Dietary Marine Lipids Suppress Continuous Expression of Interleukin-1β Gene Transcription[1]

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ABSTRACT: n-3 Polyunsaturated fatty acids abundant in marine lipids suppress certain inflammatory and immune reactions, and dietary marine lipid supplements have antiinflammatory effects in experimental and human autoimmune disease. Previous work by other investigators demonstrated that dietary marine lipid supplements suppressed production of cytokines from stimulated human peripheral blood mononuclear cells *ex vivo*. The present study further documents the ability of n-3 fatty acids to inhibit cytokine formation, and in part defines the mechanism of the inhibition of production of interleukin-1β (IL-1β) by dietary n-3 fatty acid. Female BALB/c mice were each fed a fat-free balanced diet to which was added either a refined fish oil (FO) preparation as a source of n-3 fatty acid, or beef tallow (BT), which consisted primarily of saturated and monoenoic fatty acids. After ingesting the experimental diets for periods ranging from 3 to 12 wk, spleen cell preparations were stimulated *ex vivo* with either lipopolysaccharide (LPS) or phorbol 12-myristate 13-acetate (PMA), and proIL-1β mRNA (IL-1β mRNA) was measured by northern analysis. Levels of IL-1β mRNA in both LPS- and PMA-stimulated cells from BT-fed mice were elevated to a greater extent than in cells from FO-fed mice, at most concentrations of LPS and PMA. Stability of LPS-stimulated mRNA levels after actinomycin D was similar for BT and FO groups, indicating that lower levels of IL-1 mRNA with FO groups was related to suppressed IL-1 gene transcription and not due to accelerated transcript degradation. Nuclear run-on transcription assays revealed a more transient expression of the IL-1β gene in LPS-stimulated spleen cells from FO-fed mice compared to cells from BT-fed mice. We conclude that dietary marine lipids reduce transient expression of the IL-1β gene in stimulated splenic monocytic cells. Preliminary results from nuclear run-on transcription assays indicate that n-3 fatty acids may not change the initial rate of gene transcription but may promote more rapid shutting down of transcription of this gene after induction than do alternative lipids.

Lipids 31, S-23–S-31 (1996).

Dietary n-6 and n-3 polyunsaturated fatty acids are essential for normal health in humans and other mammalian species. A large body of evidence from epidemiologic and experimental studies has also documented that the relative quantities of n-6 and n-3 fatty acids can alter a variety of cell functions and disease states (1–3). Dietary n-3 fatty acids are incorporated into tissue phospholipids, accompanied by replacement of n-6 fatty acids, including arachidonic acid. These changes are associated with reductions in the capacity of cells to produce eicosanoids, and it has been postulated that reduced production of prostaglandins and leukotrienes by cells enriched in n-3 fatty acids may contribute to their antiinflammatory effects. It is also clear, however, that alterations in eicosanoid formation cannot account for all the biological effects of n-3 fatty acids (4). In addition to inhibition of eicosanoid production, the presence of elevated levels of n-3 fatty acids may suppress cytokine formation. A study of human volunteers demonstrated that a dietary supplement of marine oil for 6 w significantly reduced quantities of interleukin-1α (IL-1α), IL-1β, and tumor necrosis factor-α (TNF-α) produced by lipo-polysaccharide (LPS)-stimulated peripheral blood mononuclear cells *ex vivo*, as measured by radioimmunoassay (5). An additional study of patients with rheumatoid arthritis ingesting a dietary supplement of fish oil (FO) found suppression of IL-1 production by LPS-stimulated peripheral blood mono-nuclear cells, compared to control patients ingesting an olive oil supplement (6). These reports raise the possibility that suppression of cytokine expression by n-3 fatty acids may contribute to antiinflammatory effects of these lipids.

Previous studies demonstrated that dietary marine lipids markedly reduced severity of the spontaneous autoimmune glomerulonephritis in murine lupus strains, which are models for the human disease, systemic lupus erythematosus (7–10). Subsequent controlled clinical trials documented modest but significant antiinflammatory effects of dietary marine lipid supplements in patients with rheumatoid arthritis, and one study reported beneficial effects of dietary marine lipids in patients with systemic lupus erythematosus (6,11–14). Mechanisms of these well-documented antiinflammatory effects of n-3 fatty acids are unclear. n-3 Fatty acids were shown to suppress formation of eicosanoids, including leukotriene B4 and other 5-lipoxygenase

[1]The IL-1β cDNA probe detects proIL-1β mRNA, which is referred to as IL-1β mRNA throughout the paper for convenience.

