Upregulation of Lipopolysaccharide-Induced Interleukin-10 by Prostaglandin A₁ in Mouse Peritoneal Macrophages

Kim, Hyo Young¹, Jae Ryong Kim², and Hee Sun Kim¹*

Department of Microbiology, ¹Biochemistry and Molecular Biology, and Aging-associated Vascular Disease Research Center, College of Medicine, Yeungnam University, Daegu 705-717, Korea

Received: October 12, 2007 / Accepted: January 5, 2008

The cyclopentenone prostaglandins (cyPGs) prostaglandin A₁ (PGA₁) and 15-deoxy-D₁₂,14-prostaglandin J₂ (15d-PGJ₂) have been reported to exhibit antiinflammatory activity in activated monocytes/macrophages. However, the effects of these two cyPGs on the expression of cytokine genes may differ. In this study, we investigated the mechanism of action of PGA₁ in lipopolysaccharide (LPS)-induced expression of interleukin (IL)-10 mRNA in mouse peritoneal macrophages. 15d-PGJ₂ inhibited expression of LPS-induced IL-10, whereas PGA₁ increased LPS-induced IL-10 expression. This synergistic effect of PGA₁ on LPS-induced IL-10 expression reached a maximum as early as 2 h after simultaneous PGA₁ and LPS treatment (PGA₁/LPS), and did not require new protein synthesis. The synergistic effect of PGA₁ was inhibited by GW9662, a specific peroxisome proliferator-activated receptor γ (PPARγ) antagonist, and Bay-11-7082, a NF-κB inhibitor. The extracellular signal-regulated kinases (ERK) inhibitor PD98059 increased the expression of PGA₁/LPS-induced IL-10 mRNA, rather than inhibiting the IL-10 expression. Moreover, PGA₁ inhibited LPS-induced ERK phosphorylation. The synergistic effect of PGA₁ on LPS-induced IL-10 mRNA and protein production was inhibited by p38 inhibitor PD169316, and PGA₁ increased LPS-induced p38 phosphorylation. In the case of stress-activated protein kinase/c-Jun NH₂-terminal kinase (SAPK/JNK), the SAPK/JNK inhibitor SP600125 did not inhibit IL-10 mRNA synthesis but inhibited the production of IL-10 protein remarkably. These results suggest that the synergistic effect of PGA₁ on LPS-induced IL-10 expression is NF-κB-dependent and mediated by mitogen-activated protein (MAP) kinases, p38, and SAPK/JNK signaling pathways, and also associated with the PPARγ pathway. Our data may provide more insight into the diverse mechanisms of action of PGA₁ on the expression of cytokine genes.

Keywords: IL-10, macrophage, PGA₁

*Corresponding author
Phone: 82-53-620-4363; Fax: 82-53-66-4628; E-mail: heesun@med.yu.ac.kr

Materials and Methods

Reagents

Trizol reagent for total RNA isolation was purchased from Invitrogen (Carlsbad, CA, U.S.A.). Dulbecco’s phosphate-buffered saline (PBS), RPMI 1640, penicillin-streptomycin, and fetal bovine serum (FBS) were purchased from Gibco/BRL (Life Technologies, Gaithersburg, MD, U.S.A.). 15d-PGJ₂, prostaglandin A₁ (PGA₁), and
GW9662 were purchased from Biomol (Plymouth Meeting, PA, U.S.A.). MAPK inhibitors, 2-amino-3-methoxyflavone (PD98059), anithral[1,9-d]-pyrazolo[2,3-d][2(1H)-one (SP600125), 4-(4-fluorophenyl)-2-(4-methoxy)phenyl]-1H-imidazole (PD169316), and NF-κB inhibitor, (E)-β-[4-(4-methylphenyl)]sulfonyl]lf-2-propenensulfinate (BSI 11-7032) were purchased from Calbiochem (San Diego, CA, U.S.A.). Nitrocellulose filter membranes were obtained from Schleicher & Schuell Biobioscience (Dassel, Germany). [α-^32P]dCTP and [α-^32P]UTP were purchased from Dupont-New England Nuclear (Boston, MA, U.S.A.). The Riboprint Multi-probe RNase Protection Assay System and the mCK-2b template set were purchased from Pharmingen (San Diego, CA, U.S.A.). Hank’s balanced salt solution (HBSS), tris(hydroxymethyl) aminomethane (Tris), sodium dodecyl sulfate (SDS), Escherichia coli trihydroxy methy l aminomethane (Tris), sodium dodecyl sulfate (San Diego, CA, U.S.A.). Hank’s balanced salt solution (HBSS), and the mCK-2b template set were purchased from Pharmingen (San Diego, CA, U.S.A.). The medium was then replaced with serum-free RPMI 1640 and the cells were cultured in the presence or absence of stimuli for the indicated times.

