Abnormal Serum Lysophospholipids in Multiple Myeloma Patients

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ABSTRACT: Lysophosphatidylcholine (LPC) and lysophosphatidic acid (LPA) mediate various kinds of biological activities and play an important role in cellular signal transduction. We analyzed serum phospholipids obtained from 16 multiple myeloma (MM) patients and observed that serum LPA level was significantly higher in MM patients (5.3 ± 0.5 nmol/mL) than in normal controls (1.7 ± 0.3 nmol/mL). LPC level was also higher than that in normal controls, and it correlated significantly with the concentration of LPA (r = 0.678, $P < 0.01$). In MM patients, palmitic acid/linoleic acid ratios in phosphatidylcholine and LPC were higher than those in normal controls. In the 12-mon follow-up study of two patients with the immune globulin G type, we recognized that the increase of LPC, LPA, and arachidonic acid/linoleic acid ratio in phosphatidylinositol corresponded with a decline in the serum albumin level and choline esterase activity.

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Multiple myeloma (MM) is characterized by malignant monoclonal lymphoplasmacytic cells that increase in bone marrow and produce an M-component [a specific immune globulin (Ig) produced by malignant cells in myeloma patients]. It is known that many kinds of malignancies involve abnormal lipid metabolism. Many MM patients have an impaired immune response, which causes a secondary high blood triglyceride level. These lipid-mobilizing activities are caused by cytokine-induced suppression of lipoprotein lipase (1) as well as by suppression of the enzyme related to the alteration of phospholipid compositions.

Lysophosphatidylcholine (LPC) and lysophosphatidic acid (LPA) mediate various kinds of biological activities and play an important role in cellular signal transduction. LPA activates its own GTP-binding protein (G-protein)-coupled receptor to trigger phospholipase C-mediated Ca^{2+} mobilization and other effector pathways such as induction of smooth muscle contraction (2), cell proliferation mediated by G-protein (3), and mediation of tumor cell invasion (4). LPC in oxidized low density lipoprotein stimulates proliferation of foam cells, which are derived from macrophages and are linked to atherosclerosis progression (5). In producing these lysophospholipids by phospholipase A_2 or C, arachidonate is released from membrane phospholipids and interacts with a G-protein (6). Arachidonate is a precursor of eicosanoids, which promote Ca^{2+} mobilization and activate protein kinase C (7).

Mills *et al.* (8) found that ascites from an ovarian cancer patient strongly induced proliferation of ovarian cancer cells. Recently, Okita *et al.* (9) found higher LPC levels in ascites from ovarian cancer patients. In this study, we analyzed serum phospholipids in MM patients and recognized increased levels of lysophospholipid levels compared to those in normal subjects.

EXPERIMENTAL PROCEDURES

Sera from 16 MM patients were obtained at the Third Department of Internal Medicine of Toyama Medical and Pharmaceutical University (Toyama, Japan). Diagnosis of MM was based on histological increase of myeloid cells on bone marrow biopsy and monoclonal hyperglobulinemia. Clinical data are shown in Table 1. Control subjects were two healthy men and 11 healthy women. Serum lipids were extracted and separated by two-dimensional thin-layer chromatography according to the procedure reported previously (9). Transmethylation of the phospholipids was carried out at 90°C for 2 h in acetylchloride/methanol (5:50 vol/vol). Fatty acid methyl esters were analyzed by gas–liquid chromatography (model GC-14A; Shimadzu, Kyoto, Japan) using margaric acid (17:0) as an internal standard. The results are expressed as the mean ± SE. For comparison of two groups of individuals, the Mann-Whitney U test was used. The coefficient of correlation (r) was calculated by the Spearman R test. P-values were two-tailed and considered significant at less than 0.05.

RESULTS AND DISCUSSION

Serum phospholipids and fatty acid compositions of the phospholipids were analyzed for each type of MM patients and compared with those of controls. No significant differences were

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Abbrevations: BJ type, Bence-Jones type; G-protein, GTP-binding protein; Ig, immune globulin; LPA, lysophosphatidic acid; LPC, lysophosphatidyl choline; MM, multiple myeloma; PC, phosphatidylcholine; PLA_2, phospholipase A_2.

