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Feedback Control of Cyclooxygenase-2 Expression through PPARγ*

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Cyclooxygenase-2 (COX-2), a rate-limiting enzyme for prostaglandins (PG), plays a key role in inflammation, tumorigenesis, development, and circulatory homeostasis. The PGD_2 metabolite 15-deoxy-$\Delta^{12,14}$ PGJ_2 (15d-PGJ_2) was identified as a potent natural ligand for the peroxisome proliferator-activated receptor-γ (PPARγ). PPARγ expressed in macrophages has been postulated as a negative regulator of inflammation and a positive regulator of differentiation into foam cell associated with atherogenesis. Here, we show that 15d-PGJ_2 suppresses the lipopolysaccharide (LPS)-induced expression of COX-2 in the macrophage-like differentiated U937 cells but not in vascular endothelial cells. PPARγ mRNA abundantly expressed in the U937 cells, not in the endothelial cells, is down-regulated by LPS. In contrast, LPS up-regulates mRNA for the glucocorticoid receptor which ligand anti-inflammatory steroid dexamethasone (DEX) strongly suppresses the LPS-induced expression of COX-2, although both 15d-PGJ_2 and DEX suppressed COX-2 promoter activity by interfering with the NF-κB signaling pathway. Transfection of a PPARγ expression vector into the endothelial cells acquires this suppressive regulation of *COX-2* gene by 15d-PGJ_2 but not by DEX. A selective COX-2 inhibitor, NS-398, inhibits production of PGD_2 in the U937 cells. Taking these findings together, we propose that expression of COX-2 is regulated by a negative feedback loop mediated through PPARγ, which makes possible a dynamic production of PG, especially in macrophages, and may be attributed to various expression patterns and physiological functions of COX-2.

Cyclooxygenase (COX)[1] has two isoforms, COX-1 and -2. COX-1 is constitutively expressed in most cells, whereas COX-2 is largely absent but is induced upon stimulation by inflammatory stimuli such as endotoxin lipopolysaccharide (LPS), suggesting that COX-2 plays a critical role in inflammation (1, 2). However, growing evidence indicates that expression of COX-2 is differently regulated in different types of cells and also plays a key role in tumorigenesis (3), development (4–6), and circulatory homeostasis (7, 8). In fact, three cis-acting elements, the NF-κB and NF-IL6 sites and the cyclic AMP response element (CRE), are differently involved in COX-2 promoter activity in different cells (2, 9–17). Anti-inflammatory steroid dexamethasone (DEX) suppresses COX-2 expression in macrophage-like differentiated U937 cells (13) but not in bovine arterial endothelial cells (BAEC) (16). This cell type-specific regulation may be physiologically important because thromboxane A_2 produced by macrophages (18) has the opposite effect of prostacyclin (PGI_2), produced by vascular endothelial cells. We have recently reported that this different effect of DEX is partly explained by differing expression levels of glucocorticoid receptor (GR) (16). Moreover, expression of PGI_2 and thromboxane A_2 synthases are inversely regulated in resident and activated peritoneal macrophages (19), where production of PGD_2 and PGE_2 is also inversely regulated (20), which suggests complex regulation of COX-2 expression as well as its physiological roles at different activated stages of macrophages.

The peroxisome proliferator-activated receptor-γ (PPARγ) is a ligand-dependent transcription factor belonging to the family of nuclear receptors that includes the estrogen receptors, thyroid hormone receptors, and GRs (21). The PGD_2 metabolite 15-deoxy-$\Delta^{12,14}$ PGJ_2 (15d-PGJ_2) was identified as a potent natural ligand for the PPARγ (22, 23). PPARγ expressed in macrophages has been postulated as a negative regulator of inflammation (24, 25) and a positive regulator of differentiation into foam cells associated with atherogenesis (26, 27). Recently, induction of COX-2 by 15d-PGJ_2 was reported in immortalized epithelial and colorectal cancer cells (28, 29), although 15d-PGJ_2 suppressed COX-2 expression in fetal hepatocytes (30). The molecular mechanisms that underlie different regulation of COX-2 expression remain to be elucidated.

