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The Isoprostane 8-iso-PGE_2 Stimulates Endothelial Cells to Bind Monocytes via Cyclic AMP- and p38 MAP Kinase-Dependent Signaling Pathways

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ABSTRACT

Increased levels of isoprostanes have been detected in human atherosclerotic lesions. To examine a possible role for 8-iso-prostaglandin E_2 (8-iso-PGE_2) in atherogenesis, we tested the effect of 8-iso-PGE_2 on adhesion of leukocytes to human umbilical vein endothelial cells (EC). We demonstrate that 8-iso-PGE_2 stimulates EC to bind monocytes, but not neutrophils. This effect was inhibited by the thromboxane A_2 receptor antagonist SQ29548. Moreover, 8-iso-PGE_2 increased levels of cyclic AMP in EC, and monocyte adhesion induced by 8-iso-PGE_2 was blocked by a protein kinase A inhibitor, H89. In addition, 8-iso-PGE_2 induced phosphorylation of p38 and extracellular signal-regulated kinase (ERK) 1/2 mitogen-activated protein (MAP) kinase and stimulated expression of EGR-1. A specific inhibitor of p38 MAP kinase (SB203580) abrogated monocyte binding, whereas an inhibitor of the ERK pathway (PD98059) did not block monocyte adhesion induced by 8-iso-PGE_2. Activation of nuclear factor-κB (NF-κB) and expression of NFκB-dependent genes intercellular adhesion molecule-1, vascular cell adhesion molecule-1, and E-selectin were not induced by 8-iso-PGE_2. Taken together, these results demonstrate that 8-iso-PGE_2 stimulates EC to specifically bind monocytes, but not neutrophils. This effect is mediated by cyclic AMP/protein kinase A- and p38 MAP kinase-dependent pathways and is independent of the classical inflammatory NFκB pathway. Thus, formation of 8-iso-PGE_2 may play an important role in chronic inflammatory diseases such as atherosclerosis by increasing adhesion and extravasation of monocytes. *Antioxid. Redox Signal.* 5, 163–169.

INTRODUCTION

ISOPROSTANES are chemically stable products of free radical-catalyzed peroxidation of arachidonic acid, structurally related to prostaglandins (15). Biological effects of isoprostanes include broncho- and vasoconstriction, as well as activation of platelets, smooth muscle cells, and endothelial cells (EC) (4, 6, 7, 11, 18, 19). The specific receptor(s) mediating effects of isoprostanes are not identified. In some cases, effects of isoprostanes are mediated by the thromboxane A_2 (TXA_2) receptor [T prostanoid (TP) receptor] (10, 13). However, other studies have suggested the existence of a specific isoprostane receptor, which is similar to, but distinct from, the TP receptor (6, 19).

The levels of isoprostanes *in vivo* are increased by oxidative stress, which accompanies many pathological conditions. In particular, increased concentrations of isoprostanes have been detected in blood of smokers (16, 21), patients with hypercholesterolemia (3, 22), and apolipoprotein E-knockout hyperlipidemic mice (20). Isoprostanes were found in oxidized low-density lipoprotein (14) and in human atherosclerotic lesions (8), suggesting that they can play a role in development of atherosclerosis. This hypothesis is further supported by the ability of antioxidants to blunt generation of

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isoprostanes and reduce atherosclerosis in a mouse model of atherogenesis (20).

One of the initiating steps in atherogenesis is attachment of circulating monocytes to EC, followed by extravasation of monocytes (12). In contrast to acute inflammation, monocytes, but not neutrophils, are the predominant cell type recruited during atherosclerosis and other types of chronic inflammation. We have shown that certain products of lipid peroxidation, such as oxidized phospholipids, cholesterol esters, and the isoprostane 8-iso-prostaglandin $F_{2\alpha}$ (8-iso-$PGF_{2\alpha}$), can selectively induce monocyte adhesion to EC, but do not stimulate adhesion of neutrophils (9, 11, 26). Here, we expand these observations by showing that another major product of nonenzymatic arachidonate oxidation, 8-iso-prostaglandin E_2 (8-iso-PGE_2), also stimulates EC to bind monocytes, but not neutrophils. Our findings indicate that 8-iso-PGE_2-induced monocyte binding is mediated by cyclic AMP (cAMP)/protein kinase A (PKA) and p38 mitogen-activated protein (MAP) kinase signaling pathways and is independent of the classical inflammatory nuclear factor-κB (NFκB) mechanism. These data provide further insight into signaling and transcriptional mechanisms of chronic inflammation, which is characteristic of atherosclerosis.

MATERIALS AND METHODS

Materials

The isoprostane 8-iso-PGE_2 and the TXA_2/prostaglandin receptor agonist and antagonist (U46619 and SQ29548, respectively) were purchased from Cayman Chemical (Ann Arbor, MI, U.S.A.). Antibodies for detection of E-selectin, vascular cell adhesion molecule-1 (VCAM-1), and intercellular adhesion molecule-1 (ICAM-1) by whole-cell enzyme-linked immunosorbent assay (ELISA) were obtained from R&D Systems (Minneapolis, MN, U.S.A.). Antibodies for Western blotting of phospho-p38 MAP kinase, total, extracellular signal-regulated kinase (ERK) 1/2, and phospho-ERK1/2 were purchased from New England BioLabs (Beverly, MA, U.S.A.). Anti-IκBα was from Santa Cruz Biotechnologies (Santa Cruz, CA, U.S.A.). SB203580, PD98059, and bisindolylmaleimide I were from Calbiochem (La Jolla, CA, U.S.A.), indomethacin was from Sigma Chemicals (St. Louis, MO, U.S.A.), and supplemented calf serum was from HyClone (Logan, UT, U.S.A.).

