Effect of Prostaglandin I_2 Analogs on Macrophage Inflammatory Protein 1α in Human Monocytes Via I Prostanoid Receptor and Cyclic Adenosine Monophosphate

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Aims: Inflammation plays critical roles in atherosclerosis. Chemokines are responsible for leukocyte trafficking and involve in inflammatory diseases. Macrophage inflammatory protein 1α (MIP-1α) has been implicated in atherosclerotic lesion formation. Prostaglandin I_2 (PGI_2) analog, used in pulmonary hypertension, has been reported to have anti-inflammatory functions. However, little is known about its role in the MIP-1α production in human monocytes.

Methods: We investigated the effects of 3 conventional (iloprost, beraprost, and treprostinil) and 1 new (ONO-1301) PGI_2 analogs, on the expression of MIP-1α expression in human monocytes. Human primary monocytes from control subjects and THP-1 cell line were treated with PGI_2 analogs, with or without lipopolysaccharide (LPS) stimulation. Supernatants were harvested to measure MIP-1α levels by enzyme-linked immunosorbent assay. To explore which receptors involved the effects of PGI_2 analogs on the expression of MIP-1α expression, I prostanoid (IP) and E prostanoid, peroxisome proliferator-activated receptor (PPAR)-α, and PPAR-r receptor antagonists were used to pretreat THP-1 cells. Forskolin, a cyclic adenosine monophosphate (cAMP) activator, was also used to further confirm the cAMP involvement on the effect of PGI_2 analogs in MIP-1α production.

Results: Three PGI_2 analogs could suppress LPS-induced MIP-1α production in THP-1 cells and human primary monocytes. ONO-1301 had a similar effect. CAY 10449, an IP receptor antagonist, could reverse the suppressive effects on MIP-1α production of iloprost. Forskolin, a cAMP activator, also suppressed MIP-1α production in THP-1 cells.

Conclusions: Prostaglandin I_2 analogs suppressed LPS-induced MIP-1α production in human monocytes via the IP receptor and cAMP pathway. The PGI_2 analog may be potential in the treatment for atherosclerosis.

Key Words: PGI_2, MIP-1α, iloprost, monocyte, beraprost, atherosclerosis

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Chemokines are a family of low-molecular-weight proteins involved in the directed migration of cells under homeostatic and pathological conditions and also play an important role in the development and progression of atherosclerosis.[1] The critical role of inflammation in the etiology of atherosclerosis makes it unsurprising that recognition of an important contribution of chemokines and their receptors is increasing in the pathology of atherosclerosis and related cardiovascular disease. Increasing attention has been focused on CCL2, CCL5, CX3CL1, and their receptors CC chemokine receptor 2 (CCR2), CCR5, and CX3CR1.[1,2] CCR5 is particularly noteworthy given the availability of an approved antagonist. The evidence is emerging supporting a role for CCR5 and its ligands CCL3 (macrophage inflammatory protein 1α [MIP-1α]) and CCL5 (RANTES) in the initiation and the progression of atherosclerosis.[2] CCR5 was paired with 3 ligands on its discovery, MIP-1α/CCL3, MIP-1b/CCL4, and regulated on activation normal T cell expressed and secreted (RANTES)/CCL5. Polymorphisms in the CCR5 genes decrease risk of coronary heart disease with coronary heart disease[3] and also protect against myocardial infarction.[4,5] Toll-like receptors (TLRs) have been recognized for their role in atherosclerotic lesion development and progression. Toll-like receptor ligands that are expressed in atherosclerotic tissues have been shown to promote atherosclerosis in animal study. During the plaque progression stage, stimulation of TLR2 and TLR4 attenuated MIP-1α and RANTES release in atherosclerotic mice.[6] In human study, high circulating levels of CCL5 may be a marker for refractory unstable angina pectoris, and plasma levels of MIP-1α may be prognostic for ischemic events.[7]

Cyclooxygenases are expressed in atherosclerotic lesions. Prostaglandins (PGs) are generated by stepwise conversion of arachidonic acid into a series of products, including PGG, PGH, PGD_2, PGE_2, $PGF_2\alpha$, and PGI_2, via the action of the cyclooxygenases and some specific enzymes.[8,9] However, the role of cyclooxygenases and individual PGs during atherosclerotic plaque progression is currently uncertain. Prostaglandins are initially regarded as proinflammatory molecules. However, PGI_2 can have an anti-inflammatory activity via I prostanoid (IP) receptor, E prostanoid (EP) receptor, and the cyclic adenosine monophosphate (cAMP) pathway.[10,11] Prostaglandin I_2 analogs and PGE_2 differ dramatically with respect to dephosphorylation of focal adhesion kinase in human aortic smooth muscle cells and inhibition of

