Docosahexaenoic acid changes lipid composition and interleukin-2 receptor signaling in membrane rafts

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Abstract Polyunsaturated fatty acids, including docosahexaenoic acid (DHA, 22:6n-3), modulate immune responses and exert beneficial immunosuppressive effects, but the molecular mechanisms inhibiting T-cell activation are not yet elucidated. Lipid rafts have been shown to play an important role in the compartmentalization and modulation of cell signaling. We investigated the role of DHA in modulating the lipid composition in lipid rafts and membrane subdomain distribution of interleukin-2 (IL-2) receptor signaling molecules. We found that DHA altered lipid components of rafts and modified the IL-2-induced Janus kinase-signal transducer and activator of transcription (STAT) signaling pathway by partially displacing IL-2 receptors from lipid rafts. We fractionated plasma membrane subcellular compartments and discovered that certain amounts of STAT5a and STAT5b existed in detergent-resistant plasma membrane fractions of T-cells. After DHA treatment, STAT5a and STAT5b were not detected in lipid raft fractions and were located in detergent-soluble fractions. These data demonstrate for the first time that DHA alters the lipid composition of membrane microdomains and suppresses IL-2 receptor signaling in T-cells. Thus, our data provide evidence for a functional modification in lipid rafts by DHA treatment and explain PUFA-mediated immunosuppressive effects.—Li, Q., M. Wang, L. Tan, C. Wang, J. Ma, N. Li, Y. Li, G. Xu, and J. Li. **Docosahexaenoic acid changes lipid composition and interleukin-2 receptor signaling in membrane rafts. *J. Lipid Res.* 2005.** 46: **1904–1913.**

Supplementary key words polyunsaturated fatty acids • lipid rafts • fatty acid composition • Janus kinase-signal transducer and activator of transcription signaling pathway

Polyunsaturated fatty acids, particularly the n-3 series, modulate the immune response and especially affect T-cell function (1). Therefore, they are used clinically as immunosuppressive agents (2) for various inflammatory diseases (3–6). Moreover, they have also been associated with the concept of immunonutrition. Their presence in cell membrane phospholipids can increase membrane fluidity and regulate the functions of membrane receptors and enzymes. The immunomodulatory effects of PUFAs are associated with their ability to inhibit T-cell activation (7, 8). However, this molecular alteration of PUFA-induced T-cell inhibition has not yet been elucidated.

Lipid rafts are specialized in membrane microdomains, which are insoluble in nonionic detergents and can be isolated as detergent-resistant membrane domains. A large number of signaling proteins are targeted to lipid rafts. Therefore, lipid rafts serve as signaling platforms to facilitate efficient and specific signal transduction in living cells. Some findings have indicated that several proteins involved in the signaling pathway via the T-cell receptor, B-cell receptor, and IgE receptor are within lipid raft compartments (9, 10). Many of the critical signaling components involved in the T-cell receptor-mediated signal pathway are localized in rafts, including Fyn, Lck, and LAT Src family kinases (10–12). G-proteins (13) and endothelial nitric oxide synthase (14) are also enriched in rafts. PUFA supplementation inhibited T-lymphocyte activation by modifying detergent-insoluble membrane domains and displacing signaling proteins from lipid rafts in T-cells (15).

The interleukin-2R (IL-2R) subunits complex (α, β, and γ_c) is involved in both proliferative and activation-induced cell death signaling of T-cells. The high-affinity IL-2 receptors consist of the IL-2Rα chain, a 55 kDa transmembrane glycoprotein with 251 amino acids, and IL-2Rβ and IL-2Rγ_c, which contribute to IL-2 binding and mediate signal transduction. Binding of IL-2 to the IL-2R results in the activation of Janus kinase-1 (JAK1) and JAK3 and their phosphorylation and activation (16). Subsequent phosphorylation

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Abbreviations: DHA, docosahexaenoic acid, 22:6n-3; EPA, eicosapentaenoic acid; IL, interleukin; JAK, Janus kinase; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; SM, sphingomyelin; STAT, signal transducer and activator of transcription.

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of tyrosine residues in receptor chains results in the recruitment of the signal transducer and activator of transcription (STAT) (17). JAK-mediated phosphorylation of STAT proteins leads to their translocation to the nucleus, where STAT proteins regulate gene transcription. Because signals mediated through IL-2R are so important in the activation of T-cells, in our previous studies we revealed that IL-2Rα, IL-2Rβ, and IL-2Rγ_c are associated with lipid rafts of T-cells and that eicosapentaenoic acid (EPA) could disrupt lipid raft integrity and alter the distribution of IL-2Rα, IL-2Rβ, and IL-2Rγc in the plasma membrane and impair IL-2R signaling (our unpublished data).

In the present study, we examined the immunomodulatory effects of docosahexaenoic acid (DHA, 22:6n-3), the longest, most unsaturated fatty acid commonly found in membranes, by analyzing the raft lipid composition and the expression of key molecules of IL-2R signaling in both rafts and soluble fractions. Thus, our data provide evidence for a functional modification in lipid rafts by DHA treatment and explain PUFA-mediated immunosuppressive effects.

EXPERIMENTAL PROCEDURES

Materials and antibodies

Cis-4,7,10,13,16,19-DHA and stearic acid (18:0) were purchased from Sigma-Aldrich, Inc. (St. Louis, MO), and 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 from Invitrogen, Inc. (Grand Island, NY). Human IL-2 (hIL-2), BSA (fraction V), protease inhibitor cocktail tablets, and the BM chemiluminescence Western blotting kit were obtained from Roche Diagnostics, Inc. (Indianapolis, IN). Goat anti-human IL-2Rα antibody, IL-2Rβ-specific goat anti-human IL-2Rβ antibody, and goat anti-human common γ chain (IL-2Rγ_c) antibody from R&D Systems, Inc. (Minneapolis, MN), were used for Western blotting. Rabbit polyclonal antibodies of JAK1, JAK3, STAT5a, and STAT5b as well as anti-phosphotyrosine and phosphotyrosine STAT5 antibodies were purchased from Upstate, Inc. (Lake Placid, NY). Anti-caveolin-1 antibody was from BD Transduction Laboratories (Lexington, KY). The rabbit anti-goat IgG (heavy and light) horseradish peroxidase-conjugated antibody was obtained from Chemicon International, Inc. (Temecula, CA). Anti-rabbit IgG horseradish peroxidase-labeled secondary antibody was from Roche Diagnostics, Inc. PC5-conju-

TABLE 1. Fatty acid composition of raft and soluble fractions from control cells (18:0) and DHA-treated T-cells

