

INCORPORATION, DISTRIBUTION, AND TURNOVER OF  
ARACHIDONIC ACID WITHIN MEMBRANE PHOSPHOLIPIDS  
OF B220<sup>+</sup> T CELLS FROM AUTOIMMUNE-PRONE  
MRL-*lpr/lpr* MICE

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The composition and turnover of arachidonic acid (AA)<sup>1</sup> within T lymphocyte membranes vary with the state of cellular activation and influence the functional capacity of a cell. An early response to ligand-receptor binding of T cells is the release of arachidonate from (1) and the redistribution of arachidonate within (2) membrane phospholipids. Changes in the content of membrane fatty acids, particularly AA, have been shown to modify transmembrane transport processes (3), membrane enzyme activity (4), and the cytotoxic efficiency of T cells (5). AA serves as a precursor of eicosanoids (6), stimulates T cell mitogenesis (7), and promotes the activation of protein kinase C (PKC) (8).

Arachidonate comprises ~25% of all fatty acids within T cell membranes. It is primarily found within T cell membranes esterified to the phosphoglycerides: phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylinositol (PI), and phosphatidylethanolamine (PE). Intact, resting T cells incorporate trace amounts of radiolabeled arachidonate into membrane phospholipids in a highly predictable pattern (9). PC is the major recipient of arachidonate. Lesser amounts are incorporated into PI, PE and PS. 3-5% of labeled AA remains unesterified.

The incorporation and distribution of arachidonate in T cells is a complex process that in part depends upon the activities of two membrane-bound enzymes. Acyl-coenzyme A (CoA) synthetase catalyzes the ATP-dependent production of arachidonoyl-CoA (10). Acyl-CoA:lysophosphatide acyltransferase promotes the transfer of AA into lysophospholipids (9). The reaction catalyzed by acyl-CoA transferase is reversible, CoA-requiring, but ATP-independent and favored if a suitable acceptor is present (9). AA may also be incorporated into phospholipids by a CoA-independent

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<sup>1</sup> *Abbreviations used in this paper:* AA, arachidonic acid; CoA, coenzyme A; NL, neutral lipid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PKC, protein kinase C; PLA<sub>2</sub>, phospholipase A<sub>2</sub>; PS, phosphatidylserine.

mechanism in which a phospholipase releases arachidonate from one lipid and transfers the fatty acid to a lysolipid acceptor. The ultimate distribution of AA within the phospholipid pool depends upon the availability of lysophospholipid substrate, which in turn is governed by the rate of turnover of individual phosphoglycerides.

The turnover of AA-containing phospholipids reflects the balance between the gain in arachidonate through the remodeling activity of acyl-CoA transferase (9) and the release of arachidonate after metabolism of phospholipids (1). The cleavage of arachidonate from phospholipids is highly selective. The membrane enzyme phospholipase A<sub>2</sub> (PLA<sub>2</sub>), which shows a nonpreference for PI, acts mainly on PC and PE to produce free AA and lyso-PC and lyso-PE, respectively (11). In contrast, a specific phospholipase C (PLC) (reviewed in reference 12) initiates the degradation of PI, and in concert with diacylglycerol (DAG) and monoacylglycerol lipases (13), liberates AA.

Products of phospholipid metabolism may impact significantly on transmembrane signal transduction in T cells. The best characterized phospholipid ligand system is comprised of phosphoinositides. Stimulation of T cells with specific antigens or mitogenic lectins is accompanied by the hydrolysis of phosphoinositol-4,5-bisphosphate (PIP<sub>2</sub>) by PLC (12), which results in the formation of inositol triphosphate (IP<sub>3</sub>), and DAG (reviewed in references 14, 15). IP<sub>3</sub> mobilizes Ca<sup>2+</sup> from intracellular stores (15). DAG promotes the translocation of PKC (15). PKC, activated in the presence of Ca<sup>2+</sup>, DAG, and phospholipid, phosphorylates cellular substrates such as the IL-2 receptor. Lyso-PC, formed by PLA<sub>2</sub> cleavage of PC, stimulates the production of cyclic GMP (16), a nucleotide implicated in T cell mitogenesis (17). More recently, lyso-PC has been shown to directly augment the activity of PKC (18).

Because the movement of AA within phosphoglycerides is linked to ligand-receptor binding and the biochemical pathways that lead to the generation of second messengers, aberrations in the metabolism of arachidonate-containing phospholipids may be associated with and potentially contribute to abnormal T cell function. To test this hypothesis, we have examined the incorporation, distribution, and turnover of AA within the membrane phosphoglycerides of T cells that are known to exhibit immunoregulatory disturbances. B220<sup>+</sup> T cells from autoimmune MRL-*lpr/lpr* mice were used as the cellular model. These T cells, which massively accumulate in mice that are homozygous for the *lpr* gene (19, 20), are Thy-1<sup>+</sup>, Ly-1<sup>+</sup>, L3T4<sup>-</sup>, Lyt-2<sup>-</sup> (21) and express the B220 antigen normally present on pre-B cells and B cells (22). B220<sup>+</sup> T cells exhibit defective lymphokine production and responsiveness upon lectin stimulation (23-25) and have been reported to demonstrate reduced formation of inositol phosphates and DAG after exposure to T cell mitogens (26). The current study shows that in the absence of stimulation with exogenous antigens, B220<sup>+</sup> T cells manifest a marked preference for distributing labeled arachidonate within PI. The predilection of B220<sup>+</sup> T cells to form arachidonoyl-PI results from greatly increased constitutive PI turnover which, in turn, appears to be due to excessive PLA<sub>2</sub>-like activity.

### Materials and Methods

*Mice.* MRL-+/+ (H-2<sup>k</sup>), MRL-*lpr/lpr* (H-2<sup>k</sup>), C3H/HeN (H-2<sup>k</sup>), and BALB/c (H-2<sup>d</sup>) mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Mice were studied at 12-14 wk of age.

