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Lysophosphatidylcholine: a chemoattractant to human T lymphocytes

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Abstract. Various cell stimuli act through activation of phospholipase A_2 resulting in the release of arachidonic acid, the precursor of eicosanoids, from the sn-2 position of cell membrane phospholipids. A byproduct of phospholipase A_2 activity is the lysophospholipids which have been found to potentiate T-lymphocyte activation. The purpose of the present study was to determine whether the various lysophospholipids modulate the migration of peripheral normal human T lymphocytes in vitro. It was found that lysophosphatidylcholine (lysoPC) induced T-lymphocyte migration in the concentration range 10^{-7} to 10^{-4} *M* with a maximum at 10^{-6} *M* (mean chemotactic index, 2.06). The migration was due to chemotaxis rather than chemokinesis. In contrast, lysophosphatidylethanolamine (lysoPE) and lysophosphatidylinositol (lysoPI) did not exhibit chemotactic properties towards T lymphocytes. Further studies showed that the length of the fatty acids in the sn-1 position as well as the presence of double bonds modulated the chemotactic ability. The lysoPC compound with the highest chemotactic activity was lysoPC;1-palmitoyl (C = 16:0). The results demonstrated that lysoPC, a phospholipase A_2-generated hydrolysis product of phosphatidylcholine, induced T-lymphocyte chemotaxis in vitro. Because phosphatidylcholine is the major phospholipid in the epidermis, the activation of phospholipase A_2 may result in the release of lysoPC in concentrations capable of inducing migration of T lymphocytes into the epidermis.

Key words: Lysophosphatidylcholine – Chemotaxis – Lysophospholipids – T lymphocytes

T lymphocytes play an essential role in the initiation and maintenance of immunological and inflammatory processes. T-lymphocytic infiltrates are characteristic for many inflammatory skin diseases, including psoriasis. In order to induce their effects, T lymphocytes must migrate through the capillary vessels and enter the dermis and epidermis, as is observed in the early stages of the development of psoriatic lesions [15].

Several T-lymphocyte chemoattractant cytokines [4] and eicosanoids [3] have been detected in psoriatic skin lesions. The rate-limiting step in eicosanoid metabolism is the release of arachidonic acid from its ester bond in cell membrane phospholipids by the action of phospholipase A_2. While the arachidonic acid metabolites such as leukotriene B_4 are well characterized, much less is known about the effects of the phospholipase A_2-generated hydrolysis products of phospholipids (lysophospholipids). One of the major lysophospholipid classes lysophosphatidylcholine (lysoPC) exhibits chemotactic activity towards monocytes, but not towards neutrophils [13]. LysoPC D- and L isomers have the same monocyte chemotactic activity, indicating a lack of stereospecificity at the receptor level. Using lysoPC analogues, it has been found that a hydrolysable binding vicinal to the phosphocholine group is required for the expression of chemotactic activity [14]. Furthermore, lysoPC induces the expression of adhesion molecules for mononuclear cells, VCAM-1 and ICAM-1, in cultured rabbit and human arterial endothelial cells [10]. With regard to T lymphocytes, lysoPC greatly potentiates the activation of protein kinase C in resting human T lymphocytes [1].

The purpose of this study was to determine whether lysophospholipids with different bases in the sn-3 position (lysoPC, lysophosphatidylethanolamine, lysoPE; lysophosphatidylinositol, lysoPI) are chemoattractants for T lymphocytes, and whether the fatty acid in the sn-1 position has any influence on the chemotactic properties.

Materials and methods

Reagents

Phosphatidylcholine (PC, 99%), lysoPC;1-caproyl (99%), lysoPC; 1-stearoyl (99%), lysoPI; 1-palmitoyl (99%), lysoPE; 1-palmitoyl (99%) and coomasie brillant blue were all from Sigma Chemicals, (St. Louis, Mo.). LysoPC;1-palmitoyl was from Larodan, Malmø, Sweden. Isopaque Ficoll was purchased from Pharmacia LKB, Biotechnology, Uppsala, Sweden.

