Lysophosphatidylcholine up-regulates CXCR4 chemokine receptor expression in human CD4 T cells

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Abstract: Oxidized low-density lipoprotein (OxLDL) is an inflammatory modulator in the atherosclerotic plaque. We examined the effect of lysophosphatidylcholine (lysoPC), a main phospholipid component of OxLDL, on inflammatory responses in human CD4 T cells. We found that lysoPC dose- and time-dependently increased expression of CXCR4, the chemokine receptor on CD4 T cells. This increase was inhibited by caffeic acid phenethyl ester or SN50, nuclear factor-κB inhibitors, and also by suppression of G2A expression, the specific receptor for lysoPC, using antisense oligonucleotide. LysoPC enhanced CD4 T cell chemotaxis in response to stromal cell-derived factor-1 (SDF-1), the exclusive ligand for CXCR4. LysoPC also enhanced SDF-1-stimulated production of inflammatory cytokines interleukin-2 and interferon-γ by CD4 T cells activated by anti-CD3 immunoglobulin G. In conclusion, this study demonstrates that lysoPC directly modulates inflammatory responses in human CD4 T cells. The data suggest that the presence of lysoPC and SDF-1 in atherosclerotic lesions may trigger inflammatory responses mediated by CD4 T cells, which may play an important role in progression of atherosclerosis. J. Leukoc. Biol. 76: 195–202; 2004.

Key Words: SDF-1 · atherosclerosis · lysophosphatidyl-choline · CXCR4 · CD4 · CD4 T cells

INTRODUCTION

Low-density lipoprotein (LDL) is subject to oxidative modification when retained by extracellular matrix proteins in the arterial wall [1]. Oxidized LDL (OxLDL) is a potent inflammatory modulator that triggers innate and adaptive immune responses in the atherosclerotic lesion. The innate immune response is the first to occur, which includes the activation of macrophages and vascular resident cells (vascular smooth muscle cells and endothelial cells) and production of various proinflammatory molecules. Activation of CD4 T cells by OxLDL is part of the subsequent adaptive immune response and involves cytokine production and expression of surface molecules that initiate activation of B cells. OxLDL is recognized by scavenger receptors and the Toll-like receptor family in antigen-presenting cells (APCs) and is phagocytosed by APCs. The complex of oxidized epitopes and major histocompatibility complex class II molecules that are presented on the cell surface activates CD4 T cells to become effector T cells that are able to secrete inflammatory cytokines such as interferon-γ (IFN-γ) [2, 3]. CD4 T cells producing T helper type 1 (Th1) cytokines such as IFN-γ [2, 4] predominate in advanced atherosclerotic lesions, suggesting the CD4 T cells with Th1 characteristics are recruited to the vascular wall and may play a pivotal role in the development of atherosclerosis. In support of this hypothesis, atherosclerosis-prone mice that lack the IFN-γ receptor developed less atherosclerosis [5], and exogenous IFN-γ accelerated formation of atherosclerotic plaques [6].

Infiltration and accumulation of T cells into the vascular wall occur at the earliest stage of atherosclerosis [2]. More than two-thirds of T lymphocytes in atherosclerotic plaques were identified as memory (CD45RO+) T cells [7]. Unactivated CD4 T cells are also found in atherosclerotic plaques, although their role is not known. It appears CD4 T cell functions may be modulated directly by oxidized epitopes and chemokines. Treatment of anti-CD3-activated CD4 T cells with lysophosphatidylcholine (lysoPC), a main phospholipid component in OxLDL, was shown to enhance CD40 ligand (CD40L) expression [8] and cytokine-induced IFN-γ expression [9] in vitro in the absence of APCs.

CD4 T cells express CXCR4, a member of the CXC chemokine receptor family. Stromal cell-derived factor-1 (SDF-1), an exclusive ligand for CXCR4, was shown to be highly expressed in atherosclerotic lesions [10]. SDF-1 is a chemoattractant for CD4 T cells and stimulates chemotaxis in vitro [11]. SDF-1 was shown to enhance cell membrane CD40L expression and enhance production of inflammatory cytokines IFN-γ and interleukin (IL)-12 in anti-CD3-activated CD4 T cells [12].