TABLE 1
Clinical Data of the Study Group[a]

	Control	Myeloma		
		IgG	Bence-Jones	IgA
Number (sex)	2(M),11(F)	2(M),8(F)	1(M),3(F)	1(M),1(F)
Age (yr)	51 ± 1	65 ± 3	63 ± 9	53
BMI		22.2 ± 1.0	22.1 ± 1.8	21.4
Total protein (g/dL)		9.4 ± 0.5[ab]	6.3 ± 0.2[b]	6.7[a]
Albumin (g/dL)	4.5 ± 0.1	3.6 ± 0.2[a]	4.1 ± 0.1	4
A/G		0.7 ± 0.1	1.9 ± 0.1	1.4
Immune globulin (Ig)				
IgG (mg/dL)		4043 ± 384	1599 ± 1234	415
IgA (mg/dL)		36 ± 14	42 ± 32	1317
IgM (mg/dL)		22 ± 8	16 ± 11	46
RBC ($\times 10^4$/μL)		325 ± 28	266 ± 25	297
WBC (/μL)		4079 ± 831	4563 ± 516	5160
Platelets ($\times 10^4$/μL)		15.7 ± 3.2	22.1 ± 1.4	12

[a]BMI, body mass index; A/G, albumin/globulin ratio; RBC, red blood cells; WBC, white blood cells. Data reported as mean ± SE. [a], $P < 0.01$; [b], $P < 0.05$.

recognized in the serum phospholipid concentrations and fatty acid compositions among the types of disease. Serum LPC levels were significantly higher in MM (193.2 ± 17.3 nmol/mL) than in controls (135.0 ± 8.4, Fig. 1). Serum LPA levels were also significantly higher in MM (5.3 ± 0.5 nmol/mL) than in controls (1.7 ± 0.3 nmol/mL). Serum LPC concentrations correlated with those of LPA ($r = 0.678$, $P < 0.01$, Fig. 2). There were no significant differences in the serum phosphatidylcholine (PC), phosphatidylethanolamine, and phosphatidylinositol concentrations between controls and MM patients.

The Ig type differs with each MM type. Malignancy induces monoclonal Ig production and suppression of other Ig types. The serum albumin level decreases in the IgG and IgA types but not in the Bence-Jones (BJ) type. To indicate the progression of each type of disease, we calculated the albumin/globulin (A/G) ratio. The ratio decreased in the IgG type but increased in the BJ type with disease progression. In the IgG type, LPC concentration and A/G correlated negatively ($r = -0.685$, $P < 0.05$) and positively in the BJ type ($r = 1.000$, $P = 0.05$, Fig. 3).

Fatty acid composition of phospholipids is shown in Table 2. In MM sera, the percentages of the saturated palmitic acid (16:0) in PC and stearic acid (18:0) in phosphatidylethanolamine were higher than those in normal subjects. However, linoleic acid (18:2n-6) in PC and LPC, and arachidonic acid (20:4n-6) in phosphatidylinositol were lower in MM. In comparing the palmitic acid/linoleic acid molar ratio in phospholipids, the ratio in MM patients was significantly higher for PC and LPC than in controls (Fig. 4).

