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Endothelial Lipase Modulates Monocyte Adhesion to the Vessel Wall

A POTENTIAL ROLE IN INFLAMMATION*

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Endothelial lipase (EL), a new member of the lipoprotein lipase gene family, plays a central role in high density lipoprotein metabolism. Previous studies indicated that EL is expressed in endothelial cells, macrophages, and smooth muscle cells in atherosclerotic lesions in human coronary arteries. However, the functional role of EL in the local vessel wall remains obscure. In this study, we evaluated the ability of EL to modulate monocyte adhesion to the endothelial cell surface. EL mRNA and protein levels were markedly increased in tissues of the mouse model of inflammation induced by lipopolysaccharide injection. Adhesion assays *in vitro* revealed that overexpression of EL in COS7 or Pro5 cells enhanced monocyte bindings to the EL-expression cells. Heparin or heparinase treatment inhibited EL-mediated increases of monocyte adhesion in a dose-dependent manner. Moreover, *ex vivo* adhesion assays revealed that the number of adherent monocytes on aortic strips was significantly increased in EL transgenic mice and decreased in EL knock-out mice as compared with wild-type mice. These results suggest that EL on the endothelial cell surface can promote monocyte adhesion to the vascular endothelium through the interaction with heparan sulfate proteoglycans. Thus, the up-regulation of EL by inflammatory stimuli may be involved in the progression of inflammation.

The lipoprotein lipase gene family plays a crucial role in the lipid metabolism for the regulation of circulating lipoprotein levels and affects the process of atherosclerotic vascular diseases (1–4). The lipoprotein lipase gene family includes lipoprotein lipase (LPL)[1] and hepatic lipase (HL), both of which are synthesized and secreted by non-endothelial cells. We and other groups have reported a new member of the lipase gene family, endothelial lipase (EL). EL is mainly synthesized and secreted by vascular endothelial cells (5, 6). Previous studies have demonstrated that EL showed preferential substrate specificity for high density lipoprotein (HDL) (7). EL knock-out mice (*LIPG*−/−) showed an elevated plasma HDL-cholesterol (HDL-C) level. In contrast, human EL transgenic mice (h*LIPG*Tg) or overexpression of human EL in mice by adenovirus vectors showed decreased plasma HDL-C and apoA-I levels (6, 8). These data suggest that EL is a determinant of HDL levels in mice.

In addition to their functional role in lipoprotein metabolism at the endothelial cell surface, lipases possess non-enzymatic functions in the vascular wall. LPL within the vessel wall increases lipoprotein retention within the subendothelial cell matrix (9, 10) and in the aortic segment (11, 12). LPL can also enhance monocyte adhesion to endothelial cells (13, 14). The local expression of LPL in the vascular wall has been implicated in the accumulation of lipoproteins and the acceleration of atherosclerosis progression through this bridging function (15). Macrophage-derived LPL in the vessel wall is known to act as a proatherogenic molecule, determining susceptibility to atherosclerotic lesion formation (16, 17).

EL expression is regulated under various conditions. Inflammatory cytokines increased EL expression and enzymatic activity in cultured endothelial cells (18, 19). The EL expression is also detected in macrophages and smooth muscle cells within the atheromatous plaque of human coronary arteries (20). Interaction between the vessel wall and circulating monocytes is central for the initiation of atherosclerosis. Enhanced adhesion of monocytes to the vascular endothelium is believed to represent one of the earliest events in atherogenesis. Because of its expression in vessel wall and up-regulation by inflammatory cytokines, EL is likely to modulate the development of the atherosclerotic process. The aim of this study was to examine whether EL expression is regulated under inflammatory conditions *in vivo* and to clarify whether EL fulfills a "bridging function" between endothelial cells and monocyte/macrophages. In this study, we demonstrated that EL enhances human monocyte adhesion to the vascular endothelium and that EL expression is strongly up-regulated in the mouse systemic inflammation model. Our findings would further expand the functional role of EL in the vascular wall and provide new insights into endothelium-lipoprotein/blood cell interaction.

MATERIALS AND METHODS

Cell Culture—THP-1, U-937, and COS7 cells were purchased from the American Type Culture Collection (Manassas, VA) and cultured according to the manufacture's recommendations. COS7 cells that stably overexpress c-Myc-tagged human EL were generated as described

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[1] The abbreviations used are: LPL, lipoprotein lipase; EL, endothelial lipase; HDL, high density lipoprotein; HDL-C, HDL-cholesterol; HL, hepatic lipase; hEL, human EL; h*LIPG*Tg, hEL transgenic mice; hLPL, human LPL; HSPG, heparan sulfate proteoglycan; *LIPG*−/−, EL knock-out mice; LPS, lipopolysaccharide; WT, wild-type.

