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Comparative effects of eicosapentaenoic acid and docosahexaenoic acid on proliferation, cytokine production, and pleiotropic gene expression in Jurkat cells

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Abstract

Comparative effects of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) acid on Jurkat T cells were investigated. The following parameters were evaluated: concanavalin A (Con A) induced proliferation, production of interleukin-2 (IL-2), IL-4, IL-10, and interferon-γ (INF-γ), and expression of pleiotropic genes by macroarray technique (83 genes in total). DHA inhibiting effect on Con A–induced proliferation was more pronounced than that of EPA. The decrease in IL-2 and INF-γ production was observed for both fatty acids, whereas the production of IL-10 was decreased by EPA only. The expression of a significant proportion of genes was altered by the fatty acids; 30% for DHA (25 genes) and 26.5% for EPA (22 genes). DHA and EPA markedly affected the expression of genes clustered as cytokines and related receptors, signal transduction pathways, transcription factors, cell cycle, defense and repair, apoptosis, DNA synthesis, cell adhesion, cytoskeleton, and hormone receptors. Therefore, the effect of fatty acids on T-lymphocyte function involves regulation of expression of important genes. Marked differences were observed between the effects of EPA and DHA, indicating that it is an over-simplification to generalize the effects of n-3 fatty acids.

Keywords: Jurkat cells; Macroarray; Eicosapentaenoic acid; Docosahexaenoic acid; Cytokines; Cell proliferation

1. Introduction

T lymphocytes play a critical role in defining the type and magnitude of the immune response. The cytokines that T cells produce regulate immune function. The mechanism leading to the propagation of T lymphocyte involves their activation, which leads to IL-2 production and subsequent proliferation, referred to as clonal expansion. Depending on the mode of activation, T lymphocytes differentiate into either a helper Th-1 or Th-2 cells (CD4+ T lymphocytes), or become cytotoxic T lymphocytes (CD8+ cells). The Th-1 cell produces primarily IL-2, INF-γ, and tumor necrosis factor–α (TNF-α), resulting in enhanced cell-mediated or cytotoxic responses. The Th-2 cell produces IL-4, IL-5, IL-6, and IL-10, generating a humoral or antibody-mediated immune response.

Several studies have demonstrated that nonesterified fatty acids inhibit T lymphocyte proliferation in response to cytokines in vitro [1]. The long chain n-3 polyunsatured fatty acids (PUFA), eicosapentaenoic acid (20:5 n-3) (EPA), and docosahexaenoic acid (22:6 n-3) (DHA) appear to be particularly potent inhibitors of lymphocyte proliferation. These fatty acids can also modulate inflammatory response, being used in the treatment of clinical conditions such as autoimmune diseases [2]. n-3 PUFA decrease IL-2 production [3], an effect associated with lowered expression of IL-2 receptor [2,4]. n-3 Fatty acids, either given in the diet or added to culture medium, can also induce apoptosis in lymphocytes [5–7].

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The mechanisms involved in the effects of fatty acids on T cell function still remain to be fully elucidated [3,8]. Although n-3 fatty acids modulate the amount and types of eicosanoids produced [9], some of their effects occur by eicosanoid-independent mechanisms, including actions upon receptors [10], intracellular signaling pathways, and transcription factors activity [11,12]. These fatty acids also modulate calcium signaling [13,14], ceramide production [15], phospholipase C activation, and subsequent generation of inositol-1,4,5-triphosphate and diacylglicerol [16–18] in lymphocytes. These fatty acids influence the production of a range of lipid-derived second messengers in lymphocytes and other leukocytes [14,19]. In addition to the mechanisms described above, the n-3 fatty acids have been shown to regulate gene expression in lymphocytes [20]. DHA and EPA can reduce the expression of adhesion molecules such as L-selectin and leukocyte function-associated antigen–1 [21]. These fatty acids have been shown to suppress the expression of IL-2 receptor α mRNA in splenic T lymphocytes [19]. DHA has been shown to alter the expression of CD8 [22]. These data demonstrate the selective effects of EPA and DHA on genes expression.

To determine precisely the effects and the mechanism of action of fish oil–derived n-3 fatty acids, it is imperative to carry out experiments with EPA and DHA separately. Fowler et al. [23] found that low dose, short term dietary supplementation with highly purified EPA or DHA can modulate select functional and signaling responses in murine splenic lymphocytes. Hung et al. [24] compared the effect of two fish oil preparations with different EPA and DHA contents on immunoglobulin productivity by lymphocytes. They found that EPA in relation to DHA has higher antiallergic activity.