Macrophages were cultured overnight in complete medium at 37 °C for 60 min and then treated with DNase I at 37 °C for 30 min. The mixture was then extracted with phenol and chloroform. Extracted RNA was precipitated with ethanol, collected by centrifugation at 4 °C, and then resuspended in 50 µl of hybridization buffer and diluted to 3×10^6 cpm/µl. Two µl of resuspended probe was used for the reaction. The RNA samples (10 µg RNA/sample) were dried in a vacuum evaporator and resuspended in 8 µl of hybridization buffer. The RNA was annealed to the probe by successive incubation at 95°C for 3 min and at 56°C overnight in a total volume of 10 µl. Nase was added to each sample and incubated at 30°C for 45 min to remove single-stranded RNA; the protected RNA duplexes were then purified by phenol/chloroform extraction and ethanol precipitation. After the pellet RNA was resuspended in 5–6 µl of gel loading buffer and incubated at 95°C for 5 min, the RNA was quickly quenched on ice and analyzed by electrophoresis on 5% polyacrylamide/8 M urea gels. The gel was adsorbed to filter paper, dried under vacuum, and exposed on X-ray film (Agfa-Gevaert N.V., Belgium) with intensifying screens at ~70°C.

Real-Time Polymerase Chain Reaction (Real-Time PCR)
Real-time PCR for IL-10 in mouse peritoneal macrophages was performed using the LightCycler (Roche, Mannheim, Germany). Real-time PCR was performed in triplicate in a total volume of 20 µl of LightCycler FastStart DNA SYBR Green 1 Mix was obtained from Roche (Mannheim, Germany), and pERK, pp38, pSAPK/JNK, phospho-IκBα, and γ-tubulin antibodies were obtained from Cell Signaling Technology (Danvers, MA, U.S.A.). Other reagents were pure-grade commercial preparations.

Mice
Specific pathogen-free, inbred C57BL/6 mice, 8 to 10 weeks of age, were purchased from Hyo-Chang (Daegu, Korea). The utmost precautions were taken to ensure that the mice remained infection-free; thereby ensuring that the degree of spontaneous activation of tissue macrophages would be minimal. The mice were cared for in accordance with the Guide to the Care and Use of Experimental Animals of the Yeungnam Medical Center.

Preparation of Mouse Peritoneal Macrophages
Thioglycollate-elicited peritoneal macrophages were obtained as previously described [18]. Briefly, macrophages in complete medium (RPMI 1640 supplemented with penicillin, streptomycin, and 10% FBS) were plated in 100-mm tissue culture dishes, incubated for 2 h at 37°C in a 5% CO2 atmosphere, and then washed three times with HBSS to remove any non-adhering cells. Typically, this resulted in 95% macrophages as determined by morphological criteria. The macrophages were cultured overnight in complete medium at 37°C in 5% CO2. The medium was then replaced with serum-free RPMI 1640 and the cells were cultured in the presence or absence of stimuli for the indicated times.

