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Absence of unsaturated fatty acid synthesis in murine T lymphocytes

THOMAS M. BUTTKE*, STEVEN VAN CLEAVE, LINDA STEELMAN, AND JAMES A. MCCUBREY

Department of Microbiology and Immunology, East Carolina University, School of Medicine, Greenville, NC 27858

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ABSTRACT **Stearic acid is toxic for T lymphocytes *in vitro* but has little effect on B lymphocytes. To investigate the molecular basis for this difference, purified murine T and B lymphocytes were compared for their abilities to incorporate and metabolize stearic acid. Unstimulated T and B cells incorporated identical amounts of stearic acid into six different phospholipids and four neutral lipids. After mitogen stimulation, fatty acid uptake was increased in both lymphocyte types, but cell-specific differences were seen in the distribution of stearic acid among the various cellular lipids. Doses of stearic acid that selectively inhibited T-cell proliferation resulted in a 5-fold greater accumulation of distearoylphosphatidylcholine in T cells than in B cells. Whereas T cells did not desaturate the exogenously derived stearic acid, up to 25% of the saturated fatty acid was converted to oleic acid in B cells. These findings suggested a deficiency of stearoyl-CoA desaturase (acyl-CoA, hydrogen-donor:oxygen oxidoreductase, EC 1.14.99.5) activity in T cells, which was confirmed by subsequent studies. Cell-free extracts from B cells displayed nearly 20-fold more stearoyl-CoA desaturase activity than T-cell extracts, and the level of stearoyl-CoA desaturase mRNA was 30-fold higher in B cells. Collectively, our data indicate that murine T cells are deficient in unsaturated fatty acid synthesis. The deficiency of stearoyl-CoA desaturase in T cells may represent the basis for the differing sensitivities of T and B lymphocytes to inhibition by saturated fatty acids.**

Saturated and unsaturated fatty acids markedly affect lymphocyte function both *in vivo* and *in vitro* (for review, see refs. 1–3). In general, saturated fatty acids and high levels (>20 μM) of unsaturated fatty acids are inhibitory, whereas low amounts (<10 μM) of unsaturated fatty acids enhance lymphocyte growth and function (4–8). The mechanisms by which fatty acids alter lymphocyte function are not fully understood. Polyunsaturated fatty acids are converted to various icosanoids having potent immunopharmacological properties (9, 10). By contrast saturated and monounsaturated fatty acids have been proposed to act by changing the organization or physical properties of immune cell membranes, although this remains controversial (3, 10, 11).

In studies carried out *in vivo* (3, 12, 13) and *in vitro* (8, 14), fatty acids have been shown to have a greater effect on T cells and cell-mediated immunity than on B cells and humoral immunity. For example, using highly purified murine T and B lymphocytes, we showed that stearic acid potently inhibited T-cell proliferation but had much less effect on B-cell proliferation (8). At the same concentration (50 μM), oleic acid was without effect; however, when added simultaneously with stearic acid, oleic acid could obviate the inhibitory effects of the saturated fatty acid (8, 15). Subsequent studies regarding the effects of free fatty acids on *in vitro* primary antibody responses provided results consistent with our earlier findings where stearic acid was shown to inhibit thymus-dependent but not thymus-independent antibody responses (14). More recently we have obtained additional evidence implicating T helper cells as the target for saturated fatty acid inhibition in primary antibody responses (S. Pourbohloul and T.M.B., unpublished observations).

The differential effect of saturated fatty acids on T and B cells suggests that the two lymphocyte types differ either in fatty acid uptake and utilization or in their abilities to tolerate changes in membrane fatty acid composition. To address this question, we have compared purified B and T lymphocytes for their abilities to incorporate and metabolize exogenous stearic acid. Because the initial results of this work indicated that T cells were unable to desaturate stearic acid to yield oleic acid, subsequent studies were undertaken to compare B and T cells for their levels of stearoyl-CoA desaturase (acyl-CoA, hydrogen-donor:oxygen oxidoreductase, EC 1.14.99.5). The results reported here demonstrate a fundamental difference in lipid metabolism between B and T lymphocytes.

