DIETARY FISH OIL MODULATES MACROPHAGE FATTY ACIDS AND DECREASES ARTHRITIS SUSCEPTIBILITY IN MICE

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The prolonged bleeding times (1) and protection against thrombosis found in Eskimos (2) is thought to be related to a marine diet rich in ω-3–fatty acids (3). Recently, fish oil diets have been shown to modify the course of rheumatoid arthritis (4). In animal models of disease, diet enrichment with eicosapentaenoic acid (EPA),[1] an ω-3–fatty acid present in fish oil, protected the NZB/NZW mouse from glomerular nephritis (5), though augmenting susceptibility of rats to type II collagen–induced arthritis (CIA) (6).

CIA is suspected of being immunologically mediated, in that anticollagen antibodies seem to be a necessary, if not sufficient, requirement for disease induction (7). Antigenic recognition of collagen, and amplification of the response requires a complex interplay of chemical signals and cell-cell interactions (8). Evidence is accumulating that prostaglandins (PG) are among such intercellular mediators operating at several levels of the response (9). Macrophages, besides initiating the immune response, also produce large quantities of PG (10, 11), and are unique in that they contain high levels of esterified arachidonic acid (AA) (12), the most abundant of the PG precursors. We have examined the changes taking place in this precursor pool and the resulting PG profile when arthritis-susceptible mice are fed a diet enriched with EPA, a less common PG precursor. We find that this diet produces marked enrichment of ω-3–fatty acids in macrophage phospholipids, depresses production of PG of the 2 series, and decreases mouse susceptibility to CIA.

Materials and Methods

Diet. The composition of the diets used in these experiments is given in Table I. The level of dietary fat was 5% of dry weight supplied as either corn oil (Mazola; Best Foods,

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[1] *Abbreviations used in this paper:* AA, arachidonic acid; CIA, collagen-induced arthritis; DHA, docosahexaenoic acid; DPA, docosapentaenoic acid; DTA, docosatetraenoic acid; EPA, eicosapentaenoic acid; FAME, fatty acid methyl esters; HBSS, Hank's balanced salt solution; LT, leukotriene; PG, prostaglandin; TX, thromboxane.

TABLE I
Composition of Basal Diet

Ingredient	Weight (%)
Fat*	5.00
Vitamin-free casein	18.00
Corn starch	33.55
Sucrose	33.55
Cellulose	5.00
D-L-Methionine	0.30
Choline chloride	0.10
Salt mix (AIN-76)	3.50
Vitamin mix‡	1.00

* Fat was either corn oil or fish oil.
‡ Composition of vitamin mix (per kilogram): Vitamin A acetate, 150,000 IU; vitamin D, 15,000 IU; vitamin E, 3,000 IU; menadione sodium bisulphite, 200 mg; biotin, 20 mg; folacin, 200 mg; insositol, 2,380 mg; niacin, 3,000 mg; calcium pantothenate, 1,600 mg; riboflavin, 700 mg; thiamin, 600 mg; vitamin B6, 700 mg; vitamin B12, 1 mg.

Union, NJ) or fish oil (Maxepa; R. P. Scherer, N. A., Troy, MI). Diets were made fresh weekly and stored refrigerated. After 3 wk of storage, the fatty acid composition of the diet was not altered. Before being fed a fish or corn oil diet, the mice were fed rodent laboratory chow (5001; Purina, St. Louis, MO).

Mice. B10.RIII and B10.G mouse colonies were maintained in our lab after breeding pairs had been kindly donated by Dr. Chella S. David of Mayo Medical School, Rochester, MN. Mice were segregated by sex, housed four or five to a cage, and allowed access to food and water ad libitum. Fresh food was given every day.

In the first experiment, 24 B10.RIII mice (8 wk old, 12 males and 12 females) were fed either the corn or fish oil diet. Four males and four females from the two dietary groups were sacrificed after 1, 5, or 9 wk of the diet. Peritoneal cells from each group were pooled in pairs, and macrophages were isolated by adherence and by separation on Ficoll-Paque. Macrophages were analyzed for fatty acids present in their phospholipid pool, (*a*) immediately after isolation on Ficoll-Paque, (*b*) after being isolated by adherence to plastic for 2 h, and (*c*) after 24 h of incubation following adherence.

