The effects of dietary n-3 polyunsaturated fatty acids and cyclic AMP-elevating agents on tumor necrosis factor production by murine-resident and thioglycollate-elicited peritoneal macrophages

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Tumor-necrosis factor (TNF), prostaglandin (PG) E_2, and 6-keto $PGF_{1\alpha}$ production by peritoneal macrophages was monitored following incubation with various cAMP-elevating agents and stimulation with lipopolysaccharide (LPS). Resident and thioglycollate (TG)-elicited macrophages were obtained from mice maintained for 4 weeks on diets containing 3 wt % n-6 fatty acids; 1.0 wt % n-3 polyunsaturated fatty acids (PUFA) +1.5 wt % n-6 fatty acids; or 1.5 wt % n-3 PUFA + 1.5 wt % n-6 fatty acids. Increasing the n-3 PUFA content of the diet increased TNF production and decreased PG production by the resident peritoneal macrophages. The cAMP analog, 8-br cAMP, and the phosphodiesterase inhibitor, 3-isobutyl-1-methylxanthine, decreased TNF production by resident peritoneal macrophages from mice consuming all diets. The adenylate cyclase (AC) activators, cholera toxin and forskolin, only decreased TNF production by resident macrophages from mice consuming the higher level of n-3 PUFA. The effects of dietary n-3 PUFA on TNF production by the resident peritoneal macrophages appear not to be mediated by cAMP, as the cAMP-elevating agents did not eliminate the dietary effects on TNF production. TG-elicited macrophages produced more TNF and less PG than the resident peritoneal macrophages. Adding n-3 PUFA to the diet did not affect TNF nor PG production by TG-elicited macrophages. The cAMP-elevating agents decreased TNF production by TG-elicited macrophages from mice consuming diets with or without n-3 PUFA. These findings show that cAMP-elevating agents decrease TNF production by both resident and elicited peritoneal macrophages, but may not be involved in the effect of n-3 PUFA on TNF production by the resident peritoneal macrophages.

Keywords: tumor necrosis factor; n-3 fatty acids; polyunsaturated fatty acids; prostaglandins; cAMP

Introduction

Tumor necrosis factor (TNF) is a macrophage-derived cytokine that mediates antitumor effects[1,2] and may be

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responsible for certain symptoms of septic shock[3] and may be involved in causing cachexia.[4] It enhances resistance to bacterial infection[5,6] and may play a beneficial role in angiogenesis,[7] peritonitis,[8] and wound healing.[9] Acute systemic release of TNF causes septic shock and tissue injury, whereas low levels may be beneficial in mediating enhanced host defense against pathogens and in modulating normal tissue remodeling.[10]

Dietary n-3 polyunsaturated fatty acids (PUFA) enhance TNF production by resident peritoneal macrophages from mice[11–13] and rats[14] but do not affect TNF production by macrophages elicited by complete Freund's adjuvant (CFA).[14,15] The effect of dietary n-3 PUFA on TNF production by thioglycollate (TG)-elicited macrophages has not been reported.

Prostaglandin (PG) E_2 suppresses TNF mRNA accumulation[16] and TNF protein release from lipopolysaccharide (LPS)-stimulated macrophages[12,17,18] and prostacyclin (PGI_2) suppresses interleukin (IL)-1 production by monocytes[19] and peritoneal macrophages.[17] PG synthesis by peritoneal macrophages is decreased by dietary n-3 PUFA.[20,21] In a previous report we suggested that the decreased PG production may be partially responsible for the increased TNF production by macrophages from mice consuming dietary n-3 PUFA, but that other factors are also involved.[12]

Adenosine 3′:5′ cyclic monophosphate (cAMP) suppresses LPS-induced TNF production[22] and TNF mRNA synthesis.[23,24] PGE_2 and PGI_2 elevate cAMP levels in murine peritoneal macrophages,[25–27] with TG-elicited macrophages being more sensitive to exogenously added PGE_2 than resident macrophages.[26] cAMP may therefore mediate the inhibitory effects of PG on TNF production. Dietary cod liver oil, in addition to decreasing PG production, decreases the responsiveness of adenylate cyclase (AC) to stimulation[28,29] and therefore decreases cAMP production by two separate mechanisms, which may have additive effects on TNF production.

The present study was conducted to investigate the effect of increasing dietary n-3 PUFA on TNF production by both resident and TG-elicited peritoneal macrophages, and the role cAMP plays in mediating the effect.