*To whom correspondence should be addressed at the Arthritis Unit, Bulfinch 165, Massachusetts General Hospital, 32 Fruit St., Boston, MA 02114. Abbreviations: BT, beef tallow; FO, fish oil; IL-1, interleukin-1; LPS, lipopolysaccharide; LT, leukotriene, PG, prostaglandin; PMA, phorbol 12-myristate 13-acetate; TNF-α, tumor necrosis factor-α.

products (6,15). Cyclooxygenase products may also be inhibited by n-3 fatty acids (4), but this effect seems unlikely to account for beneficial effects of n-3 fatty acids on murine lupus, since a cyclooxygenase inhibitor did not alter the course of autoimmune disease in one of these strains (16).

Several lines of evidence indicate that cytokines play an important role in the pathogenesis of inflammatory reactions (17–19). Elevated levels of mRNA for IL-1β and TNF-α are present in kidneys of mice with autoimmune glomerulonephritis, and administration of recombinant IL-1α and TNF-α may aggravate murine autoimmune disease under certain conditions (20,21). Synovial tissues from patients with rheumatoid arthritis contain IL-1, TNF-α, and other cytokines, and these and other observations led to the conclusion that these cytokines have an important role in the pathogenesis of this disease (18,22).

To clarify mechanisms by which n-3 fatty acids suppress cytokine production, we have investigated the effects of a dietary marine lipid on the levels of IL-1β mRNA in murine spleen cells that have been stimulated to produce cytokines *ex vivo*. Results of these studies demonstrate that dietary n-3 fatty acids reduce steady-state IL-1β mRNA levels in isolated spleen cells that have been stimulated with either LPS or phorbol 12-myristate 13-acetate (PMA). Experiments with actinomycin D–treated cells as well as nuclear run-on transcription assays demonstrated that dietary n-3 fatty acids reduce IL-1β production by depressing the total amount of gene transcription. Furthermore, preliminary examination of nuclear run-on kinetics suggests a mechanism for reduced IL-1β gene transcription involving an effect targeted not at the activation of gene transcription, but alternatively at the distal regulatory repression that generates the transient transcriptional response usually observed for this gene. Consequently, n-3 fatty acids, in contrast to alternative fatty acids, could result in more efficient activation of the transcriptional clamp previously described (23).

METHODS

Diets. Experimental diets consisted of a fat-free powder (85 wt%) to which was added one of two lipid preparations (15%). The fat-free powder (ICN Nutritional Biochemicals, Cleveland, OH) provided a balanced diet consisting of casein 21%, cellulose 15.6%, sucrose 58.5%, and a balanced salt mixture and essential vitamins, including 50 mg/kg of α-tocopherol. Lipids were stabilized with 0.02% *t*-butylhydroquinone. Female BALB/c mice were fed with experimental diets beginning at age 2 mo. Diets were given *ad libitum*, except for brief restrictions occasionally to maintain mean weights of each dietary group within 5% of each other. Fatty acid composition of dietary lipids has been published elsewhere (24). Beef tallow (BT) (ICN Nutritional Biochemicals) primarily consisted of saturated (48%) and monoenoic (47%) fatty acids, with 4.7% n-6 fatty acid in the form of linoleic acid and essentially no (<1%) n-3 fatty acids. The FO preparation (Nissui 28; Nippon Suisan Kaisha, Ltd., Tokyo) contained 55% n-3 fatty acid, 3.5% n-6 fatty acid, with the remainder (40%) as saturated and monoenoic fatty acids.

Cell preparation and stimulation. Groups of mice were sacrificed by cervical dislocation after feeding with experimental diets for varying time intervals. Spleens were rapidly removed and minced, and erythrocytes were hemolyzed with NH_4Cl. Spleen cells were washed three times with RPMI 1640 (Cellgro; Mediatech Inc., Herndon, VA) and filtered through fine mesh gauze. Cell viability was over 95%, based on trypan blue dye exclusion. Spleen cells (10^8) were cultured in 5 mL RPMI 1640 medium with or without varying doses of LPS (*E. coli* 055:B5, Sigma Chemical Co., St. Louis, MO) or PMA (Sigma Chemical Co.) for 4 h or other time intervals as indicated. Analysis of a representative spleen cell preparation fluorescence-activated cell sorter utilizing monoclonal antibodies to immunoglobulin G and Thy-1 revealed 38% Ig^+ cells, 29% Thy-1^+ cells. The small percentage of monocytes could not be determined accurately by the cell sorter using an anti-Mac-1, but histologic analyses of Wright–Giemsa-stained smears revealed 3–5% monocytes, with no differences in percent monocytes between FO and BT groups in two experiments. Others have reported that percentages of lymphocytes and monocytes in murine spleen cell preparations similar to ours were not altered by dietary n-3 fatty acid (25).