It is likely that CD4 T cells in atheromatous plaques can become activated as a result of simultaneous exposure to SDF-1 and OxLDL, as macrophages in the plaque express SDF-1 and induce oxidative modification of LDL. In the present study, we found that OxLDL and its main component lysoPC up-regulated CXCR4 expression in CD4 T cells. The treatment of CD4 T cells with lysoPC enhanced SDF-1-stimu-
lated chemotaxis and inflammatory cytokine production in CD4 T cells. These data indicate that lysoPC is a positive regulator of inflammatory responses in human CD4 T cells.

MATERIALS AND METHODS

Fresh whole blood was obtained from healthy donors. Whole blood (3 ml) in 3 mM EDTA was layered on Picoll Hypaque (1:1 v/v, d=1.077 g/ml, Sigma Chemical Co., St. Louis, MO). The mononuclear cellular layer was cellular after centrifugation (600 g, 22°C, 15 min) and twice washed with excess phosphate-buffered saline (PBS) containing 0.1% bovine serum albumin (BSA) and 0.2% EDTA (400 g, 4°C, 15 min). Untouched CD4 T cells were immediately isolated using the mini-magnetic cell sortor microbead cell isolation system (Miltenyi Biotec, Auburn, CA). The purity of isolated CD4 T cells was ~90%, as estimated by flow cytometry with fluorescein isothiocyanate-labeled monoclonal antibody against human CD4 (PharMingen, San Diego, CA), and the number of CD14(+) monocytes in the preparation was less than 1%

Isolated CD4 T cells were cultured in RPMI-1640 medium (Irvine Scientific, Santa Ana, CA) containing 10% human lipoprotein-deficient serum (LPDS) and antibiotics (penicillin, 100 units/ml, and streptomycin, 100 µg/ml, Irvine Scientific) for up to 72 h. The cell concentration was maintained lower than 5 x 10^6 cells/ml, and the proportion of dead cells was less than 5% during the maintenance of cells.

Preparation of lipoproteins

Human LDL (d=1.033-1.063 g/ml) were isolated from human plasma by ultracentrifugation as described previously [13] and stored at 4°C in 1 mM EDTA. OxLDL, was prepared by incubation of EDTA-free LDL (100 µg protein/ml) in Ham’s F-10 medium with 10 µM CuSO4 for 24 h at 37°C. Non-OxLDL, contained less than 0.3 nmol thiobarbituric acid-reactive substances (TBARS)/mg protein, as determined by fluorometric assay [14]. OxLDL preparations contained 5 nmol TBARS/mg protein. 1-Palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphorylcholine (POVPC) was a kind gift from Dr. Mi-Kyung Chang (University of California, San Diego). Lipoprotein preparations did not contain more than 0.1 ng/ml endotoxin, as determined using a timed gel formation assay kit (Sigma Chemical Co.).

Analysis of CXCR4 transcription

Total RNA was isolated from human circulating CD4 T cells by guanidine thiocyanate-phenol-chloroform extraction [15], and cDNA was generated by reverse transcription using Superscript II (Roche Diagnostics GmbH, Germany). The amount of CXCR4 cDNA was estimated by semiquantitative polymerase chain reaction (PCR) amplification using the sense primer 5’-CTCTGGCCACCATTCTACTC-3’ and the antisense primer 5’-TCCTGTGATTCCAGGAG-3’. As an internal standard, human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was amplified using the sense primer 5’-TGGCACTCAAGGGTGGTGGTA-3’ and the antisense primer 5’-ATGGACTGTCAGACTGCTTCTC-3’. The PCR reaction was performed for 30 cycles, and the condition of each cycle was as follows: 94°C for 1 min, 58°C for 1 min, and 72°C for 2 min. To estimate the relative amount of transcripts, real-time PCR with SYBR Green I was performed using a LightCycler rapid thermal cycler system (Roche Diagnostics Ltd., Lewes, UK) following the manufacturer’s instructions. These PCR's were performed for 40 cycles, and each cycle was set as follows: 95°C for 5 s, 60°C for 5 s, and 72°C for 5 s. Detection of the fluorescent product was performed at end-point of the 72°C extension period, and the Ct value (the cycle number at which emitted fluorescence exceeded an automatically determined threshold) was recorded. Data represent mean ± SD of values, which were adjusted Ct values for CXCR4 that were corrected by Ct values for GAPDH.

