

Immunosuppressive effects of polyunsaturated fatty acids on antigen presentation by human leukocyte antigen class I molecules

Saame Raza Shaikh¹ and Michael Edidin

Department of Biology, Johns Hopkins University, Baltimore, MD 21218

Abstract Dietary supplementation with polyunsaturated fatty acids (PUFAs) has immunosuppressive effects; however, the molecular targets of PUFAs and their mode of action remain unclear. One possible target is antigen presentation to T cells through the human leukocyte antigen (HLA) class I pathway. Here we show that incorporation of PUFAs lowers target cell susceptibility to lysis by effector T cells. Treatment of B lymphoblast targets with the ω -6 PUFA arachidonic acid (AA) or ω -3 docosahexaenoic acid lowered their susceptibility to lysis by alloreactive CD8⁺ T cells by ~20–25%. HLA class I surface levels and their rate of endoplasmic reticulum (ER)-Golgi traffic were also reduced by PUFA treatment. Calibration experiments showed that the ~15% reduction in surface HLA I was not sufficient to completely account for the decreased lysis. However, PUFAs significantly lowered antigen-presenting cell-T cell conjugate formation, by ~30–40%. Taken together, our data show for the first time that an ω -6 and an ω -3 PUFA affect the HLA class I pathway of B lymphoblasts. Our findings suggest that elimination of self- and pathogen-derived peptides by effectors may be compromised by dietary PUFA supplementation. In addition, PUFA-mediated changes in ER-Golgi trafficking point to a new area of PUFA modulation of immune responses.—Shaikh, S. R., and M. Edidin. Immunosuppressive effects of polyunsaturated fatty acids on antigen presentation by human leukocyte antigen class I molecules. *J. Lipid Res.* 2007. 48: 127–138.

Supplementary key words arachidonic acid • cytotoxicity • docosahexaenoic acid • membrane microviscosity • surface expression • trafficking

Polyunsaturated fatty acids (PUFAs) have attracted considerable attention because of their potential health benefits in a variety of immune disorders (1, 2). Specific PUFAs of the ω -3 and the ω -6 series exert immunosuppressive effects (1, 3–5). A number of studies have shown that PUFAs exert their effects on inflammatory diseases through changes in cytokine production or T cell function (4). However, little is known about how PUFAs influence molecular targets that are not directly involved in

mediating the symptoms associated with autoimmunity or inflammation.

A great deal of our understanding of the immunosuppressive effects of PUFAs comes from studies on T cell proliferation, activation, and signaling (4). It has become apparent that PUFAs can inhibit T cell proliferation by modulating various signaling proteins activated upon engagement of the T cell receptor (TCR) and costimulatory molecules (6–11). A recent report even shows that PUFA modulation of T cells can disrupt the formation of the immunological synapse (12). On the other hand, far less is known about the effect of PUFAs on antigen-presenting cells (APCs), the targets of T cells. A few studies have shown that ω -3 PUFAs can lower the surface expression of human leukocyte antigen (HLA) class II molecules, with concomitant reductions in antigen presentation (13, 14). These findings have clinical relevance because a variety of inflammatory disorders are characterized by upregulation of class II surface molecule expression (15).

Little attention has been given to understanding how PUFAs may modulate antigen presentation through the HLA class I pathway. HLA class I molecules present self- or pathogen-derived peptides to CD8⁺ cytotoxic T lymphocytes (CTLs), which, upon recognition of class I molecules through the TCR and costimulatory molecules, can lyse the APC. To the best of our knowledge, only one laboratory has studied how PUFAs affect HLA class I proteins. Jenski and coworkers (17) found that murine HLA class I expression changed upon dietary intake or fusion of lymphocyte membranes with the ω -3 PUFA docosahexaenoic acid (DHA). An important finding from this study was that the expression of one HLA class I epitope, measured by

Abbreviations: AA, arachidonic acid; APC, antigen-presenting cell; BFA, Brefeldin A; BHT, butylated hydroxytoluene; CTL, cytotoxic T lymphocyte; DHA, docosahexaenoic acid; ER, endoplasmic reticulum; FACS, fluorescence-activated cell sorting; HBSS, Hanks' balanced salt solution; HLA, human leukocyte antigen; NL, neutral lipid; PA, palmitic acid; PC, phosphatidylcholine; PUFA, polyunsaturated fatty acid; PVP, polyvinylpyrrolidone; TCR, T cell receptor; 7-AAD, 7-amino-actinomycin D.

¹To whom correspondence should be addressed.
e-mail: saameshaikh@gmail.com

Manuscript received 16 August 2006 and in revised form 30 October 2006.
Published, JLR Papers in Press, October 30, 2006.
DOI 10.1194/jlr.M600365-JLR200

Copyright © 2007 by the American Society for Biochemistry and Molecular Biology, Inc.

This article is available online at <http://www.jlr.org>

monoclonal antibody binding, increased, whereas another decreased, suggesting changes in conformation of class I molecules (17). Cells enriched with DHA-containing phospholipids were also more susceptible to T cell cytotoxicity than were controls. However, a major limitation of the *in vitro* functional experiments by Jenski was the lack of saturated lipid controls and a nonphysiological method of lipid delivery.

In our study, we compare the effects of the ω -6 PUFA arachidonic acid (AA), the ω -3 PUFA DHA, and the saturated fatty acid palmitic acid (PA) on susceptibility to T cell-mediated lysis through HLA class I molecules of human B lymphoblasts, JY cells. We first confirm that PUFA treatment significantly alters the acyl chain composition of JY cells upon incorporation into membrane lipids. We then show that incorporation of either the ω -6 or the ω -3 PUFA reduces JY susceptibility to lysis by alloreactive CD8⁺ T cells. This global effect may have several underlying mechanisms. We show that PUFA modification alters the surface expression of HLA class I molecules as a result of a reduced rate of forward trafficking of newly synthesized molecules from the endoplasmic reticulum (ER) to the Golgi, but the reduction in surface HLA class I molecules is not sufficient to completely account for reduced lysis. We also show that PUFAs significantly lower APC-T cell conjugate formation, sufficient to account for the reduction in lysis.

MATERIALS AND METHODS

Cells

JY B lymphoblasts (18), a gift from J. Strominger, Harvard University (Cambridge, MA), were grown in RPMI 1640 1 \times (Mediatech Inc., Herndon, VA) supplemented with 10% FBS and 5% glutamine. JY cells stably transfected with an HLA-A2-yellow fluorescent protein (YFP) construct also contained 300 μ g/ml G418 for plasmid selection in the medium. Cells were cultured at 37°C in a 95% O₂/5% CO₂ atmosphere. Cell viability was ~97%.

Fatty acid treatment

To modify membrane lipids, JY cells were incubated overnight at 37°C (12 h) with FFAs. FFAs (Nu Check Prep, Elysian, MN) were delivered as FFA/BSA (3:1) (fatty acid-free BSA; Roche Biochemicals, Indianapolis, IN) complexes that were prepared as described by others (19, 20). FFA/BSA complexes were added to serum-free medium (LGM-3; Cambrex Bio Science, Walkersville, MD) for overnight incubation. All preparations of PUFA-containing lipids were made under a gentle stream of nitrogen gas in low-light conditions using degassed reagents to prevent oxidation. Oxidation of FFA/BSA stocks was assessed with ultraviolet/visible spectroscopy. Fresh fatty acid stocks were utilized for all experiments in order to prevent the formation of micelles. FFA stocks were stored with 10 μ M antioxidant butylated hydroxytoluene (BHT) (Roche Biochemicals). Cell viability was confirmed with trypan blue exclusion and 7-amino-actinomycin D (7-AAD) staining.

Polyvinylpyrrolidone-phospholipid treatment

With slight modifications, we used the polyvinylpyrrolidone (PVP) method developed by Muller and coworkers (21) to alter

plasma membrane structure. Instead of using egg phosphatidylcholine (PC), we used 950 μ g of 1-palmitoyl-2-arachidonoyl-*sn*-glycerophosphatidylcholine (16:0-20:4PC) and 1-palmitoyl-2-docosahexaenoyl-*sn*-glycerophosphatidylcholine (16:0-22:6PC) (Avanti Polar Lipids, Alabaster, AL) dissolved in ethanol, and added it to a solution of 3.5% PVP (10,000 MWt; Sigma Chemical Co., St. Louis, MO) supplemented with 0.5% glucose, and 1% BSA in RPMI 1640 1 \times medium. Cells (1–2 \times 10⁶) were then incubated for 2 h at room temperature with mixing. After treatment, cells were washed and used for further experimentation. Cell viability was ~90%.

Column chromatography

Total cellular lipids of JY cells spiked with 1 μ Ci/ml of [³H]PA or [³H]AA, or 1.5 μ Ci/ml [¹⁴C]DHA (American Radiolabeled Chemicals, St. Louis, MO) (22) were extracted using the method of Bligh and Dyer (23). Lipids were separated into three classes [polar lipid, FFA, and neutral lipid (NL)] with column adsorption chromatography as described (24). Briefly, 100 mg of glass aminopropyl beads were placed in a Pasteur pipette between fiberglass plugs and eluted in succession with 3 ml of chloroform-isopropanol (2:1; v/v), 3 ml of diethyl ether-acetic acid (98:2; v/v), and 4 ml of methanol (24). Adequate separation of the three lipid classes with column chromatography was verified using thin-layer chromatography with known lipid standards. Aliquots of eluted fractions were counted in triplicate using a scintillation counter.