Preparation of mRNA and northern blot analysis. Total cellular RNA was prepared by guanidinium thiocyanate lysis (26). Gel electrophoresis of RNA was carried out using 1% agarose gels containing 6% formaldehyde, loading the gels with 10 mg RNA/lane (27). Ethidium bromide was added to the loading buffer, and the 28S and 18S ribosomal RNA bands were located with ultraviolet light. RNA was then transferred to nitrocellulose filters (Schleicher & Schull, Keene, NH). Prehybridization, hybridization, and autoradiography were performed according to standard procedures (28). The murine IL-1β probe was a 1600-base pair fragment of the full-length cDNA (29). The β-actin probe (provided by Dr. Mary Goldring) was a 700-base pair fragment of the full-length cDNA (30). cDNA Probes were labeled with [α-^{32}P]dCTP using a random primer method (31).

Nuclear isolation and transcriptional run-on assay. For run-on transcription experiments, spleen cells were incubated with 1 ng/mL LPS for 0 to 3 h. Nuclei were isolated from spleen cells after incubation with LPS by lysis in 0.05% NP 40 (Boehringer Mannheim Biochemicals, Indianapolis, IN) as previously described (32–34). Isolated nuclei were stored at –80° in a glycerol buffer until the run-on reaction was carried out. For the run-on transcription assay, $6–8 \times 10^7$ spleen cell nuclei were incubated with 150 mCi [α-^{32}P]uridine triphosphate for 30 min at 26–28°C. The reaction was stopped by addition of 100 mg tRNA and 100 μg/mL DNase. Proteinase K was added and incubated for 30 min at 42°C. RNA was extracted with phenol-chloroform, passed through G-50 sephadex spun columns (28) (Pharmacia Biotech Inc., Piscataway, NJ), and precipitated with 5% trichloroacetic acid at 0°C. RNA was resuspended and treated with 0.2 M NaOH for 15 min at 0°C. After neutralization, the RNA was precipitated with ethanol and resuspended in the hybridization mix. Denaturation was achieved by heating at

85–90°C for 10 min. Hybridization took place on nitrocellulose filters with each dot containing 5 mg plasmid DNA (35,36). Within each experiment, the same number of counts of nascent RNA, 1–1.6 x 10^7 cpm, was added to each dot.

Fatty acid analysis of spleen cell phospholipids. Spleen cell lipids were extracted with chloroform/methanol (2:1) using a modified Folch method (35). Total phospholipids were isolated by thin-layer chromatography on silica gel G with a solvent consisting of petroleum ether/ether/acetic acid, 70:30:1 (36). The phospholipid band that remained at the origin was scraped from the plate, and phospholipid fatty acids were transmethylated using BF_3. Fatty acid methyl esters were analyzed by capillary gas chromatography as described by Ackman, with minor modifications (37).

RESULTS

Analysis of LPS- and PMA-stimulated IL-1β mRNA. Spleen cells were obtained from mice fed with either BT or FO diets

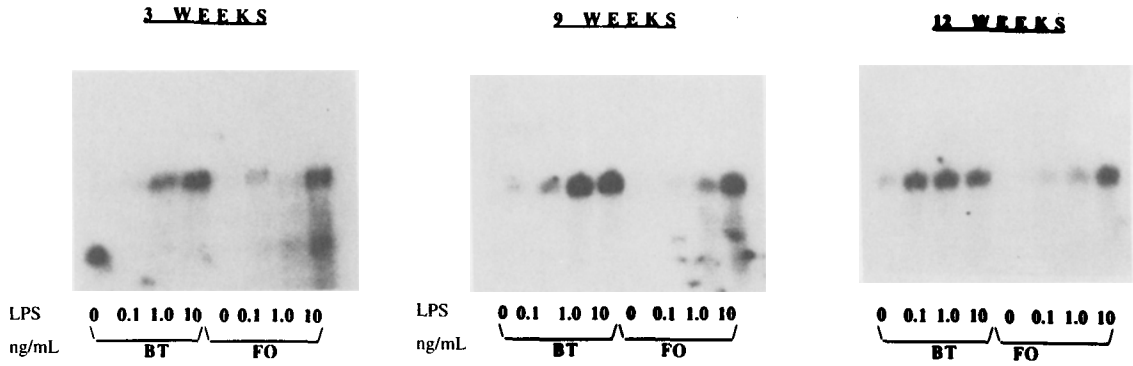

FIG. 1. Northern analysis of total RNA extracts from spleen cells isolated from mice fed for varying time intervals with diets containing either beef tallow (BT) or fish oil (FO) as their lipid components. Cells were stimulated with lipopolysaccharide (LPS) for 4 h prior to RNA extraction. RNA was transferred to nitrocellulose membranes and hybridized with a cDNA probe for murine proIL-1β. Equal quantities of RNA (10 μg) were applied to each lane. Arrows indicate the location of 18S and 28S RNA.