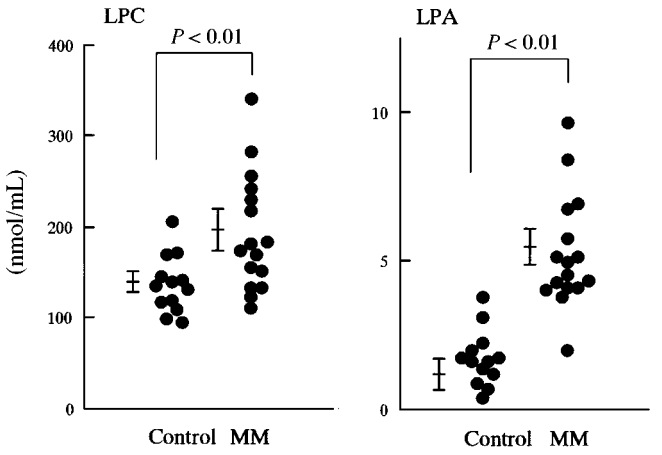

FIG. 1. Serum lysophosphatidylcholine (LPC) and lysophosphatidic acid (LPA) levels in multiple myeloma (MM) patients and control subjects. Significant differences are recognized in LPC ($P < 0.01$) and LPA concentrations ($P < 0.01$). Vertical lines show mean ± SE for 13 controls and 16 MM patients.

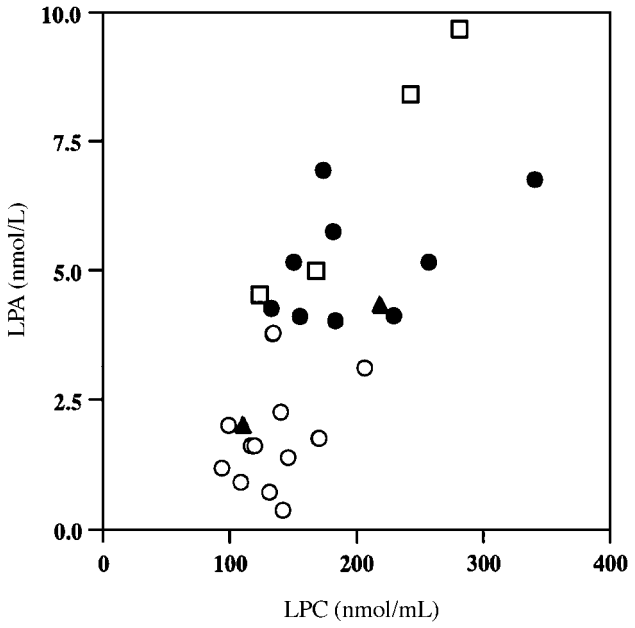

FIG. 2. Correlation of LPC and LPA concentrations. Data points indicated are controls (○), immune globulin G (IgG) type (●), Bence-Jones type (□), and IgA type (▲). Significant correlations are recognized between LPC and LPA concentrations ($r = 0.678$, $P < 0.01$). For abbreviations see Figure 1.

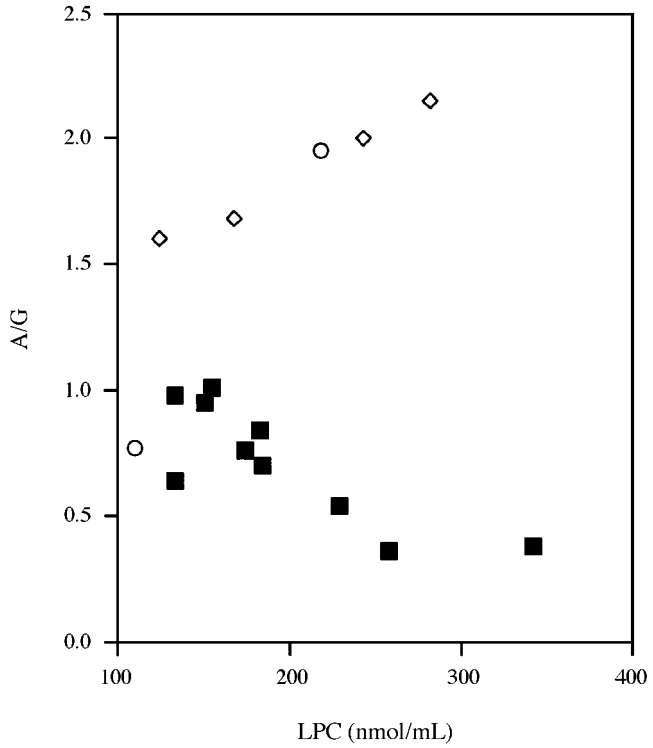

FIG. 3. Correlation of LPC concentration and albumin/globulin (A/G) ratio. Data points indicated are IgG type (■), Bence-Jones type (◇), and IgA type (○). In the IgG type, LPC and A/G ratio correlated negatively ($r = -0.685$, $P < 0.05$) and they correlated positively in the Bence-Jones type ($r = 1.000$, $P = 0.05$). For other abbreviations see Figures 1 and 2.

