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ORIGINAL CONTRIBUTION

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Polyunsaturated eicosapentaenoic acid changes lipid composition in lipid rafts

Summary *Background* Polyunsaturated fatty acids (PUFAs) modulate immune responses particularly by affecting T cell function and are applied clinically as adjuvant immunosuppressants in the treatment of various inflammatory diseases. However, the molecular mechanisms of PUFA-induced immunosuppressive effects are not yet elucidated. Membrane lipid rafts are functional plasma membrane microdomains characterized by a unique lipid environment. Since lipid interactions are crucial for the formation of lipid rafts, the immunomodulatory effects of PUFAs may be due to changes of fatty acid composition in lipid rafts. *Aim of the study* We investigated the effects of eicosapentaenoic acid (EPA, 20:5 n-3) supplementation on modulating lipid composition and fatty acyl substitution in their cytoplasmic and exoplasmic lipid leaflet in lipid rafts. *Methods* The human Jurkat E6-1 T cells were cultured in EPA-supplemented medium and the cells treated with stearic acid served as a control. Lipid rafts were isolated by discontinuous sucrose density gradient ultracentrifugation. The lipids in raft and soluble fractions from EPA-treated and control T cells were extracted and separated by gas chromatography. Raft phospholipids were analyzed by mass spectrometry. *Results* Our results showed that EPA treatment could alter lipid composition resulting in a considerable increase of unsaturated fatty acyl chains in lipid rafts from EPA-treated T cells compared with control cells. Effective incorporation of EPA to rafts was not only in the exoplasmic but also in the cytoplasmic membrane lipid leaflet. EPA treatment altered the lipid environment in lipid rafts. EPA presented an inhibiting effect on Jurkat T cells proliferation and inhibited IL-2Rα expression on the surface of T cells. *Conclusions* Our data provided evidence for an important modification in lipid composition of membrane lipid rafts and T cell function by EPA supplementation.

Key words polyunsaturated fatty acids – eicosapentaenoic acid – lipid rafts – lipid composition

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Abbreviations

PUFAs	polyunsaturated fatty acids
EPA	eicosapentaenoic acid
PC	phosphatidylcholine
PE	phosphatidylethanolamine
PS	phosphatidylserine
PI	phosphatidylinositol
SM	sphingomyelin

Introduction

Polyunsaturated fatty acids (PUFAs) can modulate immune responses and exert beneficial effects in a variety of inflammatory diseases. Because of the immunosuppressive function of PUFAs, they are used clinically as immunosuppressive agents and have clinical effects on rheumatoid arthritis, inflammatory bowel disease, sepsis and systemic inflammatory response syndrome [1, 2]. Recent research shows that n-3 PUFAs play important

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roles in immune response particularly by modification of T cell signaling function [3]. Human and animal studies also suggest that n-3 PUFAs have great immunomodulatory actions *in vivo* [4, 5]. Moreover, their presence in cell membrane phospholipids can increase membrane fluidity and regulates T lymphocyte proliferation [6, 7]. However, the molecular alterations of the immunosuppressive effects have not yet been elucidated in detail.

Membrane lipid rafts have been identified as distinct membrane subdomains, which are insoluble in nonionic detergents and can be isolated as detergent-resistant membrane subdomains. It is reported that n-3 PUFAs are incorporated into lipids of the cytoplasmic leaflet in rafts and can change the lipid composition of rafts, which would potentially affect the function of the lipid rafts [8, 9]. Meanwhile, a large number of signaling proteins are concentrated within lipid rafts, which can serve as signaling platforms to facilitate efficient and specific signal transduction in living cells [10]. Some results have also indicated that several proteins involved in signaling pathway via T-cell receptor (TCR), B-cell receptor (BCR), IgE receptor (FcεRI) are within lipid raft compartments [11–13]. In general, PUFAs supplementation inhibits T lymphocyte activation by modifying detergent-insoluble membrane domains and displacing these signaling proteins from lipid rafts [14, 15].

Lipid interactions are very important for the formation of lipid rafts. These effects are hypothesized to be mediated by modification of raft structure and composition. In our study, eicosapentaenoic acid (EPA (20:5, *n*-3)), one of the n-3 series PUFAs, was used to treat Jurkat T cells. We isolated the detergent-soluble and detergent-insoluble fractions. The alteration of lipid compositions in rafts of EPA-treated T cells was observed. It was demonstrated that treatment of Jurkat T cells with EPA considerably increased the lipid unsaturation in rafts by increasing the level of n-3 PUFAs. EPA treatment also altered the lipid environment in their cytoplasmic and exoplasmic lipid leaflet in lipid rafts. Thus, our data provide strong evidence for a functional modification of lipid rafts by EPA treatment and explain PUFA-mediated immunosuppressive effects.