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T-lymphocyte purification

Venous blood (100 ml) was obtained from healthy young individuals. Mononuclear cells were separated on Isopaque Ficoll, washed once with Hank's medium and centrifuged at 1300 rpm for 10 min. The T-lymphocyte fraction was obtained by the nylon wool method for T-lymphocyte purification as previously described [9]. The T lymphocytes had a purity of > 95% as determined by FACS analysis.

Chemotactic assay

The chemotactic activity of the T lymphocytes was analysed using a 48-well multiwell Boyden chemotaxis chamber as previously described [8]. The chemoattractants were dissolved in ethanol and then serially diluted in RPMI-1640 containing 100 U/ml penicillin, 100 µg/ml streptomycin, 20 m*M* L-glutamine and 0.5% bovine serum albumin (BSA) leaving the ethanol concentration at a maximum of 2% in the lower chambers. A volume of 27 µl was added to the lower wells with three wells for each concentration tested. The T lymphocytes were resuspended to a concentration of $5 \times 10^{6/7}$ cells/ml in the medium and 50 µl was added per well in the upper chambers. The filter (polyvinylpyrrolidone-free polycarbonate membrane with a 5-µm pore size; Nucleopore, Pleasanton, Calif., USA) was placed between the upper and lower chambers with the side coated with collagen IV (mouse type IV collagen; Gibco (BRL, Gaithersburg, Md., USA)) facing downward. The chambers were incubated at 37°C in an atmosphere containing 5% CO_2 for 2 h. For the time-course study the incubation period varied from 15 min to 120 min. The cells that had moved to the lower surface of the membrane were fixed in 70% methanol, dried and stained with coomasie brilliant blue. Two representative grid areas were chosen per well, and the cells were counted manually. Each assay was performed six times. The chemotactic activity was expressed as the chemotactic index (CI), defined as the number of cells migrating in response to the test substance divided by the the number of cells migrating when only control medium is present. In order to examine whether migration was due to chemokinesis rather than chemotaxis, a modified checkerboard analysis was done. In this analysis the top and bottom wells contained equal concentrations of test substance. As the gradient over the membrane was abolished in this situation any migration in relation to the control was the result of chemokinesis.

Cell viability assay

T-lymphocytes ($n = 3$) were separated as previously described [8]. The T lymphocytes were diluted to 1×10^6 cells/ml in 96-well trays to give 100 µl cells suspension per well in RPMI-1640 supplemented with 2% FCS, 6.06 mg/ml penicillin, 10 mg/ml streptomycin and 2.5 mg/ml gentamicin. A series of dilutions (10^{-7}–10^{-4} *M*) of the chemoattractants (lysoPI;1-palmitoyl, lysoPE;1-palmitoyl, lysoPC;1;caproyl, lysoPC;1-palmitoyl, lysoPC;1-oleyl, lysoPC;1-stearoyl, PC) used in the chemotactic assays were added to the wells. The trays were incubated at 37°C in an atmosphere containing 5% CO_2 for 2 h after which 25 µl of a 5 mg/ml stock solution of MTT (3-(4,5-dimethyl-2-yl)-2,5-diphenyltetrazolium bromide, Sigma) was added to each well and cultured for 2 h under the conditions mentioned previously. Extraction buffer (100 µl) was added (12.5% SDS, 45% *N,N*-dimethyl formamide, PBS) to each well and the trays were incubated for 24 h. The optical density was measured at 570 nm using the medium as a negative control and 70% ethanol as a positive control. An ethanol concentration of 2% without the chemoattractants was also tested for toxicity.

Statistical analysis

For each concentration of test substance the mean and standard deviation (SD) were determined and compared with the controls. If the data were distributed parametrically as assessed by the normality and equal variance test a paired *t*-test was performed. In cases where they were not, a Mann-Whitney rank sum test was used. A *P* value below 0.05 (one-tailed) was considered significant.

