Lysophosphatidylcholine posttranscriptionally inhibits interferon-γ-induced IP-10, Mig and I-Tac expression in endothelial cells

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Abstract

Objective: Lysophosphatidylcholine (lysoPC) is abundant in atherosclerotic lesions and has potential immunomodulatory activities. This study is aimed to investigate effects of lysoPC on the interferon (IFN)-γ-induced gene expression, focusing on T cell-directed CXC chemokines relevant to atherosclerosis.

Methods and Results: Effects of lysoPC on the IFN-γ-induced gene expression of IFN-inducible protein of 10 kDa (IP-10), IFN-inducible T cell A chemotactant (I-Tac), and monokine induced by IFN-γ (Mig) were evaluated in cultured endothelial cells. Northern blotting showed that lysoPC transiently and dose-dependently inhibited the IFN-γ-induced accumulation of IP-10, Mig and I-Tac but not p48, interferon regulatory factor-1 and guanidine binding protein-1. Nuclear run-off assays showed that lysoPC did not inhibit IP-10, Mig and I-Tac gene transcription. An analysis of the degradation of IP-10, Mig and I-Tac mRNA revealed it to be enhanced by lysoPC.

Conclusion: LysoPC selectively inhibits IFN-γ-induced IP-10, I-Tac and Mig expression in endothelial cells, at least in part, by reducing mRNA stability. Thus, lysoPC might regulate T cell-mediated immunity by affecting IFN-γ-mediated activation of endothelial cells in atherosclerotic lesions.

Keywords: IP_10; Interferon-γ; Lysophosphatidylcholine; Endothelial cells; mRNA stability

1. Introduction

The oxidative modification of LDL in the arterial wall has been suggested to play a key role in atherogenesis [1]. During the oxidation, phosphatidylcholine is converted to lysophosphatidylcholine (lysoPC) by phospholipase A2-like activity [2], which is accumulated in atherosclerotic and inflammatory lesions [3]. Previous reports have shown that lysoPC has potential immunomodulatory functions. LysoPC induces the expression of multiple genes relevant to atherosclerosis and inflammation in endothelial cells [4–8], macrophages [9], T cells [10], and vascular smooth muscle cells. Products of lysoPC-induced genes in endothelial cells include endothelial–leukocyte adhesion molecules, such as vascular cell adhesion molecule (VCAM)-1 and intercellular adhesion molecule (ICAM)-1 [6,7], which support the accumulation of mononuclear leukocytes in the lesion. LysoPC also functions as a chemoattractant for T cells and macrophages [11,12]. Moreover, lysoPC increases IFNγ production and CD40 ligand expression on activated T cells [10,13]. Inasmuch as CD40 ligand–CD40 interactions are required for T cell-mediated B cell differentiation and the generation of the IFNγ-producing Th1 subset of CD4+cells, these effects of lysoPC suggest the involvement of lysoPC in the selection of the Th1/Th2 response. Interestingly, lysoPC is also implicated in the pathogenesis of the autoimmune disease systemic lupus erythematosus [14–16].

IFNγ is a proinflammatory cytokine secreted by Th1 cells and promotes immune and inflammatory responses
through the activation of various types of cells, including macrophages, smooth muscle cells, and endothelial cells. IFNγ potentiates VCAM-1 expression induced by proinflammatory cytokines, such as tumor necrosis factor α (TNFα) in endothelial cells [17]. IFNγ also induces the expression on endothelial cells of CD40, which interacts with the CD40 ligand expressed on CD4+ T cells to induce the production of adhesion molecules, E-selectin, VCAM-1, and ICAM-1 on endothelial cells [18]. These effects of IFNγ suggest its involvement in the accumulation of immune cells through the activation of endothelial cells in inflammatory lesions, including atherosclerotic lesions. Furthermore, IFNγ induces the expression of activated T cell-directed chemokines IP-10, Mig, and I-Tac in endothelial cells, macrophages, and smooth muscle cells [19]. These chemokines interact with CXCR3 of activated T cells, predominantly Th1, which secretes IFNγ [20,21]. Recent in vivo studies have provided evidence that IFNγ secreted by Th1 has proatherogenic properties [22,23], and that IP-10, Mig, and I-Tac are expressed in endothelial cells, macrophages, and smooth muscle cells in atherosclerotic lesions [19]. Therefore, IFNγ secreted by Th1 could promote the recruitment of Th1 through induction of IP-10, Mig, and I-Tac expression in a paracrine manner and potentiate atherogenesis.