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migration, which might be of relevance for their respective functions in atherosclerosis.[12] Prostaglandin I_2 is a lipid mediator with vasodilatory and antithrombotic effects, and PGI_2 analogs have been used in the treatment of vasoconstrictive/ischemic diseases, including pulmonary hypertension. It has been reported that PGI_2 has pleiotropic effects that are anti-inflammatory and also anti-atherogenic, and beraprost sodium is a stable, orally active PGI_2 analog with antiplatelet and vasodilating properties.[13]

Because MIP-1α regulation is important for the induction of inflammation in inflammatory cardiovascular disease, and PGI_2 analogs show obviously anti-inflammatory effect, it is reasonable to evaluate the effect of PGI_2 analogs on the MIP-1α in monocytes and explore the associated mechanisms. In the present study, we investigated whether 3 conventional and 1 new PGI_2 analogs could modulate the lipopolysaccharide (LPS)–induced MIP-1α expression in monocytes and related mechanisms. Prostaglandin I_2 analogs may be benefit in cardiovascular disease, such as atherosclerosis, myocardiac ischemia, and related diseases.

METHODS

Cell Preparation

The human monocytic cell line THP-1 cells (American Type Culture Collection, Rockville, MD) were cultured in RPMI 1640 medium (Sigma Chemical Co, St Louis, MO) supplemented with 10% fetal bovine serum, 100 U/mL of penicillin, and 100 μg/mL of streptomycin at 37°C and 5% CO_2. THP-1 cell was centrifuged and resuspended in fresh media in a 24-well plate at a concentration of 10^6/mL for 24 hours. The study for the collection of blood from human healthy subjects was approved by the institutional review board of Kaohsiung Medical University, Taiwan. After informed consent was obtained, peripheral blood samples were obtained from healthy individuals who had no personal or family history of allergy (n = 3). Peripheral blood mononuclear cells were isolated by density-gradient centrifugation (Lymphoprep, Oslo, Norway), and human primary monocytes were isolated from peripheral blood mononuclear cells by magnetic bead sorting with anti-CD14 monoclonal antibody (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany). The cells were pretreated with iloprost, beraprost, or treprostinil, ONO-1301 (Sigma Chemical Co), or forskolin (a cyclic AMP activator) for 2 hours before LPS (0.2 Hg/mL) (*Escherichia coli*; Sigma Chemical Co) stimulation. Supernatant was collected at different time points after LPS stimulation. To examine whether the effect of PGI_2 analog on MIP-1> expression of THP-1 cells via IP, EP, or peroxisome proliferator-activated receptor (PPAR), THP-1 cells were pretreated with CAY 10449, a IP receptor antagonist, EP1 receptor antagonist (SC19220), EP2 receptor antagonist (AH-6809) or EP4 receptor antagonist (GW627368X), PPAR-α antagonist (GW6741) or PPAR-γ antagonist (GW9662) 1 hour before the treatment of the cells with iloprost treatment, and then stimulated with LPS 2 hours after iloprost. I prostanoid, EP receptor, and PPAR antagonists were purchased from Cayman Chemical Company (Ann Arbor, MI). The production of MIP-1α in the culture supernatants was determined by enzyme-linked immunosorbent assay (ELISA).

ELISA Assay

The MIP-1α concentrations of cell supernatants were determined using commercially available ELISA-based assay systems (R&D System, Minneapolis, MN). Assays were performed using the protocols recommended by the manufacturer.

Statistical Analyses

Differences between experimental and control groups were analyzed by using the Mann-Whitney *U* test. $P < 0.05$ was considered indicative of significant between-group differences.

RESULTS

Suppressive Effect of PGI_2 Analogs on MIP-1α Expression in THP-1 Cells and Human Primary Monocytes

Macrophage inflammatory protein 1α participates in the pathogenesis of plaque vulnerability and subsequent plaque rupture. To test whether PGI_2 analogs play an important role in the formation of atherosclerotic plaques, the effect of PGI_2 analogs on MIP-1α expression of THP-1 cells was investigated. Iloprost significantly decreased LPS-induced MIP-1α production in THP-1 cells at 12, 24, and 48 hours (Fig. 1, A–C). To further confirm the suppressive effect of PGI_2 analogs on MIP-1α production in primary cells, human $CD14^+$ primary monocytes were isolated from 3 control volunteers. As shown in Fig. 1, D–F, iloprost could also suppress MIP-1α production in human $CD14^+$ primary monocytes. Even very low concentrations (10^{-9} M) of iloprost had a suppressive effect on MIP-1α production in human $CD14^+$ primary monocytes. These data suggested that primary monocytes were more sensitive to iloprost. The medium doses of treprostinil (10^{-8} to 10^{-7} M) had a suppressive effect on MIP-1α production at 12-hour time point (Fig. 2A). Only higher concentration (10^{-7} to 10^{-5} M) of treprostinil could suppress MIP-1α production in THP-1 cells at 24-hour time points (Fig. 2B). However, even very lower concentration (10^{-9} M) of treprostinil had a suppressive effect on MIP-1α production at 48-hour time point (Fig. 2C). Treprostinil could also suppress MIP-1α production in human $CD14^+$ primary monocytes (Fig. 2, D–F) even in very low concentrations (10^{-9} M). Only a higher concentration (10^{-7} to 10^{-5} M) of beraprost had a suppressive effect at 12- and 24-hour time points (Fig. 3, A and B). However, beraprost (10^{-8} M) had a suppressive effect on MIP-1α production at 48-hour time point (Fig. 3C). The most powerful effect of suppression in all 3 PGI_2 analogs could be found at 48-hour time point in the present study. Beraprost could also suppress MIP-1α production in human $CD14^+$ primary monocytes.