	18:0		DHA		DHA vs. 18:0	
Fatty Acids	Raft	Soluble	Raft	Soluble	Raft	Soluble
10:0	0.14 ± 0.20	0.27 ± 0.08	0.00 ± 0.0	0.00 ± 0.0		[a]
12:0	1.95 ± 0.08[b]	2.85 ± 0.08	1.50 ± 0.39	0.99 ± 0.36		[c]
14:0	1.81 ± 0.78[d]	11.0 ± 1.50	2.88 ± 0.23[e]	9.72 ± 0.93	[f]	
16:0	18.65 ± 0.47[d]	28.35 ± 1.13	24.66 ± 0.26	26.58 ± 5.31	[g]	
16:1n-7	3.08 ± 1.54	11.66 ± 5.53	3.92 ± 0.17[e]	6.82 ± 0.29		
18:0	21.18 ± 4.81[b]	9.46 ± 3.38	9.83 ± 2.38	6.54 ± 1.56	[g]	
18:1n-9	29.08 ± 0.06[d]	16.73 ± 1.13	16.56 ± 0.92[e]	11.77 ± 0.84	[g]	[a]
cis-18:1n-9	6.53 ± 0.09[d]	1.54 ± 0.67	4.58 ± 0.55[e]	1.18 ± 0.24	[h]	
18:2n-6	1.89 ± 0.03[b]	8.16 ± 1.57	2.37 ± 1.10[e]	8.88 ± 1.81		
18:3n-6	0.00 ± 0.00	0.00 ± 0.0	0.00 ± 0.00	0.00 ± 0.00		
18:3n-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	0.00 ± 0.00	0.00 ± 0.00		
20:2n-6	1.03 ± 0.07[d]	0.00 ± 0.00	1.81 ± 0.75[e]	15.65 ± 4.43		[c]
20:3n-6	6.66 ± 3.34	3.09 ± 1.30	10.39 ± 1.72[e]	0.82 ± 0.21		
20:3n-3	0.06 ± 0.01	0.00 ± 0.00	0.37 ± 0.05	0.29 ± 0.10	[h]	
20:4n-6	6.25 ± 0.61[d]	1.80 ± 0.66	4.17 ± 0.67[e]	0.77 ± 0.25		
22:0	0.10 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.0		
20:5n-3	0.58 ± 0.19	0.00 ± 0.00	1.18 ± 0.31[e]	0.00 ± 0.00		
22:5n-3	0.30 ± 0.12[b]	0.68 ± 0.01	1.00 ± 0.15[e]	0.00 ± 0.0	[g]	
DHA	0.31 ± 0.14[i]	2.96 ± 1.42	15.30 ± 0.48	14.97 ± 2.70	[h]	[c]
Saturated fatty acid	44.13 ± 2.62[b]	53.82 ± 0.95	38.86 ± 1.06	43.84 ± 8.17		
Monounsaturated fatty acids	38.70 ± 0.98[b]	29.93 ± 3.74	25.06 ± 1.74[e]	19.76 ± 0.79	[h]	[a]
PUFAs	16.74 ± 5.40	16.95 ± 4.69	36.58 ± 6.03	41.39 ± 10.30	[h]	[c]
∑(n3)PUFA	0.93 ± 0.55[d]	3.22 ± 1.16	17.85 ± 0.05	15.26 ± 2.99	[h]	[c]
∑(n6)PUFA	15.83 ± 2.11	13.04 ± 3.52	18.73 ± 0.87[e]	26.13 ± 1.55		[c]
(n-3)/(n-6)	0.05 ± 0.04[d]	0.24 ± 0.02	0.95 ± 0.05[e]	0.58 ± 0.06	[h]	[j]

DHA, docosahexaenoic acid, 22:6n-3; 18:0, stearic acid. Jurkat T-cells were treated with DHA or stearic acid, and lipid rafts were isolated by discontinuous sucrose density gradient ultracentrifugation. Fatty acid composition was analyzed from three experiments and is expressed in mol% (means ± SEM).

[a] Significant difference between soluble fractions of DHA- and control-treated T-cells ($P < 0.01$).
[b] Significant difference compared with soluble fractions of control-treated cells ($P < 0.01$).
[c] Significant difference between soluble fractions of DHA- and control-treated T-cells ($P < 0.001$).
[d] Significant difference compared with soluble fractions of control-treated cells ($P < 0.001$).
[e] Significant difference compared with soluble fractions of DHA-treated cells ($P < 0.001$).
[f] Significant difference between rafts of DHA- versus control-treated T-cells ($P < 0.05$).
[g] Significant difference between rafts of DHA- versus control-treated T-cells ($P < 0.01$).
[h] Significant difference between rafts of DHA- versus control-treated T-cells ($P < 0.001$).
[i] Significant difference compared with soluble fractions of control-treated cells ($P < 0.05$).
[j] Significant difference between soluble fractions of DHA- and control-treated T-cells ($P < 0.05$).

gated mouse anti-human CD25 (IL-2Rα) monoclonal antibodies and isotypic control were from Immunotech Coulter Co.

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 and penicillin/streptomycin (100 U/ml and 100 μg/ml, respectively) at 37°C with 5% CO_2. For the modification of cellular lipids, the cells were cultured for 2 days in serum-free Iscove's modified Dulbecco's medium supplemented with 0.4% (w/v) BSA (fraction V, containing <3 μM total fatty acids), with the addition of 50 μM either DHA or stearic acid from stock solutions in ethanol (final concentration ≤ 0.5%). Stearic acid served as a control; it is considered the most suitable fatty acid to be added to control cultures because nonessential fatty acids are highly abundant in human serum, and this fatty acid was shown previously not to influence cellular PUFA content and the membrane subdomain distribution of proteins compared with controls treated with vehicle only (18). The other cells were treated with 1.5% methyl-β-cyclodextrin for 1 h at 37°C to disrupt lipid rafts. After stimulation with 400 U/ml IL-2 for 30 min, cells were pelleted and lysed.

Isolation of lipid rafts

Lipid rafts were isolated from Jurkat T-cells as described (19) by discontinuous sucrose density gradient ultracentrifugation. The cells (2.5×10^7 cells/ml) were washed in Hanks' balanced salt solution three times and lysed in TKM buffer (50 mM Tris, pH 7.4, 25 mM KCl, 5 mM $MgCl_2$, and 1 mM EDTA) containing 1% Brij-58 and protease inhibitor cocktail tablets (0.12 mg of antipain-HCl, 20 μg of bestatin, 40 μg of chymostatin, 0.12 mg of E-64, 20 μg of leupeptin, 20 μg of pepstatin, 0.12 mg of phosphoramidon, 0.8 mg of pefabloc, and 40 μg of 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). Fractions of 1 ml were collected from the top of the gradients one by one.

Flow cytometry

The cells were washed twice with 10 mM PBS buffer (pH 7.2), suspended with 50 μl of PBS buffer, and incubated with Cy5-labeled CD25 (the IgG is conjugated to a tandem dye constituted of R-phycoerythrin covalently linked to cyanin 5.1 at 1 mol of PC5 per mole of IgG) in the dark at room temperature for 20 min.

Negative control cells were incubated with isotypic control antibody. Finally, the cells were washed twice in PBS and assessed by flow cytometry on FACScalibur (Becton Dickinson, Franklin Lakes, NJ). Data were then analyzed with CellQuest software (Becton Dickinson).

