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Differential effects of ω-6 and ω-3 fatty acids on interleukin-2 production and mRNA expression by EL-4.IL-2 cells

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Our studies with lupus-prone (NZBxNZW)F_1 (B/W) female mice have indicated that dietary ω-3 lipids (menhaden oil) significantly extend the life span and delay the onset of autoimmune disease, while ω-6 lipids (corn oil) shorten the life span by accelerating the onset and progression of autoimmune disease, probably by altering the cell and subcellular membrane fatty acid composition. To understand the mechanisms through which ω-6 (linoleic acid, 18:2, and arachidonic acid, 20:4) and ω-3 (eicosapentanoic acid, 20:5, and docosahexanoic acid, 22:6) lipids exert their differential effects, we have studied the effects of these fatty acids in vitro *on cell proliferation, peroxidation, interleukin-2 (IL-2) production, IL-2 mRNA levels, and surface IL-2 receptor (IL-2R) expression in an IL-2 producing mouse lymphoma cell line (EL-4.IL-2; EL-4). When EL-4 cells were cultured in the presence of individual ω-6 and ω-3 fatty acids (at a final concentration of 10 μg/mL), the respective fatty acid was found to incorporate into the cells at a significant level, and no adverse effects were noted either on the viability of the cells or on the* de novo *DNA synthesis. In addition, lipid peroxidation, as measured by the generation of thiobarbituric acid-reactive substances, was significantly higher ($P < 0.05$) in cells incubated with 20:4ω-6 as compared with control cells (to which no fatty acid was added). Also, 20:4ω-6 significantly inhibited ($P < 0.05$) IL-2 production when compared with other fatty acids. Northern blot analysis revealed that this inhibition in IL-2 production by 20:4ω-6 was at the gene level, as seen by an inhibition in phorbol-12-myristate-13-acetate induced IL-2 mRNA levels by 20:4ω-6. Compared with saturated fatty acids, both ω-6 and ω-3 lipids induced higher IL-2R surface expression, as seen by flow cytometry. These studies suggest that dietary ω-3 lipids lower membrane lipid peroxidation, and thereby may preserve normal immunological functions that may delay the course of autoimmune disease in B/W mice.* (J. Nutr. Biochem. 6:467–473, 1995.)

Keywords: ω-6 fatty acids; ω-3 fatty acids; proliferative response; lipid peroxidation; IL-2 levels; IL-2 mRNA; IL-2R expression; EL-4

Introduction

Dietary ω-3 lipids, as compared with ω-6 lipids, delay the onset of autoimmune disease and prolong the survival of autoimmune lupus-prone NZW and (NZBxNZW)F_1 (B/W) mice by maintaining an improved immune surveillance.[1–3] Splenocytes from B/W mice fed ω-3 lipids exhibit a higher proliferative response to antigens and superantigens, have altered cytokine expression, and express certain oncogenes

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at a lower level as compared with mice fed an ω-6 lipid-rich diet.[4,5] The spleen cells of 6.5-month-old B/W mice receiving a diet enriched with ω-3 lipids (fish oil; FO) exhibited significantly higher proliferative response and produced higher levels of interleukin-2 (IL-2) when compared with mice fed similar levels of ω-6 lipids (corn oil; CO).

Omega-3 and ω-6 fatty acids mediate specific effects on immune functions in vivo as well as in an in vitro system by modulating membrane fatty acid composition and thereby further altering the lipid turnover, membrane fluidity, permeability, cell surface receptor-ligand interactions,[6–13] and activity of several membrane-bound and cytosolic enzymes.[11–13] Fatty acids also serve as precursors of such biologically active substances as prostaglandins and leukotrienes, which in turn influence cellular metabolism and function[14–16]. Some of these fatty acids may enhance susceptibility of subcellular membranes to oxidative stress in an immune-compromised host and may lead to several pathological conditions.[17,18]

Omega-3 lipids are generally known to decrease the levels of proinflammatory cytokines (IL-1, IL-6, tumor necrosis factor α [TNFα], etc.) and increase anti-inflammatory cytokines (e.g., IL-2) in mice.[4,5] In an earlier communication, we had reported the effects of ω-6 and ω-3 fatty acids on the proliferation, IL-2 production, and thiobarbituric acid reaction products (TBARS) generation of EL-4 cells.[19] Since T-cell functions are regulated mainly by IL-2 and IL-2R levels,[12] and cytokine imbalance is one of the contributing factors in autoimmune diseases and cancers, we have investigated the effects of individual ω-6 and ω-3 fatty acids on fatty acid composition, lipid peroxidation, and functional characteristics, such as proliferative response, IL-2 production, IL-2 mRNA expression, and IL-2 receptor levels in a mouse lymphoma cell line (EL-4.IL-2).