Suppressive Effect of PGI_2 Analogs on MIP-1α Expression in Human $CD14^+$ Primary Monocytes

The effects of a new PGI_2 analog (ONO-1301) on MIP-1α expression in human $CD14^+$ primary monocytes were also investigated. As shown in iloprost, treprostinil, and beraprost, the new PGI_2 analog had a similar effect and could also suppress MIP-1α production in human $CD14^+$ primary monocytes (Fig. 4). In very low concentrations (10^{-9} M), ONO-1301 had a suppressive effect only at 48-hour time points. Therefore, the new PGI_2 analog ONO-1301 had no better effect than the other 3 conventional PGI_2 analogs.

Suppressive Effect of PGI_2 Analogs on MIP-1α Expression Via cAMP and IP Receptor, But Not EP or PPAR-α or PPAR-γ Receptor

Prostaglandin I_2 analogs exert its function through the IP or EP receptor and lead to increased levels of intracellular cAMP.[11,13,14] So THP-1 cells were pretreated with IP receptor antagonist CAY 10449 to see whether CAY 10449 could reverse the effects of iloprost on the MIP-1α expression in THP-1 cells. As shown in Figure 5A, CAY 10449 could reverse the suppressive effect of on LPS-induced MIP-1α production in THP-1 cells. To examine whether the effect of PGI_2 analog on cytokine expression of THP-1 cells via EP receptor, THP-1 cells were pretreated with EP1 receptor antagonist (SC19220), EP2 receptor antagonist (AH-6809), or EP4 receptor antagonist (GW627368) 1 hour before the treatment of the cells with iloprost treatment and then stimulated with LPS 2 hours after iloprost. EP1, EP2, and EP4