Gas chromatography

Lipids were extracted using the Bligh and Dyer method (23) and dried under a gentle stream of nitrogen gas. Methylation and gas chromatography (GC) analysis were done by Avanti Polar Lipids. Toluene (0.2 ml) and 1% H₂SO₄ in methanol (0.4 ml) were added to the lipid extracts and heated at 70°C for 30 min. Methylated samples were cooled, and 1.0 ml hexane and 1.0 ml water were added to the samples, which were then vortexed and centrifuged. The upper hexane phase containing fatty acid methyl esters (FAMES) was collected. Hexane was evaporated under nitrogen gas, and FAMES were redissolved in hexane to an appropriate concentration. GC data were acquired on a Hewlett Packard 5890 Series II equipped with a J and W DB-225 capillary column (30 m length, 0.25 mm inner diameter, 0.25 μ m thickness). Column temperature was 220°C, pressure was 15 psi, and nitrogen was used as the carrier gas. Retention times and identifications were calibrated with standards from Nu-Check Prep.

Assessment of membrane microviscosity with fluorescence polarization

JY cells (1 \times 10⁶) were washed and labeled with 2 \times 10⁻⁶ M 1,6-diphenylhexatriene (DPH) in Hanks' balanced salt solution (HBSS) (Mediatech Inc., Herndon, VA) for 5 min at 37°C (25). Under these conditions, the signal from DPH fluorescence arises primarily from the plasma membrane surface (26). Subsequently, cells were washed and resuspended in HBSS, and fluorescence anisotropy was measured on an SLM-48000 spectrofluorometer (SLM Instruments, Urbana, IL). The DPH probe was excited at 355 nm, and emission was recorded at 425 nm. Fluorescence anisotropy was determined by the following relation: anisotropy = ($I_{VV} - G I_{VH}$) / ($I_{VV} + 2 G I_{VH}$) where I_{VV} and I_{VH} are the fluorescence intensities measured, respectively, parallel (vertical-vertical) and perpendicular (vertical-horizontal) to the direction of polarization of the emitted light, and G is an instrumentation correction factor defined as $G = I_{HV} / I_{HH}$.

Chromium release assay

JY B lymphoblasts were γ irradiated (2,000 rad) for 11 min and subsequently mixed with naive T lymphocytes, which were cultured for 4 days in RPMI 1640 $1\times$ medium supplemented with interleukin-2 (10 U/ml) (Roche Biochemicals) to prime the alloreactive T cells (provided by M.E.). Target JY cells were lipid modified as described above, pelleted, and incubated with 100 μ Ci ^{51}Cr (Perkin Elmer, Boston, MA) for 1 h in the appropriate lipid-containing medium, followed by three washes to remove excess ^{51}Cr . The effector and target cells were then cultured in 96-well plates at differing ratios for 4 h at 37°C in a final volume of 200 μ l. Cells were pelleted, and 100 μ l of the supernatant was assayed for activity with a γ counter. Maximal ^{51}Cr release was induced by the addition of 1 M HCl. Specific lysis was calculated as (sample cpm – spontaneous cpm)/(maximal release cpm – spontaneous release cpm) \times 100.

Serine esterase release assay

JY cells were treated overnight with BSA or FFA/BSA complexes and then washed with medium and cultured with CTLs at an effector-to-target (E/T) ratio of 2/1 for 4 h at 37°C in a final volume of 200 μ l. Cells were pelleted, and 100 μ l of the supernatants were collected and placed in polystyrene tubes. One milliliter of substrate solution (Calbiochem, San Diego, CA) (0.20 mM N- α -benzyloxycarbonyl-L-lysine thiobenzyl ester substrate, 0.22 mM 5,5'-dithio-bis(2-nitrobenzoic acid) coloring agent, 0.01% Triton X-100 in PBS) was added and incubated for 30 min in a 37°C water bath. The reaction was inhibited with the addition of 1 ml of 1 mM PMSF on ice. Maximal enzyme release was achieved by the addition of 100 μ l of 1% Triton X-100. Serine esterase activity was assessed by absorbance measurements at 412 nm. Percent enzyme release was calculated as (sample release – spontaneous release)/(maximal release – spontaneous release) \times 100.

Antibodies

For studies of HLA class I expression, we primarily utilized the mouse monoclonal antibody Ke2 (anti-monomorphic epitope) (27) conjugated to Cy5, or Ke2 Fab-Cy3, which recognized HLA-A2, HLA-B7 on JY cells. We also used anti-HLA-A2 (PA2.1) (28) conjugated to Cy5. Ke2 and anti-HLA-A2 antibodies were purified from hybridoma supernatants using protein-A affinity chromatography. Ke2 and anti-HLA-A2 were conjugated to dye using a standard kit (Amersham Biosciences, Piscataway, NJ). For fluorescence-activated cell sorting (FACS) experiments, cell samples not expected to bind a given primary antibody were used as controls. An additional control was the use of excess unlabeled antibody to block binding of fluorescently labeled antibody. For all experiments, background autofluorescence was established with unstained cells.

Fluorescence recovery after photobleaching microscopy

Fluorescence recovery after photobleaching (FRAP) measurements were conducted as described previously by our laboratory (29). Briefly, lateral diffusion measurements were conducted with Cy3-labeled Ke2 Fab. An attenuated laser beam was focused through a 63 \times objective on a 0.6–0.8 μ m spot size. Fluorescence was recorded from the spot followed by a 4 ms bleach with full laser power. The mobile fraction of HLA class I molecules was then calculated as the ratio of observed FRAP relative to the initial fluorescence.

Flow cytometry

Measurements were made on an FACSCalibur flow cytometer (Becton Dickinson, San Jose, CA) equipped with a 488 nm argon

laser and a 647 nm diode laser. For all experiments, cell numbers between different samples were equalized to 1×10^5 cells/sample. Samples were washed in PBS twice and stained with saturating levels of fluorescent antibody for 30 min on ice, followed by three additional washes in PBS. Data were acquired on 1×10^4 gated live cells. Dead cells were stained with 7-AAD or propidium iodide. Apoptosis measurements with Annexin V-Cy5 were performed according to the manufacturer's protocol (BD Biosciences Pharmingen, San Diego, CA).

Trafficking and stability studies

JY cells were washed twice in HBSS, and HLA class I mouse antibody binding sites were blocked with saturating amounts of unlabeled Ke2 for 30 min. The cells were then washed in serum-free medium and treated with either BSA, AA, or DHA for up to 12 h. FACS measurements were performed at varying time points with Ke2-Cy5 antibody to assess the presence of new class I molecules on the surface of the plasma membrane. We also used 15 or 20°C temperature shifts to block transport of class I molecules from the ER to the Golgi or the Golgi to plasma membrane, respectively (30, 31). For these experiments, JY cells were lipid treated as described above, except that samples were placed in a 15 or 20°C water bath for 3 h, blocked with antibody, and then incubated at 37°C. FACS measurements after the temperature block were conducted at varying time points with Ke2-Cy5 antibody to assess the expression of new class I molecules. For surface stability measurements, JY cells were treated with FFAs as described above and with 5 μ g/ml Brefeldin A (BFA) to block ER-to-plasma membrane transport. Decay in the surface expression of HLA class I was assessed by staining with Ke2-Cy5 at varying time points after BFA treatment. For all measurements, dead cells were excluded in the analysis with 7-AAD staining.

Conjugation assay

Alloreactive T cells were loaded with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate (DiD) (Invitrogen, Carlsbad, CA) for 1 h in HBSS at 37°C and then mixed at a 1.5:1 ratio with control or lipid-modified JY cells stably transfected with HLA-A2-YFP. T cells/JYs were incubated at 37°C in HBSS for 1–15 min, and conjugate formation was inhibited by adding cold HBSS and placing the samples on ice. Formation of conjugates, which were double positive for DiD and YFP, was determined by FACS.

Analysis

FACS data were analyzed with Cell Quest software. Data were plotted and fitted in Origin (7.0) or GraphPad Prism (4.0). Statistical significance was established against a common control (BSA) by performing a one-way ANOVA followed by a Dunnett's *t*-test (12). *P* values < 0.05 were considered significant.

RESULTS

Cell uptake and incorporation of fatty acids

For most of our experiments, human JY B lymphoblasts were treated with 100 μ M PA, AA, or DHA complexed to BSA for 12 h in serum-free medium. We selected this concentration and the method of delivery (FFA/BSA complexes) to mimic physiological conditions (32, 33). Depending on the cell type, PUFAs are well known to induce apoptosis at high concentrations (34). Therefore, we measured cell viability and apoptosis of JY cells at 0–100 μ M FFA. Trypan blue exclusion, 7-AAD, and AnnexinV stain-

ing showed that treatment of JY cells with 100 μ M FFAs resulted in 80–85% cell viability, with the remaining cells as either dead or apoptotic at 12 h of treatment (data not shown). However, longer periods of incubation or higher concentrations lowered cell viability considerably.

FFAs were incorporated into membrane lipids of JY cells. Cells treated with 100 μ M PA, AA, or DHA were spiked, respectively, with either [3 H]PA, [3 H]AA, or [14 C]DHA for 12 h in order to localize the radiolabeled FFAs into three different lipid classes: NL, unesterified FFA, and PL, using column adsorption chromatography (**Fig. 1**). Because added FFA can be incorporated into endomembranes, we extracted total cellular lipids. With all lipid treatments, most of the FFAs were incorporated into either NLs or PLs with very little unesterified fatty acid (<6%). [3 H]PA and [3 H]AA showed a slight preference for phospholipids over NLs, with ~66% and 57% of the [3 H]PA and [3 H]AA, respectively, localizing to the polar lipid fraction. In contrast, [14 C]DHA displayed a small preference for the NL fraction, with ~55% in the NL fraction.