Methods and materials

Animals

Female BALB-C mice, weighing 18–20 g, were purchased from Charles River Laboratory (Wilmington, MA USA). They were maintained in cages (five mice per cage) in a room with a 12-hour light/dark cycle and a temperature of 25° C. The mice were allowed to adjust to new conditions for 1 week during which they received Prolab diet (Agway, Syracuse, NY USA). Because chow contains 18:3 n-3 and our experimental diets contain n-6 fatty acids with or without n-3 PUFA, the mice were then placed on a fat-free diet (ICN Biochemicals, Cleveland, OH USA) for 1 week. The mice (10 per group) were then fed the experimental diets for 4 weeks. Three experimental diets were used in study I and two in study II. In study II the mice were injected (intraperitoneally) with 0.5 mL 3% TG 3 days before they were sacrificed to elicit macrophages to the peritoneum. The TG contained less than 0.1 ng endotoxin/mL as determined by the Limulus amebocyte lysate assay (Sigma, St. Louis, MO USA).

Diets and study design

All diets contained 10 wt % fat. The n-6 fatty acid in the diets, linoleic acid (18:2 n-6), was supplied by safflower oil (75% linoleic acid) (Hollywood Foods, Los Angeles, CA USA), and the n-3 PUFA were supplied by sardine oil (Nippon Suisan Kaisha, LTD., Tokyo, Japan), containing 5.4% 18:4 n-3, 27.1% eicosapentaenoic acid (20:5 n-3), 2.5% docosapentaenoic acid (22:5 n-3), 12.0% docosahexaenoic acid (22:6 n-3), and minimal amounts of 18:2 n-6 (1.28%). The filler oils consisted of equal amounts of high oleic sunflower oil (85% 18:1) (SVO, Eastlake, OH USA) and tripalmitin (99% 16:0) (Sigma).

In study I the n-6 diet contained 3 wt % n-6 fatty acids; the low n-3 diet contained 1.0 wt % n-3 PUFA and 1.5 wt % n-6 fatty acids; and the high n-3 diet contained 1.5 wt % n-3 PUFA and 1.5 wt % n-6 fatty acids. In study II the n-6 diet contained 3 wt % n-6 fatty acids, and the n-3 diet contained 1.5 wt % n-3 PUFA and 1.5 wt % n-6 fatty acids. *Table 1* shows the fatty acid composition of the diets, measured by gas chromatography, in mol percent.

The remainder of the diet was composed of vitamin-free casein (187 g/kg), alphacel (147 g/kg), sucrose (520 g/kg), salt mixture USP XIV (36 g/kg), and ICN vitamin mix (19.8 g/kg) with supplemental choline chloride (5 g/kg). Diets were prepared in bulk and daily portions packed in whirl-pak bags, flushed with nitrogen, sealed, and stored at 4° C. Water and food were provided ad libitum, and fresh diets were provided daily to minimize exposure to air prior to consumption. Uneaten food was discarded and the feedcups were washed daily.

Isolation and activation of macrophages

The mice were killed by ether inhalation, and the peritoneal cells were collected in phosphate buffered saline (PBS), without calcium or magnesium, containing less than 0.1 ng endotoxin/mL, as determined by the Limulus amebocyte lysate assay (Sigma). The peritoneal cells were washed twice with PBS and suspended in KC 2000 (Hazelton Biologics, Lenexa, KA USA), a serum-independent medium. Cells were counted on a hemacytometer and differential counts were obtained following staining with Diff-Quik Stain Set (Baxter, McGaw Park, IL USA). Cell viability, assessed by Trypan blue exclusion, was greater than 95%. The peritoneal cell concentration was adjusted to 1×10^6 cells/mL and 0.5 mL plated per well in 24-well plates (Corning, Corning, NY). After 2-hr incubation at 37° C and 5% CO_2 nonadherent cells were removed by aspiration, counted (~20% of the total cell number), and discarded. The adherent cells were washed twice with PBS and incubated in 0.5 mL KC-2000 with or without one of the following: 8-bromo cAMP (1 mM), 3-isobutyl-1-methylxanthine (IBMX) (100 μM), forskolin (100 μM), or cholera toxin (10 μg/mL) from Sigma. A few minutes later they were stimulated with LPS (2 μg/mL) (*E. coli* 055:B5) (Calbiochem, La Jolla, CA USA). After 6-hr incubation the supernatants were removed and analyzed for PG and TNF.

