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Depression of Humoral Responses and Phagocytic Functions *in Vivo* and *in Vitro* by Fish Oil and Eicosapentanoic Acid

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Previous studies have demonstrated that eicosapentanoic acid (EPA) has anti-inflammatory properties in both humans and experimental animals and may also depress humoral immunity in experimental animals. Our investigations showed that the addition of eicosapentanoic acid to human peripheral blood mononuclear cell cultures inhibited B cell responses to mitogenic stimulation and depressed the expression of interleukin 2 receptors in pokeweed mitogen-stimulated lymphocytes. Neutrophils were also affected in their ability to release the contents of primary and secondary granules, particularly when stimulated with antigen–antibody complexes. Similar depressions of B cell responses and neutrophil functions were observed in a normal volunteer who ingested 6 g/day of a commercially available fish oil extract (equivalent to 2.1 g of EPA/day) during a 6-week period. Phagocytosis, enzymatic release, circulating immunoglobulin levels, and the response to tetanus toxoid both *in vivo* and *in vitro* were depressed during ingestion of fish oil. Most parameters showed a trend toward normalization 6 weeks after the suspension of fish oil supplementation. These effects of fish oil extracts and EPA on phagocytosis and humoral responses may be advantageously used in the therapy of chronic inflammatory diseases and autoimmune diseases but could be a cause for concern when these compounds are used for longer periods of time and with minimal medical supervision for the prophylaxis of atherosclerosis.

INTRODUCTION

The publication of epidemiological evidence linking high fish consumption to low incidence of ischemic heart disease (1, 2) promoted considerable interest in the possibility of lowering the risk for the development of occlusive arterial disease through dietary supplementation with fish, fish oil, or fish oil-derived Ω-3 fatty acids. Clinical studies have shown that dietary supplementation with fish oil extracts causes changes in serum lipid and lipoprotein levels (particularly a depression of plasma triglycerides and VLDL), changes in the fatty acid composition of a variety of cells, a depression of platelet aggregability often associated with prolonged bleeding times, reduced synthesis of PGE_2, thromboxane, and LTB4, and an impairment of neutrophil chemotaxis and of the superoxide burst (4–11).

The interference of fish oil and eicosapentanoic acid (EPA) with prostaglandin and leukotriene metabolisms is considered as a possible basis for an anti-

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inflammatory effect of fish oil and its components (4, 12, 13). This effect has been demonstrated in animal models of inflammatory joint disease and in patients with rheumatoid arthritis. Mice whose fat source was exclusively fish oil produced less PGE_2 and PGI_2 and showed decreased susceptibility to the development of collagen-induced arthritis (14). Subjective improvement and decreased neutrophil leukotriene B_4 production were observed in a group of patients with rheumatoid arthritis after 14 weeks of administration of 2.7 g EPA and 1.8 g of docosahexanoic acid (DHA)/day (15). A decrease of both cycloxygenase and lipooxygenase activities in neutrophils obtained from healthy volunteers receiving fish oil supplementation for 6 weeks (4) and decreased chemiluminescence and superoxide production by phagocytosing polymorphonuclear (PMN) leukocytes obtained from individuals that had also ingested cod liver oil for 6 weeks (16) can be considered as objective evidence for the anti-inflammatory properties of fish oils.

Of considerable interest were the observations made in MRL-*lpr* mice demonstrating that dietary supplementation with fish oil would suppress the spontaneous development of lupus in those animals (17, 18). This effect was attributed to the anti-inflammatory properties of fish oil, but it also coincided with decreased levels of circulating retroviral gp70 immune complexes (17). The reduction in retroviral IC could reflect a depressive effect of fish oil on the humoral immune response.

In humans there is data suggesting that EPA may have a wider range of effects over the immune system. In asthmatic adults receiving 4 g/day of purified EPA for 8 weeks, a significant inhibition of leukocyte chemotaxis and an increased response of peripheral blood lymphocytes to phytohemmaglutinin (PHA) were observed (19). Our investigations were carried out with the purpose of better characterizing the effects of fish oil extracts and EPA on neutrophil and B cell functions. *In vitro* studies with pure EPA were complemented with the longitudinal follow-up of a normal healthy volunteer under dietary supplementation with a fish oil extract. Both types of studies suggest that EPA and fish oil extracts may have strong inhibitory effects on PMN leukocytes and B lymphocytes.