previously (5). To generate COS7 cells that stably overexpress human LPL with an epitope tag (c-Myc) at the carboxyl terminus, reverse transcription PCR was performed with the RETROscript first strand synthesis kit (Ambion, Austin, TX), using the primers 5′-GGGAATTC-CCCACCATGGAGAGCAAAGCCCTGCTCC-3′ (forward) and 5′-GGTC-TAGACTCGAGTCACAGATCTTCTTCGGAGATAAGCTTCTGTTCGC-CAGACTTCTTCAGAGACTTGTC-3′ (reverse). The c-Myc-tagged LPL cDNA was cloned into the EcoRI-XhoI site of the pcDNA3.1 vector (Invitrogen) and sequenced by the dideoxy method. The c-*myc*-LPL expression construct was transfected into COS7 cells with Lipofectamine (Invitrogen). The cells were selected in the presence of 500 μg/ml G418. Levels of EL and LPL protein were determined by Western blotting using anti-c-Myc monoclonal antibodies (MBL, Nagoya, Japan). A mouse yolk sac endothelial cell line, Pro5 (21), was transfected with human EL cDNA using the Lipofectamine reagent and then selected in the presence of 500 μg/ml G418. The EL expression in the transfectants was determined by Western blotting using anti-human EL monoclonal antibodies (22). Mock (pcDNA3.1 vector)-transfected cells were used as a control.

Mouse Model of Endotoxemia—Male C57BL/6 mice (~20–25 g) were obtained from the Japan Charles River (Osaka, Japan). h*LIPG*Tg and *LIPG*−/− mice were described previously (8). A mouse model of lipopolysaccharide (LPS)-induced endotoxin shock was generated as described previously (23, 24). Briefly, mice were injected with LPS (50 mg/kg, intraperitoneal) from *Escherichia coli*, serotype 055:B5 (Sigma). Control animals were injected with normal saline (vehicle). Twelve or twenty-four hours later, mice were sacrificed with an overdose of pentobarbital, and tissues were excised for mRNA or protein extraction. In a set of experiments for lipid analysis, mice were injected with heparin (100 units/kg; Sigma). Thirty minutes later, whole plasma was obtained by cardiac puncture. Post-heparin plasma was obtained after centrifugation at 3000 × *g* for 10 min at 4 °C. Concentrations of total cholesterol, triglyceride, HDL-C, and phospholipid in the mouse plasma were determined using commercial kits (WAKO, Tokyo, Japan) (8). All animal experiments were conducted according to the Guidelines for Animal Experiments at Kobe University Graduate School of Medicine.

Northern Blot Analysis, RNase Protection Assay, and Immunoblot Analysis—Total RNA was extracted from mouse tissues and cultured cells using the Isogen reagent (Nippon Gene, Tokyo). Northern blot was performed using a ^{32}P-labeled mouse EL cDNA probe as described previously (5). For the RNase protection assay, cDNA fragments of human and mouse EL were obtained by reverse transcription PCR using the primers 5′-AGCTCTTGCTGCCTCTCTTG-3′ and 5′-TGA-CAGCCTTCTACACAGGG-3′ for human EL and 5′-TGATGGTTGCTA-CAGTGCTC-3′ and 5′-ACTACTAAAGGGTGTCTCGG-3′ for mouse EL. The cDNA fragments were cloned into the pCR II vector (Invitrogen) and linearized with BamHI. [^{32}P]UTP-labeled antisense riboprobe was synthesized with T7 RNA polymerase, and RNase protection assays were performed as described (25). Protection of human EL transcripts resulted in a labeled fragment of 203 nucleotides, and protection of mouse EL transcripts resulted in a labeled fragment of 144 nucleotides. Expression of cyclophilin was used for the housekeeping gene. Protection of cyclophilin transcripts resulted in a labeled fragment of 103 nucleotides. For immunoblot analysis, mouse tissues were homogenized in lysis buffer (10 mmol/liter Tris/HCl (pH 7.4), 150 mmol/liter NaCl, 2 mmol/liter $CaCl_2$, 1% Nonidet P-40, 1% Triton X-100, 1 mmol/liter phenylmethylsulfonyl fluoride, 40 units/ml aprotinin, and 15 g/ml leupeptin). Western blotting was performed using anti-EL polyclonal antibodies (26).

In Vitro Monocyte Adhesion Assay—COS7 or Pro5 cells were propagated on 6-well culture plates to form confluent monolayers. For monocyte labeling, THP-1 or U-937 cells were suspended in phosphate-buffered saline (1×10^6/ml) containing 1 μM calcein-AM (Molecular Probes, Eugene, OR) and incubated for 15 min at 37 °C. Labeled THP-1 cells were washed two times with phosphate-buffered saline and suspended in Hanks' buffered salt solution. THP-1 or U-937 cells (5×10^5/ml) were then added to monolayers of COS7 or Pro5 and incubated for 60 min with gentle rotation. Unbound cells were removed by gently washing with Hanks' buffered salt solution, and the number of binding monocytes was counted under fluorescent microscopy. In some experiments COS7 cells were incubated with monocytes in the presence of heparin (1–5 units/ml) to remove EL bound to their surfaces. To disintegrate cell surface heparan sulfate proteoglycans (HSPGs), COS7 and/or monocytes were treated with 1 unit/ml heparinase I for 30 min at 37 °C prior to the adhesion assay.

Ex Vivo Monocyte Adhesion Assay—Thoracic aorta was isolated from h*LIPG*Tg, *LIPG*−/−, and age-matched C57BL/6 (WT) mice. The surrounding tissue was gently cleaned. The aortas were opened longitudinally and fixed with fine needles on a slide glass. The *ex vivo* adhesion assay was performed as described previously (27). Cell suspension of fluorescein-labeled THP-1 cells (5×10^5/segment) was added on the aortic strip and incubated for 20 min at 37 °C. After non-adherent cells were gently washed with phosphate-buffered saline, the number of adherent cells was counted under fluorescence microscopy.

