Lysophosphatidylcholine Enhances Cytokine Production of Endothelial Cells via Induction of L-Type Amino Acid Transporter 1 and Cell Surface Antigen 4F2

Wakako Takabe, Yoshikatsu Kanai, Arthit Chairoungdua, Noriyuki Shibata, Sono Toi, Makio Kobayashi, Tatsuhiro Kodama, Noriko Noguchi

Objective—A diverse range of lipid oxidation products detected in oxidized low-density lipoprotein (oxLDL) and atherosclerotic lesions are capable of eliciting biological responses in vascular cells. We performed DNA microarray experiments to explore novel responses of human umbilical vein endothelial cells (HUVECs) to oxLDL and its components.

Methods and Results—cDNA microarray analysis showed that oxLDL, lysophosphatidylcholine (LysoPC), 4-hydroxy-2-nonenal, and oystersterols altered gene expression specifically, but some genes were commonly induced in HUVECs. Solute carrier family 3 member 2 and family 7 member 5, encoding the heavy chain of the cell surface antigen 4F2 (4F2hc) and the L-type amino acid transporter 1 (LAT1), respectively, were induced by oxLDL and many oxidation products. LAT1 requires 4F2hc to form a heterodimeric functional complex to transport neutral amino acids into the cell. LysoPC increased membrane protein levels of LAT1 confirmed by Western blot analysis and also uptake of L-[14C]leucine, which was inhibited by a competitive inhibitor for LAT1. The release of interleukin 6 (IL-6) and IL-8 was increased in LysoPC-treated cells and was attenuated by the LAT1 inhibitor.

Conclusions—These findings suggest that an increase in uptake of neutral amino acids induced by LysoPC results in enhancement of inflammatory responses of endothelial cells. (Arterioscler Thromb Vasc Biol. 2004;24:1640-1645.)

Key Words: amino acid transporter ■ atherosclerosis ■ cytokine ■ HUVEC ■ LysoPC
Upregulated Genes by oxLDL and its Components

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1 HUVECs were treated with oxLDL (200 μg/mL), LysoPC (30 μmol/L), 4HNE (5 μmol/L), 22(Δ9)-hydroxycholesterol (22(R)OH, 10 μmol/L), 25-hydroxycholesterol (25OH, 10 μmol/L), and 7-ketocholesterol (7keto, 10 μmol/L) for 4 hours. The numbers of fold change are shown as mean±SD of three independent experiments.

Methods

Cell Culture and Treatment

Human umbilical vein endothelial cells (HUVECs) and human aortic endothelial cells (HAECs) were obtained from a commercial source (Clonetics, San Diego), and all experiments were conducted in 4 passages. HUVECs and HAECs were grown in endothelial cell growth factor–containing medium–2 (EGM-2; Clonetics) with 2% FBS (Clonetics) at 37°C in a 5% CO2 atmosphere. After reaching confluence, the medium was changed to EGM-2 with 2% FBS containing 200 μg/mL oxLDL, 10 μmol/L 7-ketocholesterol (Steraloids Inc), 10 μmol/L 22(R)-hydroxycholesterol (Sigma), 10 μmol/L 25-hydroxycholesterol (Sigma), 30 μmol/L a-palmitoyl-LysoPC (Sigma), or 5 μmol/L 4HNE (Cayman Chemical). All chemicals were dissolved in ethanol (EtOH; Wako) and diluted with EGM-2, resulting in a final EtOH concentration of 0.01%. The control cells were cultured in EGM-2 containing 0.01% EtOH in the absence of oxidation products.

Details for methods of measurement of lipid oxidation products in oxLDL, Northern blot and real-time polymerase chain reaction (PCR) analysis, Western blot analysis, L-leucine uptake, measurement of cytokines, immunohistochemical study, adhesion molecule measurement, and others are in the expanded Methods section available online at http://atvb.ahajournals.org.

Results

Effect of oxLDL and Its Components on Gene Expression in HUVECs

In preliminary experiments, concentrations of lipid oxidation products were first assessed from samples of oxLDL (200 μg/mL) oxidized for 18 hours with 100 μmol/L copper. This treatment results in extensively oxidized LDL and allows for a feasible assessment of the maximum concentrations of individual components likely to stimulate a biological response. No toxicity was evident under any conditions for 24 hours as assessed by crystal violet staining and trypan blue exclusion method (data not shown).

