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Phospholipid Base Exchange Activity in the Leukocyte Membranes of Patients With Inflammatory Disorders

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Phospholipid base exchange and cholinephosphotransferase (CPT) and ethanolaminephosphotransferase (EPT) activities were assessed in the membranes of neutrophils or lymphocytes from patients with various inflammatory disorders. Ethanolamine exchange activity was significantly enhanced in both neutrophils and lymphocytes from patients with active Behçet's disease, active systemic lupus erythematosus (SLE), and severe bacterial infections and slightly enhanced in those from patients with active rheumatoid arthritis (RA), compared with healthy controls. No abnormal findings were found in CPT, EPT, or serine or choline base exchange activities in the leukocytes from any of the diseased groups tested or in the ethanolamine exchange activity of patients with severe viral infections and inactive SLE, RA, and Behçet's disease. The authors have recently demonstrated the enhancement of transmethylation and phospholipase A_2 activity in human leukocyte membranes at the height of inflammatory disease states, as well as the activation of leukocyte ethanolamine exchange by bioactive stimulants. These data postulate that phosphatidylethanolamine synthesis by the base exchange reaction may be the precursor of transmethylation and its subsequent activation of phospholipase A_2, leading to the induction of arachidonic acid cascade. (Am J Pathol 1987, 127:317–326)

CELL ACTIVATION has been proposed to be, in part, induced by enzymatic transmethylation of plasma membrane phospholipids, which reduces membrane viscosity and enhances membrane fluidity[1–3] by methylating phosphatidyl ethanolamine (PE) to phosphatidyl choline (PC). After these biochemical changes, phospholipase A_2 is activated to mediate further degradation of PC to lyso-PC and arachidonic acid. This hypothesis predict that phospholipid transmethylation in the membranes of neutrophils and lymphocytes is fundamental to many inflammatory processes, reflecting biochemical and/or immunopathologic changes in sites of inflammation. Until recently, however, there were no reported studies of phospholipid methylation in the cell membrane of neutrophils or lymphocytes in any human disorder, probably because of the relatively low enzyme activities of human leukocyte membranes and the limited amount of blood that can ethically be drawn from any one individual subject. However, we have recently developed an assay for measuring phospholipid transmethylation in the membrane fractions of human leukocytes,[4] and with this assay we have demonstrated the enhancement of methyltransferase and phospholipase A_2 activities in the leukocyte membranes of patients with inflammatory disorders.[5–7] Meanwhile, in addition to this quantitatively minor pathway (methylation and subsequent production of arachidonic acid through the mediation of phospholipase A_2), there are two *de novo* pathways in the biosynthesis of phospholipids, including the CDP-choline or -ethanolamine pathways (major pathways) and the base exchange reactions (the third pathway), as shown in Figure 1. The major pathway enzymes include cholinephosphotransferase (CPT) and ethanolaminephosphotransferase (EPT); these enzymes mediate structural phospholipid production.[8–11] In the base exchange reactions, each phospholipid is produced from a corresponding phospho-

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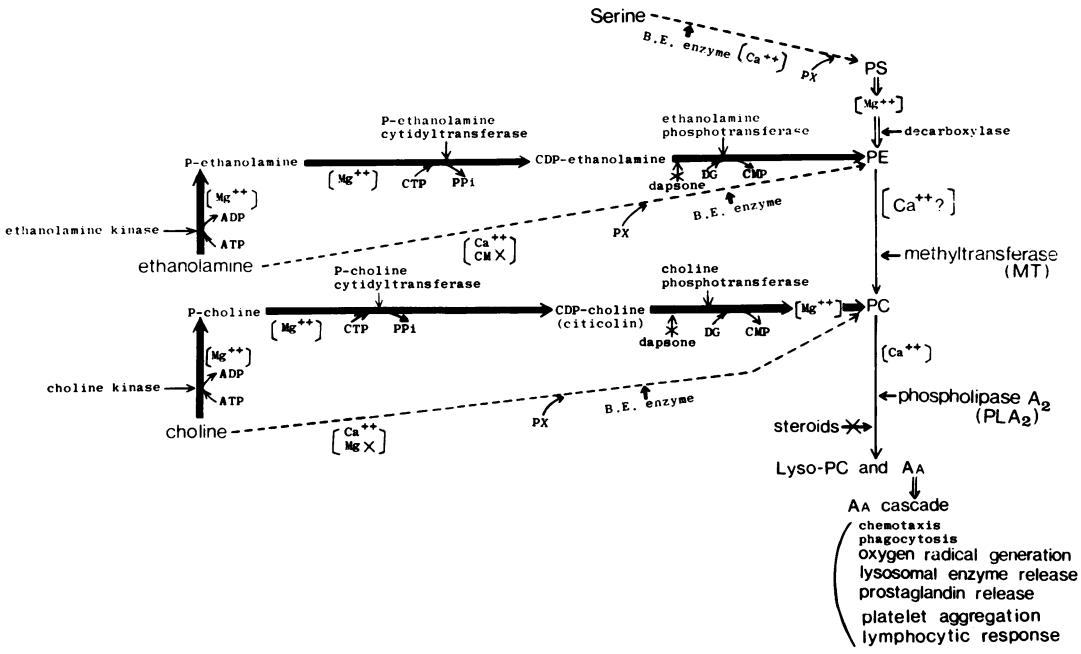