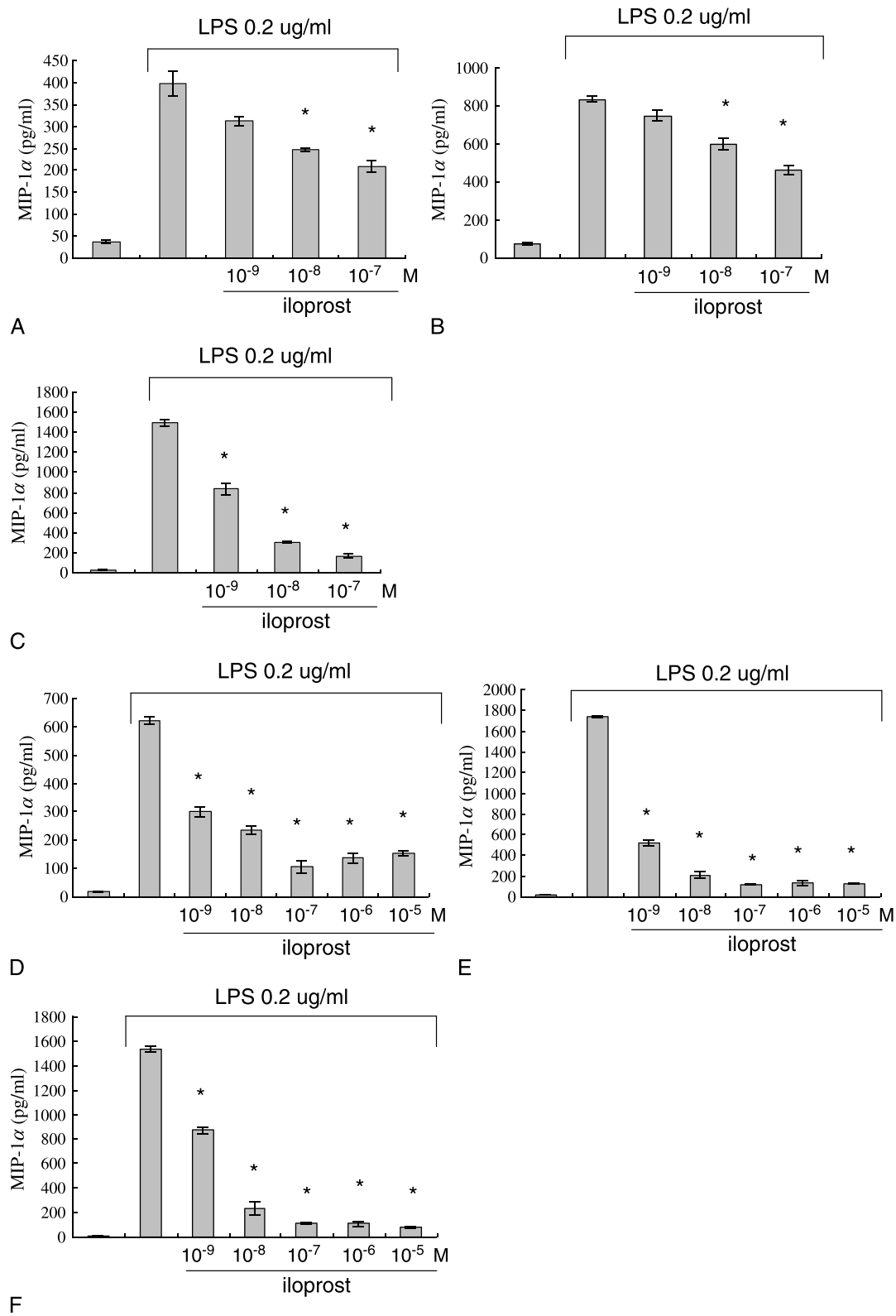

FIGURE 1. Iloprost decreased LPS-induced MIP-1α production in THP-1 cells at 12 hours (A), 24 hours (B), and 48 hours (C). Iloprost decreased LPS-induced MIP-1α production in human primary monocytes at 12 hours (A), 24 hours (B), and 48 hours (C) (all data are presented in pg/mL per 10^6 cells; *$P < 0.05$).