MATERIALS AND METHODS

1. Isolation and Stimulation of PMN Leukocytes

PMN leukocytes were isolated by a modification of the method of Guidicelli *et al.* (20, 21). Blood was collected in citrate and layered on 63 and 72% Percoll in Hank's buffered saline solution (HBSS) without calcium or magnesium. After centrifugation at 800*g*, for 26 min at 20°C, the PMN were recovered from a band just below the 63–72% interface. The PMN so recovered were washed three times in ice-cold Hank's buffered saline. The homogeneity of the preparations was found to be better than 90% by Giemsa's staining and the viability better than 95% by trypan blue exclusion.

2. Stimulation of PMN Leukocytes

Purified PMN leukocytes at a concentration of 1 to 5×10^6/ml were stimulated by incubation in immune complex (IC)-coated tissue culture dishes and by addi-

tion of opsonized zymosan, f-Met–Leu–Phe (FMLP), ionophore A23187, and phorbol myristate acetate (PMA).

Zymosan was prepared at 5 mg and opsonized by incubation with freshly drawn normal human serum; f-Met–Leu–Phe was purchased from Sigma and used at a final concentration in the culture of 1–10 μM; ionophore A23187 (Sigma) was used at a final concentration of 0.1–1.0 μM; and phorbol myristate acetate (Sigma) was used at concentrations ranging from 0.1 to 2.0 ng/ml. Further details about the protocols of activation with these different agents can be found in Refs. (22–25).

IC-coated tissue culture dishes were prepared by coating 48-well microtiter plates with KLH and incubating the KLH-coated plates with the IgG fraction obtained from a rabbit anti-KLH antiserum produced in our laboratory. The concentration of KLH used for coating was 5 μg/well, and the plates were incubated with KLH for 60 min at 37°C. Unbound sites were blocked with 1% BSA. Rabbit IgG was extracted from anti-KLH serum by precipitation with octanoic acid (26); 50 μg of purified IgG was added to each well and incubated for 60 min at 37°C. The plates were washed three times with PBS–BSA (1% w/v) prior to use.

The effects of these activating conditions were assessed by measuring the levels of extracellularly released enzymes, including lysozyme, elastase, and lactoferrin. Lysozyme was measured according to Osserman and Lawlor (27). Elastase was measured by determining the release of ^3H from tritiated elastin, prepared according to Lent and Franzblau (28).

3. Phagocytosis

The phagocytic capacity of PMN leukocytes was determined using opsonized fluorescent latex beads (Fluoresbrite fluorescent monodisperse carboxylated microspheres, Polysciences Inc., Warrington, PA) and opsonized sheep red blood cells (SRBC). Fifteen-microliter aliquots of fluorescent latex beads were opsonized with a 1/10 dilution of guinea pig complement (Anderson Labs., Fort Worth, TX) in veronal–borate buffer containing Ca^{2+} and Mg^{2+}, washed three times with tyrodes-Ca^{2+}, Mg^{2+}, and added to 0.5 ml of PMN suspension (1 to 5 $\times$ 10^6/ml). The PMN–latex beads mixture was then incubated for 30 min at 37°C in a rocking platform. At the end of the incubation the cells were sedimented by centrifugation at 500*g* for 2 min, the cell pellet was washed twice with PBS–EDTA, pH 7.35, resuspended, and examined at the fluorescent microscope to determine the total number of PMN and the number of PMN showing ingestion of more than four fluorescent beads. From this data we calculated a phagocytic index: (No. of positive cells/total number of cells) $\times$ 100.

Antibody-coated RBC were prepared by incubating 3 $\times$ 10^8 SRBC with 23 ng of IgG anti-SRBC (Cordis Labs., Miami, FL) in HBSS–1% BSA for 30 min at 37°C. After incubation, the cells were washed three times and suspended in HBSS–1% BSA and 7.5 $\times$ 10^6 IgG-coated SRBC were incubated with 5 $\times$ 10^5 PMN for 30 min at 37°C. Duplicate assays were performed for each experimental condition. At the end of this incubation, PMN and uningested RBC were sedimented by centrifugation, the cell-free media were harvested, and uningested RBC were lysed by the addition of 0.85% ammonium chloride. The remaining PMN were washed once

with HBSS–BSA, resuspended in the same buffer, and examined under a phase contrast microscope to determine the number of PMN having ingested one or more SRBC. Phagocytic indices were calculated as in the case of fluorescent beads, except that in the case of SRBC, ingestion of a single red cell was considered as positive.

4. Isolation of Peripheral Blood Mononuclear Cells (PBMNC)

PBMNC were separated from venous blood collected in citrate anticoagulant by centrifugation of a 1:3 dilution of blood in RPMI over a cushion of an equal volume of Ficoll–Paque (Pharmacia Fine Chemicals) (29). The viability of the isolated cells was assessed by trypan blue dye exclusion.