Tissue culture

Human aortic EC were cultured as described previously (1). Human umbilical vein EC were prepared and cultured as described (27). Blood monocytes were obtained from a large pool of healthy donors as described previously (5). U937 and HL-60 cells, which were shown to behave like peripheral blood monocytes and neutrophils in adhesion experiments (9, 17), were obtained from American Type Culture Collection (Manassas, VA, U.S.A.).

Leukocyte adhesion assays

EC were incubated with test medium for 4 h at 37°C. In some experiments, EC were preincubated for 1 h with inhibitors and then incubated for 4 h with a combination of agonists and inhibitors. The test medium was removed, cells were extensively washed, and a suspension of human monocytes, U937 cells, or HL-60 cells was added. After 12–15 min, nonadherent cells were removed by gentle washing/aspiration. Bound monocytes, U937, or HL-60 cells were counted microscopically.

Cell ELISA

EC were plated into 96-well plates and treated with stimuli for 4 h. After washing, cells were fixed with 0.1% glutaraldehyde in phosphate-buffered saline. Antibodies specific for E-selectin, VCAM-1, and ICAM-1 were added for 1 h at 37°C and, after washing, detected with goat anti-mouse IgG conjugated to peroxidase. *o*-Phenylenediamine (Sigma) was used for development, and the reaction was stopped by an equal volume of 0.5 *M* H_2SO_4. Optical density was read at 492 nm.

Western blotting

Experiments were performed exactly as described by us previously (2). To confirm equal protein loading, all blots were stripped and stained for total ERK1/2 (both phospho and non-phospho forms).

cAMP assay

Cells were cultured in six-well dishes and pretreated for 10 min with 0.5 m*M* isobutyl-1-methylxanthine. Then 8-iso-PGE_2 or forskolin was added and cells incubated for 2 h. After washing with phosphate-buffered saline containing 4 m*M* EDTA, cells were scraped from the dish in the same buffer. After centrifugation, the pellet was resuspended and, after three cycles of freeze-thawing, sonicated. Coagulated macromolecules were removed by centrifugation (13,000 *g*, 2 min). cAMP levels were determined in the supernatant using a cAMP EIA kit (Cayman Chemical).

Statistics

Statistical analysis was performed using one-way ANOVA. The data in the figures are expressed as means ± SDs and are representative of two to five independent experiments (see figure legends).

RESULTS

In this study, we investigate a possible role of 8-iso-PGE_2 in atherogenesis and chronic inflammation by testing its effects on adhesion of leukocytes to vascular EC. Treatment of EC with 2.5–20 μ*M* 8-iso-PGE_2 for 4 h induced binding of unstimulated monocytes to EC in a concentration-dependent manner (Fig. 1A). For all further experiments, 8-iso-PGE_2 was used at a concentration of 10 μ*M*, which induced a reproducible twofold increase in monocyte binding.

Accumulating data indicate that isoprostanes exert biological action through the TXA_2/prostaglandin H_2 (TP) receptor and through an additional isoprostane-specific receptor (11). TP receptors and putative isoprostane receptors are probably structurally similar, as evidenced by the ability of the com-

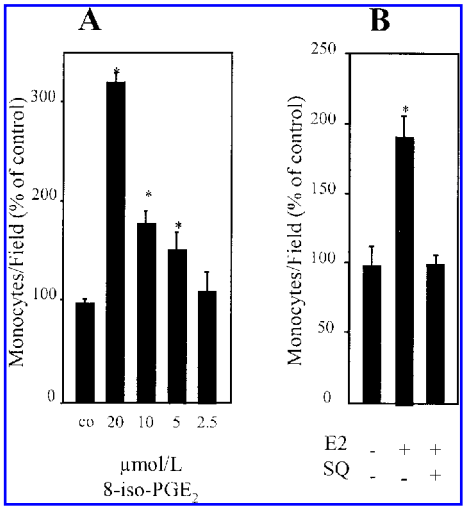

FIG. 1. 8-iso-PGE$_2$ concentration-dependently stimulates EC to bind monocytes through isoprostane/TXA$_2$ receptor. (**A**) EC grown in 48-well dishes were incubated with the indicated concentrations of 8-iso-PGE$_2$ for 4 h at 37°C and then tested for monocyte adhesion. (**B**) SQ29548 (SQ) was added together with 8-iso-PGE$_2$ (E2) (both at 10 μ*M*). The data are representative of five independent experiments. *$p < 0.05$ compared with cells treated with medium only (control).