Analysis of CXCR4 protein levels by flow cytometry

CD4 T cells were washed twice with 3 ml ice-cold PBS containing 0.1% BSA and 0.01% sodium azide (w/v, buffer A). To determine the amount of CXCR4 protein on the cell surface, 10^5 cells in 100 µl buffer A were incubated with 0.5 µg phycoerythrin (PE)-labeled mouse immunoglobulin G (IgG) against CXCR4 (PharMingen) at 4°C for 30 min. Cells were washed with 3 ml buffer A, and cell-associated fluorescence was measured and analyzed using a FACScan with CellQuest software (Becton Dickinson, San Jose, CA). In control experiments, PE-conjugated, nonspecific mouse IgG was used to measure nonspecific binding. The relative surface expression of CXCR4 was estimated by subtracting the mean fluorescence intensity (MFI) of control cells from that of cells labeled with anti-CXCR4 antibody.

In a subset of experiments, isolated CD4 T cells were pretreated with antisense and sense sequences for G2A, the lysoPC receptor. Those sequences were selected from near the 5’ end of the G2A mRNA sequence and did not bind to other mRNAs to form secondary structures. The sequences of antisense and sense oligomers were 5’-ATTGGGCACTCATCATTCT-3’ and 5’-AGAATTGAATGCCCCATT-3’, respectively, and were purified by high-pressure liquid chromatography before use (Bioneer, Taejeon, South Korea). Cells in RPMI 1640 containing 5% fetal bovine serum were incubated with 1.0 µM sense or antisense oligonucleotide overnight, after which cells were incubated with 10 µg/ml lysoPC for 24 h.

Chemotaxis assay

CD4 T cell chemotaxis in response to SDF-1 was measured using 48-well microchemotaxis Boyden chambers (Neuroprobe, Gaithersburg, MD) as described previously [16]. Briefly, 10 nM SDF-1 in chemotaxis buffer [Tyrodes’ salt buffer (Sigma Chemical Co.) with 1% NaHCO3, and 0.1% BSA, pH 7.4] was added to the lower chamber, and the microporous polycarbonate membrane (5 µm pore size, Portex) was placed between the upper and lower chambers. After prewarming the assembled chemotaxis unit for 30 min at 37°C in a 5% CO2 humidified incubator, 10^5 CD4 T cells (2 x 10^6 cells/ml) in chemotaxis buffer were added to the upper chamber. After incubation for 150 min at 37°C in a 5% CO2 incubator, migrated cells bound to the polycarbonate membrane were fixed with 2% glutaraldehyde in PBS and stained with 0.01% crystal violet. Cells were counted in four random high-power fields (x400). In control experiments, lower chambers did not contain SDF-1.

Measurement of cytokine levels

CD4 T cells were placed in 24-well plates coated with anti-CD3 mouse IgG (UCHT1 clone, PharMingen; 104 cells/well) for 24 h in the presence or absence of lysoPC (10 µg/ml). Cells were then stimulated with SDF-1 (1–10 nM) for 6 h, and the culture media was harvested and kept frozen at ~70°C until further analysis. Enzyme-linked immunosorbent assay (ELISA) kits (R&D Systems, Minneapolis, MN) were used to measure levels of cytokines (IL-2, IFN-γ, and IL-4) in the harvested medium. ELISA kits assays did not cross-react with other cytokines, and the endotoxin level in the cell culture medium was less than 0.1 ng/ml.