**Materials.** Fatty acid-free BSA was obtained from Boehringer Mannheim Biochemicals (Indianapolis, IN). Arachidonic acid (20:4), myristic acid (14:0), palmitic acid (16:0), and stearic acid (18:0), CoA sodium salt, ATP, MgCl<sub>2</sub>, MgSO<sub>4</sub>, NaCl, CaCl<sub>2</sub>, KCl, NaH<sub>2</sub>PO<sub>4</sub>, NaOH, mannitol, glucose, Tris(hydroxymethyl)-aminomethane (7-9) (Tris), EGTA, PMSF, and BME amino acids were obtained from Sigma Chemical Co. (St. Louis, MO). Fatty acid methyl ester standard (PUFA-2) was purchased from Supelco (Bellefonte, PA). All solvents used were HPLC grade. Goat anti-mouse IgG was purchased from Tago, Inc. (Burlingame, CA). RA3-6B2 was obtained from American Type Culture Collection (Rockville, MD). Fluoresceinated anti-Thy-1.2 was purchased from Becton-Dickinson & Co. (Mountain View, CA). Fluorescein-conjugated MARK-1, a murine antibody to rat  $\kappa$  chains, was obtained from AMAC, Inc. (Westbrook, ME). PC, 1-stearoyl,2-arachidonoyl [5,6,8,9,11,12,14,15-<sup>3</sup>H(N)] ([<sup>3</sup>H] AA-PC) (91 Ci/mmol), [5,6,8,9,11,12,14,15-<sup>3</sup>H]arachidonic acid ([<sup>3</sup>H] AA) (94 Ci/mmol), [arachidonoyl-1-<sup>14</sup>C] arachidonoyl coenzyme A ([<sup>14</sup>C]arachidonoyl-CoA) (53.4 Ci/mol), [choline-methyl-<sup>14</sup>C]-sphingomyelin (50 Ci/mol) ([<sup>14</sup>C]Sph), and inositol, myo-[2-<sup>3</sup>H(N)] ([<sup>3</sup>H]myoinositol) (15 Ci/mmol) were obtained from New England Nuclear (Boston, MA). Lyophilized PC, PS, PI, PE, phosphatidic acid (PA), Lyso-PC, Lyso-PS, Lyso-PI, and Lyso-PE were purchased from Avanti Polar Lipid, Co. (Birmingham, AL). Silica Gel G TLC plates were obtained from Analtech Inc. (Newark, DE). Silica Gel 60 TLC plates were purchased from Merck (Darmstadt, FRG).

**Preparation of Cells.** Single cell suspensions prepared from lymph nodes were freed of erythrocytes by osmotic lysis. T cell-enriched preparations were obtained by suspending cells (10<sup>7</sup>/ml) in Eagle's Basal Medium (BME) plus 0.02% NaN<sub>3</sub> and 5% FCS and incubating the mixture on 100 × 15 mm plastic petri dishes (Falcon Labware, Oxnard, CA) precoated with 5  $\mu$ g of anti-mouse Ig at 4°C for 70 min to eliminate B cells. The nonadherent cells were recovered, washed, and the procedure was repeated. The preparations contained 92-96% Thy-1.2<sup>+</sup> cells by immunofluorescence and cytofluorographic analyses (27). In MRL-*lpr/lpr* mice, 92 ± 4% of the cells expressed the B220 antigen as assessed by staining with RA3-6B2 mAb (22). MRL-*lpr/lpr* lymph node T cells are referred to as B220<sup>+</sup> T cells hereafter. Nonspecific esterase staining indicated that the preparations contained between 1 and 3% macrophages.

**Quantification of [<sup>3</sup>H]Arachidonate within Membrane Glycerolipids.** Lymph node T cells (10<sup>7</sup>/ml) were incubated in BME at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub> for varying periods of time with 0.5  $\mu$ Ci/ml of [<sup>3</sup>H]AA complexed to BSA. The suspensions were centrifuged at 400 g, the supernatant was removed, and the cells were resuspended in PBS. The suspensions were then spiked with [<sup>14</sup>C]Sph (100,000 dpm; 0.9 nm) to assess recoveries, and membrane lipids were extracted by the method of Bligh and Dyer (28). Organic and aqueous phase radioactivity were quantified by liquid scintillation counting using a Beckman Instruments Inc. (Fullerton, CA) LS 7500 liquid scintillation counter. The organic phase was dried under a stream of nitrogen, membrane lipids were resuspended in chloroform/methanol (3:1), and spotted onto silica gel G plates. The individual phospholipid molecular species were resolved by TLC using a solvent system consisting of chloroform/methanol/2-propanol/0.25% aqueous KCl/triethylamine (90:27:75:18:54). The radioactive TLC profiles were visualized using a Bioscan System 200 Imaging Scanner (Washington, DC). Areas corresponding to authentic sphingomyelin (R<sub>F</sub> 0.19), PC (R<sub>F</sub> 0.22), PS (R<sub>F</sub> 0.34), PI (R<sub>F</sub> 0.46), PA (R<sub>F</sub> 0.50), PE (R<sub>F</sub> 0.54), AA (R<sub>F</sub> 0.77), and neutral lipid (R<sub>F</sub> 0.86) were scraped from the plate, and the radioactivity was quantified by liquid scintillation counting. The overall recovery of radioactive phospholipid (67-72%) was not significantly different in cells from MRL-*+/+* and MRL-*lpr/lpr* mice.