In the second experiment, 165 mice (85 male and 64 female B10.RIII and 8 male and 8 female B10.G aged 8 wk) were placed on a corn or fish oil diet. After 26 d on this diet, four mice in each group were killed, macrophages were prepared from each individual mouse by adherence, then incubated for 24 h. The incubation medium was stored frozen (−20°C) for subsequent PG analysis. The remaining B10.RIII mice were immunized intradermally at the base of the tail with 100 μg type II collagen in 50 μl of 0.5 M acetic acid, emulsified in 50 μl of complete Freund's adjuvant.

Arthritis Assessment. Mice were examined every 4 or 5 d, and the number of mice with CIA, the severity of the CIA, and its time of onset in each animal were recorded. Severity of arthritis was assessed clinically by scoring each of four paws on a 0–4 scale: 0, none; 1, probable swelling; 2, definite but minimal; 3, moderate; 4, very severe; (maximum possible severity score, 16). The highest score attained by each arthritic mouse was used to calculate the mean peak severity. Time of onset was the time from immunization at which a positive arthritis score was first observed. Incidence was calculated as a percentage of immunized mice with CIA at the time of scoring.

Collagen Preparation. Native type II collagen was isolated from fetal bovine nasal septa or articular cartilage using a modification of the procedure described by Trentham et al. (13). Amino acid composition of this material was consistent with that reported for purified type II collagen and yielded a single band on sodium dodecyl sulfate electrophoretic gels.

Macrophage Preparation. Mice were killed by cervical dislocation. An incision was made

through the abdomen, and 6 ml of Hank's balanced salt solution (HBSS) was injected intraperitoneally. The abdomen was massaged, and the abdominal fluid (~5.5 ml) was withdrawn through a fresh needle. Samples where bleeding into the cavity occurred were discarded. Cells were pelleted (400 *g* for 6 min), washed twice with 2 ml of HBSS, and resuspended in a total volume of 1 ml of RPMI 1640 medium containing 10% of fetal calf serum. Cells were gently resuspended, counted, adjusted to 1.5×10^6 cells/ml, and 1 ml aliquots were placed in plastic titer wells (Falcon Labware, Oxnard, CA). After 2 h incubation at 37°C under 5% CO_2 and 95% air, the nonadherent cells were removed. The remaining cells were washed twice with 2 ml followed by 1 ml of HBSS, and the nonadherent cells were counted. Either phospholipids were immediately extracted from the adherent cells, or the cells were incubated 24 h more in 1 ml RPMI 1640 medium containing 2.5% fetal calf serum.

A second method of preparing macrophages was used in which the peritoneal cells were layered over 3 ml of Ficoll-Paque (14). Centrifugation (30 min at 4°C, 400 *g*) resulted in a cell layer at the interface which was aspirated, washed twice with HBSS, and extracted for phospholipids. By morphologic and phagocytic criteria, this cell layer and the cells prepared by adherence contained >90% macrophages.

