Lysophospholipids increase ICAM-1 expression in HUVEC through a G\textsubscript{i}- and NF-\kappaB-dependent mechanism

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Lysophospholipids increase ICAM-1 expression in HUVEC through a G\textsubscript{i}- and NF-\kappaB-dependent mechanism. Am J Physiol Cell Physiol 287: C1657–C1666, 2004. First published August 4, 2004; doi:10.1152/ajpcell.00172.2004.—Lysophosphatidic acid (LPA) and sphingosine 1-phosphate (S-1-P) are both low molecular weight lysophospholipid (LPL) ligands that are recognized by the Edg family of G protein-coupled receptors. In endothelial cells, these two ligands activate Edg receptors, resulting in cell proliferation and cell migration. The intercellular adhesion molecule-1 (ICAM-1, CD54) is one of many cell adhesion molecules belonging to the immunoglobulin superfamily. This study showed that LPA and S-1-P enhance ICAM-1 expression at both the mRNA and protein levels in human umbilical cord vein endothelial cells (HUVECs). This enhanced ICAM-1 expression in HUVECs was first observed at 2 h postligand treatment. Maximal expression appeared at 8 h postligand treatment, as detected by flow cytometry and Western blotting. Furthermore, the effects of S-1-P on ICAM-1 expression were shown to be concentration dependent. Prior treatment of HUVECs with pertussis toxin, a specific inhibitor of G\textsubscript{i}, ammonium pyrrolidinedithiocarbamate and BAY 11–7082, inhibitors of the nuclear factor (NF)-\kappaB pathway, or Clostridium difficile toxin B, an inhibitor of Rac, prevented the enhanced effect of LPL-induced ICAM-1 expression. However, pretreatment of HUVECs with exoC3, an inhibitor of Rho, had no effect on S-1-P-enhanced ICAM-1 expression. In a static cell-cell adhesion assay system, pretreatment of LPL enhanced the adhesion between HUVECs and U-937 cells, a human mononucleated cell line. The enhanced adhesion effect could be prevented by preincubation with a functional blocking antibody against human ICAM-1. These results suggest that LPLs released by activated platelets might enhance interactions of leukocytes with the endothelium through a G\textsubscript{i}, NF-\kappaB-, and possibly Rac-dependent mechanism, thus facilitating wound healing and inflammation processes.

lyso phosphatidic acid; sphingosine 1-phosphate; inflammation; intercellular adhesion molecule-1; nuclear factor-\kappaB; human umbilical cord vein endothelial cells

LYSOPHOSPHATIDIC ACID (LPA) and sphingosine 1-phosphate (S-1-P), low molecular weight LPLs with diverse biological activities (18, 33, 44), are generated by enzymatic cleavage of membrane phospholipids. Recent evidence suggests that autoxin, a previously identified exoenzyme, might be responsible for the generation of these two phospholipids (4). In serum, LPA and S-1-P reach micromolar concentrations and account for much of the cellular growth effects of serum (12, 46, 55).

LPA and S-1-P are enriched in activated platelets, injured cells, and ovarian cancer cells, suggesting potential roles in inflammatory, wound-healing, and tumor-formation events (18, 43, 47, 52, 54, 55).

The cellular signals of LPA and S-1-P are transduced by two subfamilies of G protein-coupled receptors (GPCRs) encoded by endothelial differentiation genes (Edg Rs; see Refs. 2, 5, 21, 34, 45). Human Edg1 (S-1-P1), Edg3 (S-1-P3), Edg5 (S-1-P5), Edg6 (S-1-P4), and Edg8 (S-1-P5) transduce signals for S-1-P. Human Edg2 (LPA1), Edg4 (LPA2), and Edg7 (LPA3) transduce signals for LPA. Multiple signaling pathways are activated by Edg receptors, which have been characterized in heterologous expression systems, including ras-dependent activation of Erk 1/2, increases in intracellular Ca\textsuperscript{2+} concentration, and recruitment of rho GTPase and its downstream targets (2, 5, 21, 34, 45).