Immunoblotting

The proteins in fractions isolated from sucrose density gradients were separated electrophoretically by SDS-PAGE on a Bio-Rad minigel apparatus (Bio-Rad, Inc., Hercules, CA) and transferred to polyvinylidene difluoride membranes (Bio-Rad). Immunoblot analysis of IL-2Rα, IL-2Rβ, and IL-2Rγ_c was performed using goat anti-human IL-2Rα, IL-2Rβ, and IL-2Rγ_c antibodies and rabbit anti-goat IgG (H&L) horseradish peroxidase-conjugated antibody. Immunoblot analysis of JAK1, JAK3, STAT5a, STAT5b, and anti-phosphotyrosine was performed using rabbit anti-JAK1, anti-JAK3, anti-STAT5a, anti-STAT5b, anti-phosphotyrosine antibodies, phosphotyrosine STAT5, and anti-rabbit horseradish peroxidase-labeled antibodies. Membranes were developed according to standard immunoblotting procedures, detection was performed with the BM chemiluminescence Western blotting kit, and the membranes were exposed to films (Kodak BioMAX XAR; Eastman Kodak Co., Rochester, NY).

TABLE 2. PC fatty acid-containing raft lipids from control cells and DHA-treated T-cells

PC		18:0		DHA		DHA vs. 18:0	
m/z	Lipids	Raft	Soluble	Raft	Soluble	Raft	Soluble
718	32:0 (16:0/16:0)	2.71 ± 0.73[a]	0.73 ± 0.21	8.30 ± 1.38[b]	1.37 ± 0.26	[c]	
742	34:2 (16:0/18:2)	4.78 ± 0.73	1.97 ± 0.65	5.39 ± 0.70[b]	0.87 ± 0.11		[d]
744	34:1 (16:0/18:1)	12.10 ± 5.17[a]	4.67 ± 1.33	16.23 ± 3.84[b]	1.89 ± 0.48		[d]
764	36:3 (16:0/20:3, 18:1/18:2)	0.75 ± 0.18	0.53 ± 0.01	11.74 ± 0.24[e]	0.41 ± 0.03	[f]	[g]
766	36:4 (16:0/20:4, 18:2/18:2)	1.10 ± 0.31	0.77 ± 0.13	1.61 ± 0.37[b]	0.37 ± 0.00		[h]
768	36:3 (16:0/20:3,18:1/18:2)	2.01 ± 1.04	2.16 ± 0.70	2.47 ± 0.34[b]	0.45 ± 0.08		[g]
770	36:3 (18:0/18:2)	6.58 ± 3.03[i]	2.56 ± 1.98	7.12 ± 0.98[b]	0.90 ± 0.23		
790	38:6 (18:1/20:5, 16:0/22:6, 18:2/20:4)	0.96 ± 0.33	0.52 ± 0.09	4.32 ± 0.72[b]	0.72 ± 0.23	[c]	
792	38:5 (18:1/20:4, 18:2/20:3, 16:0/22:5)	0.90 ± 0.36	0.94 ± 0.38	1.94 ± 0.17[b]	0.47 ± 0.04	[j]	
794	38:4 (18:0/20:4, 18:1/20:3, 18:2/20:2)	1.43 ± 0.62	0.67 ± 0.37	2.26 ± 0.03[b]	0.33 ± 0.09		
818	40:6 (18:1/22:5, 18:0/22:6, 20:2/20:4)	1.24 ± 0.69	0.65 ± 0.37	2.55 ± 0.09[b]	0.46 ± 0.14	[f]	
820	40:5 (18:0/22:5)	0.98 ± 0.34	0.43 ± 0.13	1.34 ± 0.26[b]	0.34 ± 0.08		

PC, phosphatidylcholine. Jurkat T-cells were treated with 50 μM DHA or 18:0, which was used as a control. Lipid rafts were isolated by discontinuous sucrose density gradient ultracentrifugation. Raft lipids were analyzed by electrospray ionization (ESI)-MS and are expressed in nanograms of 1,2-dimyristoyl-sn-glycero-3-phosphocholine (DMPC) equivalents per nanogram of phosphorous (means ± SEM).

[a] Significant difference in rafts compared with soluble fractions of control-treated cells ($P < 0.05$).
[b] Significant difference in rafts compared with soluble fractions of DHA-treated cells ($P < 0.001$).
[c] Significant difference between rafts of DHA- versus control-treated T-cells ($P < 0.001$).
[d] Significant difference between soluble fractions of DHA- versus control-treated T-cells ($P < 0.05$).
[e] Significant difference in rafts compared with soluble fractions of DHA-treated cells ($P < 0.01$).
[f] Significant difference between rafts of DHA- versus control-treated T-cells ($P < 0.05$).
[g] Significant difference between soluble fractions of DHA- versus control-treated T-cells ($P < 0.001$).
[h] Significant difference between soluble fractions of DHA- versus control-treated T-cells ($P < 0.01$).
[i] Significant difference in rafts compared with soluble fractions of control-treated cells ($P < 0.01$).
[j] Significant difference between rafts of DHA- versus control-treated T-cells ($P < 0.01$).

TABLE 3. SM fatty acid-containing raft lipids from DHA-treated T-cells and control cells

SM		18:0		DHA		DHA vs. 18:0	
m/z	Lipids	Raft	Soluble	Raft	Soluble	Raft	Soluble
685	34:2	1.06 ± 0.35	0.55 ± 0.09	1.57 ± 0.25[a]	0.35 ± 0.22		[b]
687	18:1/18:0	15.57 ± 7.88	3.37 ± 0.62	23.14 ± 10.34[c]	2.43 ± 0.63		
739	38:3	0.53 ± 0.16	0.34 ± 0.13	1.08 ± 0.10[d]	0.71 ± 0.01	[e]	[b]
745	38:0	0.68 ± 0.22	0.33 ± 0.14	0.89 ± 0.35[d]	0.31 ± 0.04		
815	43:0	3.74 ± 2.01	1.51 ± 0.52	4.77 ± 2.97[d]	0.61 ± 0.26		

SM, sphingomyelin. Jurkat T-cells were treated with 50 μM DHA or 18:0, which was used as a control. Lipid rafts were isolated by discontinuous sucrose density gradient ultracentrifugation. Raft lipids were analyzed by ESI-MS and are expressed in nanograms of DMPC equivalents per nanogram of phosphorous (means ± SEM).

[a] Significant difference in rafts compared with soluble fractions of DHA-treated cells ($P < 0.001$).

[b] Significant difference between soluble fractions of DHA- versus control-treated T-cells ($P < 0.05$).

[c] Significant difference in rafts compared with soluble fractions of DHA-treated cells ($P < 0.01$).

[d] Significant difference in rafts compared with soluble fractions of DHA-treated cells ($P < 0.05$).

[e] Significant difference between rafts of DHA- versus control-treated T-cells ($P < 0.01$).