PG and Thromboxane (TX) Analysis. PG and TX were assayed radioimmunologically, according to a standard procedure (15, 16) developed by Levine et al. (17). The sample, or known amounts of the particular PG or TX (0.1 ml), the labelled ligand (PG or TX; 0.1 ml, 12,000 cpm, 100 Ci/mM), and a known titer of rabbit antibody against the particular PG (0.1 ml) were incubated at 37°C for 1 h. Normal rabbit serum (0.1 ml, 1:100 dilution) was then added, and the antibody-bound labeled ligand was separated from the free labeled ligand by addition of a second antibody, goat anti-rabbit gamma globulin (0.1 ml). After incubation overnight at 4°C, the precipitate was separated by centrifugation, dissolved in 0.2 ml of 0.1 N NaOH and counted. The percentage inhibition of the binding of the labeled ligand to the antibody was calculated for a series of PG or TX standards. The unknown amount of PG or TX in the sample was then calculated from the percent inhibition of binding of the labeled ligand to the antibody. The 6-keto-$PGF_{1\alpha}$ (a measure of PGI_2) antibody (Seragen, Inc., Boston, MA) crossreacted (<0.01%) with TXB_2, PGD_2, PGA_2, PGB_2, and metabolites of PGE_2 and $PGF_{2\alpha}$. The crossreaction with $PGF_{2\alpha}$ was 2.2%, 0.4% with PGE_2, 10.7% with $PGF_{1\alpha}$, and 0.06% with PGE_2. The TXB_2 (a measure of TXA_2) antibody (Seragen, Inc.) crossreacted (<0.1%) with PGD_2, PGE_2, $PGF_{2\alpha}$, PGA_2, PGB_2, PGE_1, $PGF_{1\alpha}$, PG metabolites, and with 6-keto $PGF_{1\alpha}$. The PGE_2 antibody, prepared in rabbits in the laboratory, crossreacted (<0.5%) with PGA_2, PGB_2, $PGF_{2\alpha}$, and with metabolites of PGE_2, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$. The crossreaction with PGD_2 was 2%. These three antibodies used in combination were sufficient to determine the type of PG or TX present in the medium. Since crossreactions with the 3 series of PG were not tested, due to the unavailability of these compounds, data were expressed as total PGE, PGI, and TXA, without specifying the series of PG and TX measured. However, the 10% crossreaction of the PGE_2 antibody with PGE_1 indicated that this antibody was discriminating with respect to the number of double bonds in the side chain.

Fatty Acid Analysis. Macrophages prepared by separation on Ficoll-Paque or by adherence were used for total lipid extraction according to the method of Folch et al. (18). The cells were pelleted, and 12 ml of 2:1 chloroform/methanol were added. After sonicating on ice for 20 min, 2.5 ml of 0.88% KCl was added, and the mixture was centrifuged for 10 min at 1,000 *g*. The upper phase was discarded, and the lower phase washed twice with 5 ml of methanol/water (1:1). The lower phase was dried under nitrogen and stored in 1 ml chloroform. The neutral lipids, free fatty acids, and glycolipids were removed from the total lipid extract by passage through a silicic acid column (Unisil; Clarkson Chemical Company, Williamsport, PA), and the phospholipid fraction was eluted with methanol (19). After alkaline methanolysis (20), the fatty acid methyl esters (FAME) were extracted into the lower phase. This phase was washed, dried down under nitrogen, and stored at 4°C for subsequent analysis by gas chromatography (FAME fraction). The total fatty acids in the two diets were similarly converted into their methyl esters.

Gas Chromatography. For a starting population of 10^6 cells, the FAME fraction was dissolved in 20 μl of hexane, and 2–4 μl injected. The FAME was eluted from the column (OV 225) with a linear thermal gradient of 170–220°C over 25 min, and held at the final temperature for a further 25 min. To separate palmitic from palmitoleic, stearic from oleic, and docosapentaenoic (DPA) from docosahexaenoic acid (DHA), the FAME fraction was also chromatographed over a second column (OV 1) with a linear thermal gradient of 170–220°C as above. The detector was of the flame ionization type.

Results

Fatty Acid Composition of Diets. An analysis of the two diets showed that a fish compared to a corn oil diet contained little of the ω-6 essential fatty acids, linoleic acid plus AA (2% vs. 65%, respectively). Of the fatty acids in fish oil, 29% were the ω-3 fatty acids, EPA and DHA, both of which were absent in the corn oil diet. (Table II).

Fish Oil Diet and Fatty Acid Composition of Macrophage Phospholipids. The fatty acid composition of the phospholipid pool from macrophages taken from female mice fed either a rodent chow, fish oil (Table III), or corn oil diet (Table IV) for 1–9 wk was compared. Macrophages from male mice showed a similar fatty acid composition, and identical changes in such composition over time. Macrophage neutral lipids and glycolipids were devoid of AA and EPA (data not shown).

