Effects of fish oil on cytokines and immune functions of mice with murine AIDS

Side Xi,* Donald Cohen,† and Linda H. Chen[1,*,§]

Nutritional Sciences Program,* Departments of Microbiology and Immunology,† and Nutrition and Food Science,§ University of Kentucky, Lexington, KY 40506-0054

Abstract The effects of fish oil, which is rich in n–3 fatty acids, on cytokine levels in a murine model of acquired immune deficiency syndrome (AIDS) were studied. Thirty-two C57BL/6 female mice were divided into two dietary groups and fed either a corn oil diet or a fish oil diet. After 4 weeks, each diet group was further divided into two subgroups, and mice in one subgroup were injected i.p. with LP-BM5 murine retrovirus (MAIDS) stock. After 4 weeks, all mice were killed, blood samples were collected, and the spleens and the livers were excised. Splenocytes were isolated immediately and cultured in RPMI-1640 medium and stimulated by either lipopolysaccharide (LPS) or Concanavalin A (ConA) for 24 h. The supernatant was collected for cytokine assays. The results showed that MAIDS infection increased the levels of tumor necrosis factor-α (TNF-α) and interleukin-1-beta (IL-1β), while fish oil partially prevented this elevation. MAIDS infection depressed interleukin-2 (IL-2) and interferon-gamma (IFNγ), while fish oil partially prevented the depression of IL-2. In addition, MAIDS infection depressed LPS- and ConA-stimulated cell proliferation, while fish oil partially prevented the depression. The results suggest that fish oil may slow down the progression of murine AIDS by modulating levels of cytokines including TNF-α, IL-1β, and IL-2.—Xi, S., D. Cohen, and L. H. Chen. **Effects of fish oil on cytokines and immune functions of mice with murine AIDS. *J. Lipid Res.* 1998.** 39: **1677–1687.**

Supplementary key words fish oil • TNFα • IL-1β • IL-2 • IFNγ • LTB_4 • arachidonic acid

Cytokines are proteins of low molecular weight which are involved in the regulation of local or systemic immune or inflammatory responses (1). They are produced under physiological and pathological conditions for the regulation of numerous processes such as cell activation, inflammation, immunity, and tissue repair. Cytokines are distinct from each other by their specificity on target cell and biological effects. However, different cytokines may induce similar effects. The functional redundancy among cytokines constitutes the basis of effective immune regulation and immune damage as well. Tumor necrosis factor (TNF) and interleukin-1 (IL-1) are associated with many pathological processes especially inflammation and autoimmune reactions (2). Interleukin-2 (IL-2) is a primary growth factor for T cells and also acts on B cells and macrophages to promote growth and differentiation. Interferon-γ (INFγ) is a potent activator of macrophages and can enhance the cytotoxicity of natural killer cells. As an antiviral agent, IFNγ can interrupt viral entrance, viral uncoating, and the synthesis of mRNA or proteins of the virus. Because IL-2 and IFNγ are produced by T cells, a decline in their levels may adversely affect cell-mediated immune functions.

Acquired immune deficiency syndrome (AIDS) is caused by HIV infection and is characterized by eventual damage to immune functions especially $CD4^+$ T lymphocyte function. AIDS is a major public health problem in the world due to its epidemic nature, increasing incidence, and high cost of therapy. Studies have shown that T lymphocyte damage during HIV infection is preceded by extensive alterations of cytokines, such as an increase of TNF and IL-1 (3, 4) and the decrease of cytokines produced by T cells, IL-2, and IFNγ (5, 6). Further studies have demonstrated that TNF-α and IL-1β can stimulate the HIV expression in infected cells (7, 8), while the antibody to TNF can block the constitutive expression of HIV and partially block the induced expression in certain cell lines (9). A correlation between the progression of AIDS and the serum levels of TNF has been reported by Mintz et al. (10) in which they demonstrated that 79% of patients with progressive encephalopathy have elevated levels of TNF, as compared to only 8% of patients without neurologic involvement. Additionally, Ito and coworkers (11) demonstrated that TNF antagonizes the inhibitory effect of azidothymidine (AZT) on HIV replication in vitro.

Abbreviations: AIDS, acquired immunodeficiency syndrome; MAIDS, murine AIDS; HIV, human immunodeficiency virus; ROS, reactive oxygen species; IL, interleukin; TNF, tumor necrosis factor; 20:4, arachidonic acid; PLA_2, phospholipase A_2; CO, cyclooxygenase; LO, lipoxygenase; EPA, eicosapentanoic acid; DHA, docosahexanoic acid; LTB_4, leukotriene B_4; PGE_2, prostaglandin E_2; TXA_2, thromboxane A_2; PGI_2, prostaglandin I_2; ConA, concanavalin A; LPS, lipopolysaccharide.

[1]To whom correspondence should be addressed.

These studies have clearly indicated the etiologic roles of cytokines involved in the inflammatory process, especially TNF-α, in the expression of HIV. The present study used murine AIDS as the animal model for human AIDS. Murine AIDS, produced by the infection of murine LP-BM5 leukemia retrovirus, has been found to have immunological changes similar to those in human AIDS. Studies have shown that MAIDS infection, as in human HIV infection, causes a significant increase of the production of TNF and IL-1 (12, 13), and a decrease of IL-2 and IFNγ (13, 14).

Modulation of cytokines may have therapeutic potential for human AIDS. However, because of the complicated interactions of cytokines, modulation of an individual cytokine has not produced very impressive results. Ideal agent(s) should be able to regulate a range of cytokines. Fish oil, which is rich in n–3 fatty acids, may be used for this purpose based on its anti-inflammatory effects in many diseases. The anti-inflammatory effect of fish oil is attributed to its intervention on arachidonic acid (20:4, n–6) metabolism by n–3 fatty acids. By inhibiting the production of 20:4 metabolites such as leukotriene B_4 (LTB_4) or prostaglandin E_2 (PGE_2), fish oil has been shown to decrease the production of TNF-α and IL-1β, or to increase IL-2 and IFNγ in many other diseases, such as autoimmune diseases (15–17). In addition, Fernandes et al. (18) has shown that dietary fish oil, when compared with corn oil, significantly prolongs (by 30%) the life span of mice infected with MAIDS retrovirus.

The effects of fish oil on cytokines have been frequently reported in many other diseases, such as autoimmune diseases; however, only a few studies have been performed in AIDS or murine AIDS. Fernandes et al. (18) have reported that fish oil can delay the appearance of lymphadenopathy and increase IL-2 in murine AIDS. The present study was undertaken to examine the effects of fish oil on cytokines involved in the inflammatory process (TNF-α, IL-1β) and cytokines produced in T cells (IL-2, IFNγ) in murine AIDS. An additional indicator of immune function, proliferative response of splenocytes to mitogens, was also determined. In addition, the serum levels of IgG and IgM were determined in order to assess the progression of murine AIDS. In order to determine the mechanism of the effects of fish oil, the levels of leukotriene B_4, LTB_4, a metabolite of 20:4, were also assayed.

MATERIALS AND METHODS

Animals and diets

C57BL/6 female mice at 6–8 weeks of age were purchased from Harlan Sprague Dawley, Inc. and were caged individually in sterilized microisolator cages. Animals were monitored by the Division of Laboratory Animal Resources at the University of Kentucky according to the Animal Welfare Act. The mice were fed autoclaved lab chow and sterile water ad libitum for 1 week as the adaptation period, then were randomly assigned to four groups. The experimental diets were prepared in pellet form by the ICN company (St. Louis, MO) upon our request, sealed under nitrogen, and delivered in dry ice. Upon arrival, the diets were repackaged into small bags (each bag for 1 day/group) under nitrogen and stored at −20°C immediately. The diets were available to mice ad libitum daily and the leftover from the previous day was discarded. The compositions of the diets, modified from AIN76A diet (AIN, 1977), are shown as follows: casein 20%, corn starch 34.34%, corn oil 20% in the control group or 17% fish oil plus 3% corn oil in the fish oil group, cellulose 5%, AIN salt mixture 4%, AIN vitamin mixture 1.2%, dl-methionine 0.3%, butylated hydroxyquinone (BHQ) 0.02%, and additional dl-α-tocopheryl acetate to each diet to make the total vitamin E content at the level of 250 IU/kg diet. The contents of eicosapentaenoic acid (EPA, 20:5, n–3) and docosahexaenoic acid (DHA, 22:6, n–3) in menhaden fish oil were 16.03% and 10.85%, respectively (data provided by the ICN Company).