5. Culture and Stimulation of PBMNC

PBMNC at concentrations ranging from 1 to 1.5 $\times$ 10^6/ml were cultured for 11 days in 10 $\times$ 75-mm polystyrene test tubes loosely capped and were maintained vertical and stationary for the length of the culture (30). The cultures were carried out in RPMI medium (GIBCO) supplemented with 10% heat-inactivated FCS (GIBCO).

Pokeweed mitogen (PWM) stimulation was carried out by adding pokeweed mitogen (GIBCO) in an optimal dose, determined by dose–response studies (50 μl of a 1:5 dilution in RPMI). Antigenic stimulation with tetanus toxoid (TT) was achieved by adding 25 ng/ml of purified TT, generously provided by Wyeth Laboratories, Inc. (31).

6. Assay of Immunoglobulins and Specific Antibodies

The supernatants from PBMNC were harvested at Day 11 and processed as described in detail in an earlier publication (30). IgM and IgG were assayed by laser nephelometry using antisera pretested to exclude reactivity with FCS. The assays were calibrated with known reference standards adjusted to concentrations ranging from 16 to 0.5 μg/ml of IgG and from 22 to 0.7 μg/ml of IgM.

Specific anti-TT levels were determined by an ELISA assay developed in our laboratory (31) adjusted to the low concentrations expected in cell culture supernatants by using calibrators with concentrations ranging from 0.100 to 0.001 U/ml of anti-TT antibody.

7. Determination of the Proportion of Lymphocytes Expressing Interleukin 2 (IL-2) Receptors

The expression of IL-2 receptors was determined at Day 3 of culture by cytofluorography using anti-TAC obtained from Ortho Diagnostics.

8. Study of the Effects of EPA on Neutrophils and Lymphocytes

EPA was dissolved either in HBSS or in absolute ethanol. The stock solution was made at 4 mg/ml, and the highest concentration tested in PBMNC was 40 μg/ml, corresponding to the addition of 10 μl of stock solution to 1-ml cultures.

Lower concentrations were obtained by adding adequate volumes of stock dilutions in RPMI. EPA remained in the medium for the duration of the experiments. As controls, we used identical concentrations of ethanol (when ethanol was used to dissolve EPA) or of arachidonate (Sigma).

9. Human Studies

A normal healthy volunteer participated in a longitudinal study of the effects of supplementation with 6 g/day of one of the commercially available fish oil extracts, equivalent to a daily dose of 2.1 g of EPA and 0.9 g of DHA. The supplementation was maintained for a period of 6 weeks, and studies were performed before the onset of supplementation, at 3 and 6 weeks of supplementation, and 6 weeks after termination of the experiment. During this period the volunteer was carefully monitored clinically and laboratorially and the only side effect referred to was increased gum bleeding. The immunological studies included determination of immunoglobulin levels, *in vitro* and *in vivo* responses to tetanus toxoid, phagocytosis of fluorescent latex beads, and release of lysozyme by PMN stimulated with antibody-coated RBC and with *f*-Met–Leu–Phe. Immunoglobulin levels were determined by immunonephelometry in the Diagnostic Laboratories. The other parameters were determined with the methodologies outlined for studies of EPA effects *in vitro*, except that in this case no EPA was added to the volunteer's cells. Whenever possible, samples were stored and all the parameters were measured simultaneously after the end of the experiment in order to minimize the effect of run-to-run variation. Informed consent was granted by the volunteer in which these studies were carried out.

10. Statistical Analysis

The results of *in vitro* experiments in which we tested the effects of EPA on phagocytosis and lymphocyte functions were analyzed by the Wilcoxon signed rank test.

RESULTS

In Vitro Studies of the Effects of EPA on Neutrophils

Using neutrophils separated from normal donors, we investigated the effects of exogenously added EPA on the degranulation reaction after stimulation by four different mechanisms: immobilized immune complexes, FMLP, calcium ionophore A23187, and PMA. As a rule, EPA caused a general depression of degranulation, which was not seen when arachidonic acid (AA) was added to neutrophils in identical concentrations. The depression was more obvious for lysozyme release, particularly in response to stimulation via the Fc receptor with immobilized IC (Fig. 1). The release of elastase in response to IC, A23187, and FMLP was also inhibited (Figs. 2 and 3), but the differences between cultures stimulated in the presence of EPA and control cultures did not reach statistical significance. The release of lysozyme induced by PMA stimulation was also inhibited by EPA, but statistically significant differences were only observed when relatively high doses of PMA were used to induce maximal release (Fig. 4). These inhibitory effects