Results

The percentage of cells which migrated in response to the medium was only 8% of the total cell number added to the upper wells. The average cell count of a grid area where cells had migrated towards the medium was 110 ± 32 (mean ± SD). The checkerboard analysis showed that the cell migration observed was due to chemotaxis and not to chemokinesis (Table 1). Cell viability was assessed by measuring the mitochondrial dehydrogenase activity

Table 1. Chemotactic and chemokinetic activities of lysoPC forms

$\Delta_{conc.}$ (*M*)	LysoPC; 1-oleyl (CI)	LysoPC; 1-stearoyl (CI)	LysoPC; 1-palmitoyl (CI)
10^{-4}	1.08 ± 0.15	1.30 ± 0.26	1.55 ± 0.32
0	0.98 ± 0.09	1.10 ± 0.10	1.00 ± 0.12
10^{-5}	1.40 ± 0.06	1.50 ± 0.30	1.67 ± 0.41
0	0.91 ± 0.08	0.98 ± 0.07	0.88 ± 0.22
10^{-6}	1.68 ± 0.19	1.94 ± 0.43	2.06 ± 0.29
0	0.93 ± 0.08	1.00 ± 0.09	0.95 ± 0.05
10^{-7}	1.20 ± 0.07	1.66 ± 0.42	1.67 ± 0.38
0	0.85 ± 0.09	0.88 ± 0.10	1.10 ± 0.08
10^{-8}	1.13 ± 0.17	1.25 ± 0.28	1.43 ± 0.52
0	0.88 ± 0.07	0.89 ± 0.05	0.94 ± 0.10

$\Delta_{conc.}$, Concentration of test substance (*M*) in lower chamber – concentration of test substance (*M*) in upper chamber; CI, chemotactic index

The values for the modified checkerboard analysis ($\Delta_{conc.} = 0$) are mean ± SEM of three experiments performed in triplicate

Table 2. Results of the MTT assay with the test substance at a concentration of 10^{-6} *M*

Agonist	Absorbance at 570 nm (arbitrary units)
Medium	0.255
Etanol 70% in medium	0.073
LysoPI; 1-palmitoyl	0.251
LysoPE; 1-palmitoyl	0.247
LysoPC; 1-caproyl	0.248
LysoPC; 1-palmitoyl	0.252
LysoPC; 1-oleyl	0.244
LysoPC; 1-stearoyl	0.252
PC	0.245

The absorbance at 570 nm had a value of 0 of an empty chamber and a value of 0.255 when the chamber contained T-lymphocytes in medium. At the other concentrations tested (10^{-10}–10^{-4} *M*) the results were similar, with a maximum cytotoxicity at 4.30%. Values are the means of three experiments performed in triplicate. The difference between the mean value and the results obtained by each of the three independent experiments was less than 10% with all agonists at all concentrations

(MTT assay) in the concentrations used for the chemotactic assays (10^{-10}–10^{-4} *M*) [12]. All the chemoattractants tested were non-toxic in this concentration range (Table 2). The culture medium containing 2% ethanol was also negative.

T-lymphocyte chemotactic activity of different lysoPC classes

Figure 1 shows the T-lymphocyte chemotactic properties of different lysophospholipid classes (lysoPC, lysoPI, lysoPE). LysoPC proved to be the only lysophospholipid exhibiting chemotactic properties. It was chemotactic in the concentration range 10^{-7} to 10^{-4} *M* with a maximum at 10^{-6} *M*, where the CI was on average 2.06. LysoPI and lysoPE caused only minor fluctuations in T-lymphocyte chemotaxis (Fig. 1). In contrast to lysoPC, PC was not T-lymphocyte chemotactic in concentrations from 10^{-10} to 10^{-4} *M* (data not shown). For comparison we tested the T-lymphocyte chemotactic properties of fMLP at 10^{-8} *M*, where the CI was a maximum (CI, 2.48; data not shown).