Methods and materials

Cell lines

EL-4.IL-2 (EL-4) and IL-2 dependent cytotoxic T-cells (CTLL-2) were obtained from American Type Culture Collection (ATCC, Rockville, MD USA). The EL-4 cell line is derived from C57BL/6 mice and is a subline of EL-4 (ATCC TIB 39) cells. EL-4 cells produce IL-2 when stimulated with phorbol-12-myristate-13-acetate (PMA) (20 ng/mL) and the IL-2 is biologically indistinguishable from normal spleen cell derived IL-2.[20]

Reagents

Dulbecco's modified Eagle's medium (DMEM) was obtained from GibcoBRL (Grand Island, NY USA). Fatty acid esters, borontrifluoride-methanol, PMA, and MTT (3-[4,5-dimethylthiozol-2-yl]-2,5-diphenyl-tetrazolium bromide) were obtained from Sigma Chemical C. (St. Louis, MO USA). Thymidine, [methyl-^{3}H] ([^{3}H]-TdR; 6.7 Ci/mmol) was obtained from New England Nuclear (Wilmington, DE USA), [α-^{32}P]dCTP (3000 Ci/mmol) from Amersham (Arlington Heights, IL USA), and recombinant mouse IL-2 (rmIL-2) from Pharmingen (San Diego, CA USA).

Culturing conditions

EL-4 cells were grown in DMEM supplemented with glucose (4.5 g/L), penicillin, streptomycin, and heat-inactivated horse serum at 10%, in 25 cm^2 tissue culture flasks in 5% CO_2:95% air in a humidified atmosphere. Fatty acid esters were dissolved in ethanol and stored at −80°C, with working dilutions prepared in medium just before use. Cells were seeded at 1×10^6 cells/mL, individual fatty acid (30 to 34 μM) and 20 ng/mL, of PMA, and the cells were then grown in DMEM with 1% serum.

Lipid extraction and fatty acid analyses

Cultured cells were washed twice with cold PBS, the cell pellet was resuspended in 1 mL of PBS and transferred to glass tubes with Teflon lined screw caps; lipids were extracted with 5 mL of chloroform-methanol (2:1, vol/vol). The lower phase was removed, washed once with water, dried under a stream of nitrogen, and resuspended in 1 mL of hexane. Fatty acid methyl esters were obtained by transesterification with 1.5 mL of borontrifluoride-methanol reagent (90°C, 2 hr). One milliliter of water was then added, the mixture was vortexed, and the top clear layer containing methyl esters was removed.[21] After solvent evaporation by a stream of nitrogen, the lipids were resuspended in a small volume of hexane and analyzed on a Perkin Elmer-8420 gas chromatograph employing a fused silica capillary column (DB-225: 30 m × 0.25 mm; J & W Scientific, Folsom, CA USA), as described.[22] Chromatographic conditions were: column oven temperature, 205°C; injection port temperature, 250°C; flame ionization detector, 250°C; and carrier gas helium at 0.9 mL/min. A fatty acid ester standard (Nu-Check-Prep; Elysian, MN USA) was utilized for peak identification.

Proliferation assay

EL-4 cells were cultured at 1×10^4 cells/0.2 mL of DMEM with 5% serum. The cells were grown either in the presence or absence (control) of individual fatty acids (10 μg/mL) for 24, 48, and 72 hr. The wells were pulse labeled with 0.5 μCi of [^{3}H]TdR for the last 4 hr and harvested onto filter paper. [^{3}H]TdR incorporation was determined in a beta scintillation counter,[23] and the results from triplicate wells were expressed as mean ± SEM.