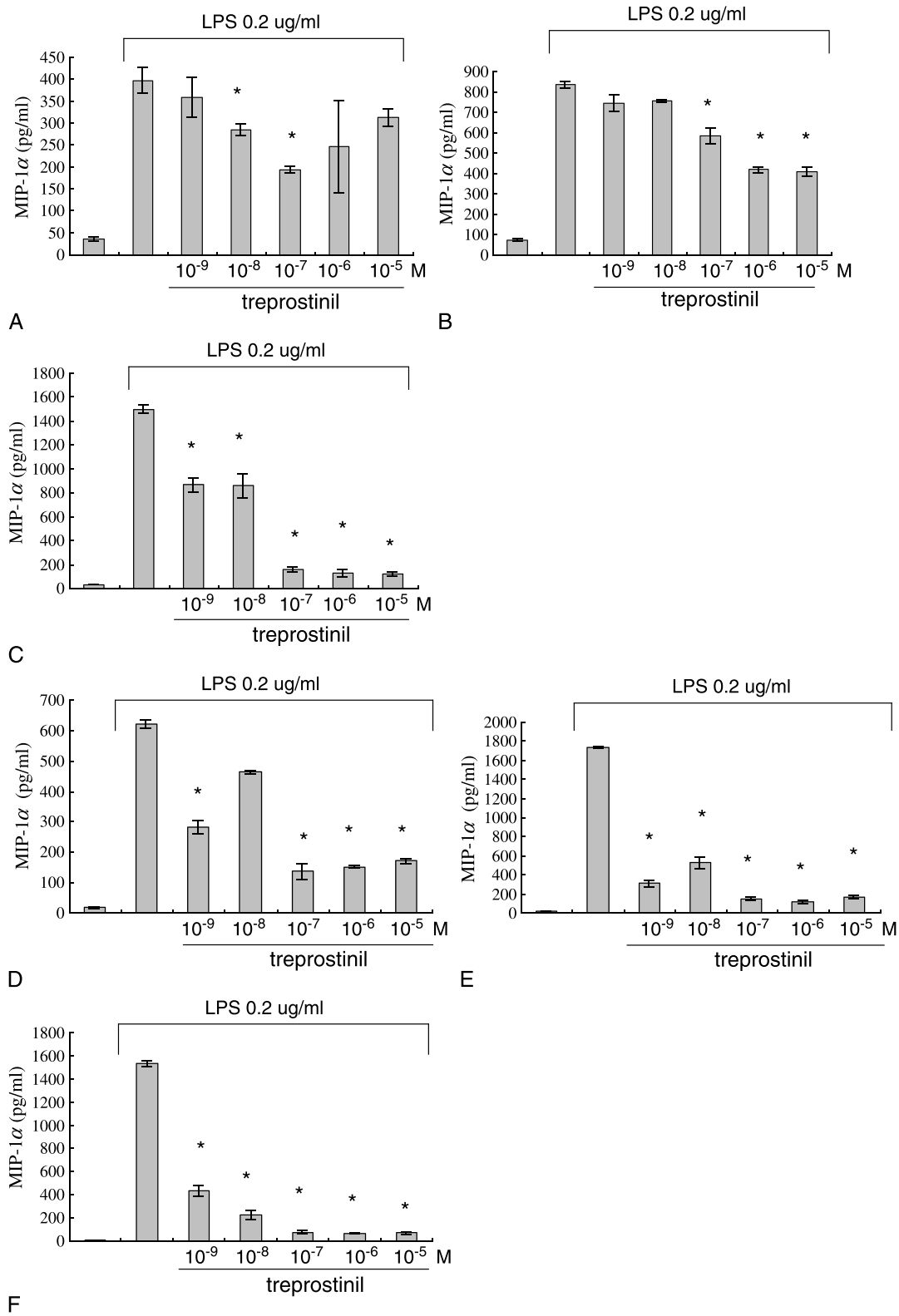

FIGURE 2. Effect of treprostinil (10^{-9} to 10^{-5} M) on LPS-induced MIP-1α production in THP-1 cells at 12 hours (A), 24 hours (B), and 48 hours (C). Effect of treprostinil (10^{-9} to 10^{-5} M) on LPS-induced MIP-1α production in human primary monocytes at 12 hours (A), 24 hours (B), and 48 hours (C) (all data are presented in pg/mL per 10^6 cells; $^*P < 0.05$).