Materials and methods

Materials

Cis-5,8,11,14,17-eicosapentaenoic acid (EPA (20:5, *n*-3)) and stearic acid (18:0) were purchased from Sigma-Aldrich Inc. (St. Louis, MO). All other chemicals were also from Sigma unless stated otherwise. RPMI 1640 medium, bovine calf serum, and serum-free Iscove's modified Dulbecco's medium were all from Invitrogen Inc. (Grand Island, NY). Bovine serum albumin (fraction V), protease inhibitor cocktail tablets were obtained from Roche Diagnostic Inc. (Indianapolis, IN).

Cell culture and fatty acid treatment

The human T cell line Jurkat E6-1 (American Type Culture Collection, Manassas, VA) was grown under standard conditions in RPMI 1640 medium, supplemented with 10 % heat-inactivated bovine calf serum, penicillin/streptomycin (100 U/ml and 100 μg/ml, respectively) at 37 °C with 5 % CO_2. For modification of cellular lipids, the cells were cultured for 2 days in serum-free Iscove's modified Dulbecco's medium (no fatty acid detectable), supplemented with 0.4 % (w/v) bovine serum albumin (fraction V, containing less than 3 μmol/l total fatty acids), with addition of 50 μmol/l of either EPA (20:5 *n*-3) or stearic acid (18:0) from stock solutions in ethanol (final concentration ≤ 0.5 %). Stearic acid served as a control and previously this fatty acid was shown not to influence cellular PUFA content and membrane subdomain distribution of proteins compared with controls treated with vehicle only [14]. After having been treated with EPA for 2 days, cells were pelleted and lysed.

Cell proliferation assay

Jurkat T cells (3.5×10^4 per well) were cultured in 96-well microtiter plates in RPMI 1640 medium, supplemented with 10 % heat-inactivated bovine calf serum, penicillin/streptomycin (100 U/ml and 100 μg/ml, respectively) in the presence of PHA (0.5 μg/ml) at 37 °C in humidified air with 5 % CO_2. Cells were treated for 48 h with EPA (12.5, 25, 50, 75, 100, 150 μmol/l) in serum-free Iscove's modified Dulbecco's medium, supplemented with 0.4 % (w/v) bovine serum albumin (fraction V). ^{3}H-thymidine (0.8 μCi per well) was added and then the cells were incubated for 8 h. Subsequently, the cells were harvested onto glass fiber paper discs (Whatman, Maidstone, England). The filter discs were transferred into scintillation vials, dried overnight, and covered with 4 ml/vial scintillation fluid. The radioactivity was recorded and analyzed by liquid scintillation counter (Beckman-Coulter). Cell proliferation was assessed by ^{3}H-thymidine uptake.

Flow cytometry

The cells were grown in RPMI 1640 medium, supplemented with 10 % heat-inactivated bovine calf serum, penicillin/streptomycin (100 U/ml and 100 μg/ml, respectively) at 37 °C with 5 % CO_2 and then were cultured for 2 days in serum-free Iscove's modified Dulbecco's medium, supplemented with 0.4 % (w/v) bovine serum

albumin (fraction V), with addition of 5, 12.5, 25, 50, 75 μmol/l of EPA from stock solutions in ethanol. After having been stimulated with 400 U/ml IL-2 for 30 min, the cells were washed twice with 0.01 mol/l PBS buffer (pH 7.2) and suspended with 50 μl of PBS buffer, and incubated with FITC-conjugated mouse anti-human CD25 (IL-2Rα) mAbs in the dark at room temperature for 20 min. The cells of negative control were incubated with isotype-matched non-reactive mouse IgG_1 antibody. Finally, the cells were washed twice in PBS and assessed by flow cytometry on FACScalibur (Becton Dickinson). Data were then analyzed with the CellQuest software. CD25 expression was assessed as a percentage of cells exceeding background fluorescence determined using isotype-matched control.

