Cholesterol, phospholipids, and fatty acids of normal immature neutrophils: comparison with acute myeloblastic leukemia cells and normal neutrophils

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Abstract The lipid composition of immature myeloid cells from the bone marrow of normal persons and myeloblasts from patients with acute myeloblastic leukemia was studied and compared with the lipid composition of normal mature human neutrophils. Total cholesterol, phospholipid, and fatty acid composition was determined on each cell type. The leukemic cells showed decreased total cholesterol and cholesterol-to-phospholipid ratio, increased phosphatidylcholine and phosphatidylinositol, decreased phosphatidylethanolamine, and an increased percentage of unsaturated fatty acids when compared to normal mature neutrophils. A nearly identical pattern was seen in the normal immature myeloid precursors from normal bone marrow. We conclude that the altered lipid composition of acute myeloblastic leukemia cells is related to unexplained factors related to cell age and not to malignancy per se.—**Klock, J. C., and J. K. Pieprzyk.** Cholesterol, phospholipids, and fatty acids of normal immature neutrophils: comparison with acute myeloblastic leukemia cells and normal neutrophils. *J. Lipid Res.* 1979. **20:** 908–911.

Supplementary key words polymorphonuclear leukocyte · myeloblast

Acute myeloblastic leukemia (AML) in humans is a proliferative disorder in which the progeny of certain primitive myeloid cells fail to differentiate and accumulate in the body of the host. Such cells do not share the morphologic or functional characteristics of their mature counterparts, the neutrophils. When compared to neutrophils in vitro, leukemic myeloblasts show an increase in cellular rigidity (1), a low adhesiveness to glass and plastic, a low propensity to aggregate, a slow rate of cell spreading, and a very diminished ability to form pseudopodia and to move and ingest particles (2). These same in vitro characteristics of AML cells have also been observed in normal immature marrow myeloid cells (3, 4).

AML cells differ from normal neutrophils in their lipid composition, having a decreased cholesterol content, a decreased cholesterol-to-phospholipid molar ratio, a decrease in phosphatidylcholine (PC), and an increase in sphingomyelin (SM) (5–7). If the abnormal lipid pattern of AML cells is peculiar to these cells and is not shared by their normal counterparts, then it is possible that the alteration in lipid content may be a determining characteristic in their abnormal growth and development. With these questions in mind we have developed methods for the isolation of normal immature myeloid cells from bone marrow and have examined their lipid composition and compared it with myeloblasts from patients with AML and with normal mature neutrophils.

MATERIALS AND METHODS

Cell isolation

Normal mature neutrophils were isolated by dextran sedimentation and Ficoll–Hypaque gradient centrifugation as previously described (8). The cells were over 95% pure neutrophils. AML cells were purified from the blood of ten patients with acute myeloblastic leukemia. The patients were 22–54 years of age, and all had peroxidase-positive leukemia cells and peripheral blast counts over 30,000/μl. The blood was anticoagulated in heparin (10 U/ml) and the AML cells were isolated by centrifugation on Ficoll–Hypaque as described above. Cells were over 90% myeloblasts and promyelocytes. Normal bone marrow myeloid precursors were isolated from normal bone marrow anticoagulated with heparin (20 U/ml). Adenosine diphosphate (Sigma Chemical Co., St. Louis, MO) was

Abbreviations: PMN, neutrophil; AML, acute myelogenous leukemia; BM, normal immature myeloid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS–PI, phosphatidylserine–phosphatidylinositol; SM, sphingomyelin.

added to a final concentration of 10^{-5} M to aggregate platelets, and the mixture was poured into an inverted plastic syringe which was packed with nylon wool fiber (3 denier, Fenwal Laboratories, Deerfield, IL). The syringe was placed at 37°C for 30 min, and the non-adherent cells were eluted with 10 volumes of 0.15 M NaCl. Eluted cells were placed on a Ficoll–Hypaque gradient as above and centrifuged at 440 *g* at room temperature for 45 min. Cells at the Ficoll–NaCl interface were removed by aspiration, and cell counts were performed. Differential counting of cells revealed 65% of the cells were myeloblasts, promyelocytes, and myelocytes. Approximately 25% were metamyelocytes, and the remainder were megakaryocytes, lymphocytes, and monocytes.