Figure 1—Synthetic and metabolic pathways of phospholipids. The solid, thick line represents the major pathway (———); the solid, thin line, the minor pathway (———.); and the dotted line, base exchange reaction (-----). [Ca^{2+}], calcium ion dependent; [Mg^{2+}], magnesium ion dependent. PS, phosphatidylserine; PE, phosphatidylethanolamine; PC, phosphatidylcholine; AA, arachidonic acid; CDP, cytidine diphosphate; P-ethanolamine, phosphorylethanolamine; P-choline, phosphorylcholine, CTP, cytidine triphosphate; CMP, cytidine monophosphate; DG, diacylglycerol; B.E. enzyme, base exchange enzyme.

lipid by the exchange of bases located in the C-3 position; the physiologic and biologic significance of the base exchange reactions has not been well clarified. Ca^{2+}-dependent, energy-independent phospholipid base exchange reactions to produce phosphatidylserine (PS), PE, and PC have been observed in various tissues and cells.[12-15] In mammals, PS has been reported to be synthesized predominantly by this reaction, while base exchange reactions to produce PE and PC are considered to be quantitatively insignificant.[12] In addition, decreased PS synthesis has been reported during the phagocytic process of neutrophils.[16] More recently, we[11] reported methods for assaying the phospholipid enzyme activities of EPT, CPT, and the base exchange reactions in normal human leukocytes. In that report, we suggested some correlation of the ethanolamine base exchange but no relationship of CDP-choline or -ethanolamine pathways to the cell activation; we further speculated regarding the role of PE formation by base exchange reaction in the induction of inflammatory processes such as activation of transmethylation, arachidonic acid production, and the subsequent generation of inflammatory mediators.

In the present study, we have assayed all these membrane phospholipid enzyme activities in neutrophils and lymphocytes at the height of inflammation in diseased patients. The changes of these enzyme activities in inflammatory disorders and the correlation of the various phospholipid synthetic and metabolic pathways with disease activity are discussed.

Materials and Methods

The present series consisted of 7 patients with Behçet's disease (4 male, aged 24–45; 3 female, 35–45 years old), 6 with systemic lupus erythematosus (SLE) (all female, 20–31 years old), 12 with rheumatoid arthritis (RA) (3 male, 39–45 years old, 9 female, 25–44 years old), 8 with acute and severe bacterial infections (5 male, 18–52 years old; 3 female, 30–49 years old), and 6 adults with acute and severe viral infections (2 male, 40–54 years old; 4 female, 21–52 years old). Ten sex- and age-matched healthy volunteers served as controls. Bacterial infections included pneumonia, peritonsillar abscess, appendicitis, and bacterial sepsis. Viral infections included varicella, measles, influenza, and rubella. All of the patients with Behçet's disease, SLE, and RA were in the active stage of their disease, and all of those with bacterial or viral infections manifested severe symptoms (such as high fever, generalized skin rash, etc.). The diagnosis of each disease and the assessment of disease activity were performed according to American Rheumatism Association,[17] Shiokawa,[18] and our previous studies.[5,7,19,20] None of the subjects had received aspirin, indometh-

acin, glucocorticosteroids, or colchicine for 72 hours prior to the experiments; these drugs are known to affect biochemical changes in cell membranes.[1,21-23]

Preparation of Microsomal Fractions

This was carried out as previously described.[4,5,11] Briefly, neutrophils or lymphocytes were separated from venous blood by Ficoll–Hypaque centrifugation. In order to obtain the greatest yield of microsomes, we suspended each washed cell fraction in 0.25 M sucrose and sonicated it at 24 W for 10 seconds on ice with a Branson Sonifier Cell Disrupter 200. The crude homogenates were centrifuged at 14,000*g* for 10 minutes at 4 C. The microsomal fraction was then recovered from the supernatants by centrifugation at 105,000*g* for 60 minutes at 4 C. In order to confirm whether membrane fraction is well prepared or not in our base exchange assay, the marker enzyme 5′-nucleotidase activity was assessed in our harvested microsomal fraction as previously described.[4] As the result, 5′-nucleotidase activity was found to be high in our microsomal fraction. Therefore, a considerably greater amount of plasma membrane was considered to be contained in the microsomes. For this reason, we refer to crude membrane fractions used in this study as the "microsomal" fraction.

Assays of CPT and EPT

CPT (CDP-choline-1, 2-diacyl-*sn*-glycerol cholinephosphotransferase, EC 2.7.8.2) and EPT (CDP-ethanolamine-1, 2-diacyl-*sn*-glycerol ethanolaminephosphotransferase, EC 2.7.8.1.) activities were determined with the use of cytidine-5′-diphosphate [^{14}C-methyl]-choline ([^{14}C]-CDP-choline) and cytidine-5′-diphosphate [1-^{3}H]-ethanol-2-amine ([^{3}H]-CDP-ethanolamine), respectively. [^{3}H]-CDP-ethanolamine was synthesized enzymatically from ^{3}H-ethanolamine by a two-step reaction[24,25] in our laboratory as previously described.[11] The standard assay mixture contained 100 mM Tris-HCl (pH 8.0), 0.1 mM EGTA, 5 mM $MgCl_2$, 50 μM [^{14}C]-CDP-choline (0.1 μCi), or 50 μM [^{3}H]-CDP-ethanolamine (0.1 μCi) and 20–50 μg protein of above-obtained microsome fraction in a total volume of 0.2 ml, and was incubated at 37 C for 30 minutes. The reaction was terminated by the addition of 0.6 ml of 0.25 M HCl and 3 ml of chloroform/methanol (1 : 2, vol/vol). In order to extract the phospholipids, the chloroform phase, which had been partitioned by the addition of 1 ml of 1% KCl and 1 ml of chloroform, was washed with 1 ml of 50% methanol containing 0.5% KCl and then with 1 ml of 60% methanol. Then the chloroform phase was removed and transferred to a counting vial; the radioactivity was measured after drying at 80 C in an oven and the addition of 10 ml of liquid scintillation cocktail. Enzyme activities were expressed as picomoles of each radioactive substrate incorporated into phospholipid per minute per milligram protein. Control tubes, lacking the microsomal fractions, were treated identically throughout the assay.