Experimental design

A 2 × 2 factorial experimental design was used in the present study. Factors consisted of diet (corn oil or fish oil) and infection (infected or non-infected). A total of 32 mice were randomly distributed into four groups. Two groups were fed the corn oil diet and two groups were fed the fish oil diet. One group from each diet group was infected with LP-BM5 retrovirus stock by intraperitoneal (i.p.) injection after 4 weeks; and the same amount of sterilized saline solution was injected i.p. into the control groups. The mice were maintained on the same diets for another 4 weeks. Body weights were recorded at the beginning and the end of the experiment. Daily food consumption was recorded for 3 consecutive days before killing the animals. Mice were killed with carbon dioxide and exsanguinated by cardiac puncture prior to removal of the livers and spleens. The weights of the spleens were also recorded. Blood samples were collected and centrifuged to separate serum, and the serum samples were frozen at −70°C until IgG and IgM levels were determined. The livers were frozen for the analysis of fatty acids, and splenocytes were prepared immediately for cytokine analysis, determination of proliferative response to mitogens, and LTB_4 assay.

Virus infection

LP-BM5 is a mixture of murine leukemia virus containing disease-causing defective retrovirus of murine AIDS (MAIDS). Virus stock was prepared by the method of Mosier, Yetter, and Morse (19). Briefly, the constitutionally infected cell line (SC-1) was maintained by biweekly subculture in Dulbecco's modified minimal essential medium supplemented with 10% fetal bovine serum. Cell-free filtrates were prepared from culture medium collected after 3–4 days of growth, centrifuging the culture fluid at 3,000 rpm for 10 min, and passing the supernatant through a 0.22-μm Millipore filter. One ml of filtered supernatant was injected i.p. into each mouse. Viral infection was verified at the time of killing by the occurrence of splenomegaly as we have previously described (20, 21).

Fatty acid analysis

Lipids in 10% liver homogenate were extracted by the method of Metcalf and Schitz (22). Neutral lipids and phospholipids were separated by using Sep-pak (Waters: WAT051900). Sep-pak was attached to a glass syringe and washed with methanol, then with chloroform. The lipid extract was added to the syringe, and pushed through the syringe. Neutral lipids were eluted with chloroform, and phospholipids were then eluted by methanol. After evaporating the solvents under nitrogen, fatty acid methyl esters were prepared by using boron trifluoride in methanol. After extracting with hexane and washing with water, the samples were evaporated under nitrogen, then redissolved in 100 μl hexane and transferred to microvials. The samples were analyzed on a Hewlett Packard Model 5890 gas chromatograph equipped with HP FFAP 0.2 mm × 10 m column. The conditions consisted of an

oven temperature of 210°C, an injection temperature at 220°C, a detector temperature at 250°C, and a carrier gas helium at 10 ml/min.

Splenocyte culture

Immediately after the spleens were excised, splenocyte suspension was prepared by gently teasing the spleens with forceps in a culture medium (RPMI-1640) containing 10% fetal bovine serum, 2 mmol/l glutamine, 100 mg/l penicillin–streptomycin, and then washing twice with the culture medium. Red blood cells were lysed by the addition of a lysis buffer (0.16 m ammonium chloride in Tris buffer, pH 7.2) at 37°C for 2 min. The final splenocyte concentration was adjusted to 2×10^5 cells/well in a 96-well plate and stimulated with either ConA or lipopolysaccharide (LPS) (10 mg/l) depending on the assay. Cells were cultured at 37°C in a 5% CO_2 humidified incubator for 24 h, and the supernatant was collected and frozen at −70°C for the assays of cytokines and LTB_4.

Serum IgG and IgM levels

Serum IgG and IgM levels were determined by using commercial ELISA kits (Pharmingen Co., San Diego, CA). The concentrations of IgG and IgM were read from the respective standard curve.

Cytokine levels

Cytokine levels of the cell-free supernatant from splenocytes cultured with either LPS (TNF-α, IL-1β) or ConA (IL-2, IFNγ) were determined using commercial ELISA kits (BioSource International, Camarillo, CA). The procedures for these four cytokines were similar except that different antibodies and incubation temperatures were used. The concentrations of cytokines in the samples were read from the respective standard curve.

Proliferative response to mitogens

The proliferation of splenocytes was determined by incorporation of [^{3}H]thymidine as described by Wang et al. (23). Briefly, splenocytes isolated from each mouse were cultured in triplicate in 100 μl of RPMI-1640 culture medium at 2×10^6 cells/ml concentration in a 96-well flat-bottom culture plate. ConA or LPS (10 mg/l) was added and incubated at 37°C in a 5% CO_2 incubator for 48 h. Each well was pulsed with [^{3}H]thymidine (37 kBq/well) and the plate was incubated at 37°C for another 4 h. The radiolabeled cells were harvested, and radioactivity indicating the incorporation of [^{3}H]thymidine into DNA was counted using a liquid scintillation counter, Matrix 96 Direct Beta Counter (Packard Instruments).

LTB_4 production

LTB_4 levels of cell-free supernatant from splenocytes cultured with LPS (10 mg/l) for 24 h were determined by using a commercial ELISA kit for LTB_4 (Neogen Co., Lexington, KY). The concentrations of LTB_4 in the samples were read from the standard curve.

Statistical analysis of data

Data were analyzed by two-way analysis of variance. When analysis of variance indicated significant differences, the treatment means were compared in pairs using Fisher's least significant difference procedure (24). Statistical probability of $P < 0.05$ was considered significant.

RESULTS

Splenomegaly is an apparent consequence of MAIDS infection. The spleen weights were compared among the four groups of mice. MAIDS infection increased the spleen weights 5- to 7-fold and fish oil decreased the spleen weights in MAIDS infected group by about 10–20%, suggesting that splenomegaly was less severe in the fish oil/MAIDS group than in the MAIDS group (data not shown). Fish oil alone (without infection) did not affect spleen weights.

Composition of fatty acids

Fatty acid pattern indicates the incorporation of dietary fatty acids into the membrane. Fatty acid compositions of the livers of mice are presented in two tables. **Table 1** shows the fatty acid composition of neutral lipids in the livers of mice. Fish oil significantly increased the content of n–3 fatty acids and decreased the content of n–6 fatty acids in neutral lipids when compared with corn oil. In **Table 2**, a similar pattern for phospholipids in the livers was observed. There were a significant increase of n–3 fatty acids and a decrease of n–6 fatty acid contents, and the increase of n–3 fatty acids in phospholipids was higher than that in neutral lipids. There was a significant decrease of 20:4 (n–6) and a significant increase of eicosapentaenoic acid (EPA, 20:5, n–3) and docosahexaenoic acid (DHA,

TABLE 1. Fatty acid composition in liver neutral lipids

Fatty Acids	Corn Oil Group	Corn Oil/ MAIDS Group	Fish Oil Group	Fish Oil/ MAIDS Group
		% of total fatty acids		
C16:0	24.49 ± 2.85^b	24.0 ± 1.59^b	37.50 ± 14.27^a	25.23 ± 4.62^b
C16:1, n–7	2.51 ± 0.48^b	2.28 ± 1.10^b	5.43 ± 1.44^a	5.43 ± 0.22^a
C18:0	1.97 ± 0.09	2.06 ± 0.25	2.10 ± 0.58	2.30 ± 0.58
C18:1, n–9	22.82 ± 1.88^a	23.16 ± 3.01^a	12.96 ± 3.38^b	12.63 ± 1.50^b
C18:2, n–6	28.90 ± 4.10^a	31.73 ± 5.96^a	9.34 ± 1.95^b	10.48 ± 0.72^b
C18:3, n–3	0.27 ± 0.05^b	0.28 ± 0.06^b	0.65 ± 0.17^a	0.67 ± 0.07^a
C20:4, n–6	1.12 ± 0.01^a	1.20 ± 0.16^a	0.37 ± 1.07^b	0.39 ± 0.04^b
C20:5, n–3	0.92 ± 0.65^b	1.56 ± 1.10^b	6.04 ± 2.59^a	4.64 ± 0.80^a
C22:6, n–3	0.07 ± 0.05^b	0.08 ± 0.06^b	4.43 ± 0.80^a	3.53 ± 0.36^a
Total n–6	30.02	32.93	10.51	10.87
Total n–3	1.26	2.02	11.12	8.84

Data represent mean ± SD of 4 mice from each group except last two rows. Minor fatty acids are not listed. Means with different letters are significantly different at $P < 0.05$.