After 1 wk on the fish oil diet, the macrophage phospholipids contained EPA, the precursor of PG of the 3 series, as well as significant quantities of DPA and DHA. These three fatty acids represented 24% of the total fatty acid, compared to the small amount (<8%), present either in the macrophage phospholipids of mice fed standard laboratory chow, or mice fed a corn oil diet for 1 wk. After 9

TABLE II
Fatty Acid Content of Basal Diet

Fatty acid	Total fatty acid (% ± SD)	
	Fish diet	Corn diet
14:0, myristic	7.7 ± 0.4	None
16:0, palmitic	18.3 ± 0.3	10.8 ± 0.6
16:1, palmitoleic	9.6 ± 0.5	None
Unidentified	2.2 ± 0.8	None
18:0, stearic	3.4 ± 0.1	1.7 ± 0.1
18:1, oleic	13.9 ± 2.2	22.0 ± 0.6
18:2, linoleic	2.0 ± 1.4	65.3 ± 0.6
18:3, linolenic	Trace	Trace
Unidentified	3.2 ± 0.2	None
20:0, arachidic	0.5 ± 0.2	Trace
20:1, erucic	1.8 ± 0.1	Trace
20:3, eicosatrienoic	1.0 ± 0.1	None
20:4, arachidonic	Trace	None
20:5, eicosapentaenoic	16.7 ± 1.8	None
22:1, docosaenoic	1.8 ± 0.1	None
22:4, docosatetraenoic	Trace	None
22:5, docosapentaenoic	2.7 ± 0.4	None
22:6, docosahexaenoic	12.2 ± 1.1	None
24:1, nervonic	Trace	None

The fatty acids were determined on freshly prepared diet.

TABLE III

Fatty Acid Content of Macrophage Phospholipids in Mice Fed Standard Rodent Chow or Fish Oil Diet for 1–9 Wk

Fatty acid	Total fatty acid (% ± SD)			
	Chow*	Fish (1 wk)‡	Fish (5 wk)‡	Fish (9 wk)‡
14:0	None	None	None	None
16:0	20.3 ± 1.7	19.1 ± 2.3	20.0 ± 1.5	17.6 ± 4.7
16:1	1.1 ± 0.3	0.8 ± 1.0	1.9 ± 0.3	2.1 ± 0.7
18:0	19.4 ± 1.2	20.2 ± 1.6	17.4 ± 0.1	16.5 ± 0.8
18:1	12.0 ± 0.5	14.2 ± 2.1	19.6 ± 3.1	22.9 ± 3.3
18:2	9.4 ± 0.3	4.2 ± 0.7	2.1 ± 0.1	1.5 ± 0.1
18:3	None	None	None	None
20:0	None	None	None	None
20:1	Trace	3.2 ± 2.4	Trace	Trace
20:3	2.6 ± 0.3	Trace	Trace	Trace
20:4	19.4 ± 1.5	12.2 ± 1.6	11.3 ± 2.3	10.5 ± 0.1
20:5	None	3.8 ± 0.4	4.9 ± 0.1	5.4 ± 0.6
22:1	None	None	None	None
22:4	7.1 ± 0.4	1.5 ± 1.8	2.3 ± 2.1	Trace
22:5	0.8 ± 0.6	10.0 ± 1.4	9.7 ± 0.5	12.5 ± 0.1
22:6	7.0 ± 1.3	10.1 ± 1.3	9.6 ± 2.0	9.8 ± 0.4

* Chow-fed B10.RIII mice were killed at 8 wk of age, macrophages were harvested by adherence to plastic for 2 h, and phopholipid fatty acids were quantitated.

‡ At 8 wk of age, mice were transferred to either a fish or corn oil diet (Table IV), and after 1, 5, or 9 wk, they were killed, their macrophages were prepared, and phospholipid fatty acids were quantitated. Each value represents a mean of determinations ± SD on two female mice.