The uptake of FFAs altered the acyl chain composition of cell membrane lipids. Total cellular lipids were extracted and analyzed with GC, and results of the analysis are summarized in **Table 1**. Relative to the BSA control, supplementation of the medium with PA (16:0) resulted in a significant increase in the percentage of 16:0 and 16:1 acyl chains. Addition of AA and DHA also caused significant changes in the acyl chain composition of JY cells relative to BSA. Both PUFAs decreased the levels of 18:0 and 18:1. AA (20:4) treatment resulted in an increase in

TABLE 1. FFA/BSA treatment alters the acyl chain composition of JY B lymphoblasts

Fatty Acid	BSA	PA (16:0)	AA (20:4)	DHA (22:6)
14:0	1.5 \pm 0.1	1.2 \pm 0.5	1.7 \pm 0.3	1.6 \pm 0.1
16:0	14.1 \pm 2.0	24.0 \pm 2.1 ^b	12.1 \pm .03	14.7 \pm 1.4
16:1	5.3 \pm 0.6	10.0 \pm 1.8 ^b	3.0 \pm 0.5	3.7 \pm 0.5
18:0	17.9 \pm 1.1	14.9 \pm 2.0	11.5 \pm 1.0 ^b	14.1 \pm 1.2
18:1	35.3 \pm 2.4	28.6 \pm 0.5	16.9 \pm 1.5 ^b	21.0 \pm 0.9 ^b
18:2	2.8 \pm 0.3	2.2 \pm 0.3	1.4 \pm 0.3	2.2 \pm 0.2
18:3	2.3 \pm 1.2	2.2 \pm 0.8	1.7 \pm 0.9	1.6 \pm 0.8
20:0	2.7 \pm 1.4	2.6 \pm 0.9	1.9 \pm 1.1	1.9 \pm 1.0
20:1	0.6 \pm 0.3	0.7 \pm 0.2	0.5 \pm 0.1	0.5 \pm 0.1
20:2	3.2 \pm 0.8	2.4 \pm 1.0	1.4 \pm 0.3	1.8 \pm 0.4
20:3 ω -6	1.4 \pm 0.4	0.5 \pm 0.2	0.8 \pm 0.3	0.7 \pm 0.2
20:3 ω -3	3.2 \pm 1.0	2.9 \pm 0.8	2.3 \pm 1.3	2.2 \pm 1.2
20:4	3.5 \pm 1.6	1.8 \pm 0.9	18.1 \pm 4.0 ^c	1.9 \pm 0.5
22:0	3.0 \pm 1.5	2.7 \pm 0.8	2.1 \pm 2.5 ^b	2.0 \pm 1.1
22:4 ω -6	0.8 \pm 0.9	0.0 \pm 0.0	20.3 \pm 4.9 ^b	0.0 \pm 0.0
22:4 ω -3	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	2.1 \pm 0.8 ^a
22:5 ω -6	0.0 \pm 0.0	0.0 \pm 0.0	2.2 \pm 1.1	0.0 \pm 0.0
22:5 ω -3	0.0 \pm 0.0	0.0 \pm 0.0	0.0 \pm 0.0	1.4 \pm 0.3 ^b
22:6	0.1 \pm 0.1	0.8 \pm 0.2	0.8 \pm 0.6	25.2 \pm 5.0 ^b
24:0	2.3 \pm 1.2	1.0 \pm 0.7	0.0 \pm 0.0	1.6 \pm 0.9
Σ Saturated	41.7 \pm 1.9	47.7 \pm 2.8	30.8 \pm 4.0 ^a	35.8 \pm 0.4
Σ MUFA	41.3 \pm 1.6	39.4 \pm 2.5	20.4 \pm 1.7 ^b	25.1 \pm 0.5 ^b
Σ PUFA	17.0 \pm 0.4	12.9 \pm 0.2	48.8 \pm 5.2 ^b	39.1 \pm 0.1 ^b

MUFA, monounsaturated fatty acid; PUFA, polyunsaturated fatty acid; JY cells were treated with BSA alone or 100 μ M FFAs [palmitic acid (PA), arachidonic acid (AA), and docosahexaenoic acid (DHA)] complexed to BSA for 12 h in serum-free medium. Total cellular lipid was extracted and analyzed with gas chromatography as described in the text. Data are the average \pm SE from three to four independent measurements. Values are expressed as a percentage of total fatty acids.

^a $P < 0.05$.

^b $P < 0.01$.

^c $P < 0.001$.

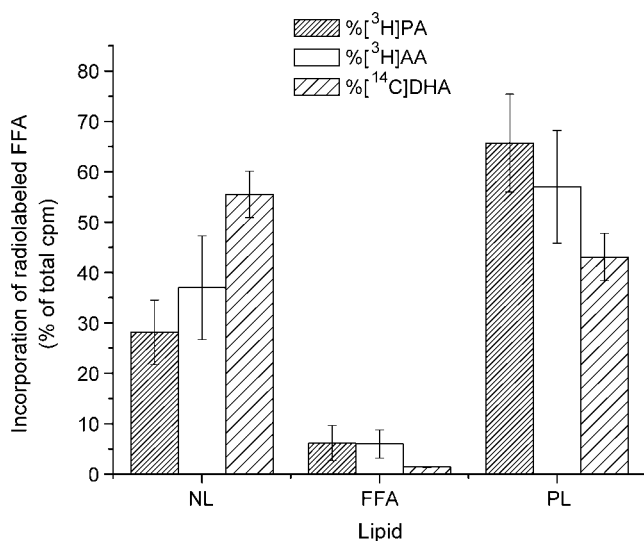


Fig. 1. Exogenous FFAs are incorporated into neutral and polar lipid fractions. Column chromatography analysis of JY B lymphoblasts treated for 12 h in serum-free medium with 100 μ M FFAs complexed to BSA spiked with either [3 H]palmitic acid ([3 H]PA), [3 H]arachidonic acid ([3 H]AA), or [14 C]docosahexaenoic acid ([14 C]DHA). Lipids were extracted using the method of Bligh and Dyer (23) and separated into neutral lipid (NL), FFA, and polar lipid (PL) fractions. Percent incorporation was determined by scintillation counting. Data (average \pm SE) are from at least three independent measurements.

20:4 and its elongation product 22:4 ω -6. Addition of DHA (22:6) significantly increased 22:6 levels and slightly increased 22:4 ω -3 and 22:5 ω -3, suggesting that DHA underwent some modification upon incorporation into membrane lipids. Overall, there was a significant increase in the amount of PUFAs in AA- and DHA-treated cells, with a substantial reduction in the total saturated and monounsaturated acyl chains (Table 1). AA treatment resulted in a larger increase in the total PUFA content relative to DHA.

A significant decrease in the microviscosity of the plasma membrane was associated with the incorporation of FFAs into membrane lipids, compared with the BSA control. Membrane microviscosity, measured in terms of fluorescence anisotropy of DPH, was reduced after cells were treated with PA and further reduced after treatment with AA and DHA (**Table 2**). The change in anisotropy values is nearly identical to that shown in a previous report on PA and PUFAs and membrane fluidity in CHO cells (35). The decrease in membrane viscosity relative to the BSA control was more pronounced with AA than with DHA, consistent with the GC data showing a higher amount of PUFAs in AA-treated cells.

PUFA-modified cells are less susceptible to lysis by CTLs than are controls

We compared the ability of activated alloreactive CD8⁺ T cells to lyse target JY cells without (BSA control) or with

TABLE 2. Comparison of PUFA-induced changes in membrane microviscosity, HLA I expression, and specific lysis between FFA/BSA and PVP-phospholipid methods of lipid delivery

Lipid Treatment	Fluorescence Anisotropy	Change in HLA Class I Surface Expression (Relative to Control)	Change in Specific Lysis (Relative to Control)
BSA/FFA			
BSA	0.153 ± 0.002	—	—
PA	0.130 ± 0.001 ^b	↓ 0.8 ± 1.2	↓ 6.2 ± 5.2
AA	0.100 ± 0.002 ^b	↓ 17.7 ± 1.3 ^b	↓ 21.9 ± 5.4 ^a
DHA	0.110 ± 0.003 ^b	↓ 11.3 ± 0.9 ^b	↓ 22.2 ± 3.6 ^b
PVP			
EtOH	0.167 ± 0.003	—	—
16:0-20:4PC	0.154 ± 0.001 ^a	↑ 14.4 ± 2.5 ^b	No change
16:0-22:6PC	0.152 ± 0.003 ^b	↑ 13.1 ± 1.4 ^b	No change

JY cells were treated with either 100 μM FFA complexed to BSA for 12 h or with heteroacid phospholipids for 2 h in the presence of polyvinylpyrrolidone (PVP). Fluorescence anisotropy, changes in surface HLA class I levels with Ke2-Cy5, and changes in specific lysis were calculated as described in the text. All values reported are averages ± SE from a minimum of three independent measurements.

^a $P < 0.05$.

^b $P < 0.01$.