RESULTS

lysoPC enhances CXCR4 expression in human CD4 T cells

Human CD4 T cells were cultured for up to 48 h in the presence or absence of OxLDL (up to 20 µg protein/ml) in RPMI-1640 medium containing 1% human LPDS, and CXCR4 surface expression levels were determined by flow cytometry. The purity of the isolated CD4 T cells was ~90%, and red blood cells were the largest contaminant. About 30% of CD4(+) cells were shown to express CXCR4. Subsequent flow cytometry analysis with CXCR4(+) CD4 T cells showed that CXCR4 surface expression levels were enhanced by incubation with OxLDL as a time- and dose-dependent manner (Fig. 1A), and the proportion of CXCR4(+) cells in CD4 T cells was stationary. The treatment of CXCR4(+) CD4 T cells with 20 µg protein/ml OxLDL for 48 h maximally enhanced
CXCR4 surface expression levels fourfold (Fig. 1B). Steady-state CXCR4 mRNA levels in CD4 T cells were estimated by reverse transcriptase (RT)-PCR and real-time PCR and compared with that of the housekeeping gene GAPDH. The treatment of CD4 T cells with OxLDL significantly increased the amount of CXCR4 transcripts in a dose-dependent manner (Fig. 1C).

lysoPC is a main component of OxLDL. We found that like OxLDL, lysoPC (up to 10 μg/ml for 24 h) increased CXCR4 amounts of proteins and transcripts in CXCR4(+) CD4 T cells in a dose-dependent manner (Fig. 2). Real-time PCR analysis showed that lysoPC increased CXCR4 transcript expression up to fourfold in CD4 T cells. By contrast, the main phospholipid in mild OxLDL such as POVPC and freshly prepared non-OxLDL did not change CXCR4 surface expression (Fig. 3) nor CXCR4 mRNA amounts (data not shown). Similarly, a 24-h incubation with 15-deoxyPGJ2, which is a nonspecific ligand for peroxisome proliferator-activated receptor (PPAR)y, or BRL49653, a specific PPARy ligand, did not change CXCR4 surface expression. As described previously [17], CXCR4 protein on the cell surface of CD4 T cells was rapidly internalized by incubation with PMA (Fig. 3).

lysoPC-stimulated CXCR4 expression involves the nuclear factor (NF)-κB pathway

Pretreatment of human CD4 T cells with a NF-κB inhibitor, caffeic acid phenethyl ester (CAPE; 10 μg/ml), almost completely abolished the positive regulatory effect of lysoPC on CXCR4 surface expression. Treatment of the isolated CD4 T cells with NF-κB SN50, a permeable peptide that inhibits the translocation of NF-κB active complex into the nucleus (Calbiochem, San Diego, CA), also inhibited lysoPC-stimulated CXCR4 surface expression (Fig. 4). Flow cytometry analysis of cells after propidium iodide staining showed that a 24-h incubation with CAPE did not affect cell viability. Inhibition of PKC by calphostin (100 nM) or tyrosine kinases by genistein (10 μM) did not reduce lysoPC-stimulated CXCR4 expression. The treatment with genistein enhanced the surface-expression level of CXCR4 in a dose-dependent manner (Fig. 4) without changing the CXCR4 mRNA levels (data not shown). Pretreatment with C. difficile toxin, U0126, or TMB-8 to inhibit the activity of Rho proteins MEK and intracellular calcium release, respectively, did not affect lysoPC-stimulated CXCR4 expression (Fig. 4).

**Fig. 1.** OxLDL up-regulates CXCR4 expression. (A) CD4 T cells were incubated with OxLDL and were labeled with PE-conjugated mouse IgG specific for CXCR4 (Anti-CXCR4 IgG). PE-conjugated, nonspecific mouse IgG (Control IgG) was used to measure nonspecific binding. Histograms representing distribution of cell-associated fluorescence in CXCR4(+) CD4 T cells are shown. (B) The cell-associated MFI of OxLDL-treated CXCR4(+) CD4 T cells was measured by flow cytometry. Data shown represent the relative surface expression of CXCR4, which was estimated by subtracting the MFI of control cells from that of cells labeled with antibodies detecting CXCR4. ***, P < 0.01, versus control, as determined by a Mann-Whitney U-test. (C) Total RNA was harvested from the CD4 T cells incubated with OxLDL for 24 h, and cDNA was generated by reverse transcription. The amount of CXCR4 cDNA was estimated by semiquantitative PCR and real-time PCR methods as described in Materials and Methods. Real-time PCR data represent mean ± SD values of ΔCt. †P < 0.05 versus control, as determined by a Mann-Whitney U-test.
lysoPC-stimulated CXCR4 expression involves the G2A receptor