TBARS generation

In order to assess the extent of lipid peroxidation, generation of TBARS was carried out by the thiobarbituric acid (TBA) assay[24] with slight modification. After incubation with selected fatty acid, the cells were washed twice with PBS and resuspended in 1 mL of PBS. One milliliter of 0.67% thiobarbituric acid reagent and 0.1 mL of 100% trichloroacetic acid were added to the cell suspension in screw-capped glass tubes. The tubes were heated for 20 min in a boiling water bath then cooled by immersion in ice. After centrifugation for 15 min at 1,200 rpm, the absorbance of the supernatant containing MDA-thiobarbituric complexes was measured at 535 nm. Malondialdehyde tetramethyl acetal (Aldrich Chemical Company Inc., Milwaukee, WI USA) was used as a reference standard.

IL-2 production

EL-4 cells were grown in the presence of individual fatty acids. Culture supernatants were harvested 24 hr later. These supernatants were frozen, thawed, titrated by serial dilutions, and assayed for the presence of IL-2 by their ability to support the proliferation of an IL-2 dependent murine cell line, CTLL-2.[25] CTLL-2 cells (5×10^3 CTLL cells/well) were seeded in triplicate, with or without diluting the supernatant. Cell proliferation was determined colorimetrically utilizing MTT.[26] Results were expressed as U/mL and were determined by comparison with a standard curve generated with rmIL-2.

RNA isolation and Northern blot analysis

Total RNA was extracted from the EL-4 cells by the acid guanidinium isothiocyanate-phenol-chloroform extraction procedure.[27] Twenty micrograms of total RNA was separated by horizontal electrophoresis in an 0.8% agarose/2.2 M formaldehyde gel, electroblotted onto nitrocellulose (Schleicher & Schuell, Keene, NH USA), and fixed to the membrane by cross-linking using a Stratagene UV crosslinker-1800 (Stratagene, La Jolla, CA USA). The blots were prehybridized, hybridized, and washed as described[28] then incubated with Kodak XAR-5 film with Dupont intensifying screens at −80°C for autoradiography. The human IL-2 cDNA[29] used in this study cross-hybridizes with mouse IL-2 mRNA (0.70 kb, Pst I fragment, Oncor, Gaithersburg, MD USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH)[30] (1.0 kb *Bam*HI-Pst I fragment, ATCC) was used as an internal control. The cDNA probes were labeled with [α^{32}P]dCTP by random primer labeling kit (Boehringer Mannheim, Indianapolis, IN USA) to a specific activity of ~0.8 to 1.2 × 10^9 cpm/μg. The intensity of autoradiographic bands was quantified by videoimage analysis using NIH Image 1.4 program, and the values were expressed in fold change as a ratio of the densitometric value of IL-2 to that of the GAPDH.

Immunofluorescent staining and flow cytometry

To determine the expression of IL-2R, EL-4 cells cultured with and without ω-6 and ω-3 fatty acids was examined by flow cytometry[31] using an anti-mouse FITC-conjugated IL-2R mAb (PharMingen, San Diego, CA USA). Cells were washed two times with 4 mL of phosphate-buffered saline (PBS) with 3% FBS. One million cells in 100 μL of PBS with 2.5% FBS (PBS-FBS) were incubated with 1 μg of antibody at 4°C for 45 min. The cells were then washed twice with PBS-FBS and the cell pellet was resuspended in 200 μL of PBS-FCS, fixed in 1% formaldehyde. Data analyses were performed by using the FACStar Plus flow cytometer (Beckton Dickinson, Mountain View, CA USA) using FACScan software. Dead cells, if any, were excluded by forward and side scattering.