Fatty acid analysis

Detergent-resistant and detergent-soluble fractions from DHA-treated and control T-cells were freeze-dried before adding 1 ml of boron trifluoride-diethyl ether/methanol (1:3, v/v); 10 μg of heptadecanoic acid served as an internal standard. Methanolysis was performed by incubating at 75°C for 30 min. After cooling, 0.5 ml of water and 1.5 ml of *n*-hexane were added and stirred, then subjected to centrifugation at 2,000 *g* for 15 min. The organic phase was collected, 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). The system was equipped with a flame ionization detector, an HP3398A workstation, and a HP-FFAP quartz capillary column (30 m length, 0.32 mm inner diameter, 0.25 μm layer thickness). Gas chromatography was performed at 110°C, then increased at 8°C/min for 10 min, at 6°C/min to reach 230°C for 8 min, and at 6°C/min to 250°C for 3 min with a constant flow (10 p.s.i.) of high-purity N_2. The fatty acid composition is expressed as mole percent. The significance of differences in fatty acid content between raft and soluble membrane fractions of DHA- and control-treated T-cells was calculated by paired Student's *t*-tests.

Mass spectrometry for raft phospholipid analysis

Total lipids in detergent-resistant and detergent-soluble fractions from DHA-treated and control T-cells were extracted. The samples were added with 1 ml of methanol containing 0.9% butylated hydroxytoluene and internal controls (1,2-dimyristoyl-*sn*-glycero-3-phosphocholine and 1,2-dimyristoyl-*sn*-glycero-3-phosphoethanolamine) and mixed. Four milliliters of chloroform was added and stirred with the addition of 1 ml of methanol. The final chloroform-methanol ratio was 2:1, and 1 ml of 50 mM 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 HPLC system (Agilent Technologies) was

TABLE 4. PE fatty acid-containing raft lipids from DHA-treated T-cells and control cells

PE		18:0		DHA		DHA vs. 18:0	
m/z	Lipids	Raft	Soluble	Raft	Soluble	Raft	Soluble
720	36:5 (p16:0/20:5)	4.15 ± 1.12[a]	1.64 ± 0.75	14.02 ± 2.33[b]	2.58 ± 0.31	[c]	
722	36:4 (p16:0/20:4)	33.39 ± 10.12	25.30 ± 12.06	47.22 ± 2.03[b]	6.76 ± 1.60		
736	36:5 (16:1/20:4)	2.56 ± 0.58	2.25 ± 0.76	3.36 ± 0.19[b]	1.21 ± 0.21	[d]	
738	36:4 (16:1/20:3, 18:2/18:2, 16:0/20:4)	3.76 ± 1.77	2.47 ± 0.70	6.40 ± 0.95[b]	1.51 ± 0.36		[e]
740	36:3 (16:0/20:3, 18:1/18:2)	6.00 ± 1.56	3.05 ± 1.01	5.78 ± 0.23[b]	1.25 ± 0.13		[e]
742	36:2 (18:1/18:1, 18:0/18:2)	26.44 ± 8.21	15.89 ± 5.97	23.14 ± 2.83[b]	4.04 ± 0.70		
744	36:1 (16:0/20:1, 18:0/18:1)	35.77 ± 13.54	22.70 ± 8.59	42.00 ± 2.47[b]	7.07 ± 0.93		[e]
750	38:4 (p18:0/20:4, p16:0/22:4)	52.14 ± 17.36	35.20 ± 12.96	60.04 ± 5.60[b]	6.39 ± 0.41		[e]
760	38:7 (18:1/20:6, 16:1/22:6)	0.78 ± 0.20	0.87 ± 0.41	3.97 ± 0.22[b]	0.92 ± 0.02		
762	38:6 (16:0/22:6, 18:2/20:4)	1.53 ± 0.01	0.92 ± 0.60	12.31 ± 1.78[b]	1.23 ± 0.14	[c]	
764	38:5 (18:0/20:5, 16:0/22:5, 18:1/20:4)	8.23 ± 2.43	5.01 ± 1.70	15.33 ± 1.15[b]	2.78 ± 0.55	[c]	
766	38:4 (18:0/20:4, 16:0/22:4, 18:2/20:2)	32.72 ± 9.69	23.86 ± 7.95	41.45 ± 1.68[b]	8.22 ± 2.43		[e]
778	40:4 (p20:0/20:4, p18:0/22:4)	21.79 ± 7.90	12.59 ± 5.99	15.03 ± 5.66[f]	2.09 ± 0.45		
788	40:7 (18:1/22:6)	1.17 ± 0.13	0.90 ± 0.43	13.25 ± 0.38[b]	2.07 ± 0.15	[d]	[e]
790	40:6 (20:2/20:4, 18:2/22:4, 18:0/22:6, 18:1/22:5)	4.02 ± 0.80	3.47 ± 0.55	43.07 ± 1.93[b]	7.26 ± 0.26	[c]	
792	40:5 (18:0/22:5, 18:1/22:4, 18:2/22:3, 20:1/20:4)	2.25 ± 1.15	4.56 ± 1.32	14.31 ± 2.56[b]	2.26 ± 0.34	[c]	[e]

PE, phosphatidylethanolamine. Jurkat T-cells were treated with 50 μM DHA or 18:0, which was used as a control. Lipid rafts were isolated by discontinuous sucrose density gradient ultracentrifugation. Raft lipids were analyzed by ESI-MS and are expressed in nanograms of DMPC equivalents per nanogram of phosphorous (means ± SEM).

[a] Significant difference in rafts compared with soluble fractions of control-treated cells ($P < 0.05$).

[b] Significant difference in rafts compared with soluble fractions of DHA-treated cells ($P < 0.001$).

[c] Significant difference between rafts of DHA- versus control-treated T-cells ($P < 0.001$).

[d] Significant difference between rafts of DHA- versus control-treated T-cells ($P < 0.01$).

[e] Significant difference between soluble fractions of DHA- versus control-treated T-cells ($P < 0.01$).

[f] Significant difference in rafts compared with soluble fractions of DHA-treated cells ($P < 0.01$).

TABLE 5. PS fatty acid-containing raft lipids from DHA-treated T-cells and control cells

PS		18:0		DHA		DHA vs. 18:0	
m/z	Lipids	Raft	Soluble	Raft	Soluble	Raft	Soluble
788	36:1 (18:0/18:1)	2.24 ± 1.11	0.66 ± 0.31	3.08 ± 0.36	0.45 ± 0.13		[a]
808	38:3 (18:1/20:4)	8.57 ± 5.43	2.70 ± 1.15	10.16 ± 6.11[b]	1.33 ± 0.56		
810	38:4 (18:0/20:4)	0.67 ± 0.07	0.67 ± 0.21	2.58 ± 1.16[b]	0.48 ± 0.23	[c]	[d]
832	40:7 (18:0/22:7)	5.85 ± 3.91	1.55 ± 0.31	5.18 ± 2.69[b]	0.96 ± 0.57		
834	38:6 (18:0/22:6)	0.67 ± 0.20[e]	0.27 ± 0.01	3.06 ± 0.85[b]	0.22 ± 0.00	[c]	
836	40:5 (18:0/22:5)	0.88 ± 0.41	0.85 ± 0.38	5.52 ± 2.18	0.70 ± 0.28	[c]	
838	40:4 (18:0/22:4)	1.37 ± 0.66	0.65 ± 0.26	2.16 ± 1.37[b]	0.36 ± 0.04		
909	40:6 (18:0/22:6)	2.42 ± 1.45	0.87 ± 0.24	1.83 ± 1.13[b]	0.30 ± 0.16		[d]

PS, phosphatidylserine. Jurkat T-cells were treated with 50 μM DHA or 18:0, which was used as a control. Lipid rafts were isolated by discontinuous sucrose density gradient ultracentrifugation. Raft lipids were analyzed by ESI-MS and are expressed in nanograms of DMPC equivalents per nanogram of phosphorous (means ± SEM).