TABLE 2. Fatty acid composition in liver phospholipids

Fatty Acids	Corn Oil Group	Corn Oil/ MAIDS Group	Fish Oil Group	Fish Oil/ MAIDS Group
		% of total fatty acids		
C16:0	26.59 ± 3.45^{a,b}	21.63 ± 1.49^{b}	27.58 ± 0.22^{a}	30.00 ± 1.96^{a}
C16:1, n–7	2.42 ± 1.11^{b}	1.46 ± 0.45^{b}	3.45 ± 0.24^{a}	3.12 ± 0.43^{a}
C18:0	16.04 ± 2.38^{b}	18.72 ± 0.36^{b}	22.18 ± 1.50^{a}	21.10 ± 1.78^{a}
C18:1, n–9	13.50 ± 2.42^{a}	10.96 ± 1.43^{a}	7.42 ± 0.89^{b}	7.51 ± 1.70^{b}
C18:2, n–6	20.67 ± 3.60^{a}	23.31 ± 1.82^{a}	13.24 ± 0.62^{b}	14.58 ± 1.14^{b}
C18:3, n–3	0.29 ± 0.41	1.29 ± 0.88	0.24 ± 0.17	0.62 ± 0.20
C20:4, n–6	9.52 ± 5.59^{a}	13.07 ± 1.91^{a}	4.54 ± 0.20^{b}	4.95 ± 1.23^{b}
C20:5, n–3	trace	trace	7.39 ± 1.52^{a}	5.53 ± 1.71^{a}
C22:6, n–3	2.35 ± 1.69^{b}	2.45 ± 1.04^{b}	5.75 ± 1.38^{a}	5.99 ± 3.62^{a}
Total n–6	30.19	36.38	17.78	19.53
Total n–3	2.64	3.74	13.38	12.14

Data represent mean ± SD of 4 mice from each group except the last two rows. Minor fatty acids are not listed. Means with different letters are significantly different at $P < 0.05$.

22:6, n–3) in both neutral lipids and phospholipids in the fish oil group when compared to the corn oil group.

Effects of fish oil on IgG and IgM levels

The serum levels of IgG and IgM are indicators of the progression of murine AIDS. As shown in **Fig. 1**, serum levels of IgG were significantly increased in the corn oil and MAIDS infected group (CA) ($P < 0.01$) and decreased in the fish oil group (F) ($P < 0.05$) when compared to the corn oil group (C). The group that was fed fish oil and received MAIDS infection (FA) had significantly lower serum IgG levels than the CA group ($P < 0.05$). When the C and CA groups were compared, the serum IgG levels of the CA group were 357% of that of the C group; and when the F and FA groups were compared, the serum IgG levels of the FA group were 353% of that of the F group. The pattern of the effects of fish oil on serum IgM levels (**Fig. 2**) was similar to that of serum levels of IgG, but with a greater magnitude. Serum IgM levels were the highest in the CA group (C versus CA, $P < 0.001$), lowest in the F group (C versus F, $P < 0.05$), and those in the FA group were intermediate, which was significantly lower than the CA group ($P < 0.05$). When the C and CA groups were compared, the serum IgM levels of the CA group were 236% of that of the C group, while comparison of the F and FA groups showed that the serum IgM levels of the FA group were 328% of that of the F group. The results indicating that the FA group had lower IgG and IgM levels than the CA group suggested that dietary fish oil can slow down the progression of murine AIDS.

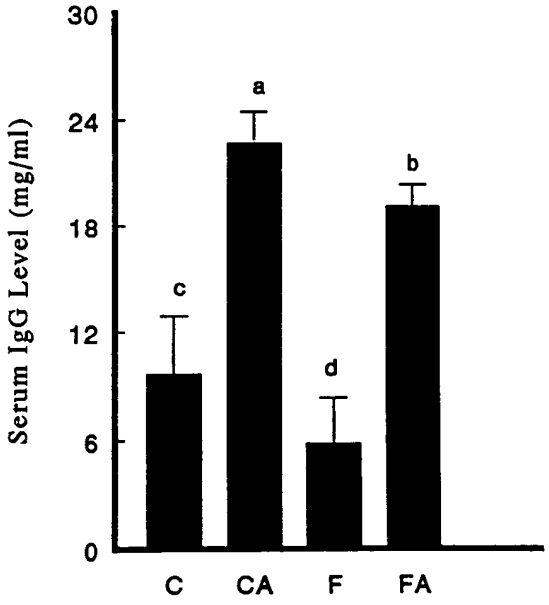

Fig. 1. Effects of fish oil on serum antibody IgG levels. Serum was diluted and assayed by ELISA method. Data represent mean ± SD of seven samples. Means with different letters are significantly different at $P < 0.05$.

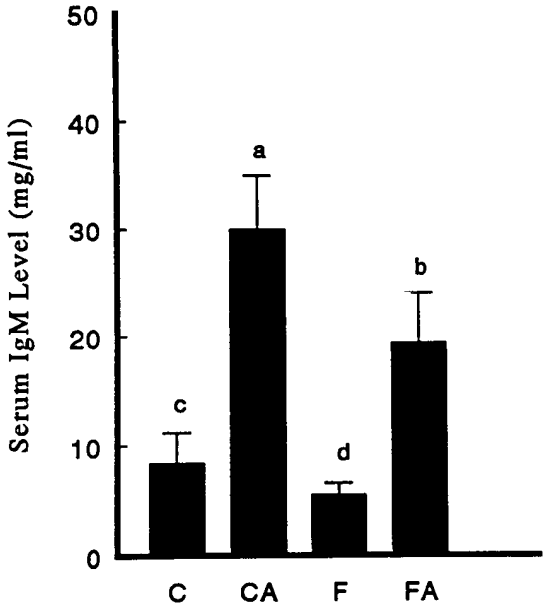

Fig. 2. Effects of fish oil on serum antibody IgM levels. Serum was diluted and assayed by ELISA method. Data represent mean ± SD of seven samples. Means with different letters are significantly different at $P < 0.05$.

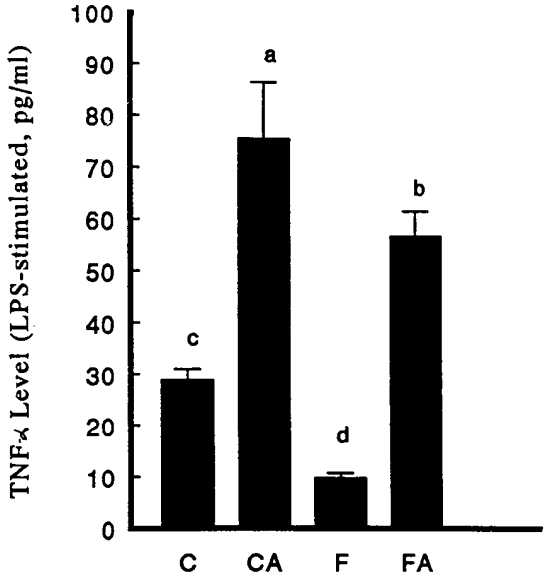

Fig. 3. Effects of fish oil on TNF-α levels in splenocytes. Cells were stimulated with LPS (10 mg/l) in RPMI-1640 medium and cultured at 37°C in a 5% CO_2 incubator for 24 h. Supernatant TNF-α levels were assayed by ELISA method. C: corn oil only, CA: corn oil plus infection, F: fish oil only; FA: fish oil plus infection. Data represent mean ± SD of four samples. Means with different letters are significantly different at $P < 0.05$.

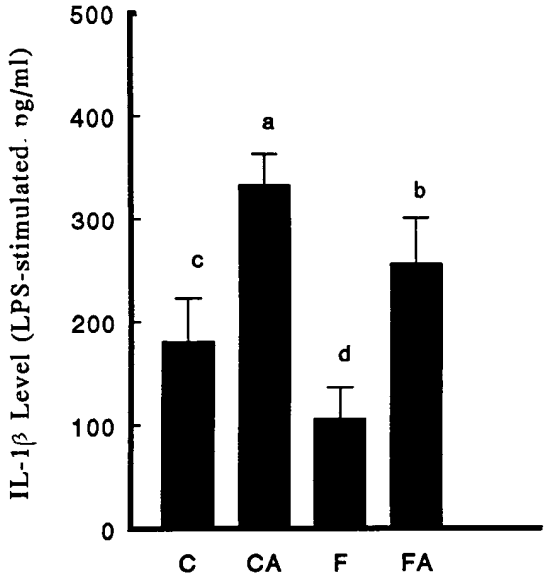

Fig. 4. Effects of fish oil on IL-1β levels in splenocytes. Cells were stimulated with LPS (10 mg/l) in RPMI-1640 medium and cultured at 37°C in a 5% CO_2 incubator for 24 h. Supernatant IL-1β levels were assayed by ELISA method. C: corn oil only, CA: corn oil plus infection, F: fish oil only, FA: fish oil plus infection. Data represent mean ± SD of four samples. Means with different letters are significantly different at $P < 0.05$.