Statistical analysis

The values are expressed as means ± SEM. Statistical analyses of the data were carried out using Statview 4.0/Super ANOVA package software (Abacus Concepts, Berkeley, CA USA). Fatty acids data and data on proliferative response were analyzed using one-way ANOVA, and the data on IL-2R expression were analyzed by two-way ANOVA. Where a significant F ratio was found ($P < 0.05$), Fisher's PLSD (protected least significant difference) test was used to describe differences in the means between group. Data on TBARS and IL-2 production were analyzed using Student's *t*-test with Bonferroni adjustment, and $P < 0.05$ was considered significant.[32]

Results

Fatty acid composition of EL-4 cells cultured with individual ω-3 and ω-6 fatty acids

The major fatty acids detected in control EL-4 cells were 18:0, 18:1, and 16:0 with lesser amounts of 18:2ω-6, 16:1, 20:4ω-6, and 14:0 with trace amounts of 14:1 and 18:3ω-3 and 24:0 (data not shown). When EL-4 cells were cultured with individual fatty acids for 48 hr respective fatty acids were found to be incorporated into the cells. Culturing the EL-4 cells with either 18:2ω-6 or 20:4ω-6 resulted in a significant increase in the 18:2ω-6 level in the cells as compared with control and other groups, while culturing with 20:4ω-6 significantly increased the level of 20:4ω-6 compared with other treatments. Culturing the cells in the presence of 20:5ω-3 resulted in a significant increase in 20:5ω-3 and 22:6ω-3 levels when compared with other groups. With 22:6ω-3 supplementation, the level of 20:5ω-3 was increased while the 22:6ω-3 level was not elevated.

Viability and proliferative response

Since higher concentrations of fatty acids (20 μg/mL) were found to be toxic in vitro (especially in case of 20:4ω-6; data not shown), the present study was carried out at 10 μg/mL. When EL-4 cells were cultured with individual fatty acids there was no significant effect on the viability of EL-4 cells as determined by trypan blue dye exclusion (95 to 110% as compared with control). However, a significant decrease in proliferative response, as assessed by [^{3}H]TdR incorporation, was observed at 24, 48, and 72 hr in cells cultured with 20:4ω-6 compared with the other groups (*Figure 1*). At 24 and 48 hr, EL-4 cells cultured with 18:2ω-6 and 20:5ω-3 did not exhibit any decrease in proliferative response when compared with the control group, while 22:6ω-3 significantly decreased proliferation compared with control, 18:2ω-6, and 20:5ω-3 groups at 48 and 72 hr.

TBARS generation

TBARS generation was measured in order to determine if changes in viability or proliferative response of EL-4 cells to indicated fatty acids is due to alteration in lipid peroxidation (TBARS generation in control: 8.52 ± 2.5; 18:2ω-6: 10.20 ± 3.0; 20:4ω-6: 17.97 ± 3.2; 20:5ω-3: 3.84 ± 2.5; and 22:6ω-3: 5.94 ± 3.1 nmol/1 × 10^3 cells). When cells were cultured with 20:4ω-6, TBARS generation was significantly higher in this group compared with control, 20:5ω-3, and 22:6ω-3 groups.

IL-2 production

EL-4 cells were cultured in the presence of individual fatty acids and IL-2 levels were measured in cell-free superna-

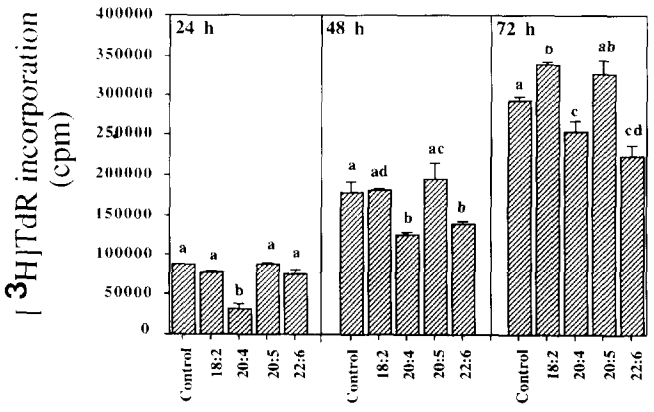

Figure 1 Time course showing the effect of individual ω-6 or ω-3 fatty acids (10 μg/mL) on the proliferative response of EL-4 cells. Values are mean ± SEM. The data were analyzed by one-way ANOVA and means with different superscripts are significantly different (at $P < 0.05$) for one time of incubation as revealed by Fisher's PLSD test.

tants. A bioassay utilizing an IL-2 responsive CTLL-2 cell line was employed. Culturing with 20:4ω-6 inhibited significantly IL-2 production, while 22:6ω-3 significantly increased IL-2 production as compared with the other groups (*Figure 2*). Culturing cells in the presence of PMA and fatty acids increased IL-2 production, but the increase was only in the case of PMA + 20:4ω-6 group compared with 20:4ω-6 group ($P < 0.001$). The data also revealed that EL-4 cells, being an IL-2 producing cell line, can produce IL-2 with or without PMA stimulation.

