Eicosapentaenoic acid and docosahexaenoic acid modulate MAP kinase (ERK1/ERK2) signaling in human T cells

Anne Denys, Aziz Hichami, and Naim Akhtar Khan[1]

UPRES Lipids and Nutrition, Department of Physiology, Faculty of Life Sciences, University of Burgundy, 6 Boulevard Gabriel, Dijon 21000, France

Abstract **This study was conducted on human Jurkat T cell lines to elucidate the role of EPA and DHA, n-3 PUFA, in the modulation of two mitogen-activated protein (MAP) kinases, that is, extracellular signal-regulated kinases 1 and 2 (ERK1 and ERK2). The n-3 PUFA alone failed to induce phosphorylation of ERK1/ERK2. We stimulated the MAP kinase pathway with anti-CD3 antibodies and phorbol 12-myristate 13-acetate (PMA), which act upstream of the MAP kinase (MAPK)/ERK kinase (MEK) as U0126, an MEK inhibitor, abolished the actions of these two agents on MAP kinase activation. EPA and DHA diminished the PMA- and anti-CD3-induced phosphorylation of ERK1/ERK2 in Jurkat T cells. In the present study, PMA acts mainly via protein kinase C (PKC) whereas anti-CD3 antibodies act via PKC-dependent and -independent mechanisms. Furthermore, DHA and EPA inhibited PMA-stimulated PKC enzyme activity. EPA and DHA also significantly curtailed PMA- and ionomycin-stimulated T cell blastogenesis. Together these results suggest that EPA and DHA modulate ERK1/ERK2 activation upstream of MEK via PKC-dependent and -independent pathways and that these actions may be implicated in n-3 PUFA-induced immunosuppression.—** Denys, A., A. Hichami, and N. A. Khan. **Eicosapentaenoic acid and docosahexaenoic acid modulate MAP kinase (ERK1/ERK2) signaling in human T cells.** ***J. Lipid Res.*** **2001.** 42: **2015–2020.**

Supplementary key words T cell receptor • protein kinase C • fatty acids

T cells are activated via aggregation of T cell receptor (TCR) by an antigenic peptide bound within a major histocompatibility molecule expressed on antigen-presenting cells (1). This event triggers a number of intracellular biochemical events that assure T cell cycle progression. One of the foremost proximal events is the engagement of TCR/CD3 in the phosphorylation and activation of several Src family protein tyrosine kinases (PTK), which are associated with the activation of downstream signaling molecules (2). Lck, Fyn, and Yes are the three Src family PTK generally expressed in most T cells. Hence, the role of Lck (known as $p56^{lck}$) in T cell activation is well documented (2, 3). Activation of $p56^{lck}$ requires dephosphorylation of Tyr-505 and phosphorylation of Tyr-394 residues (4). In fact, CD3ζ is tyrosine phosphorylated by activated $p56^{lck}$, followed by the association of Zap70/Syk family PTK with phosphorylated CD3ζ (5). These events, in association with Zap70, are responsible for the tyrosine phosphorylation of phospholipase Cγ1 (PLCγ1) (6). PLCγ1 catalyzes the hydrolysis of phosphatidylinositol into inositol trisphosphate and diacylglycerol, which, respectively, induce increases in free intracellular calcium concentration ($[Ca^{2+}]_i$) and activation of protein kinase C (PKC) (7). The increase in $[Ca^{2+}]_i$ is one of the earliest events implicated in T cell activation (8, 9). The PKC, once activated via PLCγ1, may further activate phospholipase D, which assures the completion of the T cell cycle (10). It is noteworthy that tyrosine phosphorylation in these biochemical events is controlled by the coordinated action of PTK and protein tyrosine phosphatases (PTP) such as CD45, Src homology 2 domain-containing PTP 1 (SHP1), and SHP2 (11).

Another important event triggered by TCR/CD3 aggregation is the formation of GTP-bound Ras, which is stimulated as a result of PKC-activated inhibition of Ras GTPase-activating proteins (12), and by an additional stimulatory signal, independently of PKC, involving the PTK whose activation results in the formation of complexes of adapter proteins such as Shc/Grb2/Ras/Sos, p36/Grb2/Sos, Grb2/Cbl, and Crk/C3G (13, 14). These adapter complexes link the TCR/CD3 complex to the downstream Ras/mitogen-activated protein (MAP) kinase pathway (15). In the GTP-bound position Ras stimulates the MAP kinase pathway, consisting of extracellular signal-regulated ki-

Abbreviations: ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; MEK1/2, MAPK/ERK kinase 1/2; n-3 PUFA, polyunsaturated fatty acids of the n-3 family; PKC, protein kinase C; PMA, phorbol 12-myristate 13-acetate; PTK, protein tyrosine kinase; TCR, T cell receptor.