PA, AA, or DHA treatment (Fig. 2A). The E/T ratio varied from 10/1 to 1.25/1. A significant reduction in lysis was observed with 100 μM AA or DHA treatment (Fig. 2A). The reduction in specific lysis was generally more pronounced at high E/T ratios, although differences in lysis could also be seen at low E/T ratios. On average, the decrease in lysis was ~20–25% at high E/T ratios with both PUFAs and was dose dependent (Fig. 2B). Although we observed a slightly lower lysis after treatment with 100 μM PA (Fig. 2A), the effect was within experimental error relative to the BSA control (Fig. 2B). As demonstrated previously by our laboratory, killing by peripheral blood mononuclear cells was CTL specific; it was inhibited by anti-CD8 Ab (Fig. 2C) (36).

Target cell lysis is a measure of both the state of target cells and T cell response. We assessed T cell response separately in terms of serine esterase release (Fig. 2D). We observed maximal esterase release from CTLs interacting with BSA-modified JY cells, significantly less esterase release by T cells responding to AA- or DHA-treated cells, and only a slight decrease in T cell response to PA-treated cells. The significant reduction in esterase release with PUFA treatment relative to the BSA control confirmed our findings with target cell lysis and suggested changes in the interactions between JY cells and the allo-CTLs (Fig. 2A).

PUFA treatment alters surface HLA class I expression

We speculated that the reduction in specific lysis upon PUFA enrichment into membranes was due to changes in the surface levels and/or lateral organization of HLA class I. It is well documented that addition of PUFAs to cultured cells can affect the display of cell surface molecules (4). Figure 3A illustrates the concentration-dependent decrease in surface expression of HLA class I, measured with the monoclonal antibody (MAb) Ke2 against a monomorphic HLA epitope, with a statistically significant reduction observed at 100 μM FFA corresponding to a reduction in expression by ~17% and ~11% respectively for AA- and DHA-treated cells. The ~6%

difference in expression between AA and DHA failed to reach statistical significance. In contrast, treatment of cells with PA had no effect on HLA class I expression (Fig. 3A). Percent reduction in expression was calculated by assessing the change in median fluorescence intensity (MFI) relative to the BSA control, whose MFI was set to 100% (9, 37).

The reduction in class I surface levels was not an artifact of the MAb used or the method of treatment. AA and DHA treatment significantly lowered HLA-A2 surface levels by ~22% and ~17%, respectively (Fig. 3B). The changes in surface expression upon PUFA treatment were not due to binding of MAb IgG to Fc receptors, which are abundant on the surface of B lymphoblasts. Nearly identical changes were observed with Fab (Fig. 3B). Finally, it was verified, by comparing surface expression levels in the absence or presence of BSA or BHT, that neither BSA nor the antioxidant BHT alone have any effects on HLA I expression (data not shown).

Our laboratory and others have previously demonstrated that changes in HLA class I membrane clusters are linked to changes in antigen presentation (38, 39). We assessed changes in HLA I lateral organization by measuring their mobile fractions using Ke2-Cy3 Fab with FRAP microscopy for BSA-, PA-, AA-, and DHA-treated JY cells. Mobile fractions were ~40–50% for all treatments, ruling out any changes in the lateral organization of HLA I molecules upon lipid treatment.

Comparison of HLA class I surface levels, membrane microviscosity, and specific lysis with PVP-phospholipid treatment

Muller and coworkers (21) previously showed that a decrease in membrane microviscosity increased HLA class I surface levels; they argued that these changes were due to membrane vertical phase separation. Because we observed a decrease in membrane microviscosity and a reduction in HLA class I levels (Table 2), we assessed whether the difference between our results and theirs were due to the method of lipid delivery. The method of lipid

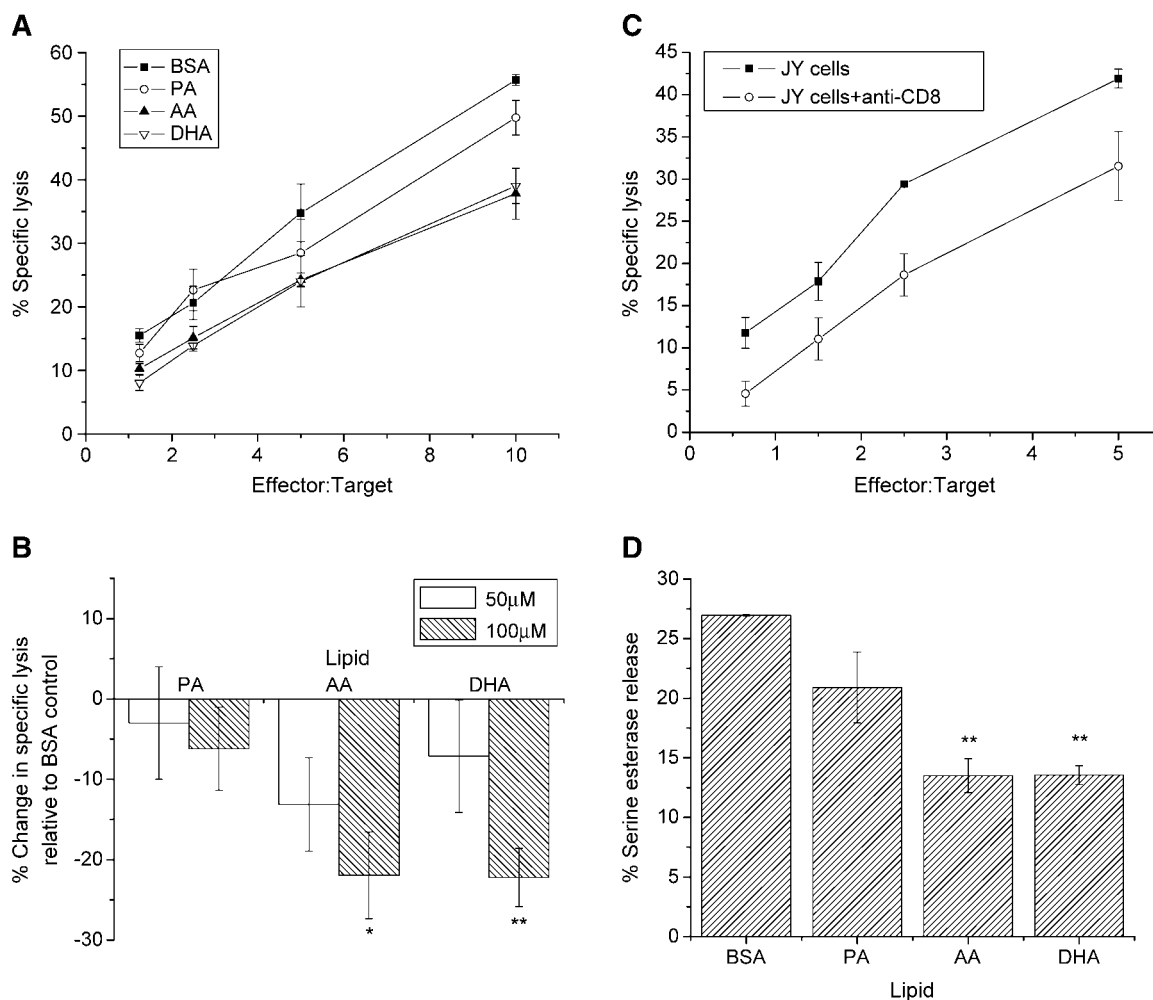


Fig. 2. HLA class I antigen presentation of JY cells is altered by polyunsaturated fatty acid (PUFA) treatment. **A:** Percent specific lysis as a function of effector-to-lipid (E/T) modified targets. Target JY cells were incubated with 100 μ M PA, AA, or DHA complexed to BSA for 12 h, followed by incubation with 51 Cr in the presence of alloreactive cytotoxic T lymphocytes (CTLs) for 4 h, at which time 51 Cr release was measured. **B:** Average change in specific lysis \pm SE for E/T = 10/1-5/1 for three to four independent measurements at 50 μ M and 100 μ M treatment. **C:** Specific lysis is CD8⁺ CTL-specific. Effector T cells were blocked with human anti-CD8 antibody for 30 min prior to incubation with 51 Cr-loaded JY cells. **D:** Percent serine esterase release at E/T = 2/1 for BSA and lipid-modified JY cells. CTLs and JY cells were incubated for 4 h as described in A followed by absorbance measurements of enzyme activity on a substrate. Data (average \pm SD) in A and C are from triplicate samples from a single experiment representative of two to four individual experiments. Data (average \pm SE) in B and D are average of triplicate samples from two to four independent measurements. Percent specific lysis and serine esterase release were calculated as described in the text. Significant differences relative to the BSA control in B and D are indicated by asterisks (* P < 0.05, ** P < 0.01).

delivery described by Muller utilized the plasma membrane expander PVP to effect incorporation of phospholipids into the plasma membrane. When we modified JY cells with either control (EtOH only), 16:0-20:4PC, or 16:0-22:6PC (heteroacid PCs with equivalent FFAs in the *sn*-2 acyl chain position), we found that indeed incorporation of these PUFA-containing phospholipids increased HLA class I surface levels by \sim 10–15% (Table 2), quantitatively similar to values reported by Muller for egg PC (21). As predicted, the change in HLA class I levels upon PVP-phospholipid treatment did correlate with a decrease in membrane microviscosity (Table 2). We did not test saturated phospholipid as a control, because it is in a gel state for these measurements and will not incorporate into the membrane. Modifying the plasma

membrane with heteroacid PUFA-containing PCs had no consequences for CTL assays. Lysis of JY cells treated with 16:0-20:4PC or 16:0-22:6PC was the same as controls (Table 2).

