Endogenous prostaglandin D_2 synthesis inhibits e-selectin generation in human umbilical vein endothelial cells

Hideyuki Negoro[1*], Hiroyuki Kobayashi[2], Yoshio Uehara[3]

[1]Harvard Medical School, Internal Medicine, Boston, USA; [*]Corresponding Author: negoroh-tky@umin.ac.jp
[2] Department of Hospital Administration, Graduate School of Medicine, Juntendo University, Tokyo, Japan;
[3]University of Tokyo Internal Medicine, Tokyo, Japan.

ABSTRACT

We examined the role of prostaglandin D_2 (PGD_2) in the formation of E-selectin following interleukin-1 (IL-1) stimulation in human umbilical vein endothelial cells (HUVEC) transfected with lipocaline-type PGD_2 synthase (L-PGDS) genes. HUVEC were isolated from human umbilical vein and incubated with 20 U/mL IL-1 and various concentrations of authentic PGD_2. The isolated HUVEC were also transfected with L-PGDS genes by electroporation. The L-PGDS-transfected HUVEC were used to investigate the role of endogenous PGD_2 in IL-1-stimulated E-selectin biosynthesis. We also used an anti-PGD_2 antibody to examine whether an intracrine mechanism was involved in E-selectin production. PGD_2 and E-selectin levels were determined by radio-immunoassay and enzyme-immunoassay, respectively. E-selectin mRNA was assessed by real-time RT-PCR. IL-1-stimulated E-selectin production by HUVEC was dose-dependently inhibited by authentic PGD_2 at concentrations greater than 10^{-6} mol/L. L-PGDS gene-transfected HUVEC produced more PGD_2 than HUVEC transfected with the reporter gene alone. IL-1 induced increases in E-selectin production in HUVEC transfected with the reporter genes alone. However, this effect was significantly attenuated in the case of IL-1 stimulation of HUVEC transfected with L-PGDS genes, and accompanied by an apparent suppression of E-selectin mRNA expression. Neutralization of extracellular PGD_2 by anti-PGD_2-specific antibody influenced neither E-selectin mRNA expression nor E-selectin biosynthesis. HUVEC transfected with L-PGDS genes showed increased PGD_2 synthesis. This increase was associated with attenuation of both E-selectin generation and E-selectin mRNA expression. The results suggest that endogenous PGD_2 decreases E-selectin synthesis and E-selectin mRNA expression, probably through an intracrine mechanism.

Keywords: Prostaglandin; E-Selectin; PGDS; Endothelial Cell

1. INTRODUCTION

Adhesion molecules play an important role in the development and progression of atherosclerosis. The hypothesis proposed by Ross and its modifications have been generally accepted as a mechanism of atherosclerosis where adhesion of circulating monocytes and lymphocytes to vascular endothelium presumably initiates a series of events toward atherosclerosis [1]. Cellular adhesion molecules mediate the adhesion, margination, and transendothelial migration of circulating mononuclear cells from the blood stream to the extravascular compartment to have an important part in the progression of atherosclerotic plaque [2]. Recent studies have elucidated further that to anchor leukocytes onto the endothelial cells, the adhesion molecules expressed on the surface of endothelial cells necessitated to bind to their ligands expressed on leukocytes. Endothelial cells are stimulated by inflammatory agents to express selectins, such as endothelial-leukocyte adhesion molecule-1 (E-selectin), which interact with carbohydrate ligands on leukocytes, and to express immunoglobulin superfamily proteins, such as vascular cell adhesion molecule-1 (VCAM-1). The adhesion molecules expressed on the endothelial cells include VCAM-1, intracellular adhesion molecule (ICAM)-1, P-selectin and E-selectin [3]. Selectins, including E-selectin and P-selectin, are in-

volved in the first step of leukocyte adhesion at sites of inflammation or injury. Selectins are characterized by rolling and tethering of leukocytes to the endothelial surface, to platelets or to other leukocytes [4]. In fact, E-selectin is demonstrated to occur in atherosclerotic lesions in the coronary artery of humans [5]. Therefore, E-selectin is believed to be a key factor for the development of immune-mediated cardiovascular injury.

Intrestngly, we have recently reported that PGD_2 attenuates inducible nitric oxide generation in vascular smooth muscle cells [6]. Endogenous prostaglandin D_2 synthesis reduces plasminogen activator inhibitor-1 generation following cytokines stimulation in bovine endothelial cells [7]. Especially, PGD_2 is synthesized in vascular components of atheromatous lesions including endothelial cells, macrophages, platelets, and mast cells [8] and lipocalin-type PGD_2 synthase (L-PGDS) is demonstrated to occur in atheromatous lesions in the cardiovascular system [9]. These data strongly suggest that L-PGDS/PGD_2 is upregulated in response to immune-related vascular lesions and in turn, the increase of L-PGDS/PGD_2 is exerted to attenuate the progression of the arterial remodeling.