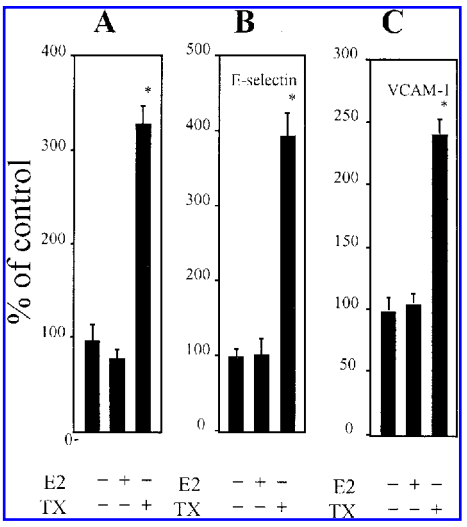

FIG. 2. 8-iso-PGE$_2$ does not stimulate adhesion of neutrophils and does not up-regulate E-selectin or VCAM-1. EC grown in 48- (**A**) or 96-well plates (**B** and **C**) were incubated with 8-iso-PGE$_2$ (E2) or TXA$_2$ receptor agonist U46619 (both at 10 μ*M*) for 4 h at 37°C. After the incubation, EC were analyzed for binding of neutrophil-like HL-60 cells (A) or for surface expression of E-selectin (B) or VCAM-1 (C). The data are representative of four ndependent experiments. *$p < 0.001$ compared with cells treated with medium only.

pound SQ29548 to block effects of both TXA$_2$ and isoprostanes. Accordingly, addition of SQ29548 abrogated the effects of 8-iso-PGE$_2$ on monocyte adhesion (Fig. 1B).

To investigate whether the action of 8-iso-PGE$_2$ was specific for induction of adhesion of monocytes, its ability to induce neutrophil–endothelial interactions was also examined. 8-iso-PGE$_2$ did not stimulate EC to bind neutrophil-like HL-60 cells (Fig. 2A). In contrast to 8-iso-PGE$_2$, the TP receptor agonist U46619 stimulated EC to bind neutrophil-like cells (Fig. 2A).

We then tested the effect of 8-iso-PGE$_2$ on the expression of the neutrophil-binding adhesion molecule E-selectin on EC. 8-iso-PGE$_2$ did not change expression of E-selectin on EC (Fig. 2B). 8-iso-PGE$_2$ also did not change expression by EC of monocyte-specific adhesion molecule VCAM-1 (Fig. 2C), suggesting that 8-iso-PGE$_2$-induced binding of monocytes to EC is mediated by adhesion molecules other than VCAM-1.

Agonists of the TP receptor, such as TXA$_2$ and U46619, are known to stimulate production of prostaglandins, which can stimulate EC in an autocrine fashion. To examine whether the effect of 8-iso-PGE$_2$ on monocyte adhesion could be mediated by induction of prostaglandin synthesis, experiments were also performed with addition of a cyclooxygenase inhibitor, indomethacin. Inhibition of cyclooxygenase did not have any effect on 8-iso-PGE$_2$-induced monocyte adhesion (data not shown). Therefore, *de novo* prostaglandin synthesis was not responsible for the increase of monocyte adhesion induced by 8-iso-PGE$_2$.

We further investigated intracellular signaling pathways mediating 8-iso-PGE$_2$-induced monocyte binding to EC. One important mechanism for accumulation of monocytes under

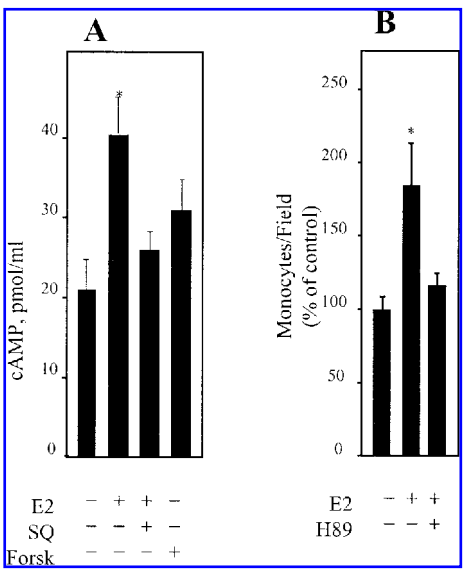

FIG. 3. 8-iso-PGE$_2$ elevates cAMP levels in EC, which is functionally important for monocyte binding. (**A**) EC grown in six-well plates were stimulated with 8-iso-PGE$_2$ (E2) or forskolin (Forsk) (both at 10 μ*M*) for 2 h at 37°C, and then processed for determination of cAMP contents. (**B**) EC grown in 48-well plates were preincubated with the PKA inhibitor H89 (10 μ*M*) for 1 h, then incubated with 8-iso-PGE$_2$ with or without H89 (both at 10 μ*M*) for 4 h at 37°C. After the incubation, EC were analyzed for binding of monocytes. The data are representative of three independent experiments. *$p < 0.01$ compared with cells treated with medium only.

FIG. 4. 8-iso-PGE_2 does not induce degradation of IκBα. EC grown in six-well plates were treated with 10 μ*M* 8-iso-PGE_2 for the indicated times in the CO_2 incubator. The cells were scraped, and contents of IκBα were determined by western blotting. The data are representative of three experiments.

conditions of chronic inflammation is cAMP-dependent cell-surface deposition of fibrinogen containing the CS-1 fragment, which is a ligand for binding of monocytes (23, 24). In order to elucidate the role of cAMP in 8-iso-PGE_2-induced monocyte adhesion, we measured the effects of 8-iso-PGE_2 on cAMP levels in EC. 8-iso-PGE_2 increased levels of cAMP approximately twofold above control, which was comparable to the action of the adenylate cyclase activator forskolin (Fig. 3A). 8-iso-PGE_2-induced cAMP generation was inhibited by addition of SQ29548. We then examined whether elevation of cAMP by 8-iso-PGE_2 was functionally important to induce monocyte binding. Addition of 25 μ*M* H89, a specific inhibitor of PKA, blocked 8-iso-PGE_2-induced monocyte binding to EC (Fig. 3B).