PUFAs lower HLA I surface expression by inhibiting ER-plasma membrane transport

A reduction in antibody binding to HLA class I molecules in the presence of PUFAs (Fig. 3), suggested either a change in conformation of class I molecules or a reduction in the number of class I molecules on the surface. A reduction in the number of class I molecules would imply an alteration in the forward or reverse trafficking rate of class I molecules, an area of PUFA modulation that is not well documented. We do not think that PUFAs

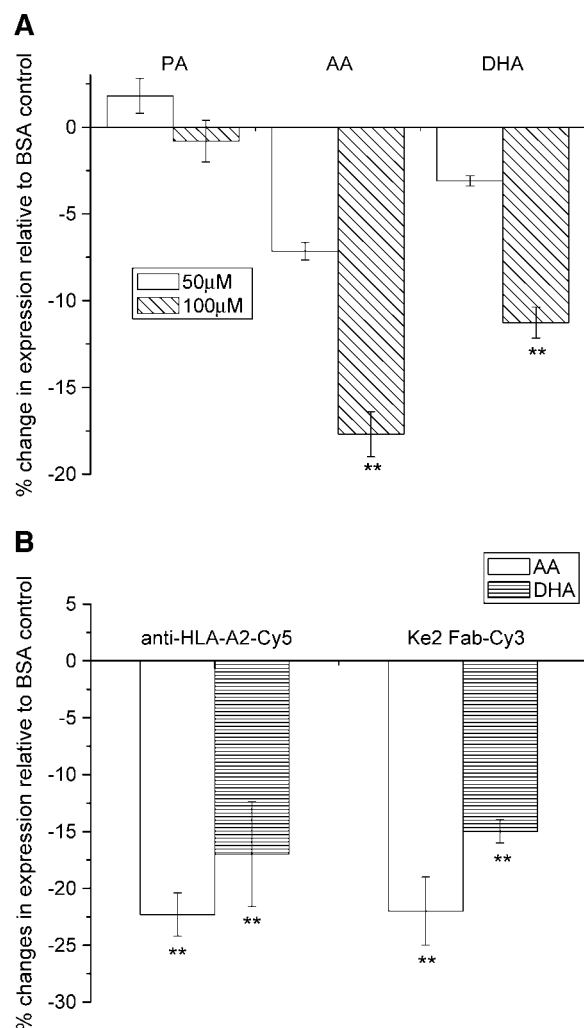


Fig. 3. PUFAs lower HLA class I surface expression. A: Percent change in HLA class I surface expression relative to the BSA control for JY cells treated with 50 μ M or 100 μ M PA, AA, and DHA. Changes in expression were assessed by Ke2-Cy5 binding, and fluorescence was measured by fluorescence-activated cell sorting (FACS). B: Percent change in HLA class I surface expression relative to the BSA control for JY cells treated with 100 μ M AA and DHA. Change in expression was assessed by anti-HLA-A2-Cy5 or Ke2 Fab-Cy3 binding. FACS data are from 10,000 live cells and are the average of three to six independent experiments \pm SE. Significant differences relative to the BSA control are indicated by asterisks (** $P < 0.01$).

change HLA class I conformation, because we had observed a reduction in surface levels with Ke2, which binds an epitope in the $\alpha 3$ domain of HLA I, and PA2.1, which binds an HLA-A2 epitope of the $\alpha 2$ domain (Fig. 3). PUFAs did not affect the affinity of antibody binding to HLA class I molecules. K_d values from Ke2 titration curves were nearly identical for PUFA treatment relative to the BSA control, on the order of 0.5 nM.

PUFA treatment did not affect class I surface stability. JY cells were treated with BFA to block ER-to-plasma membrane transport of HLA class I molecules, and surface stability was assessed in terms of loss of Ke2-Cy5 binding with time after blocking. As illustrated in Fig. 4A, there was

no change in stability of surface HLA I consequent to feeding cells PUFAs.

PUFAs do affect forward traffic of HLA class I molecules. Initially, experiments were conducted by measuring the rate of appearance of newly synthesized class I molecules on the plasma membrane surface over time after cleaving surface HLA I molecules with papain. Although we observed a reduction in the rate of appearance of surface HLA class I molecules with PUFA-treated cells (data not shown), we were not satisfied with these results, because papain in combination with PUFA treatment drastically lowered cell viability ($<50\%$). Therefore, we blocked resident surface HLA I molecules with unlabeled Ke2 antibody and measured the appearance of new Ke2 binding sites as a function of PUFA treatment. Figure 4B shows a representative experiment. It can be seen that the rate of repopulation of class I molecules on the plasma membrane surface was lowered for AA- and DHA-treated cells relative to the control. On average, the rate of surface recovery was significantly lowered, by $\sim 33\%$, with PUFA treatment relative to the BSA control (Fig. 4B, inset).

PUFAs affect trafficking from the ER to the Golgi complex. We incubated cells for ~ 3 h at 20°C , which inhibits trafficking from the Golgi to the plasma membrane, or at 15°C , which prevents ER-to-Golgi traffic (30, 31), and subsequently blocked resident HLA I molecules with unlabeled Ke2. After releasing the temperature block, we measured the rate of new Ke2 binding sites. PUFAs did not affect the rate of Golgi-to-plasma membrane trafficking (Fig. 4C). In contrast, cells released from the 15°C block showed a reduction in the rate of trafficking from the ER to the Golgi (Fig. 4D). Temperature blocks were hard on the cells, and therefore the rate of recovery was only measured for 6 h post temperature blocking.

Relationship between specific lysis and HLA class I surface levels

The lowered forward trafficking rate without a change in endocytosis (as assessed by transferrin receptor CD71 surface expression) (data not shown), explained the $\sim 15\%$ steady-state reduction in surface HLA class I levels. However, it was unclear whether the decreased T cell response to PUFA-modified cells was due to a reduction in class I surface levels. We therefore asked what change in surface expression would be required to lower lysis by ~ 20 – 25% , as observed upon PUFA modification (Fig. 2). We measured changes in specific lysis at E/T of $\sim 10/1$ after blocking a fraction of surface class I molecules on BSA-, AA-, and DHA-treated JY cells with anti-HLA-A2 antibody, which effectively blocks HLA class I-TCR interactions. As expected, a reduction in lysis was observed with increasing antibody concentration (data not shown). Using the same antibody concentrations used for the lysis experiment, we assessed the corresponding change in surface expression from the antibody titration curve for anti-HLA-A2 for BSA- and PUFA-modified cells. We then plotted the percent change in specific lysis between antibody dilutions as a function of the percent change in surface expression for BSA-, AA-, and DHA-treated cells (Fig. 5). The Xs in the