Although diacylglyceride with detergent has been occasionally used for the assessment of EPT and CPT activities,[26-28] neither diacylglyceride nor detergent was added to the present reaction mixture for the following reasons, as indicated by others[26-28]: The addition of diacylglyceride with some detergent must change the enzyme and lipid environment of the leukocyte membranes. In addition, it is very difficult to obtain the optimal concentration of the detergent with sufficient enzyme activity and stimulatory effect by diacylglyceride; since diacylglyceride with sufficient effect is water-insoluble, it requires detergent with high viscosity, which, adversely, inhibits enzyme activities if the concentration of the detergent exceeds the optimal concentration and is difficult to measure. Yet the effective range of the concentration is quite small. Further, the enzymes have a particular substrate selectivity depending on diacylglyceride species.[26-28] For these reasons, we used endogenous diacylglyceride as substrate to analyze the "subnatural state" of the leukocyte membranes.

Phospholipid Base Exchange Enzyme Assay

In the harvesting of leukocyte membranes for base exchange activity assay, some modifications were performed; the cell suspensions were made 1 mM with respect to ethylene glycol bis (β-aminoethyl ether)-N,N,N′,N′-tetraacetic acid (EGTA), and 10 mM with respect to 2-mercaptoethanol (2 ME), before sonication. In order to deplete the membrane preparations of contaminating free serine, ethanolamine, choline, and calmodulin, the membrane pellets were disrupted in a Teflon–glass homogenizer, with subsequent centrifugation at 105,000*g* for 60 minutes at 4 C, as performed in our original study.[29]

Incubations were performed in a shaking water bath at 37 C for 10 minutes. The standard assay mixture contained 50 μg membrane protein, 3 mM $CaCl_2$, 50 mM N-2-hydroxyethylpiperazine-N′-2-ethane-sulfonic acid (Hepes) buffer (pH 7.5), 0.25 mM EGTA, 2.5 mM mercaptoethanol, 62.5 mM sucrose, and 10 μM ^{3}H-serine (2 μCi), ^{3}H-ethanolamine (2 μCi), or ^{3}H-choline (4 μCi) in a total volume of 0.2 ml. Subsequent reaction procedures were performed

as in the CDP-choline pathway enzyme assay, and each enzyme activity was expressed as picomoles of free base incorporated into the corresponding phospholipid per minute per milligram protein.

Analysis of the reaction products in each base exchange was performed as previously described,[11] with the use of two-dimensional thin-layer chromatography (TLC) with high-performance silica gel glass plates. The percentage recovery rates of phospholipids, as determined by phosphorus analysis,[30] were as follows: PE, 91; PS, 93; PC, 96; lyso-PE, 87; lyso-PS, 73; and lyso-PC, 86, respectively.

Assay of Base Exchange Activities in Normal Leukocytes Following Incubation With Patients' Serum

After preincubation of normal neutrophils or lymphocytes with 20% serum derived from the patients at 37 C for 20 minutes, the cells were sonicated and assessed for each of the three base exchange activities as described above. Allogeneic serum from healthy controls was also tested for stimulation of each base exchange activity of normal cells as a control. Further, patient-derived serum was heat-inactivated at 56 C for 30 minutes to be tested for its effect on the enzymatic activities of the normal cells.

Counting efficiency of ^{3}H assessed was 31.6% and that of ^{14}C was 75.9% in our membrane assay, respectively.

Statistical significance between patients and controls was ascertained by using the Student t test or Rank's test.

Results

As shown in Figure 2, PE formation from ^{3}H-ethanolamine by base exchange reaction was markedly or significantly increased in the neutrophil membranes of patients with active Behçet, active SLE, and bacterial infections and slightly in active RA patients (bacterial, $P < 0.001$; SLE, Behçet, $P < 0.01$; RA, $0.01 < P < 0.05$) (Figure 2). In lymphocyte membranes, ethanolamine base exchange activity was also significantly enhanced in the patients with active RA and slightly in active Behçet's disease and active SLE (RA, $P < 0.01$; SLE, Behçet, $0.01 < P < 0.05$) (Figure 3). On the other hand, in the membranes of both neutrophils and lymphocytes of patients with viral infections and inactive cases of RA, SLE, and Behçet's disease, no significant difference of PE formation in base exchange was observed ($P > 0.05$) (data except for viral infection patients not shown). Neither serine nor choline exchange in either neutrophils or lymphocytes was abnormal in any of the patients. There was a statistically insignificant trend toward lower PC formation by base exchange reaction in neutrophils and lymphocytes from the RA, SLE, Behçet's, and bacterial infection patients in whom PE formation was increased ($P > 0.05$, Figures 2 and 3).