Gene expression was determined in HUVECs using the GeneChip human genome focus array, which contains 8794 genes. Expression was determined in response to 200 μg/mL oxLDL, 10 μmol/L 7-ketocholesterol, 10 μmol/L 22(R)-hydroxycholesterol, 10 μmol/L 25-hydroxycholesterol, 30 μmol/L LysoPC, and 5 μmol/L 4HNE. The concentration of each oxidation product was determined according to that of oxLDL used in the present study.

Whereas oxLDL, LysoPC, and 4HNE induced expression of 117 genes, 105 genes, and 14 genes, respectively, a few genes were responsive to different kinds of oxygenol: 0 7-ketocholesterol, 5 22(R)-hydroxycholesterol, and 1 25-hydroxycholesterol, respectively. The table shows the genes that were upregulated by 4 hours of treatment with oxLDL >2-fold. The fold change values obtained by treatment with oxidation products are also shown. Among these upregulated genes, the solute carrier family (SCL) genes were induced not only by oxLDL but also LysoPC, 4HNE, and 22(R)-hydroxycholesterol. SCL genes are known to encode transporter subunits. Another interesting gene induced by oxLDL,
LysoPC, 4HNE, and 22(R)-hydroxycholesterol was the CCAAT/enhancer-binding protein \(\beta\) (C/EBP\(\beta\)), a nuclear factor for interleukin 6 (IL-6) and IL-8 expression.

**Induction of mRNA of SLC3A2 and SLC7A5 by oxLDL**

In the next series of experiments, we verified the accuracy of the GeneChip findings by Northern blot analysis for SLC3A2 and SLC7A5. HUVECs were exposed to extensively oxidized LDL (20 or 200 \(\mu\)g/mL) for up to 24 hours. Figure 1A shows that oxLDL induced the expression of both SLC3A2 and SLC7A5 in a time- and concentration-dependent manner for up to 8 hours. The concentration of LysoPC in this oxLDL was measured and found to be 66.8 (mol/mol LDL). Thus, the concentration of LysoPC in medium was 30 \(\mu\)mol/L when 200 \(\mu\)g/mL oxLDL was added into medium. We measured other oxidation products in this oxLDL and calculated the concentration of each of them in medium as follows: 4.4 \(\mu\)mol/L 7-ketocholesterol, 2.7 \(\mu\)mol/L 22(R)-hydroxycholesterol, 5.4 \(\mu\)mol/L 25-hydroxycholesterol, and 0.5 \(\mu\)mol/L 4HNE.

**Induction of mRNA of SLC3A2 and SLC7A5 by oxLDL Components**

HUVECs were treated with 10 \(\mu\)mol/L 7-ketocholesterol, 10 \(\mu\)mol/L 22(R)-hydroxycholesterol, 10 \(\mu\)mol/L 25-hydroxycholesterol, 30 \(\mu\)mol/L LysoPC, and 5 \(\mu\)mol/L 4HNE. The microarray data showed that the fold change of SLC3A2 induced by LysoPC, 4HNE, or 22(R)-hydroxycholesterol was 1.97±0.29, 2.79±0.48, and 2.42±0.36, respectively, and that of SLC7A5 by LysoPC, 4HNE, or 22(R)-hydroxycholesterol was 4.58±2.10, 1.86±0.92, and 1.95±0.47, respectively. These results agree with the results of the Northern blot analysis (Figure 1B).

Because LysoPC is one of the most abundant oxidation products in oxLDL and induced SLC genes most extensively, the time-dependent induction of mRNA of SLC3A2 and SLC7A5 in HUVECs treated with 30 \(\mu\)mol/L LysoPC was followed for up to 4 hours by quantitative real-time PCR (Figure 1C and 1D). All data normalized by GAPDH and cyclophilin showed almost the same results. The expression of SLC3A2 and SLC7A5 was increased over time. The same experiments were performed for HAECs and showed that LysoPC induced mRNA both of SLC3A2 and SLC7A5 in aortic endothelial cells in a time-dependent manner.

**Induction of Amino Acid Transporter Protein by LysoPC**

We confirmed an increase in LAT1 (SLC7A5) protein level in membrane fraction of HUVECs after exposure to 30 \(\mu\)mol/L LysoPC for 6 hours (Figure 2). The 125-kDa protein corresponding to a heterodimeric complex of LAT1 and 4F2hc was detected under the nonreducing condition shifted to a 38-kDa protein band by the treatment of 2-mercaptoethanol (2ME).