Statistical Analysis—Results are expressed as mean ± S.E. for the indicated number of experiments. The significance of variability among the experimental group means was determined by one-way analysis of variance followed by Bonferroni's test for samples. The level of statistical significance was set at $p < 0.05$.

RESULTS

EL Expression Is Up-regulated during Endotoxemia—To examine whether EL expression is regulated *in vivo* by inflammation, a model of LPS-induced endotoxin shock was generated in C57BL/6 mice. EL mRNA expressions in mouse tissues were analyzed by Northern blotting. As shown in Fig 1*A*, EL mRNA levels were markedly up-regulated in tissues from LPS-injected mice compared with those from vehicle-treated mice. At 12 h after LPS injection, EL mRNA expression was significantly increased by 2.9-fold in the lung, 3.3-fold in the heart, 2.8-fold in the kidney, 2.1-fold in the liver, 2.5-fold in the spleen, and 3.3-fold in the aorta. We next investigated whether EL protein levels in these tissues are changed by the LPS treatment. At 24 h after LPS injection, the EL protein level in the aorta from LPS-treated mice was increased by 5-fold compared with that of vehicle-treated mice (Fig 1*B*). The increase of EL was detected after 12 h of LPS injection and peaked at 5-fold 24 h after injection (data not shown). Also, the LPS treatment significantly increased EL expression in the lung, heart, kidney, liver, and spleen by 2.1-, 2.6-, 4.2-, 1.5-, and 1.4-fold, respectively (Fig 1*B*). The secretion of EL into blood was analyzed by Western blotting using post-heparin plasma. Fig 1*C* showed that LPS administration increased the EL protein level in post-heparin plasma. Plasma EL level increased in a time-dependent manner, and after 24 h plasma levels of EL had increased 2-fold compared with those of the control groups (Fig 1*C*).

To examine whether the human EL promoter is regulated by LPS, h*LIPG*Tg mice in which human EL expression is driven by the human endogenous EL promoter were injected with LPS, and then mRNA levels of human EL, mouse EL, and cyclophilin were simultaneously determined by the RNase protection assay. As shown in Fig 1*D*, mouse EL levels in the tissues increased more in the LPS-injected mice than in the vehicle-treated mice. Also, the assay revealed a concomitant increase in human EL mRNA levels by LPS injection, suggesting that the human EL promoter is under the control of LPS *in vivo*.

To examine changes of lipid profiles by LPS treatment in our model, plasma levels of cholesterol, triglyceride, and phospholipid were measured (Table I). At 24 h after LPS treatment, the plasma total cholesterol level was increased by 31% ($p < 0.05$, $n = 12$) in WT mice. The triglyceride and phospholipid levels were also significantly increased by the LPS treatment by 321 and 25%, respectively. In contrast, the HDL-C level of WT mice was modestly but significantly decreased by 11% in response to LPS ($p < 0.05$, $n = 12$). In *LIPG*−/− mice the HDL-C level was not changed by the LPS treatment ($n = 12$). LPS administration increased plasma total cholesterol, triglyceride, and phospholipid levels in *LIPG*−/− mice.

EL Acts as a Bridging Molecule—To explore whether EL modulates interaction between the monocyte and the vessel wall, we examined the effects of EL overexpression on monocyte adhesion. Fibroblast cell lines, including the COS7 cell, did not express endogenous EL (data not shown). Therefore, we used COS7 cells constitutively overexpressing human EL (hEL-COS7) for cell adhesion assays utilizing two types of