TABLE IV

Fatty Acid Content of Macrophage Phospholipids in Mice Fed Corn Oil Diet for 1–9 wk

Fatty acid	Total fatty acid (% ± SD)		
	Corn (1 wk)*	Corn (5 wk)*	Corn (9 wk)*
14:0	None	None	None
16:0 + 16:1	21.1 ± 2.8	19.0 ± 3.8	18.2 ± 3.6
18:0 + 18:1	31.9 ± 7.7	34.7 ± 1.6	40.5 ± 5.8
18:2	7.8 ± 1.1	6.0 ± 0.8	5.0 ± 1.4
18:3	None	None	None
20:0 + 20:1	0.6 ± 0.0	0.4 ± 0.2	0.8 ± 0.2
20:4 + 20:3‡	23.8 ± 2.6	24.9 ± 1.3	22.7 ± 1.8
20:5	None	None	None
22:4	8.5 ± 0.1	12.2 ± 1.3	12.0 ± 0.1
22:5	None	None	None
22:6	6.3 ± 0.9	2.9 ± 0.6	<0.1

* See Table III legend.

‡ 20:3 comprised <15% of 20:4 and 20:3 peak.

wk of feeding a fish oil diet, these three fatty acids increased to 28% of the total, while in the corn oil–fed mice, the macrophage phospholipids now contained <0.1% of these fatty acids. DHA present only in the early stages of feeding corn oil (6.3% after 1 wk) is probably an ω-3 fatty acid derived from the original chow diet, which contains small amounts of added fish meal (1.25% EPA and 0.8%

DHA). The DHA appears in the phospholipid pool of the chow-fed mouse, and is slowly replaced by other fatty acids with continued feeding of the corn oil diet (Table IV).

Only mice receiving EPA in their diet had significant quantities of DPA in their phospholipid pool. No more than a trace was detected in the phospholipid pool of the chow-fed mice, and none in the corn oil–fed mice. Since DPA is present only in small quantities in the fish oil diet itself, it must be derived from the ω-3 fatty acid, EPA, by chain elongation. The DHA present in the macrophages of fish oil–fed mice must also be the ω-3 acid, either incorporated directly from the fish oil diet, or by further desaturation of DPA. Only the chow- and corn oil–fed mice had substantial quantities of docosatetraenoic acid (DTA) in the phosopholipids of their macrophages. The percentage of DTA increased relative to the time of feeding the corn oil. Conversely, it was rapidly depleted when mice were fed the fish oil diet. Since this acid is not present in the corn oil diet, it is probably derived from chain elongation of AA.

After 1 wk on fish oil, there was a decrease in the phosphlolipid pool of linoleic acid and AA (the precursors of PG of the 2 series), which together represented 16% of the total, compared to 29% in the corn oil–fed and chow-fed mice. This decrease, like the increase in EPA, became more marked with increased time of feeding the diets. After 5 wk, the EPA and AA pool had reached near equilibrium with the diets, whereas further depletion of the linoleic acid and DHA pools had occurred in the macrophages of the fish oil–fed and corn oil–fed mice, respectively. When these macrophages were incubated for as little as 2 h (the time necessary to isolate them by adherence) in the absence of an available source of EPA, the AA pool was increased, and the EPA and DPA plus DHA pool was reduced (Table V). This indicates that the pool of EPA present in the macrophages in vivo in fish oil–fed mice had probably been underestimated.

Fish Oil Diet and PG and TX Profile of Macrophages. In the second experiment, we studied the PG and TX profile of macrophages prepared from mice fed a

TABLE V

Fatty Acid Content of Phospholipids from Macrophages Prepared from Fish- or Corn Oil–fed Mice

Diet	Fatty acid	Total fatty acid after incubation (% ± SD)		
		0 h	2 h	26 h
Corn	20:4	21.1 ± 2.8	22.7 ± 1.8	26.7 ± 1.6
Corn	20:5	None	None	None
Corn	22:5 + 22:6	None	None	None
Fish	20:4	8.0 ± 0.1	10.5 ± 0.1	13.4 ± 0.8
Fish	20:5	8.0 ± 0.1	5.5 ± 0.6	2.5 ± 0.2
Fish	22:5 + 22:6	23.0 ± 4.7	22.2 ± 0.3	19.4 ± 3.9

At 8 wk of age, mice were transferred to either a fish or corn oil diet, and were killed after 9 wk. Phospholipids for fatty acid analysis were prepared from the same pool of macrophages isolated by Ficoll (no incubation), by adherence (2-h incubation), or after a further 24-h incubation of the adhered macrophages (26-h incubation).