Isolation of lipid rafts

Lipid rafts were isolated from Jurkat T cells as previously described [14] by discontinuous sucrose density gradient ultracentrifugation. 2.5×10^7 cells per ml were washed in Hanks' balanced salt solution three times and lysed in TKM buffer (50 mmol/l Tris, pH 7.4, 25 mmol/l KCl, 5 mmol/l $MgCl_2$, and 1 mmol/l EDTA) containing 1% Brij58 and protease inhibitor cocktail tablets (0.12 mg antipain-HCl, 20 μg bestatin, 40 μg chymostatin, 0.12 mg E-64, 20 μg leupeptin, 20 μg pepstatin, 0.12 mg phosphoramidon, 0.8 mg pefabloc, 40 μg aprotinin). Lysates were incubated on ice for 30 min, mixed with an equal volume of 80% sucrose in TKM buffer, and overlaid with 5.5 ml of 36% sucrose followed by 2.5 ml of 5% sucrose. The gradients were subjected to ultracentrifugation at 250,000 × *g* at 4 °C for 18 h with a 90 Ti rotor in an Optima L-80XP ultracentrifuge (Beckman Coulter Inc. Fullerton, CA). The 1 ml fraction was collected from the top of the gradients one by one.

Fatty acid analysis

The raft and soluble fractions from EPA-treated and control T cells were freeze-dried before adding 1 ml of boron trifluoride-diethyl ether/methanol, 1:3 (v/v), including 10 μg heptadecanoic acid, which served as the internal standard. Methanolysis was performed by incubating at 75 °C for 30 min. After cooled down, 0.5 ml of water and 1.5 ml of n-hexane were added, then subjected to centrifugation at 2,000 × *g* for 15 min. The organic phase was collected and dried by N_2 and dissolved in 25 μl of chloroform, 2 μl of which was injected and analyzed by gas chromatography on a HP4890 system (Agilent Technologies, Palo Alto, CA) equipped with flame ionization detector, HP 3398A work station and with a HP-FFAP quartz capillary column (30 m length, 0.32 mm inner diam., 0.25 μm layer thickness). Gas chromatography was operated at 110 °C, 8 °C/min for 10 min, went up at 6 °C/min to reach 230 °C for 8 min, at 6 °C/min to 250 °C for 3 min with constant flow (10 psi) of high pure N_2. The amount of fatty acid was expressed as mol %. The significance of differences in fatty acids between raft and soluble fractions or EPA- and control-treatment T cells were calculated by the paired Student's *t* test.

Mass spectrometry for raft phospholipid analysis

Total lipids in rafts from EPA-treated and control T cells were extracted. 1 ml of methanol containing 0.9% butylated hydroxytoluene with internal controls (1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) and 1,2-dimyristoyl-sn-glycero-3-phosphoethanolamine (DMPE)) were added to the sample and mixed. 4 ml of chloroform was added and stirred with addition of 1 ml methanol. The final chloroform/methanol was 2:1 and 1 ml of 50 mmol/l KCl was added, mixed and centrifuged at 2,000 × *g* for 15 min. The organic phase was collected, filtered by organic filters and evaporated-dried at 38 °C.

An HP1100 series of HPLC system (Agilent Technologies, Palo Alto, CA) was used. The LC separation was performed on a diol column (Nucleosil, 100-5 OH, Germany), 250 (mm) × 3.9 (mm, i. d.) × 5.0 (μm, particle size). The total flow rate was 0.4 ml/min. The column temperature was at 35 °C. Solvent mixture A: hexane/1-propanol/formic acid/ammonia (79/20/0.6/0.07, v/v); Solvent mixture B: 1-propanol/water/formic acid/ammonia (88/10/0.6/0.07, v/v). The mass spectrometric detection was performed on a QTRAP LC/MS/MS system from Applied Biosystems/MDS Sciex equipped with a turbo ion-spray source.

The split HPLC effluent entered the MS through a steel ES ionization needle set at 5,500 V (in positive ion mode) or 4,500 V (in negative ion mode) and a heated capillary was set to 250 °C. The ion source and ion optic parameters were optimized with respect to the positive or negative molecular related ions of the phospholipid standards. The flow rate of N_2 drying gas and turbo gas was 40 psi. The declustering potential (DP) was set at 80 psi. The other parameters as follows: EMS as survey scan (mass range *m*/*z* 450–950, scan speed 1000 Da S^{-1}, trap time 20 ms) and EPI as dependent scan (scan speed 1000 Da S^{-1}, trap time 150 ms, collision energy set at +35 eV in the positive-ion mode and –40 eV in the negative-ion mode).