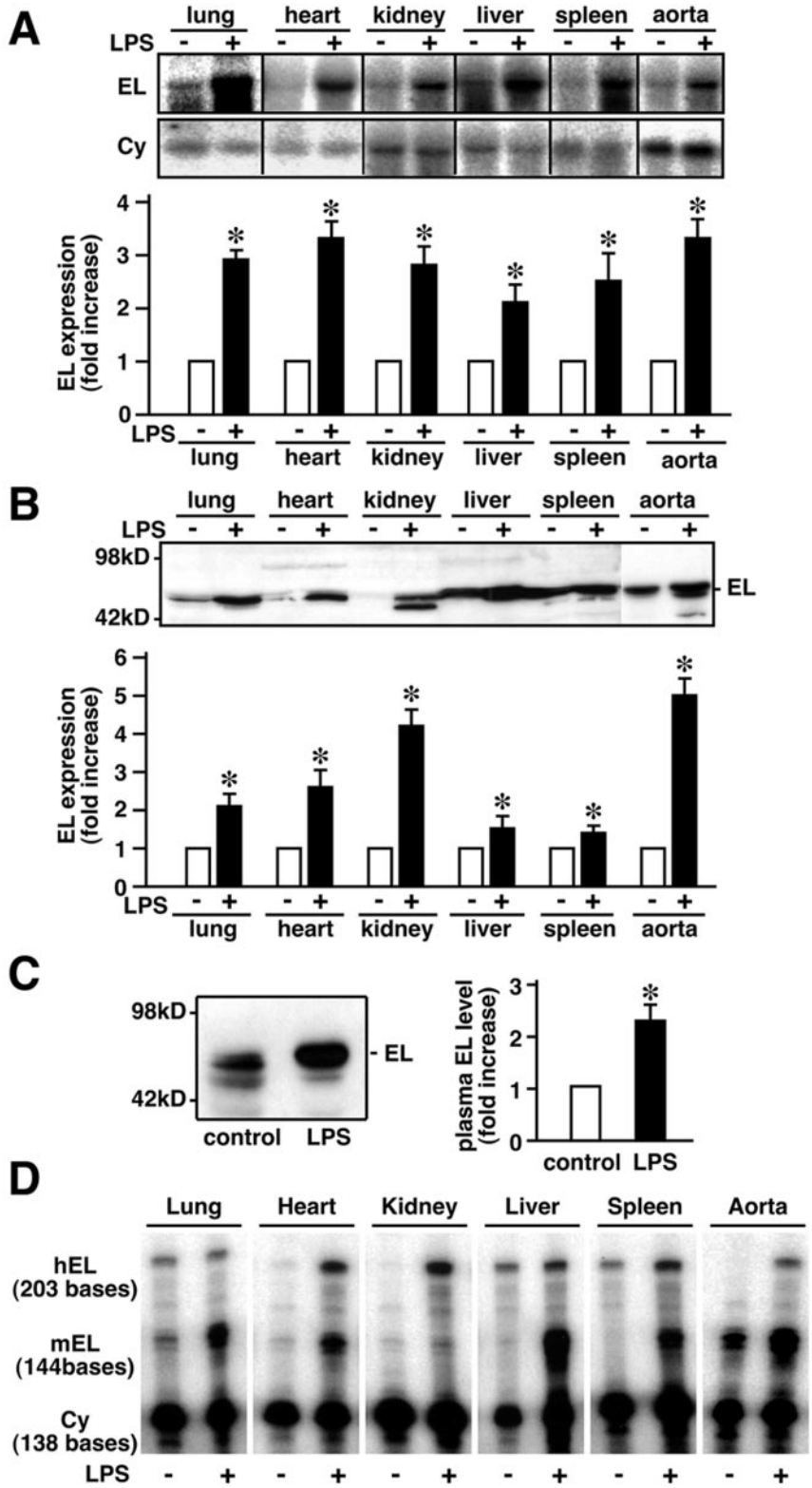

FIG. 1. **LPS up-regulates EL expression *in vivo*.** *A–C*, male C57BL/6 mice were treated with LPS (50 mg/kg, intraperitoneal). Mouse tissues were harvested after the indicated hours for EL mRNA analysis by Northern blotting (*A*). EL protein levels in mouse tissues (*B*) and post-heparin plasma (*C*) were analyzed by Western blotting using anti-EL polyclonal antibodies. LPS administration increases EL levels in various tissues and post-heparin plasma. *D*, RNase protection assay revealed that hEL expression and mouse EL (*mEL*) expression were increased by the treatment of LPS in EL transgenic mice. Values represent mean ± S.E. * $p < 0.05$ *versus* control. *Cy*, cyclophilin.

human monocyte/macrophage cell lines, THP-1 and U-937. The vector-transfected COS7 cell (mock-COS7) was used as a control. Confluent monolayers of the hEL-COS7 or mock-COS7 cells were incubated with THP-1 or U-937 cells, and the number of adherent monocytes was counted. As shown in Fig. 2, monocytes bound to the hEL-COS7 monolayer were significantly increased compared with those in mock-COS7; the number of THP-1 cells that bound to the hEL-COS7 and mock-COS7 monolayers was 78.9 ± 5.8 and 51.0 ± 2.6 cells/field, respectively ($p < 0.01$). Similar results were obtained with U-937 cells; the number of U-937 cells that bound to the hEL-COS7 and mock-COS7 monolayers was 47.4 ± 3.9 and 27.2 ± 3.4 cells/field, respectively ($p < 0.01$). These results indicate that EL overexpression can promote monocyte binding to the cell surface. It has been reported that LPL also promotes monocyte adhesion to the endothelial cell surface. To compare the abilities of these lipases in regard to monocyte adhesion, we generated COS7 cells stably overexpressing human LPL (hLPL-COS7) and employed the cell adhesion assay. The expression of EL and LPL in the transfectant was determined by

TABLE I
Lipid profile of LPS-treated mice

WT C57BL/6 and *LIPG*−/− EL knockout mice were treated with LPS or normal saline (vehicle). Concentrations of total cholesterol (Chol), triglyceride (TG), HDL-C, and phospholipid (PL) were determined by biochemical assays. Values represent mean ± S.E. (n = 12 in each group).

Mouse	Treatment	Chol	TG	HDL-C	PL
		mg/dl	*mg/dl*	*mg/dl*	*mg/dl*
WT	Vehicle	85.5 ± 5.5	38.2 ± 5.5	54.5 ± 2.1	156.3 ± 14.3
WT	LPS	112.1 ± 4.6[a]	163.6 ± 16.2[a]	48.2 ± 2.0[a]	210.0 ± 8.3[a]
LIPG−/−	Vehicle	130.9 ± 6.8[b]	58.0 ± 3.9[b]	74.4 ± 3.7[b]	235.7 ± 23.6[b]
LIPG−/−	LPS	191.8 ± 6.4[a]	480.4 ± 97.3[a]	71.9 ± 2.3	407.3 ± 14.2[a]

[a] $p < 0.05$ *versus* vehicle-treated mice.
[b] $p < 0.05$ *versus* WT.