Taken together, we proposed the hypothesis that PGD_2 regulates E-selectin expression in endothelial cells, thereby contributing to leukocyte adhesion, an integral component of the development of vascular injury. However, there had been few data investigating the crosstalk between endogenous L-PGDS/PGD_2 system and the adhesion molecule expression by cytokines. In the present study, in order to test our hypothesis that L-PGDS/PGD_2 protects the vascular wall against immune-related vascular injury, we examined the relationship between endogenous PGD_2 and E-selectin expression by endothelial cells and attempted to reveal its intracellular mechanism mediated by PGD_2 using L-PGDS gene-transfected endothelial cells in culture. We also examined whether the increases in intracellular PGD_2 synthesis influenced E-selectin mRNA expression and E-selectin biosynthesis observed following interleukin-1b (IL-1) stimulation.

2. MATERIALS AND METHODS

2.1. Materials

Eicosanoids and related compounds were purchased from Funakoshi chemicals (Tokyo, Japan). Arachidonic acid was purchased from Sigma (St. Louis, MO, USA). Recombinant murine IL-1 was purchased from R&D Systems (Minneapolis, MN, USA). Radioactively labeled materials were purchased from Amersham (Tokyo, Japan). A 3-kb gene for rat brain PGDS [(5Z, 13E)-(15S)-9a, 11a -epidoxy-15-hydroxyprosta-5,13-dienoate

D-isomerase, EC 5.3.99.2] was isolated from a rat genomic DNA library by plaque hybridization with cDNA for the PGDS enzyme, as described in our previous studies [10]. A 3-kb BamHI fragment of rat PGDS, which belongs to the lipocalin family, was inserted into a pcD2 plasmid containing the SV40 promoter, along with a polyA signal at the XhoI site (Invitrogen, Carlsbad, CA, USA) [11]. The b-galactosidase gene with a cytomegalovirus (CMV) promoter at an XbaI site was inserted into the pBluescript 2 KS+ plasmid (Stratagene, La Jolla, CA, USA).

2.2. Cell Culture

Human umbilical vein endothelial cells (HUVEC) were harvested enzymatically as described previously [12]. They were maintained in medium 199 (GIBCO BRL, Gaithersburg, MD), containing Hepes, heparin (1%), endothelial cell growth factor (50 mg/ml), L-glutamin (1%), antibiotics, and 5% fetal bovine serum (FBS). When the cells reached confluence, they were replanted onto low pyrogen fibronectin at 20,000 cells/cm^2. HUVEC which were isolated from a confluent monolayer of polygonal cells. The cells expressed von Willbrand factor as determined by their content of specific mRNA and immunoreactive protein. Cellular viability was assessed by Trypan blue exclusion.

2.3. Effect of Exogenous PGD_2 on E-Selectin Expression in Endothelial Cells

Cultures of HUVEC were treated with 20 U/ml IL-1, according to the previous study, in the presence of various concentrations of PGD_2 to be incubated for 18 h. Thereafter, the HUVEC were washed three times with FBS-free Dulbecco's phosphate-buffered saline (D-PBS; Gibco) and the cells were re-incubated in 1 ml of fresh D-PBS for 2 h. Subsequently, the cells and culture supernatants were used in various assays.

2.4. Transfection of L-PGDS Genes into HUVEC

HUVEC were transfected with L-PGDS genes using the Shimadzu GTE-10 electroporation device (Gene Transfer Equipment-10, Shimadzu Co., Ltd., Kyoto, Japan). This equipment transiently increases the permeability of plasma membranes of HUVEC, thereby facilitating translocation of genes into the cytoplasm. Briefly, the cells were washed three times with FBS-free D-PBS and 10 mg of pcD2-rat PGDS in 0.5 ml of fresh D-PBS were added to each well [13]. A transient electrical current was applied onto HUVEC growing in the culture dishes, using a 35-mm round electrode (Model FTC-

33D3, Shimadzu Co., Ltd., Kyoto, Japan), after which the cells were incubated for 30 minutes.

Following transfection, 2 ml of DMEM containing 10% FBS was added to the HUVEC and the dishes were incubated for an additional 24 hours. Endogenous PGD_2 production was stimulated by adding 10^{-6} mol/l arachidonic acid for 24 hours. Fresh medium containing 10^{-6} mol/l arachidonic acid was then added and E-selectin mRNA expression and E-selectin expression were stimulated for 18 hours by the addition of 20 U/ml IL-1. At the end of this incubation period, the HUVEC were washed three times with FBS-free D-PBS and incubated in 1 ml of fresh D-PBS for 2 hours. Finally, the cells and culture supernatants were collected for analysis.

For comparison, HUVEC were transfected with b-galactosidase genes (b-gal) by electroporation to determine the efficacy of gene transfection. Three days after the transfection, HUVEC were stained with X-gal [14], and b-galactasidase expression was measured. Transfection efficacy was estimated as the ratio of the X-gal stained area to the sectional area of the HUVEC.