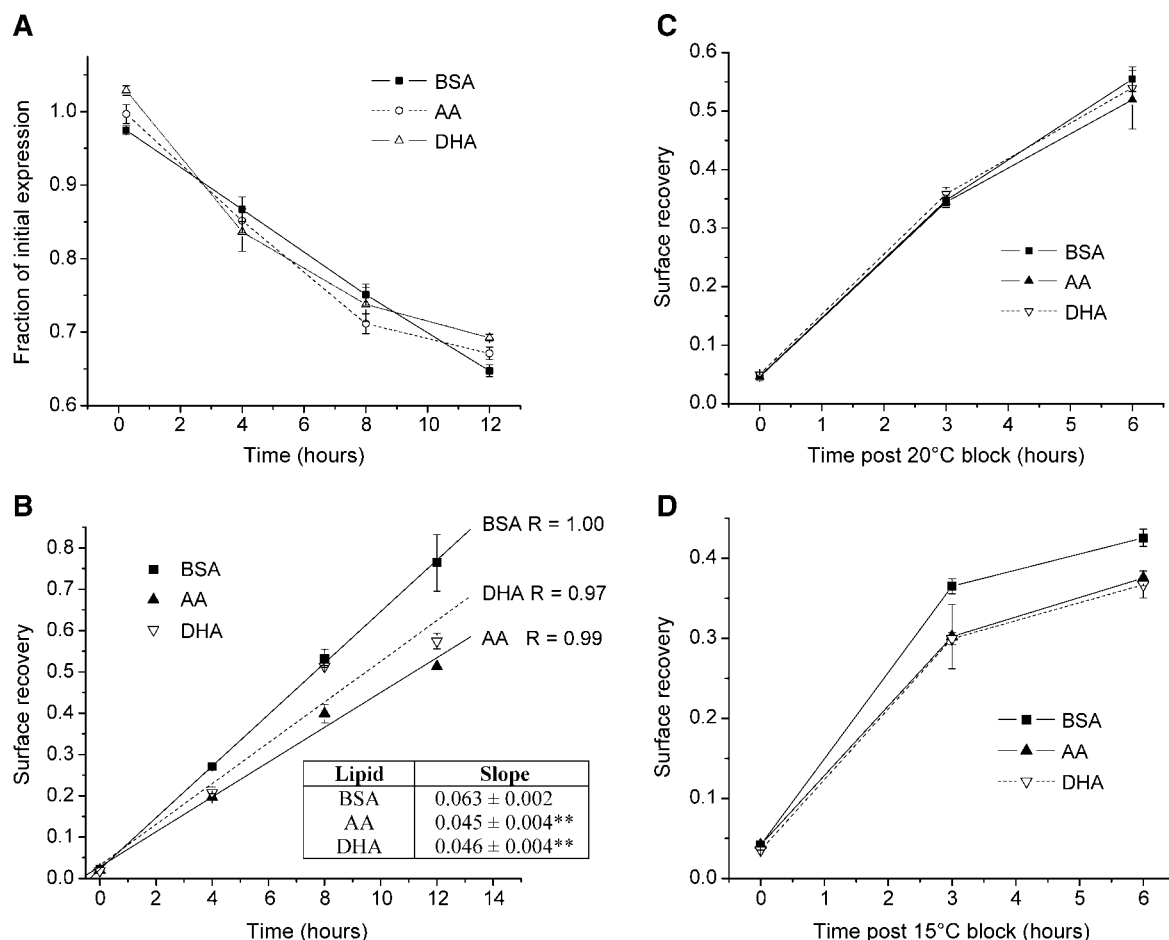


Fig. 4. PUFA treatment reduces the rate of forward traffic of HLA class I molecules from the endoplasmic reticulum (ER) to the plasma membrane. **A:** Plot of class I surface expression as a function of time after treating cells with Brefeldin A (BFA). JY cells were treated with 5 μ g/ml BFA and placed in serum-free medium supplemented with BSA, AA, or DHA. **B:** Plot of class I surface expression as a function of time after blocking with unlabeled Ke2. JY cells were treated with unlabeled Ke2 for 30 min and subsequently placed in serum-free medium with BSA, AA, or DHA. Samples were removed periodically over the next 12 h, and FACS was used to measure Ke2-Cy5 staining of new class I molecules on the plasma membrane surface. Lines were fit to data points using linear regression $y = a + bx$; R values are correlation coefficients. Inset: Changes in slope, b , for BSA-, AA-, and DHA-treated cells from three independent measurements. Statistical significance relative to BSA is indicated by asterisks ($^{**}P < 0.01$). **C:** Same as B, except that cells were treated for 3 h at 20°C to selectively inhibit trafficking from the ER to the plasma membrane. **D:** Same as B, except that cells were treated for 3 h at 15°C to selectively inhibit trafficking from the ER to the Golgi complex. Data (except inset in B) are single experiments representative of two to four independent experiments. Data are normalized to maximal surface expression at time 0.

calibration curves denote the PUFA-induced change in specific lysis (~ 20 – 25%) (Fig. 2) corresponding to a change in surface expression by $\sim 50\%$ for BSA- and AA-treated cells and $\sim 65\%$ for DHA-treated cells. This provides us with an expression-to-lysis ratio of ≥ 2 . Therefore, PUFA-modified changes in HLA class I surface levels are not solely responsible for the observed reduction in lysis.

PUFA modification of JY cells lowers conjugate formation

An alternative explanation for the lowering of lysis with PUFA modification was a reduction in conjugate formation between JY cells and CTLs. **Figure 6** presents a plot of percent change in conjugate formation relative to the BSA control. At 6 and 15 min after coincubation of JY cells with activated alloreactive CTLs, we found a significant reduction in conjugate formation with PUFA treatment

corresponding to ~ 30 – 40% change. We did not observe any significant change with PA lipid treatment.

DISCUSSION

Lipid modulation of HLA class I

In the present study, we found that AA and DHA treatment of JY cells lowers their susceptibility to CTL lysis and their rate of HLA class I trafficking to the plasma membrane. We also observed changes in conjugate formation that account for the reduction in lysis. These effects have not been described previously for HLA class I. They are consistent with data that show that class II surface expression and antigen presentation are lowered upon treatment of cells with PUFAs (13, 14, 20, 40, 41). The common

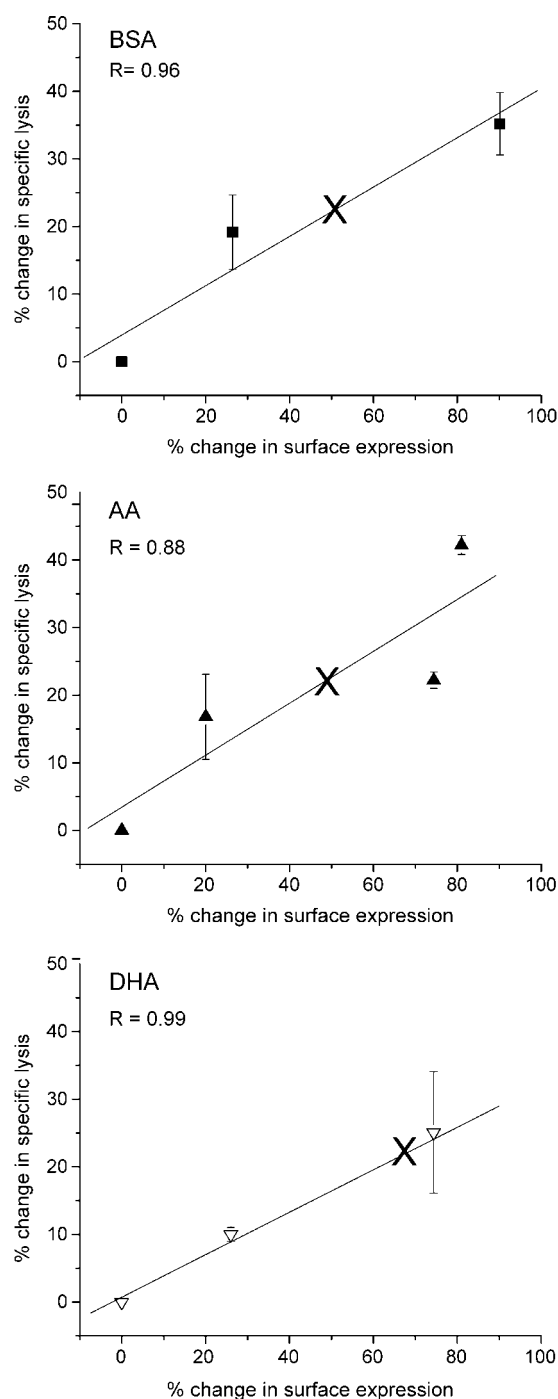


Fig. 5. Relationship between percent specific lysis and corresponding changes in surface HLA class I levels. Percent changes in specific lysis as a function of percent change in surface expression upon blocking of surface HLA class I with anti-HLA-A2 antibody for BSA-, AA-, and DHA-treated cells. Percent change in specific lysis was determined with chromium release after blocking surface HLA class I with varying dilutions of anti-HLA-A2 antibody. Percent change in surface expression was calculated from the antibody titration curves. The symbol X denotes the ~20–25% change in specific lysis (Fig. 2) observed with PUFA treatment. The lines were fit to data points using linear regression, $y = a + bx$, and the correlation coefficients, R , are indicated in the legends. Values (\pm SD) are from triplicate samples from a single experiment representative of two to three independent measurements. The slopes of the linear regression lines did not differ significantly between BSA- and PUFA-treated cells ($P > 0.05$).

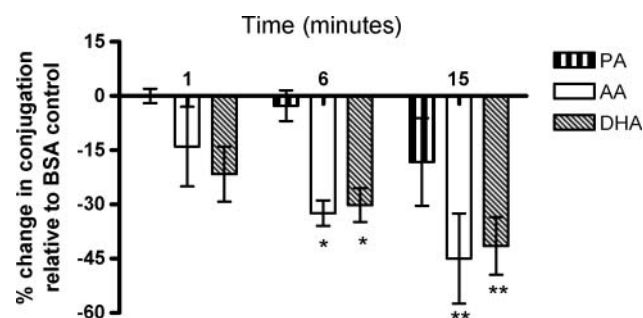


Fig. 6. PUFA treatment inhibits JY-CTL conjugate formation. Plot of percent change in JY-CTL conjugate formation upon 100 μ M FFA/BSA treatment relative to the BSA control. Target JY cells stably transfected with HLA-A2-yellow fluorescent protein (YFP) were incubated with BSA, PA, AA, or DHA for 12 h, followed by incubation with 1,1'-dioctadecyl-3,3,3',3'-tetramethylindodicarbocyanine perchlorate (DiD)-loaded alloreactive CTLs for 1, 6, or 15 min, at which time conjugate formation was determined by FACS. Conjugates were double positive for DiD and YFP fluorescence. Data are average \pm SE from three to five independent experiments. Significant differences relative to the BSA control are indicated by asterisks (* $P < 0.05$, ** $P < 0.01$).

factor may be the effect of changes in lipid composition on lipid packing density, which alters antigen presentation. This is suggested by the recent finding that infection with the intracellular parasite *Leishmania donovani* in macrophages resulted in a decrease in membrane microviscosity and a failure to present antigens to T cells, which could be corrected by making the membrane less fluid (42).

In our study, changes in membrane microviscosity (Table 2) correlate with the change in susceptibility of JY cells to CTL lysis (Fig. 2), but not in the manner described by Muller and Krueger (43). Those authors proposed that changes in membrane microviscosity altered the vertical displacement of membrane proteins and thereby altered surface expression (43). They showed that MHC class I was "antidromic"; a decrease in membrane microviscosity increased surface expression, and an increase in membrane microviscosity lowered expression (21). We observed that PUFA treatment did significantly decrease plasma membrane microviscosity, assessed by fluorescence polarization of DPH (Table 2), but this correlated with a decrease in surface HLA class I expression (Fig. 3). The difference between our measurements and those of Muller lie in methodology, because using their method of lipid delivery, we got their result (Table 2).