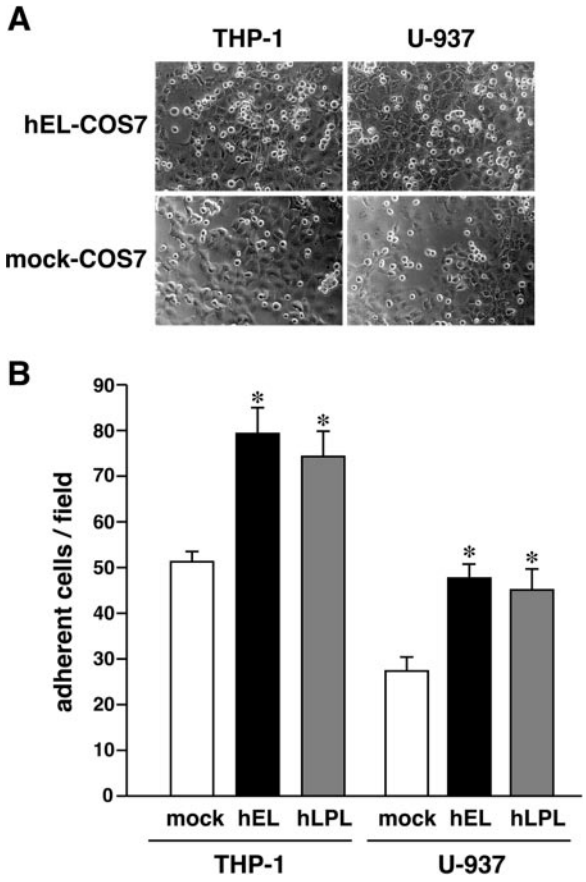

FIG. 2. **EL increases monocyte binding to COS7 cells.** Confluent monolayers of COS7 cells constitutively expressing hEL or hLPL were incubated with 5 × 10^5/ml THP-1 or U-937 cells. Unbound cells were removed by gentle washes, and the number of binding monocytes was counted under microscopy. *A*, representative images of the adhesion assay. Control was mock-COS7, representing monocyte binding to vector-transfected COS7 cells. *B*, graph representing the average number of adherent cells on confluent monolayers at eight random ×200 fields, showing that the number of adherent cells was significantly higher in hEL-COS7 and hLPL-COS7 cells compared with the number in mock-COS7 cells (*mock*). Values represent mean ± S.E. *, $p < 0.01$ *versus* mock.

Western blot using an anti-c-Myc antibody. As depicted in Fig 2*B*, the number of monocytes bound to hLPL-COS7 was significantly higher than that bound to mock-COS7, *i.e.* by 1.5-fold ($p < 0.01$). When the protein level was standardized using the c-Myc antibody, there was no significant difference in the number of adherent THP-1 or U937 cells between hEL-COS7 and hLPL-COS7 monolayers (Fig 2*B*). Thus, it was suggested that these lipases have nearly equivalent abilities in regard to monocyte binding.

It has been demonstrated that other lipase members, including LPL and HL, require HSPGs to localize distribution to the luminal surface of the blood vessel wall. Therefore, we next examined the effect of heparin and heparinase on the monocyte adhesion to EL-transfected cells. The confluent monolayers of transfectants were incubated with THP-1 in the presence of heparin or vehicle. As shown Fig 3*A*, treatment with 5 units/ml heparin did not have any effect on monocyte binding to mock-COS7 cells. However, heparin treatment completely abolished the monocyte binding to hEL-COS7 cells. The inhibitory effect of heparin was dose-dependent, and significant inhibition was achieved at a concentration as low as 1 unit/ml (Fig 3*B*). Identical results were obtained with another monocyte/macrophage cell line, U-937 (Fig. 3*A*, *right*).

To confirm the involvement of HSPGs, we examined whether degradation of HSPGs by heparinase treatment affects monocyte binding to the EL-expressing cells. As depicted in Fig 4*A*, pretreatment of hEL-COS7 cells with 1 unit/ml heparinase I completely abolished monocyte binding. In contrast, heparinase did not affect the monocyte binding to mock-COS7 cells. These results indicate that increased cell binding between monocytes and EL-expressing cells is mediated by HSPGs. To clarify whether EL-mediated monocyte binding require HSPGs on the monocyte, THP-1 cells were pretreated with 1 unit/ml heparinase I for 30 min prior to the assay. Whereas heparinase I did not show significant effects on the THP-1 binding to mock-COS7 cells, treatment of THP-1 cells with heparinase I blocked the monocyte adhesion to hEL-COS7 (Fig 4*B*). Pretreatment of both COS7-monolayers and THP-1 cells with heparinase I did not show an additional inhibitory effect on the cell binding (Fig 4*B*). Thus, EL-mediated cell binding between monocytes and EL-expressing cells requires cell surface HSPGs on both the monolayer and the monocytes.