TNF assay

Aliquots of the supernatants were frozen and stored at −70° C until analysis of immunoreactive TNF by an enzyme linked immunosorbent assay (ELISA) as described.[13]

Table 1 Fatty acid composition of the experimental diets (mol %)

	Dietary groups				
	Study I			Study II	
Fatty acid	n-6 3% n-6	low n-3 1.0% n-3/1.5% n-6	high n-3 1.5% n-3/1.5% n-6	n-6 3% n-6	n-3 1.5% n-3/1.5% n-6
14:0	nd[a]	2.29	3.20	nd	3.37
16:0	13.90	14.68	11.88	16.8	12.26
16:1	nd	3.44	4.91	nd	4.89
16:3 n-4	nd	0.65	1.04	nd	1.04
16:4 n-11	nd	1.49	2.57	nd	2.57
17:0	nd	3.58	2.21	nd	2.50
18:1	44.89	38.75	31.30	44.32	31.37
18:2 n-6	41.21	20.03	19.58	38.88	19.45
18:4 n-3	nd	1.26	2.01	nd	1.92
20:5 n-3	nd	7.86	12.47	nd	11.91
22:6 n-3	nd	3.15	4.85	nd	4.69
n-3/n-6[b]	—	0.67	1.07	—	1.04
PUFA + 18:2 n-6[c]	41.21	36.10	44.86	38.88	43.97

[a]nd: less than 0.1 mol% of the particular fatty acid. Fatty acids at concentrations less than 1 mol % in all dietary groups are not shown.
[b]n-3/n-6: Σ 18:3 n-3, 18:4 n-3, 20:4 n-3, 20:5 n-3, 21:5 n-3, 22:5 n-3, 22:6 n-3/Σ 18:2 n-6, 20:4 n-6.
[c]PUFA + 18:2 n-6: Σ 16:3 n-4, 16:4 n-11, 18:2 n-6, 18:3 n-3, 18:4 n-3, 20:4 n-6, 20:4 n-3, 20:5 n-3, 21:5 n-3, 22:5 n-3, 22:6 n-3.

Eicosanoid quantification

Portions (200 μL) of the supernatants were acidified with acetic acid and extracted three times with 1 mL ethyl acetate.[30] The pooled extract was evaporated to dryness under nitrogen, and the PG resuspended in PBS containing 0.1% gelatin. Each sample was analyzed by a radioimmunoassay for PGE_2 and 6-keto $PGF_{1\alpha}$, according to the manufacturer's instruction (Advanced Magnetics, Cambridge, MA USA). The antiserum was obtained from Advanced Magnetics, tritiated PGE_2 and 6-keto-$PGF_{1\alpha}$ from Du Pont (Wilmington, DE USA) and PGE_2 and 6-keto-$PGF_{1\alpha}$ standards from Cayman Chemical (Ann Arbor, MI USA). The cross-reactivity of PGE_3 with the PGE_2 antiserum was determined to be 26%, at half maximum binding.[12]

Fatty acid separation and analysis

Livers were perfused with cold saline (0.9%), containing EDTA (0.1%), removed from the animal, and a portion (~100 mg) homogenized in 0.8 mL saline. Pelleted peritoneal cells (2×10^6) were resuspended in 0.8 mL saline. The lipids were extracted with chloroform:methanol (1:2 vol/vol), followed by chloroform:saline (1:1 vol/vol), followed by one part chloroform (2×).[31] The pooled chloroform extracts were evaporated to dryness under nitrogen, and the lipids redissolved in 25 μL chloroform. The phospholipids were separated from the neutral lipids by thin layer chromatography using a chloroform:methanol (8:1 vol/vol) solvent system and visualized with 0.1% 8-hydroxy-1,3,6-pyrene-trisulfonic acid trisodium salt (Eastman Kodak, Rochester, NY USA) in methanol. The phospholipids were recovered from thin layer chromatography plates by scraping the appropriate bands, which were then resuspended in toluene. The phospholipids were saponified with 0.5 N KOH in methanol for 8 min at 86° C. Following acidification with 0.7 N HCl in methanol fatty acids were extracted with equal volumes of hexane (×2), evaporated under nitrogen, and methylated with ethereal diazomethane. Following evaporation, the fatty acid methyl esters were resuspended in hexane and analyzed by gas chromatography on a DB23 capillary column (0.25 mm × 30 m) (J&W Chromatography, Folsom, OH USA) with hydrogen as the carrier gas. Fatty acid composition of the diets was determined by using this method without separating phospholipids and neutral lipids.

Statistical analysis

The results are expressed as means ± SEM. The results were evaluated by Factorial Analysis of Variance and differences determined significant by Fisher's Protected Least Significant Difference at $P < 0.05$.

Results

Mice growth and cell counts

There was no difference in the body weights or relative weight gains of mice consuming the different diets over the 4-week feeding period. Cell numbers and relative portions of cells from the peritoneum of mice consuming the different diets were similar.