In the present study, we investigated the different effect of 15d-PGJ_2 on expression of the *COX-2* gene between macrophage-like differentiated U937 cells and BAEC. We provide evidence that a unique expression pattern of PPARγ is involved in this different effect. Especially in U937 cells, LPS down-regulates PPARγ mRNA but up-regulates GR mRNA, although both 15d-PGJ_2 and DEX suppressed COX-2 expression by interfering with the NF-κB signaling pathway. With additional evidence, we propose that the expression of COX-2 will be found to be regulated by a negative feedback loop mediated through PPARγ. This makes possible a dynamic production of PG especially in macrophages.

MATERIALS AND METHODS

Cell Culture—U937 cells (10) and BAEC (11) were grown in RPMI 1640 and Dulbecco's modified Eagle's medium, respectively, supplemented with 10% fetal calf serum (Flow Laboratories, Irvine, Scotland, UK), 50 μM 2-mercaptoethanol, 100 units/ml penicillin, and 100 μg/ml streptomy-

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† Deceased. This paper is dedicated to the memory of Kazuhiko Umesono, our friend and esteemed collaborator.

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[1] The abbreviations used are: COX, cyclooxygenase; PG, prostaglandin(s); LPS, lipopolysaccharide; DEX, dexamethasone; BAEC, bovine arterial endothelial cell(s); GR, glucocorticoid receptor; PPARγ, peroxisome proliferator-activated receptor-γ; 15d-PGJ2, 15-deoxy-$\Delta^{12,14}$ PGJ_2; NF-IL6, nuclear factor for interleukin-6 expression; NF-κB, nuclear factor κB; TPA, 12-*O*-tetradecanoylphorbol-13-acetate; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; CRE, cyclic AMP response element.

cin. For differentiation into monocytes/macrophages, U937 cells were treated with 100 nM TPA and allowed to adhere for 48 h, after which they were fed with TPA-free medium and cultured for 24 h prior to use.

Determination of PG Synthesis—TPA-differentiated U937 cells (5×10^5 cells/well) were cultivated on 12-well tissue culture plates with 1 ml of the culture medium. After a further 24-h of incubation, the relevant reagents were added to the medium. After 12 h of incubation, the culture medium was removed and subjected to enzyme immunoassays for PGE_2 and PGD_2 (Cayman). PGD_2 was measured as its methyl oxime after derivatization with methoxamine.

RNA Analysis—Total RNA was isolated using the acid guanidinium thiocyanate procedure. RNAs were then subjected to electrophoresis. The cDNA probes used were the 1.5-kilobase pair insert of pHEPSII17 for COX-2 (31), the 3-kilobase pair fragment of pRShGRα digested with *Kpn*I/*Xho*I for GR (32), the entire coding sequences for human PPARγ from the expression vector, and the cDNA insert (nucleotides 61–950) for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (31). The levels of mRNA were calculated on the basis of hybridization signals as measured by an image analyzer, BAS 5000 (Fuji Photo Film Co., Tokyo). Reverse transcriptase-polymerase chain reaction analysis was performed using KOD DNA polymerase (Toyobo, Osaka, Japan) as described previously (16). The primer pair for PPARγ amplification, designed to anneal to both human (33) and bovine (34) sequences, was as follows: 5′-CCAAAGTGCAATCAAAGTGGAGCC-3′ and 5′-GCAGGCTCTTTAGAAAACTCCCTTG-3′. The cycling conditions were as follows: 3 min at 96 °C, followed by 30 cycles of 94 °C, 15 s; 57 °C, 2 s; and 74 °C, 30 s. The primer pair for human PGD_2 synthase (35) had the following sequence: 5′-CCTTGGGCAGAGAAAAAGCAAG-3′ and 5′-AACATGGATCAGCTAGAGTTT-GG-3′. The cycling conditions were as follows: 3 min at 96 °C, followed by 30 cycles of 94 °C, 15 s; 58 °C, 2 s; and 74 °C, 15 s.