The lymphocyte G protein-coupled receptor, G2A, is involved in binding and uptake of lysoPC [18]. Our preliminary studies showed that lysoPC (up to 20 μg/ml for 24 h) did not affect G2A transcript levels (data not shown). To evaluate the role of G2A in the lysoPC-induced up-regulation of CXCR4 expression, CD4 T cells were incubated with G2A-specific antisense and sense oligonucleotides (1.0 μM) for 24 h, and then 10 μg/ml lysoPC was added. Pretreatment with G2A-specific antisense oligonucleotides reduced G2A expression (Fig. 5A) and inhibited the positive, regulatory effects of lysoPC on CXCR4 surface expression (Fig. 5B). Pretreatment of CD4 T cells with G2A-specific sense oligonucleotides or oligonucleotides with 20 random nucleotide sequences did not reduce G2A mRNA expression nor change lysoPC-stimulated CXCR4 expression (data not shown), suggesting the inhibition of lysoPC-stimulated CXCR4 expression is specifically a result of G2A down-regulation.

lysoPC enhances CD4 T cell chemotaxis in response to SDF-1

CD4 T cells were pretreated with lysoPC (up to 10 μg/ml) for 24 h, and the change of chemotactic activities in response to SDF-1 (10 nM) was estimated by chemotaxis assay with microchemotaxis Boyden chamber. The treatment of CD4 T cells with lysoPC significantly enhanced SDF-1-mediated chemotaxis up to 2.5-fold (Fig. 6). The calculated ED50 value of lysoPC that enhanced the SDF-1-mediated chemotactic activity of CD4 T cells was 2.0 μg/ml. The treatment of CD4 T cells with lysoPC did not increase the spontaneous migration of CD4 T cells.

lysoPC and costimulation with SDF-1 enhance secretion of proinflammatory cytokines by anti-CD3-activated CD4 T cells

Pretreatment of anti-CD3-activated CD4 T cells with lysoPC (10 μg/ml for 24 h) dose-dependently increased IL-2 and IFN-γ production as previously reported [9]. We found that pretreatment of anti-CD3-activated CD4 T cells with lysoPC (10 μg/ml for 24 h) also enhanced CXCR4 surface expression up to twofold (data not shown), SDF-1 stimulated IL-2 and
IFN-γ production, and SDF-1 did not stimulate IL-4 production (Fig. 7). Neither lysoPC nor SDF-1 alone induced cytokine secretion by CD4 T cells unless they were not activated by anti-CD3 antibody (data not shown).

**DISCUSSION**

The present study showed that OxLDL increased expression of the key chemokine receptor CXCR4 in human CD4 T cells. This study also indicated that OxLDL regulates T lymphocyte function. Furthermore, it appears that lysoPC, which reportedly has a large number of proinflammatory and proatherogenic properties, was largely responsible for the OxLDL-induced up-regulation of CXCR4 expression in CD4 T cells.

LysoPC is a major phospholipid component in OxLDL [19, 20] and is generated by oxidation and fragmentation of PC polyunsaturated sn-2 fatty acyl residues, followed by hydrolysis of shortened fatty acyl residues by LDL-associated phospholipase A2 (PLA2) [21, 22], an enzyme that is an independent predictor of coronary heart disease [23]. Although lysoPC constitutes only 1–5% of total PC in non-OxLDL, oxidative modification of LDL can raise the lysoPC proportion to as high as 40–50% [24]. That non-OxLDL did not alter CXCR4 ex-

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**Fig. 4.** LysoPC-stimulated CXCR4 expression involves the NF-κB pathway. CD4 T cells were incubated with lysoPC for 24 h in the presence or absence of genistein (a tyrosine kinase inhibitor), calphostin [a protein kinase C (PKC) inhibitor], CAPE (a NF-κB inhibitor), NF-κB SN50 (a specific, permeable NF-κB-inhibiting peptide), Clostridium difficile toxin (C; Rho inhibitor), U0126 [U; mitogen-activated protein kinase (MAPK) kinase (MEK) inhibitor], or TMB-8 (T; blocking calcium release from intracellular store). The expression level of CXCR4 on cell surface was estimated using the flow cytometry method as described in Figure 1 and was expressed as percent of CXCR4 expression in untreated control CD4 T cells. Data represent the mean ± SD of three independent experiments. *, P < 0.05; **, P < 0.01; NS, not significant, as determined by a Mann-Whitney U-test; †, P < 0.01, as determined by one-way ANOVA.