Liver and peritoneal cell phospholipid composition

Liver. Hepatic phospholipids from mice consuming diets with increasing n-3 PUFA content contained increasing amounts of n-3 PUFA and decreasing amounts of n-6 fatty acids with increasing n-3:n-6 PUFA ratio (data not shown). Total PUFA was lower in livers from mice consuming the n-6 diet than in livers from mice consuming the other diets.

Peritoneal cells. Increasing the n-3 PUFA content of the diet increased the n-3 PUFA and decreased the n-6 fatty acid concentration, and increased the n-3:n-6 PUFA ratio in phospholipids of both resident and thioglycollate-elicited peritoneal cells (data not shown). The total PUFA in phospholipids were not significantly different from the resident or the TG-elicited peritoneal cells from mice consuming the different diets.

TNF and PG production by resident peritoneal macrophages (study I)

The effect of diet. Resident peritoneal macrophages from mice consuming diets with increasing n-3 PUFA content secreted increasing amounts of immunoreactive TNF when stimulated with LPS (2 μg/mL) *(Figure 1A)*. TNF production by macrophages from mice consuming the high n-3 diet (12.2 ng/mL) was double that by macrophages from mice consuming the low n-3 diet (5.8 ng/mL), and five times more than the TNF production by macrophages from mice consuming the n-6 diet (2.4 ng/mL). In the absence of LPS, the TNF levels were 1.7–2% of that with LPS stimulation, 0.04–0.26 ng/mL.

Resident peritoneal macrophages from mice consuming diets with increasing n-3 PUFA content produced decreasing amounts of PG when stimulated with LPS *(Figure 1B and 1C)*.

The effect of 8-bromo cAMP and IBMX. To examine the role cAMP plays in mediating the effect of dietary n-3 PUFA on TNF production, a cAMP analog was added to the cultured macrophages before stimulation with LPS. Incubation of resident peritoneal cells, with 8-bromo cAMP (1 mM), followed by stimulation with LPS, resulted in significantly decreased release of immunoreactive TNF to 15–30% of that with LPS stimulation only *(Figure 1A)*. Macrophages from mice consuming diets with increasing n-3 PUFA content still produced increasing amounts of TNF when incubated with 8-bromo cAMP and LPS.

8-bromo cAMP, when added to the resident peritoneal macrophages, prior to LPS stimulation, decreased the PGE_2 production by macrophages from mice consuming the n-3 diet compared with LPS stimulation only *(Figure 1B)*. The 6-keto $PGF_{1\alpha}$ production by the resident peritoneal macrophages was not significantly changed following addition of 8-bromo cAMP with LPS, compared with LPS only *(Figure 1C)*.

Production of immunoreactive TNF by resident peritoneal macrophages was significantly decreased following addition of the phosphodiesterase (PDE) inhibitor, IBMX (100 μM), with LPS, to 20–50% of that with LPS stimulation only *(Figure 1A)*. Resident peritoneal macrophages from mice consuming diets with increasing n-3 PUFA content produced increasing amounts of TNF following addition of IBMX with LPS.

The PG production by resident peritoneal macrophages was not significantly changed following addition of IBMX with LPS compared with LPS only *(Figure 1B and 1C)*.

The effect of AC activators. To further look at the role of cAMP in the effect of diet on TNF production, AC activators were added with LPS to the macrophages. When forskolin (100 μM) or cholera toxin (10 μg/mL) were added with LPS, production of immunoreactive TNF by resident peritoneal macrophages from mice consuming the high n-3 diet decreased significantly compared with LPS only *(Figure 1A)*. The TNF levels in macrophages from mice consuming the high n-3 diet were brought to the same levels as in macrophages from mice consuming the low n-3 diet. The TNF production by macrophages from mice consuming these two diets were significantly higher than that in macrophages from mice consuming the n-6 diet.

Forskolin did not affect PG production by the resident peritoneal macrophages when added with LPS *(Figure 1B and 1C)*.

Cholera toxin significantly increased PGE_2 and 6-keto $PGF_{1\alpha}$ production by macrophages from mice consuming the low n-3 diet compared with LPS only *(Figure 1B and 1C)*. The PGE_2 production by macrophages from mice consuming the low n-3 diet was significantly higher than that by macrophages from mice consuming the high n-3 diet following addition of cholera toxin with LPS.

TNF and PG production by TG elicited peritoneal macrophages (study II)

The role of diet. There was no difference in immunoreactive TNF released by TG-elicited macrophages from mice consuming the n-6 and the n-3 diets following LPS stimulation *(Figure 2A)*. In study II, TG-elicited macrophages produced 3–14 times more TNF than the resident peritoneal macrophages in study I. TNF production by nonstimulated TG-elicited macrophages was less than 8% of that by macrophages stimulated with LPS.