**Assessment of the Molar Concentration of AA within Phospholipids.** Lipid extracts were prepared from either fresh lymph node T cells or cells cultured in BME plus 1% BSA for up to 2 h. The membrane lipids were then spotted on TLC plates as described above. Individual phospholipids were scraped from the plate, then eluted from the silica into chloroform/methanol (1:2). The silica was removed by passage through a filtration column (J. T. Baker Co., Phillipsburg, NJ), and the remaining silica was washed with chloroform/methanol (2:1). The phospholipid solution was spiked with heptadecanoic acid (17:0) (10  $\mu$ g) as an internal standard, and the organic solvents were dried under nitrogen. Fatty acid methyl esters (FAMES)

of the phospholipids were made by heating the individual phospholipids in 10% hydrochloric acid in methanol (1 ml) at 100°C for 12 min (29). The reaction mixture was cooled on ice, and the acid was neutralized using 5 N NaOH (0.5 ml). The FAMES were twice extracted from the aqueous phase into hexane (1 ml), and the FAME-hexane was dried under nitrogen. The FAMES were resuspended in hexane (50  $\mu$ l) and resolved from 140 to 220°C using a Hewlett-Packard (Palo Alto, CA) 5890A gas chromatography system equipped with a DB255 column (0.25  $\mu$ M  $\times$  30 m) (J & W Scientific, Folsom, CA). Individual FAMES were detected by a flame ionization detector and identified by comparison of  $R_F$  of known FAME standards. The quantity of arachidonoyl-methyl ester was determined relative to the heptadecanoyl-methyl ester peak area.

*Phospholipid Turnover in T Cells.* T cells ( $10^7$ /ml) were prelabeled with [<sup>3</sup>H]AA (0.5  $\mu$ Ci) for 60 min at 37°C then washed twice. Duplicate samples of labeled cells were recultured in acid-washed, glass test tubes at  $10^7$  cells/ml in BME containing 1% BSA. After varying periods of time, the suspensions were centrifuged at 400 g and the supernatant was collected for liquid scintillation counting. The cells were resuspended in PBS (1 ml), and subjected to a Bligh-Dyer extraction. The radioactivity in the aqueous and organic phases were quantified by liquid scintillation counting. The organic phase was reduced under nitrogen then spotted on TLC plates. Areas corresponding to authentic PC, PS, PI, PE, PA, AA, and neutral lipids were scraped. The percentage of radioactivity within individual glycerolipids was quantified by liquid scintillation counting. The molar concentration of [<sup>3</sup>H]arachidonoyl-phospholipid was calculated from the specific activity of labeled arachidonate (94 Ci/mmol).

*Acyl-CoA Lyso-phospholipid Transferase Activity.* Lymph node T cells ( $2.5 \times 10^6$ /ml) were suspended in a buffer (pH 7.4) consisting of mannitol (300 mM), Tris (15 mM), EGTA (5 mM), and PMSF (0.1 mM), and the cells were cavitated at 4°C using a cell disruption bomb (Parr Instrument Co., Moline, IL) (30). The homogenate was centrifuged at 48,000 g for 30 min. The supernatant was discarded and the membrane pellet was resuspended in cold PBS. Membrane protein was determined by a modification of the method of Lowry (31) using a micro BCA reagent from Pierce Chemical Co. (Rockford, IL). T cell membrane homogenates (150  $\mu$ g protein) were incubated with [<sup>14</sup>C]arachidonoyl-CoA (0.2  $\mu$ M) plus 0.25  $\mu$ M each of Lyso-PC, Lyso-PS, Lyso-PI, and Lyso-PE in PBS (1 ml) at 37°C for 60 min (32). The transferase reaction was terminated by the addition of 3.7 ml of methanol/chloroform (2.5:1.2). The extraction, separation, and quantification of radiolabeled phospholipids were otherwise as described above.

*Arachidonoyl-CoA Ligase/Acyl-CoA Lyso-phospholipid Transferase Activity.* T cell membrane homogenates (150  $\mu$ g protein) were incubated with [<sup>3</sup>H]AA (1  $\mu$ Ci), ATP (5.5 mM), MgCl<sub>2</sub> (21 mM), and CoA (65  $\mu$ M) in the presence or absence of 0.25  $\mu$ M each of Lyso-PC, Lyso-PS, Lyso-PI, and Lyso-PE in PBS (1 ml) at 37°C for 60 min (9). The reaction was terminated and the phospholipids were extracted, separated, and quantified as described above.

*Lyso-PI Formation.* Lymph node T cells ( $10^7$ /ml) were labeled for 2 h at 37°C with [<sup>3</sup>H]myoinositol (5  $\mu$ Ci/ml) in medium (pH 7.2) consisting of glucose (5 mM), NaCl (145 mM), CaCl<sub>2</sub> (1 mM), KCl (5 mM), NaH<sub>2</sub>PO<sub>4</sub> (1 mM), MgSO<sub>4</sub> (50  $\mu$ M), Hepes (10 mM), amino acids, and 1% BSA. The label was removed by three washes. The cells were resuspended ( $10^7$  cells/ml) in BME plus 1% BSA and cultured for up to 4 h at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>. At varying times, the cells were recovered, resuspended in PBS, spiked with [<sup>3</sup>H]AA-PC (50,000 dpm) to assess recoveries and with 10  $\mu$ g each of authentic PC, PI, and lyso-PI to provide a reference point for chromatographic analyses. The lipids were extracted and the organic phase was spotted onto Silica Gel 60 plates preactivated with acetone. Phospholipids were resolved in solvent systems which consisted of CHCl<sub>3</sub>/CH<sub>3</sub>OH/acetic acid (65/25/10) for the first dimension and CHCl<sub>3</sub>/CH<sub>3</sub>OH/formic acid (65/25/10) for the second dimension. Phospholipids were visualized by iodine vaporization. Areas corresponding to authentic PC, PI and lyso-PI were scraped and radioactivity was quantified by liquid scintillation counting.