Upon binding to its receptor, IFNγ activates Janus kinase (JAK)-signal transducer and activator of transcription (STAT)-1 pathway [24,25]. Homodimer of phosphorylated STAT-1α and complexes containing phosphorylated STAT-1α and p48 act as activated forms of transcription factors. Recently, it has been reported that STAT-1α and p48 are essential for IP-10 [25] and I-Tac [26] gene induction by IFNγ and are induced by IFNγ. IRF-1 is another transcription factor, which is induced and activated by IFNγ, and required for the induction of VCAM-1 [17], guanylate binding protein (GBP)-1 [27], and inducible nitric oxide synthetase.

In the light of the potential immunomodulatory role of lysoPC and the importance of IFNγ in immune response and atherogenesis, we investigated the effects of lysoPC on the endothelial expression of IFNγ-induced genes, especially the Th1-directed chemokines IP-10, I-Tac, and Mig, and the IFNγ-activated transcription factors STAT1α, p48, and IRF-1.

2. Materials and Methods

2.1. Materials

L-α-lysoPC (from egg phosphatidylcholine) was purchased from Avanti Polar Lipids. Recombinant human IFNγ and TNFα were obtained from R&D Systems (Minneapolis, MN, USA). M199 medium, penicillin G, L-glutamine, and fetal calf serum (FCS) were from Invitrogen (Carlsbad, CA, USA).

2.2. Cell culture

Human umbilical vein endothelial cells (HUVECs) were isolated from human umbilical cords using collagenase (Collaborative Research, Lakewood, NJ, USA) and cultured in M199 medium supplemented with endothelial cell growth supplement (Sigma, St. Louis, MO, USA), heparin (from porcine intestinal mucosa, Sigma), antibiotics (100 U/ml penicillin G and 100 mg/ml streptomycin), L-glutamine, and 20% FCS. Confluent cells of three to five passages were used for this study. For stimulating HUVECs, M199 medium containing 5% FCS, L-glutamine and antibiotics was used for all experiments. This study conformed to the declaration of Helsinki, and informed consent was obtained from all donors of umbilical cord.

2.3. Northern blot analysis

Northern blot analysis was performed, as previously described [28]. To prepare cDNA probes for IP-10, Mig, I-Tac, GBP-1, STAT-1α, and p48, RT–PCR was carried out using total RNA extracted from HUVECs treated with IFNγ and TNFα. The PCR products were cloned into pGEM-T or pGEM-T easy vector (Promega) and sequenced using an ABI PRISM 310 Genetic Analyzer. The following primers were used for PCR: IP-10; 5'-CTCTAAAGTTGCACTCAGAGGA and 5'-ACCCCAACAGCAGAAAGATT, Mig; 5'-AGGAACCCAGATGGAAGG and 5'-TTGTGTGAGGGATGTTGGTG, I-Tac; 5'-TTCCCCATGTTCTAAAGG and 5'-TAAAAAAAACAAAATGATGC, GBP-1; 5'-GGAAACCGAGAGGGGATAC and 5'-CTACTGCTGGTCATCTGGAA, STAT-1α; 5'-AGTGAGTAGGAGCGCGAGAC and 5'-ATCACCAACACGGCAGAGA, p48; 5'-AAGTGGAGAGTTGGGCAGTTT and 5'-AGAACCTGTGCTGCTCCTGGTG. The following cDNA fragments were also used: a 1.7-kb Sac I fragment of IRF-1 cDNA kindly provided by Dr. Tucker Collins (Brigham and Women’s Hospital, Boston, MA) and a 0.9-kb HindIII/ AccI fragment of GAPDH cDNA purchased from ATCC.

2.4. Western blot analysis

Following experimental treatment of HUVEC, whole-cell lysates were prepared using RIPA lysis buffer (1X phosphate buffer saline, pH 7.4, 1% Nonidet p–40, 0.5% sodium deoxycholate, 0.1% SDS, 10 μg/ml PMSF, 1 μg/ml aprotinin). Twenty micrograms protein of whole-cell lysate was separated by SDS–polyacrilamide gels electrophoresis under reducing conditions, transferred to nitrocellulose in 25 mM Tris, 192 mM glycine–5% methanol at 150 V for 1 h. Antibodies specific for STAT-1α, p48, and IRF-1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) were used at 1:200 dilution. Immunoreactive proteins were detected according to the enhanced chemiluminescent protocol (Amersham) using 1:1500 horseradish peroxidase-conjugated goat antirabbit secondary antibody.
2.5. ELISA

Culture supernatants were collected, and concentrations of human IP-10 and Mig were measured by sandwich ELISA using a protein detector ELISA kit (KPL, Gaithersburg, MD, USA) according to the manufacturer’s instructions. Anti-IP-10 or Mig monoclonal antibody (R&D Systems) as a capture antibody and rabbit anti-IP-10 or Mig polyclonal antibody (PeproTech, London, UK) as a detection antibody were used. Optical densities were measured with a plate reader (EAR400, SLT-Lab instruments). IP-10 and Mig concentrations were calculated after preparing standard curves using recombinant human IP-10 or Mig (R&D Systems).