There was no difference in the PG production by TG-elicited macrophages from mice consuming the two diets (n-3 and n-6) following stimulation with LPS *(Figure 2B and 2C)*. The PG production did not increase with stimulation compared with nonstimulated levels. The PGE_2 production by TG-elicited macrophages was 10–20% of the PGE_2 produced by the resident peritoneal macrophages in study I. The 6-keto $PGF_{1\alpha}$ production by TG-elicited macrophages in study II was 3–12% of the 6-keto $PGF_{1\alpha}$ produced by the resident peritoneal macrophages in study I.

The effect of 8-bromo cAMP and IBMX. 8-bromo cAMP had greater impact on the TG-elicited macrophages in study II than on the resident peritoneal macrophages in study I when added with LPS *(Figure 2A)*. The production of immunoreactive TNF by TG-elicited macrophages following 8-bromo cAMP addition was 3.6% and 4.0% of the TNF produced with LPS only. IBMX also significantly decreased TNF production by TG elicited macrophages from mice consuming the two diets to 44% and 53% of that with LPS only *(Figure 2A)*. There was no difference in the TNF production by the TG-elicited macrophages from mice consuming the two diets when 8-bromo cAMP or IBMX were added with LPS to the cells.

8-bromo cAMP and IBMX did not affect the PG production by the TG-elicited macrophages when added with LPS *(Figure 2B and 2C)*.

The effect of AC activators. Forskolin and cholera toxin significantly decreased production of immunoreactive