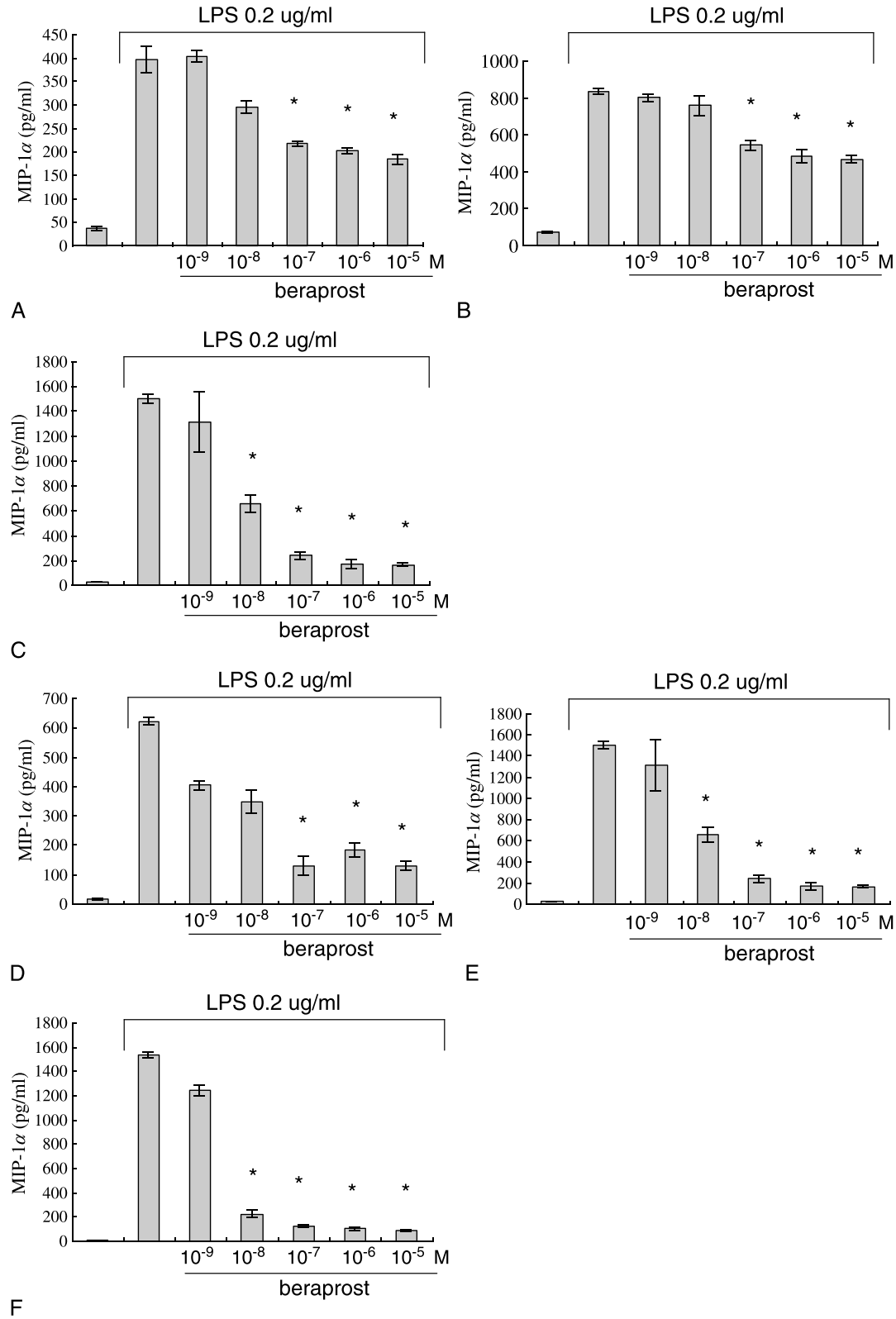

FIGURE 3. Effect of beraprost (10^{-9} to 10^{-5} M) on LPS-induced MIP-1α production in THP-1 cells at 12 hours (A), 24 hours (B), and 48 hours (C). Effect of beraprost (10^{-9} to 10^{-5} M) on LPS-induced MIP-1α production in human primary monocytes at 12 hours (A), 24 hours (B), and 48 hours (C) (all data are presented in pg/mL per 10^6 cells; *$P < 0.05$).

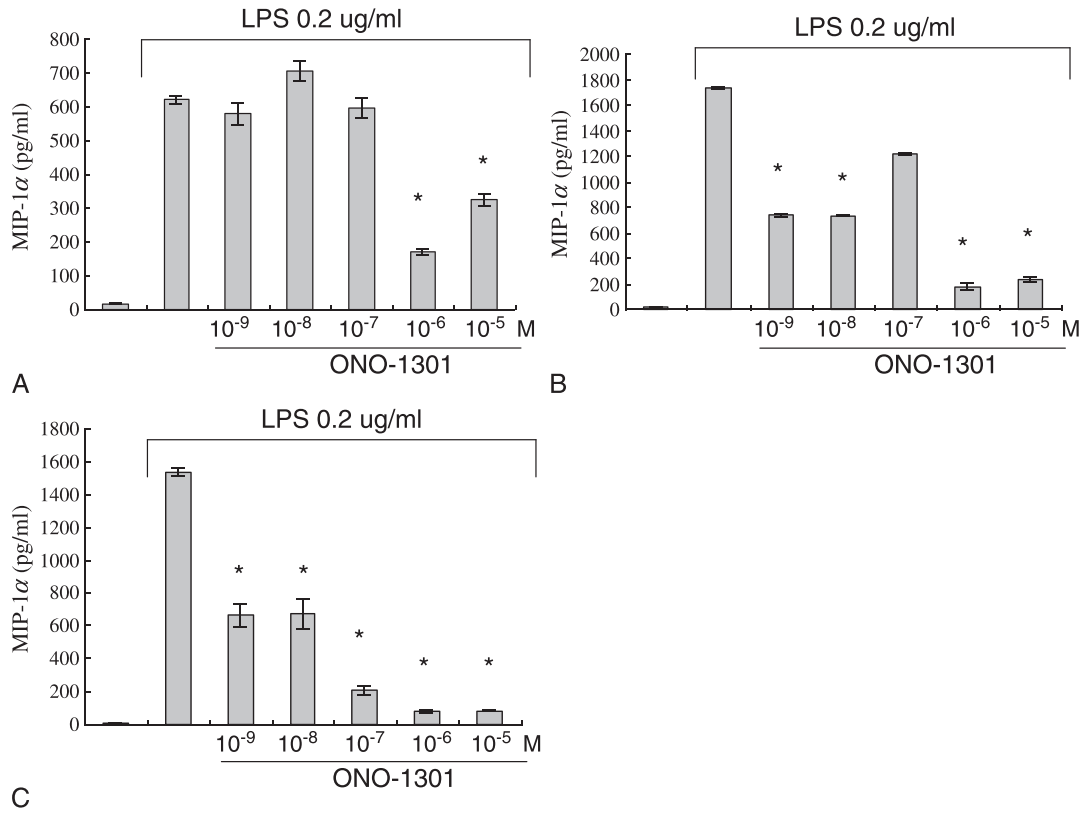

FIGURE 4. Effect of the new PGI$_2$ analog ONO-1301 (10^{-9} to 10^{-5} M) on LPS-induced MIP-1α production in human primary monocytes at 12 hours (A), 24 hours (B), and 48 hours (C) (all data are presented in pg/mL per 10^6 cells; *$P < 0.05$).

receptor antagonist could not reverse the suppressive effect on LPS-induced MIP-1α production in THP-1 (Fig. 5, B–D). Prostaglandin I_2 analog effects have been also reported via PPAR-α and PPAR-γ.[14] To examine the effect of PGI$_2$ analog on cytokine expression of THP-1 cells via PPAR-α or PPAR-γ antagonist, THP-1 cells were pretreated with PPAR-α antagonist (GW6741) or PPAR-F antagonist (GW9662) 1 hour before the treatment of the cells with iloprost treatment. As shown in Figure 5E–5F, PPAR-α or PPAR-γ antagonist could not reverse the suppressive effect on LPS-induced MIP-1α production in THP-1 cells. Next we examination whether cAMP activator forskolin had a similar effect with iloprost. Similarly, forskolin (10^{-5} M) suppressed LPS-induced MIP-1α production in THP-1 cells (Fig. 5G). These data suggest iloprost suppressed LPS-induced MIP-1α production in human monocytes via the IP receptor and cAMP pathway.