Results

T cells proliferation assay

To determine whether EPA interferes with T lymphocyte activation, we investigated the effect of EPA on PHA-me-

diated T cell proliferation. Jurkat T cells were stimulated by PHA and cultured with various concentrations of EPA for 48 h. As shown in Fig. 1, ^{3}H-thymidine incorporation with Jurkat T cells was significantly reduced by EPA treatment at 50, 75, 100, 150 μmol/l concentrations compared with the control. T cells treated with 50, 75, 100, 150 μmol/l EPA exhibited a significantly lower level of proliferation relative to control cells ($P < 0.05$). The other proliferative responses treated by 12.5, 25 μmol/l EPA were not significantly different from the control ($P > 0.05$). Therefore, EPA presented a suppressing effect on Jurkat T-cell proliferation.

EPA suppresses CD25 expression on the surface of T cells

The IL-2R in T cells is mostly mediated by induction of α chain (CD25) during activation, allowing-high affinity binding (CD25). In order to determine the effect of EPA on IL-2R expression in membrane lipid rafts of T cells, we initially detected IL-2Rα (CD25) expression on the surface of T cells by FACS analysis. After IL-2 stimulation, the positive IL-2Rα (CD25) expression cells was 39.53% in control (18:0) (Fig. 2). For Jurkat T cells treated with different levels of polyunsaturated eicosapentaenoic acid (5, 12.5, 25, 50, 75 μmol/l), the positive CD25 expression cells were 36.12, 31.30, 23.59, 16.67, 11.65%, respectively (Fig. 2). EPA inhibited IL-2Rα expression on the surface of T cells.

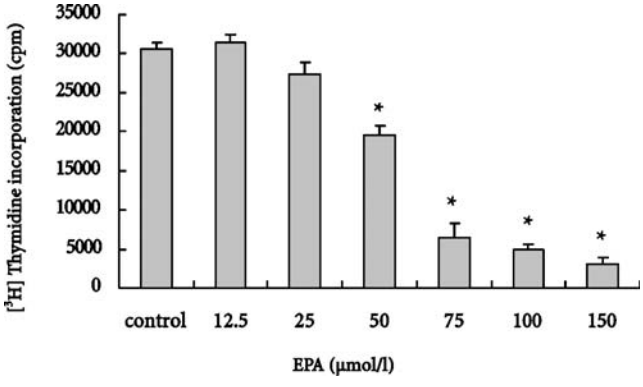

Fig. 1 Effect of EPA on the Jurkat T cells proliferation. The cells (3.5 x 10^4 per well) were cultured in the presence of PHA (0.5 μg/ml), with addition of various concentrations of EPA for 48 h and pulsed with ^{3}H thymidine, then harvested and the radioactivity of the thymidine incorporated into DNA was determined by a liquid scintillation counter as described in the Materials and methods. Values were expressed as mean ± SEM using triplicated determinations. (*) indicate $P < 0.05$ as compared with the control

EPA treatment changes fatty acid composition in raft and soluble membrane fractions

In order to investigate altered fatty acid composition in lipid rafts we isolated lipid rafts from EPA (20:5)-treated and control Jurkat T cells and analyzed fatty acyl composition. The concentration of saturated palmitic acid (16:0) was increased in rafts from EPA-treated cells compared with control cells (Table 1). The saturated stearic acid (18:0), monounsaturated oleic acid (18:1 *n*-9) and *cis*-oleic acid (*cis*-18:1 *n*-9) were markedly decreased in rafts of EPA-treated cells compared with those of control cells. EPA (20:5 *n*-3) were markedly enriched in rafts and even more its elongation product docosapentaenoic acid (22:5 *n*-3) were highly enriched in rafts from EPA-treated cells compared with control T cells. PUFAs, n-3 PUFAs and the ratio of n-3 PUFAs to n-6 PUFAs were considerably increased in rafts from EPA-treated T cells compared with control cells. EPA treatment increased the relative amount of n-3 polyunsaturated fatty acids in rafts of T cells and altered the lipid environment of membrane subdomains, which is very likely to influence the fluidity of these subdomains.