IL-2 mRNA levels

Because 20:4 ω-6 significantly inhibited IL-2 production by EL-4 cells, we sought to examine whether the inhibitory effect is at the IL-2 gene level. *Figure 3* demonstrates the effects of 20:4ω-6 on steady-state IL-2 mRNA levels as assessed by Northern blot analysis after stimulation with PMA for various time periods. Densitometric analysis of the autoradiographic bands is presented at the bottom of *Figure 3*. In control EL-4 cells, IL-2 mRNA levels were detected at a low level and remained low even after 48 hr of culture. Addition of 20:4ω-6 to the culture neither stimulated nor inhibited IL-2 mRNA, and the levels remained similar to those of control (*Figure 3*). However, PMA significantly elevated IL-2 mRNA expression at 30 hr (IL-2/GAPDH ratio, PMA: 1.69 ± 0.036; control, 0.91 ± 0.039; $n = 2$), and the level remained high even at 48 hr of incubation (IL-2/GAPDH ratio 1.88 ± 0.078 for PMA; control, 0.89 ± 0.016). When 20:4ω-6 was added in the presence of PMA, significant inhibition was noted at 30 hr (PMA: 1.69 ± 0.036; PMA + 20:4ω-6, 0.096 ± 0.056) and 48 hr (PMA − 1.88 ± 0.078; PMA + 20:4ω-6, 0.99 ± 0.017), and the levels were similar to those seen in unstimulated control cells.

Effect of 20:4ω-6, with and without PMA on the fatty acid composition of EL-4 cells cultured for 24 hr.

As the results from our previous experiments suggested that 20:4ω-6 exerted inhibitory effect on proliferation, IL-2 production, IL-2 mRNA expression, we analyzed the effect of culturing EL-4 cells with 20:4ω-6, with and without PMA, on the level of 20:4ω-6 in the cells after 24 hr of culturing. The effect of 24 hr culturing of EL-4 cells with PMA and 20:4ω-6 as main effectors on the fatty acid composition is presented in *Table 1*. Analysis by two-way ANOVA (20:4ω-6 and PMA as main effectors) revealed significant differences ($P < 0.01$ to < 0.0001) in all the fatty acids when EL-4 cells were cultured with 20:4ω-6, while 18:2ω-6 and 20:4ω-6 fatty acids showed significant differences in the presence of PMA alone. Where a significant F ratio ($P < 0.05$) was found, Fisher's PLSD was used to describe the differences in the means between the groups. PMA and 20:4ω-6 did not alter the levels of 14:0 and 14:1. Culturing the cells in the presence of 20:4ω-6 or 20:4ω-6 + PMA significantly increased the level of 16:0, 20:4ω-6, and 22:6ω-3 levels and significantly decreased the levels of 16:1, 18:1, 18:2ω-6, and 18:3 as compared with control and PMA groups.

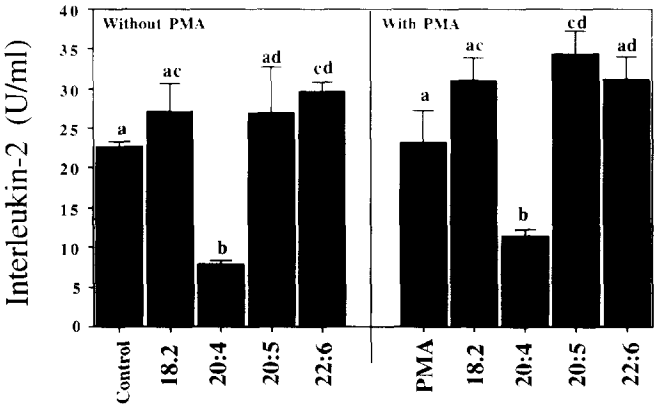

Figure 2 Effect of ω-6 and ω-3 fatty acids and PMA on IL-2 production by EL-4 cells after 24 hr of culturing. Values represent the mean ± SEM of three independent observations. Means with different superscripts are significantly different (at $P < 0.05$) as revealed by Student's *t*-test with Bonferroni adjustment