The CDP-choline or -ethanolamine pathway enzyme activities (CPT and EPT) did not show significant changes in the leukocyte membranes of the patients tested (data not shown). However, the variance of these measurements was greater in all of the patients except those with viral infections. Some patients with severe inflammation showed enhancement of the CPT and/or EPT activities, whereas the

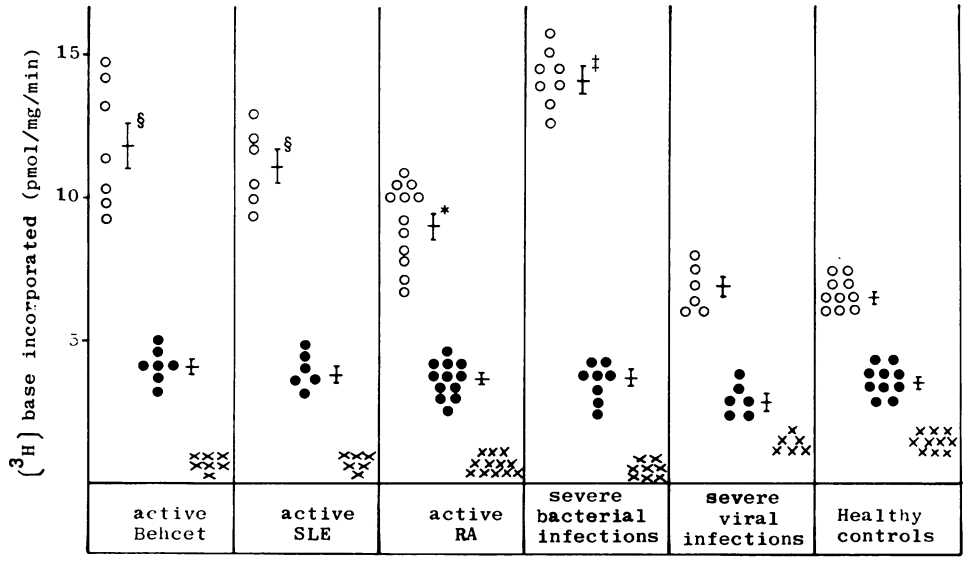

Figure 2—Incorporation of the three bases into neutrophil membrane phospholipids by base exchange reactions. Open circle (○) denotes incorporation of ^{3}H-ethanolamine; closed circle (●), ^{3}H-serine; crossed symbol (×), ^{3}H-choline. *$0.01 < P < 0.05$ versus controls, §$P < 0.01$. ‡$P < 0.001$.

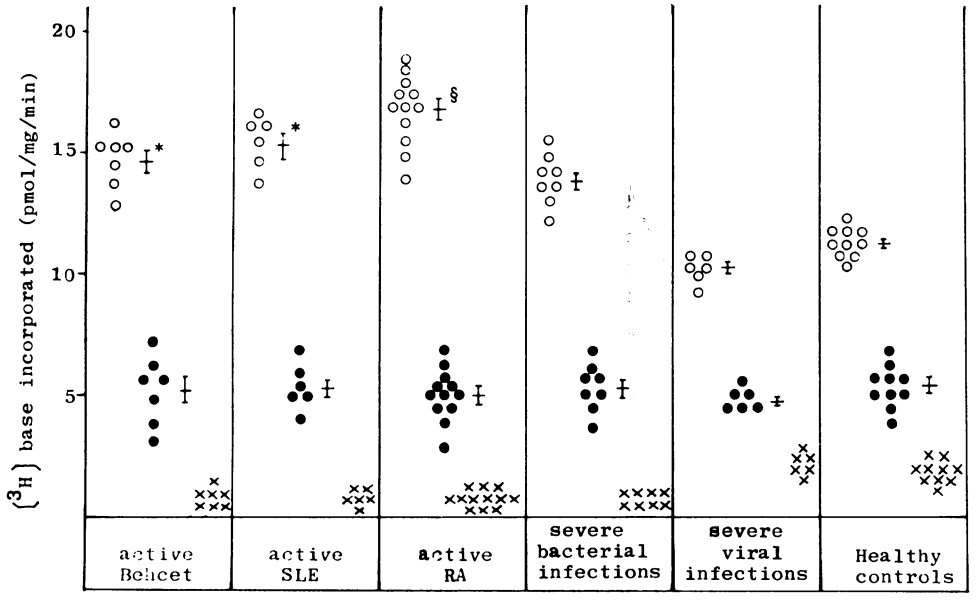

Figure 3—Incorporation of the three bases into lymphocyte membrane phospholipids by base exchange reactions. For symbols see legend for Figure 2.

activities of most of the patients fell within the normal range.

Because we had previously seen enhancement of base exchange by bioactive stimulants,[11] we investigated the effect of patients' serum on the base exchange activities. Although the serum derived from most of the patients did not exert a significant effect on normal cell enzyme activities, SLE serum raised the basal levels of PE formation in the base exchange reactions of both normal neutrophils and lymphocytes (neutrophils, $0.01 < P < 0.05$; lymphocytes, $P < 0.01$), whereas RA serum increased the PE formation of normal neutrophils ($0.01 < P < 0.05$) but not normal lymphocytes ($P > 0.05$) (Table 1). On the other hand, no activation of serine or choline exchange activity of normal leukocytes was found by preincubation with the serum from any of the patients tested (not shown). Normal cell enzyme activity preincubated with heat-treated patients' serum did not show significant change in any of the base exchange activities, including ethanolamine exchange. Allogeneic serum from healthy controls also did not affect the base exchange activities, suggesting that the activation of ethanolamine exchange with the pa-

Table 1—Ethanolamine Exchange Activity of Normal Neutrophils and Lymphocytes Stimulated With Serum From Patients With Active SLE or RA