The comparative effects of EPA and DHA on Jurkat cells were investigated in this study. Jurkat cells proliferation was determined by [^{14}C]-thymidine incorporation. Production of IL-2, IL-4, IL-10, and INF-γ was determined by ELISA. Expression of pleiotropic genes was analysed by macroarray technique and confirmed by reverse transcriptase–polymerase chain reaction (RT-PCR).

2. Methods and materials

2.1. Culture conditions and fatty acids treatment

Jurkat cells were obtained from the Dunn School of Pathology, Oxford University, England. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum. EPA and DHA did not show any sign of toxicity to Jurkat cells up to 50 μmol/L, as indicated by loss of membrane integrity and DNA fragmentation using flow cytometric analysis (FACS-Calibur, Becton Dickinson, San Jose, CA) [25]. The fatty acids were first dissolved in ethanol before emulsification in the serum proteins containing medium. The percentage of ethanol was always $<$0.05% of the total volume of culture medium. This concentration of ethanol was not toxic to the cells, as also observed by Siddiqui et al. [26].

2.2. Lipid extration and determination of DHA and EPA by HPLC

The lipids were extracted as previously described [27] from Jurkat cells cultured for 24 hours in the presence of 12.5 μmol/L DHA or EPA. The control cells were treated with ethanol in a concentration of 0.025% of the culture medium. This concentration was the same of that used in the cells treated with the fatty acids. The lipids were saponified using 2 mL of an alkaline methanol solution (1 mol per mL NaOH in 90% methanol) at 37°C for 2 hours in a shaking water bath. Afterwards, the alkaline solution was acidified to pH 3 with HCl solution (1 mol/mL). Fatty acids were then extracted three times with 2 mL hexane. After the extraction procedure and saponification [28,29,30], the fatty acids were derivatized with 4-bromomethyl-7 coumarim [31] and the analysis performed in a liquid chromatograph (Shimadzu model LC-10A, Shimadzu, Kyoto, Japan). The samples were eluted using a C8 column (25 cm $\times$ 4.6 i.d., 5 μm of particles) with pre-column C8 (2.5 cm $\times$ 4.6 i.d., 5 μm of particles), 1 mL per minute of acetonitrile/water (77:23 by vol) flow and fluorescence detector (325 nm excitation and 395 emission) [30]. The EPA, DHA, and margaric (C17:0) acid were obtained from Sigma Chemical (St. Louis, MO). This latter fatty acid was used to calculate recovery. The capacity factor (K′), elution sequence, linearity, recovery, precision, interference, and limit of detection were determined. The minimum limit of quantification of the fatty acids ranged from 1 to 10 ng. One curve of calibration for each standard, determining coefficients of correlation and regression, was obtained.

2.3. Proliferation assay

Jurkat cells (3.3×10^5 cells per mL) were plated in 96-well microtiter plates and treated for 48 hours with EPA and DHA at 12.5, 25, 50, 75, and 100 μmol/L. [^{14}C]-Thymidine (1 μCi per mL) was added to the medium at the beginning of the experiment. The plates were incubated in a humidified atmosphere of 5% CO_2 and 95% air at 37°C. Cells were harvested and the radioactivity of the [^{14}C]-thymidine incorporated into DNA was determined by using a liquid scintillation counter (Packard TRI-CARB 2100 TR counters; Downers Grove, IL). The incorporation of [^{14}C]-thymidine is expressed as total counts per minute.

2.4. Measurement of cytokines

The cells (2×10^5 cells per mL) were plated in 24 wells plate and treated for 24 hours with DHA and EPA at 12.5 μmol/L. This concentration is in the plasma physiological range [32] and it was the same as that used to study pleio-

tropic gene expression. The cells were then cultured for another 24 hours in the presence of 25 μg per mL of concanavalin A (Con A), which is a T lymphocyte mitogen [33], and the fatty acids. Afterwards, cell culture supernatant fluid was harvested to carry out the determination of secreted cytokines.

The production of IL-2, IL-10, IL-4, and INF-γ was evaluated by enzyme-linked immunosorbent assay (ELISA) using Kit OptEIA from Pharmingen (San Diego, CA). The detection limit of IL-2, IL-10, and IL-4 was 7.8 pg per mL and of INF-γ was 4.7 pg per mL according to the manufacturer.

2.5. Treatment of Jurkat cells with the fatty acids to evaluate pleitropic genes expression

Cells were resuspended at a density of 2×10^5 per mL in 25-cm^3 flasks and treated for 24 hours with DHA and EPA at 12.5 μmol/L concentration.