Classical mechanisms of induction of inflammatory adhesion molecules in EC involve stimulation of the NFκB pathway. A key event in the activation of this pathway by a variety

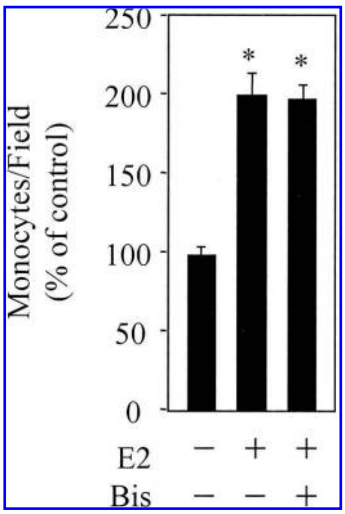

FIG. 5. Monocyte adhesion induced by 8-iso-PGE_2 is independent of PKC. EC grown in 48-well plates were pretreated with the PKC inhibitor bisindolylmaleimide I (Bis; 10 μ*M*) for 1 h, and then further incubated in the presence of 10 μ*M* 8-iso-PGE_2 (E2) for 4 h. Monocyte adhesion experiments were performed as described in Materials and Methods. The data are representative of five independent experiments. *$p <$ 0.01 compared with cells treated with medium only.

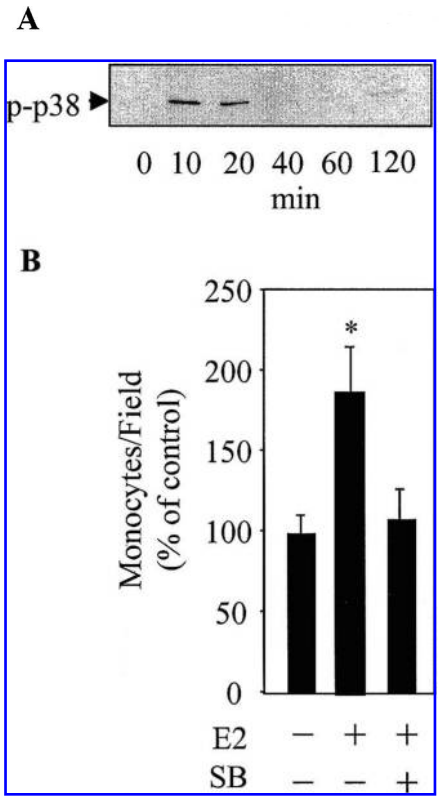

FIG. 6. 8-iso-PGE_2 induces phosphorylation of p38 MAP kinase in EC, which is important for activation of monocyte binding. (**A**) EC grown in six-well dishes were treated with 10 μ*M* 8-iso-PGE_2 for the indicated times. After the incubation, the cells were scraped off and analyzed by western blotting using an antibody specific for the phosphorylated form of p38 MAP kinase. (**B**) EC grown in 48-well plates were pretreated with SB203580 (SB; 10 μ*M*) for 1 h. After further stimulation with 10 μ*M* 8-iso-PGE_2 (E2) for 4 h at 37°C, monocyte adhesion to the EC was measured. The data are representative of three independent experiments. *$p <$ 0.01 compared with cells treated with medium only.

of inflammatory agonists includes degradation of the inhibitory subunit IκB, resulting in generation of free NFκB subunits, which are translocated into the nucleus and stimulate transcription of target genes. 8-iso-PGE_2 did not induce degradation of IκBα in EC (Fig. 4). In addition, as shown above, treatment of EC with 8-iso-PGE_2 did not stimulate expression of E-selectin, VCAM-1, and ICAM-1, which are typical NFκB-responsive genes in EC (Fig. 2B and C, and data not shown). Taken together, these results indicate that the NFκB pathway is not involved in the stimulatory action of 8-iso-PGE_2 on monocyte adhesion to EC.

Agonists of the TP receptor are known to activate protein kinase C (PKC) (10). We tested whether the PKC-dependent pathway plays a role in 8-iso-PGE_2-induced monocyte binding to EC. Inhibition of PKC by bisindolylmaleimide I (10 μ*M*) did not abrogate monocyte binding, stimulated by 8-iso-PGE_2 (Fig. 5). These results indicate that the induction of

monocyte binding by 8-iso-PGE$_2$ is independent of the PKC pathway.

We further tested the effects of 8-iso-PGE$_2$ on the activity of p38 MAP kinase, which is important for induction of adhesion molecules by various inflammatory stimuli, such as lipopolysaccharide, tumor necrosis factor-α, and oxidized phospholipids. 8-iso-PGE$_2$ stimulated phosphorylation of p38 MAP kinase with levels of phosphorylated p38 MAP kinase being sustained for up to 20 min after stimulation (Fig. 6A). Pretreatment of EC with a specific inhibitor of p38 MAP kinase (SB203580) abrogated monocyte binding induced by 8-iso-PGE$_2$ (Fig. 6B). These data suggest that the p38 MAP kinase pathway is activated by 8-iso-PGE$_2$ and is functionally important for enhancement of monocyte binding induced by this isoprostane.