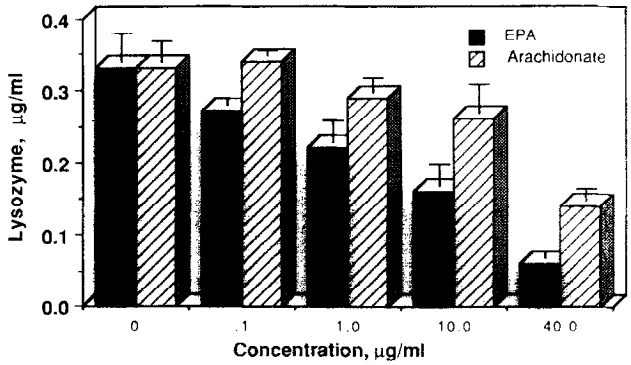

FIG. 1. *In vitro* study of the effects of different concentrations of EPA and arachidonate on the release of lysozyme by normal PMN stimulated by incubation in IC-coated tissue culture dishes. The values correspond to the mean and standard deviations for four to six experiments, depending on the points. Statistically significant reductions of lysozyme levels were seen in cultures to which we added 1 and 10 µg/ml of EPA, relative to cultures to which we added identical concentrations of AA (P = 0.03 and 0.04, respectively).

could be the result of membrane changes secondary to enrichment in EPA, but given the short incubation times used in these experiments, it appears more likely that EPA may act by disturbing the sequence of second messages that is initiated by Fc receptor crosslinking or by PMA stimulation.

In Vitro Studies of the Effects of EPA on Lymphocytes

The addition of 40 µg/ml of EPA to unfractionated PBMNC cultures resulted in a marked depression of B cell responses to PWM (Fig. 5) and of the expression of IL-2 receptors (Fig. 6). The increased expression of IL-2 receptors after PWM stimulation was also affected by the addition of arachidonic acid to the cultures.

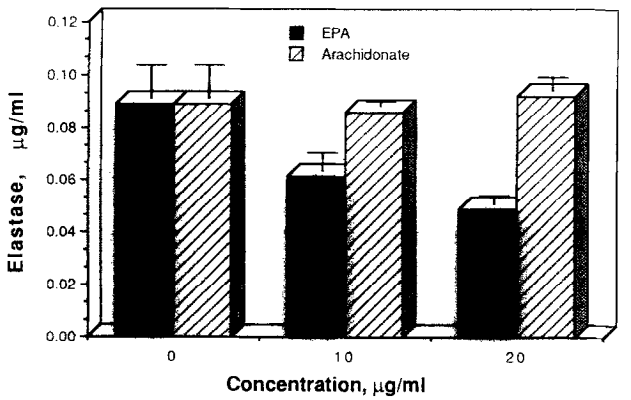

FIG. 2. *In vitro* study of the effects of different concentrations of EPA and arachidonate on the release of elastase by normal PMN stimulated by incubation in IC-coated tissue culture dishes. The values represent the means and SE from two different experiments. Although there is a definite trend toward inhibition of elastase release by EPA compared to AA, the difference did not reach statistical significance.

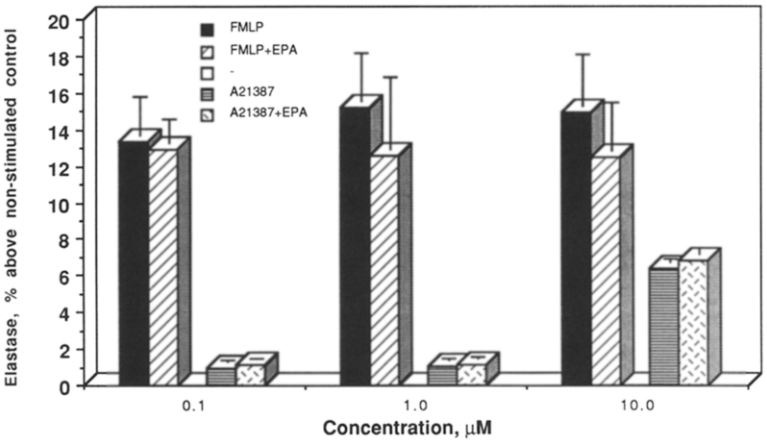

FIG. 3. *In vitro* study of the effect of the addition of 20 μg/ml of EPA to PMN stimulated with different concentrations of the chemotactic peptide f-Met–Leu–Phe and of the calcium ionophore A21387. The concentrations of released elastase are expressed as percentages over the baseline release in nonstimulated controls. The results represent the mean ± SE for a total of four experiments. The differences between EPA and medium were not statistically significant.

but to a lesser extent than when identical concentrations of EPA were added. A dose–response study of the effect of EPA on IL-2 receptor expression and immunoglobulins synthesis showed that the depression was obvious with 10 μg/ml, and even 1 μg/ml caused a slight depression of IL-2 receptor expression (Fig. 7). The specific response to TT was also found to be extremely sensitive to EPA, suppressed at concentrations of 0.1 μg/ml.