Endothelial cells form the inner lining of blood vessels and participate in important physiological processes, including materials exchange, coagulation, and wound healing. Several pathological phenotypes, including atherosclerosis, inflammation, and cancer, are associated with excessive activation or abnormalities of endothelial cells (15). Endothelial cells express at least three types of Edg receptors, including Edg1, Edg2, and Edg3 (26). LPA and S-1-P regulate several endothelial functions, including proliferation, migration, and secretion of proteases (4, 26, 36, 37). It has also been suggested that these lipids might also play a role in the regulation of angiogenesis and blood vessel integrity (4, 28, 29, 31).

The mechanisms by which vascular endothelial cells capture circulating lymphocytes are well documented, and several endothelial cell receptors responsible for these interactions have been described. Among these molecules, intercellular adhesion molecule 1 (ICAM-1; CD54) is one of the most characterized adhesion molecules expressed on endothelial cells. ICAM-1 interacts with LFA-1 and Mac-1, members of the \beta3-integrin family, which are expressed on activated lymphocytes. Interactions among LFA-1, Mac-1, and ICAM-1 are responsible for the firm interaction between leukocytes and endothelial cells and therefore are important for subsequent processes.

ICAM-1 is constitutively expressed in the microvasculature but not in large arteries or veins. However, in atherosclerotic arteries, ICAM-1 expression is elevated in the endothelium covering atherosclerotic plaques (9, 48). Abnormal interactions

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between monocytes and endothelial cells have been suggested to be one of the early events in the generation of atherosclerotic plaque and also are necessary for wound healing (17, 40). Both pretreatment of human saphenous veins with anti-ICAM-1 antibodies and pretreatment of monocytes with anti-\(\beta_2\)-integrins significantly reduced monocyte adhesion to these vessels (7). Those observations suggest that abnormal ICAM-1 expression might be an important indicator of atherosclerosis.

Expression of ICAM-1 on endothelial cells is upregulated by inflammatory cytokines such as interleukin (IL)-1\(\beta\) and tumor necrosis factor (TNF)-\(\alpha\), and also by plasma lipoprotein such as oxidized low-density lipoproteins (LDLs; see Refs. 11, 14, 24, 41). Because LPA and S-1-P stimulate the expression of these inflammatory cytokines from macrophages (27), and LPA is generated along with oxidized LDLs (13, 32, 42), we hypothesized that LPA and S-1-P may affect endothelial cell interactions with leukocytes through modulating the expression of ICAM-1. In this report, we present evidence that LPA and S-1-P enhance ICAM-1 expression in human umbilical cord vein endothelial cells (HUVECs) in a time- and concentration-dependent fashion. Furthermore, by using chemical inhibitors, we show that LPA and S-1-P enhance ICAM-1 expression through a G\(_{i/o}\), nuclear factor (NF)-\(\kappa B\)- and possibly Rac-dependent and Rho-independent mechanism, which is consistent with the signaling pathways activated by LPLs binding to Egd receptors. In addition, the adhesion between U-937 human mononucleated cells and HUVECs is enhanced by LPL treatment. This enhancement is likely because of an increase in ICAM-1 expression in HUVECs, since the enhancement is prevented by pretreatment with functional blocking antibody against human ICAM-1. These results imply that the inflammatory effects of LPL are likely mediated through the enhancement of ICAM-1 expression.

**MATERIALS AND METHODS**

**Reagents and antibodies.** S-1-P was purchased from Biomol (Plymouth, PA). LPA, fatty acid-free BSA, and *Clostridium difficile* toxin B (Toxin B) were purchased from Sigma (St. Louis, MO). Pertussis toxin (PTx) and BAY 11–7082 (BAY) were from CalBiochem (La Jolla, CA). The C3 exoenzyme was purchased from List Biological Laboratory (Campbell, CA).