MATERIALS AND METHODS

Lymphocyte Isolation and Culture. Purified T and B lymphocytes were isolated from spleens of 8- to 12-week-old BALB/c mice (Charles River Breeding Laboratories) as described (16). As demonstrated by immunofluorescence studies, the recovered B- and T-cell populations were typically >97% positive for surface immunoglobulin or Thy 1.2, respectively (16). Lymphocytes were cultured and assayed for proliferation as described (16).

Assay of Fatty Acid Uptake. [1-^{14}C]Stearic acid (59 mCi/mmol; 1 Ci = 37 GBq; New England Nuclear) was complexed to bovine serum albumin before its addition to culture (8). Fatty acid uptake was assayed with 3×10^6 cells. After extraction of cellular lipids with chloroform/methanol (17), aliquots were removed to measure total [^{14}C]stearic acid uptake.

Lipid Analyses. Lymphocyte lipids were separated into phospholipids and neutral lipids by TLC on silica gel 60 plates (Merck) developed with petroleum ether/diethyl ether/acetic acid (75:25:1) or chloroform/methanol/acetic acid/H_2O (75:45:12:5.5). Fatty acid methyl esters were separated by reversed-phase and argentation TLC (18). Radiolabeled lipids were quantified using a RITA linear radioactivity detector (Raytest, Muenster, F.R.G.).

For analysis of molecular species, radiolabeled phosphatidylcholine fractions were converted to diacylglycerides by phospholipase C digestion (19) followed by benzylation (20, 21). The benzylated (20) or dinitrobenzylated (21) diacylglycerides were separated with a Beckman model 332R HPLC fitted with a 4.6 × 45 mm precolumn and a Beckman 4.6 × 250 mm analytical column, both packed with 5-μm C_{18} Ultrasphere. Molecular species were separated by isocratic elution with acetonitrile/2-propanol (80:20; vol/vol) and a column flow rate of 1 ml/min. Separated molecular species were detected and quantified by monitoring their UV adsorption at 230 nm. Radiolabeled diacylglycerobenzoates were

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Abbreviations: LPS, lipopolysaccharide; PHA, phytohemagglutinin.
*To whom reprint requests should be addressed.

quantitated by collection of 1-ml fractions, which were dried before liquid scintillation counting. Molecular species were identified by comparing their retention volumes to those of known authentic standards, as well as by gas/liquid chromatography analysis (22) of the fatty acyl methyl esters obtained following transesterification [5% (vol/vol) HCl/methanol for 2 hr at 75°C] of the recovered molecular species.

Isolation and Assay of Stearoyl-CoA Desaturase Activity. Approximately 1×10^8 B or T lymphocytes were washed with phosphate-buffered saline (pH 7.2) and resuspended in 10 mM Tris buffer (pH 7.6) containing 0.5 mM $MgCl_2$ and phenylmethanesulfonyl fluoride at 150 μg/ml. The cell suspension was sonicated for 5 sec by using a Biosonik III sonicator (Bronwill) set at maximum output. After centrifugation of the suspension (10 min at 2000 $\times$ g, 4°C), the recovered membrane pellet was resuspended in the Tris buffer, and protein was measured by a modified Lowry procedure (23).

Stearoyl-CoA desaturase activity was measured by assaying the conversion of [1-^{14}C]stearoyl-CoA to [1-^{14}C]oleic acid (24). Equivalent amounts of T- and B-cell membrane protein were added to an assay mixture containing 0.1 M Tris (pH 7.2), 1 mM NADH, and 70 μM [1-^{14}C]stearoyl-CoA (New England Nuclear; 5.5 mCi/mmol), and the mixture was incubated for 20 min at 37°C. The reaction was stopped with 10% (wt/vol) KOH/methanol (80°C for 30 min). After acidification, fatty acids were recovered and converted to methyl esters for analysis by argentation TLC.