2.6. Nuclear run-off assay

Nuclear run-off experiments were performed, as previously reported [29] with some modifications. In brief, confluent HUVECs were stimulated with appropriate stimulants and scraped into PBS. The cell pellets were resuspended in glycerol storage buffer (10 mM Tris–HCl pH 8.0, 5 mM MgCl2, 300 mM KCl, 0.1 mM PMSF, 0.5 mM DTT, 1 mM each of ATP, CTP, and UTP, 250 U/ml RNase inhibitor (Sigma), and 0.1 mM PMSF). Then, the nuclei were resuspended in glycerol storage buffer (10 mM Tris–HCl pH 8.3, 0.1 mM EDTA, 1 mM DTT, and 40% glycerol) at 6×10^6 nuclei per 100 μl, snap frozen in liquid nitrogen. The transcription reaction was carried out by mixing nuclei with an equal volume of 2× reaction buffer (10 mM Tris–HCl pH 8.0, 5 mM MgCl2, 300 mM KCl, 0.1 mM PMSF, 0.5 mM DTT, 1 mM each of unlabelled ATP, CTP, and UTP, 250 U/ml RNase inhibitor (Promega, Madison, WI, USA) and 30 mCi [α-32P] UTP) and incubated at 30 °C for 30 min. Transcribed labelled RNA was isolated using Isogen-LS (Nippongene, Tokyo, Japan). Denatured plasmids containing cDNA fragments of IP-10, Mig, I-Tac, and GAPDH were immobilized onto nylon membrane (Hybond LX; Amersham, Piscataway, NJ, USA) and hybridized with an equal amount of newly transcribed RNA for 36 h. The membrane was autoradiographed with a Fujix Bioimage Analyzer BAS200 (Fuji Photo Film, Japan).

2.7. Preparation of IP-10 promoter/luciferase construct

A 971-bp IP-10 promoter fragment, was generated and a 616-bp proximal fragment with BglII. The 616-bp fragment has the 5’-flanking region of the human IP-10 gene from −519 to +97 and contains an IFN-stimulated response element and two NFκB sites which were required for IP-10 gene expression induced by IFNγ or a combination of IFNγ and TNFα. The 616-bp fragment was gel purified and cloned into the promoterless pGL3-basic vector (Promega) to give the plasmid Bgl IP-10. The orientation and vector insert boundaries were verified through digestion with a restriction enzyme and sequence analysis.

2.8. Transient transfection assay

HUVECs were transiently cotransfected with the IP-10 promoter/firefly luciferase construct (Bgl-IP-10) and pRL-TK plasmid as an internal control, containing the herpes simplex virus thymidine kinase promoter upstream of the Renilla luciferase gene (Promega). HUVECs on 12-well plates were transfected with 0.8 μg of DNA per well for 4 h, using Lipofectamine Plus reagent (Invitrogen) according to the manufacturer’s protocol. Forty-eight hours after transfection, cells were stimulated with IFNγ and/or lysoPC for 6 h. Then the cells were harvested, and a dual luciferase assay (Promega) was performed.

2.9. Statistical analysis

Differences were analyzed by one-way ANOVA and then by Fisher’s test. A p value of <0.05 was regarded as significant.