2.5. Neutralizing Extrinsic PGD_2 Released from HUVEC

We attempted to neutralize PGD_2 using an anti-PGD_2-specific antibody in order to investigate the effects of PGD_2 released from L-PGDS-transfected HUVEC. We estimated the amount of anti-PGD_2 antibody required to completely neutralize the secreted PGD_2 using the Scatchard analysis. The binding affinity was 0.0051 ml/pg, and the B_{max} (maximal binding capacity) was 25 pg/l [15]. Therefore, 1 liter of antibody had the capacity to bind 25 pg PGD_2. Taking into account of these results, we used 200 ml of the antibody to inhibit the receptor-mediated actions of PGD_2 in HUVEC under our culture conditions. The anti-PGD_2 antibody was raised in our laboratories using PGD_2-conjugated thyroglobulin and Freund's complete adjuvant. The antibody cross-reacted 0.003% with thromboxane B_2, 0.01% with prostaglandin E_2 (PGE_2), 0.009% with prostaglandin F_{2a} (PGF_{2a}), 0.008% with 6-keto-PGF_{1a} and 0.01% with arachidonate [16]. Antibody activity was confirmed by suppression of intracellular cyclic AMP (cAMP) following PGD_2 stimulation via PGD_2 receptor, which acts as a second messenger for PGD_2 signal transduction [17].

2.6. Eicosanoid Radioimmunoassay

Eicosanoids were determined in culture media using the direct radioimmunoassay method described previously [16]. Briefly, 0.1 ml of sample, 0.1 ml of [^{3}H] eicosanoid (5000 dpm) and 0.1 ml of the diluted antibody were mixed and incubated at 4°C for 24 hours. To separate bound from free [^{3}H] eicosanoid, 0.1 ml of dextran-coated charcoal in a 50 mmol/l phosphate buffer at pH 7.4 containing 0.1% gelatin and 100 mmol/l NaCl was added to the ice-chilled assay mixture. The mixture was vortexed and centrifuged at 3000 rpm for 5 min at 4°C. The supernatant was assayed for [^{3}H] eicosanoids bound to the antibody. Radioactivity was determined using an automatic liquid scintillation counter. The properties of the anti-6keto-PGF_{1a} antibody was described previously [15,16]. The cross-reactivity and its properties of the anti-PGD_2 antibody was detailed above. The low cross-reactivity of each antibody made it feasible to measure directly the eicosanoid in media.

2.7. E-Secletin and E-Secletin mRNA Measurements

We measured E-selectin expression by commercially available cell surface enzyme immunoassay as described previously (R&D Systems, Inc., USA).

Cultured E-selectin transcripts were detected using the real-time reverse transcription-polymerase chain reaction (real-time RT-PCR) method using real-time RT-PCR machine as described previously [18].

2.8. Statistical Analysis

All values are expressed as the mean ± SE. The differences between values were assessed by an one-way ANOVA and Duncan's multiple range test using the STATISTICA program (StatSoft, Tulsa, OK, USA) on a Gateway G6-400 computer system (Gateway Inc., N Sioux City, SD, USA) running the Windows 98 operating system. P values less than 0.05 were considered statistically significant.
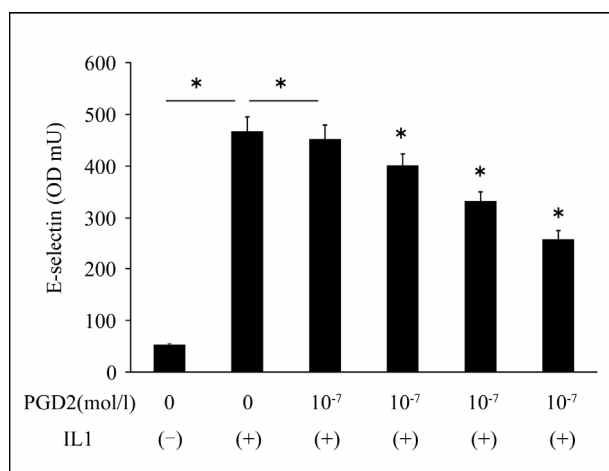
3. RESULTS

3.1. Effect of Exogenous PGD_2 on E-Selectin Expression in Endothelial Cells

Stimulation of endothelial cells with IL-1 significantly increased E-selectin expression. The increase was reduced in a dose-dependent manner by the addition of PGD_2 at concentrations ranging from 10^{-7} to 10^{-4} mol/l (**Figure 1**).