Lipid studies

Spectral grade solvents from Fisher Chemicals, Pittsburg, PA were degassed and stored under nitrogen. All material studied was extracted overnight in 20 volumes of chloroform–methanol 2:1. Extracts were filtered through glass wool and washed three times with chloroform–methanol 2:1. The aqueous phase was removed and water-soluble salts and water were removed from the organic phase by flash evaporation in the presence of Sephadex G-25 (9). Extracts were stored in Teflon-capped vials under nitrogen at −20°C until analyzed. Cholesterol determination was done according to Wybenga et al. (10) using a solution of 64% (w/v) ethyl acetate–15 N H_2SO_4 containing 30% $FeCl_3$ (w/v). After mixing with the sample and heating for 90 sec at 100°C, absorbance was read at 595 nm. Phosphorus analysis was done using a modification of the method of Ames and Dubin (11). Samples were ashed in $HClO_4$–H_2SO_4 solution for 2 hr at 180°C with added H_2O_2 and continued heating if color persisted. Three ml of a solution of 2.5 mM ammonium molybdate, 0.1 M sodium acetate, and 0.05 M ascorbic acid was used as color reagent, and absorbance was determined at 790 nm after color development at 80°C. Known phospholipid standards (Applied Science Laboratory, State College, PA) were co-chromatographed with samples for identification.

Two-dimensional thin-layer chromatography of phospholipids was performed using a modified method of Broekhuyse (12). Extracts containing 10 μg of lipid phosphorus were dried to 50-μl volumes and spotted onto precoated thin-layer plates (Silica Gel H, Applied Sciences). Plates were then developed two-dimensionally in chloroform–methanol–ammonium hydroxide 130:80:15 and chloroform–acetate–acetic acid–methanol–water 100:40:20:20:10. After drying, the plates were exposed to I_2 vapor and the spots were scraped and analyzed for phosphorus.

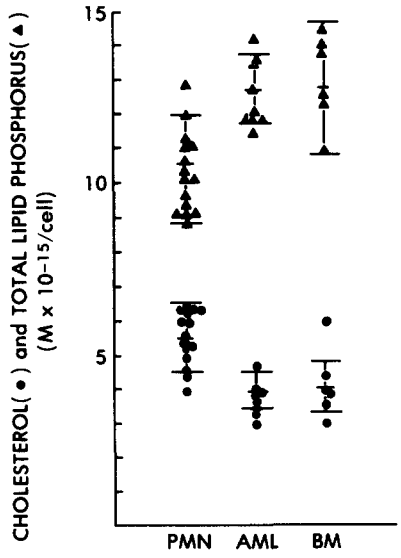

Fig. 1. Total cholesterol and phospholipid content of normal neutrophils (PMN), acute myeloblastic leukemia cells (AML), and normal immature myeloid cells (BM). Horizontal bars indicate 1 standard deviation.

Fatty acid analysis was done on methylated samples after reaction of 20–50 μg of lipid with 1% sulfuric acid in methanol at 70°C for 8 hr and extraction with hexane. Samples were dried under N_2 and dissolved in CS_2 for injection into the gas chromatograph. The