Western Blot Analysis—Cell lysates (10^5 cell equivalents) were subjected to SDS-polyacrylamide gel electrophoresis on 10% gels. The separated proteins were electroblotted onto a polyvinylidene difluoride membrane (Millipore). The membranes were probed with the human COX-2 antisera (IBL, Gunma, Japan) and visualized using the ECL Western blot analysis system (Amersham Pharmacia Biotech) according to the manufacturer's instructions.

Transcription Assays—U937 cells stably transfected with a COX-2 (nucleotide −327/+59) luciferase reporter containing NF-κB site alone and pCB6 containing a neomycin-resistant gene were made by electroporation as described previously (13). BAEC was transfected using Trans IT™-LT-1 (Mirus) (16). 0.2 μg of COX-2 reporter vector phPES2(−327/+59) (10), 2.0 μg of pRShGRα, or pCMX-hPPARγ1, and 0.02 μg of pSV-βgal (Promega) were used for transfection of each 24-well plate. pCMX-hPPARγ1 was the human PPARγ1 expression vector under control of a cytomegalovirus promoter made by Dr. S. Osada (Kyoto University). Luciferase and β-galactosidase activities were determined; luciferase activity was normalized to the β-galactosidase standard in BAEC (11), whereas it was normalized with the protein concentration in the U937 cells (13).

RESULTS

15d-PGJ_2 Inhibits Expression of COX-2 in U937 Cells but Not in BAEC—To determine the effects of 15d-PGJ_2 on the expression of *COX-2* gene, we performed Northern blot analysis using RNA derived from the differentiated U937 cells. LPS-induced expression of COX-2 mRNA (Fig. 1, *A* and *B*) and production of PGE_2 (Fig. 1*C*) were suppressed by 15d-PGJ_2 in the U937 cells. The suppressive effect of 15d-PGJ_2 was dose-dependent (Fig. 1*A*) and milder than that of DEX, *i.e.* 10 μM 15d-PGJ_2 showed 50–60% suppression (Figs. 1*B* and 2), whereas 100 nM DEX showed more than 70% (13). This was also confirmed by Western blot analysis of COX-2 protein (Fig. 1*D*). In contrast, in BAEC, 15d-PGJ_2 showed no effect on LPS-induced COX-2 mRNA expression (Fig. 2). Similar results were also obtained in human umbilical vein endothelial cells (data not shown).

Down-regulation of PPARγ by LPS in U937 Cells—DEX-mediated suppression of COX-2 expression is modulated by GR, which will explain the distinct effect of DEX on COX-2 expression between macrophages and endothelial cells (16). Similarly, we examined whether expression of PPARγ accounts for the different effects of 15d-PGJ_2. Expression of PPARγ mRNA was observed in the differentiated U937 cells (33) as well as in monocytes and macrophages (27), and that expression was down-regulated by the treatment of LPS in a time-dependent manner (Fig. 3*A* and 3C). This down-regulation was not observed by the treatment of 15d-PGJ_2 alone (data not shown). In contrast, no PPARγ mRNA was detected in BAEC (Fig. 3, *A* and *B*) and human umbilical vein endothelial cells, although PPARδ mRNA was constitutively expressed in both