3.2. Gene Transfection and Eicosanoid Generation in HUVEC

We transfected HUVEC with L-PGDS genes in order to increase endogenous PGD_2 formation. PGD_2 was assayed using radioimmunoassay. The basal levels of PGD_2 in reporter-gene-transfected HUVEC maintained in

Figure 1. Effect of exogenous PGD_2 on E-selectin generation in endothelial cells. Stimulation of endothelial cells with IL-1 significantly increased E-selectin generation (left two columns). The increase was reduced in a dose-dependent manner by the addition of PGD_2 at concentrations ranging from 10^{-7} to 10^{-4} mol/l. All experiments were performed three different times with at least six replicates. Statistical differences were analyzed by one-way ANOVA and Duncan's multiple range tests. *P < 0.01 vs the value at 0 mol/L PGD_2.

arachidonate-free media were as low as 51.5 ± 2.2 pg/10^6 cells/2 hours, and the addition of 10^{-6} mol/l arachidonate had no effect on PGD_2 synthesis. In contrast, L-PGDS gene-transfected HUVEC showed an increase (178.0%) in PGD_2 generation even in arachidonate-free media, as compared with control HUVEC carrying reporter genes alone. Furthermore, 10^{-6} mol/l arachidonate markedly stimulated PGD_2 biosynthesis by 640.7%, as compared with control HUVEC carrying vector genes alone (**Figure 2(a)**). Thereafter, PGI_2 was assayed as 6-keto-PGF_{1a} using radioimmunoassay. Basal levels of prostacyclin (PGI_2), the major eicosanoid synthesized in HUVEC, were 1488 ± 66 pg/10^6 cells/2 hours in cells having reporter genes alone under arachidonate-free conditions. The PGI_2 generation was a markedly increased by 327% when the cells were stimulated with 10^{-6} mol/l arachidonate (**Figure 2(b)**). L-PGDS gene transfection did not influence PGI_2 synthesis in HUVEC maintained under either arachidonate-free or arachidonate-stimulated conditions, as compared to HUVEC transfected with reporter genes. These data clearly suggest that the L-PGDS genes alter the phenotype of HUVEC so that the recombinant cells acquire the capacity to produce PGD_2 in response to arachidonate stimulation.

3.3. Effect of L-PGDS Gene Transfection on E-Selectin Expression in HUVEC

Using the HUVEC having L-PGDS genes, we invest-
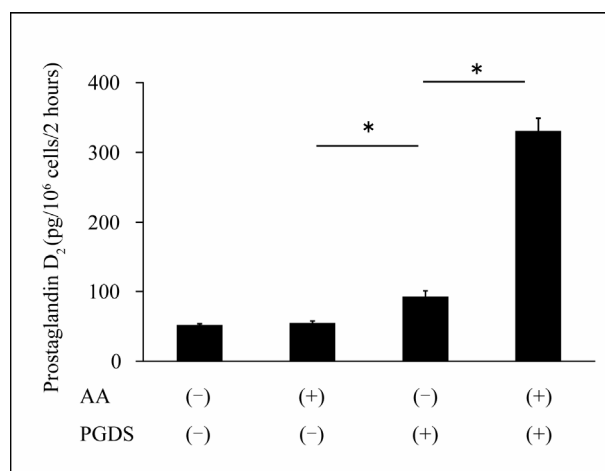
(a)
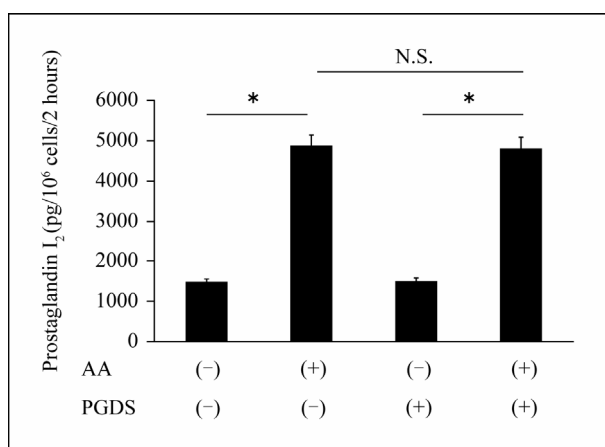
(b)

Figure 2. PGD_2 and PGI_2 generation in endothelial cells. PGD_2 was assayed using radioimmunoassay. 10^{-6} mol/l arachidonate did not stimulate PGD_2 generation in cells having reporter genes alone (two columns to the left in Graph a). However, cells carrying the L-PGDS genes acquired the ability to produce PGD_2 with or without arachidonate stimulation (two columns to the right in Graph a). (Graph a). PGI_2 was assayed as 6-keto-PGF_{1a} using radioimmunoassay. The addition of 10^{-6} mol/L arachidonate to the cultures increased PGI_2 generation; however, there were no differences in PGI_2 production between PGDS(−), AA(+) and PGDS(+), AA(+) lines. (Graph b). Statistical differences were assessed by Student's t-test (n = 6). *P < 0.01. N.S. represents not statistically significant.