## Results

*[<sup>3</sup>H]Arachidonate Incorporation in Intact T Cells.* When intact lymph node T cells from control MRL-+/+ mice and B220-enriched T cells from autoimmune MRL-

*lpr/lpr* mice were incubated with trace amounts of [ $^3\text{H}$ ]arachidonate of high specific activity, optimal incorporation of radiolabeled material occurred in both preparations at 1 h (Fig. 1, *inset*). At that time, 74% of the added label was cell-associated in MRL-*+/+* lymph node T cells and 86% in B220 $^+$  T cells from MRL-*lpr/lpr* mice. The lipid extract of cell membranes was analyzed by TLC and the radioactivity within individual glycerolipids quantified. Labeled arachidonate was incorporated primarily into PC in cells from MRL-*+/+* mice followed by PE, PI, and PS (Fig. 1). Free arachidonate comprised <5% of the label within the membrane. Neutral lipids accounted for the remaining radioactivity. This pattern was also found in lymph node T cells from age- and sex-matched, immunologically normal, C3H/HeN and BALB/c mice (data not shown). Relative to cells from MRL-*+/+* mice, B220 $^+$  T cells exhibited an unusual distribution of labeled AA within their membrane phospholipids. A marked preference for incorporating arachidonate into PI as opposed to PC was apparent (Fig. 1). No differences were found in the amount of labeled AA distributed within PS, PE, and neutral lipids in MRL-*+/+* lymph node T cells and B220 $^+$  T cells (Fig. 1).

*Specific Activity of [ $^3\text{H}$ ]Arachidonate Phospholipid Classes.* The relationship between the distribution of [ $^3\text{H}$ ]arachidonate within membrane phospholipids and the size of the phosphoglyceride AA pools was examined in lymph node T cells from MRL-*+/+* mice and B220 $^+$  T cells from MRL-*lpr/lpr* mice. In cells from both

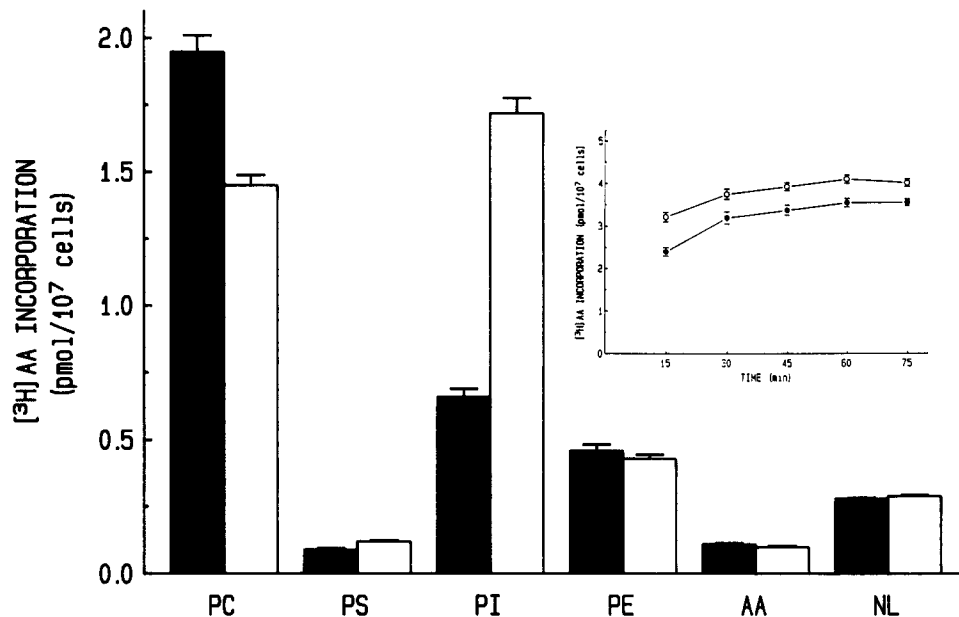


FIGURE 1. Distribution and uptake of [ $^3\text{H}$ ]arachidonate within membrane glycerolipids of MRL lymph node T cells. Lymph node T cells ( $10^7/\text{ml}$ ) from MRL-*+/+* (closed bars) and MRL-*lpr/lpr* (open bars) mice were incubated with [ $^3\text{H}$ ]AA ( $0.5 \mu\text{Ci}/\text{ml}$ ) for 60 min at  $37^\circ\text{C}$ . Membrane lipids were extracted, resolved by TLC, and glycerolipids were quantified by liquid scintillation counting. (*Inset*) The kinetics of [ $^3\text{H}$ ]arachidonate ( $0.5 \mu\text{Ci}/\text{ml}$ ) uptake in lymph node T cells ( $10^7/\text{ml}$ ) from MRL-*+/+* (closed circles) and MRL-*lpr/lpr* (open circles) mice is shown. Data are the mean  $\pm$  SE of seven experiments. NL, neutral lipids.

MRL strains, PC was found to contain the largest molar quantity of arachidonate followed by PE and PI (Fig. 2). The amount of AA within each phospholipid molecular species was comparable in T cells from MRL-*+/+* and MRL-*lpr/lpr* mice.

The ratio of the amount of labeled AA incorporated into a phospholipid and the molar quantity of arachidonate within that phospholipid provides a specific activity (33). Specific activity analysis was used to estimate the movement of arachidonate within the phosphoglycerides of B220<sup>+</sup> T cells and control lymph node T cells. Cells were cultured in the presence or absence of labeled arachidonate for 60 min then harvested. Phospholipid classes were separated by TLC and AA content was measured by gas chromatography. In membranes from MRL-*+/+* T cells, PC contained the highest labeled/unlabeled ratio (21.2 nCi/nmol) of arachidonate, while PI contained the highest specific activity (33.5 nCi/nmol) of [<sup>3</sup>H]arachidonate in B220<sup>+</sup> T cells (Fig. 3). Although the molar concentration of arachidonic acid in PI was less than that in either PE or PC in MRL-*+/+* membranes (Fig. 2), the specific activity of [<sup>3</sup>H]arachidonate in PI (13.9 nCi/nmol) was greater than twice that in PE (5.3 nCi/nmol) and approached that in PC (Fig. 3). Despite the high specific activity of PI in MRL-*+/+* membranes, for every nmol of cold AA within PI, B220<sup>+</sup> T cells incorporated 2.5 times as much labeled AA as did control cells (Fig. 3). Thus, incorporation of [<sup>3</sup>H]AA into T cell membranes of MRL-*+/+* and MRL-*lpr/lpr* mice was, in the main, unrelated to the size of their arachidonate-phospholipid pools. The marked preference for MRL-*lpr/lpr* cells to distribute labeled arachidonate into PI and for MRL-*+/+* lymph node T cells to incorporate [<sup>3</sup>H]arachidonate into PC most likely reflected differences between cells from each strain in the rates of turnover of one phosphoglyceride over another.