**Effect of LysoPC on L-[\(^{14}\)C]Leucine Uptake in HUVECs**

The effect of LysoPC on amino acid transport in HUVECs was examined using L-[\(^{14}\)C]leucine (Figure 3). LysoPC increased L-[\(^{14}\)C]leucine uptake significantly after 6 hours of incubation, the effect of which was almost completely inhibited by 1 mmol/L BCH, a selective inhibitor of system L amino acid transporter (LAT1, LAT2, and LAT3) under Na’-free conditions. The GeneChip data showed that SLC7A8 encoding LAT2 was not expressed in HUVECs and
by LysoPC was not induced by LysoPC (1.30±0.30). \textit{SLC43A1} (named prostate cancer overexpressed gene 1) encoding LAT3 was expressed slightly in HUVECs but was not induced by LysoPC (0.85±0.13). To confirm it, the following experiment was performed. Because LAT3 has been shown not to transport L-histidine or L-tryptophan, a competitive experiment was performed using 1 mmol/L L-leucine, L-histidine, and L-tryptophan. The uptake of L-[\textsuperscript{14}C]leucine induced by LysoPC was competitively inhibited by these amino acids. These results suggest that the increase in L-[\textsuperscript{14}C]leucine uptake induced by LysoPC was attributable to pronounced LAT1 activation.

**Contribution of SLC3A2 and SLC7A5 Expression to Cytokine Production in HUVECs**

It has been reported that LAT1 is upregulated in malignant tumors, and its expression is related to the growth and proliferation of tumor cells. Therefore, we investigated whether cell proliferation would be enhanced after exposure of HUVECs to LysoPC. An enhancement of cell proliferation by LysoPC was not observable, at least as assessed by crystal violet assay and trypan blue assay (data not shown).

To find consequences of amino acid uptake into cells, cytokines released into the culture medium were measured using a BioPlex cytokine analyzer, which has the capacity to measure 17 distinct cytokines. HUVEC exposure to 30 \textmu mol/L LysoPC for 24 hours significantly increased the release of IL-6 and IL-8 into medium (Figure 4). Release of IL-6 and IL-8 from HUVECs after exposure to LysoPC was inhibited by BCH by \approx 40% and 50%, respectively. BCH did not affect basal levels of IL-6 and IL-8 in the absence of LysoPC, suggesting that a substantial part of the increase in production of IL-6 and IL-8 induced by LysoPC was attributable to LAT1. IL-6 and IL-8 might also be produced using endogenous intracellular pools of amino acids in HUVECs on stimulation by LysoPC, and thus would not be inhibited by the transporter inhibitor. No release of the cytokines IL-1\beta, IL-2, IL-4, IL-5, IL-7, IL-10, IL-12p70, IL-13, IL-17, macrophage inflammatory protein 1\- \beta, interferon \gamma (INF-\gamma), granulocyte-colony stimulating factor (CSF), granulocyte-macrophage CSF, tumor necrosis factor \alpha, or monocyte chemoattractant protein 1 was detected after exposure of HUVECs to LysoPC (data not shown).

**Detection of LAT1 in LDL Receptor Knockout Mouse Aorta**

To test induction of LAT1 expression in atherogenic animals, immunohistochemical study using a monoclonal antibody against LAT1 was performed for aortas of LDL receptor knockout mice fed with high-fat or normal diet (Figure 5). LAT1 was detected predominantly in endothelial cells and macrophages but not smooth muscle cells in ascending aortas of mice on a high-fat diet compared with mice on a normal diet.

**Discussion**

OxLDL contains a variety of lipid oxidation products derived from phosphatidylcholine, CE, fatty acids, and cholesterol. LysoPC is thought to be one of the major oxidation products of oxLDL, and its biological function in vascular cells has been investigated extensively.\textsuperscript{13–16} A number of studies have shown that LysoPC stimulates endothelial cells to promote expression of adhesion molecules\textsuperscript{24–26} and release cytokines.\textsuperscript{27,28} A large-scale analysis of gene expression in HUVECs after exposure to oxLDL and lipid oxidation products including LysoPC revealed the unexpected finding that oxLDL and its components such as LysoPC affect the functions of endothelial cells by enhancing amino acid transport into cells.

\textit{SLC3A2} and \textit{SLC7A5} are translated into the heavy chain of 4F2hc and LAT1, respectively. These proteins form a functional complex for the transport of large neutral amino acids into the cell. Because LAT1 has been shown to be expressed...
highly in certain cancer cell lines\textsuperscript{18} as well as malignant tumors,\textsuperscript{22} its contribution to cell proliferation is strongly implicated. The enhancement of proliferation is more important in the smooth muscle cells than endothelial cells in the vascular wall but may also play a role in the balance between endothelial cell growth and apoptosis in response to injury. Because LAT1 activity regulation has become a target in cancer therapy, a specific inhibitor for LAT1 is now under active investigation. This article reports evidence for a novel function of oxLDL and its components in atherogenesis and suggests that LAT1 may prove to be a useful target molecule in inflammatory diseases.