Preparation of RNA and Ribonuclease Protection Assay (RPA)
Total RNA was extracted using Trizol reagent according to the manufacturer’s instructions. The quantity of RNA obtained was determined by measuring its optical density (OD) at 260 and 280 nm. RPA for nine cytokines, IL-12p35, IL-12p40, IL-10, IL-1α, IL-1β, IL-1α, IL-8B/GF, IL-6, IFN-γ, MIF, L32, and GAPDH, were performed according to the instructions of the Multi-probe RNase Protection Assay System using Riboprint. Briefly, mCK-2b was used to obtain radiolabeled antisense RNA probes. In vitro transcription was carried out by incubation in a transcription buffer containing 10 mM ATP, 10 mM CTP, 10 mM GTP, 2.50 µCi [α-^32P]UTP, and 77 RNA polymerase. The mixture was incubated at 37°C for 60 min and then treated with DNase I at 37°C for 30 min. The mixture was then extracted with phenol and chloroform. Extracted RNA was precipitated with ethanol, collected by centrifugation at 4°C, and then resuspended in 50 µl of hybridization buffer and diluted to 3×10^6 cpm/µl. Two µl of resuspended probe was used for the reaction. The RNA samples (10 µg RNA/sample) were dried in a vacuum evaporator and resuspended in 8 µl of hybridization buffer. The RNA was annealed to the probe by successive incubation at 95°C for 3 min and at 56°C overnight in a total volume of 10 µl. Nase was added to each sample and incubated at 30°C for 45 min to remove single-stranded RNA; the protected RNA duplexes were then purified by phenol/chloroform extraction and ethanol precipitation. After the pellet RNA was resuspended in 5–6 µl of gel loading buffer and incubated at 95°C for 5 min, the RNA was quickly quenched on ice and analyzed by electrophoresis on 5% polyacrylamide/8 M urea gels. The gel was adsorbed to filter paper, dried under vacuum, and exposed on X-ray film (Agfa-Gevaert N.V., Belgium) with intensifying screens at ~70°C.

Enzyme-Linked Immunosorbent Assay (ELISA) for IL-10 Production
The IL-10 protein levels in cell media were measured with an ELISA kit that was obtained from eBioscience (San Diego, CA, U.S.A.). All procedures were performed in accordance with the manufacturer’s instructions.