tigated the effects of endogenous PGD_2 on the expression of E-selectin with or without IL-1 stimulation. The E-selectin expression was significantly increased upon IL-1 stimulation in endothelial cells transfected with transporter genes. This increase in E-selectin with or without IL-1 stimulation was significantly blunted in HUVEC transfected with L-PGDS genes that produced indeed endogenous PGD_2 in response to arachidonate stimulation (**Figure 3**).
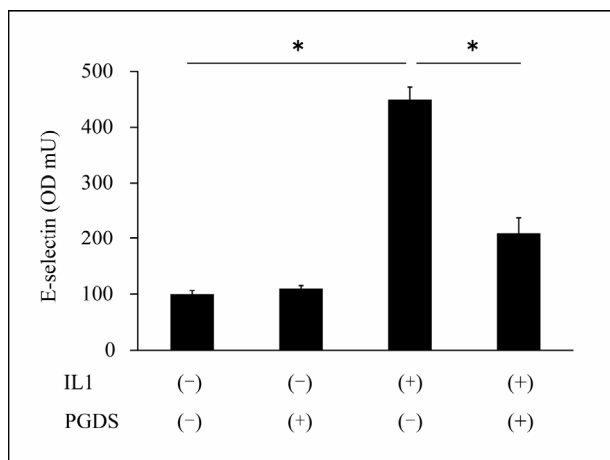
Figure 3. Effect of PGD₂ synthase gene transfection on E-selectin expression in endothelial cells. Under normal (un-stimulated) conditions, endothelial ells bearing L-PGDS genes (IL-1(−), PGDS(+)) expressed less E-selectin than those bearing reporter genes alone (IL-1(−), PGDS(−)). E-selectin expression increases observed following IL-1 stimulation were significantly attenuated in cells carrying L-PGDS genes in response to arachidonic acid stimulation (IL-1(+), PGDS(+), AA(+)), though there was no attenuation of E-selectin expression in cells carrying L-PGDS genes but not stimulated by arachidonic acid (IL-1(+), PGDS(+), AA(−)), compared to endothelial cells having only reporter genes (IL-1(+), PGDS (−)). The experiment was carried out in the presence of 10^{-6} mol/l arachidonate. Statistical differences were assessed by Student's t-test (n = 6). *P < 0.01.

3.4. Intracellular Effects of PGD₂ on E-Selectin Expression

In order to assess the role of PGD₂ receptor-mediated signal transduction in E-selectin expression, we studied changes in cAMP, a second messenger of PGD₂ signal transduction in HUVEC transfected with transporter gene alone. Intracellular cAMP was unaffected by L-PGDS transfection per se. However, intracellular cAMP was increased by exogenous PGD₂ stimulation [19]. This response was completely abrogated by addition of anti-PGD₂ antibody to the media, the amount of which was more than the concentrations sufficient to neutralize PGD₂ in the media (**Figure 4(a)**). Using such a dose of anti-PGD₂ antibody enough to inhibit the receptor-mediated cyclic AMP rising, we investigated contribution of PGD₂ receptor-mediated signal transduction to the PGD₂-mediated E-selectin expression, and determined E-selectin expression in the supernatants following neutralization with an anti-PGD₂-specific antibody. The addition of anti-PGD₂ antibody to the media did not influence E-selectin expression following IL-1 stimulation in the endothelial cells transfected with L-PGDS genes (**Figure 4(b)**).
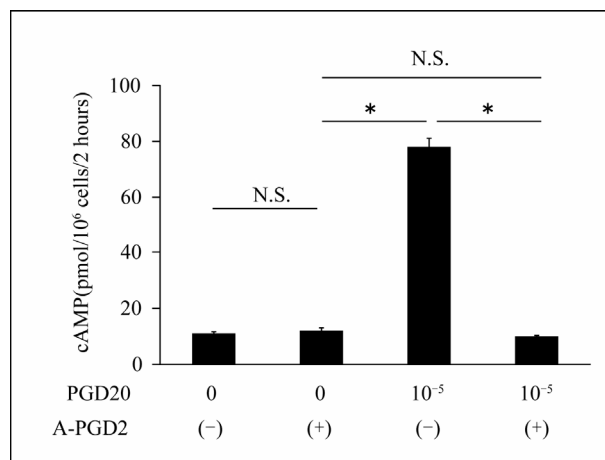
(a)
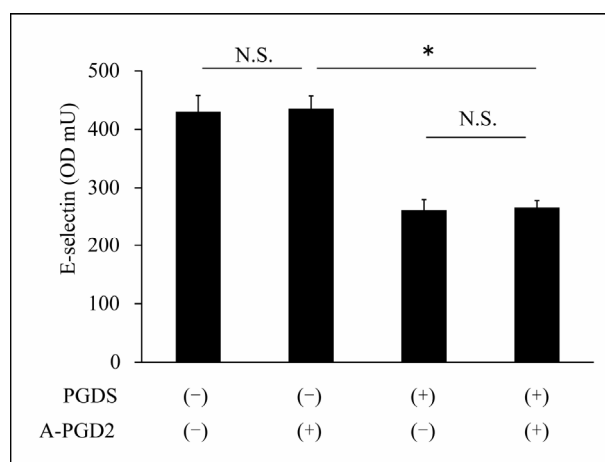
(b)