[a] Significant difference between soluble fractions of DHA- versus control-treated T-cells ($P < 0.001$).
[b] Significant difference in rafts compared with soluble fractions of DHA-treated cells ($P < 0.05$).
[c] Significant difference between rafts of DHA- versus control-treated T-cells ($P < 0.05$).
[d] Significant difference between soluble fractions of DHA- versus control-treated T-cells ($P < 0.05$).
[e] Significant difference in rafts compared with soluble fractions of control-treated cells ($P < 0.05$).

used. The LC separation was performed on a diol column (Nucleosil 100-5 OH) of 250 mm × 3.9 mm (inner diameter) × 5.0 μm (particle size). The total flow rate was 0.4 ml/min, and the column temperature was 35°C. Solvent mixture A consisted of hexane-1-propanol-formic acid-ammonia (79:20:0.6:0.07, v/v), and solvent mixture B consisted of 1-propanol-water-formic acid-ammonia (88:10:0.6:0.07, v/v). 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 mass spectrometer through a steel electrospray 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 rates of nitrogen drying gas and turbo gas were both 40 p.s.i. The declustering potential was set at 80 p.s.i. The other parameters were as follows: Enhanced Mass Scan as survey scan (mass range m/z 450–950, scan speed 1,000 Dalton per second, trap time 20 ms) and Enhanced Product Ion Scan as dependent scan (scan speed 1,000 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

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

To investigate whether the displacement of IL-2R from membrane rafts might be attributable to altered fatty acid composition in lipid rafts, we isolated rafts form DHA- and control-treated Jurkat T-cells. When T-cells were treated with DHA, the n-3 polyunsaturated fatty acids [linolenic acid (20:3n-3), docosapentaenoic acid (22:5n-3), and DHA] were increased significantly in raft fractions compared with those of control cells (**Table 1**). The concentrations of saturated myristic acid (14:0) and palmitic acid (16:0) were also increased in raft fractions of DHA-treated cells compared with control cells. Rafts from DHA-treated cells contained significantly less fatty acids [saturated stearic acid, monounsaturated oleic acid (18:1n-9), and *cis*-oleic acid (*cis*-18:1n-9)] compared with rafts of control cells. The PUFAs, n-3 PUFAs, and the ratio of n-3 PUFAs to n-6 PUFAs were considerably increased in rafts from DHA-

TABLE 6. PI fatty acid-containing raft lipids from DHA-treated T-cells and control cells

PI		18:0		DHA		DHA vs. 18:0	
m/z	Lipids	Raft	Soluble	Raft	Soluble	Raft	Soluble
857	36:4 (16:0/20:4)	0.62 ± 0.17	0.50 ± 0.20	0.97 ± 0.23[a]	0.28 ± 0.04	[b]	
861	36:2 (18:0/18:2)	3.52 ± 1.59	1.17 ± 0.22	3.99 ± 1.01[a]	0.70 ± 0.13		[c]
863	36:1 (18:0/18:1)	1.70 ± 0.65	0.83 ± 0.10	2.65 ± 0.26[a]	0.64 ± 0.10	[d]	
883	38:5 (18:1/20:4)	3.09 ± 1.21[e]	1.05 ± 0.14	3.93 ± 0.87[f]	0.66 ± 0.20	[d]	
885	38:4 (18:0/20:4)	15.65 ± 8.23	5.58 ± 0.20	17.02 ± 2.11[a]	2.21 ± 0.48		[g]
909	40:6 (18:0/22:6)	0.48 ± 0.19	0.38 ± 0.10	5.56 ± 0.78[a]	0.73 ± 0.24	[d]	
911	40:6 (18:0/22:5)	0.82 ± 0.27	0.65 ± 0.32	2.27 ± 0.51[a]	0.45 ± 0.03	[d]	
913	40:4 (18:0/22:4)	1.52 ± 0.63	0.79 ± 0.05	1.46 ± 0.58[f]	0.31 ± 0.14		[g]

PI, phosphatidylinositol. Jurkat T-cells were treated with 50 μM DHA or 18:0, which was used as a control. Lipid rafts were isolated by discontinuous sucrose density gradient ultracentrifugation. Raft lipids were analyzed by ESI-MS and are expressed in nanograms of DMPC equivalents per nanogram of phosphorous (means ± SEM).

[a] Significant difference in rafts compared with soluble fractions of DHA-treated cells ($P < 0.001$).
[b] Significant difference between rafts of DHA- versus control-treated T-cells ($P < 0.01$).
[c] Significant difference between soluble fractions of DHA- versus control-treated T-cells ($P < 0.05$).
[d] Significant difference between rafts of DHA- versus control-treated T-cells ($P < 0.05$).
[e] Significant difference in rafts compared with soluble fractions of control-treated cells ($P < 0.05$).
[f] Significant difference in rafts compared with soluble fractions of DHA-treated cells ($P < 0.01$).
[g] Significant difference between soluble fractions of DHA- versus control-treated T-cells ($P < 0.001$).

treated T-cells compared with rafts from control cells. DHA treatment increased the relative amount of polyunsaturated fatty acids in raft fractions in T-cells and altered the lipid environment of membrane microdomains

DHA treatment alters fatty acyl substitution of raft lipids

As shown in Table 1, DHA treatment changes the raft lipid environment. Therefore, we analyzed the fatty acyl composition of different phospholipids, including phosphatidylcholine (PC), sphingomyelin (SM), phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI), in lipid rafts, and the phospholipid species were identified in lipid extracts by QTRAP LC/MS/MS. DHA treatment significantly increased the concentrations of PC species substituted with 32:0 (16:0/16:0), 36:3 (16:0/20:3, 18:1/18:2), 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 compared with control cells (**Table 2**). Substitution of SM with 38:3 acyl chains was significantly increased in rafts of DHA-treated cells compared with control cells (**Table 3**). In DHA-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), and 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 (**Table 4**). The concentrations of PS species substituted with 38:4 (18:0/20:4), 38:6 (18:0/20:6) and 40:5 (18:0/22:5) acyl chains were increased (**Table 5**). PI species substituted with 36:4 (16:0/20:4), 40:6 (18:0/22:6), and 40:5 (18:0/22:5) acyl chains were more abundant in rafts of DHA-treated cells compared with control cells, and those substituted with 36:1 (18:0/18:1) and 38:5 (18:1/20:4) acyl chains were increased (**Table 6**). DHA treatment resulted in the alternation of unsaturated lipid composition in lipid rafts and fatty acyl composition of raft phospholipids.