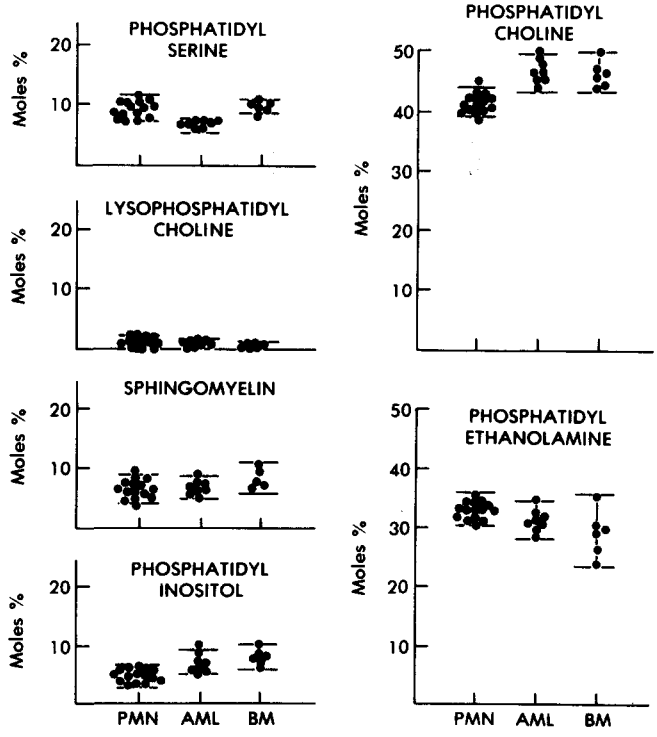

Fig. 2. Phospholipid distributions in normal neutrophils (PMN), acute myeloblastic leukemia cells (AML), and normal immature myeloid cells (BM). Horizontal bars indicate 1 standard deviation.

TABLE 1. Total fatty acid composition of AML, BM, and PMN lipids[a]

	14:0	14:1	16:0	18:0	18:1	18:2	18:3	20:4
AML cells (n = 8)	4 ± 1	2 ± 1	22 ± 9	22 ± 4	24 ± 1	11 ± 5	8 ± 6	16 ± 8
BM cells (n = 6)	2 ± 2	3.5 ± 2	22 ± 5	17 ± 3	27 ± 3	12 ± 7	15 ± 1	11 ± 6
PMN cells (n = 8)	2 ± 1	3 ± 5	27 ± 5	28 ± 4	26 ± 2	8 ± 5	2 ± 1	3 ± 2

[a] The following fatty acids were present but in amounts less than 2 mol%: 20:1, 22:0, and 24:1.

gas chromatographic column was 5% DEGS-PS (Supelco, Inc, Bellefonte, PA); carrier gas was N_2, and the column temperature was 180°C. Quantitation was done by measuring peak areas; identification of fatty acids was done by comparing retention times with those of the following fatty acid standards: 14:0, 14:1, 16:0, 16:1, 18:0, 18:1, 18:2, 18:3, 20:0, 20:1, 20:5, 22:0, 22:1, 22:5, 22:6, 24:0, 24:1 (Supelco, Inc.). Student's *t* test was used for tests of significance.

RESULTS

Results of lipid analyses of neutrophils (PMN), AML cells, and normal immature myeloid cells (BM) are shown in **Figs. 1** and **2.** Fig. 1 shows that total lipid phosphorus was higher in AML and BM cells ($P > 0.1$), and total cholesterol was lower in AML and BM cells ($P < 0.01$) when compared to PMN cells. Cholesterol-to-phospholipid molar ratios were 0.52 ± 0.1 for PMN and 0.30 ± 0.12 for AML and BM cells, respectively ($P < 0.05$). Less than 10% of the cholesterol was in the esterified form (data not shown).

Fig. 2 shows the phospholipid composition of the three cell types. AML and BM cells had significantly increased proportions of PC when compared to PMN ($P < 0.025$). AML and BM cells also showed increased phosphatidylinositol (PI) and decreased phosphatidylethanolamine (PE), but with these small numbers the values are not significant ($P > 0.2$). Specific analyses for plasmalogens and lysobisphosphatidic acid were not done (13).

Fatty acid analyses on the total phospholipid extract are shown in **Table 1.** The major finding is that most of the fatty acids in PMN are saturated (58%), whereas AML cells and BM cells had 49% and 41% saturated