2.6. Total RNA extraction

Total RNA was obtained from 0.5–1 $\times$ 10^7 cells using Trizol reagent (Life Technologies, Rockville, MD). Briefly, the cells were lysed using 1 mL Trizol reagent (Life Technologies). After 5 minutes of incubation at room temperature, 200 μL chloroform were added to the tubes and centrifuged at 12,000 $\times$ *g*. The aqueous phase was transferred to another tube and the RNA was pelleted by centrifugation (12,000 $\times$ g) with cold ethanol and dryed in air. RNA pellets were eluated in RNase-free water and treated with DNase I. After, RNAs were stored at −70°C until to the time of the experiment. The RNA was quantified by measuring absorbance at 260 nm. The purity of the RNAs was assessed by the 260/280 nm ratio and on a 1% agarose gel stained with ethidium bromide at 5 μg per mL [34]. These samples were used for macroarray and RT-PCR analysis.

2.7. Synthesis of cDNA probes

The cDNA probes were synthesized using the pure total RNA labeling system Atlas Kit according to manufacturer's recommendations (Clontech Laboratories, New Jersey). Briefly, 10 μg of total RNA and 2 μL primers mix "CDs" (a mixture of primers specific for each different type of Atlas Array) were heated at 70°C for 5 minutes in a TechneGenius Thermal cycler (Oxford, UK). The temperature was decreased to 50°C and 13.5 μL of the mix of the following reagents were added: 4 μL reaction buffer 5x concentrated, 0.5 μL 100 mmol/L DTT, 2 μL 10x concentrated dNTP mix (dCTP, dGTP, dTTP), 5 μL of [α-^{33}P] ATP (at 10 μCi/μL), and 2 μL of reverse transcriptase enzyme (Life Technologies). The reaction was incubated for 25 minutes at 50°C and stopped by using 2 μL Termination Mix. The ^{33}P-labeled probe was purified from unincorporated nucleotides by passing the reaction mixture through a push column (NucleoSpin Extraction Spin Column, Clontech Laboratories). Experiments using 20 μg total RNA were also performed and the results indicated saturation of the hybridization reactions (data not shown).

2.8. Macroarray hybridization

All solutions for hybridization were obtained from Clontech Laboratories. The listing of genes represented on the macroarray membranes is available on the Clontech website (www.bdbiosciences.com/clontech/atlas/genelists/k1840-1_HuTrial.pdf). The membrane was pre-hybridized for 30 minutes at 68°C in Express Hyb containing 50 μg freshly denaturated salmon sperm DNA. Subsequently the membrane was hybridized during 18 hours at 68°C with 2×10^6 cpm per mL ^{33}P-labeled denaturated probe. The membrane was washed twice at 68°C with 1 $\times$ SSC, 0.1% SDS; followed by two washings in 1 $\times$ SSC, 1% SDS, then exposed to phosphor screen for 48 hours and scanned in the Storm 840 (Molecular Dynamics, Sunnyvale, CA). The results were obtained from two membranes used just once.

2.9. Analysis of macroarray results

Changes in the genes expression induced by the fatty acids were analyzed by comparison with untreated cells using the software Array-Pro Analyzer, version 4 (Media Cybernetics, Silver Spring, MD). Local ring background was subtracted from the density value of each spot to obtain a "net" value. Spots with a mean intensity of greater than 1.2 times the mean local background intensity were further considered as "measurable spots." Normalization was done by calculating total intensity ratios and using the housekeeping gene β-actin (the same used for RT-PCR analysis) present in the membrane. Duplicate hybridizations using separate sets of nylon membranes were performed for all conditions. Only those signals that differed from the control by at least 2-fold in the two independent experiments were considered as significant. The procedure was similar to that used by Yamazaki et al. [35].

2.10. RT-PCR

RT-PCR using specific primers was performed to confirm the differential expression of the mRNAs detected with the macroarray analysis. This technique has been used to confirm the results of macroarray analysis [36–39]. The sequences of the primers were designed using information contained public database in GeneBank of the National Center for Biotechnology Information (NCBI).

The RT-PCR was performed using parameters described by Innis and Gelfand [40]. The number of cycles used was selected to allow quantitative comparison of the samples in a linear way. For semi-quantitative PCR analysis, the housekeeping β-actin gene was used as reference. The primer sequences and their respective PCR fragment lengths are

Table 1
The standardized conditions for RT-PCR analysis. The sequences of the primers, the PCR fragment lengths, the temperature and the number of cycles are shown for each gene under study.