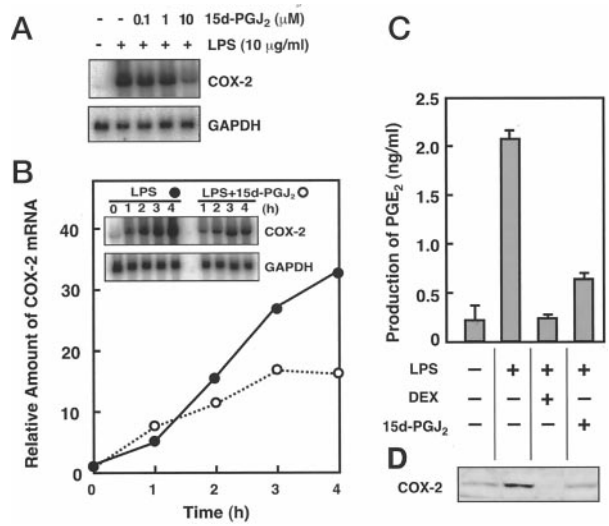

FIG. 1. **Inhibition of COX-2 gene expression and production of PGE_2 by 15d-PGJ_2 in differentiated U937 cells.** *A*, macrophage-like differentiated U937 cells were treated for 5 h with LPS in the presence or absence of the indicated concentrations of 15d-PGJ_2. Total RNA (10 μg) was isolated from the U937 cells and subjected to Northern blot analysis using specific COX-2 and GAPDH cDNA probes. *B*, time course of COX-2 mRNA expression in the U937 cells treated with LPS in the presence or absence of 10 μM 15d-PGJ_2. The relative amount of COX-2 mRNA was measured by an image analyzer after normalization with that of GAPDH. Values represent the means ± standard deviations of three separate dishes. *C*, PGE_2 in the culture medium was measured by enzyme immunoassays after treatment of the cells with LPS (10 μg/ml) and/or DEX (100 nM) or 15d-PGJ_2 (10 μM) for 12 h. Values represent the means ± standard deviations of three separate wells. *D*, cells treated with reagents described in *C* were collected, and proteins were examined by Western blot analysis using antisera specific for COX-2. Similar results were obtained in two additional experiments.

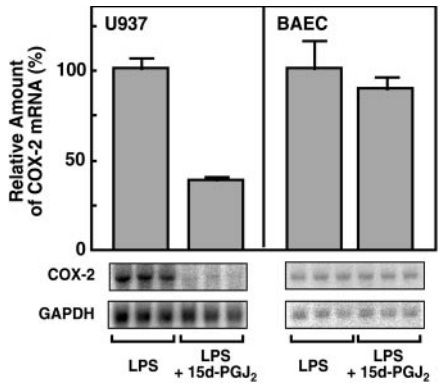

FIG. 2. **Different effects of 15d-PGJ_2 between differentiated U937 cells and BAEC.** Macrophage-like differentiated U937 cells and BAEC were treated with LPS for 5 h in the presence or absence of 10 μM 15d-PGJ_2. Isolated total RNA (10 μg) was examined by Northern blot analysis for expression of COX-2 mRNA. The relative amount of COX-2 mRNA was measured by an image analyzer after normalization with that of GAPDH, and LPS-induced amount of COX-2 mRNA was indicated as 100% because expression of COX-2 mRNA was very low in both cells without the LPS treatment. The results represent the mean ± standard deviations of three separate dishes. The GAPDH expression level in the U937 cells is higher than that in BAEC, although ethidium bromide staining intensities of 28 S RNA were equal between them, as measured by an image analyzer FLA2000. Similar result was also obtained using a bovine COX-2 cDNA probe instead of the human probe.

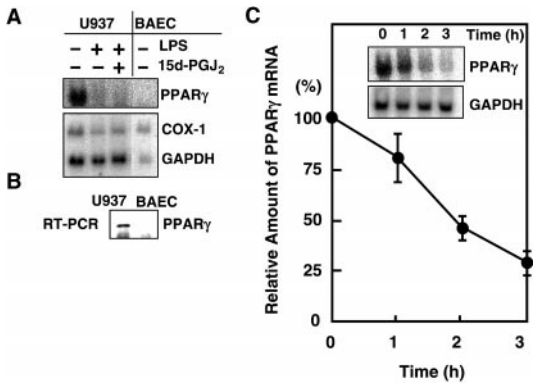

FIG. 3. **Different expression patterns of PPARγ mRNA between U937 cells and BAEC.** Macrophage-like differentiated U937 cells and BAEC were treated with LPS for 5 h in the presence or absence of 10 μM 15d-PGJ_2. *A*, isolated total RNA (10 μg) was examined by Northern blot analysis using radiolabeled probes for COX-2, PPARγ, COX-1, and GAPDH, respectively, after stripping each probe in this order. *B*, RNA samples (1 μg each) extracted from U937 cells and BAEC were subjected to reverse transcriptase-polymerase chain reaction analysis (*RT-PCR*) to confirm the relative expression levels of PPARγ, as described under "Materials and Methods." *C*, U937 cells were treated with LPS (10 μg/ml), and at the indicated times, total RNA was isolated and examined by Northern blot analysis using radiolabeled probes for PPARγ and GAPDH. The relative amount of PPARγ mRNA was measured by an image analyzer after normalization with that of GAPDH and relative amount of PPARγ before treatment with LPS was indicated as 100%. Values represent the means ± standard deviations of three separate dishes. This down-regulation of PPARγ mRNA was observed after treatment with LPS but not with 15d-PGJ_2 (10 μM) alone. Similar results were obtained in two additional experiments.