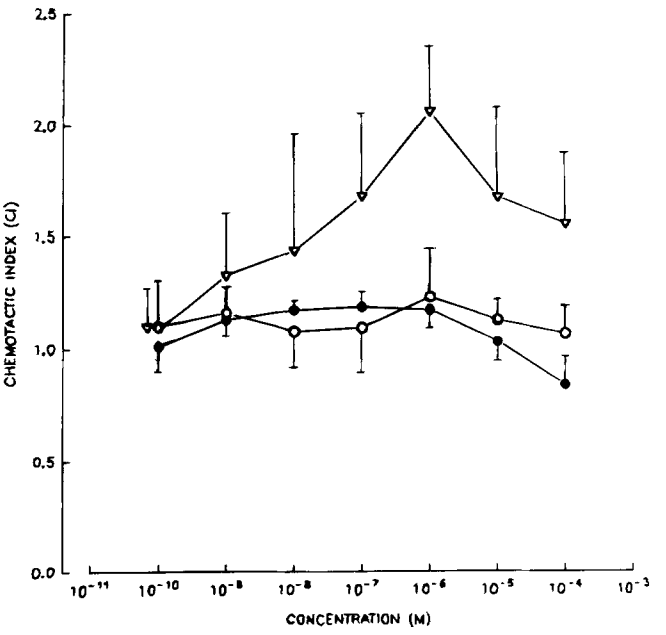

Fig. 1. T-lymphocyte chemotactic activity of various lysophospholipid classes. ●, LysoPE; ○, lysoPI; ▽, lysoPC. T lymphocytes were incubated in a Boyden chamber for 2 h. Data are mean ± SEM of six experiments performed in triplicate

Influence of fatty acid length on lysoPC chemotactic ability

In the experiments described above, the fatty acid in the sn-1 position of lysoPC was palmitoyl (C = 16:0). The following experiments compared the T-lymphocyte chemotactic activity of lysoPCs with fatty acids of different length in the sn-1 position (Fig. 2). LysoPC;1-caproyl (C = 6:0) did not possess chemotactic properties in the concentration range 10^{-10} to 10^{-4} *M*. For lysoPC;1-stearoyl (C = 18:0) chemotaxis was observed in the concentration range 10^{-8} to 10^{-4} *M* and was a maximum at 10^{-6} *M* (CI, 1.94). At all concentrations tested, the CI for lysoPC;1-stearoyl was lower than for lysoPC;1-palmitoyl, but the difference was not statistically significant.

Influence of double bonds on the chemotactic activity of lysoPCs

Comparison of lysoPC;1-oleyl (C = 18:1), which possesses a double bond in the ω-9 position, and lyso-PC;1-stearoyl (C = 18:0), which is saturated, showed that the presence of a double bond weakened the chemotactic properties of the molecule, although this difference was not statistically significant (Fig. 2). The maximum CI of lysoPC;1-oleyl was 1.68 (10^{-6} *M*), and the concentration range inducing chemotaxis was narrow, which was reflected in the sharp rise from 10^{-7} to 10^{-6} *M*.

It would have been appropriate to test lysoPCs with other fatty acid lengths and lysoPCs with more double bonds. However, such lysoPCs were not commercially available.

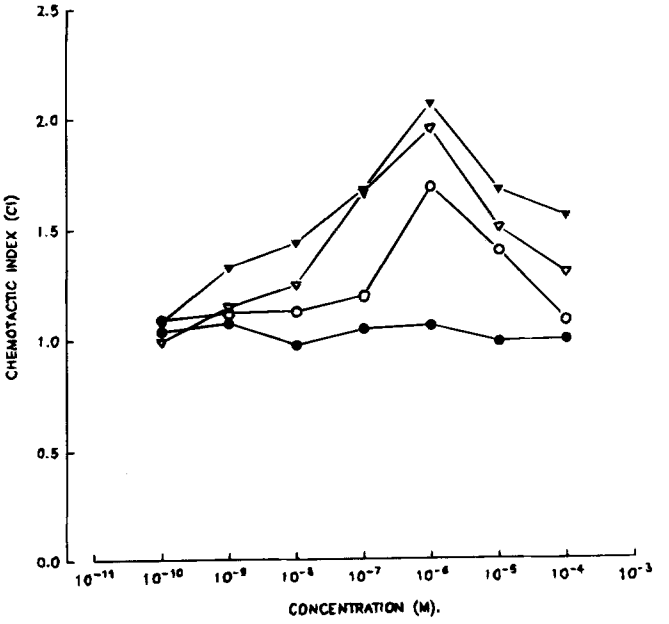

Fig. 2. Influence of sn-1 fatty acid length and double bond on lysoPC chemotactic abilities. LysoPC with sn-1 fatty acids of different lengths (●, caproyl C = 6; ▼, palmitoyl C = 16; ▽, stearoyl C = 18) and of different degrees of saturation (○, oleyl C = 18(9)) were tested for chemotactic activity in a Boyden chamber. The incubation time was 2 h. Each experiment was performed in triplicate ($n = 6$). The SEMs for the chemotactic lysoPC forms (10^{-8}–10^{-4} *M*) can be seen in Table 2. The remaining SEMs did not exceed 0.4