Genes	Sense primer	Antisense primer	Anneling temperature (°C)	PCR fragment lengths (bp)	Number of cycles
MAPKK3	5′-AGGCGAATTAT AGCGTTCAGCC-3′	5′-GTTGATGCTGG TGTAGGACCC-3′	57	436	35
PLA_2	5′-AGCCCGTAGG TCATCTTGG-3′	5′-GCTTCAGCTTC GTCTCCTTGG-3′	56	559	30
PKC-beta	5′-CCATCAAATGC TCCCTCAACCC-3′	5′-TTGCCAAAGCT GCCTTTCCC-3′	57	424	35
Myc proto-oncogene	5′-TACCCTCTCAA CGACAGCAGCT-3′	5′-CTTGACATTCTCC TCGGTGTCC-3′	60	455	40
Kruppel related zinc finger protein*	5′-GCAAAGCATTT AGCCAGCCTTC-3′	5′-TCTCTCCAGTG TGCATCCTCG-3′	56	313	35
TOP1	5′-CGTACAATGCC TCCATCACGC-3′	5′-ACTTCTTGCA CCAAGCCACTG-3′	56	436	30

For all genes 1.5 mM $MgCl_2$ was used. * For kruppel related zinc finger protein RT-PCR, formamide was used at 2.5% concentraiton. MAPKK3, MAP Kinase Kinase 3; PLA_2, phospholipase A_2; PKC-beta, Protein Kinase C type beta I; TOP1, DNA Topoisomerase 1.

shown in the Table 1. Published guidelines were followed to guard against bacterial and nucleic acid contamination [41].

2.11. Analysis of PCR products

The analysis of PCR amplification products was performed in 1.5% agarose gels containing 0.5 μg per mL ethidium bromide and electrophoresed for 1 hour at 100 V. The gels were photographed using a DC120 Zoom Digital Camera System from Kodak (Life Technologies, Inc., Rockville, MD). The images were processed and analyzed in the software Kodak Digital Science 1D Image Analysis (Life Technologies).

PCR band intensities were expressed as optical density (OD) normalized for β-actin expression. The data are presented as the ratio with the respective controls, which received an arbitrary value of 1 in each experiment.

2.12. Statistical analysis

The results of proliferation assay and measurement of the production of cytokines were expressed as mean ± SEM of six determinations from three experiments. Comparisons with control (ethanol) and between DHA and EPA treatments were performed by analysis of variance (ANOVA). Significant differences were found by using the Tukey-Kramer method (INStat–Graph Pad Software, Inc., San Diego, CA).

3. Results

3.1. Determination of fatty acid composition by HPLC

Fatty acid analysis showed that cells cultured in the presence of EPA or DHA (12.5 μmol/L) for 24 hours were subsequently enriched with the corresponding n-3 fatty acid. The proportion of EPA was increased by 6.1-fold and of DHA by 5.7-fold. These findings confirm the incorporation of the fatty acids in to the cells. Also, these findings support the proposition that EPA is not converted into DHA in Jurkat cells.

3.2. Con A–induced Jurkat cell proliferation

[^{14}C]-Thymidine incorporation by Jurkat cells was decreased by both EPA and DHA (Fig. 1). The decrease in proliferation was 17%, 32%, and 59% by EPA at 50, 75, and 100 μmol/L concentrations, respectively. DHA decreased

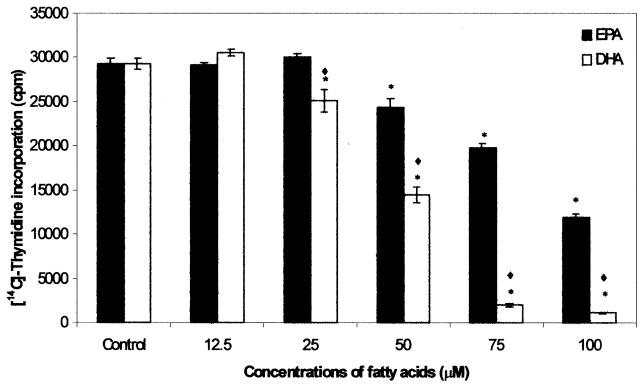

Fig. 1. Effect of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) on Jurkat cells proliferation. Cells (3.3×10^5 cells per mL) were cultured in the presence of 12.5, 25, 50, 75, and 100 μmol/L EPA or DHA for 48 hours. Cells were pulsed with 0.2 μCi/200 μL [^{14}C]-thymidine and harvested, and the radioactivity of the [^{14}C]-thymidine incorporated into DNA was determined using a liquid scintillation counter. Incorporation of [^{14}C]-thymidine is expressed as total counts per minute. Values are mean ± SEM of six determinations from three experiments. *$P < 0.05$ as compared with corresponding controls; $^{\blacklozenge}P < 0.05$ for comparison between DHA and EPA.