EPA treatment alters acyl substitution of raft lipid

We further analyzed fatty acyl composition of different phospholipids including phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI) in lipid rafts and the phospholipid species were identified by QTRAP LC/MS/MS system (Table 2). EPA treatment significantly increased the concentrations of PC species substituted with 36:3 (16:0/20:3), 36:4 (16:0/20:4), 38:6 (18:1/20:5, 16:0/22:6, 18:2/20:4), 38:5 (18:1/20:4, 18:2/20:3, 16:0/22:5) and 40:6 (18:1/22:5, 18:0/22:6, 20:3/20:3, 20:2/20:4) acyl chains in rafts from EPA-treated T cells compared with control cells. There were no significant differences for acyl chain substitution of sphingomyelin species (data not show). In EPA-treated cells, PE species substituted with 36:5 (16:0/20:5), 36:5 (16:1/20:4), 38:6 (16:0/22:6, 18:2/20:4), 38:5 (18:0/20:5, 16:0/22:5, 18:1/20:4), 40:7 (18:1/22:6), 40:6 (20:2/20:4, 18:2/22:4, 18:0/22:6, 18:1/22:5), 40:5 (18:0/22:5, 18:1/22:4, 18:2/22:3, 20:1/20:4) acyl chains were significantly enriched in lipid rafts compared with control cells. The concentrations of PS species substituted with 38:4 (18:0/20:4), 38:6 (18:0/22:6) and 40:5 (18:0/22:5) acyl chains were increased. PI species substituted with 36:4 (16:0/20:4), 36:1 (18:0/18:1), 38:5 (18:1/20:4), 40:6 (18:0/22:6), 40:5 (18:0/22:5) acyl chains were abundant in rafts of EPA-treated cells compared with control cells. EPA treatment altered unsaturated lipid environment and fatty acyl composition of phospholipids in lipid rafts.