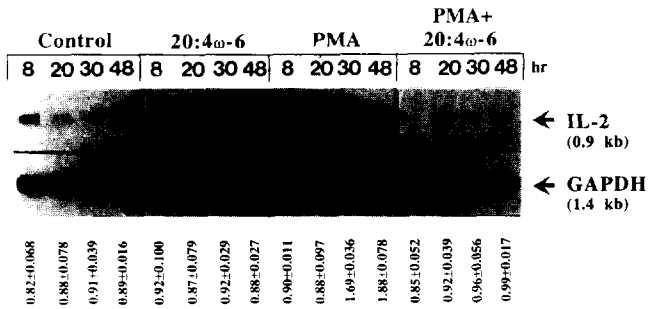

Figure 3 Effects of 20:4ω-6 and/or PMA on IL-2 mRNA in EL-4 cells by Northern blot analysis (a representative autoradiogram was shown). Twenty micrograms of total RNA per lane was electrophoresed, electroblotted onto nitrocellulose, and fixed by UV irradiation. The blot was probed with [α-^{32}P]dCTP-labeled IL-2 cDNA, stripped, and reprobed with [α-^{32}P]-labeled GAPDH cDNA. mRNA size was determined in comparison to the relative mobility of 28S and 18S rRNA and to that of the mRNA ladder (0.24 to 9.5 kb; GibcoBRL). Densitometric analysis (mean ± SEM) of autoradiographic bands from two independent experiments was shown in the bottom. Values shown are the ratio of the numbers obtained for IL-2 divided by those for GAPDH.

IL-2R levels by flow cytometry

The EL-4 cells were cultured for 24 hr in the presence of individual fatty acids, without and with PMA, to determine their effects on IL-2R expression. FACS analysis revealed that the percentage of IL-2R positive cells was very low (2%) when the cells were grown in the presence of saturated (16:0 and 18:0) fatty acids (*Figure 4*). When analyzed by two-way ANOVA, significant main effects were found with fatty acids and PMA ($P < 0.0001$). Where a significant ($P < 0.05$) F ratio was found, Fisher's PLSD was used to describe the differences in the means between the groups. Culturing with 18:2ω-6 caused a marginal but significant increase in the number of IL-2R positive cells while cell cultured with 20:4ω-6, 20:5ω-3, and 22:6ω-3 resulted in a highly significant increase in IL-2R positive cells. Addition of PMA alone increased significantly the number of IL-2R

Table 1 Effect of 20:4ω-6 supplementation (with or without PMA) on fatty acid composition of EL-4 cells cultured for 24 hr

Fatty acids	Control	Fatty acid or PMA added to cell culture			Level of significance (Main effects)	
		20:4ω-6	PMA	PMA + 20:4ω-6	20:4ω-6	PMA
		(Fatty acid composition of cells, percent of total)				
14:0	2.45 ± 0.44^{a}	2.79 ± 0.07^{ac}	2.52 ± 0.01^{a}	2.97 ± 0.15^{bc}	†	NS
14:1	1.93 ± 0.19^{ad}	1.57 ± 0.37^{bd}	1.92 ± 0.32^{bc}	1.37 ± 0.01^{b}	†	NS
16:0	16.8 ± 0.45^{ce}	23.8 ± 0.23^{a}	16.47 ± 1.24^{de}	22.31 ± 0.61^{b}	‡	NS
16:1	3.78 ± 0.10^{bd}	1.89 ± 0.17^{a}	3.93 ± 0.50^{cd}	2.24 ± 0.07^{a}	†	NS
18:0	23.55 ± 0.43^{a}	23.32 ± 0.09^{a}	24.57 ± 0.07^{c}	22.56 ± 0.17^{b}	‡	NS
18:1	33.97 ± 1.00^{b}	19.19 ± 0.89^{a}	31.59 ± 0.97^{c}	19.85 ± 0.05^{a}	‡	NS
18:2ω-6	15.4 ± 0.46^{bd}	13.13 ± 0.58^{a}	16.79 ± 0.24^{cd}	13.28 ± 0.4^{a}	‡	†
18:3	0.66 ± 0.13^{b}	0.46 ± 0.01^{a}	0.65 ± 0.01^{b}	0.51 ± 0.01^{a}	‡	NS
20:4ω-6	1.46 ± 0.18^{ce}	10.32 ± 0.56^{a}	1.56 ± 0.13^{ce}	11.44 ± 0.25^{b}	‡	†
22:6ω-3	ND^{b}	3.56 ± 0.70^{a}	ND^{b}	3.48 ± 0.09^{a}	‡	NS