DHA suppresses CD25 expression on the surface of T-cells

To determine the effect of DHA 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, 79.19% of the control cells (stearic acid) were CD25-positive. For Jurkat T-cells treated with DHA (25, 50, and 75 μM), the positive CD25 expression levels were 74.05, 59.75, and 10.98%, respectively (**Fig. 1**).

IL-2R displacement from lipid rafts by DHA treatment

It was reported recently that PUFAs inhibit T-cell signal transduction by removing the Src kinases Fyn and Lck

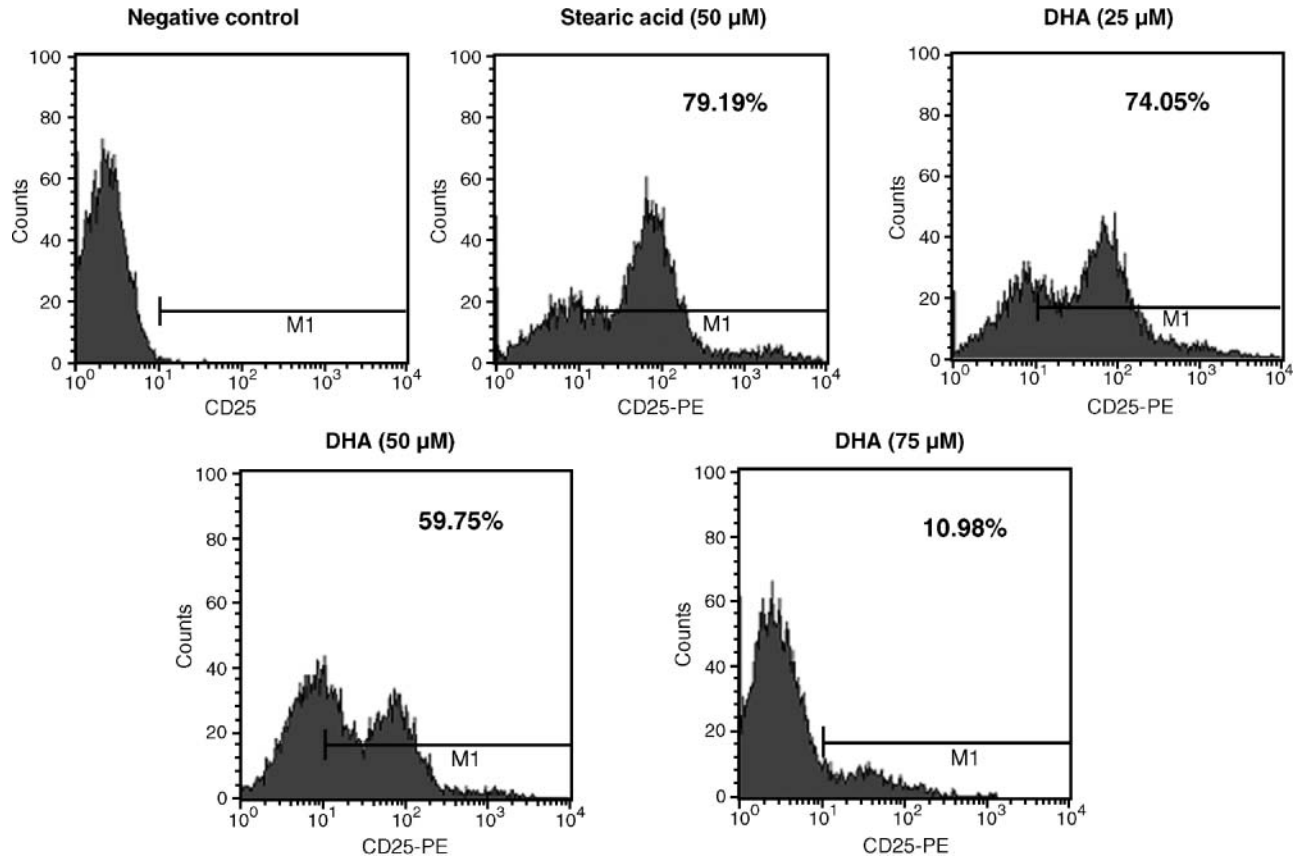

Fig. 1. Effect of docosahexaenoic acid (DHA, 22:6n-3) on CD25 expression on the surface of Jurkat T-cells by FACS analysis. The cells were cultured in the absence [stearic acid (18:0)] or presence of DHA (25, 50, or 75 μM) for 48 h and stained with FITC-labeled anti-CD25 antibody. T-cells were analyzed by FACS analysis.

from the biochemically by its presence in soluble membrane fractions (20, 21). Caveolins could be used to indicate lipid rafts on the plasma membrane, and caveolin-1 is the most abundant form of caveolin in lipid rafts. Using immunoblot analysis, we revealed the location of caveolin-1 in lipid rafts (**Fig. 2**). After methyl-β-cyclodextrin treatment, lipid rafts were disrupted significantly, as indicated by the dislocation of caveolin-1 in discontinuous density gradient separation (Fig. 2). We investigated whether IL-2R subunits were associated with lipid rafts and whether DHA affected the location of IL-2R in lipid rafts. The cells were stimulated for 30 min with 400 U/ml IL-2 before lysis (22). The association of a protein with membrane rafts is defined, and membranes were fractionated by sucrose density gradient ultracentrifugation. Immunoblot analysis of fractions from these gradients showed that IL-2Rα was detected in low density, detergent-resistant membrane fractions of T-cells after solubilization with the nonionic detergent Brij-58 (Fig. 2). The IL-2Rβ and IL-2Rγ_c chains were also detected consistently in the lipid raft fractions but not in the soluble membrane fractions (Fig. 2). IL-2R chains were enriched in lipid rafts and localized within lipid raft microdomains. Lipid rafts play an important role in the regulation of the IL-2R signaling pathway. After DHA treatment, IL-2Rα, IL-2Rβ, and IL-2Rγ_c were detected not only in raft fractions but also in soluble membrane fractions (Fig. 2), which indicated that IL-2R was partly displaced from lipid rafts after this treatment. Our results revealed that DHA could disrupt lipid raft integrity and alter the distribution of IL-2Rα, IL-2Rβ, and IL-2Rγ_c in the plasma membrane and impair IL-2R signaling.

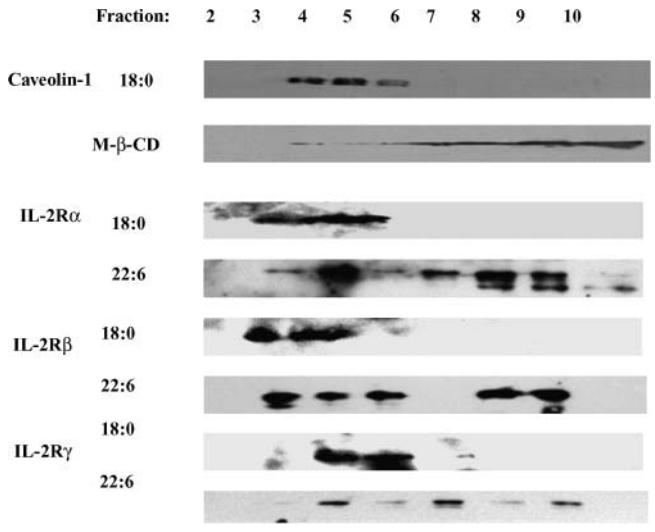

Fig. 2. Effect of DHA treatment on the subcellular distribution of interleukin-2Rα (IL-2Rα), IL-2Rβ, and IL-2Rγ_c in the plasma membrane of human Jurkat T-cells. T-cells were treated with or without methyl-β-cyclodextrin (M-β-CD). The cells were cultured with 50 μM saturated 18:0 or DHA for 48 h. Membranes were solubilized by the nonionic detergent Brij-58 and fractionated on a density gradient. The proteins were separated by SDS-PAGE. Localization of IL-2Rα, IL-2Rβ, and IL-2Rγ_c chains was analyzed by Western blotting with anti-IL-R2α, anti-IL-2Rβ, and anti-IL-2Rγ_c chain antibodies.