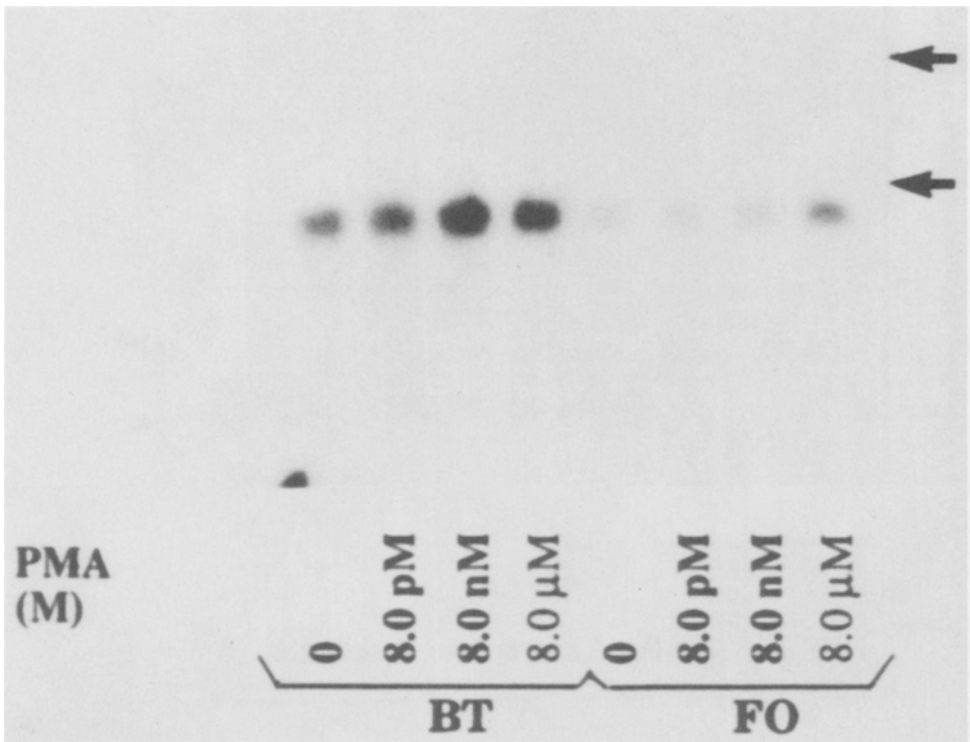

FIG. 2. Northern analysis of proIL-1β mRNA from phorbol 12-myristate 13-acetate (PMA)-stimulated murine spleen cells. Cells were obtained after administration of experimental diets for 12 wk. Abbreviations as in Figure 1.

for varying time periods and were stimulated *ex vivo* to produce IL-1β by either LPS or PMA. In the first experiment (Fig. 1), groups of mice were fed with experimental diets for either 3, 9, or 12 wk prior to stimulation of cells with LPS. In the next experiment (Figs. 2 and 3), mice were fed for 12 wk prior to stimulation of the cells with either LPS or PMA. In each experiment, the amount of LPS-inducible 1.7 kB proIL-1β mRNA derived from FO-fed mice cells was less than that of the BT controls, at most concentrations of LPS or PMA used. In the first experiment (Fig. 1), after 12 wk of feeding, a maximal intensity of the IL-1β mRNA band occurs at 0.1 ng/mL LPS with BT cells. In contrast, only faint bands occur at the lower two concentrations of LPS with FO cells, but at the highest concentration of LPS of 10 ng/mL, intensity of the band is similar to bands with stimulated cells from the BT group. These results are consistent with a lower sensitivity of FO cells than BT cells to LPS, but levels of IL-1β mRNA in most cases are similar in both groups of cells at the highest concentration of LPS.

Results in Figure 2 demonstrate that after PMA stimulation the maximum response of cells from the BT group occurs at 8 nM PMA, whereas in cells from the FO group only the highest concentration of PMA of 8 micromolar produces a signal above background level, and the response observed is less than that seen with the BT group. Aliquots of cells from this experiment were also stimulated with LPS (Fig. 3), with similar results to those seen at 12 wk in Figure 1. This membrane was also hybridized with a human β-actin probe as an internal control for cell numbers and the state of cell differen-