Effects of fish oil on TNF-α and IL-1β

The effects of fish oil on TNF-α levels in the supernatant of LPS-stimulated cultured splenocytes are shown in **Fig. 3**. MAIDS infection significantly increased TNF-α levels (C versus CA, $P < 0.005$), while fish oil partially prevented the elevation of TNF-α levels. When the C and CA groups were compared, the TNF-α levels of the CA group were 262% of that of the C group; and when the F and FA groups were compared, the TNF-α levels of the FA group were 580% of that of the F group. **Fig. 4** shows that MAIDS infection significantly increased IL-1β levels (C versus CA, $P < 0.01$), while fish oil again partially prevented the elevation. The comparison of the C and CA groups showed that the IL-1β levels of the CA group were 185% of that of the C group; while the comparison of the F and FA groups showed that the IL-1β levels of the FA group were 243% of that of the F group. The inhibitory effects of fish oil on these two cytokines (Fig. 3 and Fig. 4) were observed in both the infected (CA versus FA, $P < 0.05$) and non-infected groups (C versus F, $P < 0.05$). The results indicating that the TNF-α and IL-1β levels were lower in the FA group than in the CA group suggested that these two cytokines were decreased in the rats fed fish oil diet when compared to those fed corn oil diet after MAIDS infection.

Effects of fish oil on IL-2 and IFNγ

The production of IL-2 and IFNγ is critical for the generation of an effective immune response. **Figure 5** shows that MAIDS infection significantly suppressed the levels of IL-2 levels (C versus CA, $P < 0.001$). The fish oil group (F) and the corn oil group (C) without infection had similar IL-2 levels. Fish oil significantly increased IL-2 levels in the infected group (FA) when compared to the corn oil group with infection (CA) ($P < 0.05$). When the C

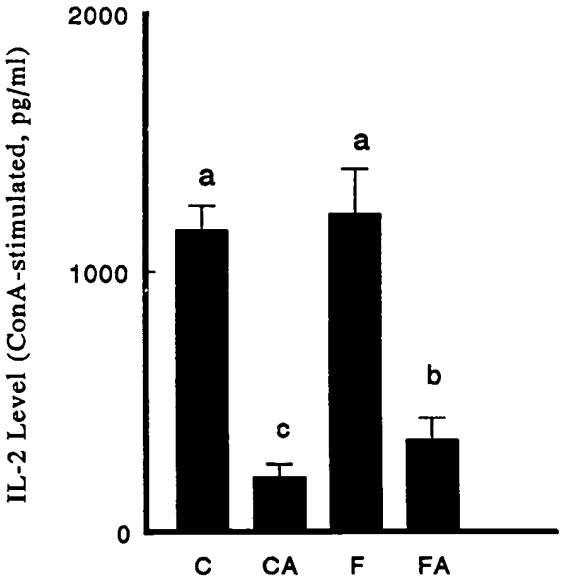

Fig. 5. Effects of fish oil on IL-2 levels in splenocytes. Cells were stimulated with ConA (10 mg/l) and cultured in RPMI-1640 medium at 37°C in a 5% CO_2 incubator for 24 h. Supernatant IL-2 levels were assayed by ELISA method. C: corn oil only, CA: corn oil plus infection, F: fish oil only, FA: fish oil plus infection. Data represent mean ± SD of four samples. Means with different letters are significantly different at $P < 0.05$.

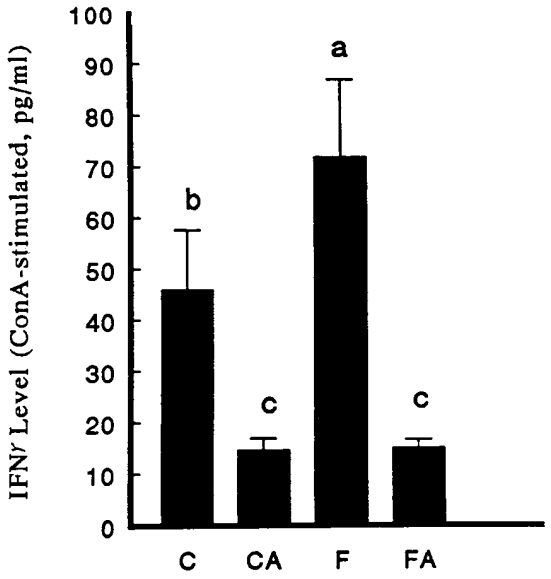

Fig. 6. Effects of fish oil on IFNγ levels in splenocytes. Cells were stimulated with ConA (10 mg/l) and cultured in RPMI-1640 medium at 37°C in a 5% CO_2 incubator for 24 h. Supernatant IFNγ levels were assayed by ELISA method. C: corn oil only, CA: corn oil plus infection, F: fish oil only, FA: fish oil plus infection. Data represent mean ± SD of four samples. Means with different letters are significantly different at $P < 0.05$.

and CA groups were compared, the IL-2 levels of the CA group were 17.9% of that of the C group; the comparison of the F and FA groups showed that the IL-2 levels of the FA group were 28.9% of that of the F group. On the other hand, as shown in **Fig. 6**, fish oil increased the production of IFNγ levels in the non-infected group (C versus F, $P < 0.05$), but did not affect IFNγ levels in the infected group.

Effects of fish oil on proliferative response to mitogens

The proliferative responses of splenocytes to LPS and ConA stimulation from the four groups of mice are presented in **Fig. 7**. LPS is a B lymphocyte stimulator, while ConA is a T lymphocyte stimulator. In both treatments, MAIDS infection significantly decreased cell proliferation (C versus CA: LPS, $P < 0.01$; ConA, $P < 0.001$), while dietary fish oil partially prevented the depression of cell proliferation caused by MAIDS infection (CA versus FA: LPS, $P < 0.05$; ConA, $P < 0.01$). With LPS stimulation, cell proliferation of the CA group was decreased to 43% of the C group; while cell proliferation of the FA group was decreased to 80% of that of the F group. With ConA stimulation, cell proliferation of the CA group was decreased to 10% of that of the C group; while cell proliferation of the FA group was decreased to 46% of that of the F group. Results indicating that the FA group had higher cell proliferation than the CA group with either LPS or ConA stimulation suggested that fish oil can improve splenocyte proliferation after MAIDS infection, when compared to corn oil.

Effects of fish oil on LTB_4 levels

In an attempt to explain the possible mechanism of the effects of fish oil, the levels of LTB_4 were also determined in the cell-free supernatant of cultured selenocytes stimulated with LPS. This metabolite for 20:4 has been reported to increase the production of TNF-α and IL-1. In **Fig. 8**, it can be seen that MAIDS infection caused a significant increase in LTB_4 levels (C versus CA, $P < 0.01$), while fish oil completely prevented the elevation of LTB_4 levels (CA versus FA, $P < 0.01$). When the C and CA groups were compared, the LTB_4 levels of the CA group were 171% of that of the C group; while the comparison of the F and FA groups indicated that the LTB_4 levels of the FA groups were 116% of the F group. To further elucidate the effects of LTB_4 on cytokines involved in inflammation, two in vitro experiments were performed using isolated splenocytes from normal mice. As shown in **Fig. 9**, addition of exogenous LTB_4 resulted in increased levels of TNFα when compared to the control ($P < 0.05$); while PGE_2, another metabolite of 20:4, did not affect the levels of this cytokine. In **Fig. 10**, a similar effect of exogenous LTB_4 to increase IL-1β levels was observed. Prostaglandin E_2 (PGE_2) again did not affect IL-1β levels.