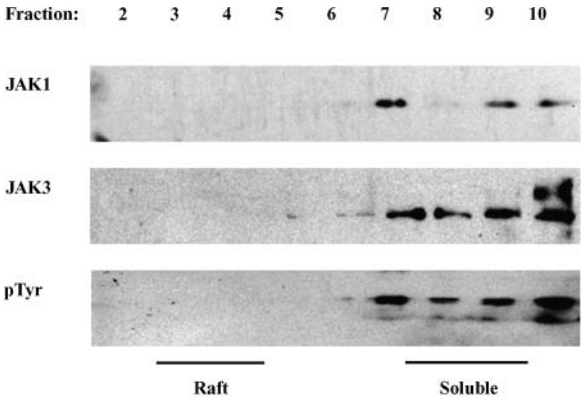

Fig. 3. Janus kinase-1 (JAK1), JAK3, and phosphotyrosine (pTyr) are localized in detergent-soluble membranes. Jurkat T-cells were treated with 50 μM saturated 18:0 for 48 h and stimulated for 30 min with IL-2. Membranes were solubilized by the nonionic detergent Brij-58 and fractionated by sucrose density gradient ultracentrifugation. The proteins were separated by SDS-PAGE, and immunoblotting was performed using anti-JAK1, anti-JAK3, and anti-phosphotyrosine-specific antibodies.

JAK1, JAK3, and tyrosine phosphorylation in soluble membrane fractions

After stimulation by IL-2, JAK1 and JAK3 kinases are associated with the IL-2R chains and can induce tyrosine phosphorylation of JAK1 and JAK3 molecules, which is critical for IL-2R signal transduction. We analyzed the localization of JAK1, JAK3, and IL-2-induced tyrosine phosphorylation. In the immunoblot shown in **Fig. 3**, JAK1, JAK3, and phosphotyrosine were detected in soluble membrane fractions. After DHA treatment, we found the presence of JAK1, JAK3, and phosphotyrosine in the soluble fraction, but their abundance was decreased compared with that in control cells (**Fig. 4**).

Subcellular distributions of STAT5a and STAT5b in T-cells

STAT5a and STAT5b transcription factors signal from the plasma membrane to the nucleus by cytokine induc-

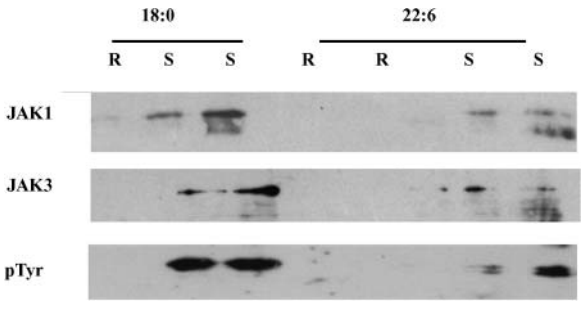

Fig. 4. DHA treatment suppresses the accumulation of JAK1, JAK3, and phosphotyrosine (pTyr) in detergent-soluble membranes. Jurkat T-cells were cultured in the absence (18:0) or presence of DHA (50 μM) for 48 h and stimulated for 30 min with IL-2. The membranes were solubilized by the nonionic detergent Brij-58, and lipid rafts (R) as well as detergent-soluble membranes (S) were fractionated by sucrose density gradient ultracentrifugation. The proteins were resolved by SDS-PAGE and immunoblotted with anti-JAK1, anti-JAK3, and anti-phosphotyrosine-specific antibodies.

tion. In this study, we investigated whether lipid rafts are involved in IL-2-activated STAT signaling. The data in **Fig. 5** show that large amounts of STAT5a and STAT5b were associated with soluble membrane fractions and that small portions of STAT5a and STAT5b were localized in plasma membrane raft fractions. However, the tyrosine phosphorylation of STAT5 was barely detected in rafts. After DHA treatment, STAT5a and STAT5b were not detected in detergent-resistant lipid raft fractions and were present in detergent-soluble fractions. STAT5a and STAT5b proteins were displaced from raft fractions to soluble membrane fractions in DHA-treated T-cells (Fig. 5, second and fourth panels), and the tyrosine phosphorylation of STAT5 was decreased as well (Fig. 5, sixth panel).

DISCUSSION

There is overwhelming evidence for the effects of n-3 PUFAs on inflammatory diseases, and they exert beneficial effects in various inflammatory disorders (23). DHA is one of the primary effector molecules (24). Recent studies have demonstrated that several signaling proteins are enriched in lipid rafts and can be displaced from membrane rafts by EPA (15, 25). The raft lipid composition is also altered after EPA supplementation. However, there is still debate regarding the biochemical alteration of the lipid environment in rafts by PUFA treatment, which might be the molecular basis for PUFAs displacing proteins from membrane rafts. To date, it has not been determined that DHA changes the fatty acyl composition in lipid rafts and alters the lipid environment. To provide biochemical evidence for the displacement of proteins in IL-2R signaling from lipid rafts in DHA-treated T-cells, we analyzed the fatty acid composition and fatty acyl substitution in phospholipids in detergent-resistant rafts and soluble membrane fractions of DHA-treated and control T-cells. When T-cells were treated with DHA, the n-3 PUFAs (linolenic acid, docosapentaenoic acid, and DHA) were significantly enriched in raft fractions compared with those of control cells. DHA treatment resulted in a considerable increase of unsaturated fatty acyl chains in rafts from DHA-treated T-cells compared with control cells. Distinct saturated fatty acids, such as myristic acid and palmitic acid, were also increased in raft fractions of DHA-treated cells compared with control cells; however, stearic acid, oleic acid, and *cis*-oleic acid were markedly decreased. This alteration indicated that DHA-treated cells aim to maintain lipid composition in rafts by changing the saturated or monounsaturated fatty environment. Unfortunately, this compensatory mechanism is still unclear.