Human ICAM-1 antibody (clone M19), FITC-conjugated mouse anti-human ICAM-1 monoclonal antibody (clone 6.5B5), and functional blocking anti-human ICAM-1 monoclonal antibody (clone 15.2) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Horseradish peroxidase-conjugated goat anti-mouse IgG was obtained from Boehringer Mannheim (Indianapolis, IN). FBS and M199 were purchased from Hyclone (Logan, UT). RPMI-1640 medium and trypsin were purchased from Gibco-BRL (Grand Island, NY). Endothelial cell growth medium (EGM) was purchased from Endothelial Cell Technologies (Manas-
with double-distilled water, mounted with Fluoromount G (Electron Microscopy Sciences, Washington, PA), and examined using a confocal microscope.

Confocal microscopy. To examine the distribution of ICAM-1, immunostained cells were mounted with Immunomount G. Cells were observed using a laser-scanning confocal microscope (Leica model TCS SP2) with a Leica Mellis-Griot ×63 numerical aperture oil immersion objective, with a pinhole of 1.5 and an electronic zoom of 1.5 or 2. Cy3 was excited using a 543-nm argon/krypton laser and detected with a 550- to 620-nm band-pass filter. Images were manipulated with a Leica TCS SP2 scanner.

Assay for U-937 adhesion to treated endothelium. HUVECs cultured in six-well plates were stimulated for 8 h with 5 μM LPA or S-1-P. After being gently washed three times with M199, U-937 monocytes (1 ml, 5 × 10⁶ cells/ml) were added to the cultures and then incubated at 37°C for 1 h. In the inhibitory experiments, anti-human ICAM-1 functional blocking antibodies (10 μg/ml) or normal mouse IgG was added before the addition of U-937 monocytes. Cultures were then washed by placing 1 ml of M199 gently on the cultures three to five times until no visible suspension of U-937 cells was observed. After being washed, cell cultures were imaged by microscopy with a Kodak digital camera, and the number of monocytes per culture was counted.

Statistical analysis. Significant differences between treatment groups were tested using ANOVA followed by Duncan’s new multiple range test (StatView; Abacus Concept, Berkeley, CA). Each experiment was repeated at least three times. A value of $P < 0.05$ was considered statistically significant.

RESULTS

LPA and S-1-P increase ICAM-1 mRNA in HUVECs. By DNA array analysis, we found that several genes that participate in inflammatory processes are upregulated by both LPA and S-1-P treatments. To confirm these observations, we determined the expression of ICAM-1 mRNA in LPL-treated HUVECs by RT-PCR. As shown in Fig. 1A, middle, and Fig. 2A, middle, ICAM-1 mRNA levels increased in both LPA- and S-1-P-treated samples in a concentration (Fig. 1A)- and time (Fig. 2A)-dependent manner. The expression patterns of GAPDH, which was used as the loading control, did not differ significantly in either LPA- or S-1-P-treated samples (Figs. 1A and 2A).

![Fig. 1. Lysophosphaticid acid (LPA) and sphingosine 1-phosphate (S-1-P) upregulate intercellular adhesion molecule (ICAM)-1 expression in human umbilical vein endothelial cells (HUVECs) in a dose-dependent manner. A: HUVECs were incubated with LPA or S-1-P for 4 h at various concentrations, as indicated. RNAs from treated cells were harvested and subjected to RT-PCR reactions using specific primer sets for human ICAM-1 or GAPDH. The reaction products were separated on 2% agarose gels and photographed. TNF-α was used as the positive control. Similar experiments were repeated three times, and a representative result is shown. Histograms represent quantification of RT-PCR corrected with GAPDH and analyzed by PhosphorImager using ImageQuaNT software. All data are relative multiples of expression compared with untreated cells. B: HUVECs were incubated with LPA (top) and S-1-P (bottom) for 8 h at various concentrations, as indicated. Total cell lysates were isolated, and ICAM-1 protein expression levels were monitored by Western blotting. Similar experiments were repeated three times, and a representative result is shown.](http://ajpcell.physiology.org/)