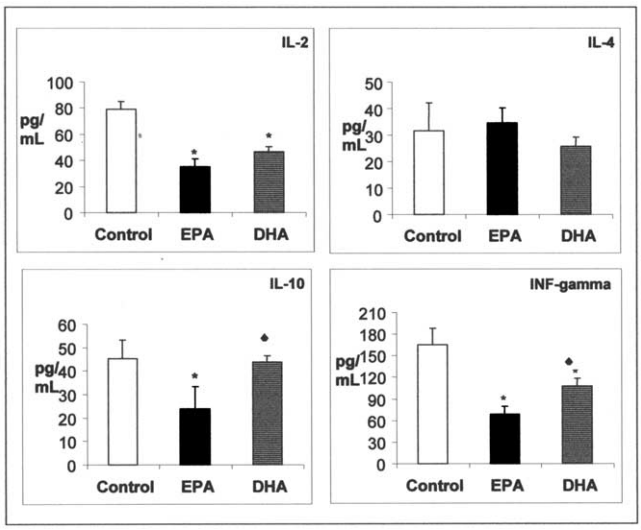

Fig. 2. Effect of fatty acids on cytokine production by Jurkat cells. Cells were cultured in the presence of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (12.5 μmol/L) for 24 hours. Cells were then cultured for another 24 hours in the presence of 25 μg/mL of concanavalin A (Con A). Afterward the supernatant fluid was used for determination of the cytokines (by ELISA) as described in Methods and materials section. Values are mean ± SEM of six determinations. $^{*}P < 0.05$ compared with corresponding controls; $^{\blacklozenge}P < 0.05$ for comparison between DHA and EPA.

[^{14}C]-thymidine incorporation by 14%, 51%, 93%, and 96% at concentrations of 25, 50, 75, and 100 μmol/L, respectively. The values of radioactivity incorporated were lower in cells treated with DHA (>μmol/L concentrations) as compared to EPA treatment. Thus, the inhibiting effect of DHA on Jurkat cells proliferation was more intense than that of EPA.

3.3. Production of cytokines

Both EPA and DHA decreased the production of cytokines, in particular of IL-2 and INF-γ (Fig. 2). EPA decreased the production of IL-2 (55%), IL-10 (47%), and INF-γ (58%). DHA caused a significant decrease in the production of IL-2 (41%) and INF-γ (35%). Neither fatty acid affected the production of IL-4. The comparison between EPA and DHA showed significant differences in the production of IL-10 and INF-γ.

3.4. Pleiotropic genes expression

The comparative effect of DHA and EPA on expression of genes involved with several aspects of T lymphocyte function is shown in Table 2. There are common and uncommon genes regulated by DHA and EPA treatment (Fig. 3). To validate the results of the macroarray analysis, six genes were selected for confirmation by RT-PCR (Fig. 4). Although the magnitude of changes was not identical, the direction of changes induced by the FA was the same for both macroarray and RT-PCR analysis. Therefore, macroarray analysis performed in duplicate using pooled cells from two experiments provided reliable results as also reported by others [36–39].

Of the surveyed genes (83 in total), 39 were modified by at least one of the fatty acids tested. The proportions of genes changed by the fatty acids were 30% for DHA and 26.5% for EPA. DHA raised the expression of 24 genes and reduced that of one only. EPA up-regulated the expression of 13 genes and down-regulated 9 gene expressions. DHA caused remarkable increase (>10-fold) of the following genes: tumor necrosis factor receptor–β (TNFR-β), v-erbA related ear-2 gene, cyclin-dependent kinase 4 inhibitor 2 (CDK4I), Bcl2-binding component 6 and FN1. EPA raised by at least 10-fold the expression of the following genes: CD27LG or CD70 and NBK apoptotic inducer protein or bcl-2 interacting killer (BIK). EPA also reduced by a great magnitude (>10-fold) the expression of glutathione S-transferase pi (GSTP1) and microsomal glutathione S-transferase 1.

3.5. RT-PCR results

The genes selected to perform RT-PCR were those altered by the treatments with DHA and EPA, as indicated by macroarray analysis. DHA increased the expression of mitogen-activated protein kinase kinase–3 (MAPKK3), phospholipase A_2, Kruppel-related zinc finger protein, and DNA topoisomerase 1 (TOP1). EPA raised the expression of protein kinase C–beta (PKC-β) type I and Myc proto-oncogene.

4. Discussion

Fish oil is suggested as being involved in aspects of cell function as an anti-inflammatory and immunosuppressive agent [42–44]. This oil has been repeatedly documented to reduce inflammation in diseases such as rheumatoid arthritis [45]. The inflammation that occurs in these diseases is due in part to hyper-proliferation of T cells and the subsequent production of pro-inflammatory cytokines. The active components of fish oil are DHA and EPA. In most studies, these two fatty acids have shown similar effects on immune cells. For instance, both fatty acids inhibit MAP kinase activation during antigen challenge [46]. However, these two fatty acids presented remarkable differences on their effects to regulate functional parameters and gene expression of Jurkat cells.