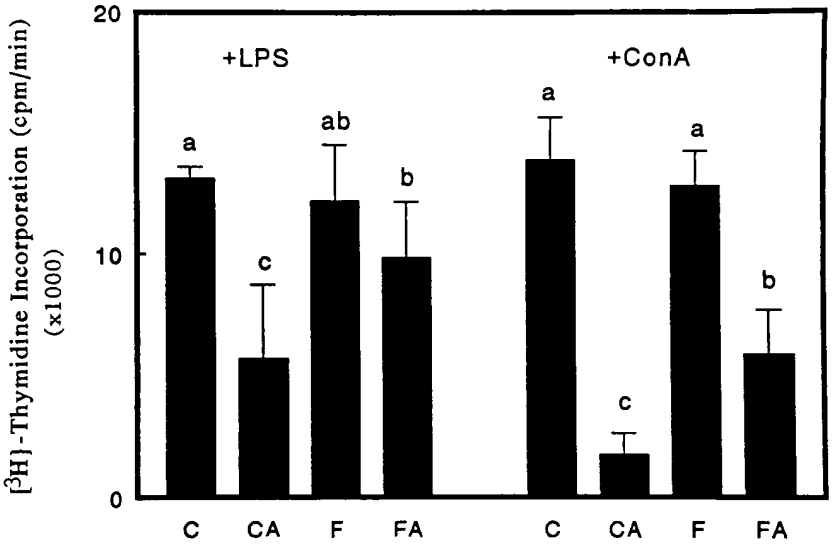

Fig. 7. Effects of fish oil on cell proliferation in splenocytes. Cells were stimulated with either LPS or ConA (10 mg/l) and cultured in RPMI-1640 medium at 37°C in a 5% CO_2 incubator for 48 h, and then pulsed with [^{3}H]thymidine for 4 h. C: corn oil only, CA: corn oil plus infection, F: fish oil only, FA: fish oil plus infection. Data represent mean ± SD of four samples. Means with different letters are significantly different at $P < 0.05$ with each stimulating agent.

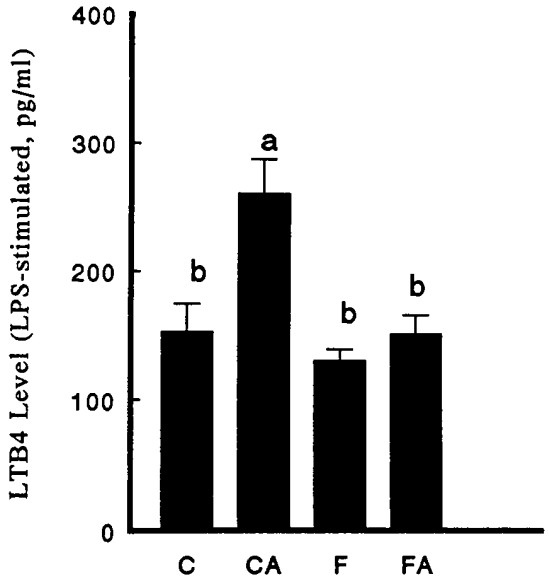

Fig. 8. Effects of fish oil on LTB_4 levels in splenocytes. Cells were stimulated with LPS (10 mg/l) and cultured in RPMI-1640 medium at 37°C in a 5% CO_2 incubator for 24 h. Supernatant LTB_4 levels were assayed by ELISA method. C: corn oil only, CA: corn oil plus infection, F: fish oil only, FA: fish oil plus infection. Data represent mean ± SD of four samples. Means with different letters are significantly different at $P < 0.05$.

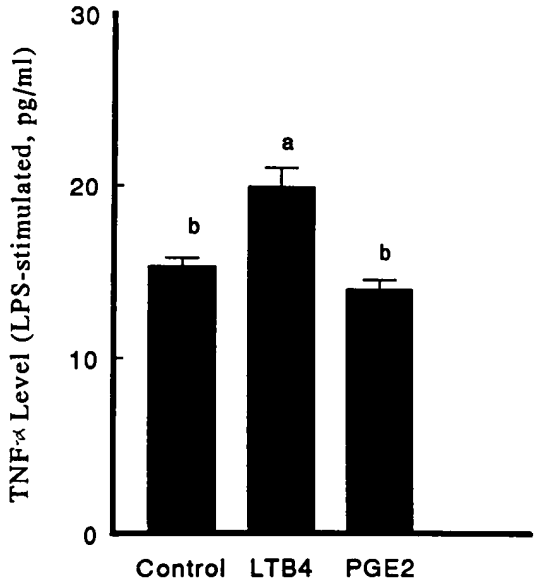

Fig. 9. Effects of arachidonic acid metabolites on TNF-α levels in cultured splenocytes. Cells were stimulated with LPS (10 mg/l) and cultured in RPMI-1640 medium containing none, 0.1 μm LTB_4 or 5 μm PGE_2, respectively, at 37°C in a 5% CO_2 incubator for 24 h. Supernatant TNF-α levels were assayed by ELISA method. Data represent mean ± SD of four assays for each treatment. Means with different letters are significantly different at $P < 0.05$.

DISCUSSION

Murine AIDS displays a number of immunological changes that are observed in human AIDS. Both diseases cause early spontaneous proliferation of T and B lymphocytes, and eventually the loss of both cell-mediated and antibody-mediated immunity (25, 26). Both diseases cause hypergammaglobulinemia because of the polyclonal activation of B lymphocytes (27–29), decreased mitogen-stimulated responses from T or B lymphocytes (19, 28, 29), and decreased cytotoxic functions of lymphocytes and natural killer cells (26). Consequently, these abnormalities in AIDS or murine AIDS lead to the loss of host resistance to pathogens, resulting in opportunistic infections. Therefore, murine AIDS has been used as an animal model to study the pathogenesis of human AIDS (30).

Fish oil, the rich source of n–3 fatty acids, has been studied for its beneficial effects in many diseases, such as cardiovascular diseases (31) and rheumatoid arthritis (32). The clinical improvement in these diseases caused by increasing fish consumption or fish oil supplementation is associated with the increase of n–3 fatty acids in membrane lipids. Fish oil has been shown to affect cell functions in a variety of ways, such as membrane fluidity, the amount and type of immunoregulatory eicosanoids production (16, 33), activity of components of signal transduction systems (34), immune cell populations (35), and gene expression (34). The effects of n–3 fatty acids on immune functions have evoked much attention because of their potential therapeutic roles in many immune dysfunction diseases such as autoimmune diseases. Beneficial effects of fish oil supplementation have been observed in many diseases of this category including rheumatoid arthritis (32), systemic lupus erythematosus (SLE) (36, 37), psoriasis (38), ulcerative colitis (39), and asthma (40).

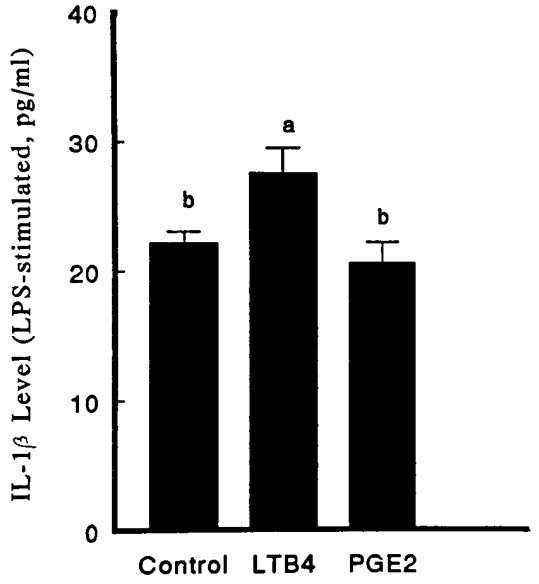

Fig. 10. Effects of arachidonic acid metabolites on IL-1β levels in cultured splenocytes. Cells were stimulated with LPS (10 mg/l) and cultured in RPMI-1640 medium containing none, 0.1 μm LTB_4 or 5 μm PGE_2, respectively, at 37°C in a 5% CO_2 incubator for 24 h. Supernatant IL-1β levels were assayed by ELISA method. Data represent mean ± SD of four assays for each treatment. Means with different letters are significantly different at $P < 0.05$.