EL-4 cells were cultured for 24 hr at 37°C in the presence of 20:4ω-6 (at a final concentration of 10 μg/mL), with or without PMA (20 ng/mL). Values are the mean ± SEM of three independent experiments. Data were analyzed by two-way ANOVA with 20:4ω-6 and PMA as main effectors with significance at †$P < 0.01$ and ‡$P < 0.001$. Means with two different superscripts in the same row are significantly different at $P < 0.05$ as revealed by Fisher's PLSD test.

positive cells, and with the exception of 20:4ω-6, all the other unsaturated fatty acids tested acted in synergy with PMA in inducing IL-2R expression (*Figure 4*). However, saturated fatty acids did not synergize with PMA in inducing IL-2R expression (*Figure 4*).

Discussion

Specific dietary lipid interventions delay autoimmune diseases and the incidence and severity of certain types of cancers.[33–35] We and others have indicated earlier that ω-3 lipid-rich FO dramatically extends the life span and delays the onset of autoimmune disease in B/W mice, while ω-6 lipid-rich CO shortens the life span and accelerates the disease.[5,36] We have previously reported that dietary lipids alter IL-2 and PGE_2 production by spleen cells.[10,13] In an attempt to understand further the mechanisms of action of ω-3 lipids on immune functions, we investigated the effects of individual ω-6 and ω-3 fatty acids in an in vitro system utilizing IL-2 secreting EL-4 cell line.

Culturing EL-4 cells in the presence of individual fatty acids significantly increased the respective level of fatty acid in the cells with the exception of 20:6ω-3; this indicated that it is possible to alter the fatty acid composition of EL-4 cells by adding specific fatty acid to the culture medium. Since higher concentrations of certain fatty acids had toxic effects on the cells (data not presented), all experiments reported in the present study were carried out at a concentration of 10 μg/mL. At this concentration, the cells appeared healthy and their viability was similar to that of control cells (>95% by trypan blue dye exclusion). When EL-4 cells were cultured for 48 hr, 18:2ω-6 increased [^{3}H]TdR incorporation, and 20:4ω-6 and 22:6ω-3 decreased [^{3}H]TdR incorporation into the cells as compared with the control, indicating the differential effects of polyunsaturated fatty acids on cell proliferation. It has been suggested that fatty acid metabolism, intracellular lipid second messengers (including eicosanoids), and antioxidant status contribute to the regulation of cell proliferation.[37]

Addition of 20:4ω-6 to EL-4 cells significantly decreased IL-2 production as compared with control. Peroxidation, as measured by TBARS generation, was significantly higher in cells cultured with 20:4ω-6 as compared with the other fatty acids, suggesting that lipid peroxidation may modulate IL-2 production. 20:4ω-6 was reported earlier to inhibit IL-2 production in a dose-dependent manner, and incubation of cells with fatty acids not associated with PG precursors did not affect mitogen-induced IL-2 production by peripheral mononuclear cells.[38] Omega-6 fatty acids may also inhibit IL-2 production through secondary mediators such as lipoxygenase pathway products. IL-2 production can be reduced markedly by small changes in cellular

