 30 min prior to the addition of 15d-PGJ₂ (20 μM) or TRO (50 μM) and were subsequently stimulated with IL-1β (100 pg/ml) for 18 h. Western blot analysis revealed that GW9662 dose-dependently relieved the suppressive effect of 15d-PGJ₂ (Fig. 6C, last three lanes).

PPARγ-DN on its own had no significant effect on IL-1β-induced mPGES-1 promoter activation (bar 2 versus bar 3), it relieved the suppressive effects of both 15d-PGJ₂ (20 μM) (bar 4 versus bar 6) and TRO (50 μM) (bar 5 versus bar 7) (Fig. 5B), suggesting that the inhibition of IL-1β-induced mPGES-1 expression by 15d-PGJ₂ and TRO is mediated by PPARγ.
mediated activation of mPGES-1 promoter. As shown in Fig. 7, the activity of the mPGES-1 promoter was enhanced by cotransfection with a human Egr-1 expression plasmid (bar 1 versus bar 2). However, the activation of the mPGES-1 promoter by Egr-1 was significantly attenuated by cotransfection with the human PPARγ/H9253 expression plasmid (bar 2 versus bar 3). The activity of mPGES-1 promoter was also reduced by either 15d-PGJ2 (5 μM) (bar 2 versus bar 4) or TRO (10 μM) (bar 2 versus bar 5). Moreover, the suppressive effect of PPARγ was further enhanced in the presence of 15d-PGJ2 (bar 3 versus bar 6) or TRO (bar 3 versus bar 7).

Next, we sought to confirm that the inhibition of Egr-1 transcriptional activity is essential in the suppression of mPGES-1 by PPARγ. To this end, we analyzed the effect of PPARγ on the transcriptional activation of a synthetic luciferase reporter construct containing three tandem repeats of the putative Egr-1 binding sequence, pEgr-1 × 3-TK-Luc (31). As shown in Fig. 7B, overexpression of the human Egr-1 cDNA induced a robust increase in the transcriptional activity of the above construct (bar 1 versus bar 2). This activation was attenuated by cotransfection with the human PPARγ expression plasmid (bar 2 versus

**Fig. 4.** 15d-PGJ2 and TRO inhibit the transcriptional activity of mPGES-1 promoter. HSFs were cotransfected with 1 μg/well of the human mPGES-1 promoter (~538/-28) construct ligated to luciferase and 0.5 μg of the internal control pSV40-β-galactosidase, using FuGENE 6 transfection reagent. The next day, transfected cells were incubated with increasing concentrations of 15d-PGJ2 (A) or TRO (B) in the absence or presence of IL-1β (100 pg/ml) for 14 h. Luciferase activity values were determined on cell extracts and normalized to β-galactosidase activity. Results are expressed as -fold induction, considering 1 as the value of unstimulated cells and represent the mean ± S.E. of four independent experiments. *, p < 0.05; compared with cells treated with IL-1β alone (control).

**Fig. 5.** Effect of PPARγ and dominant negative PPARγ on 15d-PGJ2- and TRO-mediated suppression of mPGES-1 promoter activity. HSFs were cotransfected with the mPGES-1 promoter (1 μg/well), the internal control pSV40-β-galactosidase (0.5 μg/well), and 0.5 μg of vectors expressing PPARγ (A) or DN-PPARγ (B). The total amount of transfected DNA was kept constant by addition of empty vector. The next day, cells were treated with the indicated concentration of 15d-PGJ2 or TRO in the absence or presence of 100 pg/ml IL-1β for 14 h. Luciferase activity values were determined on cell extracts and normalized to β-galactosidase activity. Results are expressed as -fold induction, considering 1 as the value of unstimulated cells and represent the mean ± S.E. of four independent experiments. *, p < 0.05; compared with cells treated with IL-1β alone (control).
from HSFs and a radiolabeled oligonucleotide corresponding to the Egr-1 binding sites in the mPGES-1 promoter. As shown in Fig. 8A, the binding of Egr-1 was strongly induced by IL-1β (lane 1 versus lane 2). When the cells were treated with 15d-PGJ2 (lanes 3–5) or TRO (lanes 6–8) the formation of the Egr-1-DNA complex decreased in a dose-dependent manner. This binding was specific, because it could be completely abolished by coincubation with a 100-fold molar excess of unlabeled probe (lane 9). Coincubation with a 100-fold molar excess of the mutant probe, did not affect Egr-1 DNA-binding activity (lane 10). The specificity of this interaction was further observed by the supershift assays, showing a further retardation in the electrophoretic mobility of the Egr-1-DNA complex in the presence of a specific anti-Egr-1 antibody (lane 11). These results suggest that PPARγ ligands inhibit IL-1β-induced mPGES-1 expression by reducing Egr-1 DNA-binding activity to the promoter sequence.