Electrophoretic Mobility Shift Assay (EMSA)
Nuclear extracts were prepared as previously described [16]. Cells were washed three times with cold PBS, and then scraped and harvested by centrifugation. Cell pellets were resuspended and incubated on ice for 15 min in 400 µl of hypotonic buffer A (10 mM HEPES, 10 mM KCl, 1.5 mM MgCl2, 0.5 mM DTT, 0.1 mM PMSF, 10 µg/ml pepstatin, 10 µg/ml leupeptin, 10 µg/ml antipain, and 10 µg/ml aprotinin). Nonidet P-40 was then added to a final concentration of 2.5% and the cells were vortexed for 10 s. Nuclei were separated from the cytosol by centrifugation at 12,000 × g for 15 s. Pellets were resuspended in 40 µl of hypotonic buffer C (20 mM HEPES, 2.5% glycerol, 0.4 M NaCl, 1 mM EDTA, 1 mM EGTA, 0.5 mM EDTA, 1 mM EGTA, 0.5 mM EDTA). The mixture was then extracted with phenol and chloroform. Extracted RNA was precipitated with ethanol, collected by centrifugation at 4°C, and then resuspended in 50 µl of hybridization buffer and diluted to 3×10^6 cpm/µl. Two µl of resuspended probe was used for the reaction. The RNA samples (10 µg RNA/sample) were dried in a vacuum evaporator and resuspended in 8 µl of hybridization buffer. The RNA was annealed to the probe by successive incubation at 95°C for 3 min and at 56°C overnight in a total volume of 10 µl. Nase was added to each sample and incubated at 30°C for 45 min to remove single-stranded RNA; the protected RNA duplexes were then purified by phenol/chloroform extraction and ethanol precipitation. After the pellet RNA was resuspended in 5–6 µl of gel loading buffer and incubated at 95°C for 5 min, the RNA was quickly quenched on ice and analyzed by electrophoresis on 5% polyacrylamide/8 M urea gels. The gel was adsorbed to filter paper, dried under vacuum, and exposed on X-ray film (Agfa-Gevaert N.V., Belgium) with intensifying screens at ~70°C.
DTT, 0.1 mM PMSF, 10 µg/ml pepstatin, 10 µg/ml leupeptin, 10 µg/ml antipain, and 10 µg/ml aprotinin). Samples were sonicated at level 3-4 for 2-3 s, and then centrifuged for 10 min at 4°C. Nuclear protein concentration was measured using the Bradford assay (Bio-Rad, Richmond, CA, U.S.A.). A consensus sequence for the NF-κB DNA binding site (5'-AG TTGAGGGGACTTT AGGC-3') (sc-2505, Santa Cruz Biotechnology) was labeled with [α-32P]dCTP using a random-primed DNA labeling kit (Roche, Germany). A mutant binding sequence for NF-κB was identical to sc-2505 except for a "G→C" substitution in the NF-κB DNA binding motif (sc-2511, Santa Cruz Biotechnology). Labeled DNA was purified over a 200-HP column (Pharmacia, Piscataway, NJ, U.S.A.) to remove unbound nucleotides. Nuclear extracts were incubated at room temperature for 20 min with approximately 50,000 cpm of labeled oligonucleotide suspended in binding buffer [200 mM HEPES, 500 mM KCl, 10 mM EDTA, 50% glycerol, 10 mM DTT, 1 mg/ml BSA, 1 µg/µl poly(dI-dC)]. Following this incubation, samples were resolved on 4% polyacrylamide gels at 140 V and exposed to film.

Protein Extraction and Western Blot Analysis
Total lysates were prepared as described by Kim et al. [17]. The protein concentrations were determined by the Bradford assay (Bio-Rad, Richmond, CA, U.S.A.) using bovine serum albumin as a standard. Nuclei were separated from the cytosol by centrifugation at 12,000 × g for 5 min. Pellets were resuspended in 50 µl of buffer C (20 mM HEPES, 0.4 M NaCl, 1 mM EDTA, 1 mM DTT, 10% PMSF, 10% NP-40). Samples were centrifuged at 12,000 × g for 10 min. Thirty-µg samples of protein were separated on 10% SDS-polyacrylamide gels, and then transferred to nitrocellulose membranes. The membranes were soaked in 5% nonfat dried milk to remove unbound nucleotides. Nuclear extracts were incubated at room temperature for 20 min with approximately 50,000 cpm of labeled oligonucleotide suspended in binding buffer [200 mM HEPES, 500 mM KCl, 10 mM EDTA, 50% glycerol, 10 mM DTT, 1 mg/ml BSA, 1 µg/µl poly(dI-dC)]. Following this incubation, samples were resolved on 4% polyacrylamide gels at 140 V and exposed to film.

Statistical Analysis
Data are expressed as mean±SEM. Statistical differences were analyzed by Wilcoxon signed-rank test. A level of P<0.05 was considered statistically significant.

RESULTS
Effect of PGA1 on LPS-Induced Cytokine Gene Expression in Mouse Peritoneal Macrophages
We first compared the effect of PGA1 on LPS-induced cytokine gene expression to that of 15d-PGJ2. After thioglycollate-elicited peritoneal macrophages (TG-PeMφ)
were stimulated with LPS (100 ng/ml), LPS plus PGA₁ (25 µM), or LPS plus 15d-PGJ₂ (10 µM) simultaneously for 2 h, RPA was performed. As shown in Fig. 1A, 15d-PGJ₂ had an inhibitory effect on LPS-induced expression of IL-12p35, IL-12p40, IL-10, IL-1α, IL-1β, and IL-6 mRNA. However, the effects of PGA₁ differed from those of 15d-PGJ₂ on the expression of these cytokine genes, with the exception of IL-12p40. PGA₁ especially increased the expression of LPS-induced IL-10 mRNA. This synergistic effect of PGA₁ on IL-10 expression was confirmed by real-time PCR and ELISA for IL-10 production (Figs. 1B and 1C).