![Fig. 2. LPA and S-1-P upregulate ICAM-1 expression in HUVECs in a time-dependent manner. A: HUVECs were incubated with LPA (5 μM) or S-1-P (5 μM) for various times, as indicated. RNAs were analyzed as in Fig. 1A. Similar experiments were repeated three times, and a representative result is shown. Histograms represent quantification of RT-PCR corrected with GAPDH analyzed by PhosphorImager using ImageQuaNT software. All data are relative multiples of expression compared with untreated cells. B: HUVECs were incubated with 5 μM LPA (top) or S-1-P (bottom) at various times, as indicated. Total cell lysates were isolated, and ICAM-1 protein expression levels were monitored by Western blotting. Similar experiments were repeated three times, and a representative result is shown.](http://ajpcell.physiology.org/)
shown in Figs. 1A and 2A, top. The maximum stimulatory effects were first observed with treatment at a concentration of 0.5 μM and peaked at 5 μM for both LPA and S-1-P treatments. Significant increases in ICAM-1 mRNA levels were first observed as early as 1 h after the treatments were added, peaked at 4 h, and decreased thereafter. These results confirmed our previous observations from DNA array experiments that both LPA and S-1-P enhance ICAM-1 mRNA expression in HUVECs.

LPA and S-1-P increase ICAM-1 protein expression. Because mRNA levels were increased by LPLs, we further investigated whether the elevated mRNA levels of ICAM-1 were also correlated to protein expression levels. The total ICAM-1 protein levels in LPL-treated cells were detected by Western blotting. After 8 h of treatment, both LPA and S-1-P enhanced total ICAM-1 protein expression in a concentration (Fig. 1B)- and time (Fig. 2B)-dependent manner. Consistent with the RT-PCR results, the enhancement effects of LPA and S-1-P on ICAM-1 expression in HUVECs peaked at a concentration of 5 μM. In the time course experiments, the enhancement effects of both LPA (Fig. 2B, top) and S-1-P (Fig. 2B, bottom) peaked at 8 h after treatment and decreased thereafter.  

Fig. 3. Immunocytochemical analysis of ICAM-1 expression on human endothelial cells. HUVECs were stimulated with medium, LPA (5 μM), or S-1-P (5 μM) for 8 h. ICAM-1 expression staining by anti-ICAM-1 FITC-conjugated monoclonal antibody is shown on left, cell nuclei stained by 4’,6’-diamidino-2-phenylindole (DAPI) are shown in middle, and merged images are given on right. All images were visualized by confocal microscopy.
By confocal microscopy, we also observed a significant enhancement of ICAM-1 expression in LPA- and S-1-P-treated HUVECs (Fig. 3). By FACscan analysis, which detects the antigenic activities of proteins on cell surfaces, we observed a significant increase in cell-surface ICAM-1 levels in LPL-treated HUVECs (Fig. 4). These results indicated that the enhancement effects of LPLs on ICAM-1 also occur at the surface protein level, which might affect interactions between leukocytes and the endothelium during wound-healing and inflammatory processes.

In the time course experiments, we showed that both LPA (Fig. 5A) and S-1-P (Fig. 5B) enhancement of ICAM-1 protein expression on the surface of HUVECs also occurred in a time-dependent manner. LPL-enhanced HUVEC surface ICAM-1 expression was first observed at 4 h postligand treatment and peaked at 8 h postligand treatment. After 16 h, the effects of LPL on ICAM-1 expression levels of HUVECs returned to basal levels. These results indicate that both LPA and S-1-P are potent stimulators of ICAM-1 expression on HUVECs.

PTx, PDTC, and BAY, but not exoC3, blocked LPA and S-1-P effects on ICAM-1 expression. Because LPA and S-1-P are ligands for Edg receptors, and at least three different Edg receptors are expressed on HUVECs (26, 30), we further investigated if the effects of LPLs on ICAM-1 expression are mediated through these receptors. We addressed this question by using chemical inhibitors known to impede signaling processes of activated Edg receptors. PTx, a specific inhibitor for G_{i/o} proteins that has been shown to inhibit G_{i/o}-dependent LPL...

Fig. 4. LPA and S-1-P increase ICAM-1 cell surface expression on HUVECs. HUVECs were treated with 5 μM LPA (top) or S-1-P (bottom) for 8 h. Treated cells were dissociated by trypsinization. The dissociated cells were incubated with FITC-labeled mouse anti-human ICAM-1 monoclonal antibody for 30 min and analyzed by FACscan. Fluorescence label 1 (FL1) represents intensities of FITC labeling.