Among the 17 cytokines measured, IL-6 and IL-8 were induced by LysoPC treatment in HUVECs. There is a binding site for C/EBP\textbeta in the promoter region of IL-6 and IL-8.\textsuperscript{29–33} The induction of C/EBP\textbeta mRNA by LysoPC was examined by a GeneChip experiment (Table, fold change 6.59±2.54), and the evident genetic activity may account for a substantial part of this increased production of IL-6 and IL-8. C/EBP\textbeta mRNA was also increased by oxLDL, 4HNE, and 22(\textgamma)-hydroxycholesterol. In addition, GeneChip analysis revealed that the mRNA level of IL-6 was significantly increased by LysoPC (fold change 2.35±0.35). However, a significant induction of IL-8 mRNA could not be confirmed because its basal level was too low to evaluate and allow statistical analysis. According to computer analysis of the ideal transcription factor binding sites in the promoter region, 4 C/EBP\textbeta binding sites were found within 2000 bp in the promoter region of both SLC3A2 and SLC7A5. The molecular mechanisms by which amino acid transporter gene expression is enhanced by LysoPC have yet to be elucidated, but overexpression of the transporter may account in part for the proinflammatory effects of LysoPC. It has been reported that the promoter region of SLC3A2 displays sequence homologies with IL-2 and the IL-2 receptor \( \alpha \) chain, the induction of which is important for T-cell activation.\textsuperscript{34,35} In contrast to cytokines, a relationship between amino acid transport and adhesion molecule expression was not observed (see online supplement).

Computer analysis suggests that in the promoter region, there are certain other transcription factor-binding sites such as AP-1, cAMP response element-binding protein (CREB), SREBP, and specificity protein 1 (Sp1) for SLC3A2; and in addition to them, there is a nuclear factor \( \kappa B \) (NF-\( \kappa B \)) site in that of SLC7A5. Several signaling pathways active in endothelial cells have been identified after exposure to LysoPC. Phosphorylation of CREB by LysoPC is reported in bovine arterial endothelial cells,\textsuperscript{36,37} and NF-\( \kappa B \) activation has been shown to occur in response to LysoPC and prevented by protein tyrosine kinase inhibitors but not by cAMP-dependent protein kinases or PKC inhibitors.\textsuperscript{26} However, Sugiyama et al report that NF-\( \kappa B \) activation by LysoPC is concentration-dependent, biphasically regulated, and PKC activation might be involved in part in the LysoPC-induced NF-\( \kappa B \) activation in HUVECs.\textsuperscript{38} They also have shown that LysoPC increases the activities of AP-1 and CREB but not Sp1 and that only AP-1 activation in their experiments was PKC dependent. In addition to the PKC pathway, the mitogen-activated protein (MAP) kinases (ERK1/2) and the c-Jun N-terminal kinases are known to act as AP-1 activators.\textsuperscript{13,38,39} The overexpression of dual-specificity phosphatase 1 suggests activation of MAP-kinase pathways (Table).\textsuperscript{40} The signaling pathway crucial for the LysoPC-induced expression of SLC3A2 and SLC7A5 is under continuing investigation.

Acknowledgments

This work was supported in part by a grant-in-aid of Special Coordination Funds for Promoting Science and Technology from the Ministry of Education, Culture, Sports, Science and Technology. We thank Drs. Yasukazu Yoshida, Satoshi Yamada, and Koichi Sumi for helping measurements of LysoPC, HNE-adduct, and cytokines, respectively. We also thank Akashi Izumi, Akiko Kikuchi, and Chizuru Nagao for excellent technical assistance.

References


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Arterioscler Thromb Vasc Biol. 2004;24:1640-1645; originally published online June 3, 2004;
doi: 10.1161/01.ATV.0000134377.17680.26

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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In the September 2004 issue of *Arteriosclerosis, Thrombosis, and Vascular Biology*, in the article entitled “Lysophosphatidylcholine Enhances Cytokine Production of Endothelial Cells via Induction of L-Type Amino Acid Transporter 1 and Cell Surface Antigen 4F2” by Takabe et al (*Arterioscler Thromb Vasc Biol*, 2004;24:1640–1645), Figure 5 was erroneously published in black and white. The correct color figure is shown.