Effects of Fish Oil Ingestion in a Normal Volunteer

Phagocytic functions were severely depressed in this volunteer, particularly at the end of the 6-week period of dietary supplementation with a fish oil extract.

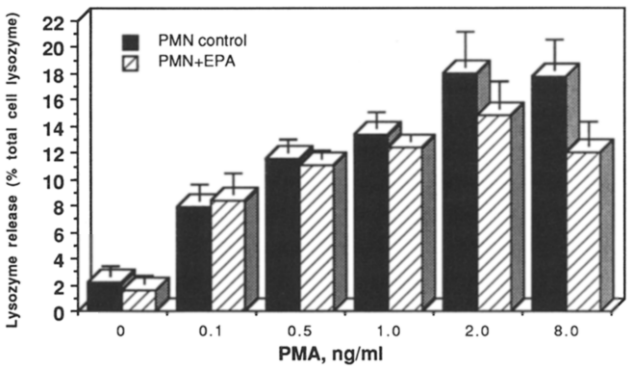

FIG. 4. *In vitro* study of the effects of the addition of 20 μg/ml of EPA to PMN stimulated with different concentrations of PMA. The concentrations of released lysozyme are expressed as percentages relative to the levels of lysozyme measured in supernatants of Triton X-treated cells. The values shown in the figure correspond to the mean ± SE for a total of eight experiments. The difference between the addition of 8 ng/ml of PMA or EPA was statistically significant ($P = 0.034$).

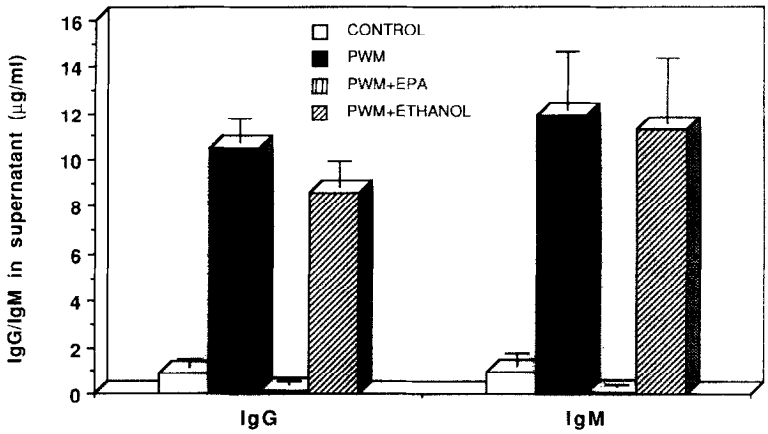

FIG. 5. *In vitro* inhibition of B cell responses with EPA. Unfractionated PBMNC from a normal, healthy volunteer were stimulated with PWM. The addition of 40 μg/ml of EPA to the culture totally abrogated the B cell responses. Controls to which ethanol (used to dissolve EPA) was added showed comparable responses to PWM-stimulated cultures. Viability counts were similar in all cultures. The values shown in the figure correspond to mean ± SE for a total of five experiments. Statistically significant differences were observed between cultures stimulated with PWM in the presence or absence of EPA for IgG and IgM ($P = 0.003$) as well as between cultures stimulated with PWM in the presence of ethanol or EPA for IgG and IgM ($P = 0.003$). No significant difference existed between IgM released by cultures stimulated with PWM or with PWM + ethanol, but a significant difference ($P = 0.019$) was seen between the levels of IgG released by cultures stimulated with PWM or with PWM + ethanol.