Figure 4. Effect of endogenous PGD₂ on E-selectin expression in endothelial cells. cAMP levels were measured to assess the antibody inhibition of PGD₂ receptor-mediated signal transduction in the cells with transporter gene alone (Graph a). 10^{-5} mol/l PGD₂ greatly increased cAMP formation in the cells in the absence of anti-PGD₂ antibody (anti-PGD₂(−)). This increase was completely abolished to the basal levels by the addition of anti-PGD₂ antibody in the media. Using such a dose of anti-PGD₂ antibody, we examined the effects of neutralization of PGD₂ in media on E-selectin expression (Graph b). E-selectin expression following IL-1 stimulation was significantly reduced in endothelial cells having L-PGDS genes and without anti-PGD₂ antibody (PGDS(+), anti-PGD₂(−)), compared to endothelial cells with reporter genes alone (PGDS(−), anti-PGD₂(−)). The reduction in E-selectin expression was unaffected when PGD₂ in the culture was neutralized with an anti-PGD₂-specific antibody (PGDS(+), anti-PGD₂(+)). These studies were carried out in the presence of 10^{-6} mol/l arachidonate. Statistical differences were assessed by Student's t-test. *P < 0.01. N.S. represents not statistically significant.

These results clearly indicated that anti-PGD₂-specific antibody inhibited the PGD₂ receptor-mediated signal transduction and that PGD₂-mediated reduction in

Openly accessible at http://www.scirp.org/journal/HEALTH/

E-selectin expression was not due to the PGD_2 receptor-mediated events.

3.5. L-PGDS Genes and Expression of E-Selectin mRNA

We demonstrated that L-PGDS gene transfection onto HUVEC brought about increases in PGD_2 formation and decreases in E-selectin expression using real-time RT-PCR (**Figure 5**). The expression of E-selectin mRNA following IL-1 stimulation was significantly less in the HUVEC carrying L-PGDS genes. Expression was normalized to β-actin. Values are expressed as fold change compared to untreated controls.

4. DISCUSSION

Both PGD_2 and adhesion molecules play a very important part in the process of atherosclerosis. In the present study, we demonstrated that PGD_2 regulates E-selectin generation in HUVEC following IL-1 stimulation. More interestingly, we demonstrated that endogenous PGD_2 production in HUVEC transfected with L-PGDS genes brought about a decrease of E-selectin expression. This effect was observed even if the PGD_2-mediated cAMP increase was reversed to basal levels using anti-PGD_2-specific antibody. These findings strongly suggest that PGD_2 exerts inhibitory effects on E-selectin expression via an intracellular mechanism as well as the well-known receptor-mediated mechanism [20].

In this context, recent studies have demonstrated that the orphan nuclear receptor of peroxysome proliferator-activated receptor (PPAR)-g, a member of the nuclear receptor superfamily of ligand-dependent transcription factors, binds to PGD_2 metabolites and thereby regulates adipocyte differentiation and glucose homeostasis [21, 22].

PGD_2 is converted quickly to PGJ_2, delta 12-PGJ_2, and 15-deoxy-delta12,14 PGJ_2 in plasma [23]. 15-deoxy-delta12,14 PGJ_2 inhibits inhibitor of kB kinase that phosphorylates another inhibitor of kB after activation with cytokines and also affects the DNA-binding domains of nuclear factor-kB (NF-kB) subunits [24]. Because the genes involved in E-selectin expression include the NF-kB binding site in its promoter regions [25], it is presumable that PGD_2 and its metabolites inhibit cytokine induced E-selectin expression, at least in part, through inhibition of NF-kB translocation.

It is reported that E-selectin mRNA and E-selectin are much expressed in vascular lesions such as atherosclerosis [5]. Different cell lines like macrophages, platelets or lymphocytes work together to secrete cytokines in response to the inflammatory events, and in turn, the secreted cytokines stimulate the endothelial cells, thereby
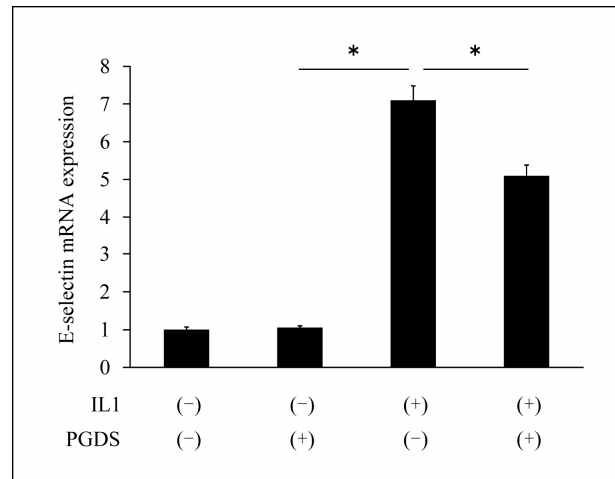

Figure 5. Effect of PGD_2 synthase gene transfection on E-selectin mRNA. Changes in E-selectin mRNA levels were investigated using real-time RT-PCR. The expression of E-selectin mRNA following IL-1 stimulation was significantly less in the HUVEC carrying L-PGDS genes. Expression was normalized to β-actin. Values are expressed as fold change compared to untreated controls. Statistical differences were assessed by Student's t-test. *$P < 0.01$.

increasing E-selectin mRNA and E-selectin expression in the atherosclerotic lesions. In fact, it is well postulated that L-PGDS is highly expressed in the stenotic lesions and in the lipid core of advanced atherosclerotic plaques in patients with stable angina [9]. Moreover, PGD_2 reduces inducible nitric oxide synthase formation. These actions of PGD_2 and metabolites on vasoactive substances regulated by cytokines are in favor of vascular protection against vascular injury [6].