DHA presented a more pronounced inhibiting effect on Con A–induced Jurkat cell proliferation than EPA. In fact, the more unsaturated the fatty acid is more potent is its inhibitory effect on proliferation [47]. It has been reported that n-3 fatty acids present a marked effect on IL-2 production by T-lymphocytes [3,4]. Both fatty acids caused a marked decrease of IL-2 and INF-γ production, whereas

Table 2
Modifications in Jurkat cell gene expression after treatment with docosahexaenoic (DHA) and eicosapentaenoic (EPA) acids.

Cluster/GAN*	Gene name	DHA	EPA
1. Cytokines and related receptors			
L08096; S69339	CD27 ligand (CD27LG); surface antigen CD70	−	+68.1
M32315	Tumor necrosis beta factor receptor (TNFR-beta)	+32.1	+5.6
L41690	TNF receptor 1-associated death domain protein (TRADD)	+5.2	+2.0
2. Signal transduction pathways			
L36719	Mitogen-Activated Protein Kinase Kinase 3 (MAPKK3)	+2.2	−
L36870	MAP Kinase Kinase 4 (MAPKK4)	+3.9	−
X06318	Protein Kinase C beta I (PKC-beta)	−	+5.0
M31158	cAMP-dependent protein Kinase type II beta regulatory subunit	−	+5.4
L05624	MAP kinase kinase 1 (MAPPKK1)	+2.3	+3.9
L26318	c-jun N-terminal Kinase 1 (JNK-1)	+3.8	−
U39657	MAP Kinase Kinase 6 (MAPKK6)	+8.4	−
M86400	Phospholipase A2 (PLA_2)	+3.4	−
3. Transcription factors and related genes			
V00568	Myc proto-oncogene	−	+2.6
X12794	v-erbA homology-like 2	+18.9	
J04111	c-jun proto-oncogene	−	+2.3
Z12020; M92424	p53-associated gene; p53-associated mRNA	−	+2.0
X07282; Y00291	Retinoic acid receptor epsilon protein (RAR-epsilon); retinoic acid receptor beta 2 (RAR-beta2)	+4.5	−
M14752	c-abl proto-oncogene	+2.4	−2.8
L11672	Kruppel related zinc finger protein	+2.7	−
M15400	Retinoblastoma-associated protein 1 (RB1); phosphoprotein 110 (pp110)	−	−5.9
4. Cell cyle			
L33264	Cyclin-dependent Kinase 10 (CDK10)	+2.4	−
M68520	Cyclin-dependent protein kinase 2 (CDK2)	−	−7.7
M81933	Cell division cycle 25 homolog A (CDC25A)	+2.4	−
L27211	Cyclin-dependent kinase 4 inhibitor 2 (CDK4I)	+22.4	−
M73812	G1/S-specific cyclin E (CCNE)	+6.4	−
5. Defense and repair			
M60974	Growth arrest and DNA-damage-inducible protein (GADD45)	+2.5	+3.3
X15722	Glutathione reductase (EC 1.6.4.2)	+2.4	−
J03746	Microsomal Glutathione S-transferase 1	−	−33
X08058	Glutathione S-transferase pi (GSTP1)	−	−10.2
M25627	Glutathione S-transferase A1 (GSTA1)	+2.1	−2.3
6. Apoptosis			
L22474	BCL-2-associated X protein membrane (BAX)	−	+2.6
X89986; U34584	NBK apoptotic inducer protein; Bcl-2 interacting killer (BIK)	−	+10.2
U66879	Bcl2-binding component 6 (bbc6)	+27	−
U43746	Breast cancer type 2 susceptibility protein (BRCA2)	+5.3	−2.2
7. DNA synthesis			
J03250	DNA Topoisomerase I (TOP1)	+3.2	−
L04088	DNA topoisomerase II alpha (TOP2A)	−3.3	−2.3
M63488	Replication protein A 70-kDa subunit (RPA70)	−	−5.5
8. Cell adhesion, cytoskeleton, and related genes			
X02761	Fibronectin 1 (FN1)	+25	−
9. Hormone Receptors			
X04434	Insulin-like growth factor I receptor (IGFIR)	−	+2.6
M10051; X02160	Insulin receptor (INSR); insulin receptor precursor	+7.3	−

Jurkat cells were treated for 24 hours with DHA and EPA (12.5 μM) acids. Total RNA was isolated, retrotranscribed, ^{33}P-labelled and hybridized to the cDNA array presenting 83 transcripts of known genes. The signals were then analyzed by pro-analysis software Array-Pro™ Analyzer, version 4 (Media Cybernetics®, Silver Spring, MD, USA) and expressed as fold of increase with respect to untreated cells. Data are presented as mean of two different experiments. Only signals that differed from untreated cells by at least 2-fold were considered as significant. Changes are indicated as (+) increase and (−) decrease as compared to untreated cells. * GAN – GeneBank accession number.