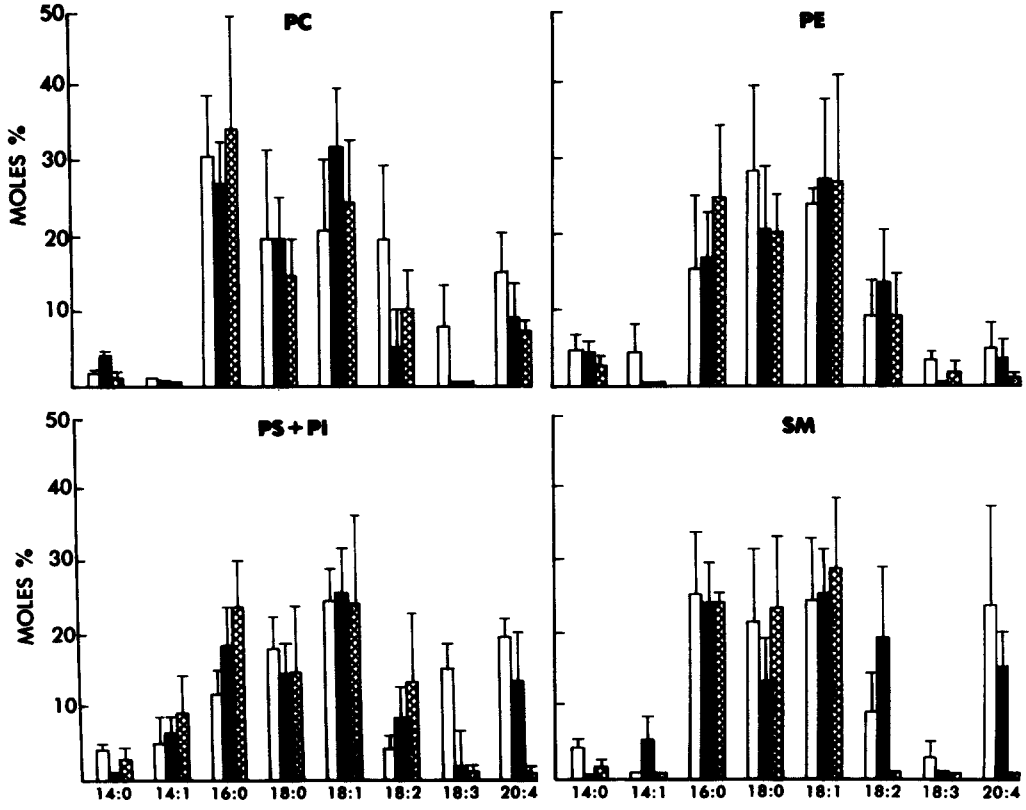

Fig. 3. Fatty acid distribution in phospholipids of normal neutrophils (PMN) ▨, acute myeloblastic leukemia cells (AML) □, and normal immature myeloid cells (BM) ■. Horizontal bars indicate 1 standard deviation.

fatty acids, respectively ($P < 0.05$). This was mostly due to an increase in 16:0 in PMN PE and a decrease in 20:4 and 18:2 in PMN SM, and an increase in 18:2, 18:3, and 20:4 in AML and BM SM, PS, and PI as shown in **Fig. 3.**

DISCUSSION

This work describes a method for the purification of an enriched fraction of immature normal myeloid precursors from normal bone marrow which has allowed us to study the significance of the altered lipid composition of AML cells. The data on AML cells and PMN agree with previously published data (5–7, 13, 15). Gottfried (6) previously suggested that the alterations in lipid composition of leukemia cells might be related to their maturational age. In contrast to those studies in which analyses were done on normal transformed lymphocytes and myeloid leukemic cell lines maintained in long-term culture, we have performed analyses on the normal immature counterpart of the leukemic cell. This study has confirmed that the alterations in neutral and phospholipids in AML cells are shared by normal immature myeloid cells, and the data are in keeping with recent results of similar studies in lymphocytes (14). The reason for the altered lipid patterns in the immature myeloid cells (AML and BM cells) is not clear from these studies. It is possible that the differences are reflections of subcellular organelle and membrane composition, e.g., the increase in saturated fatty acids in PMN may be explained on the basis of an increase in the number and kind of intracellular granules which are known to be enriched in such fatty acids (13, 15).

We do not know whether the altered cholesterol, phospholipid, and fatty acid composition of the young neutrophil has anything to do with its decreased deformability, decreased adhesion to surfaces, low propensity to aggregate, and diminished locomotion and ingestion. However it seems unlikely that the lipid changes in leukemic cells are related to their abnormal growth and development. It was initially felt that the abnormal lipid composition in leukemic cells was directly related to their malignant potential (16, 17); however, recent studies now suggest that some of these changes may be related to the growth cycle of the leukemic cell (18). Differences in glycoprotein and/or glycolipid composition of these cells might also explain behavioral differences; nevertheless, the results of our studies underscore the necessity for correlating abnormal biochemical findings in AML cells with the results obtained from similar studies in normal immature myeloid cells.

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