The mechanisms of the effects of fish oil on these diseases are still under active research. However, the effects of n–3 fatty acids on cytokines and eicosanoids are considered to be the major contributors of the beneficial effects of fish oil on autoimmune diseases. By incorporating into the cell membrane, n–3 fatty acids replace some of the membrane 20:4, an n–6 fatty acid. After being released from the cell membrane upon stimulation of phospholipase A_2 (PLA_2), 20:4 is metabolized by the cyclooxygenase (CO) and lipooxygenase (LO) pathways (41, 42). The CO pathway yields many biologically active metabolites such as prostaglandins, thromboxanes, and prostacyclins; while the LO pathway produces a family of leukotrienes. Among the leukotrienes, LTB_4 is one of the most important mediators of the inflammatory process (43). It is involved in the processes of chemotaxis, degranulation, the recruitment of more inflammatory cells, and the secretion of cytokines involved in the inflammatory process (43, 44). The in vitro experiments in the present study also demonstrated that exogenous addition of LTB_4 stimulated the production of TNF-α and IL-1β in splenocytes. As n–3 fatty acids are metabolized by the same enzymes of 20:4 by competitive mechanism to produce less potent metabolites, the incorporation of the n–3 fatty acids into the membrane decreases LTB_4 production, and therefore, decreases the production of cytokines involved in the inflammatory process. The consistent decreases of LTB_4, TNF-α, and IL-1β by dietary fish oil in the present study seem to follow this logic. Other studies showed that inhibitors of LO suppressed both the in vivo and in vitro formation of TNF and IL-1 (44, 45). In addition, it was reported that LTB_4 receptor antagonist decreased the production of cytokines, which further demonstrated the causal relationship between LTB_4 and cytokines (46). Our results are in agreement with several other groups indicating that fish oil decreased the production of TNF and IL-1 in normal mice (47), autoimmune lupus-prone NZB/NZW F_1 (b/W) female mice (15), and healthy human subjects (48, 49). Caughey et al. (49) reported that TNF-α and IL-1β production decreased as cellular EPA increased to 1% of total fatty acids, and there was a significant inverse exponential relationship between TNF-α or IL-1β synthesis and the content of eicosapentaenoic acid (EPA) in mononuclear cells. Studies by Chandrasekar and Fernandes (15) and Renier et al. (50) showed that the suppression of these cytokines by fish oil occurred at the mRNA levels. As TNF and IL-1 are major mediators of inflammation, the inhibitory effects of fish oil on their overproduction of TNF and IL-1 may explain the protective role of fish oil as an anti-inflammatory agent. However, Somers and Erickson (51) reported that 3 weeks feeding of 10% fish oil diet, when compared to safflower oil, caused a higher production of TNF-α from macrophages after in vitro stimulation with LPS. The reason for the controversial reports of the effects of fish oil on cytokine production is not immediately apparent. It is likely due to the large variations in the protocols used in the studies. For example, the discrepancy of results can be interpreted by the differences in experimental period (feeding time and infection time). The shorter time with a lower amount of dietary fish oil used by Somers et al. (51) was not sufficient to observe the inhibitory effects of fish oil on the production of these cytokines. Other factors that may affect the results include the vitamin E/antioxidant levels, the types of cells studied, the samples collected, the species of animals, the ages of the animals, experimental periods, the agents used for eliciting peritoneal macrophages, the agent used to stimulate the cells in culture, and the nature of the culture medium. The large diversity of the protocols used by different researchers have made the direct comparison between any two studies difficult.

IL-2, secreted mainly from Th1 subtype of T lymphocytes, was decreased in the MAIDS-infected mice in this study. This is consistent with an earlier study by Wang and Watson (13) using the same animal model. The decrease of IL-2 is very likely related to the effects of virus infection on the Th1 subtype. As IL-2 is an important stimulator for T lymphocyte proliferation, the increase of IL-2 is beneficial in reversing the progressive decline of $CD4^+$ T lymphocytes in quantity and functions. Furthermore, IL-2 can also stimulate the cytotoxic T lymphocytes ($CD8^+$) which kill the virus in the infected cells by recognizing viral antigen-MHC I protein complexes on the cell surface. Therefore, the increase of IL-2 levels should improve the T lymphocyte-mediated immunity that is damaged by HIV infection. In clinical trials with AIDS patients, administration of IL-2 combined with antiviral drugs produced substantial and sustained increases in $CD4^+$ cells when compared with the antiviral drugs only (52). In another human study, IL-2 enhanced the depressed natural killer cells and CMV-specific cytotoxic activating lymphocytes from AIDS patients (53). Thus, the recovery of T lymphocytes during HIV infection may be achieved by administering exogenous IL-2 or inducing endogenous IL-2 production. However, the administration of exogenous IL-2 may be limited in its use because of its extensive side effects. Fish oil, which partially but significantly increased the levels of IL-2, may provide an alternative pathway for IL-2 treatment. Similar results were reported by Fernandes et al. (17, 18) who observed that fish oil supplementation could prevent the rise in B cells and the loss of $CD8^+$ T cells, and could maintain higher IL-2 levels in mice with murine AIDS. Higher levels of IL-2 with dietary fish oil were also reported in autoimmune disease-prone mouse models (54). On the other hand, Jolly et al. (55) reported that IL-2 levels decreased in healthy mice after they were treated with EPA or DHA for 10 days. Endres et al. (56) also reported that fish oil decreased the in vitro production of IL-2 from stimulated peripheral blood mononuclear cells of healthy volunteers who consumed 18 g fish oil/day for 6 weeks. In these two studies, no additional vitamin E or antioxidant was added to the diet (55) or ingested by the subjects (56), and the extent of lipid peroxidation was not assessed. The inconsistency might result from the difference in the type and status of the cells stimulated in vitro, the level of fish oil or vitamin E/antioxidant administered, or the length of the experimental period. An increased intake of fish oil or n–3 fatty acids without adequate antioxidant protection

could result in increased ROS production and lipid peroxidation, leading to a reduction in T cell function (57). In addition, the immune functions of the healthy and infected subjects are different.

Like IL-2, much of IFNγ is also secreted by T lymphocytes, especially Th1 subtype. Johnson, Russell, and Torres (58) suggested that 20:4 and its lipoxygenase metabolites play a role in the mediation of helper signals for INFγ production. MAIDS infection significantly decreased the levels of IFNγ in the present study. This is consistent with the previous study in MAIDS by Wang, Ardestani, and Liang (59). Research in AIDS patients (5) also showed that mitogen-induced production of IFNγ by mononuclear cells was impaired in 70% of the patients when compared with heterosexual controls matched by age and sex. Even more remarkable was the virtual absence of IFNγ in response to the microbial antigens used in the study. In the present study, fish oil supplementation did not reverse the decline of IFNγ production in infected mice, although fish oil supplementation to uninfected mice did increase IFNγ levels when compared with the corresponding corn oil group. This result is consistent with that of Fritsche, Feng, and Berg (60) that dietary fish oil, when compared to soybean oil, did not alter ConA-stimulated IFNγ levels in splenocytes of rats infected with *Listeria monocytogenes*. The reason why fish oil did not stimulate IFNγ in the infected mice is not clear. It is possible that the function of IFNγ secretion is more readily damaged by the virus infection at the early stage.

Unlike TNF and IL-1, the stimulatory effects of fish oil on IL-2 and IFNγ in noninfected mice are more likely to be regulated by PGE_2 rather than LTB_4. PGE_2 is reported to be immune suppressive to T lymphocytes and IL-2 production (61). It was observed that PGE_2 production by peripheral monocytes of human AIDS (61) or by splenocytes in murine AIDS was increased (18). Therefore, fish oil probably exerted stimulatory effects on T lymphocytes (both Th1 and Th2 subtypes) by inhibiting the production of PGE_2.

Our result indicating that fish oil prevented the depression of ConA-stimulated splenocyte proliferation produced by MAIDS infection is consistent with the report of Fernandes et al. (18) in autoimmune-prone mice. In addition, Payan and Goetzel (40) reported that fish oil increased the proliferation of ConA-stimulated peripheral blood mononuclear cells in asthma patients, and Wu et al. (62) observed that EPA/DHA also increased the proliferation of same cells in cynomolgus monkeys. On the other hand, Yaqoob, Newsholme, and Calder (63) administered high doses of all fatty acids (saturated and unsaturated) to normal rats for 10 weeks which resulted in a suppression of T lymphocyte proliferation. Three additional studies also reported suppressive effects of fish oil or n–3 fatty acids on cell proliferation (48, 55, 56). In these studies, Jolly et al. (55) used healthy mice, and Endres et al. (56) and Meydani et al. (48) used healthy human volunteers as subjects. As stated earlier, no additional vitamin E or antioxidant was ingested by the subjects in the first two studies, and the subjects in the study by Meydani et al. (48) had only 6 IU per day vitamin E for 2.4 g EPA/DHA ingested per day. The inconsistent results from these studies could be due to healthy versus infected state, as the immune function of subjects is different in these two states, or the level of fish oil/EPA/DHA or vitamin E/antioxidant ingested.