To verify the EL effect on cell adhesion in the other cell type, Pro5 cells were transfected with human EL cDNA. This cell line is considered to be an embryonic endothelial progenitor and expresses EL. The cells were grown to confluent monolayers, and the cell adhesion assays were performed using THP-1 cells. As is shown in Fig. 5, hEL overexpression resulted in a significant 52% increase in monocyte adhesion to the cell. These findings suggest that EL levels may modulate the binding activity of monocytes to the endothelial cell surface.

Up-regulation of EL in the Vessel Wall Promotes Monocyte Adhesion—To address whether EL regulates monocyte binding to the vessel wall, we evaluated monocyte binding to the blood vessel from h*LIPG*Tg mice and *LIPG*−/− mice by an *ex vivo* adhesion assay. h*LIPG*Tg mice were shown to overexpress human EL on the endothelial cell surface of thoracic aorta. The thoracic aortas were isolated from h*LIPG*Tg, *LIPG*−/−, or WT mice and incubated with fluorescein-labeled THP-1 cells. Unbound cells were gently washed, and the number of adherent cells was counted under fluorescent microscopy. The number of THP-1 cells that bound to the aortic strips from h*LIPG*Tg mice was increased 68% compared with that from WT mice. In contrast, the number of THP-1 cells that bound to the aortic strip from *LIPG*−/− mice was decreased compared with that in WT mice (Fig. 6).

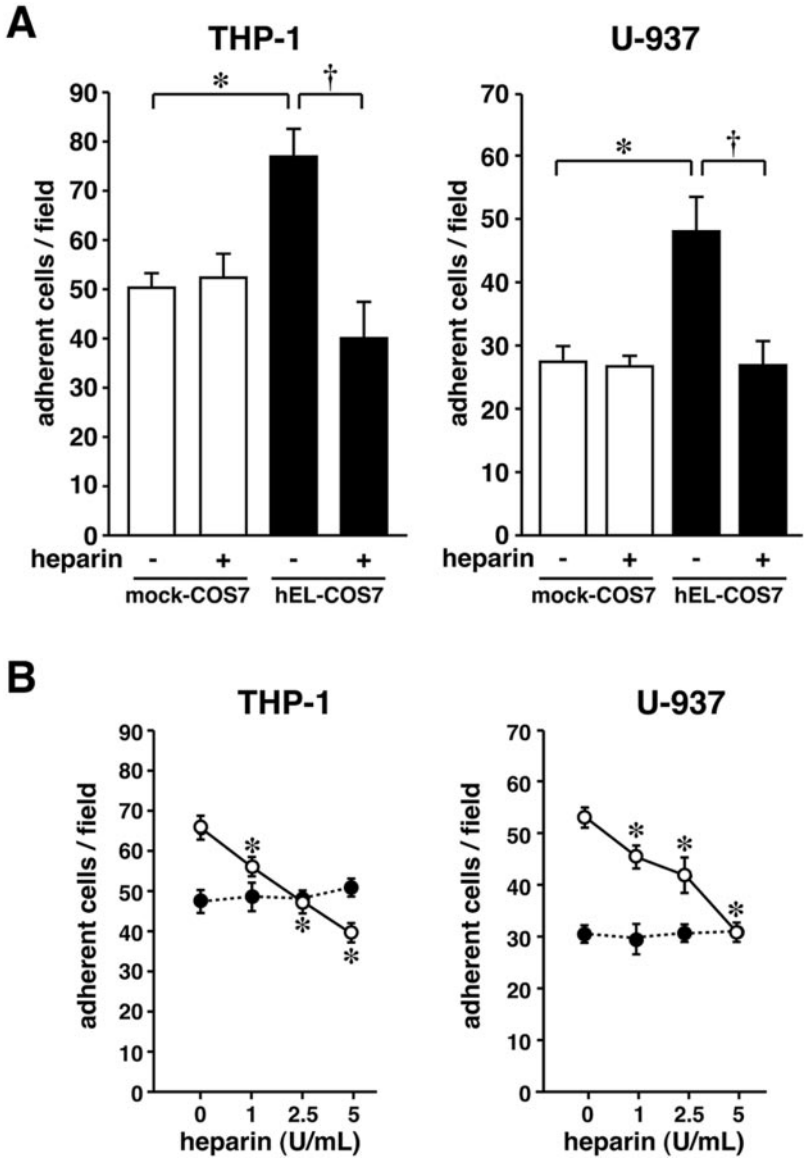

FIG. 3. **Heparin treatment inhibits monocyte binding to EL-expressing cells.** Confluent monolayers of COS7 cells constitutively expressing human EL (hEL-COS7) were treated with heparin (1–5 units/ml) or vehicle and incubated with THP-1 or U-937 cells. Control was mock-COS7, representing monocyte binding to vector-transfected COS7 cells. *A*, heparin treatment (5 units/ml) significantly diminished the augmented cell adhesion to hEL-COS7 cells. *, $p < 0.01$ *versus* mock without heparin treatment; †, $p < 0.01$ *versus* vehicle treatment. *B*, graphs showing the dose-dependent effect of heparin treatment on the cell adhesion to hEL-COS7 cells (*open circle*) or mock-COS7 cells (*closed circle*). Values represent mean ± S.E. *, $p < 0.01$ *versus* vehicle treatment.