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**Fig. 5.** LysoPC-stimulated CXCR4 expression involves G2A. (A) CD4 T cells were pretreated overnight with 1.0 μM sense or antisense oligonucleotides specific for the G2A sequence. G2A expression was then estimated by semiquantitative RT-PCR. GAPDH was used as an internal standard. Control, Untreated human CD4 T cells. (B) CD4 T cells pretreated with sense or antisense oligonucleotides specific for the G2A sequence were further treated with 10 μg/ml lysoPC for 24 h. CXCR4 surface expression was estimated using the flow cytometry method described in Figure 1 and was expressed as percent of CXCR4 expression in untreated control CD4 T cells. Data represent the mean ± SD of three independent experiments. *, P < 0.05, as determined by a Mann-Whitney U-test.
pression in the present study suggests the amount of lysoPC in LDL preparations was too low to affect cells. It is interesting that other OxLDL derivatives, such as POVPC, did not affect CXCR4 expression. POVPC, a type of oxidized 1-palmitoyl-2-ara-chidonoyl-sn-glycero-3-phosphorylcholine (PAPC), is the main oxidized phospholipid in mildly oxidized LDL. The bioactivity of POVPC is abolished by further oxidative processes, i.e., hydrolysis with PLA2 [25]. Therefore, our study results suggest that the lysoPC, a main component of fully oxidized LDL, is mainly responsible for CXCR4 up-regulation in CD4 T cells. Unlike oxidized PAPCs, there is little evidence that lysoPC activates PPARs. We confirmed that nonoxidized derivatives of PPARγ agonists (PGJ2 and BRL49653) did not induce CXCR4 up-regulation, suggesting the effect of OxLDL is not through PPARγ activation.

Recently, a subfamily of G protein-coupled receptors that bind lysophospholipids, i.e., G2A and GPR4, was cloned in T cells. G2A and GPR4 have also been cloned in CD4 T cells [18], and lysoPC was found to preferentially bind G2A with high affinity [18, 26]. lysoPC-induced G2A activation reportedly modulates T cell function, possibly affecting atherosclerotic lesion formation. T cells isolated from G2A-knockout mice exhibited hyperproliferative responses to antigen receptor stimulation in vitro [27]. Activation of G2A with lysoPC triggered RhoA-mediated actin polymerization [28, 29] and directly induced chemotactic migration of G2A (+) Jurkat T cells [18]. The present study suggests binding and uptake of lysoPC through G2A may be involved in CXCR4 up-regulation, as functional ablation of G2A with specific antisense oligonucleotides inhibited lysoPC-stimulated CXCR4 expression. However, it is yet to be determined whether transduction signals elicited by lysoPC-induced G2A activation may directly induce CXCR4 up-regulation. A previous report described that ligand-induced activation of G2A triggered intracellular calcium and the activation of Rho small G protein via Gα13 [28]. Extracellular-regulated kinase and MAPK were mainly activated in G2A-transfected cells after treatment with lysoPC [18]. However, in the present study, inhibition of intracellular calcium release, MEK activity, and the activity of Rho proteins did not affect lysoPC-stimulated CXCR4 expression in CD4 T cells. Instead, we found that lysoPC-stimulated CXCR4 expression was mediated by signaling pathways involving NF-κB. Although the binding of lysoPC to G2A does not directly trigger basal NF-κB-luciferase activity [30], cytosolic lysoPC directly induces dissociation of NF-κB from inhibitor of κB and subsequent translocation to the nucleus in a macrophage cell line [31]. Taken together, the amount of lysoPC in the cytosol, rather than G2A-derived signal transduction per se, may be an important factor determining CXCR4 levels in CD4 T cells. We unexpectedly found that blocking tyrosine kinase activities profoundly increased CXCR4 surface expression. Others report that tyrosine kinases are involved in CXCR4-mediated T cell migration [32] and that tyrosine-specific phosphorylation of CXCR4 in monocytes/macrophages results in cytokine-

**Fig. 6.** The effect of lysoPC on chemotactic activity of CD4 T cells, which were preincubated with lysoPC for 24 h, and their migration in response to 10 nM SDF-1 or spontaneous migration in the absence of SDF-1 (Buffer only) was estimated in a microchemotaxis Boyden chamber as described in Materials and Methods. Counting cells in four random high-power (400×) fields determined the number of migrated cells attached to the filter underside. Data represent the mean ± sd of three independent experiments, *, P < 0.05, as determined by a Mann-Whitney U-test.