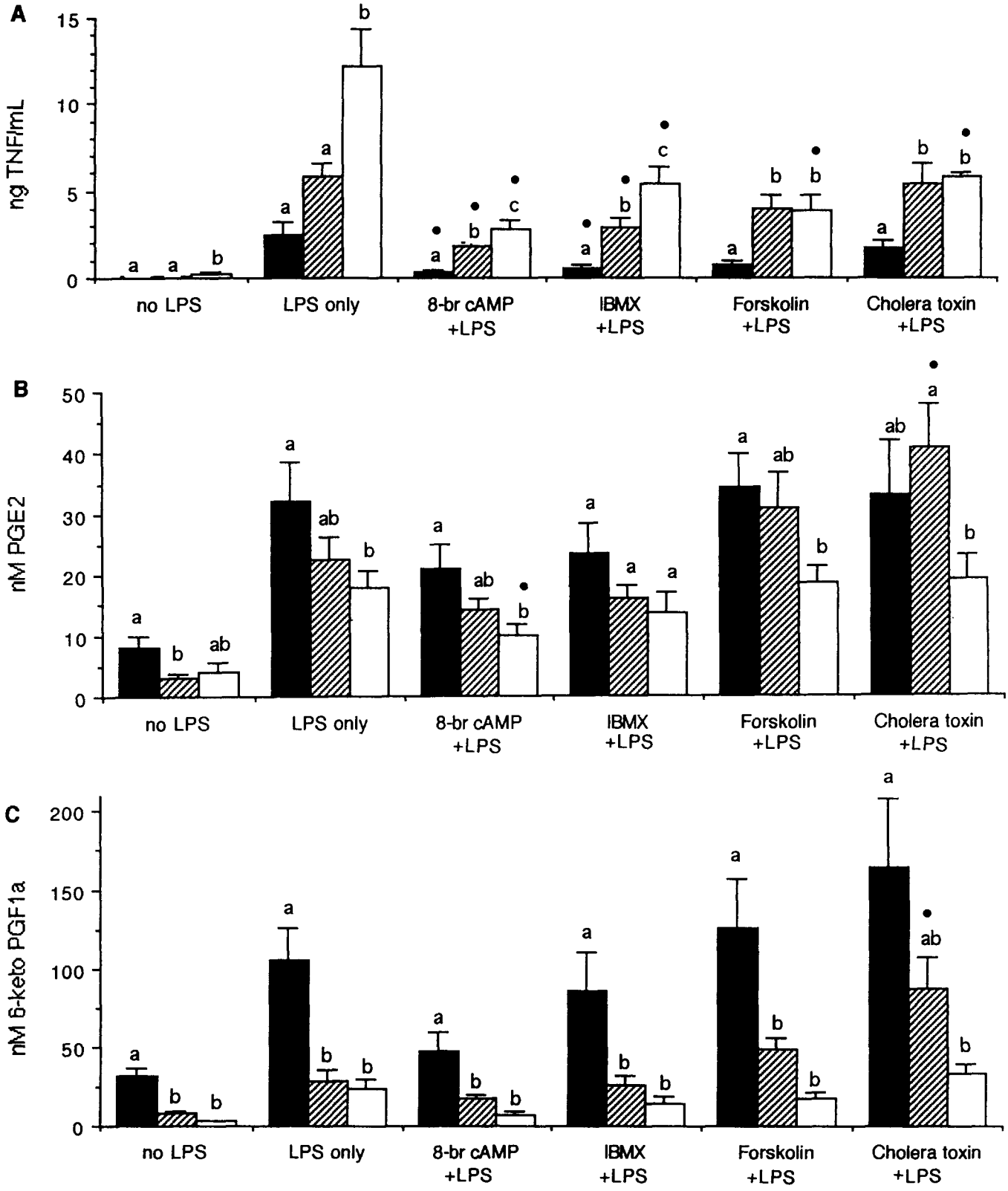

Figure 1 The effect of diet on (A) immunoreactive TNF, (B) PGE_2, and (C) 6-keto-$PGF_{1\alpha}$ production by resident peritoneal macrophages (study I). Macrophages from mice consuming experimental diets were incubated with one of the following: 8-bromo cAMP (1 mM), 3-isobutyl-1-methylxanthine (IBMX) (100 μM), forskolin (100 μM), or cholera toxin (10 μg/mL), and stimulated with LPS (2 μg/mL). After 6 hours the supernatants were collected and TNF measured by an ELISA method. Dietary groups: closed bars, n-6 diet (3% n-6); striped bars, low n-3 diet (1.0% n-3 + 1.5% n-6); open bars, high n-3 diet (1.5% n-3 + 1.5% n-6). Values are mean ± SEM for 10 animals. Bars with different letters are significantly different ($P < 0.05$) for each treatment (Fisher's Protected Least Significant Difference). Bars with a dot are significantly different ($P < 0.05$) from that with LPS stimulation only, for each diet (Fisher's Protected Least Significant Difference).