In conclusion, dietary fish oil when compared with corn oil produced a significant decrease of 20:4 and a significant increase of n–3 fatty acids in tissue neutral lipids and phospholipids. MAIDS infection caused an elevation of TNF-α and IL-1β, a depression of IL-2 and IFNγ, and also a depression of proliferative response to mitogens. Dietary fish oil when compared to corn oil partially prevented the elevation of TNF-α and IL-1β levels, partially prevented the depression of IL-2 levels, and also partially prevented the decline in proliferative response to mitogens. These effects of fish oil might be through modulation of LTB_4 levels. However, as PGE_2 and some other metabolites of 20:4 such as thromboxane A_2 (TXA_2) and prostaglandin I_2 (PGI_2) were not measured in the present study, the potential roles of these metabolites on cytokines cannot be ruled out from in vivo situation. Indeed, sporadic reports have shown that TXA_2 and PGI_2 are also stimulatory to IL-1 and TNF production (64). These results suggest that fish oil may have a potential for preventive or adjunct therapy for human AIDS by modulating the production of cytokines and immune functions. In addition, fish oil supplementation can correct long chain fatty acid deficiency, especially the n–3 series, which has been reported in AIDS patients (65, 66).

This work was supported by the Kentucky Agricultural Experiment Station. We thank Joel Reynolds for his technical assistance in the preparation of splenocyte suspension and the determination of cell proliferation.

Manuscript received 13 February 1998 and in revised form 15 May 1998.

REFERENCES

1. Lee, J., and J. Vilcek. 1987. Tumor necrosis factor and interleukin-1: cytokines with multiple overlapping biological activities. *Lab. Invest.* **56:** 234–248.
2. Arai, K., R. Lee, A. Miyajima, S. Miyatabe, N. Arai, and T. Yokotu. 1990. Cytokines: coordinators of immune and inflammatory processes. *Annu. Rev. Biochem.* **59:** 783–836.
3. Lahdevirta, J., C. P. J. Maury, A. M. Teppo, and H. Repo. 1988. Elevated levels of circulating cachectin tumor necrosis factor in patients with acquired immunodeficiency syndrome. *Am. J. Med.* **85:** 289–291.
4. Hess, G., S. Rossol, R. Rossol, and K-H. Meyer Zum Buxchenfelde. 1991. Tumor necrosis factor and interferon as prognostic markers in human immunodeficiency virus (HIV) infection. *Infection.* **19:** S93–S97.
5. Murray, H. W., B. Y. Rubin, H. Masur, and R. B. Roberts. 1984. Impaired production of lymphokines and immune (gamma) interferon in the acquired immunodeficiency syndrome. *N. Engl. J. Med.* **310:** 883–889.
6. Prince, H. E., V. Kermani-Arab, and J. L. Fahey. 1984. Depressed interleukin-2 receptor expression in acquired immune deficiency and lymphadenopathy syndromes. *J. Immunol.* **133:** 1313–1317.
7. Fauci, A., S. Schnittman, G. Poli, S. Koenig, and G. Pantaleo. 1991. NIH conference. Immunopathogenetic mechanisms in human immunodeficiency virus infection. *Ann. Intern. Med.* **114:** 678–693.
8. Rosenberg, Z. F., and A. Fauci. 1990. Immunopathologenic mechanism of HIV infection: cytokine induction of HIV expression. *Immunol. Today.* **11:** 176–180.
9. Poli, G., A. Kinter, and J. S. Justement. 1990. Tumor necrosis factor

alpha functions in an autocrine manner in the induction of human immunodeficiency virus expression. *Proc. Natl. Acad. Sci. USA.* **87:** 782–785.
10. Mintz, M., R. Rapaport, J. M. Oleske, E. M. Connor, M. R. Koenigsberger, T. Denny, and L. G. Epstein. 1989. Elevated serum levels of tumor necrosis factor are associated with progressive encephalopathy in children with acquired immunodeficiency syndrome. *Am. J. Dis. Child.* **143:** 771–774.
11. Ito, M., M. Baba, S. Moris, K. Hirabayashi, F. Tanbe, S. Shigeta, and E. Declercq. 1990. Tumor necrosis factor antagonizes inhibitory effect of azidothymidine on human immunodeficiency virus (HIV) replication in vitro. *Biochem. Biophys. Res. Commun.* **166:** 1095–1101.
12. Cheung, S. C., S. K. Chattopadhyay, H. C. Morse, and P. M. Pitha. 1991. Expression of defective virus and cytokine genes in murine AIDS. *J. Virol.* **65:** 823–828.
13. Wang, Y., and R. R. Watson. 1994. Ethanol, immune response and murine AIDS: the role of vitamin E as an immunostimulant and antioxidant. *Alcohol.* **11:** 75–84.
14. Gazzinelli, R. T., M. Makino, S. K. Chattopadhyay, and C. M. Snapper. 1992. Preferential activation of Th_2 cells during progression of retrovirus-induced immunodeficiency in mice. *J. Immunol.* **148:** 182–188.
15. Chandrasekar, B., and G. Fernandes. 1994. Decreased proinflammatory cytokines and increased antioxidant enzyme gene expression by ω–3 lipids in murine lupus nephritis. *Biochem. Biophys. Res. Commun.* **200:** 893–898.
16. Endres, S., R. Ghorbani, V. E. Kelley, K. Georgilis, G. Lonnemann, J. W. M. van der Meer, J. G. Cannon, T. S. Rogers, M. S. Klempener, P. C. Weber, E. J. Schaefer, S. M. Wolff, and C. A. Dinarello. 1989. The effect of dietary supplementation of n–3 fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N. Engl. J. Med.* **320:** 265–271.
17. Fernandes, G. 1989. Effect of dietary fish oil supplement on autoimmune disease: changes in lymphoid cell subsets, oncogene mRNA expression and neuroendocrine hormones. *In* Health Effects of Fish and Fish Oils. R. Chandra, editor. ARTS Biomedical Publications, St. John's, Newfoundland, Canada. 409–433.
18. Fernandes, G., V. Tomer, N. Mohan, and J. T. Venkatraman. 1992. Potential of diet therapy on murine AIDS. *J. Nutr.* **122:** 716–722.
19. Mosier, D. E., R. A. Yetter, and H. C. Morse. 1985. Retroviral induction of acute lymphoproliferative disease and profound immunosuppression in adult C57B1/6 mice. *J. Exp. Med.* **161:** 766–784.
20. Huang, C. Y., L. H. Chen, Y. Osio, and D. A. Cohen. 1994. Effect of diet composition on liver antioxidant defense and detoxifying enzymes in mice with murine AIDS. *Nutr. Res.* **14:** 1841–1851.
21. Chen, L. H., S. Xi, and D. A. Cohen. 1996. Liver endogenous antioxidant defenses in mice fed AIN-76A diet and infected with murine AIDS. *Chemico-Biol. Interact.* **99:** 17–28.
22. Metcalf, L. D., and A. A. Schitz. 1961. The rapid preparation of the fatty acid esters for gas chromatographic analysis. *Anal. Chem.* **33:** 363–364.
23. Wang, D., D. S. Huang, B. Liang, and R. R. Watson. 1994. Nutritional status and immune responses in mice murine AIDS are normalized with vitamin E supplementation. *J. Nutr.* **124:** 2024–2032.
24. Snedecor, A. A. 1974. Statistical Methods. 7th ed., Iowa State press, Ames, IA.
25. Hartley, J. W., T. N. Fredrickson, R. A. Yetter, M. Makino, and H. C. Morse. 1989. Retrovirus-induced murine acquired immunodeficiency syndrome: natural history of infection and differing susceptibility of inbred mouse strains. *J. Virol.* **63:** 1223–1231.
26. Watson, R. R. 1989. Minireview of murine model for acquired immune deficiency syndrome. *Life Sci.* **44:** 3–4.
27. Buller, R. M. L., R. A. Yetter, T. N. Fredrickson, and H. C. Morse. 1987. Abrogation of resistance to severe mouse pox in C57BL/6 mice infected with LP-BMS murine leukemia viruses. *J. Virol.* **61:** 383–387.
28. Siegal, F. P., C. Lopez, and G. S. Hamer. 1981. Severe acquired immunodeficiency in homosexual males, manifested by chronic perianal lesions. *N. Engl. J. Med.* **305:** 1439–1444.
29. Gottlieb, M. S., R. Schroff, and H. M. Schander. 1981. Pneumocytis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *N. Engl. J. Med.* **305:** 1425–1431.
30. Basham, T., T. Holdener, and T. Merigan. 1991. Intermittent, alternating, and concurrent regiments of zidovudine and 2–3 dideoxycytidine in the LP-BM5 murine induced immunodeficiency model. *J. Infect. Dis.* **163:** 869–872.
31. Kromhout, D., E. B. Bosschieter, and C. Coulander. 1985. The inverse relation between fish consumption and 20-year mortality from coronary heart disease. *N. Engl. J. Med.* **312:** 1205–1209.
32. Kremer, J. M., W. Jubiz, A. Michalek, R. I. Rynes, L. W. Bartholomew, J. Bigaquette, M. Timchalk, D. Besler, and L. Lininger. 1987. Fish oil fatty acid supplementation in active rheumatoid arthritis: a double blinded, controlled crossover study. *Ann. Int. Med.* **106:** 497–502.
33. Brouard, C., and M. Pascaud. 1990. Effects of dietary supplementation with n–3 fatty acids on macrophage and lymphocyte phospholipids and macrophage eicosanoid synthesis in the rat. *Biochim. Biophys. Acta.* **1047:** 19–28.
34. Fernandes, G., C. Bysani, J. T. Venkatraman, V. Tomar, and W. Zhao. 1994. Increased TGF-beta and decreased oncogene expression by omega-3 fatty acids in the spleen delays the onset of autoimmune disease in B/W mice. *J. Immunol.* **152:** 5979–5987.
35. Huang, S. C., M. L. Misfeldt, and K. L. Fritsche. 1992. Dietary fat influences Ia antigen expression and immune cell populations in the murine peritoneum and spleen. *J. Nutr.* **122:** 1219–1231.
36. Kelly, V. E., A. Farritti, S. Izui, and T. B. Strom. 1985. A fish oil diet rich in eicosapentaenoic acid reduces cycloxygenase metabolites and suppresses lupus in MRL/lpr mice. *J. Immunol.* **134:** 1914–1919.
37. Fernandes, G. 1994. Dietary lipids and risk of autoimmune disease. *Clin. Immunol. Immunopathol.* **72:** 193–197.
38. Bittiner, S. B., W. F. Tucker, I. Cartwright, and S. S. Bleehen. 1988. A double blind, randomized, placebo-controlled trial of fish oil in psoriasis. *Lancet.* **1:** 378–380.
39. Hawkey, C. J., Y. R. Mahida, and A. B. Hawthorne. 1992. Therapeutic interventions in gastrointestinal disease based on an understanding of inflammatory mediators. *Agents Actions.* Special Conference Issue, C22–C26.
40. Payan, D. G., and E. J. Goetzel. 1983. Specific suppression of human T-lymphocyte function by leukotriene B_4. *J. Immunol.* **131:** 551–553.
41. Bakhle, Y. S., and M. A. Dphil. 1983. Synthesis and metabolism of cycloxygenase products. *Br. Med. Bull.* **39:** 214–218.
42. Taylor, G. W., and H. R. Morris. 1983. Lipoxygenase pathways. *Br. Med. Bull.* **39:** 219–232.
43. Ma, B. 1983. The pharmacology and pathology of leukotriene B_4. *Br. Med. Bull.* **39:** 249–254.
44. Rola-Pleszcynski, M., and I. Lemaire. 1985. Leukotrienes augment interleukin-1 production by human monocytes. *J. Immunol.* **135:** 3958–3961.
45. Hopkins, S. J., and A. Meager. 1988. Cytokines in synovial fluid: II. The presence of tumor necrosis factor and interferon. *Clin. Exp. Immunol.* **73:** 88–92.
46. Rola-Pleszcynski, M., M. Thivierge, N. Gagnon, C. Lacasse, and J. Stankova. 1993. Differential regulation of cytokine and cytokine receptor genes by PAF, LTB_4 and PGE_2. *J. Lipid Mediat.* **6:** 175–181.
47. Yaqoob, P., and P. Calder. 1995. Effects of dietary lipid manipulation upon inflammatory mediator production by murine macrophages. *Cell. Immunol.* **163:** 120–128.
48. Meydani, S. N., S. Endres, M. M. Woods, B. R. Goldin, C. Soo, A. Morrill-Labrode, C. Dinarello, and S. L. Gorbach. 1991. Oral n–3 fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. *J. Nutr.* **121:** 547–555.
49. Caughey, G. E., E. Mantzioris, R. A. Gibson, L. G. Cleland, and M. J. James. 1996. The effect on human tumor necrosis factor-α and interleukin-1β production of diets enriched in n–3 fatty acids from vegetable oil or fish oil. *Am. J. Clin. Nutr.* **63:** 116–122.
50. Renier, G., E. Skamene, J. DeSanctis, and D. Radzioch. 1993. Dietary n–3 polyunsaturated fatty acids prevent the development of atherosclerotic legions in mice, modulation of macrophage secretory activities. *Arterioscler. Thromb.* **13:** 1515–1524.
51. Somers, S. D., and K. L. Erickson. 1994. Alteration of tumor necrosis factor-alpha production by macrophages from mice fed diets high in eicosapentaenoic and docosahexaenoic fatty acids. *Cell. Immunol.* **153:** 287–297.
52. Kovacs, J. A., S. Vogel, J. M. Albert, J. Falloon, R. T. Davey, R. E. Walker, M. A. Polis, K. Spooner, J. A. Metcalf, M. Baseler, G. Fyfe, and C. Lane. 1996. Controlled trial of interleukin-2 infusions in