**Fig. 7.** Cytokine production by anti-CD3-activated CD4 T cells after costimulation with lysoPC and SDF-1. CD4 T cells were immobilized overnight on plates coated with anti-human CD3 antibody, in the presence (solid bars) or absence (shaded bars) of 10 μg/ml lysoPC. The cells were further stimulated with given concentrations of SDF-1 (1 or 10 nM) for 6 h, and the culture media was harvested for measurement of IL-2 (A), IFN-γ (B), and IL-4 (C) by ELISA. *, P < 0.05; ***, P < 0.01, as determined by a Mann-Whitney U-test.
induced endocytosis of CXCR4 [33]. We are currently investigating whether ligand-independent endocytosis of CXCR4 in CD4 T cells is dependent on tyrosine kinase activity.

Lysophosphatidylcholine (lysoPC) is found abundantly in atheromas [34, 35] and is a well-known, inflammatory mediator. LysoPC itself is a chemottractant for monocytes and T cells [36, 37] and induces growth factor gene expression in monocytes [38] and proliferation of macrophages [39]. Lysophosphatidylcholine inhibits arterial relaxation induced by endothelium-derived relaxing factor [40] and stimulates expression of adhesion molecule and growth factor genes by endothelial cells [41, 42]. Our study showed that up-regulation of adhesion molecule and growth factor genes by macrophages [39]. LysoPC inhibits arterial relaxation induced by endothelial cell-derived relaxing factor gene expression in monocytes [38] and proliferation of monocytes and T cells [36, 37] and induces growth factor gene expression in macrophages [10, 35], exposure to lysoPC may help recruited CD4 T cells migrate to and colocalize with APCs in the inflammatory lesion. Second, we found that lysoPC enhanced SDF-1-mediated chemotaxis of CD4 T cells may not contribute to CD4 T cell recruitment from the circulation into atherogenic vessel walls in vivo. Instead, as SDF-1 and lysoPC in atherosclerotic plaques are strongly associated with areas abundant in macrophages [10, 35], exposure to lysoPC may help recruited CD4 T cells migrate to and colocalize with APCs in the inflammatory lesion. Second, we found that lysoPC enhanced SDF-1-mediated chemotaxis of CD4 T cells with lysoPC was shown previously to induce IFN-γ production [9]. The present study further shows the inflammatory role of lysoPC, which potentiated the SDF-1-mediated production of inflammatory cytokines IL-2 and IFN-γ by stimulated anti-CD3-activated CD4 T cells. This effect appeared specific, as IL-4 production was not affected. Most T cells in atherosclerotic lesions are CD3+CD4+ T cell receptor αβ+ cells and are largely of the Th1 (helper) subtype, which secretes IFN-γ, IL-2, and tumor necrosis factor α and β [7, 43]. Abundant, proinflammatory signals generated from Th1-like CD4 T cells in part are known to aggravate inflammation in the atherosclerotic lesion by activating macrophages and resident vascular cells [2, 3]. Therefore, the costimulation with lysoPC and SDF-1 may change the characteristics of recruited, naïve CD4 T cells similar to CD4 T cells with Th1 characteristics and may eventually exacerbate the process of atherogenesis.

In summary, our results provide novel evidence that lysoPC up-regulates CXCR4 expression in CD4 T cells. LysoPC enhances SDF-1-stimulated chemotaxis and inflammatory cytokine production by CD4 T cells. These data may describe a mechanism by which immunologically naïve T cells in atherosclerotic lesions are activated and produce inflammatory molecules. The colocalization of lysoPC and SDF-1 in atherosclerotic lesions may amplify proinflammatory responses through activation of CD4 T cells and may eventually contribute to atherogenesis.

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