[1] To whom correspondence should be addressed.
e-mail: Naim.Khan@u-bourgogne.fr

nases 1 and 2 (ERK1 and ERK2) (16). Hence, active GTP-bound Ras directly binds and promotes the activation of protein kinase Raf-1, a Ser/Thr kinase (17). Active Raf-1, by phosphorylating at two serine residues, activates the dual-specificity protein kinase MEK1/2 [MAP kinase (MAPK)/ERK kinase 1/2], which, in turn, phosphorylates and activates ERK1/ERK2 (18). In human T cells, the Ras/Raf-1/MEK1/2/MAP kinase pathway plays a major role in the transmission of signals toward the nucleus in order to regulate the transcription of genes involved in T cell cycle progression (19). In human Jurkat T cells, the translocation of NF-κB, involved in the transcription of the interleukin 2 gene, into the nucleus is also dependent on the Ras/Raf-1/MEK1/2 pathway (20).

PUFA modulate T cell functions (21). PUFA of the n-6 family (n-6 PUFA), such as arachidonic acid (20:4n-6), have been considered as immunostimulators whereas those of the n-3 family, such as EPA (20:5n-3) and DHA (22:6n-3), have been reported to be potent immunosuppressors (22). PUFA of the n-3 family (n-3 PUFA), abundantly present in diets containing fish oil, exert curative effects in autoimmune diseases such as rheumatoid arthritis (23) and multiple sclerosis (24). The precursor of n-3 PUFA is linolenic acid (18:3n-3), which is metabolized to DHA, the most unsaturated fatty acid, in microsomes via a number of reactions involving the desaturases and chain elongation steps (25). EPA is not only an intermediate fatty acid during the metabolism of DHA but also a parallel product if compared with the metabolism of arachidonic acid. The mechanism of action of n-3 PUFA, implicated in T cell activation, is not well understood. It is possible that these fatty acids may modulate T cell functions by interfering with the second-messenger cascades. We have shown that DHA exerts immunosuppressive effects by modulating calcium signaling in human Jurkat T cells (26). Hence, DHA induces increases in $[Ca^{2+}]_i$ by recruiting calcium from intracellular pool and by opening of calcium release-activated calcium channels in these cells (26). It is also possible that the n-3 PUFA may modulate the phosphorylation of MAP kinases activated during an antigenic challenge. As far as DHA and EPA are concerned, to our knowledge, no study is available, except one report by Yang et al. (27), in which EPA has been found to inhibit MAP kinase activation in carotid artery endothelial cells. As far as T cells are concerned, the role of n-3 fatty acids in the modulation of the MAP kinase pathway has not yet been studied. Therefore, it was thought worthwhile to elucidate the effects of two n-3 PUFA (EPA and DHA), potent immunosuppressors, on the activation of MAP kinases in human Jurkat T cells.

MATERIALS AND METHODS

Chemicals

Anti-phosphorylated MAP kinase (ERK1/ERK2) antibodies were obtained from New England BioLabs (Beverly, MA). [^{3}H]thymidine (specific activity, 20 Ci/mmol) and [γ-^{32}P]ATP (specific activity, 4,000 Ci/mmol) were purchased from Amersham (Arlington Heights, IL). GF109203X was obtained from Calbiochem (La Jolla, CA). Anti-CD3 antibodies were obtained from Dako (Carpinteria, CA). The PKC detection kit SignalTeck was purchased from Promega (Madison, WI). All other chemicals including phorbol 12-myristate 13-acetate (PMA), DHA (22:6n-3), and EPA (20:5n-3) were procured from Sigma (St. Louis, MO). U0126 was a generous gift from Promega.

Cell culture and preparation of extracts

The Jurkat T cells were routinely cultured in RPMI 1640 medium supplemented with L-glutamine and 10% fetal calf serum at 37°C in a humidified chamber containing 95% air and 5% CO_2. Cell viability was assessed by trypan blue exclusion test. Cell numbers were determined with a hemocytometer.

n-3 PUFA, dissolved in ethanol at a concentration of 0.01% (v/v), were kept under nitrogen in order to minimize their oxidation in accordance with several investigators (27–29). Before determining MAP kinase activation, cells were incubated for 6 h in RPMI 1640 medium without serum. Control cells received vehicles that did not affect MAP kinase activity. Before stimulation with PMA, Jurkat T cells (5×10^6/ml) were incubated for 5 min in the presence of n-3 PUFA and then with PMA (200 nM) for 30 min, essentially according to Nel et al. (30). The time of incubation for PUFA is indicated in the figure legends. After incubation at 37°C, cells were lysed with 50 μl of buffer containing the following: HEPES, 20 mM, pH 7.3; EDTA, 1 mM; EGTA, 1 mM; NaCl, 0.15 mM; Triton X-100, 1%; glycerol, 10%; phenylmethylsulfonyl fluoride, 1 mM; sodium orthovanadate, 2 mM; antiprotease cocktail, 2 μl in 1 ml of buffer. After centrifugation (2,500 *g* for 1 min), cell lysates were used immediately, or stored at −80°C, for Western blot detection of phosphorylation. The protein contents were determined with a Sigma Bradford reagent kit.