Fig. 5. LPA and S-1-P increase ICAM-1 protein levels expressed on HUVEC surfaces in a time-dependent manner. HUVECs were treated with 5 μM LPA (A) or S-1-P (B) at various times as indicated, and treated cells were dissociated by trypsinization. The dissociated cells were incubated with FITC-labeled mouse anti-human ICAM-1 monoclonal antibody for 30 min and analyzed by FACscan.
effects in different cell systems, including HUVECs (26, 30), was used in our assay. Pretreatment with 15 ng/ml PTx for 16 h had no significant effect on the basal level of ICAM-1 expression in HUVECs (Fig. 8, A and B, top left). However, the stimulatory effects of 5 μM LPA or S-1-P on ICAM-1 expression in HUVECs were totally suppressed by the toxin treatment (Fig. 6, A and B, top left). On the other hand, pretreatment with 200 μM PDTC, an inhibitor of the NF-κB-dependent pathway (8), had no effects on basal ICAM-1 expression but significantly inhibited LPL-enhanced ICAM-1 expression on HUVECs (Fig. 6, A and B, bottom left). Moreover, pretreatment with 10 μM BAY, another specific inhibitor of the NF-κB pathway that selectively inhibits phosphorylation of inhibitory factor-κB-α, had no effects on basal ICAM-1 expression but significantly inhibited LPL-enhanced ICAM-1 expression on HUVECs (Fig. 6, A and B, bottom right). Pretreatment with 1 μg/ml exoC3, a specific inhibitor of Rho (26, 30), had no effects on either basal or LPL-enhanced ICAM-1 expressions on HUVECs (Fig. 6, A and B, top right). These results suggest that the enhancement effects of LPL on ICAM-1 expression are G_{12/13}- and NF-κB-dependent, but independent of the function of Rho. Similar results were also observed at the RNA level, as detected by RT-PCR (Fig. 7).

LPA and S-1-P enhanced U-937 cell adherence to treated HUVECs. Because ICAM-1 expression on endothelial cells is responsible for their firm adhesion to leukocytes, we tested the hypothesis that LPL treatments enhance endothelial cell and monocyte adhesion by an in vitro adhesion assay. Five micromolars of LPA or S-1-P treatment on endothelial cells enhanced cell adhesion by the human monocytic cell line U-937 (Fig. 8). Furthermore, preincubation with the functional blocking antibody against human ICAM-1 significantly inhibited the enhancement effect of LPL on U-937 cell adhesion. On the contrary, a nonspecific antibody had no effects in these assays (Fig. 8, A and B).

Fig. 6. LPA and S-1-P increases in ICAM-1 are mediated through a G_{12/13} and nuclear factor (NF)-κB-dependent but Rho-independent mechanism. HUVECs were treated with control media alone, 15 ng/ml pertussis toxin (PTx), 1 μg/ml C3 exotoxin (exoC3), 200 μM PDTC, or 10 μM BAY 11–7082 (BAY) for 1 h. Treated cells were then treated with 5 μM LPA (A) or S-1-P (B) for 8 h. The treated cells were dissociated by trypsinization, incubated with FITC-labeled mouse anti-human ICAM-1 monoclonal antibody for 30 min, and analyzed by FACscan.