DISCUSSION

Atherosclerosis affects large and medium elastic and muscular arteries and underlies a large proportion of cardiovascular disease morbidity and mortality.[15] Chemokines are instrumental in the initiation and progression of atherosclerotic lesions. Recent advances in genomic technologies and the recognition of atherosclerosis as an inflammatory disease address the relevance of chemokines for the clinically manifest stages of atherosclerosis. The critical role of inflammation and immune cells in atherosclerosis makes it unsurprising that many chemokines and chemokine receptors have been linked to this disease. CC chemokines have been widely implicated in atherosclerotic plaque development and has also been found to involve atherosclerosis in ApoE$^{-/-}$ mice.[16] CC chemokines have been linked to saphenous vein graft disease, which shares similarity to native vessel atherosclerosis. Studies in mouse models reveal CCR5 ligands MIP-1α/CCL3 and CCL5 to be linked with atherosclerotic plaque progression,[17] and MIP-1α in plasma levels may be the prognostic marker for ischemic events[7] CCR5 and its ligands CCL3, CCL4, and CCL5 have been identified in human and mouse vasculature and have been detected in human atherosclerotic plaque. Levels of CCL3, CCL4, and CCL5 have all been linked to coronary atherosclerosis,[18,19] whereas CCL3 and CCL5 have been shown to correlate with congestive heart failure.[20] Distinct roles for chemokine-receptor systems in atherogenesis have been proposed, with CCR5 likely to be critical in recruitment of monocytes to developing plaques.[2]

Because PGI$_2$ is very unstable, PGI$_2$ analogs with more chemical stability have been used in clinical application. Iloprost, a stable PGI$_2$ analog, is awell-accepted medication for pulmonary arterial hypertension. Beraprost sodium could lower circulating vascular cell adhesion molecule 1 concentration and has been used for the prevention and treatment of atherosclerosis in patients with type 2 diabetes mellitus.[21] In the present study, iloprost was more effective in the suppression of MIP-17α production by monocytes in all 4 PGI$_2$ analogs. Therefore, iloprost may be the candidate for in vivo study and clinical trials. A new PGI$_2$ analog (ONO-1301) with a highly potent and selective IP receptor effect has been developed. We here also investigated the effect of the new PGI$_2$ analog, ONO-1301, on the LPS-induced MIP-1α/CCL3 expression in monocytes to be the guide for clinical selection.[22] However, the suppressive effect of ONO-1301 on the LPS-induced MIP-1α expression in monocytes was not better than other conventional PGI$_2$ analogs. In the present study,