Western blot detection of phosphorylated MAP kinases

Denatured proteins (20 μg) were separated by SDS-PAGE (10%) and transferred to polyvinylidine difluoride membranes, and immunodetection was performed by using anti-phosphorylated anti-rat ERK1/ERK2 antibodies (2 μg/ml). After treating the membranes with peroxidase-conjugated goat anti-rat secondary antibodies, peroxidase activity was detected with ECL reagents (Amersham).

PKC assays

After the incubation of Jurkat T cells with n-3 PUFA and/or other agents (see the figure legends), the cells were washed twice with phosphate-buffered saline, pH 7.4, and the reaction was stopped by addition of extraction buffer: Tris-HCl, 25 mM; EGTA, 0.5 mM; EDTA, 0.5 mM; 2-mercaptoethanol, 10 mM; phenylmethylsulfonyl fluoride, 10 mM; leupeptin, 10 μg/ml; pH 7.5. The cells were sonicated by two cycles at 4°C and the enzyme activity was determined by incorporation of $^{32}P_i$, according to the instructions furnished with the kit.

T cell blastogenesis

Jurkat T cells (10×10^4) were cultured in 96-well flat-bottom tissue culture plates (Nunc, Roskilde, Denmark) in the presence of PMA (10 nM) plus ionomycin (500 nM). Cells were distributed in quadruplicate as follows: 140 μl, cell suspension; 20 μl, mitogen or medium; and 20 μl, DHA or EPA (as indicated in the figure legends). Plates were incubated at 37 ± 2°C (90% humidity). After 36 h, 20 μl of [^{3}H]thymidine (20 Ci/mmol, 0.8 μCi/well) was added and, 12 h later, the cells were harvested with a cell harvester [Dynatech (Acterna), Burlington, MA], trapping their DNA onto glass filtermats. Dried filter circles were placed in plastic minivials (Packard, Downers Grove, IL), 3.5 ml of Optifluor-O (Packard) was added, and the radioactivity was recorded in a scintillation counter (Beckman, Fullerton, CA). The index

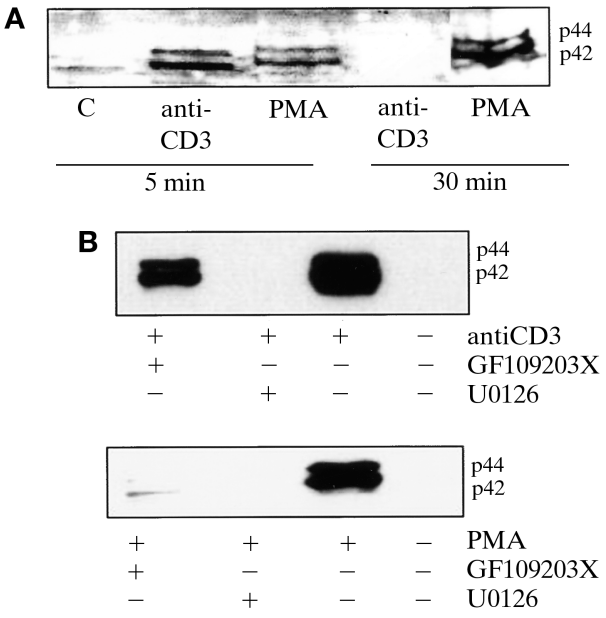

Fig. 1. Effects of anti-CD3 antibodies and PMA on ERK1/ERK2 phosphorylation in Jurkat T cells. Jurkat T cells (5×10^6 cells/ml), before determination of MAP kinase phosphorylation, were incubated for 6 h in RPMI 1640 medium without serum, and treated or not (control) with anti-CD3 antibodies (10 μg/ml) or PMA (200 nM) for 5 and 30 min, respectively, at 37°C (A). In (B), the cells, after serum starvation, were treated or not (control) with GF109203X (500 nM) and U0126 (1 μM) for 5 min. Later on, these cells were treated or not treated with anti-CD3 antibodies (10 μg/ml) and PMA (200 nM), respectively, for 5 and 30 min, at 37°C. Cells were lysed and the detection of phosphorylated MAP kinases was performed as described in Materials and Methods.

of T cell stimulation (IS) was calculated as counts per minute in the presence of mitogens divided by counts per minute of unstimulated cultures.

Statistical analysis

Results are shown as means ± SD for quadruplicate assay samples, reproduced independently at least three times. Statistical analysis of data was carried out with Statistica (version 4.1; Statsoft, Paris, France). The significance of the differences between mean values was determined by one-way analysis of variance, followed by a least significant difference test.