Northern (RNA) Analyses of T and B Cells. Cellular RNA was isolated from $\approx 1 \times 10^8$ B or T cells after washing them twice with Ca^{2+}- and Mg^{2+}-free phosphate-buffered saline. The cell pellet was lysed in guanidinium isothiocyanate (25) and loaded onto a cushion of 5.7 M CsCl containing 100 mM EDTA. The RNA was collected by ultracentrifugation at 30,000 $\times$ g for 48 hr. After ethanol precipitation, the RNA pellet was resuspended in Tris/EDTA buffer (10 mM Tris, pH 7.6/1 mM EDTA). Poly(A)$^+$ RNA was isolated, the concentration was determined by UV spectrophotometry, and 2–5 μg of poly(A)$^+$ RNA was denatured with formaldehyde/formamide and electrophoresed in 1.2% agarose gels containing formaldehyde as described (26). Blotting of RNA gels was performed by standard procedures (26). The size of mRNA transcripts encoding stearoyl-CoA desaturase and β-actin were determined by linear regression analysis, with use of an ethidium bromide-stained 0.24- to 9.5-kilobase (kb) RNA ladder (BRL).

A cDNA probe for stearoyl-CoA desaturase was provided by Philipp Strittmatter (University of Connecticut, Farmington) (27), and a chicken β-actin probe was purchased from Oncor (Gaithersburg, MD). The probes were labeled by random priming (28) with [α-^{32}P]dCTP (New England Nuclear; 3000 Ci/mmol). Prehybridization was performed for 4 hr at 42°C in a solution consisting of 50% deionized formamide, 4× SSC (1× SSC = 0.15 M sodium chloride/0.015 M sodium citrate), 5× Denhardt's solution (1× Denhardt's solution = 0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin), denatured salmon sperm DNA at 25 μg/ml, 0.05 M sodium phosphate buffer (pH 7), sodium pyrophosphate at 0.5 mg/ml, and 1% SDS. Hybridization was performed in the same solution except that the Denhardt's solution was 1×, and $\approx 2.5 \times 10^7$ cpm of denatured probe was added. Hybridization was allowed to occur for 16–20 hr at 42°C. After hybridization, the filters were washed (final wash was at 65°C in 0.1× SSC/0.1% SDS), wrapped in plastic wrap, and put in a DuPont Cronex cassette with Lightning Plus intensifying screens for 1–3 days at −70°C. Intensities of hybridization were determined by scanning autoradiographs with a Zeineh soft laser densitometer (Biomed Instruments, Fullerton, CA).

Statistical Analyses. Differences in lipid composition between unstimulated versus stimulated lymphocytes or between stearic acid-treated and -untreated lymphocytes were tested for significance ($P < 0.05$) by two-tailed, paired Student's t tests.

RESULTS

Incorporation of [1-^{14}C]Stearic Acid into B- and T-Cell Lipids. Time-course studies of [1-^{14}C]stearic acid uptake by T and B lymphocytes were initially carried out to determine whether activated T cells incorporated more stearic acid than either activated B cells or resting (unstimulated) T or B cells. Purified T and B lymphocytes were incubated in the presence of 45 μM [1-^{14}C]stearic acid and either stimulated with phytohemagglutinin (PHA) or lipopolysaccharide (LPS), respectively, or left unstimulated. Selection of this fatty acid dose was based on our previous studies showing that 45 μM stearic acid inhibited T-cell proliferation by >90%, while having much less effect on B-cell proliferation (8). At various times, aliquots were removed from the cultures, and the amounts of [1-^{14}C]stearic acid incorporated into T- and B-cell phospholipids and neutral lipids were determined. Fig. 1 shows that resting B and T cells incorporated similar amounts of [1-^{14}C]stearic acid into the various phospholipids and neutral lipids. In both lymphocyte types, phosphatidylcholine was the major (60–65%) phospholipid labeled, whereas fatty acids were the major (60–65%) neutral lipid species labeled. The nearly identical incorporation of [1-^{14}C]stearic acid into the resting T- and B-cell lipids is consistent with our previous finding that stearic acid is not toxic for unstimulated lymphocytes of either type (22).