DISCUSSION

The adhesion of monocytes to the arterial endothelium is a common feature linking the inflammation and development of atherosclerosis (28–30). The present study demonstrated for the first time evidence that EL expression was strongly up-regulated by LPS stimulation *in vivo* and that EL may act as a bridging molecule between the vessel wall and circulating monocytes. Overexpression of EL in cultured monolayers enhanced the binding of monocytes to EL expression cells. The release of EL from the cell surface by heparin completely abolished the ability of EL to enhance monocyte adhesion. Degradation of HSPGs by treatment with heparinase also suppressed the EL-mediated monocyte adhesion. These data indicate that EL-mediated cell adhesion requires interaction with HSPGs. The bridging function of the lipase family has been extensively characterized in LPL. The present results indicate that the effect of EL on monocyte adhesion is nearly equivalent to that of LPL. Furthermore, the aortic endothelium from h*LIPG*Tg mice showed an increased binding to monocytes, and *LIPG*−/− mice showed decreased monocyte binding, indicating that the bridging function of EL is relevant *in vivo*.

Treatment of mice with LPS increased EL mRNA and protein levels in the aorta, lung, heart, kidney, liver, and spleen. EL up-regulation in mouse tissues was accompanied by an EL increase in post-heparin plasma. This is the first report demonstrating that EL expression was increased during acute inflammation *in vivo*. This up-regulation of EL by inflammation is one of the unique characteristics of EL, because the expression of LPL and HL is known to be down-regulated by inflammatory cytokines (31, 32). The physiological significance of increased EL expression in inflammation remains speculative. However, given that EL may act as a bridging molecule, we speculate that the increase of EL levels may play a role in the progression of inflammation by the recruitment of monocyte macrophages through its bridging function. In addition, it has been reported that inflammatory cytokines up-regulate EL enzymatic activities in vascular endothelial cells (19). During acute inflammation, affected organs require energy to repair damaged tissues. Damaged cells may require cholesterol for new membrane synthesis during cell repair and regeneration. Under inflammatory conditions, furthermore, angiogenesis occurs to supply blood flow to repair the tissue, and endothelial cell proliferation is essential for the process of angiogenesis. EL is known to be increased in tube-forming endothelial cells (5). Throughout these cellular responses to inflammation the increased expression of EL may supply fatty acids generated from triglyceride and the phospholipids of lipoproteins through its lipase activity as a fuel source to injury tissues.

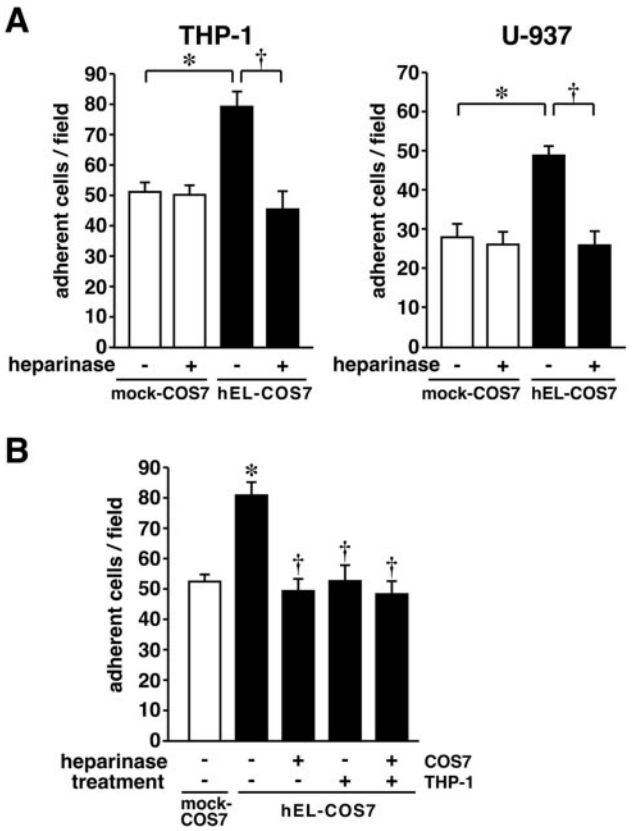

FIG. 4. **Heparinase treatment abolished the increased monocyte binding by EL.** *A*, confluent monolayers of COS7 cells constitutively expressing human EL (hEL-COS7) were treated with heparinase I (1 unit/ml) prior to the adhesion assay with THP-1 or U-937 cells. Control was mock-COS7, representing monocyte binding to vector-transfected COS7 cells. Values represent mean ± S.E. *, $p < 0.01$ *versus* mock without heparinase treatment; †, $p < 0.01$ *versus* vehicle treatment. *B*, confluent monolayers of hEL-COS7 cells and/or THP-1 cells were treated with heparinase I (1 unit/ml) prior to the adhesion assay. Values represent mean ± S.E. Treatment of COS7 or monocytes with heparinase I totally suppressed the ability of EL to enhance monocyte adhesion to hEL-COS7 cells, suggesting the involvement of EL though heparan sulfate proteoglycans. *, $p < 0.01$ *versus* mock; †, $p < 0.01$ *versus* vehicle treatment.