EPA decreased also IL-10 production by Jurkat cells. Reduction of INF-γ production was also observed by Fritsche et al. [48] in n-3-PUFA–fed mice.

The comparative effects of DHA and EPA on pleitropic genes expression by Jurkat cells were then examined by using a macroarray technique. To validate the results of the macroarray analysis, six genes were selected for confirmation by RT-PCR (Fig. 3). Although the magnitude was not the same, the changes induced by the fatty acids were observed by both macroarray and RT-PCR. Therefore, mac-

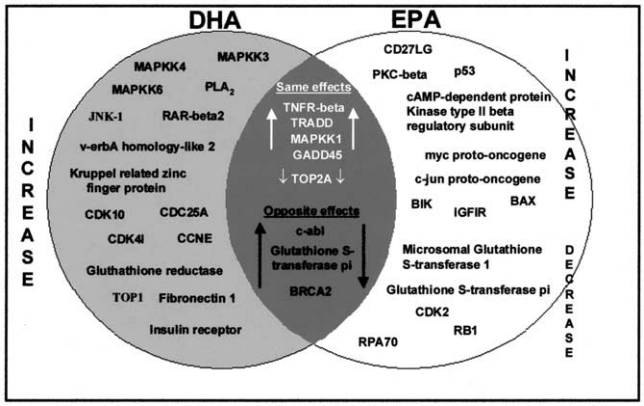

Fig. 3. Diagram with common and uncommon genes regulated by docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) treatment. Genes regulated by DHA only are shown on left. Genes regulated by EPA only are shown on right. Genes regulated by both fatty acids are in intersection. ↑ Indicates increase and ↓ indicates decrease in gene expression. TNFR-beta-tumor necrosis factor beta receptor; MAPKK 1, 3, 4, 6 = mitogen-activated protein kinase kinase 1, 3, 4, 6; PKC-beta = protein kinase C type beta I; JNK-1 = c-jun N-terminal kinase 1; PLA_2 = phospholipase A_2; RB1 = retinoblastoma-associated protein 1; CDK10 = cyclin-dependent protein kinase 10; CDK2 = cyclin-dependent protein kinase 2; CDC25A = cell division cycle 25 homolog A; CDK4I = cyclin-dependent kinase 4 inhibitor 2; CCNE = G1/S-specific cyclin E; GADD45 = growth arrest and DNA-damage–inducible protein; GSTP1 = glutathione S-transferase pi; GSTA1 = glutathione S-transferase A1; TRADD = TNF receptor–associated death domain protein; retinoic acid receptor epsilon protein (RAR) = epsilon-retinoic acid receptor epsilon protein; RAR-beta2 = retinoic acid receptor beta 2; BAX = BCL-2–associated X protein membrane; BIK = bcl-2 interacting killer; bbc6 = Bcl2-binding component 6; BRCA2 = breast cancer type 2 susceptibility protein; TOP1 = DNA topoisomerase 1; TOP2A = DNA topoisomerase II alpha; IGFIR = insulin-like growth factor I receptor; INSR = insulin receptor.