Fig. 7. Effects of a Gi inhibitor (PTX), Rho inhibitor (exoC3), and NF-κB inhibitor (PDTC) on lysophospholipid (LPL)-stimulated ICAM-1 mRNA expression on HUVECs. Top: histograms representing quantification by PhosphorImager of LPL-stimulated ICAM-1 mRNA expression using ImageQuant software (*P < 0.05). NS, no significance was observed. All data are relative multiples of expression compared with untreated cells. Similar experiments were repeated three times, and a representative result is shown.
Toxin B blocked the enhancement effects of LPA and S-1-P on ICAM-1 expression. Rac has been shown to play important roles in regulating ICAM-1 expression in human endothelial cells (1). In addition, LPA has also been shown to be able to activate Rac in neuroblastoma cells (49). Therefore, we intended to determine whether Rac also participate in the enhancement effects of LPL on ICAM-1 expression on HUVEC. Toxin B, an inhibitor of the small GTPases Rho and Rac (16), was used in our assay. Pretreatment with 0.5 nM Toxin B for 2 h had no significant effect on the basal level of ICAM-1 protein expression in HUVECs, as detected by FACS. However, the stimulatory effects of 5 μM LPA or S-1-P on ICAM-1 expression in HUVECs were partially suppressed by Toxin B treatment (Fig. 9). Rho has no significant effect in the stimulatory property of LPA and S-1-P on ICAM-1 expression, suggesting that Rac might be involved in the enhancement effects of these LPLs on ICAM-1 expression on HUVEC.

DISCUSSION

The results of this report show evidence that LPLs generated from activated platelets or other cell regions might enhance ICAM-1 expression by surrounding endothelial cells. Previous work suggested that LPA induces the expression of ICAM-1 on endothelial cells (35). In this report, we show that S-1-P, an LPL that binds receptors belonging to the Edg family, has a similar effect on ICAM-1 expression on HUVECs. The enhancement effects might be mediated through a G_{i/o}-, NF-κB-, and Rac-dependent mechanism, which is consistent with the fact that Edg receptors activate these pathways. Furthermore, the time- and concentration-dependent activation of ICAM-1 by LPLs is also consistent with a receptor-mediated mechanism. It has been shown that both LPA and S-1-P receptors are expressed on HUVEC (26, 30). Therefore, the effects of LPA and S-1-P on these cells are expected to be mediated through these receptors.

In our previous study, we showed that LPLs are wound-healing factors in the endothelium (26). Another report also suggested that LPA facilitates wound healing in in vivo systems (3). Because ICAM-1 is an important regulator of interactions between leukocytes and endothelial cells, this suggests that the facilitating effects of LPLs on wound healing might partially be mediated through enhancement of ICAM-1 expression. From DNA array analysis and also by results reported by others, we know that LPA also enhances the expression of endothelial-leukocyte adhesion molecule (ELAM) on endothelial cells (28, 29). ELAM is responsible for the initial rolling effect during interactions between leukocytes and endothelial cells. These results suggest that the effects of LPLs on leukocyte-endothelial cell interactions during wound healing occur at multiple levels. This is consistent with the fact that, when tissue encounters a mechanical wound, LPLs are released from activated platelets, which might act as one of the initiating signals for the subsequent wound-healing processes.

With confocal microscopy, we also observed that LPL-enhanced ICAM-1 expression formed significant capping on HUVECs. The aggregation of cell-surface molecules upon antibody cross-linking or association with other cell types has been described in several papers (20, 22). The capping effects...
In addition, binding of LPA to LPA1 also activates Rac (49) in regulating ICAM-1 expression in human endothelial cells (1). Currently, it is unclear how physiological roles of this capping by adhesion molecules are mediated through a Rac-dependent mechanism. HUVECs were treated with control media alone, 0.5 nM Clostridium difficile toxin B for 16 h. Treated cells were then treated with 5 μM LPA (A) or S-1-P (B) for 8 h. Treated cells were dissociated by trypsinization, incubated with FITC-labeled mouse anti-human ICAM-1 monoclonal antibody for 30 min, and analyzed by FACscan.

Fig. 9. LPA and S-1-P increases in ICAM-1 are mediated through a Rac-dependent mechanism. HUVECs were treated with control media alone, 0.5 nM Clostridium difficile toxin B for 16 h. Treated cells were then treated with 5 μM LPA (A) or S-1-P (B) for 8 h. Treated cells were dissociated by trypsinization, incubated with FITC-labeled mouse anti-human ICAM-1 monoclonal antibody for 30 min, and analyzed by FACscan.

are likely because of surface-expressed ICAM-1 associated with the intracellular cytoskeletal structure. However, the physiological roles of this capping by adhesion molecules is currently unclear.