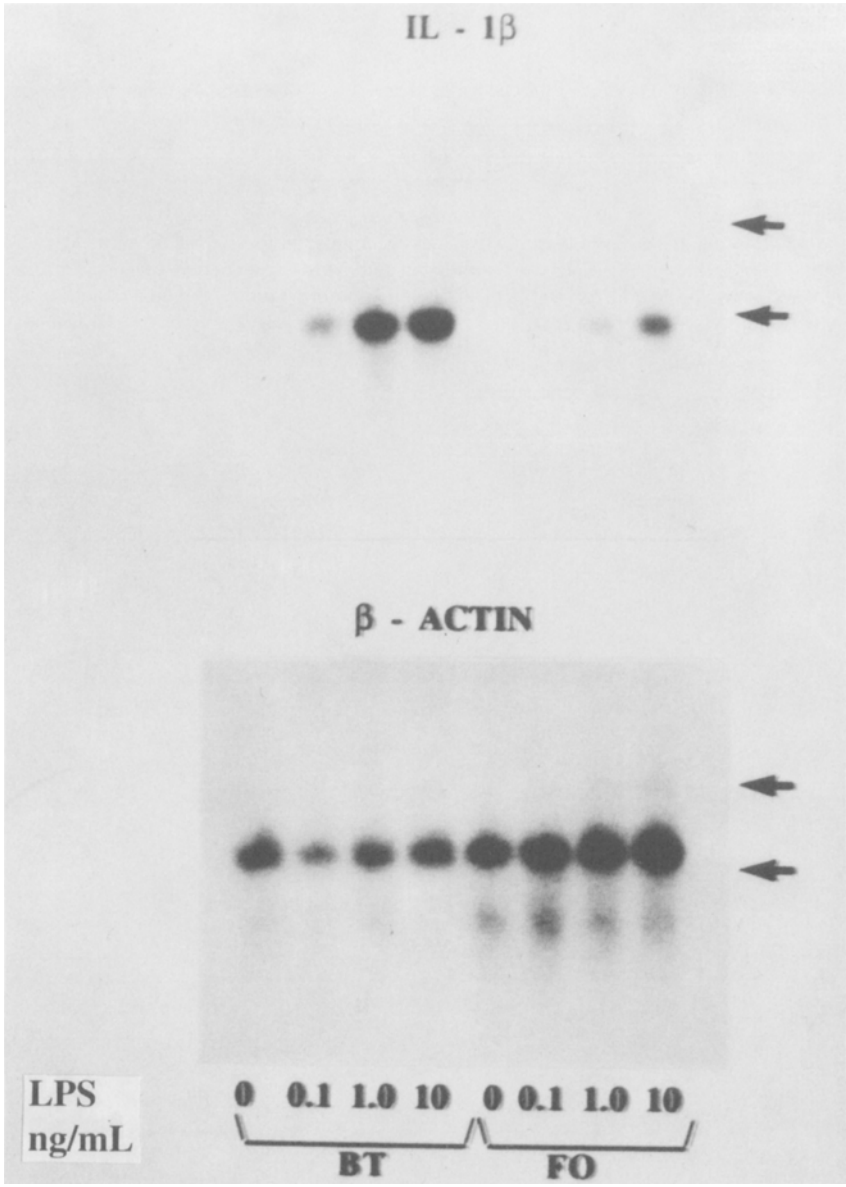

FIG. 3. Analysis of RNA extracts from LPS-stimulated murine spleen cells for proIL-1β and β-actin mRNA. Cells were aliquots of the same preparation used for PMA stimulation in Figure 2. Abbreviations as in Figures 1 and 2.

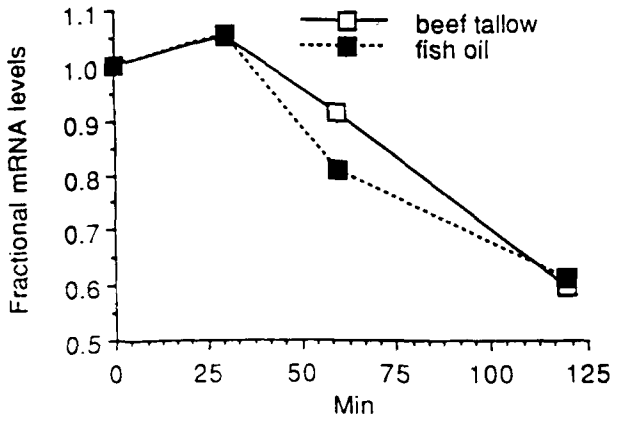

FIG. 4. Degradation rates of IL-1β mRNA from LPS-stimulated murine spleen cells. Cells were stimulated with LPS at 10 ng/mL for 5 h, after which actinomycin D was added to replicate cultures. After incubating with actinomycin D (5 mg/mL) for time periods indicated, each culture was subjected to northern analysis. Quantities of IL-1β mRNA were determined by scanning densitometry. Quantities of mRNA are expressed as the ratios of the densities at each time interval to the densities at the time of addition of actinomycin D (t = 0). Abbreviation as in Figure 1.

tiation. In contrast to results with the IL-1β probe, the β-actin band intensities with LPS stimulation are slightly more intense with the FO group than with the BT group, demonstrating the specificity of IL-1β mRNA effects.