In a previous study, we have shown that 8-iso-PGF$_2$ activated ERK1/2 in EC and that activation of these kinases was necessary for 8-iso-PGF$_2$-induced monocyte adhesion to EC (11). 8-iso-PGE$_2$ also induced phosphorylation of ERK1/2 in EC, which was sustained for up to 40 min after stimulation (Fig. 7A). Phosphorylation of ERK1/2 induced by 8-iso-PGE$_2$ was inhibited by the TXA$_2$ receptor antagonist SQ29548, but was insensitive to inhibitors of PKC (bisindolylmaleimide) and PKA (H89) (Fig. 7B). In addition, 8-iso-PGE$_2$ stimulated expression of the transcription factor EGR-1 (Fig. 7C). It is known that transcription of the *Egr-1* gene is activated by the ERK kinase cascade and that induction of this protein by oxidized phospholipids can be inhibited by the compound PD98059, known to inhibit the ERK pathway (2). In order to elucidate the functional importance of the ERK1/2/EGR-1 cascade for stimulation of monocyte binding by 8-iso-PGE$_2$, we checked whether this induction could be blocked by the MEK1/2 inhibitor PD98059, which acts upstream of ERK1/2. Inhibition of the ERK pathway by PD98059 did not

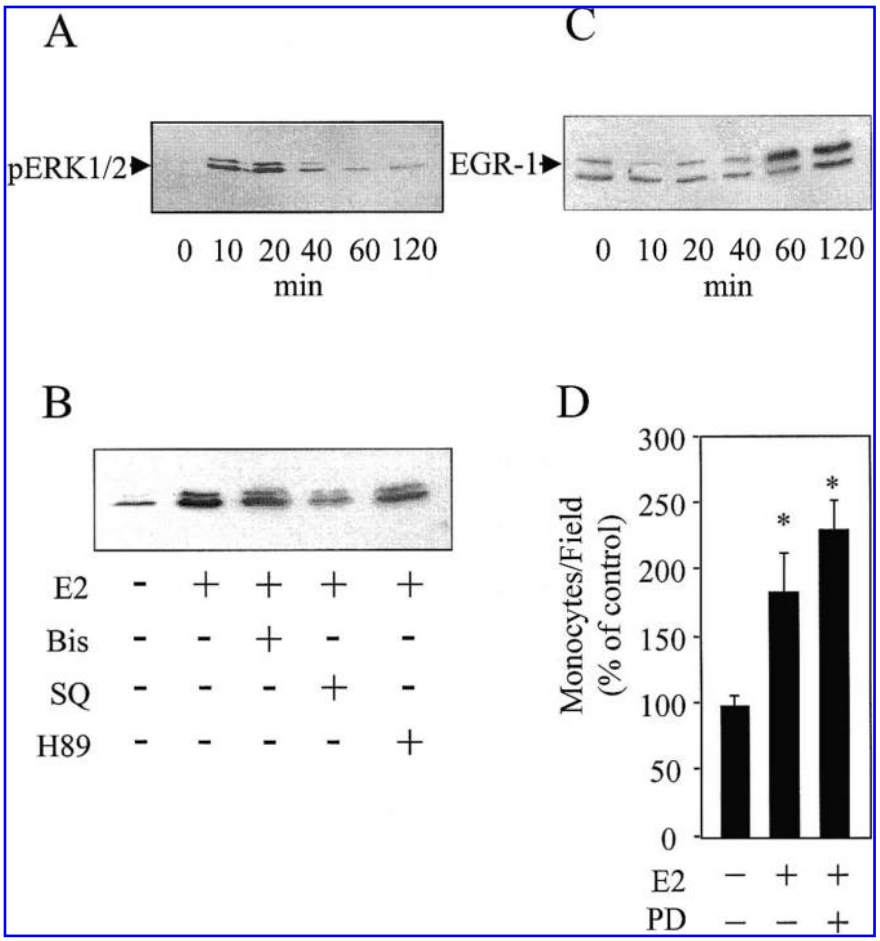

FIG. 7. 8-iso-PGE$_2$ stimulates phosphorylation of ERK1/2 MAP kinases and expression of EGR-1 in EC. (**A**) EC grown in six-well plates were stimulated with 10 μ*M* 8-iso-PGE$_2$ for the indicated times. Afterwards, the cells were scraped and analyzed by western blotting using an antibody specific for the phosphorylated forms of ERK1/2. (**B**) EC were stimulated for 20 min with 10 μ*M* 8-iso-PGE$_2$ (E2) in the presence or absence of bisindolylmaleimide I (Bis), SQ29548 (SQ), or H89 (all at 10 μ*M*), and then were analyzed for phosphorylated ERK1/2. (**C**) EC, stimulated with 10 μ*M* 8-iso-PGE$_2$, were analyzed for expression of the EGR-1 protein using western blotting. (**D**) EC grown in 48-well plates were pretreated for 1 h with a specific inhibitor of MEK1/2 [PD98059 (PD)] then stimulated for 4 h with 10 μ*M* 8-iso-PGE$_2$, and analyzed for monocyte adhesion. Data are representative of two to four independent experiments. *$p < 0.01$ compared with cells treated with medium only.

block 8-iso-PGE_2-induced monocyte binding, demonstrating an interesting and unexpected difference in mechanism of 8-iso-PGE_2 and 8-iso-PGF_2 (Fig. 7D).