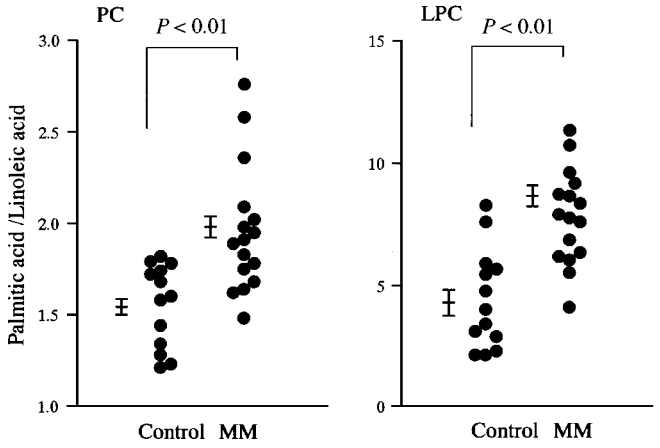

FIG. 4. Palmitic acid/linoleic acid ratio in serum phosphatidylcholine (PC) and LPC. Significant differences are recognized between the palmitic acid/linoleic acid ratio in PC ($P < 0.01$) and LPC concentrations ($P < 0.01$). Vertical lines show mean ± SE for 13 controls and 16 MM patients. For abbreviations see Figure 1.

We observed two patients with the IgG type (61- and 64-yr-old females) for 12 mon. Markedly higher levels of LPC and LPA were observed at the start of follow-up compared with those of normal subjects. These levels increased slightly after 12 mon, corresponding to a decline in serum albumin and choline esterase activity. The arachidonic acid molar ratio in phosphatidylinositol increased with disease progression (Fig. 5).

LPA was produced rapidly in thrombin-activated platelets (10). LPA concentration in heparinized plasma, compared to that in serum, was 3.21 and 6.39 nmol/mL, respectively, in a MM patient with IgG type, whereas these values averaged 0.29 and 1.45 nmol/mL, respectively, in three controls (data not shown). Therefore, LPA production in MM may be higher than that in controls during blood clotting. LPA has been reported to be produced from phosphatidic acid by phospholipase A_2 (PLA_2) (11). LPA plays a role in the growth of ovarian and breast cancer cells (3). LPA stimulates proliferation of Jurkat cells in serum-free medium or in a low concentration of fetal bovine serum. Biological activities differ in each LPA species: oleoyl-LPA has a higher activity, followed by arachidonoyl-LPA, and linoleoyl-LPA which constitute the unsaturated group. LPA can mobilize cellular Ca^{2+}, and palmitoyl-LPA induces maximal activity, on the order of 10^{-6} M (12). LPA concentrations in both serum and plasma in MM are considered sufficient to induce Ca^{2+} mobilization *in vitro*. LPA induces mitogenic responses in fibroblasts, or inhibition of proliferative activity of myeloma cells (13). Imagawa *et al.* (14) reported that LPA induced varying degrees of cell proliferation in different cell types, mediated through pertussis toxin-sensitive or -insensitive G-protein. On the basis of these facts (this research; 3,12–14) we suggest that LPA may be potentially active in the plasma of MM patients.