*Turnover of Phospholipids in T Cells from MRL Mice.* The constitutive turnover of arachidonate within membrane phospholipids was next investigated in lymph node T cells from MRL-*+/+* mice and B220<sup>+</sup> T cells in MRL-*lpr/lpr* mice. Cells were cultured in BME plus lipid-free BSA for up to 2 h. At various times the supernatants and cell pellets were harvested, glycerolipids were resolved by TLC and quantified by liquid scintillation counting. Initial studies revealed that the amount of radiolabel lost over time was comparable in cells from MRL-*+/+* and MRL-*lpr/lpr* mice (Fig. 4). During the culture period, the molar quantities of cold arachidonate contained

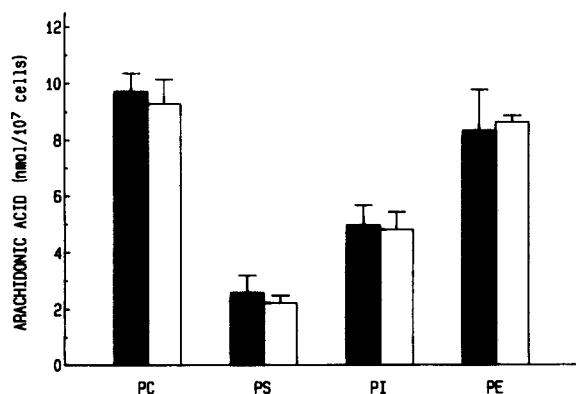


FIGURE 2. AA content within phospholipids of MRL lymph node T cells. Lipid extracts were prepared from lymph node T cells of MRL-*+/+* (closed bars) and MRL-*lpr/lpr* (open bars) mice. Phospholipids were isolated by TLC, fatty acids were derivatized to methyl esters and arachidonoyl-methyl ester content was determined by gas chromatography. Data are the mean  $\pm$  SE of four experiments.

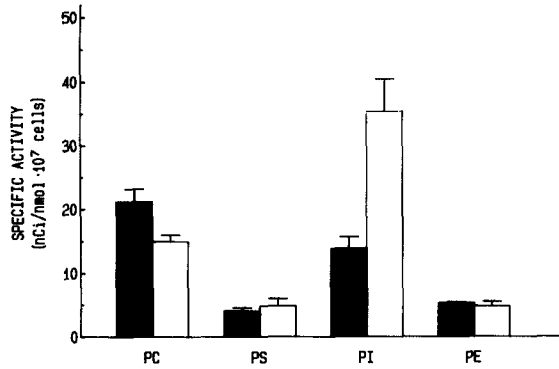


FIGURE 3. Specific activity of lymph node T cells. Lymph node T cells ( $10^7$ /ml) from MRL-+/+ (closed bars) and MRL-lpr/lpr (open bars) mice were incubated for 60 min at  $37^\circ\text{C}$  with and without [ $^3\text{H}$ ]AA ( $0.5 \mu\text{Ci/ml}$ ). Membrane lipids were extracted from each group and phospholipids were resolved by TLC. In unlabeled cells, the molar concentration of arachidonic acid within individual phospholipid molecular species was quantified by gas chromatography following derivation of fatty acids to methyl esters. In labeled cells, the concentration of AA within various phospholipids was measured by liquid scintillation counting. Data are the

ratio of the nCi [ $^3\text{H}$ ]AA incorporated within an individual phosphoglyceride over a 60-min culture period relative to the nanomolar concentration of arachidonate within that phospholipid. Results at the mean  $\pm$  SD of two experiments performed in duplicate.

within phosphoglycerides remained constant (data not shown). As expected, loss of [ $^3\text{H}$ ]arachidonate-PC was significantly greater in lymph node T cells from MRL-+/+ mice than in B220<sup>+</sup> T cells from MRL-lpr/lpr mice (Fig. 5, left). In contrast, cells from MRL-lpr/lpr mice exhibited a marked increase in the loss of arachidonic acid-containing PI when compared to those from MRL-+/+ mice (Fig. 5, right). No differences were found in the turnover of PS, PE, or neutral lipids (data not shown).

**[ $^3\text{H}$ ]Arachidonate Incorporation in MRL-+/+ T Cell Membranes.** As stated earlier, AA may be incorporated into membrane phospholipids by the combined activities of two membrane-bound enzymes. Acyl-CoA synthetase catalyzes the ATP-dependent formation of arachidonoyl-CoA (10). This rate-limiting step is then followed by the ATP-independent transfer of arachidonoyl-CoA into membrane lysophospholipids

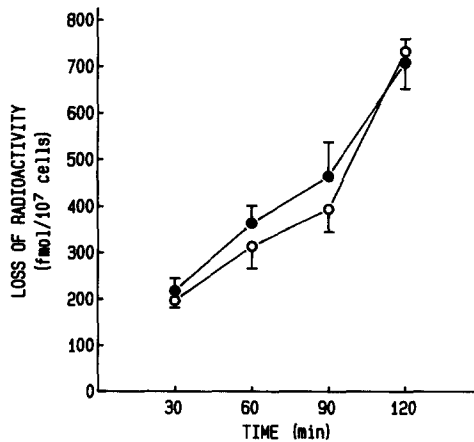


FIGURE 4. Loss of radioactivity from lymph node T cells. Lymph node T cells ( $10^7$ /ml) from MRL-+/+ (closed circles) and MRL-lpr/lpr (open circles) mice were prelabeled with [ $^3\text{H}$ ]AA ( $0.5 \mu\text{Ci/ml}$ ) at  $37^\circ\text{C}$  for 60 min. The cells were harvested, washed, and recultured ( $10^7$ /ml) at  $37^\circ\text{C}$  in BME containing 1% lipid-free BSA for up to 120 min. At various times, the supernatants were harvested and examined for their content of radioactivity. Data are the mean  $\pm$  SE of four experiments.