The endothelial cells exhibited a striking increase in PGI_2 generation, but not PGD_2, following arachidonate stimulation, suggesting that these cells have a large capacity to synthesize PGI_2, but not PGD_2. In spite of this, L-PGDS gene transfection greatly enhanced PGD_2 synthesis in EC. This was particularly apparent when eicosanoid expression was stimulated with its precursor, arachidonate. The alteration of phenotype of these cells reduced E-selectin mRNA expression and consequently E-selectin expression. This genetic procedure would provide a new strategy against vascular lesions. These activities have relevance to the in vivo situation, and remain to be clarified.

In conclusion, the introduction of PGD_2 synthase genes into endothelial cells increased endogenous PGD_2 generation. This brought about a reduction in E-selectin expression and a decrease in E-selectin mRNA expression following IL-1 stimulation. The inhibitory effects of PGD_2 on E-selectin expression were due to an intracrine mechanism rather than to any receptor-mediated events. Since suppression of the E-selectin system is postulated

to be protective, an increase in endogenous PGD_2 synthesis might represent a novel strategy to prevent cardiovascular injury in humans.

5. ACKNOWLEDGEMENTS

REFERENCES

[1] Joris, I., Zand, T., Nuunari, J.J., Krolikowski, F.J. and Majno, G. (1983) Studies on the pathogenesis of atherosclerosis, I: Adhesion and emigration of mononuclear cells in the aorta of hypercholesterolemic rats. *American Journal of Pathology*, **113**, 341-358.

[2] Rao, R.M., Yang, L., Garcia-Cardena, G. and Luscinskas, F.W. (2007) Endothelial-development mechanisms of leukocytes recruitment to the vascular wall. *Circulation Research*, **101**, 234-247. doi:10.1161/CIRCRESAHA.107.151860b

[3] Cybulsky, M.I. and Gimbrone, M.A.Jr. (1991) Endothelial expression of a mononuclear leukocyte adhesion molecule, in rabbit aortic endothelium. *Science*, **251**, 788-791. doi:10.1126/science.1990440

[4] Tousoulis, D., Antoniades, C., Bosinakou, E., Kotu, M., Tsoufis C., Marinou, K., *et al.* (2007) Differences in inflammatory and thrombotic markers between unstable angina and acute myocardial infarction. *International Journal of Cardiology*, **115**, 203-207. doi:10.1016/j.ijcard.2006.03.011

[5] Wal, van der A.C., Das, P.K., Tigges, A.J. and Becker, A.E. (1992) Adhesion molecules on the endothelium and mononuclear cells in human atherosclerotic lesions. *American Journal of Pathology*, **141**, 1427-1433.

[6] Nagoshi, H., Uehara, Y., Kanai, F., Maeda, S., Ogura, T., Goto, A. and Omata, M. (1998) Prostaglandin D_2 inhibits inducible nitric oxide synthase expression in rat smooth muscle cells. *Circulation Research*, **82**, 204-209.

[7] Negoro, H., Shin, W.S., Taguchi, R.H., Eguchi, N., Urade, Y., Toyo-oka, T., Fujita, T., Omata, M. and Uehara, Y., (2002) Endogenous prostaglandin D_2 synthesis reduces an increase in plasminogen activator inhibitor-1 following interleukin stimulation in bovine endothelial cells. *Journal of Hypertension*, **20**, 1347-1354. doi:10.1097/00004872-200207000-00021

[8] Watanabe, T., Narumiya, S., Shimizu, T. and Hayaishi, O. (1982) Characterization of the biosynthetic pathway of prostaglandin D_2 in human platelet-rich plasma. *Journal of Biological Chemistry*, **257**, 14847-14853.

[9] Eguchi, Y., Eguchi, N., Oda, H., Seiki, K., Kijima, Y. and Matsuura, Y. (1997) Expression of lipocalin-type prostaglandin D synthase (beta-trace) in human heart and its accumulation in the coronary circulation of angina patients. *Proceedings of National Academy of Sciences of the United States of America*, **94**, 14689-14694. doi:10.1073/pnas.94.26.14689

[10] Urade, Y., Nagata, A., Suzuki, Y., Fujii, Y. and Hayaishi, O. (1989) Primary structure of rat brain prostaglandin D synthase deducted from cDNA sequence. *Journal of Biological Chemistry*, **264**, 1041-1045.