patients infected with human immunodeficiency virus. *N. Engl. J. Med.* **335:** 1350–1356.
53. Rook, A. H., H. Masur, and L. C. Lane. 1983. Interleukin-2 enhances the depressed natural killer and CMV-specific cytotoxic activation of lymphocytes from patients with the acquired immunodeficiency syndrome. *J. Clin. Invest.* **72:** 398–403.
54. Izui, S., G. Fernandes, I. Hara, P. J. McConahey, F. C. Jensen, F. J. Dixon, and R. A. Good. 1981. Low-calorie diet selectively reduces expression of retroviral envelope glycoprotein Gp70 in sera of (NZB × NZW) F1 hybrid mice. *J. Exp. Med.* **154:** 1116–1124.
55. Jolly, C. A., Y. H. Jiang, R. S. Chapkin, and D. N. McMurry. 1997. Dietary n–3 polyunsaturated fatty acids suppress murine lymphoproliferation, interleukin-2 secretion, and the formation of diacylglycerol and ceramide. *J. Nutr.* **127:** 37–43.
56. Endres, S., S. N. Meydani, R. Ghorbani, R. Schindler, and C. A. Dinarello. 1993. Dietary supplementation with n–3 fatty acids suppresses interleukin-2 production and mononuclear cell proliferation. *J. Leukocyte Biol.* **54:** 599–603.
57. Meydani, S. N. 1996. Effects of n–3 polyunsaturated fatty acids on cytokine production and their biologic function. *Nutrition*. **12 (1 suppl):** S8–S14.
58. Johnson, H. M., J. K. Russell, and B. A. Torres. 1986. Second messenger role of arachidonic acid and its metabolites in interferon-γ production. *J. Immunol.* **137:** 3035–3056.
59. Wang, Y., S. K. Ardestani, and B. Liang. 1994. Administration of interferon-gamma and monoclonal anti-interleukin-4 retards development of immune dysfunction and cytokine dysregulation during murine AIDS. *Immunology.* **83:** 383–389.
60. Fritsche, K. L., C. Feng, and J. N. Berg. 1997. Dietary fish oil enhances circulating interferon-γ in mice during Listeriosis without altering in vitro production of this cytokine. *J. Interferon. Cytok. Res.* **17:** 271–277.
61. Razzini, E., and G. F. Baronzio. 1993. Omega–3 fatty acids as coadjuvant treatment in AIDS. *Med. Hypotheses.* **41:** 300–305.
62. Wu, D., S. K. Meydani, M. Meydani, M. G. Hayek, P. Huth and R. J. Nicolosi. 1996. Immunologic effects of marine- and plant-derived n–3 polyunsaturated fatty acids in non-human primates. *Am. J. Clin. Nutr.* **63:** 273–280.
63. Yaqoob, P., E. A. Newsholme, P. C. Calder. 1994. The effect of dietary lipid manipulation on rat lymphocyte subsets and proliferation. *Immunology.* **82:** 603–610.
64. Kuhn, D. C., J. L. Stauffer, L. J. Gaydos, S. L. Lacey, and L. M. Demers. 1993. An inhibitor of thromboxane production attenuates tumor necrosis factor release by activated human alveolar macrophages. *Prostaglandins.* **46:** 195–205.
65. Begin, M. E., M. S. Manku, and D. Horrobin. 1989. Plasma fatty acid levels in patients with Acquired Immune Deficiency Syndrome and in controls. *Prostaglandin Leukotriene Essent. Fatty Acids.* **37:** 135–137.
66. William, L. L., D. M. Doddy, and L. A. Horrocks. 1988. Serum fatty acid composition of plasma from AIDS patients and normal individuals. *Arch. AIDS Res.* **23:** 981–988.