We subsequently examined the dose effect and time course of the synergistic effect of PGA₁ on LPS-induced IL-10 expression. TG-PeMφ were treated with LPS (100 ng/ml) or LPS plus PGA₁ (5, 12, 25, or 50 µM) simultaneously (PGA₁/LPS) for 2 h. For the time course of PGA₁ synergistic effect, TG-PeMφ were treated with LPS or LPS plus PGA₁ (12 µM) simultaneously (PGA₁/LPS) for 0.5, 2, or 4 h. PGA₁ at 5 or 12 µM had the most synergistic effect on LPS-induced IL-10 expression (Fig. 2A). Maximum synergistic expression of PGA₁/LPS-induced IL-10 mRNA occurred at 2 h after treatment, but disappeared at 4 h after treatment (Fig. 2B).

If the synergy of PGA₁/LPS on IL-10 mRNA expression is due to newly synthesized protein, the synergistic action would be blocked in macrophages cotreated with a protein synthesis inhibitor such as cycloheximide (CHX). To test this possibility, TG-PeMφ were treated with LPS alone or in combination with PGA₁ in the presence or absence of CHX (10 µg/ml) for 2 h. The PGA₁/LPS-induced synergistic effect was not attenuated by CHX; rather,
the PGA1/LPS-induced IL-10 expression was increased remarkably by CHX (Fig. 2C).

**Synergistic Effect of PGA1 on LPS-Induced IL-10 Expression is Mediated by PPARγ and NF-κB Activation**

To evaluate whether the mechanism of the synergistic effect of PGA1 was mediated by PPARγ, GW9662, a PPARγ antagonist, was tested in mouse peritoneal macrophages. TG-PeMφ were stimulated with PGA1/LPS in the presence or absence of GW9662 (10 µM) for 2 h. GW9662 remarkably inhibited the synergistic effect of PGA1 on LPS-induced IL-10 expression (Fig. 3A) and inhibited IL-10 protein production (Fig. 3B). Therefore, the synergistic increase in IL-10 mRNA expression following PGA1/LPS treatment is related to PPARγ activation.

Next, the role of NF-κB activation on PGA1/LPS-induced IL-10 expression was investigated. Bay-11-7082 selectively blocks the phosphorylation of IκBα, thus preventing the activation and nuclear translocation of NF-κB. After TG-PeMφ were treated with PGA1, and/or LPS in the presence or absence of Bay-11-7082 (10 µM) for 2 h, real-time PCR and ELISA for IL-10 gene expression and protein production were performed. Bay-11-7082 blocked the synergistic effect of PGA1 on LPS-induced IL-10 mRNA expression remarkably (Fig. 4A) and also inhibited the production of PGA1/LPS-induced IL-10 protein (Fig. 4B). To further confirm the association between NF-κB activity and IL-10 expression, EMSA for binding activity of NF-κB and Western blotting for IκBα phosphorylation were performed. In cells treated with PGA1/LPS, we could not detect strong increase of NF-κB activity; however, IκBα...
phosphorylation was increased compared with those in cells treated with LPS alone (Figs. 4C and 4D). We therefore concluded that the synergistic effect of PGA₁ on LPS-induced IL-10 expression is mediated via NF-κB activation.