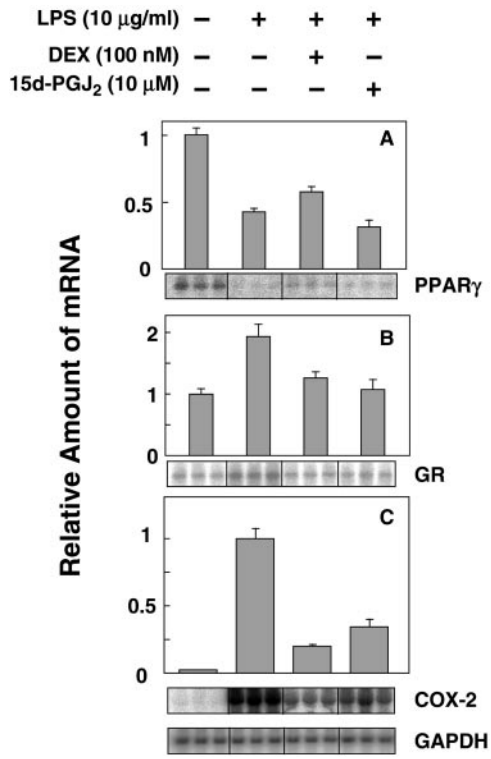

FIG. 4. **Inverse expression patterns of PPARγ and GR in the differentiated U937.** Macrophage-like differentiated U937 cells were treated with the indicated reagents for 5 h. Isolated total RNA (10 μg) was examined by Northern blot analysis using radiolabeled probes for COX-2, PPARγ, GR, and GAPDH, respectively, after stripping each probe in this order. The relative amounts of PPARγ (*A*), GR (*B*), and COX-2 (*C*) mRNAs were measured by an image analyzer after normalization with that of GAPDH. Values represent the means ± standard deviations of three separate dishes. Similar results were obtained in two additional experiments.

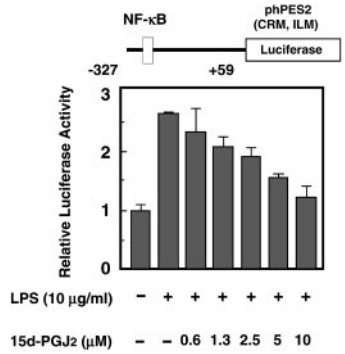

FIG. 5. **Suppressive effect of 15d-PGJ_2 on COX-2 transcription in the differentiated U937 cells.** U937 cells stably transfected with a −327/+59 COX-2 reporter gene, *phPES2*(*CRM, ILM*), consisting of only an NF-κB site, were differentiated into the macrophage-like cells as described under "Materials and Methods." The cells were treated with LPS (10 μg/ml) and the indicated concentrations of 15d-PGJ_2 and analyzed for luciferase activity 5 h later. Results are represented as fold increases in luciferase activity/μg of protein relative to the control. Values represent the means ± standard deviations of three separate wells. Similar results were obtained in two additional experiments.

cells as well as in U937 cells (data not shown). In aortic smooth muscle cells (36), the PPARα activators inhibit the inflammatory response. However, in the U937 cells as well as in activated macrophages (24), no expression of PPARα was observed by Northern blot analysis; a selective PPARα activator, Wy-14643 (100 μM), showed no effect on COX-2 mRNA expression in the U937 cells (data not shown).

Inverse Expression of PPARγ and GR by LPS in U937 Cells—As described previously, the suppressive effect of 15d-PGJ_2 on COX-2 expression was milder than that of DEX in the U937 cells. To address this question, we examined the expression levels of GR after various treatments (Fig. 4). LPS increased GR mRNA about 2-fold, which shows an inverse expression pattern between GR and PPARγ. Moreover, DEX partly restored the suppressive expression of PPARγ by LPS. This inverse expression pattern between GR and PPARγ is explained in part by the milder suppressive effect of 15d-PGJ_2 than of DEX, suggesting that different roles of GR and PPARγ on COX-2 expression.