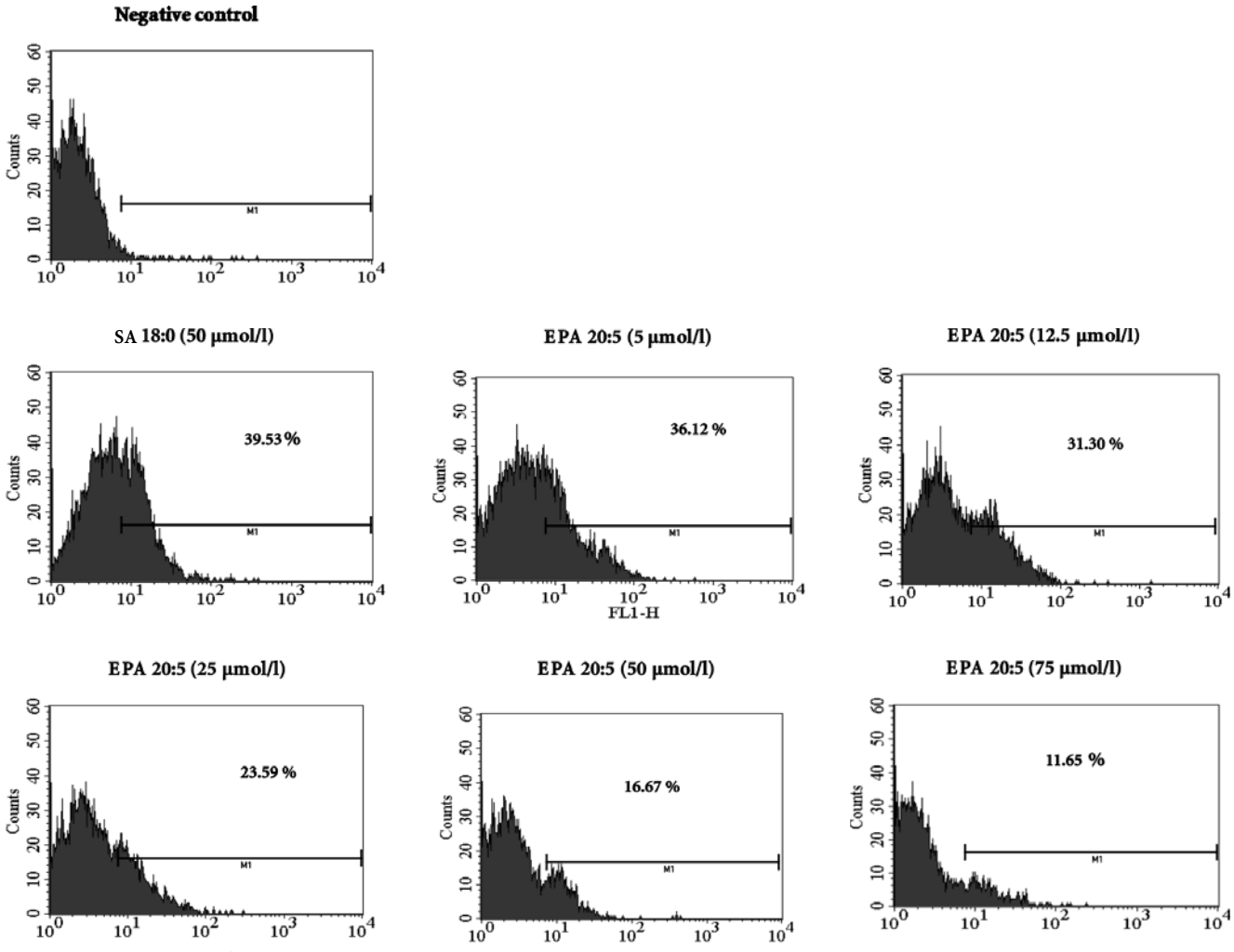

Fig. 2 Effect of EPA on CD25 expression on the surface of Jurkat T cells. The cells cultured in the absence (18:0) or presence of EPA (5, 12.5, 25, 50, 75 µmol/l) for 48 hours. After having been stimulated with 400 U/ml IL-2 for 30 min, cells were washed and stained with FITC-labeled anti-CD25 or IgG_1 isotype control. T cells were analyzed by FACS analysis

Discussion

The polyunsaturated EPA is considered as one of the primary effecting molecules on inflammatory diseases, which show great modulations on the immune response most probably by altering raft lipid environment. To date, it has not been clearly elucidated whether EPA could change fatty acyl composition and lead to alteration of the unsaturated lipid environment in the raft lipid. In this study, we confirmed that EPA could mediate suppression of T-cell proliferation (Fig. 1) and suppress IL-2Rα (CD25) expression on the surface of T cells by flow cytometry (Fig. 2). Our results indicate consistently that EPA resulted in downregulation of IL-2Rα (CD25) expression on the surface of T cells and T-cell proliferation. We analyzed fatty acid composition and fatty acyl substitution of phospholipids in rafts and soluble membrane fractions of EPA-treated and control T cells. It was demonstrated that EPA treatment could alter raft lipid composition. The *n*-3 PUFAs (eicosapentaenoic acid 20:5; docosapentaenoic acid 22:5) were significantly enriched in raft fractions compared with those of control cells (Table 1). Distinct saturated fatty acids, e. g. palmitic acid (16:0), were also enriched in raft fractions of EPA-treated cells compared with control cells. However, stearic acid (18:0), monounsaturated oleic acid (18:1 *n*-9) and *cis*-oleic acid (*cis*-18:1 *n*-9) were markedly decreased in rafts of EPA-treated cells. Addition of EPA markedly increased their proportion in parallel with a decrease of monounsaturated fatty acids. This partial lipid alteration indicated that EPA-treated cells aimed at maintaining lipid composition in rafts by changing a saturated or monosaturated fatty environ-

Table 1 Fatty acid composition in raft and soluble fractions from control and EPA treated T cells

Fatty acids	18:0		20:5 (n–3)		20:5 vs 0.18:0	
	Raft	Soluble	Rafts	Soluble	Rafts	Soluble
10:0	0.14±0.20	0.27±0.08	0.66±0.19	0.00±0.00		**[d]
12:0	1.95±0.08**[a]	2.85±0.08	1.70±0.28	2.02±0.23		**
14:0	1.81±0.78***	11.00±1.5	3.76±0.16**[b]	6.88±0.20		***
16:0	18.65±0.47***	28.35±1.13	23.96±1.54***	29.72±0.82	**[c]	
16:1 (*n*-7)	3.08±1.54	11.66±5.53	3.33±1.33	5.35±0.48		
18:0	21.18±4.81**	9.46±3.38	11.33±1.60	9.82±0.12	**	
18:1 (*n*-9)	29.08±0.06***	16.73±1.13	15.25±0.34	14.79±1.66	**	
cis–18:1 (*n*-9)	6.53±0.09***	1.54±0.67	4.22±0.44**	2.22±0.57	***	
18:2 (*n*-6)	1.89±0.03**	8.16±1.57	2.38±0.52***	6.49±0.22		**
18:3 (*n*-6)	0.00±0.00	0.00±0.0	0.05±0.03**	0.33±0.06		**
18:3 (*n*-3)	0.03±0.04	0.26±0.25	0.00±0.00	0.00±0.00		
20:0	0.30±0.27	1.86±0.81	0.18±0.06**	0.30±0.01		
20:2 (*n*-6)	1.03±0.07***	0.00±0.00	1.19±0.78**	4.10±0.03		***
20:3 (*n*-6)	6.66±3.34	3.09±1.30	6.85±5.97	5.94±0.38		***
20:3 (*n*-3)	0.06±0.01	0.00±0.00	0.00±0.00	0.50±0.27		
20:4 (*n*-6)	6.25±0.61***	1.80±0.66	2.50±0.17***	1.41±0.09	***	
22:0	0.10±0.04	0.00±0.00	0.00±0.00	0.00±0.00		
20:5 (*n*-3)	0.58±0.19	0.00±0.00	13.32±1.14***	3.74±0.18	***	***
22:5 (*n*-3)	0.30±0.12**	0.68±0.01	13.45±4.79**	4.30±0.66	***	***
22:6 (*n*-3)	0.31±0.14*	2.96±1.42	0.87±0.39**	2.11±0.70		
Saturated fatty acid	44.13±2.62**	53.82±0.95	41.58±0.32***	48.74±0.46		**
Monounsaturated fatty acids	38.70±0.98**	29.93±3.74	22.80±2.11	22.36±0.43	***	*
PUFAs	16.74±5.40	16.95±4.69	40.61±3.26**	28.91±0.03	***	**
∑(*n*-3)PUFA	1.28±0.35***	3.22±1.16	27.64±3.26***	10.66±0.59	***	***
∑(*n*-6)PUFA	15.83±2.11	13.04±3.52	12.97±10.63	18.26±0.55		
(*n*-3)/(*n*-6)	0.08±0.03***	0.24±0.02	2.13±1.8	0.58±0.05	*	***