RESULTS

Anti-CD3 antibodies and PMA induce ERK1/ERK2 phosphorylation

Figure 1 shows that anti-CD3 antibodies and PMA induce the phosphorylation of ERK1/ERK2 in Jurkat T cells. Anti-CD3 antibody-induced MAP kinase phosphorylation was transient, as it was marked at 5 min as compared with that at 30 min of incubation. On the other hand, the PMA-induced state of phosphorylation was higher at 30 min as compared with that at 5 min of incubation (Fig. 1A). GF109203X, a PKC inhibitor, abolished the PMA-induced phosphorylation of MAP kinases (Fig. 1B). The degree of inhibition of MAP kinase phosphorylation by this agent was less in anti-CD3 antibody-treated cells as compared with PMA-treated cells (Fig. 1B). However, U0126, an MEK1/2 inhibitor, abolished the MAP kinase phosphorylation, induced both by PMA and anti-CD3 antibodies in Jurkat T cells (Fig. 1B).

EPA and DHA diminish PMA-induced ERK1/ERK2 activation

We used PMA to activate MAP kinases via activation of PKC. **Figure 2A** shows that EPA and DHA alone failed to induce phosphorylation of MAP kinases in Jurkat T cells. However, both the fatty acids curtailed the degree of phosphorylation, induced by PMA. DHA inhibited MAP kinase phosphorylation at all concentrations (from 5 to 60 μM) whereas EPA exerted its inhibitory effects only at 20, 40, and 60 μM (Fig. 2A). These fatty acids also diminished anti-CD3 antibody-induced MAP kinase phosphorylation (Fig. 2B).

EPA and DHA curtail PMA-stimulated PKC activation

Figure 3 shows that DHA and EPA alone did not induce the activation of PKC. However, these fatty acids curtailed

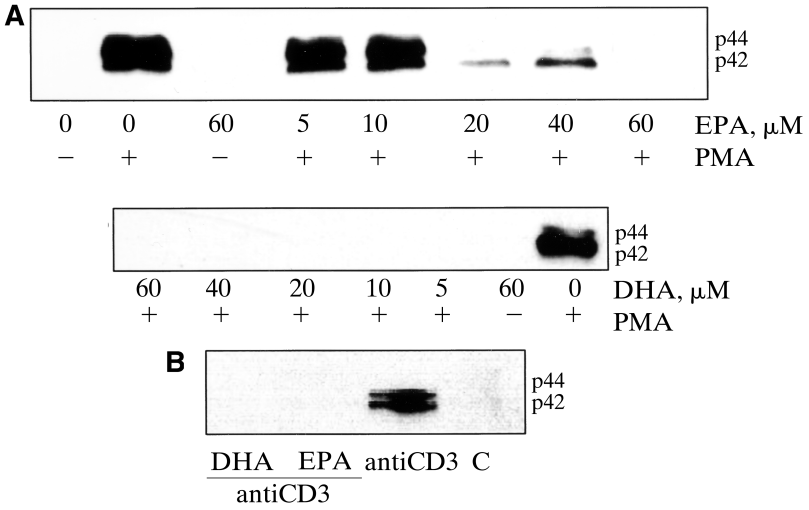

Fig. 2. Effects of n-3 PUFA on anti-CD3 antibodies and PMA-stimulated ERK1/ERK2 phosphorylation in Jurkat T cells. Jurkat T cells (5×10^6 cells/ml), before determining MAP kinase phosphorylation, were incubated for 6 h in RPMI 1640 medium without serum, and treated or not (control) with increasing concentrations (0 to 60 μM) of n-3 PUFA (A). After 5 min of incubation, cells were stimulated with PMA (200 nM) for another 30 min at 37°C. As a control, cells were also incubated with n-3 PUFA (DHA and EPA at 60 μM). In (B), the cells, after serum starvation, were treated or not (control) with n-3 PUFA (DHA and EPA at 20 μM) for 5 min. Later on, cells were treated or not (C, control) with anti-CD3 antibodies (10 μg/ml) for 5 min. After incubation at 37°C, cells were lysed and phosphorylations were determined as described in Materials and Methods.

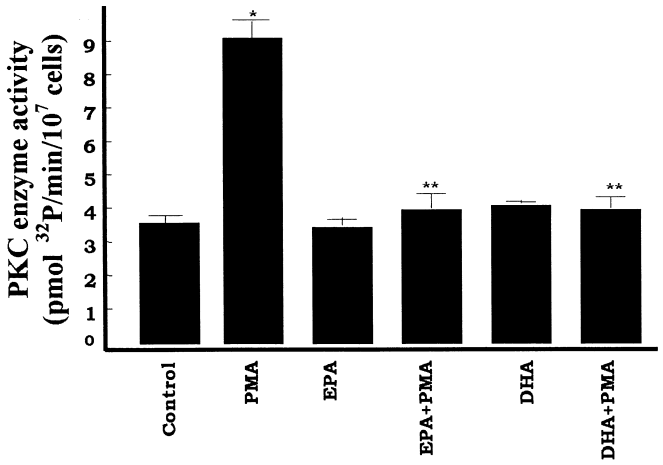

Fig. 3. Effects of n-3 PUFA on PMA-stimulated PKC enzyme activity in Jurkat T cells. Jurkat T cells (5×10^6 cells/ml), before the determination of PKC enzyme activity, were incubated for 6 h in RPMI 1640 medium without serum, and were treated or not (control) with n-3 PUFA (DHA and EPA at 20 μM) for 5 min. Later on, these cells were treated or not treated with PMA (200 nM) for 30 min. After incubation at 37°C, cells were lysed and PKC kinase enzyme activity was assayed as described in Materials and Methods. Data are significantly different as compared with control (* $P < 0.001$) and with PMA-stimulated enzyme activity (** $P < 0.001$).