Stability of LPS-stimulated IL-1β mRNA. Since it has been demonstrated that cytokine mRNA have relatively rapid turnover rates, we considered the possibility that reduced levels of IL-1β mRNA generated by cells from FO-treated mice could be achieved by an enhanced rate of decay of the message, compared to the BT group (39). To investigate this possibility, we determined rates of decay of IL-1 mRNA from LPS-stimulated spleen cells after exposure to actinomycin D. Cells were stimulated with 10 ng/mL LPS for 5 h, after which 5 mg/mL actinomycin D were added. Total RNA was isolated from these cells after incubation with actinomycin D for periods up to 2 h and subjected to northern analysis. Results in Figure 4 demonstrate that there is no significant difference in the rates of decay of IL-1β mRNA between the BT and the FO groups (t 1/2 = 2–3 h), indicating that the rate of transcription of the IL-1β gene in these LPS-stimulated cells is reduced in the FO group compared to the BT group.

Nuclear run-on transcription assays. Actinomycin D results suggest that transcriptional regulation is likely to be responsible for the steady-state differences between BT- and FO-fed animals' *ex vivo* cellular responses. Nuclear gene transcription was directly examined by nuclear run-on assay in which isolated nuclei are allowed to complete arrested transcription in the presence of radiolabelled ribonucleotide triphosphates, thus creating probes that can be assessed by hybridization to DNA targets fixed to nitrocellulose membranes. We demonstrated in three separate experiments reduced transcription from the IL-1β gene in LPS-stimulated spleen cells from mice fed the FO diet, compared to mice fed the BT diet. Results of two run-on experiments done in duplicate are shown in Figure 5. In both experiments, no signifi-

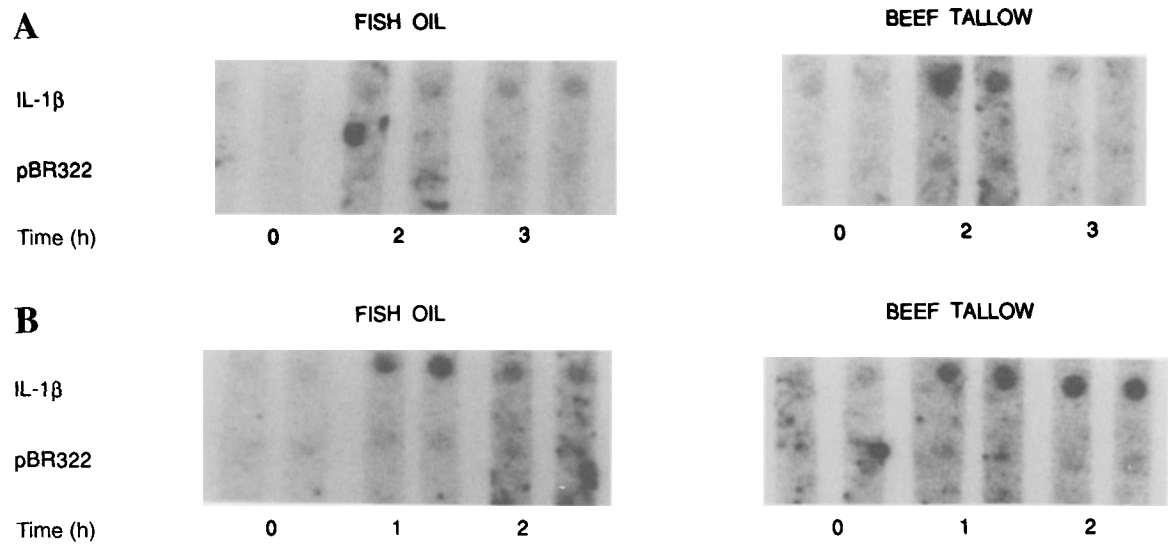

FIG. 5. Nuclear run-on transcripts after LPS stimulation. Nuclei were isolated from murine spleen cells that had been stimulated with LPS (1 ng/mL) for 0 to 3 h as indicated. Radiolabelled nascent RNA transcripts were prepared by incubating 6–8 x 10^7 nuclei with 150 mCi [a-^{32}P]uridine triphosphate. Radiolabelled RNA was isolated from the nuclei by digestion with DNase and protease K followed by phenol-chloroform extraction, G-50 sephadex spun column (Boehringer, Indianapolis, IN) purification, and trichloroacetic acid precipitation. RNA was treated with 0.2 M NaOH, and precipitated with Na acetate-ethanol. Equal counts of RNA (from 1.0 to 1.6 x 10^7 cpm per sample) were used to probe dots on nitrocellulose strips containing 5 mg denatured IL-1β cDNA, or denatured pBR322 plasmid DNA as a control: A) Spleen cells stimulated for 0, 2, and 3 h; B) Spleen cells from different mice than in experiment 1, stimulated for 0, 1, and 2 h. In both experiments, mice were fed described experimental diets for 12 wk before sacrificing to obtain spleen cells.

cant transcription was detected at 0 time, and faint signals were seen after 2 and 3 h of LPS stimulation from FO-fed mice. BT-fed mice showed more activity after 2 h of stimulation than FO-fed mice, with less activity after 3 h. No significant activity was seen with the vector control pBR322 as the target. Figure 5B reveals that there was increased transcription at 1 h of stimulation with RNA from both groups of mice, but at 2 h of stimulation, intense uptake continued with cells from BT mice, while FO cells returned to baseline. Thus, between 0 and 1 h following LPS exposure, the initial rate of IL-1β gene transcription was similar in cells from FO-fed and BT-fed mice. Beyond 1 h, however, transcription in cells from FO-fed mice shut down more rapidly than transcription from BT-fed mice. These findings indicate that dietary n-3 fatty acids reduce the levels of IL-1β mRNA by increasing the efficiency of the rapid gene shutdown associated with the transient expression usually observed for the IL-1β gene (38,50).