**Figure 5.** Detection of LAT-1 in aorta of LDL receptor knock-out mouse. The aortas of LDL receptor knockout mice that were fed a normal diet (A) and a high-fat diet (B) were stained by a monoclonal antibody raised against LAT-1. LAT-1 was expressed in endothelial cells of mice on a high-fat diet but not with a normal diet. LAT-1 was also expressed in macrophages accumulating in the intima of aortas of mice fed with high-fat diet (C).
METHODS

**LDL Preparation and Oxidation.** Human low-density lipoprotein (LDL) was isolated from the plasma of healthy volunteers by the method of Goldstein et al.\(^1\). After dialysis with EDTA-free PBS, 1.7 mg/mL LDL were oxidized with 100 µmol/L CuCl\(_2\) at 37 °C for 18 hours.

**Measurement of Lipid Oxidation Products in Oxidized LDL.** For the measurement of 7-ketocholesterol, 22(R)-hydroxycholesterol and 25-hydroxycholesterol, the lipid fraction was extracted from 1 mg/mL oxidized LDL (oxLDL) by two volumes of chloroform/methanol (2/1 by volume). The chloroform phase was dried under N\(_2\) gas atmosphere and dissolved in methanol. Oxysterols were analyzed with an HPLC-UV at 210 nm using an ODS-3 column (250 mm x 4.6 mm, 5 µm particle size, GL Sciences). Methanol was used as an eluent at a flow rate of 1.0 mL/minute.

LysoPC was measured by means of thin-layer chromatography with a flame-ionization detection (FID) system (MK-5, Iatron Laboratories) according to the literature with a slight modification\(^2\).

Histidine adducts of 4HNE were measured by using a competitive ELISA for HNE-adducts according to the literature with a slight modification\(^3\). HNE-adducts were measured by comparing the test sample with an inhibition curve generated from synthetic HNE-N\(\alpha\)-acetyl-L-histidine.
**Northern Blot Analysis for SLC3A2 and SLC7A5.** Northern blot analysis was performed using \(^{32}\text{P}\)dCTP-labeled human SLC3A2 or SLC7A5 cDNA probes as previously described\(^4\).

**DNA Chip Analysis.** An oligonucleotide microarray analysis was performed by using the GeneChip Human Genome Focus Array (Affymetrix). Fold change value was calculated as previously described\(^5\).

**Real-Time PCR Analysis.** For real-time PCR, 1 \(\mu\)g of total RNA was reverse transcribed into cDNA using a GeneAmp RNA PCR Core Kit (Applied Biosystems). The housekeeping genes GAPDH and cyclophilin were used as an endogenous control. The following primer sequences were used. SLC3A2: forward primer, 5’-GCA GAT CGA CCC CAA TTT TG-3’, reverse primer, 5’-ACG AGT TCT CAC CCC GGT AGT T-3’; SLC7A5: forward primer, 5’-CAC AGA GGA AAT GAT CAA CCC CT-3’, reverse primer, 5’- TGA TAG TTC CCG AAG TCC ACG G-3’; GAPDH: forward primer, 5’-TTT GGC TAC AGC AAC AGG GT G GTG-3’, reverse primer, 5’-ATG GTA CAT GAC AAG AGG GTG GTG-3’, GAPDH: forward primer, 5’- TGG GTA GAC AAG GTG GTG-3’; cyclophilin: forward primer, 5’-TGG AGA GCA CCA AGA ACA-3’, reverse primer, 5’- TCC CGG AGT CGA CAA TGA T-3’. The mRNA of SLC3A2 and SLC7A5 was quantified using the ABI PRISM
Western Blot Analysis. After treatment with lysoPC, cells were washed and lysed with lysis buffer (50 mmol/L Tris-HCl, pH=7.5, 150 mmol/L NaCl, 1 mmol/L EDTA, 0.5 mmol/L phenylmethylsulfonyl fluoride, 1 mmol/L sodium vanadate, 10 mmol/L sodium fluoride, 2 µg/mL leupeptin, 2 µg/mL aprotinin and 2 µg/mL pepstatin A) at 4 °C. The lysate was homogenized and centrifuged at 500,000 x g for 20 minutes at 4 °C. The pellet was resuspended in lysis buffer containing 0.5 % Triton-X. The samples were sonicated and then centrifuged at 500,000 x g for 20 minutes at 4 °C. The protein concentrations of supernatant were measured by a BCA protein assay kit (Pierce, Rockford, IL) and Western blot analysis using a monoclonal antibody against LAT1 was performed as previously described.