DISCUSSION

A large body of evidence indicates that isoprostanes are markers for increased oxidative stress. Elevated concentrations of isoprostanes were detected in patients after coronary reperfusion and angioplasty and in atherosclerotic lesions. A variety of pharmacological effects of isoprostanes strongly suggest that they are not merely markers of oxidative stress induced by these disorders, but actively participate in their pathogenesis.

Two of the most extensively studied isoprostanes produced from arachidonic acid are 8-iso-$PGF_{2\alpha}$ and 8-iso-PGE_2. Previously, we demonstrated that 8-iso-$PGF_{2\alpha}$ stimulates EC to bind monocytes. Here we demonstrate that 8-iso-PGE_2 also selectively induces monocyte–endothelial interactions, a process known to be an important initiating event in atherogenesis.

Systemic plasma concentrations of isoprostanes found in humans are in the picomolar range (3). As 8-iso-PGE_2 in the present study was active in the micromolar range of concentrations, it is unlikely that circulating isoprostanes would induce monocyte binding to EC. However, in human atherosclerotic plaques, these compounds are present in abundance (8). Immunochemically, local accumulation of isoprostanes in macrophages near the necrotic core and in smooth muscle cells has been shown.

The region of the plaque near the necrotic core is thought to be a locus for monocyte recruitment, further implicating a role for these lipid oxidation products. In addition, one has to take into account that a combination of lipid oxidation products from all lipid classes is present in atherosclerotic vessels, including various isoprostanes (11), oxidized phospholipids (26), and oxidized cholesteryl esters (9). Acting in concert, these oxidized lipids may lead to specific monocyte adhesion, and thus to the progression of the lesion.

We found that 8-iso-PGE_2 increased intracellular endothelial levels of cAMP and that inhibition of the cAMP-dependent PKA clearly attenuated 8-iso-PGE_2-induced monocyte adhesion. These results are consistent with another study, which demonstrated the ability of 8-iso-PGE_2 to elevate intracellular levels of cAMP (25). cAMP in EC is known to activate surface deposition of a splice variant of fibrinogen, which contains the CS-1 fragment (24). As we did not observe any effect of 8-iso-PGE_2 on the levels of ICAM-1, VCAM-1, or E-selectin, we propose that enhanced binding of monocytes to 8-iso-PGE_2-stimulated EC was mediated by interaction of $\alpha_4\beta_1$ integrin of monocytes with the CS-1 fragment of fibrinogen on EC. However, this question requires further experimentation.

In addition, we demonstrate that the p38 MAP kinase signaling pathway is activated by 8-iso-PGE_2 and that blocking of this pathway results in abrogation of monocyte adhesion induced by 8-iso-PGE_2 (Fig. 6). Phosphorylation of ERK1/2 was also induced by 8-iso-PGE_2, but, surprisingly, inhibition of this pathway did not block induction of monocyte binding to EC by 8-iso-PGE_2 (Fig. 7). In contrast, in our previous work we found that 8-iso-$PGF_{2\alpha}$-induced monocyte adhesion was dependent on ERK1/2 activation (11). However, we show that EGR-1 expression is induced by 8-iso-PGE_2, and this transcription factor is up-regulated by the PKC/ERK1/2 MAP kinase pathway (2).

Here we present evidence that NFκB translocation, as well as induction of NFκB-dependent genes, is not induced by 8-iso-PGE_2. This result is in accordance with other studies showing that monocyte-specific adhesion induced by oxidized lipids such as 8-iso-$PGF_{2\alpha}$, oxidized phospholipids, and oxidized cholesteryl esters is determined by induction of cAMP/PKA and MAP kinase pathways, rather than by the common inflammatory NFκB paythway (9, 11).

In summary, we have shown that 8-iso-PGE_2 specifically induces monocyte adhesion to EC through cAMP/PKA- and p38 MAP kinase-dependent signaling pathways. These results demonstrate that 8-iso-PGE_2 may play an important role in the development of the atherosclerotic lesion.

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ABBREVIATIONS

cAMP, cyclic AMP; EC, endothelial cell(s); ELISA, enzyme-linked immunosorbent assay; ERK, extracellular signal-regulated kinase; ICAM-1, intercellular adhesion molecule-1; 8-iso-PGE_2, 8-iso-prostaglandin E_2; MAP kinase, mitogen-activated protein kinase; NFκB, nuclear factor-κB; PKA, protein kinase A; PKC, protein kinase C; TP receptor, T prostanoid receptor; TXA_2, thromboxane A_2; VCAM-1, vascular cell adhesion molecule-1.