3. Results

3.1. LysoPC selectively inhibits IFNγ-induced IP-10, I-Tac, and Mig mRNA expression in HUVECs

IFNγ induced expression of IP-10, I-Tac, and Mig mRNA in HUVECs in a time-dependent manner (Fig. 1A). Increased levels of IP-10, I-Tac, and Mig mRNA were detectable at 4-h stimulation, reached a peak after 8 h, and were maintained for at least 24 h. We also examined mRNA expression of IRF-1, one of IFNγ-induced and activated transcription factors, and GBP-1, an IRF-1-dependent gene. IFNγ-induced IRF-1 and GBP-1 mRNA expression reached maximal levels after 4 h and was sustained for at least 24 h. LysoPC transiently inhibited IP-10, I-Tac, and Mig mRNA expression induced by IFNγ. These inhibitory effects of lysoPC were observed at 4 h and 8 h and almost abolished at 24 h (Fig. 1A). Dose-response experiments revealed that these inhibitory effects were also dose-dependent; the effects were observed at 40 μM, and mRNA signals became almost undetectable at 80 μM of lysoPC with 8 h of stimulation (Fig. 1B). In contrast, IFNγ-induced IRF1 and GBP-1 mRNA expression was not affected by
Fig. 1. LysoPC selectively inhibits IP-10, Mig, I-Tac, and IRF-1 mRNA expression induced by IFNγ or a combination of TNFα and IFNγ in HUVECs. HUVEC monolayers were stimulated with IFNγ (200 U/ml) in the absence or presence of lysoPC (80 μM) for 0, 4, 8, and 24 h. Total RNA was isolated, and levels of Mig, I-Tac, IP-10, IRF-1, and GBP-1 mRNA were analyzed by Northern blotting, as described in Materials and methods. (B and C) HUVECs were treated with medium alone and IFNγ (200 U/ml) in the absence or presence of the indicated concentrations of lysoPC for 8 h, and mRNA levels of Mig, I-Tac, and IP-10 (B), or STAT-1α, p48, and IRF-1 (C) were analyzed. (D) HUVECs were treated with TNFα (10 ng/ml) and IFNγ (200 U/ml) with or without lysoPC (80 μM) for 0, 4, and 8 h, and levels of IP-10, I-Tac, Mig, and IRF-1 mRNA were analyzed. (E) HUVECs were treated with medium alone or IFNγ (200 U/ml) with or without lysoPC (80 μM), phosphatidylcholine (PC; 80 μM) or lysophosphatidic acid (lysoPA; 80 μM) for 8 h, and IP-10, I-Tac, and Mig mRNA was analyzed. GAPDH mRNA served as a loading control.

Fig. 2. Effect of IFNγ pretreatment on the inhibitory effect of lysoPC. (A) HUVECs were treated for 8 h with medium alone, IFNγ alone, or a combination of IFNγ and cycloheximide (CHX; 5 μg/ml). Then, IP10, Mig, I-Tac, and IRF-1 mRNA expression was analyzed by Northern blotting, as described in Materials and methods. (B) HUVECs were first pretreated with IFNγ for 24 h to produce enough IFNγ-inducible protein and then treated for 6 h with IFNγ (200 U/ml) alone or IFNγ and lysoPC (80 μM). IP10, Mig, I-Tac, and IRF-1 mRNA expression was analyzed by Northern blotting. Ethidium bromide staining of 28S and 18S rRNA served as a loading control. (C) Western blotting to show that IFNγ induces STAT-1α, p48, and IRF-1. Whole cell lysate was prepared from HUVECs untreated or treated with IFNγ for 8 or 24 h, and then STAT-1α, p48, and IRF-1 protein expression was analyzed by Western blotting.
lysoPC even at 80 μM for up to 24 h (Fig. 1A). Furthermore, we tested whether lysoPC inhibits the gene expression of other IFNγ-inducible transcription factors, STAT-1α, and p48. As shown in Fig. 1C, lysoPC dose dependently inhibited the IFNγ-induced mRNA expression of STAT-1α but not p48 and IRF-1.

Additionally, lysoPC inhibited the mRNA expression of IP-10, I-Tac, and Mig induced by TNFα and IFNγ (Fig. 1D), whose combination can synergistically induce the expression of these chemokines (data not shown). Furthermore, we observed that other phospholipids, phosphatidylcholine, and lysophosphatidic acid did not affect IP-10, Mig, and I-Tac mRNA expression induced by IFNγ (Fig. 1E).

Because the protein synthesis inhibitor cycloheximide inhibited IFNγ-induced IP-10, Mig, and I-Tac mRNA expression (Fig. 2A), we next studied whether an inhibition of new protein synthesis of STAT1-α, other transcription factor(s) we did not examine in the present study, or factor(s) that influence posttranscriptional regulation could be involved in the inhibitory effect of lysoPC. HUVECs were first pretreated with IFNγ for 24 h to produce enough IFNγ-inducible protein and then treated with IFNγ alone or IFNγ

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**Fig. 3.** LysoPC inhibits IP-10 and Mig protein secretion induced by IFNγ in HUVECs. HUVECs were treated for 16 h, with medium alone, lysoPC alone, or IFNγ (200 U/ml) in the absence or presence of the indicated concentrations of lysoPC. Culture supernatants were collected and subjected to sandwich ELISA to measure IP-10 (left panel) and Mig (right panel). The results are shown as the mean± S.D. of four independent experiments. *p<0.01 versus IFNγ-treated cells.