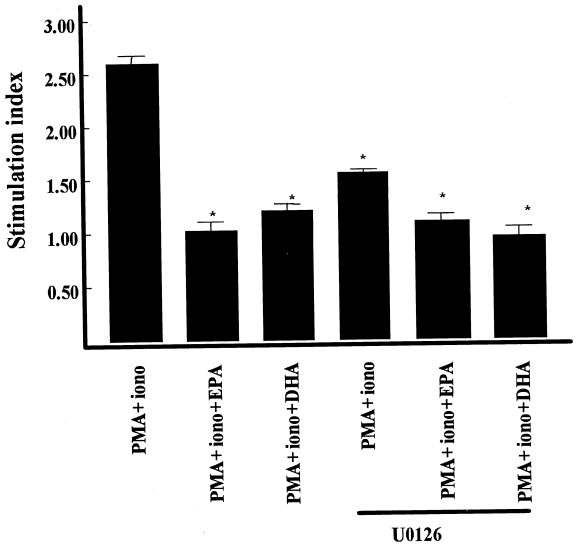

Fig. 4. Effects of n-3 PUFA on PMA plus ionomycin-stimulated proliferation of Jurkat T cells. Human (Jurkat) T cells (10×10^4) were cultured in 96-well flat-bottom tissue culture plates in the presence of PMA (10 nM) plus ionomycin (iono, 500 nM), containing or not containing n-3 PUFA (DHA or EPA, at 20 μM) with or without U0126 (10 μM) as described in Materials and Methods. Shown are the results of an identical experiment, reproduced independently three times with triplicate values. Data are significantly different as compared with PMA and ionomycin-stimulated cells (* $P < 0.001$).

significantly the PMA-stimulated PKC activity in Jurkat T cells (Fig. 3).

EPA and DHA inhibit Jurkat T cell blastogenesis

To shed light on the physiological importance of n-3 PUFA-induced inhibition of MAP kinase signaling, we stimulated Jurkat T cells with PMA plus ionomycin in the presence or absence of exogenous DHA or EPA. We observed that the two n-3 PUFA significantly curtailed Jurkat T cell proliferation. U0126 also inhibited T cell blastogenesis (**Fig. 4**).

DISCUSSION

It has been established that n-3 PUFA, particularly DHA and EPA, exert immunosuppressive effects (21). One of the mechanisms of action of n-3 PUFA is the replacement of arachidonic acid at the *sn*-2 position of plasma membrane phospholipids and, therefore, these agents will influence signal transduction mechanisms, particularly at the level of diacylglycerol production and PKC activation. Another plausible mechanism of action of n-3 PUFA is their interference with MAP kinase signaling.

Most of the studies conducted hitherto have dealt with the role of arachidonic acid, and this fatty acid has been found to enhance MAP kinase phosphorylation in a number of cell types (28–32). Surprisingly, in the present study, we observed that DHA and EPA alone failed to induce the activation of MAP kinases (ERK1/ERK2) in human Jurkat T cells. Our results partly corroborate the findings of Yang et al. (27), who have observed that EPA alone failed to stimulate basal MAP kinase enzyme activity in bovine carotid artery cells.

To stimulate MAP kinase phosphorylation in Jurkat T cells, we used anti-CD3 antibodies and PMA. We observed that anti-CD3 antibodies induced a transient MAP kinase activation, seen after 5 min of incubation, whereas that induced by PMA was maximum after 30 min of incubation. Furthermore, PMA seems to act via PKC activation as GF109203X, a PKC inhibitor, completely abolished the action of PMA on MAP kinase phosphorylation. These results are in close agreement with the observations of Nel et al. (30), who have shown that MAP kinase activation by PMA was a PKC-dependent mechanism in human T cells. During activation of T cells via TCR/CD3, PKC also seems to be a component implicated in MAP kinase activation as GF109203X diminished, but did not completely abolish, the action of anti-CD3 antibodies on ERK1/ERK2 phosphorylation. These results agree well with the findings of several authors (20, 33) who have shown that TCR ligation regulates MAP kinase signaling via both PKC-dependent and PKC-independent, but PTK-dependent, pathways in human T cells. The present study further demonstrates that PMA and anti-CD3 antibodies activate MAP kinase signaling upstream of MEK as U0126 completely abolished their effects on ERK1/ERK2 activation in human Jurkat T cells.