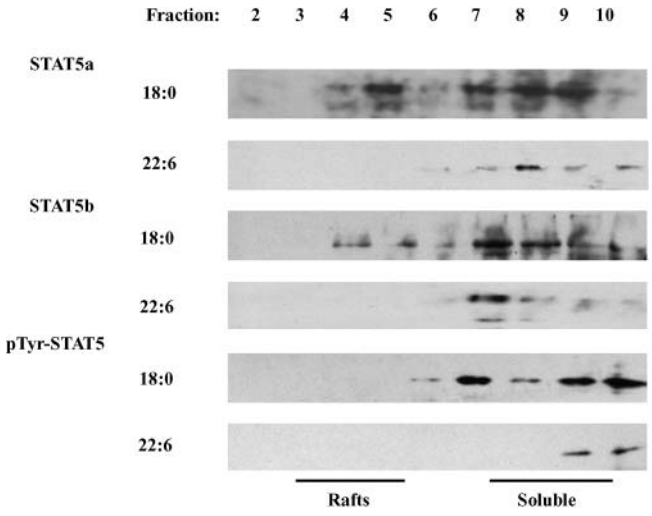

Fig. 5. Effect of DHA treatment on signal transducer and activator of transcription 5a (STAT5a), STAT5b, and phosphotyrosine (pTyr) in detergent-resistant and detergent-soluble membranes in Jurkat T-cells. Jurkat T-cells were cultured in the absence (18:0) or presence of DHA (50 μM) for 48 h and stimulated for 30 min with IL-2. Lipid rafts and detergent-soluble membranes were isolated by sucrose density gradient ultracentrifugation. The proteins were resolved by SDS-PAGE and immunoblotted by anti-STAT5a, anti-STAT5b, and anti-phosphotyrosine-STAT5 (pTyr-STAT5)-specific antibodies.

PUFAs displace proteins from rafts and affect cytoplasmic and transmembrane proteins in rafts by posttranslational regulation with fatty acyl moieties (15). The alteration in membrane fatty acyl composition should affect membrane lipids residing in the exoplasmic leaflet and also has indirect effects on the membrane subdomain distribution of acylated proteins (10). Lipid alteration of phospholipids, including PC, SM, PE, PS, and PI, in lipid rafts would have particular effects on displacing proteins from the functional lipid rafts. EPA treatment in T-cells resulted in the enrichment of PUFAs in the cytoplasmic leaflet as well as the exoplasmic leaflet of rafts (10). Therefore, we analyzed the incorporation of fatty acids in raft lipids not only in the exoplasmic lipid leaflet (PC and SM) (Tables 2, 3) but also in the cytoplasmic membrane lipid leaflet (PE, PI, and PS) (Tables 4–6) after DHA treatment. DHA treatment significantly increased the concentrations of PC species substituted with 36:3 (16:0/20:3), 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 (Table 2). SM consists of choline, ceramide, and fatty acids and is very difficult to split by electrospray ionization MS. Table 3 shows the total amounts of carbon and unsaturated bonds of fatty acids and ceramide. Substitution of SM with 38:3 acyl chains was increased significantly in rafts of DHA-treated cells compared with control cells (Table 3). Alteration of the fatty acyl composition of exoplasmic leaflet lipids (PC and SM) could indirectly affect the displacement of proteins from lipid rafts (10). 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), and 40:5 (18:0/22:5, 18:1/22:4, 18:2/22:3, 20:1/20:4) acyl chains (Table 4); PS species substituted with 38:4 (18:0/20:4), 38:6 (18:0/20:6), and 40:5 (18:0/22:5) acyl chains (Table 5); and PI species substituted with 36:4 (16:0/20:4), 40:6 (18:0/22:6), and 40:5 (18:0/22:5) acyl chains in rafts in DHA-treated cells compared with control cells (Table 6). DHA

treatment changes the fatty acyl composition of lipids from the cytoplasmic leaflet in rafts. Thus, the alterations of fatty acyl chains in the cytoplasmic and exoplasmic lipid leaflet of lipid rafts from DHA-treated cells play an important role in raft structure and its lipid environment.

In this study, we found that after IL-2 stimulation in Jurkat T-cells, IL-2Rα, IL-2Rβ, and IL-2Rγ_c chain proteins were detected in the fractions of membrane lipid rafts (Fig. 2), consistent with the data of Matkó, Bodnár, and Vereb (26). It was also confirmed using fluorescence resonance energy transfer and confocal microscopy that IL-2Rα, IL-15Rα, IL-2/15Rβ, and IL-2Rγ_c subunits formed supramolecular receptor clusters in lipid rafts (27). These findings were different from those of a previous study (28), which suggested that IL-2Rα was enriched in lipid rafts and that the IL-2Rβ and IL-2Rγ_c chains were found only in detergent-soluble membranes. Our results indicate that DHA treatment caused IL-2Rα, IL-2Rβ, and IL-2Rγ_c subunit displacement from membrane lipid rafts. The partial IL-2R chains removing from lipid rafts to soluble membrane fractions were very important for the DHA-induced suppression of the IL-2R signaling pathway.

The phosphorylated Janus family kinases (JAK1 and JAK3 kinases and phosphotyrosine) in response to IL-2 were not detected in lipid rafts but in soluble membrane fractions (Fig. 3), consistent with a previous report (26). To date, very little data are available for the influence of PUFAs on the signaling properties of cytokine receptors and JAKs in rafts (27, 29). Some results have indicated that JAKs were detected in rafts, whereas other reports showed JAKs enriched in detergent-soluble membranes (27). In addition, according to the standard model of STAT signaling upon IL-2-induced receptor activation, monomeric cytosolic STAT5s are recruited to the cytoplasmic tail of the respective plasma membrane receptor and phosphorylated by JAK family kinases, then dimerize and translocate to the nucleus to modulate gene expression. Most of reports showed that the association of STATs with plasma membrane rafts was always excluded from cellular membranes (30–35). However, in 1997, Koshelnick and colleagues (36) first found STAT1, one of the STAT families, in a low density, detergent-resistant fraction of human kidney tumor epithelial cells. Later, STAT3, activated PY-STAT3, and PY-STAT1 were also detectable in this special domain of cell membrane (35), which initiated the study of "raft-STAT signaling transduction" in many cell types (37). Furthermore, in this study, we detected the presence of STAT5a and STAT5b not only in detergent-soluble fractions but also in detergent-resistant rafts. In addition, after DHA treatment, STAT5a and STAT5b were not detected in lipid raft fractions but were located in detergent-soluble fractions. In addition, the results in Fig. 5 showed that phosphorylated STAT5 was associated with detergent-soluble fractions and decreased with DHA treatment. We suggest that STATs in membrane rafts may be a transient early stage in cytokine signaling and that membrane rafts containing STATs may contain physical sites for integrating the combinatorial effects of different cytokines and different activation pathways. Our discovery of the alteration of STATs in rafts was an extension of work characterizing the subcellular distribution of STAT proteins.

In summary, our data strongly demonstrate that altered raft lipid environment affects the membrane subdomain distribution of proteins in the IL-2R signaling pathway in DHA-treated T-cells. Such partial disorganization in lipid rafts induced by DHA treatment mediates the immunodepressive function and its immunomodulatory effect. However, the exact mechanism underlying these alterations after PUFA treatment remains obscure in most instances, whether in vivo or in a given clinical situation. This awaits further investigation.

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