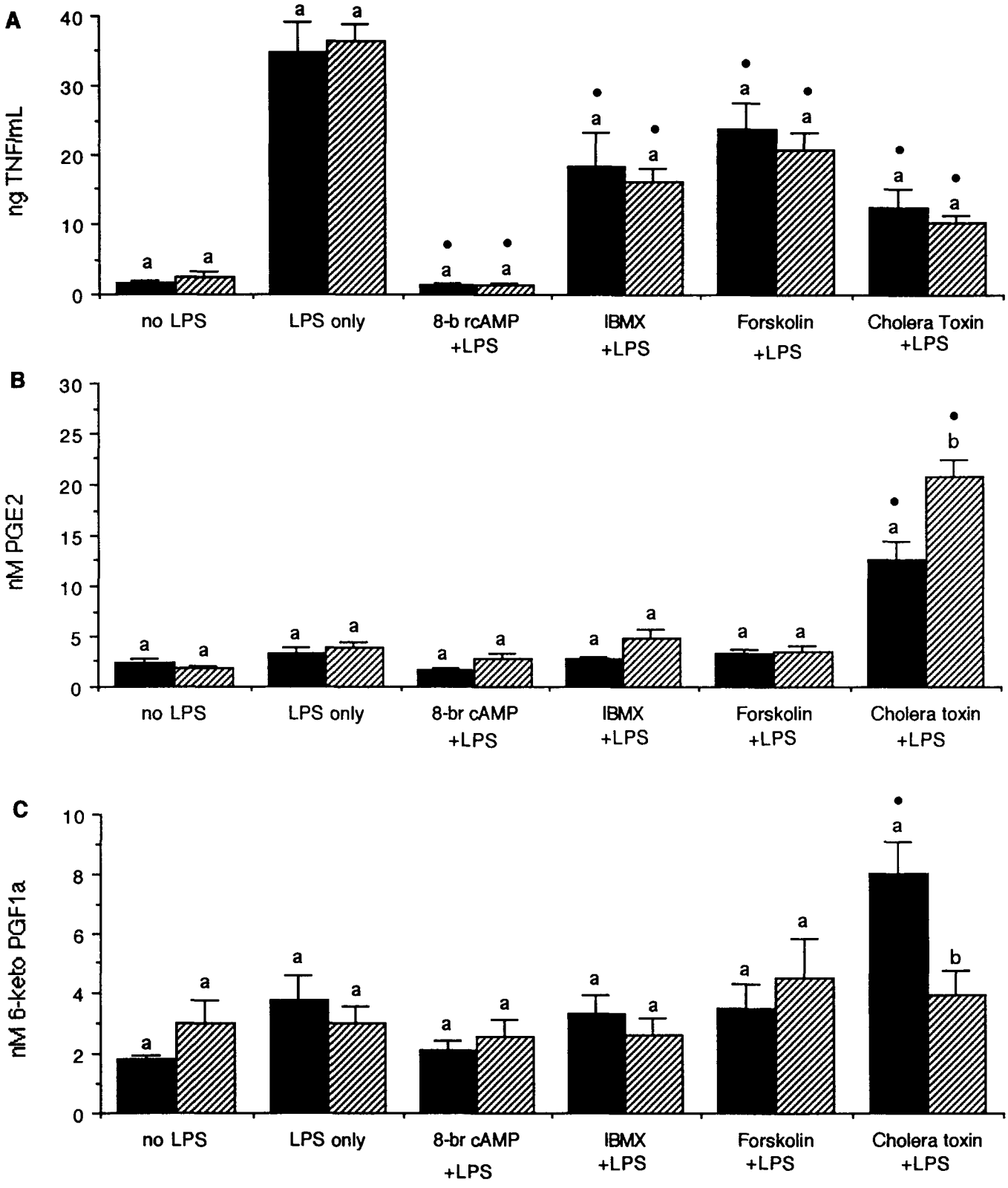

Figure 2 The effect of diet on (A) immunoreactive TNF, (B) PGE_2, and (C) 6-keto-$PGF_{1\alpha}$ production by thioglycollate-elicited peritoneal macrophages (study II). Macrophages from mice consuming experimental diets were incubated with one of the following: 8-bromo cAMP (1 mM), 3-isobutyl-1-methylxanthine (IBMX) (100 μM), forskolin (100 μM), or cholera toxin (10 μg/mL), and stimulated with LPS (2 μg/mL). After 6 hours the supernatants were collected and TNF measured by an ELISA method. Dietary groups: closed bars, n-6 diet (3% n-6); open bars, n-3 diet (1.5% n-3 + 1.5% n-6). Values are mean ± SEM for 10 animals. Bars with different letters are significantly different ($P < 0.05$) for each treatment (Fisher's Protected Least Significant Difference). Bars with a dot are significantly different ($P < 0.05$) from that with LPS stimulation only for each diet (Fisher's Protected Least Significant Difference).

TNF by TG-elicited macrophages from mice consuming both diets, compared with LPS stimulation only *(Figure 2A)*. With forskolin the TNF production decreased to 57% and 68% of that with LPS only, and with cholera toxin it decreased to 28% and 36% of that with LPS only. There was no difference in the TNF production by the TG-elicited macrophages from mice consuming the two diets following addition of cholera toxin or forskolin with LPS.

Cholera toxin, when added with LPS, enhanced PG production by the TG-elicited macrophages in study II, compared with LPS only, but forskolin had no effect *(Figure 2B and 2C)*. The PGE_2 production by TG-elicited macrophages from mice consuming the n-3 diet was significantly higher than that for macrophages from mice consuming the n-6 diet following addition of cholera toxin with LPS. The 6-keto $PGF_{1\alpha}$ production by macrophages from mice consuming the n-6 diet was significantly higher than that by macrophages from mice consuming the n-3 diet following addition of cholera toxin with LPS.

Discussion

The results demonstrate that increasing the n-3 PUFA content of the diet increased the production of immunoreactive TNF by the resident peritoneal macrophages. This is in agreement with previous studies from this laboratory in which resident peritoneal macrophages from mice consuming dietary n-3 PUFA produced more TNF than resident peritoneal macrophages from mice consuming dietary n-6 fatty acids.[11-13,15] Enhanced TNF production by resident peritoneal macrophages from rats consuming a diet high in linolenic acid (18:3 n-3), compared with macrophages from rats consuming a diet high in n-6 fatty acids, has also been reported.[14]

In the present study, resident peritoneal macrophages from mice consuming diets with increasing n-3 PUFA content produced decreasing amounts of PGE_2 and 6-keto-$PGF_{1\alpha}$. This is in agreement with other studies in which macrophages from mice consuming dietary n-3 PUFA produced less PG than macrophages from mice consuming n-6 fatty acids.[20,21] The decreased PG production may be partially responsible for the increased TNF production by macrophages from mice consuming the diets with increasing n-3 PUFA because of the inhibitory effect of PGE_2 on TNF production.[12,17,18]