We have observed that DHA and EPA, without influencing the basal activity, diminished MAP kinase activation stimulated by both anti-CD3 antibodies and PMA. DHA exerted inhibitory effects from 5 μM onward, whereas

EPA, without exerting any effect at 5 and 10 μM, inhibited PMA-induced MAP kinase phosphorylation only from 20 μM onward in Jurkat T cells. The inhibitory effects of n-3 PUFA at high concentrations were not due to their cytotoxic effects, as we checked cell viability by trypan blue exclusion test during these assays.

We were tempted to ascertain whether DHA and EPA modulate PMA-induced MAP kinase activation via their inhibitory effects on PKC. To test this notion, we assessed the effects of PUFA on PMA-stimulated PKC activity in Jurkat T cells. We observed that DHA and EPA alone did not influence basal PKC enzyme activity; rather, they significantly diminished PMA-stimulated PKC activation in Jurkat T cells. These results suggest that n-3 PUFA may exert their inhibitory action on ERK1/ERK2 activation by inhibiting PKC activity in PMA-stimulated cells. Jurkat T cells have been shown to possess different isoforms of PKC, such as α, β, δ, ε, and ζ (34). The α, β, and ε isoforms of PKC are translocated toward plasma membrane in response to PMA in these cells (34). Studies have demonstrated that PKCα and PKCε are the upstream regulators of the MAP kinase cascade (35, 36). It is possible that DHA and EPA inhibit the activation of these isoforms of PKC either indirectly via the production of diacylglycerol-containing exogenous n-3 PUFA (37) or directly by intercalation with the binding site for phosphatidylserine (38). Which PKC isoform is directly influenced by these n-3 PUFA remains to be ascertained in future; however, our results corroborate the findings of May et al. (39), who have noticed that the culture of rat lymphocytes in the presence of EPA and DHA resulted in the reduction of PKC activity in these cells. However, these two fatty acids do act on a PKC-independent pathway as they inhibited the MAP kinase phosphorylation induced by anti-CD3 antibodies that act via PKC-dependent and -independent manner (see above).

In the present study, the involvement of lipoxygenase and cyclooxygenase metabolites is unlikely, as Jurkat T cells do not possess the enzymes for these two pathways, and, therefore, these cells cannot metabolize n-3 PUFA (40, 41). Even though we used nordihydroguaiaretic acid and indomethacin, the respective inhibitors of the two pathways, as expected, the two agents did not reverse the inhibitory actions of EPA and DHA on MAP kinase phosphorylation (results not shown).

To understand the physiological relevance of the inhibition of MAP kinase activation by n-3 PUFA, we stimulated Jurkat T cells with PMA and ionomycin in the presence of DHA and EPA. We observed that these fatty acids significantly curtailed PMA-induced T cell blastogenesis. Interestingly, U0126 also inhibited T cell proliferation but failed to exert additive effects on n-3 PUFA-induced inhibition of T cell blastogenesis, suggesting that n-3 PUFA exert their effects upstream of MEK.

The results of the present study demonstrate that unesterified DHA and EPA inhibit MAP kinase activation via PKC-dependent and -independent pathways. Because anti-CD2 antibodies also stimulate phospholipase A_2 and the release of PUFA in human T cells (42), we can speculate that unesterified n-3 PUFA, released during T cell activation, possess the potential to regulate the activation of MAP kinase during an antigenic challenge and, hence, may modulate the progression of autoimmune diseases.

The authors are thankful to the Fondation pour la Recherche Médicale (Paris, France) for a contingent grant that was sanctioned to help in the creation of the "Jeune Equipe" at the University of Burgundy, Dijon. This work was also supported by a contingent grant from Burgundy Region, Dijon and Group Lipids and Nutrition (Paris, France).

Manuscript received 20 February 2001, in revised form 8 May 2001, and in re-revised form 23 August 2001.