Time course study for lysoPC induced T-lymphocyte chemotaxis

The time course study (Fig. 3) showed that migrating T lymphocytes could be detected after 15 min. The curve representing cell migration in response to medium (controls) reached a plateau after 30 min. LysoPC (10^{-6} *M*) induced a time-dependent migration of T lymphocytes, and a plateau was not reached after incubation for 2 h.

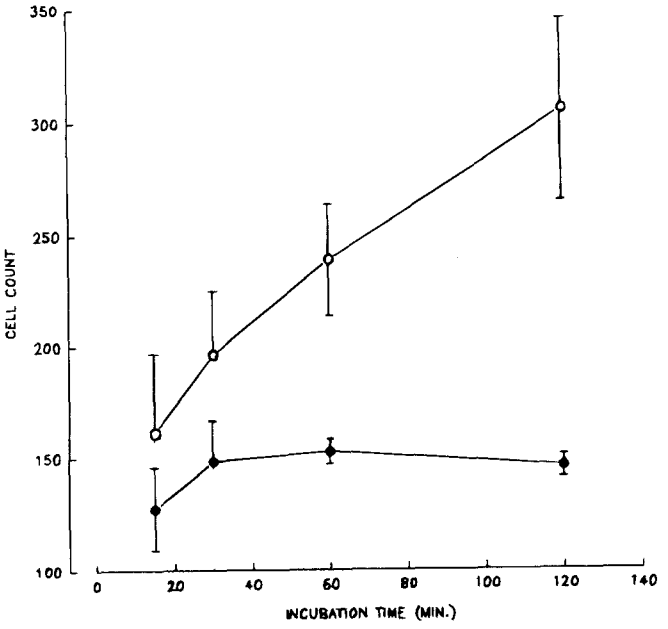

Fig. 3. Time course study. LysoPC: 1-palmitoyl at a concentration of 10^{-6} *M* was incubated in a Boyden chemotaxis chamber for various times ($n = 6$). Each experiment was done in triplicate. ●, Controls; ○, lysoPC

Discussion

Our studies involving lysophospholipids with different bases showed that lysoPC was the only one that induced T-lymphocyte chemotaxis. The biological activity of lysoPC is in accordance with other studies in which lysoPC was found to be the most potent among the lysoderivatives to potentiate activation of resting human T lymphocytes. This includes acitvation of protein kinase C and the IL-2 receptor [1]. In addition, lysoPC was the only lysophospholipid class that induced monocyte chemotaxis [14].

We found that the length of the fatty acid in the sn-1 position of the lysoPC molecule was important for T-lymphocyte chemotactic activity. A short chain fatty acid such as caproyl (C = 6:0) was ineffective, whereas the longer ones were effective exhibiting a maximum at a length of 16 C atoms. The presence of a double bond at ω-9 of the fatty acid weakened the chemotactic properties and narrowed the concentration range in which chemotaxis was present. For comparison the maximum chemotactic activity of lysoPC; 1-palmitoyl was similar to that of IL-8 (10^{-9} *M*) [15], but lower than that of fMLP at a concentration of 10^{-8} *M*.

Because PC is the quantitatively most important epidermal phospholipd, comprising approximately 54% of the phospholipids in normal epidermis [7], it is interesting that this molecule, but the action of phospholipase A_2, can be turned into a molecule that stimulates the activities of T lymphocytes, monocytes and mast cells, which all participate in inflammatory reactions in the skin [2, 11, 13].