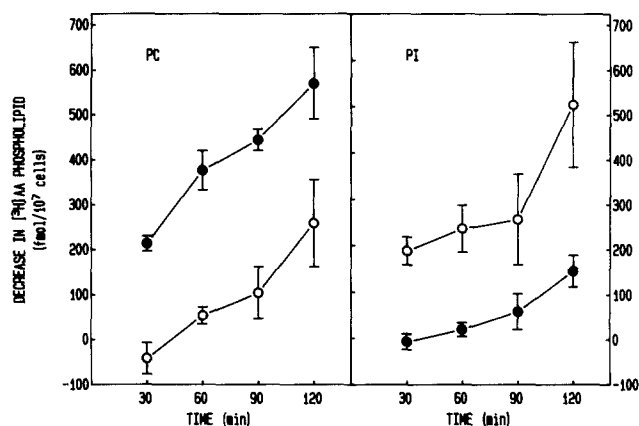


FIGURE 5. Phospholipid turnover in lymph node T cells. Lymph node T cells from MRL-+/+ (closed circles) and MRL-lpr/lpr (open circles) mice were cultured as described in the legend for Fig. 4. At the initiation of culture (zero time) and at various times thereafter the cells were harvested, lipids were extracted, and phosphoglycerides were resolved and quantified by TLC and liquid scintillation counting, respectively. The results represent the changes in the content of [<sup>3</sup>H]arachidonoyl-PC (left) and [<sup>3</sup>H]arachidonoyl-PI (right) during culture relative to that at zero time. Data are the mean  $\pm$  SE of four experiments.

by acyl-CoA transferase (9). Transferase activity is governed primarily by the amount of arachidonoyl-CoA formed and by the availability of lysophospholipid substrate. In preliminary studies we found that when lymph node T cell membranes from MRL-+/+ mice were used as a source of acyl-CoA transferase activity and given [<sup>14</sup>C]arachidonoyl-CoA plus 0.25  $\mu$ M each of lyso-PC, lyso-PS, lyso-PI, and lyso-PE, PI was the dominant acceptor of arachidonate (data not shown). When lymph node T cell membranes from MRL-+/+ mice were challenged with [<sup>3</sup>H]arachidonate plus 0.25  $\mu$ M each of lyso-PC, lyso-PS, lyso-PI, and lyso-PE, PI was again found to be the dominant acceptor species of arachidonate (Fig. 6, left). However, when membranes from MRL-+/+ lymph node T cells were incubated with [<sup>3</sup>H]arachidonate in the absence of exogenous lysophospholipids, PC became the primary acceptor of arachidonic acid (Fig. 6, right). The latter result is consistent

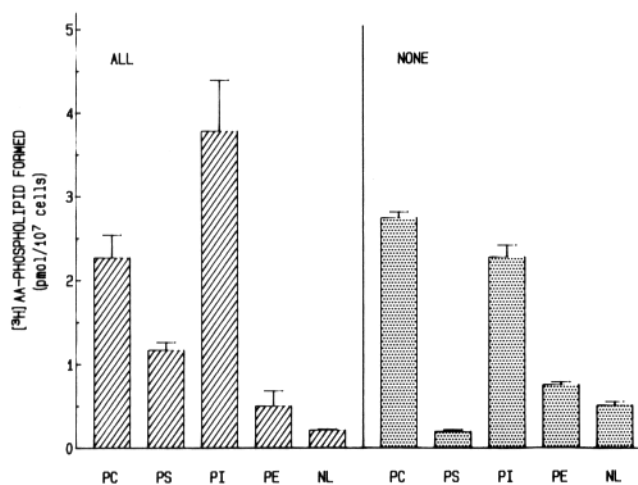


FIGURE 6. Acyl-CoA ligase/acyl-CoA lysophospholipid transferase activity in MRL-+/+ lymph node T cells. Membrane homogenates (150  $\mu$ g protein) from MRL-+/+ lymph node T cells were incubated with [<sup>3</sup>H]AA (1  $\mu$ Ci), ATP, MgCl<sub>2</sub> and CoA in the presence (left) or absence (right) of 0.25  $\mu$ M each of lyso-PC, lyso-PS, lyso-PI and lyso-PE at 37°C for 60 min. Lipids were extracted, resolved by TLC, and quantified by liquid scintillation counting. Data are the mean  $\pm$  SE of four experiments.



with the higher levels of lyso-PC characteristically found in cellular membranes when compared with that of lyso-PI (34).

***[<sup>3</sup>H]Arachidonate Incorporation in B220<sup>+</sup> T Cell Membranes of MRL-*lpr/lpr* Mice.*** We have shown that B220<sup>+</sup> T cells from MRL-*lpr/lpr* mice preferentially incorporate labeled arachidonic acid into PI, demonstrate normal levels of AA-containing PI and exhibit increased turnover of [<sup>3</sup>H]arachidonate-PI. These data suggest that there is increased metabolism of PI in B220<sup>+</sup> T cells that leads to an increase in the availability of lyso-PI for acyl-CoA transferase. If the synthetase-transferase system operates normally in B220<sup>+</sup> T cells, membrane preparations would be expected to preferentially incorporate labeled arachidonate into PI in both the presence and absence of added lysophosphatides. Consistent with this thesis, PI is the major acceptor of [<sup>3</sup>H]AA when B220<sup>+</sup> T cell membranes of MRL-*lpr/lpr* mice are cultured with [<sup>3</sup>H]arachidonate alone (Fig. 7, *right*) or with label plus an equal amount of lyso-PC, lyso-PS, lyso-PI, and lyso-PE (Fig. 7, *left*).