		Preincubated with serum from			
	No preincubation	Active SLE	Inactive SLE	Active treated*	Normals†
PE synthesis‡					
PMN§	6.53 ± 0.5	9.14 ± 0.8‖	7.14 ± 0.6	6.21 ± 0.5	6.65 ± 0.4
lymphocyte	11.2 ± 0.9	17.3 ± 1.4**	12.4 ± 1.0	10.6 ± 0.8	11.8 ± 0.9
		Preincubated with serum from			
	No preincubation	Active RA	Inactive RA	Active treated*	Normals†
PE synthesis‡					
PMN§	6.53 ± 0.5	8.97 ± 0.8‖	6.88 ± 0.7	6.15 ± 0.8	6.88 ± 0.5
lymphocyte	11.2 ± 0.9	13.0 ± 1.4	11.8 ± 1.5	10.5 ± 1.4	11.7 ± 1.1

*Active, treated denotes RA or active SLE patient-derived serum which was heat-inactivated at 56°C for 30 min.
†Donors of the serum are allogeneic to those of the cells used.
‡Expressed as pmol/min/mg protein of [^{3}H]-ethanolamine incorporated.
§PMN, polymorphonuclear leukocyte (neutrophil).
‖$0.01 < P < 0.05$.
**$P < 0.01$ versus control.

tients' serum obtained in this study is not due to HLA difference between the donors of the cells and the donors of the serum (not shown).

These findings on PE synthesis by base exchange are comparable with our previous results on the changes in transmethylation and phospholipase A_2 activity in patients with similar inflammatory disorders,[5-7] the similarity being greater to transmethylation than to phospholipase A_2 activity. In patients with viral diseases, ethanolamine exchange was unchanged; whereas in our previous report,[5] the phospholipase A_2 activity of lymphocytes was increased, but methylation was not enhanced. As for the effect of malignant RA[18] serum, PE formation by base exchange of normal neutrophils was enhanced, but that of lymphocytes was not increased. Meanwhile, transmethylation of neutrophils was stimulated, but that of lymphocytes was not enhanced.[7]

Table 2 shows the close correlation of the ethanolamine base exchange activity to the laboratory findings, clinical course, and response to treatment in the representative case of SLE, which was available for study before the start of conventional treatment. PE formation by base exchange closely paralleled disease activity. Ethanolamine exchange was elevated before treatment with corticosteroids and fell toward the normal range in response to treatment. In addition, ethanolamine exchange appears to be the most sensitive indicator of the disease activities, preceding any other laboratory abnormalities (Table 2).

Discussion

We have recently reported that leukocyte methyltransferase and phospholipase A_2 are closely correlated with the degree of disease activity in inflammatory disease patients. Our findings support the hypothesis of Hirata et al, as well as other investigators,[1-3,31-33] that N-transmethylation of PE to form PC enhances the fluidity of the cell membrane with resultant activation of phospholipase A_2, leading to the induction of the arachidonic acid cascade and subsequent production of inflammatory mediators. In previous reports[4-7] we have addressed criticisms[34-40] that have been raised against the hypothesis that implicates a role for transmethylation in membrane fluidity and the induction of inflammation. In the present study, we have shown that, in addition to transmethylation and phospholipase A_2, ethanolamine base exchange correlates with other parameters of inflammation, although the physiologic and biochemical significance of this correlation has not been clarified. With regard to the effect of patients' serum, ethanolamine base exchange also showed behavior similar to minor pathway enzyme activity (methyltransferase and phospholipase A_2).

Ethanolamine exchange, alone among the three base exchange activities, was increased in patients with inflammatory states. Although the three phospholipid base exchange reactions, serine, choline, and ethanolamine, appear to share common mechanisms, each base exchange activity has also been reported to vary independently.[11,12,16] Furthermore, this reaction paralleled the changes in the patients' clinical courses, reflecting more sensitively the level of disease activity and the influence of treatment than did the assays of either transmethylation or phospholipase A_2 activity.

Major pathway enzyme activities (EPT and CPT) were not significantly abnormal in the patients tested. This finding is consistent with the hypothesis that major pathway enzymes do not mediate cell activation, but rather the formation of structural membrane phospholipids.[8,9,11] These activities are not stimulated by chemoattractant,[10] opsonized zymosan, or concanavalin A[4,11] in the appropriate target cell populations.

We observed in this study that PE synthesis by base exchange behaved in a manner similar to the minor pathway enzymes, transmethylase and phospholipase A_2. Previous work in our laboratory likewise suggested a correlation between ethanolamine exchange and cell-activating systems such as transmethylation and phospholipase A_2,[11] based on the following data: 1) The base exchange reaction was dependent on Ca^{2+} but inhibited by Mg^{2+}, which is similar to the minor pathway, but not to CDP-choline or -ethanolamine structural phospholipid pathway enzymes (CPT and EPT).[1,4,11] 2) Every stimulant of neutrophils and lymphocytes that was tested enhanced the activity of ethanolamine exchange while having no effect on the major pathway enzyme activities (CPT and EPT).[11] 3) Optimal concentrations of these cell stimulants in the ethanolamine base exchange were quite similar to those of the transmethylation assay[4,11]; transmethylation was enhanced only when concentrations of bioactive stimulants were lower than those generally used to stimulate the activity of whole cells. 4) In contrast to the other two base exchange reactions, ethanolamine-exchange alone was not inhibited by calmodulin, on which other cell activating systems (methylation and phospholipase A_2) are dependent.[1-3,11] 5) The EGTA/$CaCl_2$ system used in preparing the membrane fraction and incubation mixtures in assaying base exchange also provides the best assay conditions for the methylation assay.[11]