**Fig. 4.** LysoPC does not affect the transcription rate of IP-10, I-Tac and Mig genes induced by IFNγ or a combination of TNFα and IFNγ. HUVECs were treated for 8 h with medium alone, IFNγ (200 U/ml) with or without lysoPC (80 μM) (A), or a combination of TNFα (10 ng/ml) and IFNγ (200 U/ml) with or without lysoPC (80 μM) (B). The nuclei were isolated and nuclear run-off experiments were performed to determine the transcription rates of the IP-10, I-Tac, Mig, and GAPDH genes, as described in Materials and methods. pGL3 plasmid was used as a negative control. Results of the densitometric analysis of (A) and (B) are shown in (C) and (D), respectively. Signal intensities of IP-10, I-Tac and Mig were normalized to that of GAPDH. Data are expressed as relative transcription rates compared to unstimulated conditions. Open bars indicate medium alone, hatched bars indicate IFNγ alone (C), or TNFα and IFNγ (D), and closed bars indicate IFNγ with lysoPC (C) or TNFα and IFNγ with lysoPC (D). Data shown are representative of two experiments.
LysoPC inhibited IFNγ-induced IP-10, Mig, and I-Tac mRNA accumulation in response to IFNγ alone or in combination with TNFα. The increase in transcription of the IP-10, I-Tac, and Mig genes was 1.3-fold, 1.1-fold, and 1.5-fold by IFNγ and TNFα, respectively, compared with medium alone (Fig. 4C, D). Unexpectedly, lysoPC did not inhibit but potentiated IP-10, I-Tac, and Mig transcription induced by IFNγ alone or in combination with TNFα (Fig. 4).

To confirm the above results, HUVECs were cotransfected with Bgl-IP-10 and pRL-TK plasmids, and luciferase activities were analyzed. As shown in Fig. 5, IFNγ slightly but reproducibly activated the IP-10 promoter. As previously reported, TNFα effectively and TNFα and IFNγ synergistically increased IP-10 promoter activity. As expected from the results of the nuclear run-off experiments, lysoPC enhanced IP-10 promoter activity in the presence of IFNγ or a combination of IFNγ and TNFα. Thus, our results indicate that lysoPC inhibits IP-10, Mig, and I-Tac not at the transcriptional level but at the posttranscriptional level.

3.4. LysoPC decreases the stability of Mig, I-Tac, and IP-10 mRNA in endothelial cells

We investigated the effect of lysoPC on the stability of IP-10, Mig, and I-Tac mRNA accumulated in response to IFNγ. The decay of the mRNA was chased for 18 h because a combination of IFNγ and actinomycin D seems toxic to HUVECs at 24 h. As shown in Fig. 5, the half-life of IP-10 mRNA was approximately 12 h, while that of Mig as well as I-Tac was more than 18 h in endothelial cells. Interestingly,
lysoPC enhanced the decay of IP-10, Mig, and I-Tac mRNA (Fig. 6), indicating a decrease in the stability of the mRNA of these chemokines.

4. Discussion

In the present study, we demonstrated that lysoPC inhibited IP-10, I-Tac, and Mig mRNA expression induced by IFNγ. In addition, lysoPC inhibited the IFNγ-induced expression of STAT1α mRNA but not IRF-1, GBP-1, and p48 mRNA. The results suggest that lysoPC selectively inhibits some of the IFNγ-inducible genes in HUVECs. STAT-1 is a well-known transcription factor playing a pivotal role in IFNγ signalling. Recently, it has been revealed that IFNγ activates both STAT1-dependent and STAT-1-independent pathways [24]. IRF-1 is a transcription factor, which is induced by IFNγ, and is reported to be involved in the induction of GBP-1 gene expression [27,28]. Because lysoPC did not inhibit IRF-1 and GBP-1 mRNA expression induced by IFNγ, lysoPC appears to inhibit IRF-1-independent pathway(s) in HUVECs. We also demonstrated in the present study that lysoPC inhibited the mRNA expression of CXC chemokines induced by IFNγ, even STAT1α mRNA expression which was highly induced by IFNγ pretreatment. This result and the result that lysoPC did not inhibit STAT1-dependent IRF-1 expression suggest that STAT1α is not involved in the inhibitory effect of lysoPC.