Fatty acid analyses of spleen cell phospholipids. Fatty acid analyses of spleen cell preparations were carried out after both 6 and 12 w feeding with each of the experimental diets. The wt% of each polyunsaturated fatty acid present in significant quantities is listed in Table 1. Administration of the FO diet was associated with large increases in the content of long-chain n-3 fatty acid in spleen cell phospholipids. Total polyunsaturated fatty acid content remained nearly constant, regardless of dietary lipid ingested or duration of experimental diets. Elevation of n-3 fatty acid content is therefore accompanied by reduction in n-6 fatty acid content, with relatively small differences in the contents of saturated and monoene fatty acids. The major n-6 fatty acid present is arachidonic acid, which is reduced in cells from the FO group to approximately one-third of the content of arachidonic acid in cells from the BT group. There are also large increases in contents of the three major n-3 fatty acids in cells from the FO group

TABLE 1
Fatty Acid Composition of Total Phospholipids Mouse Speen Cells[a]

	Six weeks[b]		Twelve weeks[b]	
Fatty acid	BT	FO	BT	FO
16.0	21.6	26.8	20.5	26.0
16:1	2.1	4.2	1.4	4.2
18:0	16.8	15.1	17.8	15.7
18:1	18.0	11.4	18.0	11.6
18:2n-6	4.1	1.2	4.1	1.2
20:4n-6	22.0	7.2	24.9	7.9
22:4n-6	2.7	0.2	3.6	0.2
22:5n-6	0.7	0.2	1.5	0.2
20:5n-3	<0.2	11.5	<0.2	11.3
22:5n-3	0.9	6.3	0.7	6.6
22:6n-3	7.3	10.5	4.7	10.3
Saturated	38.8	42.9	38.5	43.2
Monoene	20.9	16.4	20.2	16.6
n-6 PUFA	31.6	11.7	35.9	10.4
n-3 PUFA	8.4	28.8	5.6	28.9
Total PUFA	40.0	40.5	41.5	39.2

[a]Composition determined by weight percentage of fatty acids as their fatty acid methyl ester derivatives; PUFA, polyunsaturated fatty acids; BT, beef tallow; FO, fish oil.
[b]Duration of experimental diets.

DISCUSSION

Results presented in this report demonstrate that dietary n-3 fatty acids inhibit sustained transcription of the IL-1β gene in stimulated spleen cells *ex vivo* by affecting the kinetics of gene expression beyond 1 h poststimulation. Others reported previously that dietary supplements of n-3 fatty acids inhibit production of IL-1 and TNF-α by LPS-stimulated human peripheral blood mononuclear cells (5,6). Studies reported here help clarify the mechanism of cytokine inhibition by n-3 fatty acids. Our experiments demonstrate that dietary n-3 fatty acids reduce steady-state levels of IL-1β mRNA in both LPS- and PMA-stimulated murine spleen mononuclear cells *ex vivo*. Reduced levels of IL-1β mRNA in stimulated spleen cells appear to arise from suppression of IL-1β gene transcription by n-3 fatty acids, based on the reduced rate of transcription beyond 1 h of *ex vivo* cell stimulation as assessed by nuclear run-on assays. Also, similar decay rates for IL-1β mRNA in cells from either FO- or BT-fed mice support the argument for a transcriptional effect. Reduced nuclear transcription does not appear to be related to a decreased induction, but rather to a more rapid shutdown of transcription following induction, as exhibited by the altered kinetics beyond 1 h of treatment. The IL-1β gene was previously shown to be transiently transcribed in various cell lines as well as in peripheral blood monocytes (38). This transient expression is due to the immediate and rapid induction that occurs in the absence of protein synthesis, followed by an almost immediate shutdown resulting from the action of a *de novo* expressed protein, which has been referred to as the transcriptional clamp (23). These data support a model in which either n-3 fatty acids induce a more vigorous expression of the clamp protein(s) or in which BT inhibits clamp expression (compared to alternative fatty acids). Therefore, the role of FO may be to support the strong expression of a protein factor that limits IL-1β gene transcription.