As cAMP may mediate the inhibitory effect of PG or have an additional inhibitory effect on TNF production, the cells were incubated with 8-bromo cAMP and IBMX to increase intracellular cAMP levels. 8-bromo cAMP may stimulate the same physical responses as the natural nucleotide without being hydrolyzed by intracellular PDE and the PDE inhibitor, IBMX, increases cAMP levels in human monocytes[32] and rat peritoneal macrophages.[33] The decreased secretion of immunoreactive TNF by the resident peritoneal macrophages following addition of 8-bromo cAMP and IBMX in the present study is in agreement with results from others that show decreased TNF production and TNF mRNA accumulation in macrophages following addition of 8-bromo cAMP, dibutyryl cAMP,[22-24] and IBMX.[32] If n-3 PUFA mediate their effect on TNF production through cAMP, increasing intracellular cAMP levels would eliminate the dietary effects on TNF production. However, 8-bromo cAMP and IBMX did not eliminate the dietary effect on TNF production by the resident peritoneal macrophages, suggesting that the effect of dietary n-3 PUFA on TNF production by resident peritoneal macrophages is not mediated by cAMP.

Dietary n-3 PUFA decrease the responsiveness of AC to PG stimulation.[28,29] To elucidate whether decreased responsiveness to AC may be involved in the effect of dietary n-3 PUFA on TNF production, the cells were incubated with the AC activators, cholera toxin and forskolin. Forskolin and cholera toxin activate the AC enzyme by different mechanisms, with different kinetics and different effects on PG production. Cholera toxin causes ADP-ribosylation of the α subunit of guanyl nucleotide binding proteins (Gs), resulting in constitutive activation of AC,[34] whereas forskolin directly activates the AC and may, in addition, facilitate the activation of AC by the guanyl nucleotide regulatory protein.[35] Cholera toxin induces a gradual (after 1 hr) rise in cAMP generation,[36] whereas forskolin rapidly (within minutes) increases cAMP synthesis.[36,37] Cholera toxin either increases,[38] decreases,[39] or has no effect[36] on PG production by different cells, and forskolin either enhances[36] or has no effect[38] on PG synthesis. Cholera toxin suppresses accumulation of TNF mRNA[24] and inhibits LPS-induced TNF production[22] by TG-elicited macrophages. In the present study, cholera toxin and forskolin decreased TNF production by resident peritoneal macrophages from mice consuming the diet with the highest n-3 PUFA content. This may indicate decreased AC activity in macrophages from mice consuming this diet compared with macrophages from mice consuming the other diets. The AC activity may be restored by addition of the AC activators, and the resulting increase in intracellular cAMP levels may cause the decreased TNF production. The AC activators did not, however, eliminate the difference in TNF production by macrophages from mice consuming the n-6 diet and the n-3 PUFA-containing diets, which suggests that other factors are involved in the effect of dietary n-3 PUFA on TNF production.

With regard to the TG-elicited macrophages, the results show that dietary n-3 PUFA had no effect on immunoreactive TNF production by the elicited peritoneal macrophages. We and others have shown that dietary n-3 PUFA do not affect TNF production by CFA-elicited macrophages,[14,15] but their effect on TG-elicited macrophages has not previously been reported. CFA- and TG-elicited peritoneal macrophages produce more TNF than resident macrophages,[14,40,41] and in the present study the TG-elicited macrophages secreted three and 14 times more immunoreactive TNF than the resident peritoneal macrophages. Elicited peritoneal macrophages may therefore already produce maximum amounts of TNF, preventing any further enhancement by dietary n-3 PUFA.

Elicited peritoneal macrophages show diminished production of arachidonic acid oxygenation products,[42,43] and in the present study the TG-elicited macrophages produced 4–20% of the PG produced by the resident peritoneal macrophages. The decreased PG production and the lack of effect of diet on the PG production may partially explain the lack of effect of dietary n-3 PUFA on TNF production by TG-elicited peritoneal macrophages.

The differential effect of diet on resident and elicited peritoneal macrophages suggests that dietary n-3 PUFA may enhance TNF production by resident peritoneal macrophages, which produce low levels of TNF, without affecting the TNF production by TG-elicited macrophages, which produce more TNF. Increasing low levels of TNF may enhance antitumor action and antipathogenicity, and the lack of effect on high TNF levels may prevent from undesirable systemic inflammatory effects of overproduction.

In summary, the effects of dietary n-3 PUFA on TNF production by resident peritoneal macrophages appear not to be mediated by cAMP, as the cAMP analog, 8-bromo cAMP; the PDE inhibitor, IBMX; and the AC activators, forskolin and cholera toxin did not eliminate the dietary effects on TNF production in these cells.

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