[11] Chen, C. and Okayama, H. (1987) High-efficiency transformation of mammalian cells by plasmid DNA. *Molecular and Cellular Biology*, **7**, 2745-2752.

[12] Zimmerman, G.A., Whatley, R.E., McIntyre, T.M., Benson, D.E. and Prescott, S.M. (1990) Endothelial cells for studies of platelet-activating factor and arachidonate metabolites. *Methods in Enzymology*, **187**, 520-535. doi:10.1016/0076-6879(90)87059-C

[13] Ohtani, K., Nakamura, M., Saito, S., Nagata, K., Sugamura, K. and Hinuma, Y. (1989) Electroporation: Application to human lymphoid cell lines for stable introduction of a transactivator gene of human T-cell leukemia virus type 1. *Nucleic Acid Research*, **17**, 1589-1604. doi:10.1093/nar/17.4.1589

[14] Bonnerot, C., Rocancourt, D., Briant, P., Grimber, G. and Nicolas, J.F. (1987) A b-galactosidase hybrid protein targeted to nuclei as a marker for developmental studies. *Proceedings of National Academy of Sciences of the United States of America*, **84**, 6795-6799. doi:10.1073/pnas.84.19.6795

[15] Tobian, L., Uehara, Y. and Iwai, J. (1985) Prostaglandin alterations in barely hypertensive Dahl S rats. *Trans Association of the American Physiology*, **98**, 378-383.

[16] Uehara, Y., Tobian, L., Iwai, J., Ishii, M. and Sugimoto, T. (1987) Alterations of vascular prostacyclin and thromboxane A2 in Dahl genetical strain susceptible to salt-induced hypertension. *Prostaglandins*, **33**, 727-738. doi:10.1016/0090-6980(87)90038-4

[17] Koide, M., Kawahara, Y., Nakayama, I., Tsuda, T. and Yokoyama, M. (1993) Cyclic AMP-elevation agents induce an inducible type of nitric oxide synthase in cultured vascular smooth muscle cells. *Journal of Biological Chemistry*, **268**, 24959-24966.

[18] Tamasawa, N., Murakami, H., Matsui, J., Yamato, K., JingZhi, G., Imaizumi, T., Fujimoto, K., Yoshida, H., Satoh, K. and Suda, T. (2001) An oxidised derivative of cholesterol increase the release of soluble vascular cell adhesion molecule-1 from human umbilical vein endothelial cells in culture. *Biochimica et Biophysica Acta-General Subjects*, **1531**, 178-187.

[19] Negoro, H., Shin, W.S., Hakamada-Taguchi, R., Eguchi, N., Urade, Y., Goto, A., Toyo-Oka, T., Fujita, T., Omata, M. and Uehara, Y. (2005) Endogenous prostaglandin D(2) synthesis decreases vascular cell adhesion molecule-1 expression in human umbilical vein endothelial cells. *Life Sciences*, **78**, 22-29. doi:10.1016/j.lfs.2005.02.037

[20] Zidovetzki, R., Wang, J.L., Kim, J.A., Chen, P., Fisher, M. and Hofman, F.M. (1999) Endothelin-1 enhances plasminogen activator-1 production by human brain endothelial cells via protein kinase C-dependent pathway. *Arteriosclerosis Thrombosis and Vascular Biology*, **19**, 1768-1775.

[21] Forman, B.M., Tontonoz, P., Chen, J., Brun, R.P., Spiegelman, B.M. and Evans, R.M. (1995) 15-Deoxy-D12,14-prostaglandin J2 is a ligand for the adipocyte determination factor PPAR. *Cell*, **83**, 803-812. doi:10.1016/0092-8674(95)90193-0

[22] Kliewer, S.A., Lenhard, J.M., Willson, T.M., Patel, I., Morris, D.C. and Lehmann, J.M. (1995) A prostaglandin J2 metabolite binds peroxysome proliferator-activator g and promotes adipocyte differentiation. *Cell*, **83**, 813-819. doi:10.1016/0092-8674(95)90194-9

[23] Kikwa, Y., Narumiya, S., Fukushima, M., Wakatsuka, H. and Hayaish, O. (1984) 9-deoxy-d9, d12-13,14-dihydro-prostaglandin D$_2$, a metabolite of prostaglandin D$_2$ formed in human plasma. *Proceedings of National Academy of Sciences of the United States of America*, **81**, 1317-1321. doi:10.1073/pnas.81.5.1317

[24] Ricote, M., Li, A.C., Willson, T.M., Kelly, C.J. and Glass, C.K. (1998) The peroxysome proliferator-activated re-ceptor-g is a negative regulator of macrophage activation. *Nature*, **391**, 79-82. doi:10.1038/34178

[25] Yu, G., Rux, A.H., Ma, P., Bdeir, K. and Sachais, B.S. (2005) Endothelial expression of E-selectin is induced by the platelet-specific chemokine platelet factor 4 through LRP in an NF-kappaB-dependent manner. *Blood*, **105**, 3545-3551. doi:10.1182/blood-2004-07-2617