Neither the present nor the previous studies utilized a system containing a single cell type, raising the possibility that the FO effect could be exerting its effect by altering composition of cell preparations from which the cytokines were derived. In the studies reported here, we cannot determine which cell type is responsible for the major portion of IL-1β mRNA, although spleen monocytes are a likely source. We considered the possibility that dietary n-3 fatty acids altered composition of spleen cell preparations such that the percentage of cells containing major quantities of IL-1β mRNA after stimulation was reduced, but the following considerations make this possibility unlikely. No difference in numbers of B-cells and T-cells between BT and FO groups were observed

by fluorescence-activated cell sorter analysis, and no differences in monocytes were observed microscopically. Others also reported that percentages of lymphocytes and monocytes in similar murine spleen cell preparations were not altered significantly by changes in dietary lipids, including n-3 fatty acids (25). The PMA experiment demonstrates that the decreased responsiveness of FO cells is not specific for LPS induction, since the data in Figure 1 demonstrate that responsiveness of FO cells to PMA is less than that of BT cells by over three orders of magnitude.

The mechanism of inhibitory effects of n-3 fatty acids on production of IL-1β mRNA by activated mononuclear cells is unknown. The experimental diets achieved major modifications in the polyunsaturated fatty acid composition of spleen cells, with large reductions in n-6 fatty acids and reciprocal elevations in the contents of n-3 fatty acids, but we have not determined the relationship, if any, between changes in phospholipid fatty acid composition and suppression of IL-1β gene transcription. Our experiments demonstrate that this suppression applies to both a transmembrane and a soluble stimulant, each of which acts by complex, but different, mechanisms (39).

The following evidence indicates that induction of transcription of both IL-1 and TNF-α genes by LPS and PMA may be mediated by activation of protein kinase C. Induction of LPS responsiveness in U937 cells is associated with the stimulation of mRNA synthesis for the G protein Gi2 (40). It was suggested that PMA induces protein kinase C–mediated phosphorylation of endogenous substrates such as the transcriptional activator, AP-1, that could in turn induce transcription of the Gi2 gene. It was also reported that PMA induction of IL-1 by the human monocytic cell line THP-1 was inhibited by a selective protein kinase C inhibitor (41). Since protein kinase C utilizes both diacylglycerol and phospholipids as cofactors, it is possible that the changes in fatty acid composition of phospholipids associated with higher levels of dietary n-3 fatty acids could inhibit the activity of protein kinase C due to changes in the structures of its lipid cofactors.

Certain eicosanoids have been found to modify the production of cytokines. Inhibition of LPS-induced TNF-α mRNA production from murine peritoneal macrophages was observed with prostaglandin E_2 ($PGE_{1)}$ and PGE_2, but leukotriene B_4 (LTB_4), and LTC_4 had no effect (42). Conversely LTB_4 and LTD_4 enhanced production of IL-1 by human peripheral blood monocytes that had been stimulated by LPS and other agonists (43). Since dietary marine lipids reduce production of 5-lipoxygenase products by human leukocytes (6,15), it is possible that suppressed LT production by mononuclear cells from FO-treated mice could reduce IL-1 production, perhaps by interfering with a key early signaling requirement. Another study, however, reported that LTB_4 did not enhance LPS-stimulated IL-1 production by murine peritoneal macrophages (44), and thus LT enhancement of IL-1 synthesis may be limited to certain cell types. We have not yet examined effects of LT on IL-1 production by spleen cell preparations utilized in experiments reported here.

Dietary n-3 fatty acids also suppress formation of prostaglandins and thromboxanes, although production of prostacyclin in humans is not affected significantly (45–48). Although the E prostaglandins suppress TNF-α mRNA production, as noted above, and also suppress production of IL-1 protein (44,49), evidence indicates that inhibition of IL-1 production is at a post-transcriptional level, since IL-1β mRNA levels are unaffected in stimulated U937 cells by PGE_2 (49). In any event, the E prostaglandins inhibit cytokine production, and thus the usual reduction of PGE levels by n-3 fatty acids, if such inhibitory effects occurred in our experimental system, would have augmented cytokine mRNA levels.

This study demonstrates that dietary n-3 fatty acids suppress levels of IL-1 mRNA in murine spleen cells following activation by two different stimuli, and results with LPS-stimulated cells indicate that the mechanism involves inhibition of induction of IL-1 gene transcription by n-3 fatty acids. Molecular mechanisms for inhibition of IL-1 gene transcription by n-3 fatty acids remain to be determined. IL-1 gene expression depends on the integrity of an upstream LPS and PMA responsive enhancer and a tissue-specific promoter, and both of these regions bind positive factors important for gene expression. This raises the possibility that n-3 fatty acids may interfere with the function of factors necessary for maximal IL-1 gene induction.

ACKNOWLEDGMENTS

We are grateful to Dr. Mary Goldring for providing the β-actin probe. This work was supported by grants AR 03564, AR 43518, CA68544, and AI 27850 from the National Institutes of Health, and a grant from the Davis Family Foundation.

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