Although neutrophils are known to play a key role in Behçet's disease[19,41-43] and lymphocytes are closely

Table 2—The Changes of Clinical Course, Laboratory Findings, and Phospholipid Enzyme Activities in a Case of Active SLE

		1985						
		April 8	May 13	June 10	June 24	July 17	Aug 19	Sep 9
Symptoms (active rash, arthritis, etc.)		Very slight		Mild	Moderate		Severe	Mild
Therapy		[Streptomycin (1 g for 3 weeks)]		[Mefanamic acid (1 g)]			Prednisolone [30 mg	20 mg]
Temperature (C)		36.2	36.4	36.5	36.5	37.8	38.2	36.8
Leukocyte counts ($\times 10^3$/cu mm)		2.3	2.2	2.4	2.1	2.1	2.1	6.1
Erythrocyte counts ($\times 10^4$/cu mm)		375	370	374	370	340	330	350
CRP (mm)		0	0	0	0.5	2	5	1.5
ESR (mm/hr)		17	20	21	26	76	82	25
Urine: protein (mg/day)		—	—	—	—	45	80	—
Urine: sediment (per field): leukocyte		—	—	—	—	8–10	21–24	—
Urine: sediment (per field): erythrocyte		—	—	—	—	4–5	6–8	—
Urine: sediment (per field): epithelial cell		—	—	—	—	8–10	10–13	—
Histology of the skin		liquefaction (−) (+); PMNs, round cell infiltration (perivascular) (+); vascular damage (−) (+)					liquefaction (++); PMNs, round cell infiltration (perivascular) (++); vascular damage (+)	
Unstimulated Methylation (pmol/ /mg protein)	PMNs	0.70			1.0		1.68	1.29
	Lymphocytes	0.89			1.4		1.85	1.39
Phospholipase A_2 (%)	PMNs	3.4			4.8		5.5	4.7
	Lymphocytes	5.6			8.2		8.9	7.5
Ethanolamine base exchange reaction	PMNs	10.8			10.4		13.8	7.4
	Lymphocytes	16.5			15.3		17.0	11.8

related to the pathogenesis of SLE, both neutrophil and lymphocyte membrane activities were found to be enhanced in these two diseases. With both diseases, we found similar abnormalities in the two leukocyte populations in our transmethylation assay[7]; this probably can be explained by our previous studies, in which we found that abnormally enhanced B lymphocytes are involved in the pathogenesis of Behçet's disease[44] and that neutrophil-related oxygen radicals induce tissue injury and lymphocyte abnormalities in SLE.[45-47] Nevertheless, it still seems paradoxical that in SLE patients, both ethanolamine base exchange activity and transmethylation[7] are more elevated in neutrophils than in lymphocytes. This paradoxical phenomenon can be explained as follows: because neutrophils are evidently less affected by glucocorticoids than lymphocytes,[7,48] and because most SLE patients take substantial doses of corticoids, the lymphocytes themselves might be intrinsically impaired by long-term administration of steroids, despite discontinuation of steroid therapy 72 hours before blood was drawn for study.

Both in the present study (Figures 2 and 3) and in our recent study[11] we found that in leukocyte membranes the ethanolamine exchange activity was far higher than the choline exchange activity, whereas in the major pathway, which does not represent functional but structural phospholipids in the membranes,[8-11] the rate of PE synthesis was, if anything, lower than the rate of PC formation.[11] Furthermore, as shown in Figures 2 and 3, in patients with acute inflammatory disorders, PE formation by base exchange was greatly increased, in parallel with the enhancement of methylation and phospholipase A_2,[5-7] which are considered to be the source of cell activation or biologic responses,[1-3] whereas PC formation tended to be lower than normal. We speculate that these findings are notable for the following reasons: if in leukocytes in which the minor phospholipid biosynthetic pathways are activated PC formation were greater than PE formation in both normal and inflammatory states, then the minor pathway PE → PC → arachidonic acid would be inhibited by an increase in PC (Figure 1). However, it can be considered that because PC is generally contained in the membrane in greater amount than PE, PC is the substrate for PE formation in base exchange reactions. In addition, the amount of PE that can be methylated to PC and acts as the important inflammatory factor releasing arachidonic acids, is very minute, and therefore, it has been argued[33-35,37-40] that methylation has no correlation with membrane viscosity and fluidity, cell activation, or biologic responses. Further, the amount of both PE and PC which is synthesized via base exchange was found in the present experiment to be far smaller than that synthesized through major pathways. On the contrary, as hitherto described, PE synthesis by base exchange is increased, behaving in a manner similar to methyltransferase and phospholipase A_2 enzyme in the presence of bioactive stimulants[4] and in acute inflammatory situations in the leukocyte membrane of the patients.[5-7] In addition, although the amount of phospholipids (PE→PC) which participates in methylation is very minute, the association between methylation and an increase in membrane fluidity and biologic responses has also been reported.[1-3] From these two controversial concepts, one hypothesis (which would be quantitatively ruled out but functionally supported) may be proposed as follows: ethanolamine in the base exchange pathway may serve as the source of PE substrate for transmethylation, leading to the activation of phospholipase A_2.

In the present study, we have begun to examine the changes in the phospholipid enzyme activities in membrane associated events in other inflammatory situations. Further studies are needed to examine other enzyme systems known to be involved. For example, phospholipase C-induced diacylglycerol-related phosphatidylinositol turnover represents another cell-activating system, and PE formation from PS through mediation of decarboxylase represents another pathway for PE synthesis (Figure 1). Neither of these pathways was investigated in our study; the study of these and other enzyme systems in inflammatory diseases and correlation with the present findings are important subjects for future investigation.