Both the ability to ingest latex particles and antibody-coated RBC and the release of lysozyme in response to Fc receptor and f-Met–Leu–Phe stimulation were sharply depressed at the end of the 6-week period of fish oil supplementation (Figs. 8 and 9). Six weeks after ending fish oil ingestion the values of these

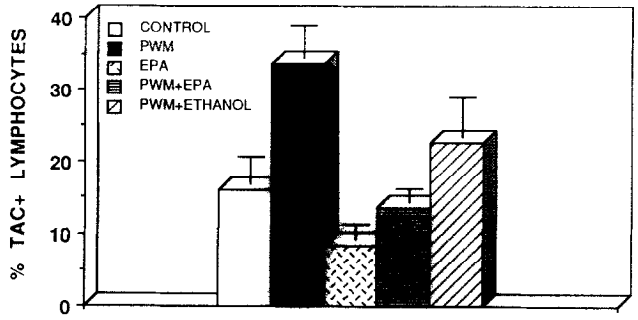

FIG. 6. Effects of EPA (40 μg/ml) on the expression of IL-2 receptor (measured by cytofluorography 3 days after initiation of the cultures). EPA caused a marked decrease in the IL-2 receptor expression of greater extent than the decreased observed in culture tubes to which we added ethanol or arachidonic acid ($P = 0.008$ between IL-2 receptors in PWM-stimulated cultures vs cultures stimulated with PWM + EPA) while no difference was seen between cultures stimulated with PWM or PWM + ethanol. The values shown in the figure correspond to the mean ± SE of four experiments. Viability counts were similar in all cultures.

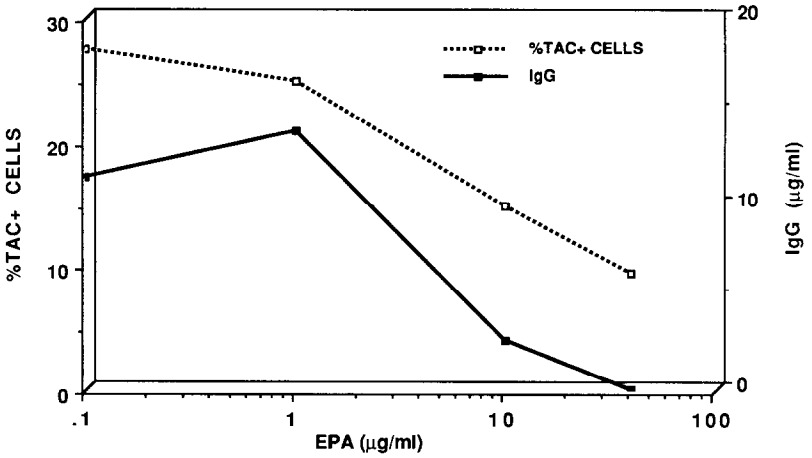

FIG. 7. Dose–response effect of EPA on the expression of IL-2 receptors and on the synthesis of IgG by PWM-stimulated PBMNC.

parameters had increased but did not reach the pretrial levels. The levels of immunoglobulins G and M also showed a depression in association to fish oil supplementation (Fig. 10), while IgA was not as affected. The levels of IgG and IgM had risen to or beyond pretrial levels 6 weeks after ending the trial. The *in vitro* response to TT was also depressed during the period of fish oil ingestion, while the baseline release of anti-TT antibody by PBMNC stimulated with TT and PWM was 20 mU/ml; no release could be detected either at 3 or at 6 weeks of fish oil supplementation (1 mU/ml being the lower limit of detection for the assay of anti-TT antibodies). The response to a booster with TT was irregular, showing a transient and small rise at Week 3 and a second increase in antibody levels 6 weeks after the end of fish oil administration (Fig. 11). The influence of EPA on

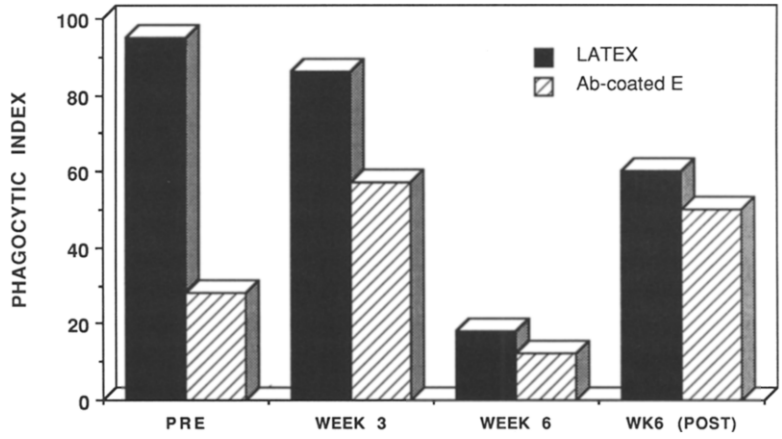

FIG. 8. Longitudinal study of the ingestion of fluorescent latex beads and antibody-coated RBC in a normal volunteer who supplemented his diet with 4 g/day of a fish oil extract for a 6-week period. The last study (post) was carried out 6 weeks after cessation of therapy.