Activation of Rac has been shown to play important roles in regulating ICAM-1 expression in human endothelial cells (1). In addition, binding of LPA to LPA1 also activates Rac (49). In this study, we showed that the enhancement effects of LPA and S-1-P on ICAM-1 expression in HUVECs could be partially blocked by Toxin B, an inhibitor of Rac. These results suggest that the effects of LPLs on ICAM-1 in HUVECs are at least partially mediated through Rac, which is consistent with the previous reports. By DNA array analysis, we also observed that LPLs enhanced both IL-1β and TNF-α expression in macrophages (27). Both IL-1β and TNF-α are cytokines that regulate the blood vessel environment, specifically endothelial cell physiology. Expression of ICAM-1 on endothelial cells is also upregulated by these cytokines (11, 14). IL-1β and TNF-α also participate in a self-augmentation induction mechanism (10), which allows a positive-feedback mechanism to amplify the effects of these cytokines within a local milieu. Furthermore, TNF-α directly and potently stimulates sphingosine kinase activity in HUVECs, inducing the generation of S-1-P, which further enhances endothelial and macrophage functions (53). These results suggest that platelet-derived LPLs might facilitate wound-healing processes through complex cell-cell interactions in the local environment.

It has been reported that concentrations of LPA and S-1-P in serum can reach micromolar levels (19). Therefore, it is likely that, at the wounded area, local concentration of LPLs may easily reach micromolar concentrations. The high concentration of LPLs at these wounded areas might generate a gradient of ICAM-1 expression, which is expected to peak at the spot of platelet activation. This might result in maximized leukocyte-endothelial interaction at these hot spots, thereby facilitating the wound-healing process.

Hypercholesterolemia is a major risk factor for atherosclerosis. Delivery of cholesterol to HUVECs results in an increase in ICAM-1 levels (56). Upregulation of vascular cell adhesion molecule-1, ICAM-1, and E-selectin in endothelial cells by inflammatory cytokines such as vascular endothelial growth factor has also been implicated in facilitating the formation and progression of atherosclerotic plaque (6, 25). Abnormal expression of adhesion molecules on endothelial cells and the large amount of trans-endothelial macrophage accumulation are two of the early events in the process of atherosclerosis generation. As mentioned earlier, ICAM-1 plays a crucial role in leukocyte-endothelial cell interactions. Previous studies also indicated that ICAM-1 is an important molecule involved in atherosclerosis (51). These results strongly suggest that LPLs may play multiple roles in the process of atherosclerosis through regulating the expression of ICAM-1 and inflammatory cytokines.

Several groups have reported the effects of LPA and S-1-P on leukocyte interactions with the endothelium. In one study, S-1-P inhibited neutrophil-endothelial cell interactions and subsequent neutrophil invasion (23), whereas in another report (39), LPA enhanced HL-60 cell adherence to human aortic endothelial cells. Apparent diversity of the effects of LPA and S-1-P on leukocyte-endothelial cell interaction may be attributed to the use of different leukocytes and endothelial cell types. In our report, we clearly showed that both LPA and S-1-P are potent enhancers of U-937 cell adhesion to HUVECs, and this is likely because of the enhancement of ICAM-1 expression. Our results suggest that platelet-derived LPLs enhanced leukocyte-endothelial cell interactions, consistent with the fact that they might be important regulators of the wound-healing process.

In summary, our results clearly indicate that LPLs increase ICAM-1 mRNA and protein levels in a time- and concentration-dependent manner. This induction is inhibited by specific inhibitors for NF-κB, Gi/o, and Rac. Furthermore, the enhancement effects of LPLs on ICAM-1 expression on HUVECs are responsible for augmentation of the adherence of U-937 cells to treated endothelium. Our results suggest that LPLs might be important physiological regulators of interactions between endothelial cells and mononuclear phagocytes. Therefore, these lipids might play an important role in the regulation of wound healing and possibly the generation of atherosclerosis.

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LYSOPHOSPHOLIPIDS ENHANCE ICAM-1 EXPRESSION

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