REFERENCES

1. Berliner JA, Territo MC, Sevanian A, Ramin S, Kim JA, Bamshad B, Esterson M, and Fogelman AM. Minimally modified low density lipoprotein stimulates monocyte endothelial interactions. *J Clin Invest* 85: 1260–1266, 1990.
2. Bochkov VN, Mechtcheriakova D, Lucerna M, Huber J, Malli R, Graier WF, Hofer E, Binder BR, and Leitinger N. Oxidized phospholipids stimulate tissue factor expression in human endothelial cells via activation of ERK/EGR-1 and Ca^{++}/NFAT. *Blood* 99: 199–206, 2002.
3. Davi G, Alessandrini P, Mezzetti A, Minotti G, Bucciarelli T, Costantini F, Cipollone F, Bon GB, Ciabattoni G, and Patrono C. In vivo formation of 8-epi-prostaglandin F2 alpha is increased in hypercholesterolemia. *Arterioscler Thromb Vasc Biol* 17: 3230–3235, 1997.
4. Davi G, Ciabattoni G, Consoli A, Mezzetti A, Falco A, Santarone S, Pennese E, Vitacolonna E, Bucciarelli T, Costantini F, Capani F, and Patrono C. In vivo formation of 8-iso-prostaglandin f2alpha and platelet activation in dia-

betes mellitus: effects of improved metabolic control and vitamin E supplementation [see comments]. *Circulation* 99: 224–229, 1999.
5. Fogelman AM, Elahi F, Sykes K, Van LB, Territo MC, and Berliner JA. Modification of the Recalde method for the isolation of human monocytes. *J Lipid Res* 29: 1243–1247, 1988.
6. Fukunaga M, Makita N, Roberts LJ, Morrow JD, Takahashi K, and Badr KF. Evidence for the existence of F2-isoprostane receptors on rat vascular smooth muscle cells. *Am J Physiol* 264: C1619–C1624, 1993.
7. Fukunaga M, Yura T, and Badr KF. Stimulatory effect of 8-Epi-PGF2 alpha, an F2-isoprostane, on endothelin-1 release. *J Cardiovasc Pharmacol* 26 (Suppl 3): S51–S52, 1995.
8. Gniwotta C, Morrow JD, Roberts LJ 2nd, and Kuhn H. Prostaglandin F2-like compounds, F2-isoprostanes, are present in increased amounts in human atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 17: 3236–3241, 1997.
9. Huber J, Boechzelt H, Karten B, Surboeck M, Bochkov VN, Binder BR, Sattler W, and Leitinger N. Oxidized cholesteryl linoleates stimulate endothelial cells to bind monocytes via the extracellular signal-regulated kinase 1/2 pathway. *Arterioscler Thromb Vasc Biol* 22: 581–586, 2002.
10. Kinsella BT, O'Mahony DJ, and Fitzgerald GA. The human thromboxane A2 receptor alpha isoform (TP alpha) functionally couples to the G proteins Gq and G11 in vivo and is activated by the isoprostane 8-epi prostaglandin F2 alpha. *J Pharmacol Exp Ther* 281: 957–964, 1997.
11. Leitinger N, Huber J, Rizza C, Mechtcheriakova D, Bochkov V, Koshelnick Y, Berliner JA, and Binder BR. The isoprostane 8-iso-PGF(2alpha) stimulates endothelial cells to bind monocytes: differences from thromboxane-mediated endothelial activation. *FASEB J* 15: 1254–1256, 2001.
12. Lusis AJ. Atherosclerosis. *Nature* 407: 233–241, 2000.
13. Mohler ER, Franklin MT, and Adam LP. Intracellular signaling by 8-epi-prostaglandin F2 alpha is mediated by thromboxane A2/prostaglandin endoperoxide receptors in porcine carotid arteries. *Biochem Biophys Res Commun* 225: 915–923, 1996.
14. Moore KP, Darley UV, Morrow J, and Roberts LJ: Formation of F2-isoprostanes during oxidation of human low-density lipoprotein and plasma by peroxynitrite. *Circ Res* 77: 335–341, 1995.
15. Morrow JD and Roberts LJ. The isoprostanes: unique bioactive products of lipid peroxidation. *Prog Lipid Res* 36:1–21, 1997.
16. Morrow JD, Frei B, Longmire AW, Gaziano JM, Lynch SM, Shyr Y, Strauss WE, Oates JA, and Roberts LJ. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. *N Engl J Med* 332: 1198–1203, 1995.
17. Parhami F, Fang ZT, Fogelman AM, Andalibi A, Territo MC, and Berliner JA. Minimally modified low density lipoprotein-induced inflammatory responses in endothelial cells are mediated by cyclic adenosine monophosphate. *J Clin Invest* 92: 471–478, 1993.
18. Parhami F, Morrow AD, Balucan J, Leitinger N, Watson AD, Tintut Y, Berliner JA, and Demer LL. Lipid oxidation products have opposite effects on calcifying vascular cell and bone cell differentiation. A possible explanation for the paradox of arterial calcification in osteoporotic patients. *Arterioscler Thromb Vasc Biol* 17: 680–687, 1997.
19. Pratico D, Smyth EM, Violi F, and FitzGerald GA. Local amplification of platelet function by 8-Epi prostaglandin F2alpha is not mediated by thromboxane receptor isoforms. *J Biol Chem* 271: 14916–14924, 1996.
20. Pratico D, Tangirala RK, Rader DJ, Rokach J, and FitzGerald GA. Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in ApoE-deficient mice. *Nat Med* 4: 1189–1192, 1998.
21. Reilly M, Delanty N, Lawson JA, and FitzGerald GA: Modulation of oxidant stress in vivo in chronic cigarette smokers. *Circulation* 94: 19–25, 1996.
22. Reilly MP, Pratico D, Delanty N, DiMinno G, Tremoli E, Rader D, Kapoor S, Rokach J, Lawson J, and FitzGerald GA. Increased formation of distinct F2 isoprostanes in hypercholesterolemia [see comments]. *Circulation* 98: 2822–2828, 1998.
23. Shih PT, Brennan ML, Vora DK, Territo MC, Strahl D, Elices MJ, Lusis AJ, and Berliner JA. Blocking very late antigen-4 integrin decreases leukocyte entry and fatty streak formation in mice fed an atherogenic diet. *Circ Res* 84:345–351, 1999.
24. Shih PT, Elices MJ, Fang ZT, Ugarova TP, Strahl D, Territo MC, Frank JS, Kovach NL, Cabanas C, Berliner JA, and Vora DK. Minimally modified low-density lipoprotein induces monocyte adhesion on endothelial connecting segment-1 by activating beta1 integrin. *J Clin Invest* 103: 613–625, 1999.
25. Tintut Y, Parhami F, Tsingotjidou A, Tetradis S, Territo M, and Demer LL. 8-Isoprostaglandin E2 enhances a receptor-activated NFkappa B ligand (RANKL)-dependent osteoclastic potential of marrow hematopoietic precursors via the cAMP pathway. *J Biol Chem* 277: 14221–14226, 2002.
26. Watson AD, Leitinger N, Navab M, Faull KF, Horkko S, Witztum JL, Palinski W, Schwenke D, Salomon RG, Sha W, Subbanagounder G, Fogelman AM, and Berliner JA. Structural identification by mass spectrometry of oxidized phospholipids in minimally oxidized low density lipoprotein that induce monocyte/endothelial interactions and evidence for their presence in vivo. *J Biol Chem* 272: 13597–13607, 1997.
27. Zhang JC, Fabry A, Paucz L, Wojta J, and Binder BR. Human fibroblasts downregulate plasminogen activator inhibitor type-1 in cultured human macrovascular and microvascular endothelial cells. *Blood* 88: 3880–3886, 1996.