Involvement of PPARγ in COX-2 Expression—Next, we examined the effect of 15d-PGJ_2 on the COX-2 promoter activity. The human COX-2 promoter region (−327/+59) contains the NF-κB and NF-IL6 sites and CRE (31). In the differentiated U937 cells expressing GR and PPARγ, the NF-κB site is involved in both LPS-induced expression of the *COX-2* gene and its suppression by DEX (13). Similarly, 15d-PGJ_2 suppressed COX-2 transcription mediated through the NF-κB site in a dose-dependent manner (Fig. 5). On the other hand, in BAEC expressing no detectable levels of GR (16) and PPARγ (Fig. 3, *A* and *B*), C/EBPδ (also known as NF-IL6β) activates COX-2 transcription mainly through CRE, whereas the NF-κB and NF-IL6 sites also contribute to the COX-2 expression (11). Transient transfection assay using the COX-2 promoter (−327/+59) showed that 15d-PGJ_2 did not suppress the COX-2 promoter activity in BAEC (Fig. 6), which is consistent with no suppression of COX-2 mRNA by 15d-PGJ_2 (Fig. 2). However, by coexpression of PPARγ, BAEC acquired the suppressive regulation of *COX-2* gene by 15d-PGJ_2 but not by DEX, whereas by coexpression of GR (16), BAEC acquired a more suppressive regulation by DEX than by 15d-PGJ_2 (Fig. 6), indicating the involvement of PPARγ in regulation of COX-2 expression by 15d-PGJ_2.

Suppression of PGD_2 Production by NS398—In the presence of albumin or serum, PGD_2 is metabolized to PGJ_2 and Δ^{12}-PGJ_2, natural ligands for PPARγ (22, 23), and these PGD_2

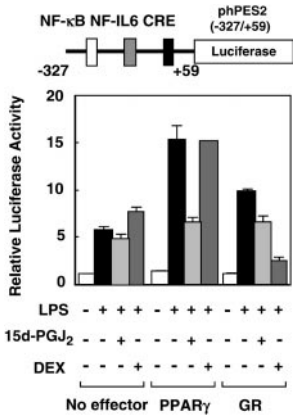

FIG. 6. **BAEC transfected with a PPARγ expression vector acquire the ability to suppress COX-2 transcription after treatment with 15d-PGJ$_2$ but not with DEX.** BAEC were transfected with a COX-2 reporter vector, *phPES2*(*−327/+59*) along with either human PPARγ expression vector (pCMX-hPPARγ1) or human GR expression vector (pRShGRα) and with pSV-βgal as an internal control for the transfection. 48 h after transfection, the cells were incubated for 5 h with no stimulant or with 1 μg/ml LPS in the presence or absence of 10 μM 15d-PGJ$_2$ or 100 nM DEX. Values represent the means ± standard deviations of three separate wells. Similar results were obtained in two additional experiments.

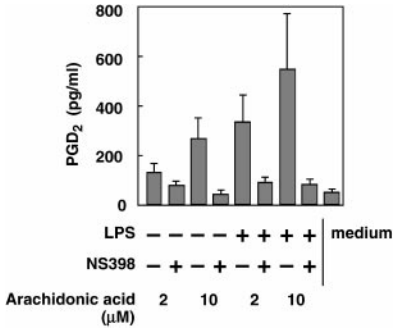

FIG. 7. **Production of PGD$_2$ through the COX-2 pathway in U937 cells.** PGD$_2$ in culture medium was measured by enzyme immunoassay after cells were treated with LPS (10 μg/ml) and/or NS-398 (1 μM) for 16 h and then stimulated with the indicated concentrations of arachidonic acid for 1 h. Values represent the means ± standard deviations of three separate wells. Similar results were obtained in two additional experiments. The concentration of PGD$_2$ was not changed by the addition of 10 μM arachidonic acid alone.