References

1. Hirata F, Axelrod J: Phospholipid methylation and biological signal transmission. Science 1980, 209:1082–1090
2. Ishizaka T, Hirata F, Ishizaka K: Stimulation of phospholipid methylation, Ca^{2+} influx, and histamine release by bridging of IgG receptors on rat cells. Proc Natl Acad Sci USA 1980, 77:1903–1906
3. Strittmatter WJ, Hirata F, Axelrod J: Phospholipid methylation unmasks cryptic beta-adrenergic receptors in rat reticulocytes. Science 1979, 204:1205–1207
4. Niwa Y, Sakane T, Taniguchi S: Phospholipid transmethylation in the membrane of human neutrophils and lymphocytes. Arch Biochem Biophys 1984, 234:7–14
5. Niwa Y, Sakane T, Yamamoto S, Kano T, Taniguchi S: Methyltransferase and phospholipase A_2 activity in the membrane of neutrophils and lymphocytes from patients with bacterial and viral infections. Inflammation 1985, 9:53–65
6. Niwa Y: Neutrophil activation and their release of mediators from the cells, Proceedings of the XVIth International Congress of Rheumatology, ILAR '85. Edited by PM Brooks, JR York. Amsterdam, Elsevier Science Publishers BV, 1985:51–56

7. Niwa Y, Miyachi Y, Kanoh T, Sakane T: Methyltransferase and phospholipase A_2 activities in the membrane of neutrophils and lymphocytes from patients with Behçet's disease, systemic lupus erythematosus, and rheumatoid arthritis. Manuscript submitted
8. Kennedy EP, Weiss SB: The function of cytidine coenzymes in the biosynthesis of phospholipids. J Biol Chem 1956, 222:193–214
9. Weiss SB, Smith SW, Kennedy EP: The enzymatic formation of lecitine from cytidine diphosphate choline and d-1, 2-diglyceride. J Biol Chem 1958, 231:53–64
10. Hirata F, Corcoran BA, Venkatasubramanian K, Schiffmann E, Axelrod J: Chemoattractants stimulate degradation of methylated phospholipids and release of arachidonic acid in rabbit leukocytes. Proc Natl Acad Sci USA 1979, 76:2640–2643
11. Niwa Y, Taniguchi S: Phospholipid base-exchange in human leukocyte membranes: Quantitation and correlation with other phospholipid biosynthetic pathways. Arch Biochem Biophys 1986, 250:345–357
12. Kanfer JN: The base-exchange enzymes and phospholipase D of mammalian tissue. Can J Biochem 1980, 58:1370–1380
13. Hübscher G: Metabolism of phospholipids: VI. The effect of metal ions on the incorporation of L-serine into phosphatidylserine. Biochim Biophys Acta 1962, 57:555–561
14. Porcellati G, Arienti G, Pirotta M, Giorgini D: Base-exchange reactions for the synthesis of phospholipids in nervous tissue: The incorporation of serine and ethanolamine into phospholipids of isolated brain microsomes. J Neurochem 1971, 18:1395–1417
15. Bjerve KS: The Ca^{2+}-dependent biosynthesis of lecithin, phosphatidylethanolamine and phosphatidylserine in rat liver subcellular particles. Biochim Biophys Acta 1973, 296:549–562
16. Tou J-S: Decreased incorporation of L-[U-^{14}C] serine into phosphatidylserine by polymorphonuclear leukocytes during phagocytosis. Biochim Biophys Acta 1979, 575:384–388
17. Bevans M, Nabel J, Demartini F, Ragan C: The systemic lesions of malignant rheumatoid arthritis. Am J Med 1954, 16:197–203
18. Shiokawa Y: Recent advance in collagen diseases, allergic diseases and auto-immune diseases, Chronic Rheumatoid Arthritis: Malignant Rheumatoid Arthritis. Edited by Y Shiokawa. Tokyo, Kanehara Shuppan, 1979, pp 33–44
19. Niwa Y, Miyake S, Sakane T, Shingu M, Yokoyama M: Auto-oxidative damage in Behçet's disease—endothelial cell damage following the elevated oxygen radicals generated by stimulated neutrophils. Clin Exp Immunol 1982, 49:247–255
20. Niwa Y, Kanoh T: Immune deficiency states and immune imbalance in systemic lupus erythematosus and other autoimmune disease. Clin Immunol Immunopathol 1979, 12:289–300
21. Oyanagui Y: Inhibition of superoxide anion production in macrophages by anti-inflammatory drugs. Biochem Pharmacol 1976, 25:1473–1480
22. Hirata F, Schiffmann E, Venkatasubramanian K, Salomon D, Axelrod J: A phospholipase A_2 inhibitory protein in rabbit neutrophils induced by glucocorticoids. Proc Natl Acad Sci USA 1980, 77:2533–2538
23. Vane JR: Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature New Biol 1971, 231:232–235
24. Weinhold PA, Rethy VB: The separation, purification and characterization of ethanolamine kinase and choline kinase from rat liver. Biochemistry 1974, 13:5135–5141
25. Schneider WC: Enzymatic preparation of labeled phosphorylcholine, phosphorylethanolamine, cytidine diphosphate choline, deoxycytidine diphosphate choline, cytidine diphosphate ethanolamine, and deoxycytidine diphosphate ethanolamine. Methods Enzymol 1969, 14:684–690
26. Kanoh T, Ohno, K: Solubilization and purification of rat liver microsomal 1, 2-diacylglycerol: CDP-choline cholinephosphotransferase and 1, 2-diacylglycerol: CDP-ethanolamine ethanolaminephosphotransferase. Eur J Biochem 1976, 66:201–210
27. Holub BJ: Differential utilization of 1-palmitoil and 1-stearoyl homologues of various unsaturated 1, 2-diacyl-*sn*-glycerols for phosphatidylcholine and phosphatidylethanolamine synthesis in rat liver microsomes. J Biol Chem 1978, 253:691–696
28. Vial HJ, Thuet MJ, Philippot JR: Cholinephosphotransferase and ethanolaminephosphotransferase activities in *plasmodium knowlesi*-infected erythrocytes: Their use as parasite-specific markers. Biochim Biophys Acta 1984, 795:372–383
29. Morikawa S, Taniguchi S, Mori K, Kumada K, Fujiwara M, Fujiwara M: Effects of calmodulin antagonists and calmodulin on phospholipid base-exchange activities in rabbit platelets. Thromb Res 1985, 37:267–278
30. Ames BN, Dubin DT: The role of polyamines in the neutralization of bacteriophage deoxyribonucleic acid. J Biol Chem 1960, 235:769–775
31. Pike MC, Kredich NM, Snyderman R: Requirement of S-adenosyl-L-methionine-mediated methylation for human monocyte chemotaxis. Proc Natl Acad Sci USA 1978, 75:3928–3931
32. Schiffmann E, Corcoran BA, Aswanikumar S: Molecular events in the response of neutrophils to synthetic N-fMET chemotactic peptides: demonstration of a specific receptor, Leukocyte Chemotaxis. Edited by JI Gallin, PH Quie. New York, Raven Press, 1978, pp 97–111
33. Crews FT, Morita Y, Hirata F, Axelrod J, Siraganian RP: Phospholipid methylation affects immunoglobulin E-mediated histamine and arachidonic acid release in rat leukemic basophils. Biochem Biophys Res Commun 1980, 93:42–49
34. Vance DE, Kruijff B: The possible functional significance of phosphatidylethanolamine methylation. Nature 1980, 288:277–279
35. Parola AH, Kaplan JH, Lockwood SH, Uzgiris EE: Activation of human lymphocytes by concanavalin A or purified protein derivative results in no alteration of fluorescence polarization of lipid probes although the electrophoretic mobility of the cells is changed. Biochim Biophys Acta 1981, 649:616–624
36. Moore JP, Smith GA, Hesketh TR, Metcalfe JC: Early increases in phospholipid methylation are not necessary for the mitogenic stimulation of lymphocytes. J Biol Chem 1982, 257:8183–8189
37. Chien MM, Ashman RF: Phospholipid transmethylation in human mononuclear cells is not influenced by mitogens. Mol Immunol 1984, 21:621–625
38. Aksamit RR, Backlund PS Jr, Gantoni GL: Chemotaxis and the synthesis of specific proteins are inhibited by 3-deaza-adenosine and other adenosine analogs in a mouse macrophage cell line. J Biol Chem 1983, 258:20–23
39. Garcia-Castro I, Mato JM, Vasabthakumar G, Weismann WP, Schiffmann E, Chiang PK: Paradoxical effects of adenosine on neutrophil chemotaxis. J Biol Chem 1983, 258:4345–4349
40. Gil MG, Alonso F, Sänchez-Crespo M, Mato JM: Inhibition of phospholipid methyltransferase during zymosan induced secretion of platelet-activating factor in human polymorphonuclear leukocytes. Biochem Biophys Res Commun 1981, 101:740–748