***Formation of Lyso-PI in Lymph Node T Cells.*** It remained to be shown that increased constitutive metabolism of PI in B220<sup>+</sup> T cells was associated with increased formation of lyso-PI. Lymph node T cells from MRL mice were labeled with [<sup>3</sup>H]myo-inositol. The cells were washed and recultured for up to 4 h in BME plus 1% BSA. At the initiation of culture and after 2 and 4 h of culture, membrane lipids were extracted, phosphoglycerides were resolved by TLC and the appearance of lyso-PI over time was determined radiometrically. The area corresponding to authentic PI comprised the major percentage of radioactivity in both B220<sup>+</sup> T cells (94 ± 3%) and in control lymph node T cells (82 ± 4%). The levels of labeled lyso-PI were significantly greater in preparations of B220<sup>+</sup> T cells versus control cells at the initiation of culture and after 2 and 4 h of culture (Table I).

## Discussion

The results presented herein demonstrate that B220<sup>+</sup> T cells from autoimmune MRL-*lpr/lpr* mice exhibit a novel pattern of distribution of newly incorporated, labeled AA within their membrane phospholipids. The preference of B220<sup>+</sup> T cells

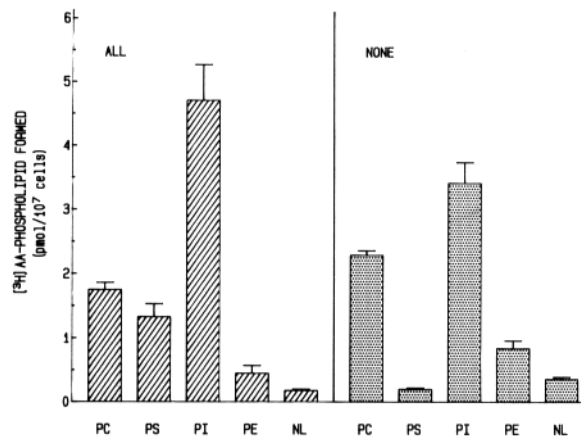


FIGURE 7. Ligase/transferase activity in B220<sup>+</sup> T cells. Membrane homogenates (150 μg protein) from B220<sup>+</sup> T cells were incubated with [<sup>3</sup>H]AA in the presence (*left*) or absence (*right*) of lysophospholipids as outlined in the legend for Fig. 6. [<sup>3</sup>H]Arachidonoyl phospholipids were resolved by TLC and quantified by liquid scintillation counting. Data are the mean ± SE of four experiments.

for forming [<sup>3</sup>H]arachidonoyl-PI over -PC was not found in lymph node T cell membranes of congenic MRL-<sup>+/+</sup> mice or in those from immunologically normal C3H/HeN and BALB/c mice (not shown) and may be a unique property of these mutant T cells that massively accumulate in mice homozygous for the *lpr* gene (19, 20).

The cause of the unusual predilection of the B220<sup>+</sup> T cell PI pool for AA remains to be fully explained. Relative to MRL-<sup>+/+</sup> lymph node T cells, B220<sup>+</sup> T cells exhibit increased turnover of AA-containing PI in association with increased formation of lyso-PI. In immunologically normal T cells, PI appears to be principally metabolized by membrane PLC (12). Inositol phosphates and DAG are products of the reaction (14). Lyso-PI is not formed in the course of PI metabolism by PLC. Our results, therefore, indicate that in B220<sup>+</sup> T cells enhanced turnover of PI cannot be fully explained by a pathway that involves PLC.

When pharmacological doses of lyso-PC, lyso-PS, lyso-PI, and lyso-PE are simultaneously given to lymph node T cell membranes from autoimmune and normal strains, lyso-PI is the dominant acceptor species for [<sup>3</sup>H]arachidonate. In the absence of exogenously added lysophospholipids, lyso-PC is the major recipient of AA in T cells from normal mice. B220<sup>+</sup> T cells, however, continue to show preferential transfer of arachidonate into lyso-PI. In resting T cells, the level of lyso-PC is generally very much greater than that of any other lysophosphatide including lyso-PI (34) and labeled arachidonate is distributed primarily into PC (9). B220<sup>+</sup> T cells exhibit a profile that suggests a relative deficiency in lyso-PC and/or an excess of lyso-PI. Alterations in lysophospholipid levels, in turn, may be intrinsic or the result of variations in the rate of phospholipid metabolism. The fact that the phospholipid AA pools are comparable in lymph node T cells from MRL-<sup>+/+</sup> mice and B220<sup>+</sup> T cells from MRL-*lpr/lpr* mice indicates that differential rates of PC versus PI turnover contribute significantly to the distribution of labeled AA within the membrane phospholipids of each strain.

Labeling experiments with [<sup>3</sup>H]myoinositol suggest that PI is giving rise to increased quantities of lyso-PI at an increased rate in B220<sup>+</sup> T cells. The most direct route for the generation of lyso-PI is through PLA<sub>2</sub>-mediated cleavage of arachidonate from the *sn*-2 position of PI. In macrophages, where significant PLA<sub>2</sub> activity exists, PI is a relatively poor substrate and PC a preferred substrate for PLA<sub>2</sub>

TABLE I  
*Lyso-PI Formation in MRL Lymph Node T Cells*

T cell source (strain)	Lyso-PI detected per 10 <sup>7</sup> cells cultured for:		
	0 h	2 h	4 h
	<i>fmol</i>		
MRL- <sup>+/+</sup>	4.6 ± 0.3	4.5 ± 0.7	2.1 ± 0.1
MRL- <i>lpr/lpr</i>	14.1 ± 0.9	10.8 ± 0.6	4.3 ± 0.3

Lymph node T cells (10<sup>7</sup>/ml) from MRL mice were incubated for 2 h with [<sup>3</sup>H]myoinositol (5 μCi/ml), washed, and cultured (10<sup>7</sup>/ml) for up to 4 h at 37°C in BME plus 1% lipid-free BSA. At the initiation (0 h) and after 2 and 4 h culture, phospholipids were resolved by TLC and quantified by liquid scintillation counting. Radioactivity in the organic phase of the lipid extract was 1,580 ± 209 cpm in MRL-<sup>+/+</sup> T cells and 12,296 ± 843 cpm in B220<sup>+</sup> T cells. Results are the mean ± SD of two experiments performed in duplicate.