Jurkat T cells were treated with EPA (20:5 *n*-3) or stearic aicd (18:0) as control and lipid rafts were isolated by discontinuous sucrose density gradient ultracentrifugation. Fatty acid composition was analyzed from three experiments and expressed in mol % (mean ± S.E)

[a] indicates the significant differences in rafts compared with soluble fractions of control-treated cells (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

[b] indicates the significant differences in rafts compared with soluble fractions of EPA-treated cells as detailed in footnote [a]

[c] indicates the significant differences between rafts of 20:5 (*n*-3) versus control-treated T cells as detailed in footnote [a]

[d] indicates the significant differences between soluble fractions of 20:5 (*n*-3) versus control-treated T cells as detailed in footnote [a]

ment. Unfortunately, this compensatory mechanism is still unclear. In addition, more original EPA supplementation was elongated to docosahexaenoic acid 22:5, which can be synthesized from EPA by T cells. Conclusively, PUFAs are effectively associated with raft lipids and change the lipid environment in rafts of T cells.

It was already known that EPA treatment of T cells resulted in enrichment of PUFAs in the cytoplasmic leaflet as well as the exoplasmic leaflet of rafts [8]. They analyzed substitution of PC and SM, which typically locate in the exoplasmic membrane lipid leaflet. But in the cytoplasmic lipid leaflet they only assessed the effects of EPA treatment on substitution of PE species. Alteration of lipids (PI, PS) from the cytoplasmic leaflet is also of particular importance. Therefore, we analyzed effective incorporation of EPA into raft lipids not only in the exoplasmic (PC, SM) but also in the cytoplasmic membrane lipid leaflet (PE, PS, PI) (Table 2). Alteration of fatty acyl composition in exoplasmic leaflet lipids (PC and SM) could indirectly affect the displacement of proteins from lipid rafts by interdigitating fatty acyl moieties [8]. In the present study, EPA treatment greatly changes fatty acyl composition from the cytoplasmic leaflet (PE, PS, and PI) in rafts. Thus, we could easily make the conclusion that alteration of fatty acyl chains in the cytoplasmic as well as in the exoplasmic lipid leaflet of rafts from EPA-treated cells plays an important role in maintaining raft structure and its lipid environment.

In conclusion, our results suggested that EPA presented a marked inhibiting effect on Jurkat cells proliferation and IL-2Rα expression on the surface of T cells.