In LPS-activated B cells and PHA-activated T cells, [1-^{14}C]stearic acid incorporation into each of the phospholipids and neutral lipids (except fatty acids) was increased within 6–8 hr after mitogen addition (Fig. 1). The activated T cells incorporated 1.5- to 2-fold more fatty acid than activated B cells. However, based on [^{3}H]thymidine uptake measurements ($\approx$95,000 cpm for untreated, LPS-activated B cells; $\approx$150,000 cpm for untreated, PHA-activated T cells), the difference in fatty acid uptake probably reflects the proportions of B or T cells activated by the two mitogens. Thus, the selective toxicity of stearic acid towards T cells could not be correlated with differences in fatty acid uptake by the two lymphocyte types.

Changes in Phospholipid Molecular Species in Stearic Acid-Treated B and T Cells. Although B and T lymphocytes incorporated nearly identical amounts of [1-^{14}C]stearic acid into the various cellular lipids, preliminary studies suggested that B and T cells incorporated the fatty acid into distinct phospholipid molecular species. Therefore, mitogen-stimulated B and T lymphocytes were recovered after overnight (16-hr) incubation in the absence or presence of 50 μM stearic acid, and their phosphatidylcholine molecular species were quantified. Mitogen-activated T and B cells incubated for 16 hr without stearic acid contained 16 different phosphatidylcholine molecular species, with palmitic acid/oleic acid and stearic acid/linoleic acid (in a ratio of 3:1) being the predominant species (Fig. 2*A*). Overnight supplementation of T cells with 50 μM stearic acid resulted in a significant ($P < 0.0001$) decrease in palmitic acid/oleic acid, with concomitant increases in stearic acid/oleic acid ($P < 0.005$) and distearoylphosphatidylcholine ($P < 0.02$) (Fig. 2*B*). B cells incubated with 50 μM stearic acid (Fig. 2*C*) also displayed a decrease in palmitic acid/oleic acid ($P < 0.003$) and a corresponding increase in stearic acid/oleic acid ($P < 0.02$), but the small increase of distearoylphosphatidylcholine observed was not significant ($P = 0.13$). Thus, after a 16-hr incubation in the presence of 50 μM stearic acid, roughly 30% of T-cell phosphatidylcholine was in the distearoyl form,