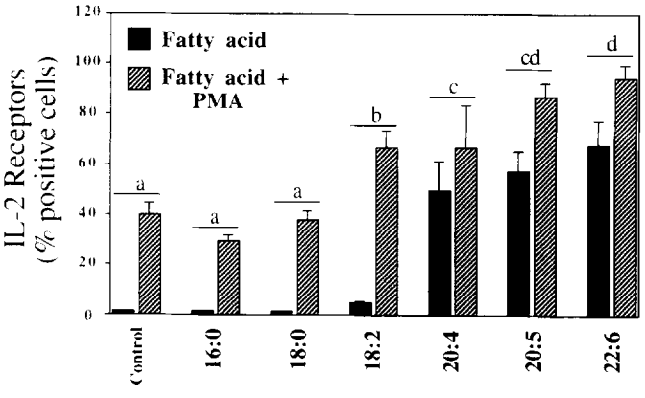

Figure 4 Effects of ω-6 and ω-3 fatty acids, with and without PMA, on IL-2 receptor expression in EL-4 cells, as determined by flow cytometry. Values are mean ± SEM of three independent experiments. Analysis by two-way ANOVA revealed significant main effects with fatty acids and PMA ($P < 0.0001$). The superscripts indicate the effects of fatty acids on IL-2 receptors and means with different superscripts are significantly different at $P < 0.05$ as revealed by Fisher's PLSD test.

fatty acids, suggesting that minimal alteration of dietary essential fatty acids may have marked effects on immune responses.[39] Another very interesting observation emerging from the present study is the indication that the surface expression of IL-2R was higher in EL-4 cells treated with ω-6 and ω-3 fatty acids compared with cells treated with saturated fatty acids. This may be another possible way through which certain fatty acids may be able to exert their specific action on the immune functions. Although 20:4ω-6 was able to inhibit IL-2 protein levels and mRNA expression, IL-2R expression was considerably higher, as compared with control.

The immunoregulatory role of fatty acids and PGs can be very crucial in the pathogenesis of diseases associated with persistent T-cell activation, such as autoimmune or inflammatory disorders. For instance, PGE_2 produced by macrophages can activate PGE_2 receptor positive lymphocytes to stimulate suppressor T cells[40] and can promote the secretion of soluble suppressor factors that are known to cause immune suppression.[41] Similar mechanisms might alter the ability of the fatty acids to influence a normal T-cell function in producing IL-2.

In addition to lowering proinflammatory cytokines, ω-3 lipids may also exert a protective action by preventing excess production and utilization of IL-2 in immune cells and may decrease TBARS generation.[5] Although results from in vitro studies need to be interpreted cautiously, it may be possible to conclude from the present studies on EL-4 cells that excess 20:4ω-6 incorporation in the absence of ω-3 lipids has deleterious effects on autoimmune disease as a result of increasing susceptibility to lipid peroxidation, thereby lowering the IL-2 production, IL-2 mRNA, and IL-2R expression. This finding is compatible with results from our laboratory and others which indicate that ω-6 fatty acids solely derived from vegetable oils accelerate autoimmune renal disease[42–44] and increase the incidence of breast cancer[45] and sepsis in experimental animal models.[46]

The results of the present study suggest that one of the mechanisms of protective action of ω-3 lipids in autoimmune diseases may be exerted through preserving IL-2 production, IL-2 mRNA levels and IL-2R density, and by lowering TBARS generation by immune cells. Because eicosanoids, cytokines, and free radicals are known to participate in the pathogenesis of inflammation, ω-3 fatty acids may delay the onset of inflammation by preserving and maintaining the delicate balance between the pro- and anti-inflammatory cytokine and lipid mediators. Because the data collected in the present study were from EL-4 cells, a thymoma cell line, the findings need to be interpreted with caution when applying to normal cells. However, our earlier in vivo studies in B/W mice fed ω-6 and ω-3 lipids indicated that spleen cells of mice fed ω-3 lipids exhibit higher proliferative response and produce more IL-2 compared with the group fed ω-6 lipids.[5] Further work is necessary to understand the mechanisms by which ω-3/ω-6 lipids influence cytokine interactions, cytokine receptor functions, and lipid mediators both in normal cells as well as in nonautoimmune disease-prone mice and humans, so that potential fatty acid–based therapeutic strategies can be formulated for the treatment of various autoimmune diseases and cancer.

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