Okita *et al.* (9) showed that the percentages of palmitoyl- and stearoyl-LPC species in plasma and ascites from ovarian cancer patients were significantly higher than those of con-

TABLE 2
Composition of Selected Fatty Acids in Serum Phospholipids (mol%)[a]

	PC		PE		PI		LPC	
	Control	MM	Control	MM	Control	MM	Control	MM
16:0	33.3 ± 0.8	37.1 ± 0.9[a]	16.8 ± 1.7	20.0 ± 1.4	9.8 ± 0.9	11.0 ± 0.5	43.9 ± 1.9	58.6 ± 0.9[b]
18:0	14.8 ± 0.3	13.3 ± 0.6	16.0 ± 1.0	21.1 ± 1.3[a]	42.3 ± 0.9	43.8 ± 1.0	20.9 ± 0.4	18.8 ± 1.0
18:1	10.6 ± 0.6	12.2 ± 0.4[b]	8.0 ± 0.5	7.1 ± 0.3	8.0 ± 0.6	7.2 ± 0.5	7.6 ± 0.4	8.4 ± 0.4
18:2n-6	21.8 ± 0.8	19.2 ± 0.6[b]	10.8 ± 0.7	9.3 ± 0.6	10.2 ± 0.7	10.2 ± 0.9	12.4 ± 1.3	7.9 ± 0.5[a]
20:4n-6	6.1 ± 0.5	5.9 ± 0.4	16.0 ± 1.1	14.5 ± 0.9	21.7 ± 1.1	18.3 ± 1.2[b]	4.7 ± 0.4	1.6 ± 0.2
20:5n-3	3.2 ± 0.7	1.7 ± 0.2	7.6 ± 1.1	4.2 ± 0.6[b]	0.5 ± 0.1	0.6 ± 0.2	0.7 ± 0.2	0.5 ± 0.1
22:6n-3	5.5 ± 0.6	5.3 ± 0.4	16.4 ± 1.7	17.4 ± 1.5	2.6 ± 0.4	2.6 ± 0.3	1.2 ± 0.3	1.0 ± 0.1

[a]PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; LPC, lysophosphatidylcholine; MM, multiple myeloma. Data presented as mean ± SE, [a], $P < 0.001$; [b], $P < 0.05$, vs. control.