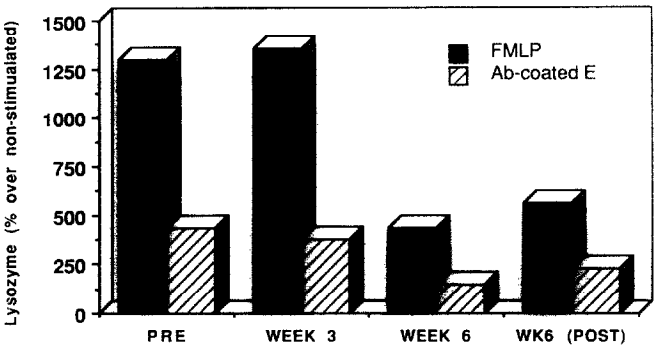

FIG. 9. Longitudinal study of the release of lysozyme by PMN leukocytes from the same healthy volunteer after stimulation with f-Met–Leu–Phe or with antibody-coated erythrocytes (E). The values are given as the percentage over background release by nonstimulated PMN.

the response of this subject's lymphocytes to PWM could not be studied since he was a nonresponder to this mitogen.

DISCUSSION

The depressive effects of essential fatty acids on the immune response have been the object of numerous studies, summarized by Johnston and Marshall (32). Prior to the onset of interest in fish oil and its active constituents, the focus of most studies had been the comparison of polysaturated vs polyunsaturated fatty acids, particularly of the Ω-6 series. Omega-6 fatty acids are the natural precursors for leukotriene B_4 and prostaglandins of the 1 and 2 series, whose capacity to induce immunosuppression has been well documented (33, 34). The administration of polyunsaturated oils rich in linolenic acid to experimental animals for extended periods of time results in decreased prostaglandin synthesis, but no consistent effects on phagocytosis or the humoral immune responses have been reported (32).

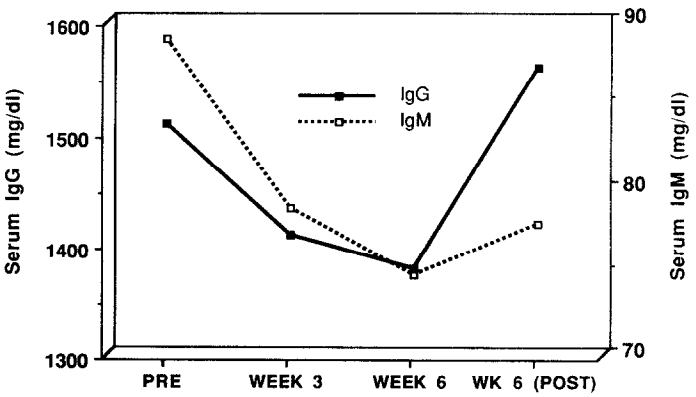

FIG. 10. Longitudinal evolution of the levels of circulating IgG and IgM in the same normal volunteer who supplemented his diet with 4 g/day of a fish oil extract. The post values were determined 6 weeks after terminating the ingestion of fish oil extract.

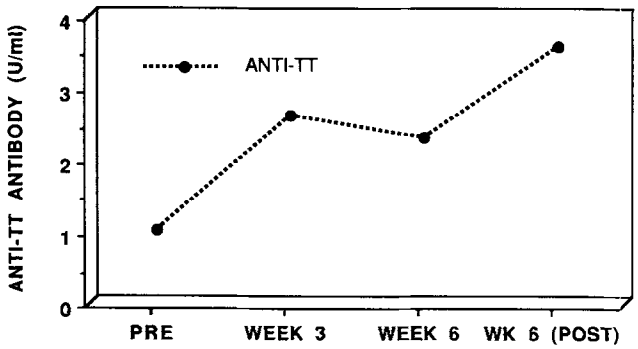

FIG. 11. Longitudinal assay of anti-tetanus antibodies in the same volunteer. A tetanus toxoid booster was given in the first day of dietary supplementation with a fish oil extract. The antibody levels were determined in the same run to minimize interassay variation.

On the other hand, the main active components of fish oil extracts are EPA and DHA, which belong to the Ω-3 series of fatty acids. *In vitro*-purified EPA inhibits prostaglandin and LTB_4 synthesis (35–37). Evidence for a depression of arachidonic acid metabolism has also been observed in humans and experimental animals receiving dietary fish oil supplementation (4, 12, 38, 39). This depression appears to result from the competition between EPA and AA as substrates for cyclooxygenase and lipooxygenase. The metabolic end products of EPA, particularly the prostaglandin endoperoxides and LTB_5, are several orders of magnitude less biologically potent than their homologous products derived from AA (4, 40).