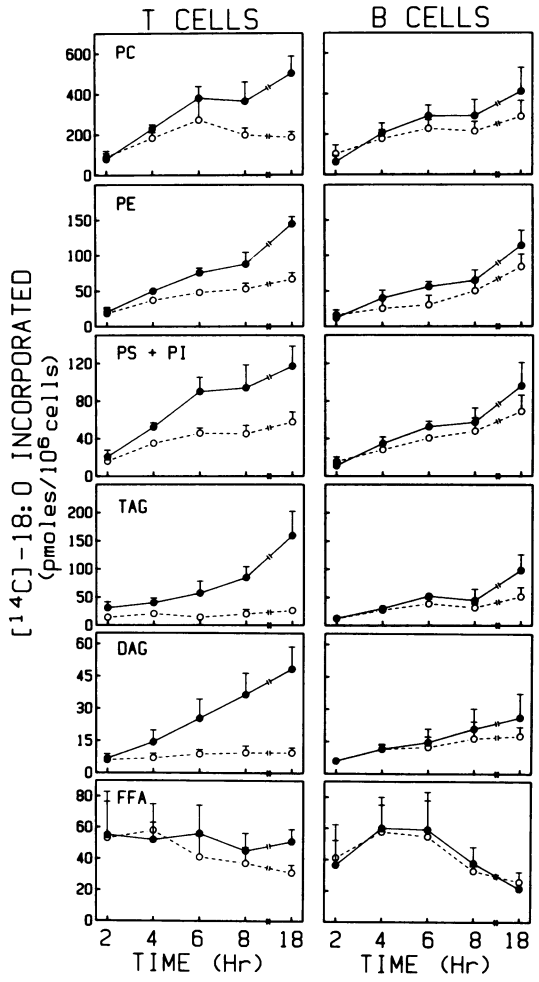

FIG. 1. Total uptake and incorporation of [1-^{14}C]stearic acid ([^{14}C]-18:0) into T- and B-cell phospholipids and neutral lipids. Purified T and B cells were incubated in the presence of 45 μM [1-^{14}C]stearic acid and stimulated with either PHA or LPS, respectively (●) or left unstimulated (○). At the times indicated, cellular lipids were extracted and separated by TLC. PC, phosphatidylcholine; PE, phosphatidylethanolamine; PS + PI, phosphatidylserine and phosphatidylinositol; FFA, free fatty acids; DAG, diacylglycerides; and TAG, triacylglycerides. Data are the mean (±SEM) of three to five separate experiments.

whereas this same species accounted for <8% of the total phosphatidylcholine molecular species of B cells.

Desaturation of Exogenously Derived Stearic Acid by B and T Cells. To determine the basis for the greater accumulation of distearoylphosphatidylcholine in T cells, T and B lymphocytes were compared subsequently for possible differences in their abilities to desaturate exogenously derived [1-^{14}C]stearic acid to yield [1-^{14}C]oleic acid. The two cell types were incubated in the presence of three concentrations of [1-^{14}C]stearic acid and either stimulated with mitogen or left unstimulated. After 7 hr, total T- and B-cell phospholipids were recovered and analyzed for their contents of radiolabeled fatty acids. Fig. 3 shows that phospholipids from B cells incubated in the presence of [1-^{14}C]stearic acid contained both [1-^{14}C]stearic acid and [1-^{14}C]oleic acid. Over the concentration range of exogenous [1-^{14}C]stearic acid provided, the proportion of [1-^{14}C]oleic acid ranged from 12% of total radiolabeled phospholipid fatty acids in cells exposed to 40 μM [1-^{14}C]stearic acid to 23% in cells treated with 160 μM [1-^{14}C]stearic acid. By contrast, T cells contained much lower amounts of [1-^{14}C]oleic acid, most notably in T cells incubated at the higher doses of [1-^{14}C]stearic acid tested,

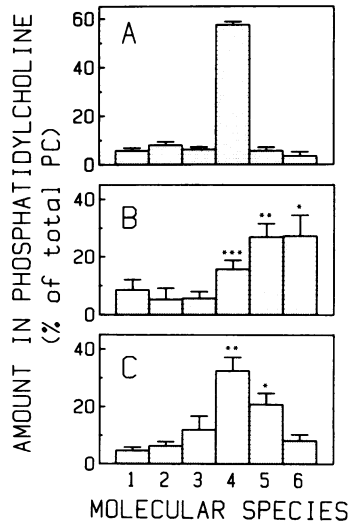

FIG. 2. Effect of stearic acid supplementation on the phosphatidylcholine molecular species of T and B lymphocytes. Purified T and B cells were left unstimulated (*A*) or were stimulated with PHA (T cells; *B*) or LPS (B cells; *C*), with or without 50 μM stearic acid. After 16 hr the cells were harvested and analyzed for their phosphatidylcholine molecular species. No differences were detected between untreated T and B cells; therefore, the data from the individual lymphocyte types were combined to yield the data of *A* for control T and B cells. Data are shown only for the major molecular species and represent the mean (+SEM) of three separate experiments. Asterisks denote molecular species significantly different from control cells: *, $P < 0.02$; **, $P < 0.005$; ***, $P < 0.0001$. Molecular species: 1, oleic acid/arachidonic acid; 2, palmitic acid/linoleic acid; 3, dioleoyl acid; 4, palmitic acid/oleic acid and stearic acid/linoleic acid; 5, stearic acid/oleic acid; 6, distearoyl acid.

wherein radiolabeled unsaturated fatty acids accounted for only 1–2% of total radiolabeled phospholipid fatty acids. The data in Fig. 3 also show that this difference was seen between resting as well as mitogen-stimulated T and B cells. Additional studies involving longer incubation times (13, 16, and 21 hr) yielded similar results (data not shown).