REFERENCES

1. Germain, R. N. 1994. MHC-dependent antigen processing and peptide presentation providing ligands for T lymphocyte activation. *Cell.* **76:** 287–299.
2. Weiss, A., and D. R. Littman. 1994. Signal transduction by lymphocyte antigen receptors. *Cell.* **76:** 263–274.
3. Veillette, A., N. Abraham, L. Caron, and D. Davidson. 1991. The lymphocyte: specific tyrosine kinase p56Lck. *Semin. Immunol.* **3:** 143–152.
4. Amrein, K. E., and B. M. Sefton. 1988. Mutation of a site of tyrosine phosphorylation in the lymphocyte-specific tyrosine kinase, p56Lck, reveals its oncogenic potential in fibroblasts. *Proc. Natl. Acad. Sci. USA.* **85:** 4247–4251.
5. Straus, D. B., and A. Weiss. 1993. The CD3 chains of the T cell antigen receptor associated with ZAP-70 tyrosine kinase are tyrosine phosphorylated after receptor stimulation. *J. Exp. Med.* **178:** 1523–1530.
6. Secrist, J. P., L. Karnitz, and R. T. Abraham. 1991. T-cell antigen receptor ligation induces tyrosine phosphorylation of phospholipase C-gamma 1. *J. Biol. Chem.* **266:** 12135–12139.
7. Park, D. J., H. W. Rho, and S. G. Rhee. 1991. CD3 stimulation causes phosphorylation of phospholipase C-gamma I on serine and tyrosine residues in a human T-cell line. *Proc. Natl. Acad. Sci. USA.* **88:** 5453–5457.
8. Khan, N. A., F. Ferrier, and P. Deschaux. 1995. Serotonin-induced calcium signalling via 5-HT1A receptors in human leukaemia (K 562) cells. *Cell. Immunol.* **165:** 148–152.
9. Khan, N. A., J-P. Meyniel, and P. Deschaux. 1996. Ca^{2+}/calmodulin and protein kinase C regulation of serotonin transport in human K 562 lymphocytes. *Cell. Immunol.* **172:** 269–274.
10. Khan, N. A., and A. Hichami. 1999. Ionotropic 5-hydroxytryptamine type 3 receptor is implicated in the activation of protein kinase C-dependent phospholipase D pathway in human T cells. *Biochem. J.* **344:** 199–204.
11. Neel, B. G. 1997. Roles of phosphatases in lymphocyte activation. *Curr. Opin. Immunol.* **9:** 405–420.
12. Downward, J., J. D. Graves, P. H. Warne, S. Rayter, and D. A. Cantrell. 1990. Stimulation of p21ras upon T-cell activation. *Nature.* **346:** 719–723.
13. Buday, L. S., S. E. Egan, P. Rodriguez, P. A. Cantrell, and J. Downward. 1994. A complex of Grb2 adapter protein, Sos exchange factor, and a 36 Kda membrane-bound tyrosine phosphorylation is implicated in Ras activation in T cells. *J. Biol. Chem.* **269:** 9019–9023.
14. Sieh, M., A. Batzer, J. Schlessinger, and A. Weiss. 1994. GRB2 and phospholipase C-gamma 1 associate with a 36- to 38-kilodalton phosphotyrosine protein after T-cell receptor stimulation. *Mol. Cell. Biol.* **14:** 4435–4442.
15. Clements, J. L., and G. A. Koretzsky. 1999. Recent developments in lymphocyte activation: linking kinases to downstream signalling events. *J. Clin. Invest.* **103:** 925–929.
16. Izquierdo, M., J. Downward, J. D. Graves, and D. A. Cantrell. 1992. Role of protein kinase C in T-cell antigen receptor regulation of p21ras: evidence that two p21ras regulatory pathways coexist in T cells. *Mol. Cell. Biol.* **12:** 3305–3312.
17. Zhang, X. F., J. M. Kyriakis, E. Takeuchi-Suzuki, E. Elledge, M. S.