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2. Nuria Matesanz, Victoria Jewhurst, Elisabeth R. Trimble, Ann McGinty, Daphne Owens, Gerald H. Tomkin, Lesley A. Powell. 2011. Linoleic acid increases monocyte chemotaxis and adhesion to human aortic endothelial cells through protein kinase C- and cyclooxygenase-2-dependent mechanisms. *The Journal of Nutritional Biochemistry* . [CrossRef]
3. Brandon J. Reeder, Dimitri A. Svistunenko, Michael T. Wilson. 2011. Lipid binding to cytoglobin leads to a change in haem co-ordination: a role for cytoglobin in lipid signalling of oxidative stress. *Biochemical Journal* **434**:3, 483-492. [CrossRef]
4. Ahmad K. Mashmoushi, Gary S. Gilkeson, Jim C. OatesThe Role of Reactive Nitrogen and Oxygen Intermediates in Systemic Lupus Erythematosus 199-211. [CrossRef]
5. Brandon J. Reeder . 2010. The Redox Activity of Hemoglobins: From Physiologic Functions to Pathologic Mechanisms. *Antioxidants & Redox Signaling* **13**:7, 1087-1123. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
6. Jim C. Oates. 2010. The biology of reactive intermediates in systemic lupus erythematosus. *Autoimmunity* **43**:1, 56-63. [CrossRef]
7. Lucia S. Graham, Farhad Parhami, Yin Tintut, Christina M.R. Kitchen, Linda L. Demer, Rita B. Effros. 2009. Oxidized lipids enhance RANKL production by T lymphocytes: Implications for lipid-induced bone loss. *Clinical Immunology* **133**:2, 265-275. [CrossRef]
8. Alma J. Nauta, Ferdi Engels, Leon M. Knippels, Johan Garssen, Frans P. Nijkamp, Frank A. Redegeld. 2008. Mechanisms of allergy and asthma. *European Journal of Pharmacology* **585**:2-3, 354-360. [CrossRef]
9. Jim C. Oates, Gary S. Gilkeson. 2006. The biology of nitric oxide and other reactive intermediates in systemic lupus erythematosus. *Clinical Immunology* **121**:3, 243-250. [CrossRef]
10. Jean-Luc Cracowski, Thierry Durand. 2006. Cardiovascular pharmacology and physiology of the isoprostanes. *Fundamental and Clinical Pharmacology* **20**:5, 417-427. [CrossRef]
11. Ginger L. Milne , Jason D. Morrow . 2006. Isoprostanes and Related Compounds: Update 2006. *Antioxidants & Redox Signaling* **8**:7-8, 1379-1384. [Abstract] [Full Text PDF] [Full Text PDF with Links]
12. Alexandra Kadl , Norbert Leitinger . 2005. The Role of Endothelial Cells in the Resolution of Acute Inflammation. *Antioxidants & Redox Signaling* **7**:11-12, 1744-1754. [Abstract] [Full Text PDF] [Full Text PDF with Links]
13. Luke J. Janssen , Adriana Catalli , Peter Helli . 2005. The Pulmonary Biology of Isoprostanes. *Antioxidants & Redox Signaling* **7**:1-2, 244-255. [Abstract] [Full Text PDF] [Full Text PDF with Links]
14. J Cracowski. 2004. Isoprostanes: an emerging role in vascular physiology and disease?. *Chemistry and Physics of Lipids* **128**:1-2, 75-83. [CrossRef]
15. Valery N. Bochkov , Norbert Leitinger . 2003. Redox Regulation of Endothelial Function. *Antioxidants & Redox Signaling* **5**:2, 145-146. [Citation] [Full Text PDF] [Full Text PDF with Links]