L-Leucine Uptake. After confluence, HUVEC were treated with 30 µmol/L LysoPC for 6 hours on 24-well plates in growth medium (EGM-2 with 2 % FBS). Cells were washed twice with Na⁺-free uptake solution (125 mmol/L choline-Cl, 5.6 mmol/L glucose, 4.8 mmol/L KCl, 1.2 mmol/L MgSO₄, 1.2 mmol/L KH₂PO₄, 1.3 mmol/L CaCl₂, 25 mmol/L HEPES, pH 7.4) and pre-incubated for 10 minutes at 37 °C. Cells were incubated for 1 minute in Na⁺-free uptake solution containing 10 µmol/L L-[¹⁴C]leucine (Amerciam Pharmacia Biotech) in the presence or absence of 1 mmol/L
2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH, SIGMA), an inhibitor of system L transporters. After washing three times with ice-cold Na\(^+\)-free uptake solution, cells were dissolved with 0.1 N NaOH and the radioactivity was counted by liquid scintillation spectrometry (Pharmacia Biotech, Barcelona, Spain). For the competition experiments, 1 mmol/L L-leucine, 1 mmol/L L-histidine, and 1 mmol/L L-tryptophan were used.

**Measurement of Cytokines.** Cytokines were measured using a Bio-Plex Cytokine Assay Kit (Bio-Rad). Briefly, HUVEC were cultured on 96-well plates in EGM-2 containing (with) 2 % FBS and exposed to 30 µmol/L LysoPC for 24 hours. The supernatant was collected and mixed with beads conjugated with antibody against 17 different cytokines and stained with streptavidin/phycoerythrin. The quantification was performed with a BioPlex assay reader (Bio-Rad).

**Determination of cell number.** The cell number and viability were determined by two methods, crystal violet staining according to the literature\(^{7,8}\) and trypan blue exclusion analysis.

**Immunohistochemical Study for Mouse aorta.** Male LDL receptor (-/-) mice, 5 weeks old (n=3-4) were obtained from the Jackson Laboratory and were fed with control diet
and high fat diet (1.25% cholesterol added) for 16 weeks as previously described. The ascending aorta was removed from each mouse under anesthesia. The primary antibodies used in immunohistochemistry were a mouse monoclonal anti-LAT-1C IgM antibody (clone name; Transgenic Inc.) at a dilution of 1:1,000. A mouse monoclonal anti-α-smooth muscle actin (α-SMA) IgG antibody (M0851; Dako) at a dilution of 1:500, and a goat polyclonal anti-CD68 IgG antibody (Santa Cruz) at a dilution of 1:500 were used as markers of smooth muscles and macrophages, respectively. Immunoreaction was visualized by the avidin-biotin-immunoperoxidase complex method using appropriate ABC kits (Vector Laboratories) for LAT-1C and CD68, and by the indirect immunoperoxidase method using an Envision-plus kit for SMA (Dako).

**Measurement of adhesion molecules** The expression of vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1), and PECAM-1 was measured using a flow cytometer as previously reported.

**Statistical Analysis.** All experiments were repeated at least three times and results are given as the mean +/- standard deviation from the mean, and Student’s t-test was used to analyze for statistical significance.
RESULTS

Expression of adhesion molecules by LysoPC on which the effect of inhibitor of amino acid transporter. We studied the effect of the inhibitor of amino acid transporter, BCH on LysoPC-induced expression of adhesion molecules. LysoPC increased the expression of ICAM-1 and PECAM-1 (data not shown) but not VCAM-1, and BCH had no effect on any of these, suggesting that there was not a relationship between amino acid transport and adhesion molecule expression. The mechanism underlying the selective utilization of amino acids for enhancement of cytokine activity is not known at present.
REFERENCES


Figure I. Expression of adhesion molecules by LysoPC on which the effect of inhibitor of amino acid transporter. HUVEC were treated with 30 µmol/L LysoPC for 60 hours and harvested. The expression of VCAM-1 and ICAM-1 was measured by flow cytometry (A). The effect of LAT-1 inhibitor (1 mmol/L BCH) on the expression of ICAM-1 induced by LysoPC was studied (B). The data were shown as the mean +/- SD (n=3).
Fig. I

A

LysoPC
- + - +
ICAM-1  VCAM-1

Expression of adhesion molecules, %

B

LysoPC
- - + +
BCH
- + - +

Expression of ICAM-1, %

*