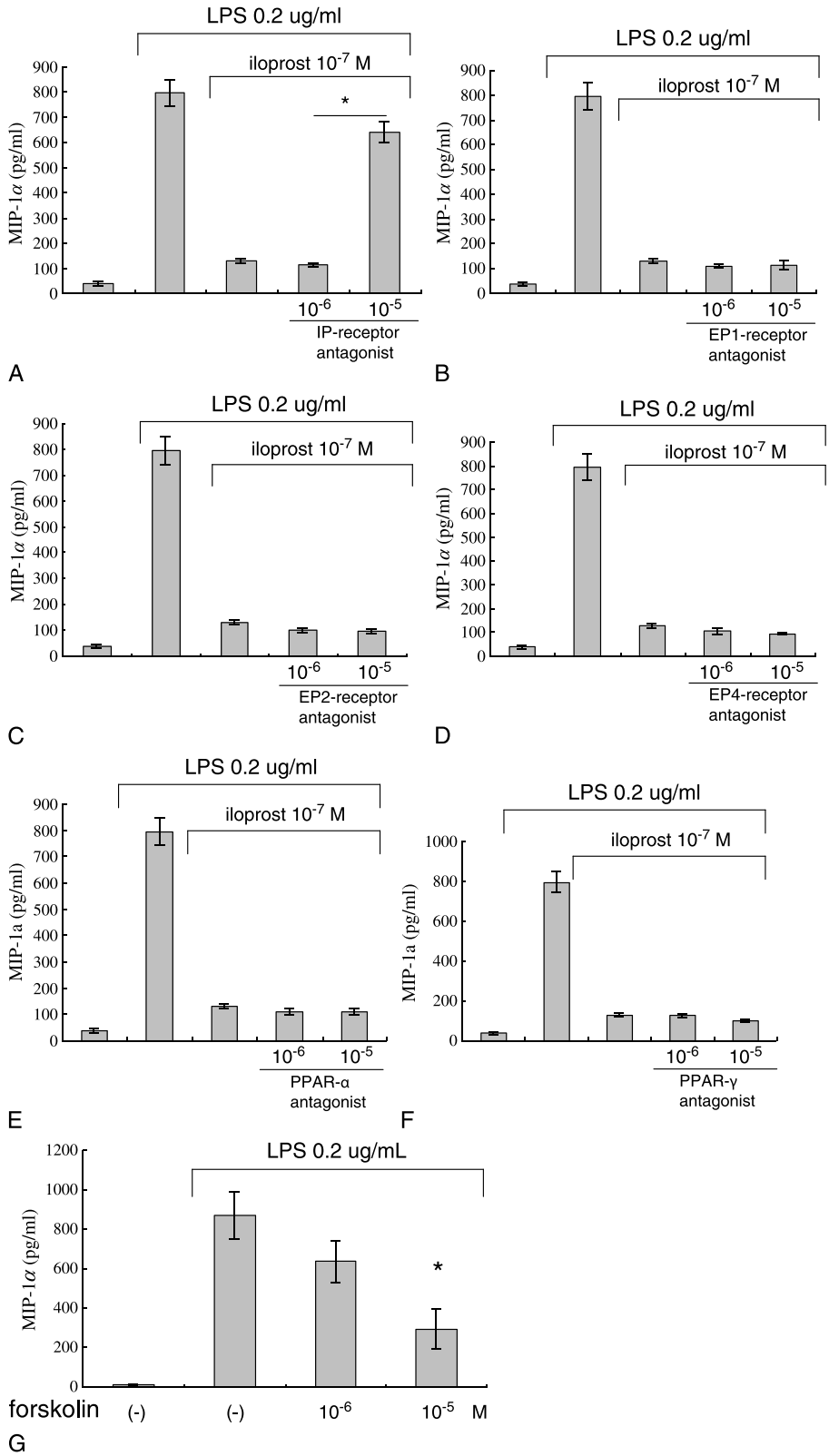

FIGURE 5. CAY 10449 could reverse iloprost-suppressed LPS-induced MIP-1α expression in THP-1 cells (A). E prostanoid receptor, including EP1 (B), EP2 (C), or EP4 (D), antagonists have no effect on the suppressive effect of iloprost on LPS-induced MIP-1α expression in THP-1 cells. PPAR-γ (E) and PPAR-α (F) could not reverse iloprost-suppressed LPS-induced MIP-1α expression in THP-1 cells. Only a higher dose (10^{-5} M) of forskolin also suppressed MCP-1 production in THP-1 cells (G) (all data are presented in pg/mL per 10^6 cells).

iloprost inhibited the production of MIP-1α production by monocytes from human peripheral blood. Forskolin, a cAMP activator, could also suppress MIP-1α expression in a dose-dependent manner in monocytes. CAY 10449, an IP receptor antagonist, could at least partly reverse the effects of iloprost on MIP-1α expression in monocytes. Other reported PGI_2 receptor antagonists including EP1, EP2, EP4, PPAR-α, and PPAR-γ antagonists could not reverse the suppression of iloprost on MIP-1α production in monocytes. These results suggested iloprost may modulate MIP-1α expression in monocytes, at least in part, via IP receptor and cAMP. Prostaglandin I_2 analogs also have other anti-inflammatory or inflammatory effect. For example, PGI_2 analogs enhanced T_H2-related chemokine MDC, but suppressed T_H1-related chemokine IP-10 expression in LPS-stimulated monocytes through IP receptor antagonist and intracellular cAMP pathway.[14] Combined with our present study, PGI_2 analogs suppressed macrophage- and T_H1-related chemokine IP-10 expression, but enhanced T_H2-related chemokine in monocytes.

In the present study, all PGI_2 analogs suppressed monocyte-produced MIP-1α. These data suggested PGI_2 analogs may be the powerful agents for prevention of plaque formation not only by suppressive MIP-1α production in monocytes, but also its profound anti-inflammatory effect. Different PGI_2 analogs should be used in different application forms. For example, iloprost should be used in inhalation, and treprostinil has been used in injection. Beraprost sodium is an orally active PGI_2 analog with antiplatelet and vasodilating properties.[13] Our data also suggested beraprost had a profound effect to suppress MIP-1α production. Therefore, beraprost may be a more potential candidate for the treatment or prevention in the antiatherosclerotic field because of its oral active property. Further animal and human in vivo translational studies are still needed to confirm the preventive effect of PGI_2 analogs on plaque formation.

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