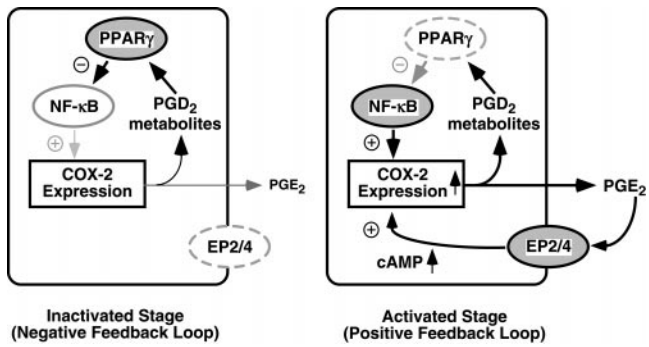

FIG. 8. **Control of COX-2 expression by positive and negative feedback loops mediated through PPARγ and PG receptors in macrophages.** In the inactivated stage, macrophages produce PGD$_2$ metabolites via the cyclooxygenase pathway using COX-2. PGD$_2$ metabolites inhibit COX-2 transcription, partly mediated through PPARγ, by interfering with the NF-κB pathway. Because of suppression of COX-2 expression, production of PGD$_2$ decreases. A low level of COX-2 expression is retained in this negative feedback loop. In the activated stage, by stimulation with endotoxin LPS, up-regulation of COX-2 is coincident with down-regulation of PPARγ, which removes the negative feedback loop. A large amount of PGE$_2$, caused by the induction of COX-2 expression, activates PGE receptor subtypes EP2 and EP4, which increases the intracellular cAMP level and enhances COX-2 transcription. This positive feedback loop can be removed by DEX, which activates GR and suppresses COX-2 expression, and a relative level of COX-2 expression may return to the inactivated stage. In fact, LPS up-regulates GR mRNA.

metabolites actively transport to cellular nuclei (37). Therefore, there is a possibility that COX-2 expression is self-regulated by PGD$_2$ metabolites, which are produced in a COX-2 dependent manner. To examine this possibility, we investigated whether U937 cells produce PGD$_2$ in a COX-2 dependent manner. An enzyme immunoassay showed that U937 cells produced PGD$_2$, and a COX-2 selective inhibitor, NS398, suppressed this production (Fig. 7). Moreover, PGD$_2$ synthase mRNA was detected by reverse transcriptase-polymerase chain reaction analysis of the U937 cells (data not shown). These results are also consistent with previous reports in bone marrow-derived macrophages (20) and specialized antigen-presenting cells (38).