Marchall, J. T. Bruder, U. Rapp, and J. Avruch. 1993. Normal and oncogenic p21 ras proteins bind to the amino acid terminal regulatory domain of c-Raf-1. *Nature.* **364:** 308–313.
18. Kyriakis, J. M., H. App, X. F. Zhang, P. Banerjee, D. L. Brautigan, U. R. Rapp, and J. Avruch. 1992. Raf-1 activates MAP kinase-kinase. *Nature.* **358:** 417–421.
19. Robbins, D. J., E. Zhen, M. Cheng, S. Xu, D. Ebert, and M. H. Cobb. 1994. MAP kinases ERKI and ERK2: pleiotropic enzymes in a ubiquitous signalling network. *Adv. Cancer Res.* **63:** 93–116.
20. Whitehurst, C. E., and D. Geppert. 1996. MEK1 and the extracellular signal-regulated kinases are required for the stimulation of IL-2 gene transcription in T cells. *J. Immunol.* **156:** 1020–1029.
21. Calder, P. C. 1996. Effects of fatty acids and dietary lipids on cells of the immune system. *Proc. Nutr. Soc.* **55:** 127–150.
22. Harbige, L. S. 1998. Dietary n-6 and n-3 fatty acids in immunity and autoimmune disease. *Proc. Nutr. Soc.* **57:** 555–562.
23. Kremer, J. M., D. A. Lawrence, W. Jubiz, R. Di Giacomo, K. Rynes, L. E. Bartolomew, and M. Sherman. 1990. Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis. *Arthritis Rheum.* **33:** 810–820.
24. Bates, D., N. E. F. Cartlidge, J. M. French, M. J. Jackson, S. Nightingale, D. A. Shaw, S. Smith, E. Woo, S. A. Hawkins, J. H. D. Millar, J. Belin, D. M. Conroy, S. K. Gill, M. Sidey, A. D. Smith, R. H. S. Thompson, K. Zilkha, M. Gale, and H. M. Sinclair. 1989. A double-blind controlled trial of long chain n-3 polyunsaturated fatty acids in the treatment of multiple sclerosis. *J. Neurol. Neurosurg. Psychiatr.* **52:** 18–22.
25. Lands, W. E. M. 1991. Biosynthesis of prostaglandins. *Annu. Rev. Nutr.* **11:** 41–60.
26. Bonin, A., and N. A. Khan. 2000. Regulation of calcium signalling by docosahexaenoic acid in human T cells: implication of CRAC channels. *J. Lipid Res.* **41:** 277–284.
27. Yang, S. P., I. Morita, and S-I. Murota. 1998. Eicosapentaenoic acid attenuates vascular endothelial growth factor-induced proliferation via inhibiting Flk-1 receptor expression in bovine carotid artery endothelial cells. *J. Cell Physiol.* **176:** 342–349.
28. Hii, C. S. T., A. Ferrante, Y. S. Edwards, Z. H. Huang, P. J. Hartfield, D. A. Rathjen, A. Poulos, and A. W. Murray. 1995. Activation of mitogen-activated protein kinase by arachidonic acid in rat liver epithelial WB cells by a protein kinase C-dependent mechanism. *J. Biol. Chem.* **270:** 4201–4204.
29. Rao, G. N., A. S. Baas, W. C. Glagow, T. E. Eling, M. S. Runge, and R. W. Alexander. 1994. Activation of mitogen-activated protein kinases by arachidonic acid and its metabolites in vascular smooth muscle cells. *J. Biol. Chem.* **269:** 32586–32591.
30. Nel, A. E., C. Hanekom, A. Rheeder, K. Williams, S. Pollack, R. Katz, and G. E. Landreth. 1990. Stimulation of MAP-2 kinase activity in T-lymphocytes by antiCD3 or antiTCR monoclonal antibody is partially dependent on protein kinase C. *J. Immunol.* **144:** 2683–2689.
31. Barry, O. P., M. G. Kazanietz, D. Pratico, and G. A. FitzGerald. 1999. Arachidonic acid in platelet microparticles up-regulates cyclooxygenase-2-dependent prostaglandin formation via a protein kinase C/mitogen-activated protein kinase-dependent pathway. *J. Biol. Chem.* **274:** 7545–7556.
32. Dulin, N. O., A. S. Sorokin, and J. G. Douglas. 1998. Arachidonate-induced tyrosine phosphorylation of epidermal growth factor receptor and Shc-Grb2-Sos association. *Hypertension.* **32:** 1089–1093.
33. Nel, A. E., C. Hanekon, and C. Hultin. 1991. Protein kinase C plays a role in the induction of tyrosine phosphorylation of lymphoid microtubule-associated protein-2 kinase. Evidence for a CD3-associated cascade that includes p56lck and that is defective in HPB-ALL. *J. Immunol.* **147:** 1933–1939.
34. Tsutsumi, A., M. Kubo, H. Fuji, J. Freire-Moar, C. W. T. Turck, and J. T. Ransom. 1993. Regulation of protein kinase C isoform proteins in phorbol ester-stimulated Jurkat T lymphoma cells. *J. Immunol.* **150:** 1746–1754.
35. Kolch, W., G. Heidecker, G. Kochs, R. Hummel, H. Vahidi, H. Mischak, G. Finkenzeller, D. Marme, and U. R. Rapp. 1993. Protein kinase C alpha activates RAF-1 by direct phosphorylation. *Nature.* **364:** 249–252.
36. Clark, K. J., and A. W. Murray. 1995. Evidence that the bradykinin-induced activation of phospholipase D and of the mitogen-activated protein kinase cascade involves different protein kinase C isoforms. *J. Biol. Chem.* **270:** 7097–7103.
37. Marignani, P. A., R. M. Epand, and R. F. Sebaldt. 1996. Acyl chain dependence of diacylglycerol activation of protein kinase C activity in vitro. *Biochem. Biophys. Res. Commun.* **225:** 469–473.
38. Nishizuka, Y. 1995. Protein kinase C and lipid signalling for sustained cellular responses. *FASEB J.* **9:** 484–496.
39. May, C. L., A. J. Southworth, and P. C. Calder. 1983. Inhibition of lymphocyte protein kinase C by unsaturated fatty acids. *Biochem. Biophys Res. Commun.* **195:** 823–828.
40. Goldyne, M. E., G. E. Burrish, P. Paubelle, and P. Borgeat. 1984. Arachidonic acid metabolism among human mononuclear leukocytes. Lipoxygenase-related pathways. *J. Biol. Chem.* **259:** 8815–8819.
41. Kurland, J. L., and R. Bockman. 1978. Prostaglandin E production by human blood monocytes and mouse peritoneal macrophages. *J. Exp. Med.* **147:** 952–957.
42. Le Gouvello, S. L., O. Colrad, I. Theodorou, G. Bismuth, N. Tarantino, and P. Debre. 1990. CD2 triggering stimulates a phospholipase A_2 activity beside the phospholipase C pathway in human T lymphocytes. *J. Immunol.* **144:** 2359–2364.