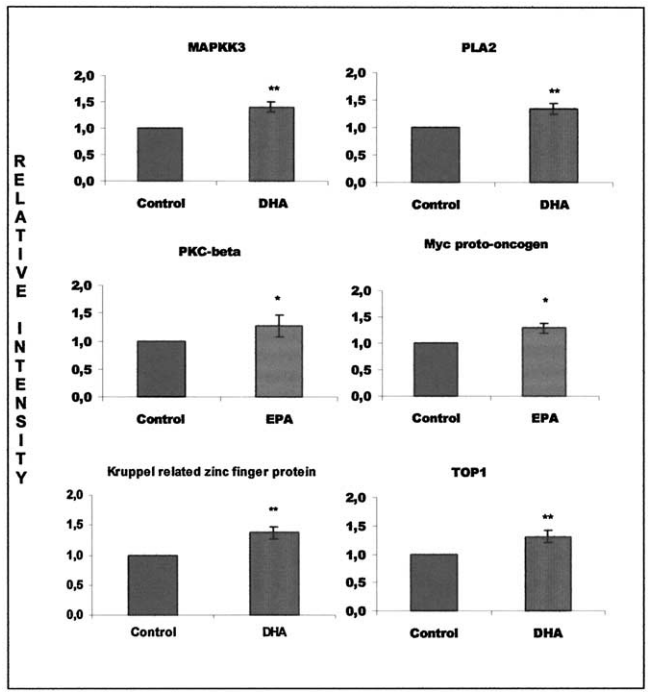

Fig. 4. Confirmation by reverse transcriptase–polymerase chain reaction (RT-PCR) of the genes modified by the fatty acids as detected by macroarray analysis (Table 2). Jurkat cells (10^7 cells per condition) were exposed for 24 hours to the following conditions: control (no fatty acids added); docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) (12.5 μmol/L). Cells were then harvested, mRNA extracted, and RT-PCR performed with the equivalent of 10^7 cells. PCR band intensities were expressed as OD normalized for β-actin expression. Data are ratio of respective controls, which received an arbitrary value of 1 in each experiment. Values are mean ± SEM for four experiments. PKC-beta = protein kinase C type beta I; PLA_2 = phospholipase A_2; TOP1 = DNA topoisomerase 1. $^*P < 0.05$; $^{**}P < 0.01$ with the corresponding control groups by paired Student t test.

roarray analysis performed in duplicate using two pools of cells from two different experiments provides reliable observations.

The fatty acids responsive genes were clustered according to main putative biological functions of their encoded protein. DHA and EPA markedly affected the expression of genes clustered as: cytokines and related receptors, signal transduction pathways, transcription factors, cell cycle, defense and repair, apoptosis, DNA synthesis, cell adhesion, cytoskeleton, and hormone receptors. However, the effects of the two fatty acids were very much different. DHA raised the expression of 62% of the genes changed by the fatty acids (39 genes), whereas EPA up-regulated 33%. DHA enhanced the expression of most genes, whereas EPA raised many genes and suppressed several others. There is substantial evidence that FA can regulate lymphocyte proliferation [49–51]. The changes in lymphocyte proliferation may be related to the effects of FA on expression of genes controlling the cell cycle. DHA up-regulated the expression of cyclin-dependent protein kinase–10 (CDK10), cyclin-dependent protein kinase–2 (CDK2), and cell division cycle 25 homolog A (CDC25A), whereas EPA down-regulated CDK2.

Oxidative stress and lipid peroxidation have been associated with impaired immune function and with the development of several diseases [52,53]. DHA presented a stimulating effect on expression of three genes related to defense and repair: growth arrest and DNA-damage–inducible protein (GADD45), glutathione reductase, and glutathione S-transferase A1 (GSTA1). EPA increased GADD45 and decreased microsomal glutathione S-transferase 1, GSTP1, and GSTA1.

In addition, EPA raised the expression of some of the genes increased by DHA: TNFR-beta, MAPKK1, GADD45, and TNF receptor–associated death domain protein (TRADD) (Fig. 3). In contrast, EPA augmented the expression of other genes such as myc proto-oncogene, c-jun proto-oncogene, and p53-associated gene. This fatty acid also inhibited the expression of a number of genes such as PKC-β and JAK-1. Therefore, the molecular mechanisms for the modulatory effect of these two fatty acids on T lymphocytes are remarkably different. In fact, other investigators have altered the ratio of EPA/DHA and have shown that EPA is more effective than DHA in suppressing the

inflammatory response [54,55]. Systematic clinical studies should be carried out to examine separately the effect of DHA and EPA on T lymphocyte function.

It is noteworthy that DHA and EPA also affected the expression of hormone receptors. EPA raised the expression of IGF1 receptor and DHA increased insulin receptor. Insulin is known to have a potent immunomodulatory effect, and diabetic patients show impaired immune function [56]. IGF-1 plays an important role in the maintenance, development, and proliferation of T-lymphocytes and protects activated T-cells from apoptosis [57,58]. The observations presented here indicate that the administration of n-3 fatty acids could be used to potentiate or suppress the action of hormones on immune function as also shown by others [59]. In fact, dietary fish oil has shown to reduce insulin resistance in rats [60].

Our study findings led us to conclude that EPA and DHA present remarkable differences in functional parameters and genes expression of Jurkat cells. The inhibiting effect of DHA on proliferation was more pronounced than that of EPA. Both DHA and EPA markedly reduced IL-2 and INF-γ production. However, the production of IL-10 was decreased by EPA only. The changes in genes expression induced by DHA were much different from those caused by EPA. DHA increased the expression of most genes, whereas EPA raised many genes and suppressed several others. Therefore, the effect of n-3 fatty acids on T-lymphocyte function involves regulation of expression of important genes. Marked differences were observed between the effects of EPA and DHA, indicating that it is an over-simplification to generalize the effects of n-3 fatty acids.

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