The chemotactic properties of lysoPC can probably be attributed to its role as an additional second messenger to phospholipase C as it acts synergistically with diacylglycerol to activate protein kinase C [1]. Another model by which lysoPC could exert its chemotactic effect is that proposed by Quinn et al. [14] who suggested that lysoPC is hydrolysed by lysophospholipase C generating monoacylglycerol which undergoes an acetylation to diacylglycerol – a known second messenger. Further studies are required to determine the relevance of these models. The activity of phospholipase A_2 is increased in psoriasis [6] and possibly also in atopic dermatitis where increased eicosanoid levels are present [5]. Whether increased activity of phospholipase A_2 leads to increased amounts of lysoPC depends on the activity of the acetyltransferases which reesterify the lysoderivative. Our results indicate that lysoPC at biologically active concentrations can participate in the immunological and inflammatory processes occurring in psoriatic lesions. It would, therefore, be of interest to examine the content of lysoPC in normal and psoriatic skin.

References

1. Asaoka Y, Oka M, Yoshida K, Nishizuka Y (1991) Lysophosphatidylcholine as a possible second messenger synergistic to diacylglycerol and calcium ion for T-lymphocyte activation. Biochem Biophys Res Commun 178:1378–1385
2. Asaoka Y, Oka M, Yoshida K, Sasaki Y, Nishiuka Y (1992) Role of lysophosphatidylcholine in T-lymphocyte activation: Involvement of phospholipase A_2 in signal transduction through protein kinase C. Proc Natl Acad Sci USA 89:6447–6451
3. Christophers E (1989) Regulative Faktoren der psoriatischen Hautreaktion. Hautarzt 40:456–460
4. Cooper KD (1990) Psoriasis. Leukocytes and cytokines. Dermatol Clin 8:737–745
5. Fogh K, Herlin T, Kragballe K (1989) Eicosanoids in skin of patients with atopic dermatitits: prostaglandin E_2 and leukotriene B_4 are present in biologically active concentrations. J Allergy Clin Immunol 83:450–455
6. Forster S, Ilderton E, Norris JFB, Summerly R, Yardley HJ (1985) Characterization and activity of phospholipase A_2 in normal human epidermis and lesion-free epidermis of patients with psoriasis or eczema. Br J Dermatol 112:135–147
7. Grøn B, Iversen L, Ziboh V, Kragballe K (1993) Monohydroxy fatty acids esterified to phospholipids are decreased in lesional psoriatic skin. Arch Dermatol Res 285:1–6
8. Jinquan T, Larsen CG, Gesser B, Matsushuisa K, Thestrup-Pedersen K (1993) Human IL-10 is a chemoattractant for CD8+ T lymphocytes and an inhibitor of IL-8-induced CD4+ T lymphocyte migration. J Immunol 151:4545–4551
9. Kragballe K, Ellegaard J (1978) ATP-ase activity of purified human normal T- and B-lymphocytes. Scand J Haematol 20: 271–279
10. Kume N, Cybulsky I, Gimbrone MA Jr (1992) Lysophosphatidylcholine, a component of atherogenic lipoproteins, induces mononuclear leukocyte adhesion molecules in cultured human and rabbit arterial endothelial cells. J Clin Invest 90:1138–1144
11. Marquardt DL, Walker LL (1991) Lysophosphatidylcholine induces mast cell secretion and protein kinase C activation. J Allergy Clin Immunol 89:721–730
12. Mossman T (1983) Rapid colorimetric assay for cellular growth and survival: application of proliferation and cytotoxity assays. J Immunol Methods 65:55–63
13. Quinn MT, Parthasarathy S, Steinberg D (1988) Lysophosphatidylcholine: a chemotactic factor to human monocytes and its potential role in atherogenesis. Proc Natl Sci USA 85: 2805–2809
14. Quinn MT, Kondratenko N, Parthasarathy S (1991) Analysis of the monocyte chemotactic response to lysophosphatidylcholine: role of lysophospholipase C. Biochem Biophys Acta 1082:293–302
15. Valdimarsson H, Baker BS, Jonsdottir I, Fry L (1986) Psoriasis: a disease of abnormal keratinocyte proliferation. Immunol Today 7:256–259