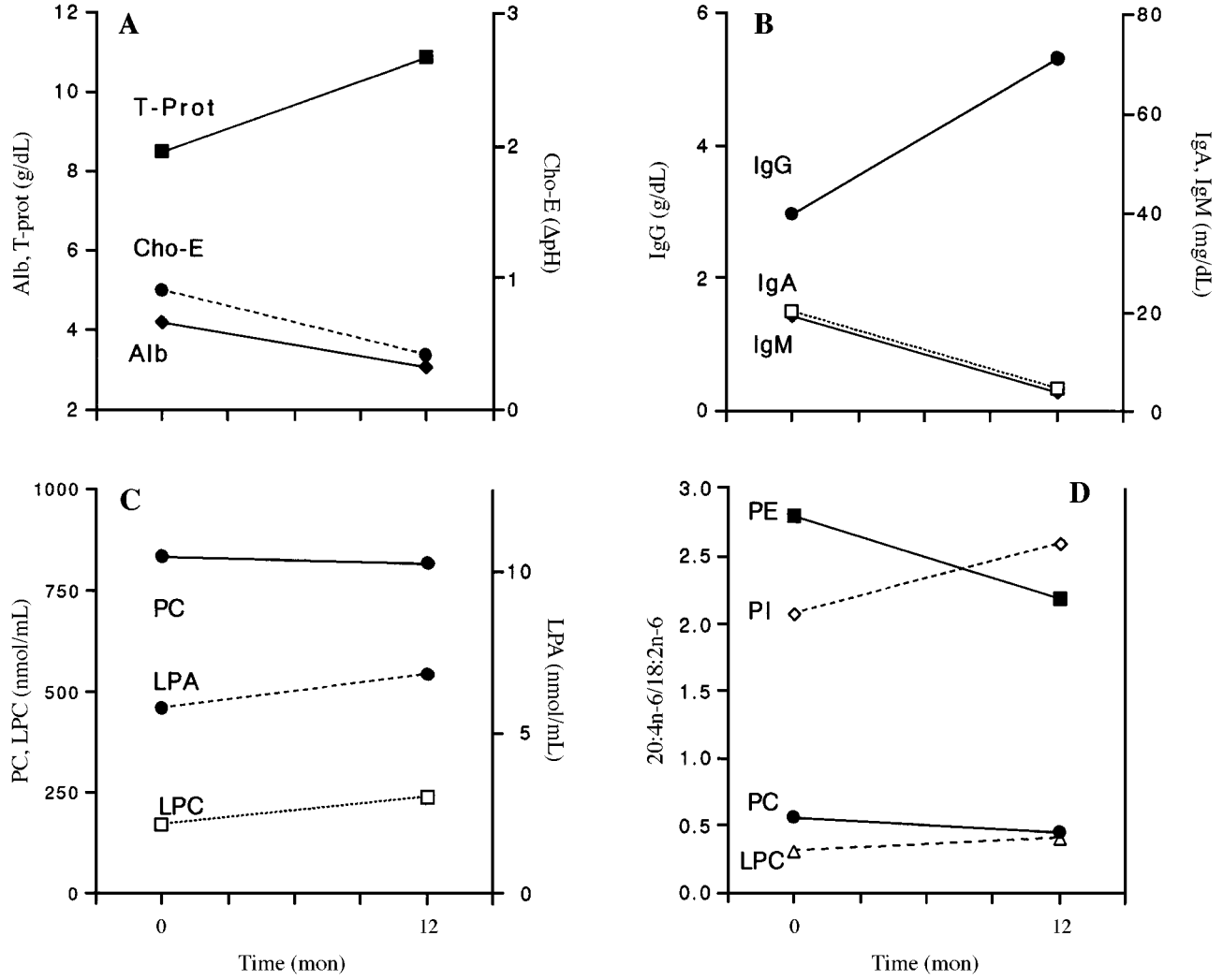

FIG. 5. Follow-up study of two patients of IgG type for 12 mon. Averages of clinical data and serum phospholipid levels are shown at 0 and 12 mon. Abbreviations: T-Prot, total protein; Alb, albumin; Cho-E, choline esterase; PE, phosphatidylethanolamine; PI, phosphatidylinositol. For other abberviations see Figures 1–3.

trols. Our data showed a higher palmitic/linoleic acid ratio of PC and LPC in serum phospholipids of MM patients compared to that in the controls (Fig. 4). Moreover, we recognized an abnormal serum albumin/globulin ratio in MM patients, a decrease in IgG type and an increase in BJ type, and a significant correlation of this ratio with the serum LPC level (Fig. 3). These results may suggest that elevated serum LPC level in MM indicates a progression of the disease. Several biological functions of LPC have been studied. Sakai *et al.* (5) reported that macrophage proliferation occurred following the LPC uptake of oxidized low density lipoprotein through the scavenger receptor. Other investigators (15,16) reported that LPC enhanced T-lymphocyte proliferation and potentiated protein kinase C activation in other cell lineages.

The source of LPC is fatty acid release from PC by PLA_2 and phospholipase A_1. Secretory group II PLA_2 ($sPLA_2$) and cytosolic group IV PLA_2 ($cPLA_2$) regulate extra- or intracellular arachidonic acid release with cross reactivity (17,18). In malignant tissue, phospholipid fatty acid remodeling was regulated through PLA_2 and acyltransferase, and this process leads to increased use of arachidonic acid (18). Another LPC-producing pathway involves transacylation of PC by lecithin cholesterol acyltransferase. Kuliszkiwicz-Janus and Baczynski (19) reported that high density lipoprotein and lecithin cholesterol acyltransferase levels decreased in MM patients, and our data also indicated decrement of these levels. From our present results, a close relation between the formations of two lysophospholipids, LPA and LPC, was observed. It may indicate involvement of lysophospholipase D in the production of LPA (20). Further investigation is needed to determine the relationship between tumor progression and lysophospholipids.

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