(11). The turnover of PC in cultured MRL-+/+ lymph node T cells and associated loss of esterified, labeled arachidonate are most likely manifestations of PLA<sub>2</sub> activity. The loss of [<sup>3</sup>H]arachidonate-PI in B220<sup>+</sup> T cells also suggests PLA<sub>2</sub>-like activity. However, in the latter case, PI and not PC appears to be the enzymatic target. To our knowledge, B220<sup>+</sup> T cells may represent the first example of a subset of cells in which PLA<sub>2</sub>-like activity is preferentially directed against PI and not another phospholipid.

The possibility that lyso-PI is being produced by acyl-CoA transferase-catalyzed exchange of arachidonic acid between PC and lyso-PI cannot be excluded. Paradoxically, the driving force behind such an exchange might be increased metabolism of PC by a PLA<sub>2</sub>, resulting in enhanced formation of lyso-PC. If this were the case, we would anticipate increased turnover of both PC and PI in B220<sup>+</sup> T cells. However, specific activity analysis and direct measurements of phospholipid turnover indicate that the loss of [<sup>3</sup>H]arachidonoyl-PC is significantly decreased in B220<sup>+</sup> T cells compared with that in control cells. It is therefore unlikely that augmented production of lyso-PI in B220<sup>+</sup> T cells is due to increased loss of PC and accelerated transfer of arachidonate from PI to lyso-PC.

The contribution of enhanced constitutive turnover of PI to the derangements in transmembrane signal transduction that have been reported in B220<sup>+</sup> T cells (26) remains to be determined. If, as the data indicate, PI is being metabolized at an enhanced rate and PC at a decreased rate, in B220<sup>+</sup> T cells by a PLA<sub>2</sub>-like activity, then formation of lyso-PI should be greater than that of lyso-PC. In contrast, immunologically normal T cells, which constitutively turn over PC more efficiently than PI, would be expected to generate more lyso-PC than lyso-PI. The biochemical properties of lyso-PC suggest that it might play an important role in cellular activation. Lyso-PC is an excellent detergent (35) and is potentially capable of increasing membrane fluidity. *In vitro* studies indicate that lyso-PC can augment PKC (18) and guanylate cyclase (17) activities. If increased metabolism of PI favors the production of lyso-PI over lyso-PC in B220<sup>+</sup> T cells, signal transduction may thus be compromised.

Another potential consequence of increased PI-PLA<sub>2</sub>-like activity is that of competition with PI-specific PLC (12) for substrate. If PI is preferentially metabolized by PLA<sub>2</sub>, as opposed to PLC, diminished production of inositol phosphates and DAG might occur. The latter second messengers appear to be required in order to fully activate PKC (15). Cellular events that are thought to be coupled to PKC activation (e.g., IL-2 synthesis, IL-2 receptor expression) could conceivably be jeopardized if PI availability for PLC is limited. Simultaneous measurements of PLC and PLA<sub>2</sub> activities in membrane preparations of B220<sup>+</sup> T cells should help to define the relative capacities of these enzymes to use PI as a substrate.

In summary, the data indicate that cells that manifest abnormal immune function may also exhibit derangements in the incorporation, distribution, and metabolism of AA within membrane phospholipids. It remains possible that disturbances in the movement of arachidonate within phosphoglycerides play a role in the expression of aberrant immunological activity.

### Summary

The metabolism of AA-containing phosphoglycerides within T cell membranes leads to the generation of second messengers that appear to play a crucial role in

transmembrane signal transduction. To test the hypothesis that aberrations in the movement of arachidonoyl-phospholipids are associated with and may potentially contribute to abnormal T cell function, the incorporation, distribution, and turnover of AA within the membrane glycerolipids of cells that are known to exhibit immunoregulatory disturbances was examined. Thy-1<sup>+</sup>, Ly-1<sup>+</sup>, L3T4<sup>-</sup>, Lyt-2<sup>-</sup>, B220<sup>+</sup> T cells from autoimmune MRL-*lpr/lpr* mice were used as the cellular model.

In contrast to control lymph node T cells, which preferentially incorporate labeled AA into phosphatidylcholine (PC), B220<sup>+</sup> T cells displayed a predilection for distributing [<sup>3</sup>H]arachidonate into phosphatidylinositol (PI). The arachidonoyl-phospholipid pools were normal in B220<sup>+</sup> T cells. The constitutive turnover of [<sup>3</sup>H]arachidonoyl-PI was significantly enhanced and that of [<sup>3</sup>H]arachidonate-PC substantially reduced in B220<sup>+</sup> T cells compared with control cells. Using membrane homogenates B220<sup>+</sup> T cells demonstrated a functional increase in the levels of lyso-PI. Intact B220<sup>+</sup> T cells prelabeled with [<sup>3</sup>H]myoinositol and cultured in the absence of stimulation with exogenous antigens or mitogens, exhibited increased production of lyso-PI.

The data indicate that the preferential formation of [<sup>3</sup>H]arachidonoyl-PI in B220<sup>+</sup> T cells is the result of greatly increased, constitutive PI turnover that appears to be due to a membrane phospholipase A<sub>2</sub> activity. It remains possible that disturbances in the movement of arachidonate within phospholipids of B220<sup>+</sup> T cells play a role in the expression of aberrant immunological activity.

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