The interference of fish oil and EPA with prostaglandin and leukotriene metabolisms is considered as a possible basis for an anti-inflammatory effect of fish oil and its components (4, 12, 13). This effect has been demonstrated in animal models of inflammatory joint disease and in patients with rheumatoid arthritis. Mice whose fat source was exclusively fish oil produced less PGE_2 and PGI_2 and showed decreased susceptibility to the development of collagen-induced arthritis (14). Subjective improvement and decreased neutrophil leukotriene B_4 production were observed in a group of patients with rheumatoid arthritis after 14 weeks of administration of 2.7 g EPA and 1.8 g of DHA/day (4).

A possible immunosuppressive effect of fish oil and its derivatives has been suggested by experimental and clinical observations. Studies with MRL-*lpr* mice which develop a spontaneous lupus-like disease demonstrated a protective effect of dietary supplementation with fish oil (17, 18). This effect was attributed to the anti-inflammatory properties of fish oil extracts, but it also coincided with decreased levels of circulating retroviral gp70 immune complexes (17). The reduction in retroviral IC could obviously reflect a depressive effect of fish oil on the humoral immune response.

Several observations suggest that a similar immunodepressive effect may take place in humans. In asthmatic adults receiving 4 g/day of purified EPA for 8 weeks, a significant inhibition of leukocyte chemotaxis and an increased response of peripheral blood lymphocytes to phytohemagglutinin, a T lymphocyte mitogen, were observed (19). Our own data suggest that EPA may have a strong inhibitory

effect over PMN leukocytes and B lymphocytes *in vitro*. During a 6-week period in which a volunteer underwent supplementation with 6 g/day of a commercially available fish oil extract, we detected a progressive decline in immunoglobulin levels, lack of *in vitro* response to tetanus toxoid, poor *in vivo* response to a tetanus toxoid booster, impairment of PMN phagocytosis, and depression of the enzymatic release reaction triggered by a variety of stimuli, particularly IgG-coated RBC and f-Met–Leu–Phe. These effects had, in general, a slow onset, some of them only evident at the end of the 6-week trial, and some of the parameters did not completely normalize 6 weeks after cessation of fish oil ingestion. This suggests that the effects of fish oil *in vivo* may be related to a progressive replacement of fatty acids in cells and tissues eventually leading to important functional abnormalities. Other functional abnormalities caused by fish oil extracts have similarly been reported to develop only after prolonged dietary intake. For example, animals and humans receiving fish oil for several weeks show a decreased synthesis of thromboxane A2, which does not occur in short-term *in vitro* experiments. Therefore, it appears as very likely that some or most of the *in vivo* effects attributed to EPA would depend on a slowly progressive change in cellular lipid composition and metabolism (41).

Whether or not the *in vitro* effects of EPA are due to similar mechanism is not known, but we were able to induce objective depression of B cell responses and PMN functions *in vitro* by adding EPA to cells obtained from normal donors. In the case of PMN, we documented a dose-dependent depression of enzymatic release in response to several stimuli, and in the case of lymphocytes we documented dose-related depressions of immunoglobulin production in response to PWM. The effect on B cell responses appears to be secondary to an inhibition of T cell activation, as reflected by a depression of IL-2 receptor expression which can be observed at concentrations of EPA as low as 1.0 μg/ml.

There is little to no available information concerning the optimal dosage of fish oil extracts. In the therapeutic trials that have been published, the dose and the mode of administration of fish oil have varied considerably. For example, the dosage of MaxEPA, a commercial product derived from cod liver oil, has varied between approximately 2.7 to 10 g/day of EPA (15, 40, 42). Little justification is given to the use of doses as large as 10 g/day of EPA. Our patient responded quite dramatically to a considerably lower dose of EPA (1.2 g/day), but indeed more data from properly controlled studies are needed to establish the range of active doses of EPA and fish oil extracts, as well as their safety.

Although our *in vivo* observations were based on a single individual, the magnitude and the nature of the longitudinal changes observed were impressive and cannot be easily attributed to extraneous factors, such as a placebo effect. The good correlation of the effects seen *in vivo* with those induced *in vitro* using cells from normal donors also reinforces the case for a true depressive effect of EPA, which in the case of humoral immunity had not been previously documented in man. Our observation suggests that individuals ingesting the usually recommended doses of fish oil may suffer a broad depression of phagocytic functions as well as a depression of the humoral immune responses. These findings raise important questions about the safety of long-term administration of fish oil and

fish oil derivatives. However, these suppressive effects could be extremely beneficial in patients with autoimmune disorders.

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