DISCUSSION

The present study has shown that 15d-PGJ$_2$ suppressed LPS-induced COX-2 mRNA in macrophage-like differentiated U937 cells but not in vascular endothelial cells. This difference comes from different expression patterns of PPARγ, *i.e.* a much higher level of expression in the U937 cells (Fig. 3) and acquisition of 15d-PGJ$_2$ sensitivity upon COX-2 expression by coexpression of PPARγ into BAEC (Fig. 6). Moreover, LPS down-regulates PPARγ mRNA but up-regulates GR mRNA, although PPARγ and GR suppressed COX-2 promoter activity by interfering with the NF-κB signaling pathway (13) (Fig. 5). On the other hand, U937 cells as well as macrophages (20) produces PGD$_2$ in a COX-2-dependent manner, and PGD$_2$ is spontaneously converted to PGJ$_2$ derivatives by non-enzymatic dehydration (37). Therefore, we propose that PGD$_2$ metabolites such as 15d-PGJ$_2$ work as intracellular signaling mediators, which retain the low expression level of COX-2 by a negative feedback loop meditated through PPARγ in macrophages (Fig. 8). After treatment with LPS, up-regulation of COX-2 was coincident with down-regulation of PPARγ (Fig. 3), which canceled the negative feedback loop. Simultaneously, a rapid increase of PGE$_2$ (Fig. 1) was observed, and cAMP enhanced the COX-2 transcription by LPS in the U937 cells (13), suggesting that COX-2 expression is enhanced by a positive feedback loop (20) mediated through PG receptors. In fact, the existence of PGE receptor subtypes EP2 and EP4, increasing the intracellular cAMP level, were reported in murine macrophage-like cell line J774.1 (39). This positive feedback loop can be suppressed by DEX because LPS up-regulates GR mRNA and will increase the sensitivity to DEX (Fig. 4). Moreover, the possibility that COX-2 has anti-inflammatory properties at the later phase of carrageenin-induced pleurisy was recently reported (40), which is also explained by the negative feedback regulation of COX-2 by PPARγ. PPARγ is activated by a range of synthetic and naturally occurring substances, including antidiabetic thiazolidinediones, polyunsaturated fatty acids, PGD$_2$ metabolites, components of oxidized low density lipoprotein, and 12/15-lipoxygenase products (41). Among these ligands, rosiglitazone (BRL49653), the most potent synthetic ligand for PPARγ, did not suppress LPS-induced expression of COX-2 mRNA in U937 cells (data not shown). Interestingly, despite the stronger binding activity of rosiglitazone *in vitro*, several reports emphasize the higher biological activity of 15d-PGJ$_2$ compared with rosiglitazone (42). In this context, 15d-PGJ$_2$, but not PPARγ agonists such as troglitazone, was recently reported to be a direct inhibitor of IκB kinase, which is responsible for NF-κB activation (43), suggesting that some biological effects of 15d-PGJ$_2$ are independent of PPARγ. On the other hand, a somatic PPARγ mutation, R288H, showed a normal response to synthetic ligands but greatly decreased response to natural ligand 15d-PGJ$_2$ (44),

implying that there are different responses of PPARγ between different ligands. Moreover, the fact that subtype U937 cells express no detectable level of PPARγ indicates a significant amount of COX-2 mRNA in the inactivated stage but no induction of COX-2 mRNA by LPS,[2] suggesting the involvement of PPARγ in COX-2 expression. Further studies are necessary to elucidate these different effects between 15d-PGJ_2 and synthetic PPARγ ligands.

TPA-differentiated U937 cells would be assumed to be responsive macrophages because of similar expression patterns of COX-2 and thromboxane A_2 synthase mRNAs in casein-elicited peritoneal macrophage (19). However, expression of PPARγ but not PPARα is observed in both undifferentiated and differentiated U937 cells; this is different than the report that PPARγ is induced upon differentiation into macrophages, whereas PPARα is already present in undifferentiated monocytes (45). This discrepancy may be attributed to heterogeneity of macrophages (19).

COX-2 expression is regulated not only in a cell type-specific but also a species-specific manner. In fact, the delayed induction of COX-2 by gonadotropin was reported in bovine granulosa cells but not in rat cells; however, the induction was observed in both species (46). The similarity of nucleotide sequences of the COX-2 promoter region between bovine and human was higher than between bovine and rat genes, although cis-acting elements for NF-κB, NF-IL6 sites, and CRE are conserved among human, bovine, rat, and mouse COX-2 promoter regions. No suppression of 15d-PGJ_2 on the LPS-induced COX-2 mRNA and no detectable level of PPARγ mRNA were observed in human umbilical vein endothelial cells or in BAEC. Therefore, there is not as much difference in the regulation of COX-2 expression at least between human and bovine endothelial cells.

PPARγ and GR mRNAs are inversely regulated by LPS in U937 cells (Fig. 4), although both 15d-PGJ_2 and DEX suppressed COX-2 promoter activity by interfering with the NF-κB signaling pathway (Fig. 5). Ligands for PPARs and DEX are reported to enhance COX-2 expression in some carcinoma cells (28, 29) and amnion cells (47), respectively. These different effects on COX-2 expression may be explained by differently regulated levels of expression of PPARs, steroid hormone receptors, and CAAT enhancer-binding proteins. In this context, estrogen-induced production of a PPAR ligand was reported in a PPARγ-expressing tissue in which induced conversion of PGD_2 to a metabolite was observed (48). Interestingly, a precise transcriptional network among these transcription factors is important for adipocyte differentiation. Therefore, it will be interesting to determine each relationship between COX-2 and the transcriptional network in physiological and pathophysiological functions.

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