Specific Lymphocyte Phospholipid Changes in Chronic Renal Failure

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I describe phospholipid fluctuations in lymphocytes of peripheral blood of patients with chronic renal failure who were undergoing maintenance hemodialysis. The findings of decreased concentration of total phospholipids, phosphatidylcholine, and phosphatidylethanolamine, and increased concentrations of phosphatidylinositol and diphosphatidylglycerol are discussed, in relation to the lymphocyte membrane enzymatic systems, and immune response.

Additional Keyphrases: maintenance hemodialysis • lymphocytes membrane • total lipids • individual phospholipids

Studies on lymphocyte lipid disturbances provide basic contributions to the understanding of various disease states (1–3). Furthermore, conditions with abnormal functioning of the immune system are closely related to leukocytes and (especially) fluctuations in lymphocyte phospholipid (4–6).

Several reports (7–9) have dealt with lipid metabolism in chronic renal failure, but phospholipid composition (10, 11)—an important lipid group in the cell membrane—in blood cells is largely unreported.

In this paper I present some observations on lymphocyte phospholipid fluctuations in the peripheral blood of patients with chronic renal failure who are undergoing maintenance hemodialysis.

Materials and Methods

The lymphocyte phospholipid composition was determined in 29 patients (ages 21–55 years) suffering from chronic renal failure, who were undergoing stable maintenance hemodialysis. Twenty-five healthy subjects (20–52 years old) were used as controls, free of any serious or chronic diseases at the time of the study. All patients had a history of chronic renal failure due to glomerulonephritis of diverse origin (diffuse chronic glomerulonephritis, 19; systemic lupus erythematosus, 10). Samples were drawn before beginning the dialysis. Patients and controls were studied simultaneously, after fasting for at least 14 h, and were coded until the analysis was completed. Statistical evaluation was done by Student's t-test.

Lymphocytes were isolated as follows. Blood samples (20 mL), drawn from an antecubital vein with a siliconized syringe, were immediately mixed with ethylenediaminetetraacetate solution (15 g/L) to prevent coagulation and diluted with an equal volume of NaCl solution (9 g/L). The diluted samples were carefully layered on 30 mL of "Lymphoprep" (Nagaard and Co., Oslo) and centrifuged for 40 min at 1700 rpm and 4 °C. Thus, three layers were formed: the upper, containing platelets and monocytes; the middle, lymphocytes; and the lower, polymorphonuclear leukocytes and erythrocytes. The lymphocytes were aspirated with a Pasteur pipette, with a circular motion, and then washed three times with isotonic saline and centrifuged for 12 min at 1700 rpm and 4 °C. The contamination of lymphocytes with other cells was expected not to exceed 5%, as the reliability of the method has been tested with blood samples (Nagaard and Co.).

Total lipids were extracted by the method of Folch et al. (12) and the lipid residue was re-extracted three times with CHCl₃/MeOH (2/1 by vol). The final lower phase was evaporated in a nitrogen atmosphere. A silicic acid column ("Special for Lipid Chromatography"; Bio-Rad Lab., Richmond, CA 94804) was used to separate the main lipid fractions: neutral lipids, glycolipids, and phospholipids (13). The individual phospholipid components were determined by two-dimensional thin-layer chromatography based on the method of Kwiterovich et al. (14) and were made visible as follows: (a) the major phospholipid components by spraying the chromatogram with an equimolecular mixture of H₂SO₄ and H₂O and heating to 160 °C for 15 min, and (b) the lipid-containing, ninhydrin-reacting portion by spraying with a ninhydrin solution (ninhydrin, 1 g/L, and pyridine, 3/1 by vol) and heating to 80 °C for 10 to 12 min. Each phospholipid class was analyzed by chromatography with commercial phospholipids as standards (Pierce Chemical Co., Rockford, IL 61105). The lipid phosphorus of each spot was estimated according to Bartlett's method (15).

Results

Table 1 shows the results of our study. Although total lipid concentration did not differ significantly between controls and patients suffering from chronic renal failure, total phospholipid concentration was significantly decreased in the patients (p < 0.01).

As for the individual phospholipid classes, a significant decrease of phosphatidylycholine (p < 0.01) and phosphatidylethanolamine (p < 0.05) was noted in patients; the concentration of phosphatidylinositol was significantly (p < 0.01) increased in the patient group.

All other phospholipids showed a slight but insignificant increase in concentration as compared with healthy controls, but the increase of diphosphatidylglycerol in patients' lymphocytes could be of interest.

Discussion

Several vital membrane functions (including active transport of Na⁺ and K⁺, oxidative phosphorylation, and selective permeability) have been associated with different requirements of phospholipid classes, and changes in their concentrations may reflect lipid alterations occurring in the cellular components and in the plasma membrane.

In the present study, the decreased concentration of lymphocyte total phospholipids in patients with chronic renal failure on maintaining hemodialysis is of interest. Previous studies (16–19) dealing with diseases that have a different

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immune response have reported reduced concentration of T-cells, especially in the case of systemic lupus erythematosus. This reduction has been attributed to the production of lymphocyte-specific cytotoxins. My finding of reduced total phospholipids could be related to the disarrangement of the lymphocyte membrane and consequently to the destruction of T-cells.

I found some interesting fluctuations in the individual phospholipid classes, namely, a decrease in phosphatidylcholine and phosphatidylethanolamine and an increase in phosphatidylserine concentrations. It is well known (20, 21) that phosphatidylcholine—the most abundant phospholipid in cell membranes—plays an important role in their structure and function, and many enzyme systems that are localized in the cytoplasm (e.g., acyl-CoA synthetase, Ca2+Mg2+ ATPase) require this phospholipid for activation. Evidently the decrease of total phospholipids should be accompanied by a decrease in the concentration of phosphatidylcholine, and quite possibly the decreased concentration of phosphatidylcholine has a functional influence on the above-mentioned enzymic systems.

The fluctuation of phosphatidylserine is of great importance, as this phospholipid is connected to major enzymic functions. Moreover, it is associated with the involvement of some membrane-bound enzymes such as Na+ + K+ -dependent ATPase, adenylcyclase (22, 23), and probably plays a modulatory role in lymphocyte receptor mechanisms. I believe that the increased concentration of phosphatidylserine observed may affect the lymphocyte’s enzyme mechanism, which possibly interferes with the cell’s immune response.

Another interesting finding is the decreased concentration of phosphatidylethanolamine in lymphocytes of patients in chronic renal failure of immunologic origin. This finding could be possibly attributed to the presence of a phospholipid enzyme-splitting system in lymphocytes, the existence of which has been already demonstrated in the degradation of phosphatidylcholine (24).

Finally, the increase of diphosphatidylglycerol, although not significant, is of interest because this phospholipid is mainly located in mitochondria (25, 26) and the cell membrane. Alterations in its concentration probably are related to the degradation of these cellular components, changing the mitochondrial respiratory chain and oxidative phosphorylation in lymphocytes.

The results of this study point to the existence of specific structural changes in lymphocytes during chronic renal failure. I hope that this stimulates more extensive research on certain phospholipid metabolic pathways in this disease. This and further studies are needed concerning fluctuations in lymphocyte phospholipid concentrations of patients with chronic renal failure free of disturbance in their immune system.

References
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