Stearoyl-CoA Desaturase Activity in B and T Cells. To confirm the apparent absence of oleic acid synthesis in T cells, cell-free extracts were prepared from unstimulated as well as mitogen-stimulated B and T cells and assayed for stearoyl-CoA desaturase activity. Cell-free extracts prepared from unstimulated and LPS-activated B cells displayed >20 times more stearoyl-CoA desaturase activity than did extracts prepared from either unstimulated or Con A-stimulated T cells (Table 1). Similar data were obtained with PHA-

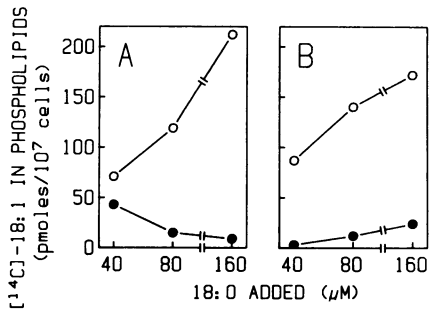

FIG. 3. Conversion of [1-^{14}C]stearic acid (18:0) to [1-^{14}C]oleic acid by T and B cells. Purified T and B cells were either mitogen-stimulated (*A*) or left unstimulated (*B*) and cultured with various concentrations of [1-^{14}C]stearic acid. After 7 hr cellular phospholipids were recovered and analyzed for their contents of radiolabeled fatty acids. Data are expressed as pmol of [1-^{14}C]oleic acid ([1-^{14}C]18:1) recovered from the total phospholipids of 10^7 B cells (○) or T cells (●).

Table 1. Stearoyl-CoA desaturase activity in B and T lymphocytes

Cell population	[^{3}H]Thymidine uptake, cpm per 5×10^5 cells	Protein, μg	Desaturase activity, pmol of labeled 18:1 per mg per 20 min
Experiment 1			
B cells	1,234 ± 533	317	755
T cells	154 ± 25	317	0
B cells + LPS	39,462 ± 6746	343	726
T cells + Con A	64,497 ± 2792	343	48
Experiment 2			
B cells + LPS	39,466 ± 1212	283	919
T cells + Con A	64,460 ± 5981	283	0

18:1, oleic acid.

stimulated T cells (data not shown). These results are consistent with the data obtained with intact cells and demonstrate a near total absence of stearoyl-CoA desaturase activity in resting and activated T lymphocytes.

Levels of Stearoyl-CoA Desaturase mRNA in B and T Cells. Poly(A)$^+$ RNA isolated from unstimulated and mitogen-activated B and T cells was subjected to Northern (RNA) analyses by using a cDNA probe for stearoyl-CoA desaturase (27). Fig. 4 shows that the desaturase cDNA probe hybridized to a single mRNA species with the expected size of ≈5 kb (29, 30). Fig. 4 also shows that desaturase mRNA was present in both resting and LPS-stimulated B cells, but was virtually absent from resting and Con A-stimulated T cells. By comparison with β-actin mRNA, which was expressed equally in B and T cells, resting and stimulated B cells contained >30 times more stearoyl-CoA desaturase mRNA than resting or activated T cells. These data are consistent with the demonstrated inability of T cells to desaturate stearic acid.

DISCUSSION

The results described in this study provide evidence that murine T cells are deficient in unsaturated fatty acid synthesis. The ability of lymphocytes to incorporate and use exogenously derived free fatty acids has been reported by numerous workers, as has enhanced fatty acid uptake after mitogen activation (5, 31–33). However, the previous studies involved unfractionated lymphocyte populations containing both B and T cells, and it was not determined whether the fatty acids were metabolized subsequent to their incorporation. By making use of highly enriched B- and T-cell populations and assaying the products of the exogenously derived stearic acid, we have demonstrated that T and B cells differ in their abilities to synthesize oleic acid. Whereas B cells converted as much as 23% of the incorporated stearic acid to oleic acid, T cells desaturated only 1–2%. A lack of desaturase activity in T cells was similarly indicated by assaying stearoyl-CoA desaturase activity in cell-free extracts prepared from B and T lymphocytes. Membrane preparations isolated from both resting and mitogen-activated B cells displayed >20-fold more desaturase activity than membranes isolated from resting or activated T cells.