Our findings are also discrepant to those of the Jenski laboratory (17) that examined lipid modulation of HLA class I. In their studies, lipid modification of T27A cells grown as ascites tumors in mice fed fish oil resulted in altered class I surface expression but an increase in cytolysis by alloreactive CTLs. They suggested that DHA-containing phospholipids could modulate the conformation of class I molecules and so modulate recognition by CTLs (16). In our work, PUFA treatment did not change the affinity of HLA class I for two different antibodies, which led us to rule out conformational changes due to lipid incorporation. We speculate that these differences are due to different

methods of lipid treatment, concentrations, duration of treatment, and cell lines.

PUFA concentrations

Our study utilized a fairly high concentration (100 μ M) of FFAs complexed to BSA for modulation of JY cell function. It is generally thought that physiological levels of PUFA are far lower (low micromolar range), and a majority of studies use 10–50 μ M PFAs complexed to BSA, which allows investigators to treat cells for 24–96 h. However, PFAs complexed to BSA can reach serum levels in the low millimolar range (D. Jump, unpublished observation) (32). We attempted to mimic these physiological conditions by using high concentrations for a short period of time (12 h). High levels of FFAs can lead to lipid cytotoxicity, which is often used as a model for studying lipid overload associated with diabetes and obesity (44). We verified that our cells were not experiencing lipid toxicity as assessed by measurements of apoptosis.

Mechanisms

PFAs lower the specific lysis of JY cells by CTLs through a variety of mechanisms, which are not mutually exclusive. Although feeding cells AA and DHA reduced surface HLA class I levels, (Fig. 3), this was not the only PUFA-induced change. PUFA treatment also altered the rate of T cell-APC conjugate formation (Fig. 6). We speculate that PFAs may exert their effects distal from HLA class I molecules, on the expression, conformation, orientation, and/or lateral organization of adhesion molecules and costimulatory receptors involved in APC-T cell interactions (45). Surface expression levels of proteins distal from HLA class I that may modulate APC-T cell interactions could be altered by changes in gene expression or eicosanoid production. It is plausible that PUFA treatment may interfere with the formation of the immunological synapse, which would require successful engagement of both adhesion molecules and the TCR with HLA class I. Our observations on conjugate formation are qualitatively similar to those found in a study by Geyerregger et al. (12), who showed that treatment of T cells with 50 μ M EPA lowered conjugate formation with APCs, a consequence of an inhibition in synapse formation (12).


Another possible mechanism is that our JY cells may release FFAs into the medium, which could directly alter the T cells. It has been shown in endothelial cells and macrophages that lipid feeding can result in release of FFAs into the cell culture medium (46, 47). A recent study by Kleinfeld and Okada (48) showed that breast cancer tissues, but not normal tissues, release unsaturated FFAs, which inhibits T cell lysis of cancerous cells. This finding has led to the hypothesis that cancer cells may evade CTL immune recognition by releasing FFAs into surrounding tissues. It is plausible that JY cells release FFAs into the medium, which may directly suppress T cell serine esterase activity (Fig. 2D). Our future studies will address this possibility.

The PUFA-induced inhibition of HLA I trafficking points to a new way by which PFAs affect the cell biology

of antigen presentation. Only one other laboratory has reported the effects of a PUFA on protein trafficking. A recent quantitative fluorescence imaging study by the Chapkin laboratory (49) showed that DHA enrichment of mouse colonocytes selectively inhibited trafficking of lipidated Ras proteins to the plasma membrane through the secretory pathway. There are various possibilities by which PFAs could inhibit trafficking, including altering vesicle fusion, modification of COPI/COPII proteins, or alterations in HLA class I peptide loading and assembly.

Physiological implications

PFAs can display a high degree of specificity in cellular studies (4), and molecular-level studies in model membranes of ω -3 versus ω -6 PFAs show sharp differences in their effects on bilayers and proteins (50, 51). However, no significant differences in lysis, HLA class I surface levels, or conjugate formation were observed between the ω -6 AA and the ω -3 DHA. This finding is consistent with the notion that both ω -3 and ω -6 PFAs can exert immunosuppressive effects (4). Our findings also provide support for an argument that the immunosuppressive effects of ω -6 PFAs are often overlooked (52).

Finally, we speculate that a reduction in the sensitivity of PUFA-modified APCs to CTLs could affect antiviral and some autoimmune responses. Although PFAs may have potential health benefits for inflammatory and autoimmune diseases, immune suppression may also render individuals more susceptible to infection. This is an important consideration, because HLA class I molecules, unlike class II, are generally not upregulated in autoimmune or inflammatory disorders. Indeed, one major drawback identified thus far of fish oil consumption has been an increased susceptibility to pathogenic infection (53). For instance, a fish oil-feeding study in mice showed an impairment in CTL cytotoxicity upon infection with influenza virus (54). Further work is clearly required in understanding the benefits and drawbacks of dietary supplementation with PFAs. 

This work was supported by National Institutes of Health Grant AI-14584 (M.E.) and National Institutes of Health Training Grant 5T32 AI-07247.

REFERENCES

1. Simopoulos, A. P. 2002. Omega-3 fatty acids in inflammation and autoimmune diseases. *J. Am. Coll. Nutr.* **21**: 495–505.
2. Calder, P. C. 2002. Dietary modification of inflammation with lipids. *Proc. Nutr. Soc.* **61**: 345–358.
3. Calder, P. C., J. A. Bond, S. J. Bevan, S. V. Hunt, and E. A. Newsholme. 1991. Effect of fatty acids on the proliferation of concanavalin A-stimulated rat lymph node lymphocytes. *Int. J. Biochem.* **23**: 579–588.
4. Stulnig, T. M. 2003. Immunomodulation by polyunsaturated fatty acids: mechanisms and effects. *Int. Arch. Allergy Immunol.* **132**: 310–321.
5. Switzer, K. C., D. N. McMurray, and R. S. Chapkin. 2004. Effects of dietary n-3 polyunsaturated fatty acids on T-cell membrane composition and function. *Lipids.* **39**: 1163–1170.