41. Matsumura N, Mizushima Y: Leukocyte movement and colchicine treatment in Behçet's disease. Lancet 1975, 2:813
42. Abdulla YH, Lehner T: The effect of immune complexes on chemotaxis in Behçet's syndrome and recurrent oral ulcers, Behçet's Syndrome—Clinical and Immunological Features. Edited by T Lehner, CG Barnes. London, Academic Press, 1979, pp 55–56
43. Shimizu T, Katsuta Y, Oshima Y: Immunological studies on Behçet's syndrome. Ann Rheum Dis 1965, 24:494–500
44. Sakane T, Ueda Y, Suzuki N, Niwa Y, Hoshino T, Tsunematsu T: OKT^{4+} and OKT^{8+} lymphocytes produce soluble factors that can modulate growth and differentiation of human B cells. Clin Exp Immunol 1985, 42:112–120
45. Niwa Y, Sakane T, Shingu M, Miyachi Y: Role of stimulated neutrophils from patients with systemic lupus erythematosus in tissue injury, with special reference to serum factors and increased active oxygen species generated by neutrophils. Inflammation 1985, 9:163–172
46. Abramson SB, Given WP, Edelson HS, Weissmann G: Neutrophil aggregation induced by sera from patients with active systemic lupus erythematosus. Arth Rheum 1983, 26:630–636
47. Hashimoto Y, Ziff M, Hurd ER: Increased endothelial cell adherence, aggregation, and superoxide generation by neutrophils incubated in systemic lupus erythematosus and Felty's syndrome sera. Arth Rheum 1982, 25:1409–1418
48. Ohara M, Shirado M, Miyata M, Sakuma H, Nanamiya M, Yoshida N, Yoshida H, Kasukawa R: Natural and antibody-dependent cellular cytotoxicity of polymorphonuclear leukocytes. Tohoku J Exp Med 1983, 140:59–66