Previous reports have shown that IFNγ regulates IP-10 and Mig mRNA expression at the transcriptional level in macrophage and fibroblast cell lines [25,30]. However, our data demonstrated that the transcriptional up-regulation of these chemokines by IFNγ alone was marginal in endothelial cells, although at the mRNA and protein level, it was induced effectively. Conversely, TNFα activated the IP-10 promoter effectively but did not induce IP-10 expression at the mRNA or protein level in HUVECs. These results suggest that IFNγ-induced IP-10 mRNA expression could involve posttranscriptional regulation. Indeed, some recent reports have shown that IFNγ can up-regulate gene expression by influencing posttranscriptional regulation, such as pre-mRNA splicing, mRNA stability, or unknown mechanisms [31,32]. The present study demonstrated that lysoPC enhanced the degradation of IP-10, Mig, and I-Tac mRNA induced by IFNγ. Taken together, it is likely that lysoPC destabilizes the mRNA of these chemokines accumulated by IFNγ via posttranscriptional mechanism(s).

Th1 cytokines, such as IFNγ and IL-2, are abundant in atherosclerotic lesions, especially early lesions, whereas very small amounts of Th2 cytokines, such as IL-4 and IL-10, are detected in advanced human lesions [33]. Recent reports have shown that Th1 cytokines are proatherogenic, and Th2 cytokines are potentially antiatherogenic. Zhou et al. [34] have demonstrated that apoE-deficient mice showed a Th1/Th2 switch at a very advanced stage in severe hypercholesterolemic conditions. Although there is no direct evidence, lysoPC-induced down-regulation of these Th1-directed CXC chemokines might contribute to the shifting of T cell responses from Th1 to Th2 in focal areas of atherosclerotic lesions. In addition, IP-10, I-Tac, and Mig are natural antagonists for CCR3 [35], a receptor for Th2-specific chemokines, including eotaxin, which is expressed in atherosclerotic lesions. Therefore, inhibition of CCR3 antagonists by lysoPC may promote Th2 cell accumulation in atherosclerotic lesions.

IP-10, I-Tac, and Mig also function as angiostatic factors [36]. Because plaque neovascularization is suggested to be an important factor promoting atherosclerotic progression and plaque rupture [37,38], inhibition of angiostatic CXC chemokines by lysoPC may promote the rupture of atherosclerotic plaques. In addition, IP-10 can induce the migration and proliferation of smooth muscle cells [39]. Therefore, the inhibition of IP-10 production by lysoPC might result in a reduction of smooth muscle cell migration and the secretion of collagen in the formation of the fibrous cap, which could lead to plaque rupture.

To our knowledge, there are few in vivo studies showing that lysoPC is involved in the development of atherosclerosis. Interestingly, lysoPC is implicated in the pathogenesis of autoimmune diseases, such as SLE [14–16], and patients with SLE exhibit a higher incidence of cardiovascular diseases [40]. In addition, recent studies suggested that autoimmunity could result from defects in the ability to scavenge apoptotic bodies [14,15]. In this regard, lysoPC is speculated to be a component of the phagocyte attraction signals that are secreted by cells undergoing apoptosis [14]. Aprahamian et al. [15] have studied the link between autoimmune disease and atherosclerosis and demonstrated that lysoPC delays the clearance of apoptotic cells, allowing cells to enter the late stages of apoptosis, and thereby promotes vascular inflammation and then the progression of atherosclerosis. Furthermore, mice lacking G2A, a receptor of lysoPC, develop lupus-like autoimmune disease [16]. So far, G-protein coupled receptors G2A and GPR4 have been identified as receptors of lysoPC [41,42]. G2A is expressed on T cells and macrophages, while GPR4 mRNA is expressed in endothelial cells (our unpublished data). To obtain evidence in vivo that lysoPC really plays a roll in the development of atherosclerosis, studies using apoE−/− mice crossed with G2A- or GPR4-deficient mice should be conducted.

In summary, the present report demonstrates, for the first time, that lysoPC selectively inhibits IFNγ-induced expression of IP-10, I-Tac, and Mig in cultured endothelial cells. Mechanisms responsible for the inhibitory effects of lysoPC involve, at least in part, a decrease in mRNA stability. The inhibition of IP-10, Mig, and I-Tac expression by lysoPC might be functionally implicated in the regulation of T cell-mediated immune responses, intima neovascularization, and fibrous cap formation during the development of atherosclerosis.
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