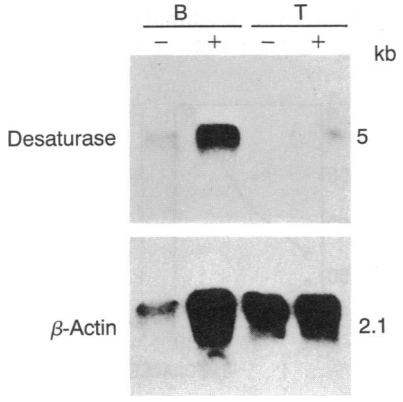

FIG. 4. Northern blot analysis of resting and activated B and T cells. One to 5 μg of poly(A)$^+$ RNA from unstimulated (−) or LPS-stimulated (+) B cells and unstimulated (−) or Con A-stimulated (+) T cells was separated on formaldehyde-agarose gels, transferred to nitrocellulose, and sequentially hybridized to ^{32}P-labeled cDNA probes for rat liver stearoyl-CoA desaturase and chicken β-actin.

Northern analysis of stearoyl-CoA desaturase mRNA yielded results that were also consistent with the apparent absence of stearoyl-CoA desaturase activity in T cells. While mRNA transcripts encoding the stearoyl-CoA desaturase were detected in both resting and activated B cells, these transcripts were virtually absent from resting or mitogen-activated T cells. Thus, in both lymphocyte types the levels of stearoyl-CoA desaturase mRNA coincided with amount of desaturase activity obtained in cell-free extracts. Expression of stearoyl-CoA desaturase in B and T cells is, therefore, regulated at the level of transcription. Similar results have been seen in rat and mouse liver (29, 34).

The inability of T cells to convert stearic acid to oleic acid may be responsible for the toxic effects of saturated fatty acids toward these cells. After an overnight incubation in the presence of 50 μM stearic acid, activated T cells contained 4–5 times more of the fatty acid in distearoylphosphatidylcholine than did activated B cells. Previous studies by Van Deenen and coworkers have shown that stoichiometric replacement of erythrocyte phosphatidylcholine with distearoylphosphatidylcholine resulted in hemolysis when 22% of native phosphatidylcholine had been exchanged (35, 36). This level is equivalent to the amount of distearoylphosphatidylcholine observed in the stearic acid-treated T cells. If the accumulation of distearoylphosphatidylcholine is, in fact, responsible for the toxicity of stearic acid to T cells, then the ability of B cells to avoid the synthesis of the toxic lipid species may stem from their ability to desaturate stearic acid to yield oleic acid. We have previously reported that the simultaneous addition of oleic acid obviates the otherwise toxic effects of the stearic acid (15), and we have recently found that T cells supplemented with both stearic and oleic acids do not accumulate distearoylphosphatidylcholine (T.M.B., unpublished observations).

The finding that murine T cells do not synthesize olefinic fatty acids is surprising but, nevertheless, agrees with reports that T cells require exogenous unsaturated fatty acids for growth *in vitro*. Several studies have shown that the addition of either albumin-associated unsaturated fatty acids (4, 6) or complex lipids containing olefinic acyl chains (37–39) to serum-free culture medium significantly improved human and murine lymphocyte proliferation *in vitro*. Indeed, unsaturated fatty acids may even be essential for PHA-induced proliferation of human T cells cultured in serum-free medium (4, 37). Thus, the demonstrated inability of T cells to synthesize unsaturated fatty acids is consistent with the previous studies.

Changes in the levels of dietary fat can profoundly alter immune responses *in vivo*, and it has been suggested that T cells are the predominant sensitive immune cell population (3). Whether T lymphocytes are also dependent upon exogenous unsaturated fatty acids for proliferation and function *in vivo* is not known. If T cells are so dependent, the ability of B cells to synthesize monounsaturated fatty acids may enable them to "fine-tune" the lipid composition of B cells, thereby preserving permissive membrane properties.

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