To determine whether the reduction of Egr-1 DNA-binding activity by PPARγ ligands in HSFs was due to inhibition of Egr-1 expression, we examined the effects of 15d-PGJ2 and TRO on IL-1β-induced Egr-1 protein expression. The cells were pretreated with increasing concentrations of 15d-PGJ2 or TRO prior to stimulation with IL-1β. In quiescent HSFs, protein levels of Egr-1 were very low. Treatment with IL-1β (100 pg/ml) caused a robust induction of Egr-1. Interestingly, neither 15d-PGJ2 nor TRO altered IL-1β-induced Egr-1 (Fig. 8B). These data suggest that 15d-PGJ2 and TRO are not general inhibitors of IL-1β-induced gene expression and that the inhibitory effect of PPARγ ligands on mPGES-1 expression does not involve inhibition of Egr-1 protein expression.

**DISCUSSION**

An expanding body of evidence indicates that PPARγ and its ligands play an important role in the regulation of multiple inflammatory processes (19, 24–28). In the present study, we have extended these observations by showing that both natural and synthetic PPARγ ligands inhibit IL-1β-induced mPGES-1 expression in HSFs. Furthermore, we elucidate the molecular mechanism underlying this effect. We demonstrate that this suppressive effect is transcriptional and PPARγ-dependent. Moreover, PPARγ activation inhibited the transcriptional and DNA binding activities of Egr-1. Taken together, our results
mPGES-1 promoter activation. Finally, pretreatment with an irreversible pharmacological PPARγ antagonist, GW9662, overcame the inhibitory effect of PPARγ ligands on mPGES-1 protein expression. However, inhibition of PPARγ (via GW9662 or a DN), almost completely restored the suppressive effect of TRO, whereas the suppressive effect of 15d-PGJ2 was only partially restored, suggesting that 15d-PGJ2 can activate other PPARγ-independent signaling pathways to inhibit mPGES-1 expression. In this context, several studies reported that 15d-PGJ2 inhibits many inflammatory responses by mechanisms that are independent of PPARγ, such as the expression of iNOS in microglial cells and astrocytes (34). This is the first evidence that PPARγ ligands inhibit Egr-1 transcriptional activity in a promoter-independent manner. This is the first evidence that PPARγ activation inhibits Egr-1 transcriptional activity in a promoter-independent manner. Several mechanisms can explain the repression of Egr-1 activities by PPARγ. One possibility is that PPARγ activation suppresses Egr-1 expression. Indeed, PPARγ ligands were reported to inhibit hypoxia-induced Egr-1 expression in mononuclear phagocytes (40). However, in our study, 15d-PGJ2 and TRO had no effect on IL-1β-induced Egr-1 expression in HSFs, suggesting that PPARγ ligands inhibit Egr-1 transcriptional and DNA binding activities in HSFs by distinct mechanisms. A second mechanism could be competition between PPARγ and Egr-1 for binding to response elements. This possibility is probable unlikely, because: (i) EMSA analysis showed no binding of PPARγ to an oligonucleotide corresponding to the Egr-1 binding sites in the mPGES-1 promoter; and (ii) human mPGES-1 promoter construct used in this study contains no consensus PPRE sequence; and (iii) PPARγ activation inhibited Egr-1 transcriptional activity in a promoter-independent manner. Alternatively, PPARγ may inhibit Egr-1 activity by directly binding to Egr-1 and inhibiting its DNA binding and/or transcriptional activity. In this context, PPARγ has
been shown to inhibit NF-κB, NF-AT, and SP-1 transcriptional activity through mechanisms that involve protein-protein interactions (26, 41, 42). Finally, PPARγ can attenuate Egr-1 activities by competing for general transcriptional coactivators such as CREB-binding protein (CBP/p300). CBP/p300 interacts with PPARγ and positively regulates PPARγ-dependent gene transcription (43, 44). Importantly, CBP/p300 also interacts with Egr-1 and enhances its transcriptional activity (45, 46). Thus, the sequestering of limiting amounts of CBP/p300 by activated PPARγ could account for the transcriptional repression effect of PPARγ ligands on Egr-1 activities and mPGES-1 expression. This is corroborated by our finding that overexpression of a PPARγ mutant lacking transcriptional coactivator recruitment activity overcomes the inhibitory effect of PPARγ ligands on mPGES-1 promoter activity.