Synergistic Effect of PGA₁ on LPS-Induced IL-10 mRNA Expression is Related to MAPK Signaling Pathways

We investigated whether MAPK signaling pathways are involved in the synergistic effect of PGA₁ on LPS-induced IL-10 expression. After TG-PeMφ were pretreated with the ERK1/2 inhibitor PD98059 (10 μM), the p38 MAP kinase inhibitor PD169316 (10 μM), or the SAPK/JNK inhibitor SP600125 (25 μM) for 0.5 h, cells were treated with PGA₁ and/or LPS for 2 h. Real-time PCR and ELISA were then performed. In addition, to further confirm these results, we investigated the phosphorylation of MAP kinases in cells treated with PGA₁/LPS. PD98059 increased the IL-10 mRNA expression in cells stimulated with PGA₁/LPS. PD98059 increased the IL-10 RNA expression in cells stimulated with PGA₁/LPS, rather than inhibiting IL-10 expression. The ELISA result showed the same pattern as the gene expression (Figs. 5A and 5B). Moreover, phosphorylation of ERK was inhibited in cells treated with PGA₁/LPS (Fig. 5C). The expression of PGA₁/LPS-induced IL-10 mRNA was decreased by the p38 MAP kinase inhibitor PD169316, and production of

**Fig. 6.** Synergistic effect of PGA₁ on LPS-induced IL-10 expression is decreased by a p38 inhibitor.

TG-PeMφ were untreated or pretreated with PD169316 (p38 inhibitor, 10 μM) for 30 min. Cells were left untreated or treated with LPS (100 ng/ml) and/or PGA₁ (12 μM) for 2 h. After total RNAs and cell supernatants were isolated, real-time PCR (A) and ELISA (B) were performed. C. TG-PeMφ were untreated or treated with LPS (100 ng/ml) and/or PGA₁ (12 μM) for 10 min. Thereafter, cell lysates were separated on 10% SDS-polyacrylamide gels and then immunoblotted with phospho-p38 antibody. Bars represent mean±SEM from three separate experiments. *P<0.05 vs. cells treated with PGA₁/LPS.

**Fig. 7.** SAPK/JNK is responsible for the synergistic effect of PGA₁ on LPS-induced IL-10 expression.

TG-PeMφ were untreated or pretreated with SP600125 (SAPK/JNK inhibitor, 25 μM) for 30 min. Cells were left untreated or treated with LPS (100 ng/ml) and/or PGA₁ (12 μM) for 2 h. After total RNAs and cell supernatants were isolated, real-time PCR (A) and ELISA (B) were performed. C. TG-PeMφ were untreated or treated with LPS (100 ng/ml) and/or PGA₁ (12 μM) for 10 min. Thereafter, cell lysates were separated on 10% SDS-polyacrylamide gels and then immunoblotted with phospho-SAPK/JNK antibody. Bars represent mean±SEM from three separate experiments. *P<0.05 vs. cells treated with PGA₁/LPS.
Prostaglandins (PGs) have diverse biologic actions depending on the target cell type and the PG type. Among PGs, cyclopentenone prostaglandins (cyPGs) can interact with specific cellular signaling molecules and transcription factors [6] and exhibit complex regulatory mechanisms on cytokine gene expression [1, 2, 11, 19].

In the present study, PGA<sub>1</sub> had a synergistic effect on LPS-induced IL-10 mRNA expression, in contrast to 15d-PGJ<sub>2</sub>. Although 15d-PGJ<sub>2</sub> had suppressive effects on the LPS-induced expression of IL-12, IL-10, IL-1, and IL-8 mRNA, PGA<sub>1</sub> did not affect IL-10 mRNA expression (Fig. 6A, 6B). Nucleus in cells treated with PGA<sub>1</sub>/LPS was also detected (Fig. 6C). In the case of SAPK/JNK, SP600125 did not inhibit IL-10 mRNA expression in cells treated with PGA<sub>1</sub>/LPS and the phosphorylation of SAPK/JNK was not increased in cells stimulated with PGA<sub>1</sub>/LPS compared with that in cells treated with LPS alone (Figs 7A and 7C). However, SP600125 inhibited IL-10 protein production remarkably (Fig. 7B). Taken together, these results suggest the upregulation of IL-10 by PGA<sub>1</sub>/LPS is mediated through p38 and SAPK/JNK signaling pathways.