6. Calder, P. C., and E. A. Newsholme. 1992. Polyunsaturated fatty acids suppress human peripheral blood lymphocyte proliferation and interleukin-2 production. *Clin. Sci. (Lond.)* **82**: 695–700.
7. Stulnig, T. M., M. Berger, T. Sigmund, D. Raederstorff, H. Stockinger, and W. Waldhausl. 1998. Polyunsaturated fatty acids inhibit T cell signal transduction by modification of detergent-insoluble membrane domains. *J. Cell Biol.* **143**: 637–644.
8. Stulnig, T. M., J. Huber, N. Leitinger, E.-M. Imre, P. Angelisova, P. Nowotny, and W. Waldhausl. 2001. Polyunsaturated eicosapentaenoic acid displaces proteins from membrane rafts by altering raft lipid composition. *J. Biol. Chem.* **276**: 37335–37340.
9. Zeyda, M., A. B. Szekeres, M. D. Saemann, R. Geyeregger, H. Stockinger, G. J. Zlabinger, W. Waldhausl, and T. M. Stulnig. 2003. Suppression of T cell signaling by polyunsaturated fatty acids: selectivity in inhibition of mitogen-activated protein kinase and nuclear factor activation. *J. Immunol.* **170**: 6033–6039.
10. Fan, Y.-Y., L. H. Ly, R. Barhoumi, D. N. McMurray, and R. S. Chapkin. 2004. Dietary docosahexaenoic acid suppresses T cell protein kinase C θ lipid raft recruitment and IL-2 production. *J. Immunol.* **173**: 6151–6160.
11. Webb, Y., L. Hermida-Matsumoto, and M. D. Resh. 2000. Inhibition of protein palmitoylation, raft localization, and T cell signaling by 2-bromopalmitate and polyunsaturated fatty acids. *J. Biol. Chem.* **275**: 261–270.
12. Geyeregger, R., M. Zeyda, G. J. Zlabinger, W. Waldhausl, and T. M. Stulnig. 2005. Polyunsaturated fatty acids interfere with formation of the immunological synapse. *J. Leukoc. Biol.* **77**: 680–688.
13. Hughes, D. A., and A. C. Pinder. 2000. n-3 Polyunsaturated fatty acids inhibit the antigen-presenting function of human monocytes. *Am. J. Clin. Nutr.* **71** (Suppl.): 357–360.
14. Sanderson, P., G. G. MacPherson, C. H. Jenkins, and P. C. Calder. 1997. Dietary fish oil diminishes the antigen presentation activity of rat dendritic cells. *J. Leukoc. Biol.* **62**: 771–777.
15. Calder, P. C., and R. B. Zurier. 2001. Polyunsaturated fatty acids and rheumatoid arthritis. *Curr. Opin. Clin. Nutr. Metab. Care.* **4**: 115–121.
16. Pascale, A. W., W. D. Ehringer, W. Stillwell, L. K. Sturdevant, and L. J. Jenks. 1993. Omega-3 fatty acid modification of membrane structure and function. II. Alteration by docosahexaenoic acid of tumor cell sensitivity to immune cytotoxicity. *Nutr. Cancer.* **19**: 147–157.
17. Jenks, L. J., L. K. Sturdevant, W. D. Ehringer, and W. Stillwell. 1993. Omega-3 fatty acid modification of membrane structure and function. I. Dietary manipulation of tumor cell susceptibility to cell- and complement-mediated lysis. *Nutr. Cancer.* **19**: 135–146.
18. Terhorst, C., P. Parham, D. L. Mann, and J. L. Strominger. 1976. Structure of HLA antigens: amino-acid and carbohydrate compositions and NH₂-terminal sequences of four antigen preparations. *Proc. Natl. Acad. Sci. USA.* **73**: 910–914.
19. Diaz, O., A. Berquand, M. Dubois, S. Di Agostino, C. Sette, S. Bourgoin, M. Lagarde, G. Nemoz, and A.-F. Prigent. 2002. The mechanism of docosahexaenoic acid-induced phospholipase D activation in human lymphocytes involves exclusion of the enzyme from lipid rafts. *J. Biol. Chem.* **277**: 39368–39378.
20. Khair-el-Din, T. A., S. C. Sicher, M. A. Vazquez, W. J. Wright, and C. Y. Lu. 1995. Docosahexaenoic acid, a major constituent of fetal serum and fish oil diets, inhibits IFN gamma-induced Ia-expression by murine macrophages in vitro. *J. Immunol.* **154**: 1296–1306.
21. Muller, C. P., D. A. Stephany, M. Shinitzky, and J. R. Wunderlich. 1983. Changes in cell-surface expression of MHC and Thy-1.2 determinants following treatment with lipid modulating agents. *J. Immunol.* **131**: 1356–1362.
22. Zahabi, A., and C. F. Deschepper. 2001. Long-chain fatty acids modify hypertrophic responses of cultured primary neonatal cardiomyocytes. *J. Lipid Res.* **42**: 1325–1330.
23. Bligh, E. G., and W. J. Dyer. 1959. A rapid method of total lipid extraction and purification. *Can. J. Biochem. Physiol.* **37**: 911–917.
24. Butler, M., N. Huzel, and N. Barnabe. 1997. Unsaturated fatty acids enhance cell yields and perturb the energy metabolism of an antibody-secreting hybridoma. *Biochem. J.* **322**: 615–623.
25. Matko, J., and P. Nagy. 1997. Fluorescent lipid probes 12-AS and TMA-DPH report on selective, purinergically induced fluidity changes in plasma membranes of lymphoid cells. *J. Photochem. Photobiol. B.* **40**: 120–125.
26. Shinitzky, M., and M. Inbar. 1974. Difference in microviscosity induced by different cholesterol levels in the surface membrane lipid layer of normal lymphocytes and malignant lymphoma cells. *J. Mol. Biol.* **85**: 603–615.
27. Schreiber, A. B., J. Schlessinger, and M. Edidin. 1984. Interaction between major histocompatibility complex antigens and epidermal growth factor receptors on human cells. *J. Cell Biol.* **98**: 725–731.
28. Brodsky, F. M., P. Parham, C. J. Barnstable, M. J. Crumpton, and W. F. Bodmer. 1979. Monoclonal antibodies for analysis of the HLA system. *Immunol. Rev.* **47**: 3–61.
29. Kwik, J., S. Boyle, D. Fooksman, L. Margolis, M. P. Sheetz, and M. Edidin. 2003. Membrane cholesterol, lateral mobility, and the phosphatidylinositol 4,5-bisphosphate-dependent organization of cell actin. *Proc. Natl. Acad. Sci. USA.* **100**: 13964–13969.
30. Hammond, A. T., and B. S. Glick. 2000. Dynamics of transitional endoplasmic reticulum sites in vertebrate cells. *Mol. Biol. Cell.* **11**: 3013–3030.
31. Pepperkok, R., J. Scheel, H. Horstmann, H. P. Hauri, G. Griffiths, and T. E. Kreis. 1993. Beta-COP is essential for biosynthetic membrane transport from the endoplasmic reticulum to the Golgi complex in vivo. *Cell.* **74**: 71–82.
32. Wilson, P. R., H. M. Kronenberg, S. Melmed, and K. S. Polonsky. 2003. Williams: Textbook of Endocrinology. 8th edition. Saunders, Philadelphia, PA.
33. Potter, B. J., D. Sorrentino, and P. D. Berk. 1989. Mechanisms of cellular uptake of free fatty acids. *Annu. Rev. Nutr.* **9**: 253–270.
34. Siddiqui, R. A., S. R. Shaikh, L. A. Sech, H. R. Yount, W. Stillwell, and G. P. Zaloga. 2004. Omega 3-fatty acids: health benefits and cellular mechanisms of action. *Mini Rev. Med. Chem.* **4**: 859–871.
35. Berlin, E., J. S. Hannah, K. Yamane, R. C. Peters, and B. V. Howard. 1996. Fatty acid modification of membrane fluidity in Chinese hamster ovary (TR715-19) cells. *Int. J. Biochem. Cell Biol.* **28**: 1131–1139.
36. Kwik, J. 2000. Immobilization of MHC class I molecules enhances target cell lysis by CD8⁺ T cells. PhD Dissertation. Johns Hopkins University, Baltimore, MD.
37. Zeyda, M., M. D. Saemann, K. M. Stuhlmeier, D. G. Mascher, P. N. Nowotny, G. J. Zlabinger, W. Waldhausl, and T. M. Stulnig. 2005. Polyunsaturated fatty acids block dendritic cell activation and function independently of NF- κ B activation. *J. Biol. Chem.* **280**: 14293–14301.
38. Bodnar, A., Z. Bacso, A. Jenei, T. M. Jovin, M. Edidin, S. Danjanovich, and J. Matko. 2003. Class I HLA oligomerization at the surface of B cells is controlled by exogenous beta(2)-microglobulin: implications in activation of cytotoxic T lymphocytes. *Int. Immunol.* **15**: 331–339.
39. Fooksman, D. R., G. K. Gronvall, Q. Tang, and M. Edidin. 2006. Clustering class I MHC modulates sensitivity of T cell recognition. *J. Immunol.* **176**: 6673–6680.
40. Fujikawa, M., N. Yamashita, K. Yamazaki, E. Sugiyama, H. Suzuki, and T. Hamazaki. 1992. Eicosapentaenoic acid inhibits antigen-presenting cell function of murine splenocytes. *Immunology.* **75**: 330–335.
41. Huang, S. C., M. L. Misfeldt, and K. L. Fritsche. 1992. Dietary fat influences Ia antigen expression and immune cell populations in the murine peritoneum and spleen. *J. Nutr.* **122**: 1219–1231.
42. Chakraborty, D., S. Banerjee, A. Sen, K. K. Banerjee, P. Das, and S. Roy. 2005. Leishmania donovani affects antigen presentation of macrophage by disrupting lipid rafts. *J. Immunol.* **175**: 3214–3224.
43. Muller, C. P., and G. R. Krueger. 1986. Modulation of membrane proteins by vertical phase separation and membrane lipid fluidity. Basis for a new approach to tumor immunotherapy. *Anticancer Res.* **6**: 1181–1193.
44. Listenberger, L. L., X. Han, S. E. Lewis, S. Cases, R. V. Farese, Jr., D. S. Ory, and J. E. Schaffer. 2003. Triglyceride accumulation protects against fatty acid-induced lipotoxicity. *Proc. Natl. Acad. Sci. USA.* **100**: 3077–3082.
45. Shaikh, S. R., and M. Edidin. Polyunsaturated fatty acids, membrane organization, T cells, and antigen presentation. *Am. J. Clin. Nutr.* Epub ahead of print. October 30, 2006.
46. Figard, P. H., D. P. Hejlik, T. L. Kaduce, L. L. Stoll, and A. A. Spector. 1986. Free fatty acid release from endothelial cells. *J. Lipid Res.* **27**: 771–780.
47. von Hodenberg, E., J. C. Khoo, D. Jensen, J. L. Witztum, and D. Steinberg. 1984. Mobilization of stored triglycerides from macrophages as free fatty acids. *Arteriosclerosis.* **4**: 630–635.

48. Kleinfeld, A. M., and C. Okada. 2005. Free fatty acid release from human breast cancer tissue inhibits cytotoxic T-lymphocyte-mediated killing. *J. Lipid Res.* **46**: 1983–1990.
49. Seo, J., R. Barhoumi, A. E. Johnson, J. R. Lupton, and R. S. Chapkin. 2006. Docosahexaenoic acid selectively inhibits plasma membrane targeting of lipidated proteins. *FASEB J.* **176**: 770–772.
50. Eldho, N. V., S. E. Feller, S. Tristram-Nagle, I. V. Polozov, and K. Gawrisch. 2003. Polyunsaturated docosahexaenoic vs docosapentaenoic acid-differences in lipid matrix properties from the loss of one double bond. *J. Am. Chem. Soc.* **125**: 6409–6421.
51. Rajamoorthi, K., H. I. Petrache, T. J. McIntosh, and M. F. Brown. 2005. Packing and viscoelasticity of polyunsaturated omega-3 and omega-6 lipid bilayers as seen by ^2H NMR and X-ray diffraction. *J. Am. Chem. Soc.* **127**: 1576–1588.
52. Sacks, F. M., and H. Campos. 2006. Polyunsaturated fatty acids, inflammation, and cardiovascular disease: time to widen our view of the mechanisms. *J. Clin. Endocrinol. Metab.* **91**: 398–400.
53. Anderson, M., and K. L. Fritsche. 2002. (n-3) Fatty acids and infectious disease resistance. *J. Nutr.* **132**: 3566–3576.
54. Byleveld, M., G. T. Pang, R. L. Clancy, and D. C. Roberts. 2000. Fish oil feeding enhances lymphocyte proliferation but impairs virus-specific T lymphocyte cytotoxicity in mice following challenge with influenza virus. *Clin. Exp. Immunol.* **119**: 287–292.