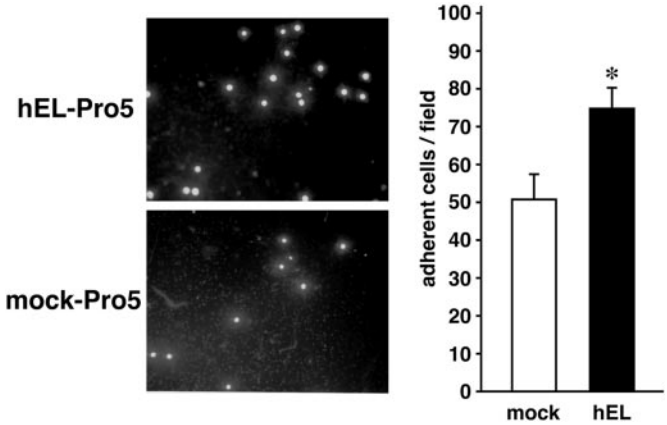

FIG. 5. **EL-overexpression in yolk sac cells enhanced monocyte adhesion.** Confluent monolayers of a yolk sac cell line (Pro5) constitutively expressing hEL were incubated with labeled 5×10^5/ml THP-1 cells. Mock represents monocyte binding to the vector transfected COS7 cells. The number of binding monocytes was counted under microscopy. Values represent mean ± S.E. *, $p < 0.01$ *versus* mock

Although the acute increase of EL levels during the inflammation may be beneficial to the host defense, a prolonged induction of EL may result in undesirable consequences. Atherosclerosis is now considered to be a chronic inflammatory process (33). The earliest events in atherosclerosis include monocyte recruitment into subendothelial spaces. Previous studies demonstrated that EL is substantially expressed in infiltrating macrophages and smooth muscle cells as well as in endothelial cells within atherosclerotic plaques of human coronary arteries (20). Moreover, it has been reported that EL mediates the uptake and binding of HDL and regulates the selective uptake of HDL-associated cholesterol esters (34). Another study reported that EL mediates the binding and uptake of LDL as well as HDL (35). The increase of EL expression and its activity at the site of chronic inflammation may also increase the uptake of lipoprotein-associated cholesteryl ester into vascular cells. Taken together, EL induction in local and chronic inflammation may contribute to monocyte/macrophage recruitment and lipoprotein accumulation in the vascular wall. Thus, the present data imply that EL may modulate the progression of vascular diseases (36).

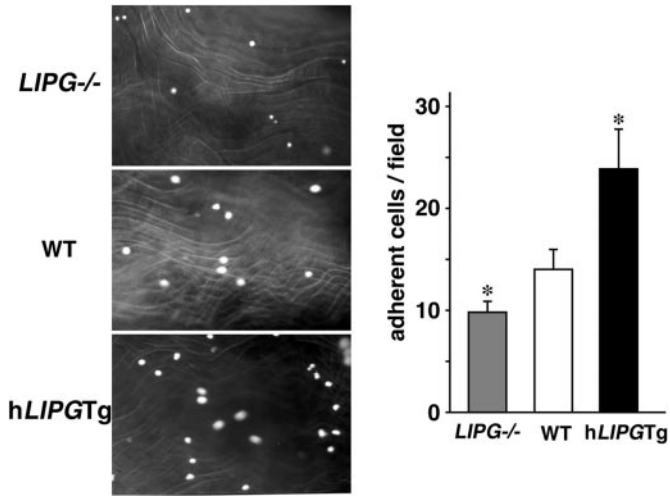

FIG. 6. **Gene dosage effect of EL expression in the vessel wall on monocyte binding.** Thoracic aortas were isolated from h*LIPG*Tg, *LIPG*−/−, and WT mice and incubated with fluorescein-labeled THP-1 cells (5×10^5/segment). The number of adherent monocytes was counted under fluorescence microscopy. Values represent mean ± S.E. *, $p < 0.05$ *versus* WT.

It has been shown that systemic inflammation can perturb lipoprotein metabolism and result in fundamental changes in the plasma concentrations of lipids and lipoproteins (37). In particular, severe infection or inflammation is associated with a decrease in the HDL-C level (38–40). Furthermore, chronic inflammatory diseases such as rheumatoid arthritis and systemic lupus are also accompanied by reduced plasma HDL-C levels (41). In the present study, plasma HDL-C levels in WT mice are decreased by 11% in the LPS-injected mice compared with those in control mice. On the other hand, HDL-C levels in *LIPG*−/− mice were not changed with LPS treatment. Considering that the overexpression of EL decreases the plasma HDL-C level in mice (6, 8), the up-regulation of EL in the present study could, at least in part, account for the reduced HDL-C levels in inflammation. A previous study showed an increase in LPL and HL expression in *LIPG*−/− mice (42). It has been reported that a number of molecules, including secretary type phospholipase A2, LPL, and HL, are regulated in LPS-induced endotoxemia. We speculate that lipid profile in LPS-induced inflammation may be regulated by the net effect of these molecules. In this context, EL may have a local role in the pathophysiology of inflammation and atherosclerosis beyond its action on plasma lipid levels.

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