**DISCUSSION**

It is widely accepted that cyPGs exert their effects on inflammatory-mediated genes in cells by either inhibiting or activating NF-κB signaling [3, 15, 23, 30]. The anti-inflammatory activity of cyPGs is mediated mainly through inhibition of NF-κB activation, but 15d-PGJ<sub>2</sub> has been also reported to upregulate IL-8 and MIP-2(CXCL2) expressions through NF-κB activation. Most of the previous studies with cyPGs have focused on 15d-PGJ<sub>2</sub>, and few studies of the signaling pathways of PGA<sub>1</sub> or its effects on NF-κB activity have been reported. In our NF-κB result, NF-κB activity was not increased remarkably in cells treated with PGA<sub>1</sub>/LPS. However, Bay-11-7082 blocked the synergistic activity of PGA<sub>1</sub>/LPS-induced IL-10 mRNA expression and protein production, and the increase of IkBα phosphorylation was detected in cells treated with PGA<sub>1</sub>/LPS. Therefore, the synergistic effect of PGA<sub>1</sub> on LPS-induced IL-10 mRNA expression is mediated by NF-κB activation. Rossi et al. [26] demonstrated an inhibitory effect of PGA<sub>1</sub> on NF-κB activation in various human cell types. However, they used PGA<sub>1</sub> at high concentration (24 μM) and different cell types (Jurkat T cell, T lymphoid cell, and HeLa cell).

Although many reports [6, 20, 28, 30, 34] have shown that cyPGs inhibit MAP kinase activation, this inhibition appears to be target gene-, cell type-, and stimulation condition-dependent. Wilmer et al. [32] reported that 15d-PGJ<sub>2</sub> dose-dependently increases ERK activity in human mesangial cells. Among MAPK signaling pathways, the ERK pathway is known to be associated with the synergistic activity of 15d-PGJ<sub>2</sub> on the expression of some cytokine genes [9, 10, 12]. In our previous MIP-2(CXCL2) study [15], p38 MAP kinase and SAPK/JNK pathways were associated with the synergistic effect of 15d-PGJ<sub>2</sub> on LPS-induced MIP-2 (CXCL2) expression. In the present study, p38 and SAPK/JNK signaling pathways were associated with the synergistic effect of PGA<sub>1</sub> on LPS-induced IL-10.
expression. PGA₁ increased the phosphorylation of p38 in cells treated with LPS. SP600125, a SAPK/JNK inhibitor, inhibited the production of IL-10 protein but did not inhibit IL-10 mRNA synthesis in cells treated with PGA₁/LPS. SP600125 seems to have no effect on the transcription of IL-10 synthesis but plays an unknown inhibitory action on the translation process. Thus, IL-10 synthesis seems to be regulated at the transcriptional and translational levels in mouse peritoneal macrophages. Therefore, it is possible that upregulation of IL-10 by PGA₁/LPS is mediated through the SAPK/JNK pathway.

This is the first report demonstrating that PGA₁ has a synergistic effect on LPS-induced IL-10 expression in mouse peritoneal macrophages. This synergistic effect is mediated by MAP kinases, p38, and SAPK/JNK signaling pathways, and through a PPARγ pathway. IL-10 is a well-known antiinflammatory cytokine and has an important regulatory role in limiting the duration and extent of acute inflammatory response. Therefore, PGA₁ may play a regulatory role as a naturally occurring feedback inhibitor of inflammation.

Acknowledgments

This work was supported by a Yeunam University research grant (Grant No. 205-A-236-001) and the Korean Science and Engineering Foundation through the Aging-associated Vascular Disease Research Center at Yeunam University [R13-2005-005-01003-0(2006)].

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activated receptor-γ block activation of pancreatic stellate cells.