In addition to Egr-1, the mPGES-1 promoter contains binding sites for transcription factors (AP-1 and SP-1) (32, 47) known to associate and/or to be down-regulated by PPARγ (24, 25, 42). Although the role of those elements in IL-1β-induced mPGES-1 transcription is still unknown, we can not exclude the possibility that PPARγ interaction with these transcription factors may be involved in the repression of mPGES-1 expression. The ability of PPARγ to repress Egr-1-mediated transcription may be of relevance for other inflammatory genes. Indeed, and as stated above, PPARγ ligands were reported to inhibit the expression of IL-1β, TNF-α, IL-2, and several chemokines. Interestingly, Egr-1 activation is involved in the transcriptional activation of these genes (48–50). Therefore, it is possible that PPARγ-mediated Egr-1 repression may be part of the mechanisms by which PPARγ down-regulates these genes. Moreover, it was recently reported that Egr-1 positively regulates expression of PPARγ. This is consistent with the suggestion that up-regulation of PPARγ expression by Egr-1 may constitute a negative feedback mechanism by which Egr-1 inhibits expression and/or signaling pathways of pro-inflammatory mediators.

Several studies demonstrated that PPARγ ligands attenuate inflammation in vivo in animal models of experimental allergic encephalomyelitis (51), inflammatory bowel disease (52), lupus nephritis (53), atherosclerosis (54), and arthritis (55, 56). Thus, inhibition of mPGES-1 could be part of a mechanism by which PPARγ inhibits inflammatory responses in vivo. In addition to inflammation, increased expression of mPGES-1 may have important consequences in other pathological conditions. For instance, ischemia-reperfusion of mPGES-1 has been described in symptomatic atherosclerotic plaques and various carcinoma and cancer cell lines (14–18). PPARγ ligands may therefore have clinical application not only in chronic inflammatory conditions but neoplastic diseases as well.

In conclusion, we show for the first time that PPARγ activation suppresses mPGES-1 expression via negative interference with Egr-1. This novel function of PPARγ ligands further supports the role of PPARγ in inflammation and suggests that the modulation of this gene expression by PPARγ ligands may constitute an additional therapeutic tool to take into account for the treatment and/or prevention of inflammatory and neoplastic diseases.

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REFERENCES

1. Crosby, C. G., and DuBois, R. N. (2003) Expert Opin. Emerg. Drugs 8, 1–7
2. Martel-Pelletier, J., Pelletier, J. P., and Fahmi, H. (2003) Semin. Arthritis Rheum. 33, 155–167
3. Smith, W. L., and Langenbach, R. (2001) J. Clin. Invest. 107, 1491–1495
Brenner, M. B., and Goldfeld, A. E. (2003) Mol. Cell. Biol. 23, 526–533
46. Silverman, E. S., Du, J., Williams, A. J., Wadgaonkar, R., Drazen, J. M., and Colline, T. (1998) Biochem. J. 336, 183–189
47. Ekstrom, L., Lyrennas, L., Jakobsson, P. J., Morgenstern, R., and Kelner, M. J. (2003) Biochim. Biophys. Acta 1627, 79–84
48. Yan, S. F., Fujita, T., Lu, J., Okada, K., Shan Zou, Y., Mackman, N., Pinsky, D. J., and Stern, D. M. (2000) Nat. Med. 6, 1355–1361
49. Yao, J., Mackman, N., Edgington, T. S., and Fan, S. T. (1997) J. Biol. Chem. 272, 17795–17801
50. Skerka, C., Decker, E. L., and Zipfel, P. F. (1995) J. Biol. Chem. 270, 22500–22506
51. Diab, A., Deng, C., Smith, J. D., Hussain, R. Z., Phanavanh, B., Lovett-Racke, A. E., Drew, P. D., and Racke, M. K. (2002) J. Immunol. 168, 2508–2515
52. Su, C. G., Wen, X., Bailey, S. T., Jiang, W., Rangwala, S. M., Keilbaugh, S. A., Flanagan, A., Murthy, S., Lazar, M. A., and Wu, G. D. (1999) J. Clin. Invest. 104, 383–389
53. Reilly, C. M., Oates, J. C., Cook, J. A., Morrow, J. D., Halushka, P. V., and Gilkeson, G. S. (2000) J. Immunol. 164, 1498–1504
54. Li, A. C., Brown, K. K., Silvestre, M. J., Wilson, T. M., Palinski, W., and Glass, C. K. (2000) J. Clin. Invest. 106, 523–531
55. Kawahito, Y., Rondo, M., Tsuuchi, Y., Hashimoto, A., Bishop-Bailey, D., Inoue, K., Kohno, M., Yamada, R., Ha, T., and Sano, H. (2000) J. Clin. Invest. 106, 189–197
56. Cuzzocrea, S., Mazzon, E., Dugo, L., Patel, N. S., Serraino, I., Di Paola, R., Genovese, T., Britti, D., De Maio, M., Caputi, A. P., and Thiemermann, C. (2003) Arthritis Rheum. 48, 3544–3556