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Table 2 Fatty acid composition for species in raft and soluble fractions from control (18:0) and PUFA (20:5 n–3)-treated T cells

m/z	Fatty acid composition	18:0	20:5 (n-3)
	phosphatidylcholine		
764	36:3 (16:0/20:3)	0.75±0.18	3.40±0.21***
766	36:4 (16:0/20:4)	1.10±0.31	1.60±0.04*
790	38:6 (18:1/20:5, 16:0/22:6, 18:2/20:4)	0.96±0.33	2.11±0.43*
792	38:5 (18:1/20:4, 18:2/20:3, 16:0/22:5)	0.90±0.36	4.45±0.56***
794	38:4 (18:0/20:4, 18:1/20:3, 18:2/20:2)	1.43±0.62	1.68±0.39
818	40:6 (18:1/22:5, 18:0/22:6, 20:3/20:3, 20:2/20:4)	1.24±069	2.48±0.92*
820	40:5 (18:0/22:5)	0.98±0.34	1.54±0.23
	phosphatidylethanolamine		
736	36:5 (16:1/20:4)	2.56±0.58	4.96±0.33**
738	36:4 (16:1/20:3, 16:0/20:4)	3.76±1.77	3.16±0.18
740	36:3 (16:0/20:3)	6.00±1.56	3.74±0.39
744	36:1 (16:0/20:1)	35.77±13.54	27.53±0.82
750	38:4 (18:0/20:4, 16:0/22:4)	52.14±17.36	43.25±2.50
760	38:7 (18:1/20:6, 16:1/22:6)	0.78±0.20	1.16±024
762	38:6 (16:0/22:6, 18:2/20:4)	1.53±0.01	7.00±0.06***
764	38:5 (18:0/20:5, 16:0/22:5)	8.23±2.43	33.45±2.38***
766	38;4 (18:0/20:4, 16:0/22:4)	32.72±9.69	20.77±1.38***
778	40:4 (20:0/20:4, 18:0/22:4)	21.79±7.90	15.74±1.51
788	40:7 (18:1/22:6)	1.17±0.13	7.15±1.60**
790	40:6 (20:2/20:4, 18:2/22:4, 18:0/22:6, 18:1/22:5)	4.02±0.80	10.98±0.02***
792	40:5 (18:0/22:5, 18:1/22:4, 18:2/22:3, 20:1/20:4)	2.25±1.15	35.85±0.92***
	phosphatidylserine		
808	38:3 (18:1/20:4)	8.57±5.43	7.57±3.41
810	38:4 (18:0/20:4)	0.67±0.07	2.41±0.85**
832	40:7 (18:0/22:7)	5.85±3.91	4.59±1.45
834	38:6 (18:0/20:6)	0.67±0.20	0.75±0.04
836	40:5 (18:0/22:5)	0.88±0.41	2.17±0.91*
838	40:4 (18:0/22:4)	1.37±0.66	5.49±1.81*
909	40:6 (18:0/22:6)	2.42±1.45	1.88±0.69
	phosphatidylinositol		
857	36:4 (16:0/20:4)	0.62±0.17	1.04±0.03**
861	36:2 (18:0/18:2)	3.52±1.59	3.39±0.11
863	36:1 (18:0/18:1)	1.70±0.65	3.28±0.70*
883	38:5 (18:1/20:4)	3.09±1.21	7.55±1.89*
885	38:4 (18:0/20:4)	15.65±8.23	5.85±1.55
909	40:6 (18:0/22:6)	0.48±0.19	1.17±0.21**
911	40:5 (18:0/22:5)	0.82±0.27	2.79±0.72**
913	40:4 (18:0/22:4)	1.52±0.63	1.18±0.20

Jurkat T cells were treated with 50 µmol/l EPA (20:5 *n*-3) or stearic acid (18:0), which served as a control. Lipid rafts were isolated by discontinuous sucrose density gradient ultracentrifugation. Rafts lipids were analyzed by ESI-MS and expressed in nanograms of DMPC equivalents per ng of phosphorous (mean ± S. E.). The significant differences between rafts of 20:5 (*n*-3) versus control-treated T was demonstrated by asterisks (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$)

In addition, EPA could modulate fatty acyl substitution of phospholipids as well as fatty acid composition in rafts of T cells, and alter raft lipid environment. Obviously, EPA treatment not only alters the fatty acid composition of the inner leaflet but also outer leaflet in the plasma membrane, which disrupts the raft structure. Because of the immunosuppressive effects of PUFAs *in vitro* and *in vivo*, the application of PUFAs has been studied for their putative clinical effects in a large variety of inflammatory diseases. However, their mechanism of this immunomodulation is still largely unknown. Our results proposed a possible mechanism for explaining the immunosuppressive function of PUFAs. Since PUFA supplementation is effectively associated with alteration of lipid environment in rafts and pronounced functional effects on rafts, their lipid composition changes appear to be clinically important for treating or preventing a broad range of inflammatory diseases.

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