Each value represents a mean of determinations ± SD on two female mice.

fish oil or a corn oil diet just before immunization with collagen. TX levels were unchanged by the fish oil diet. However, in both strains of fish oil–fed male mice, significantly less PG was found in the medium, relative to that found in macrophages from corn oil–fed mice (Table VI). Using an antibody against PGE_1 (Seragen, Inc.) little PGE of the 1 series was detected in medium from macrophages prepared from corn oil–fed mice. In the corn oil–fed mice in which no EPA and little eicosatrienoic acid was found, the PG and TX detected can be assigned to the 2 series. Because EPA was present in the macrophages of the fish oil–fed mice, there may be some crossreaction of the antibodies with the corresponding PG of the 3 series. However, our evidence is that the PGE antibody discriminates with respect to the number of double bonds on the side chain. Even if some crossreaction does occur with the 3 series of PG, this would increase the percent inhibition of binding and underestimate any reduction in PGE_2 and PGI_2 that occurred with the fish oil diet. Thus, the decrease in total PG found in the medium of macrophages from fish oil–fed mice (Table VI) is due to a reduction in PG of the 2 series.

The female mice of both strains produced more PG and TX from their macrophages than did males (data not shown). However, on fish oil, the macrophages from the female showed a similar reduction of PG, and an unchanged level of TX in the medium.

Collagen-induced Arthritis in the Mouse. Clinically, peripheral joints of front and hind limbs were visibly distended and reddened to variable degrees. Histological examination done 80 d after immunization, 48 d after the onset of CIA, and 23 d from the time of maximum severity revealed joints that were characterized by a chronic inflammatory process that was relatively organized. Advanced, healed synovitis was accompanied by thickened synovial membranes and proliferative fibrosis of the joint capsule, with extensive erosion and resorption of the articular cartilage and supporting bony surface. In many cases, the articular cartilage was obliterated by an overgrown pannus, which all but filled the synovial cavity. The pannus was composed primarily of fibroblasts and collagen, but in

TABLE VI

Prostaglandin and Thromboxane Produced from Macrophages of Male Arthritis-susceptible Mice

Mice	Diet	PG or TX produced (ng/10^6 cells/24 h ± SE)			
		PGE*	PGI*	TXA*	Total
R(4)	Corn	6.1 ± 0.9‡	11.5 ± 2.0‡	5.8 ± 0.4	23.3 ± 3.2‡
R(4)	Fish	2.3 ± 0.3	2.4 ± 0.3	5.3 ± 0.2	10.0 ± 0.5
G(3)	Corn	5.1 ± 0.3‡	12.7 ± 2.8‡	4.5 ± 0.1	22.2 ± 3.0‡
G(4)	Fish	2.9 ± 0.7	3.7 ± 0.8	6.2 ± 0.7	12.7 ± 2.0

Male B10.RIII (R) or B10.G (G) mice were fed a corn or fish oil diet for 26 d. Macrophages were isolated, incubated 24 h, and the medium was assayed for PG and TX by radioimmunoassay. Numbers in parentheses are the number of mice in each group.

* The antibodies used in the assay were prepared against the respective PG or TX of the 2 series. Since the crossreaction of the antibody with the corresponding PG or TX of the 3 series was not known, the data are reported as total PGE, PGI, and TXA, irrespective of series.

‡ Significantly different from fish oil diet ($p < 0.001$).

loosely organized areas, it was infiltrated by numerous monocytes and macrophages with foamy cytoplasm. In some joints, the bony articulations were indistinguishable due to the inflammatory fibrosis that had occurred. (Fig. 1)

Fish Oil Diet and Collagen-induced Arthritis. Arthritis was first detected, in both the fish oil–fed and corn oil–fed groups of mice, 19 d after immunization with collagen. However, the mean time of onset of CIA in the fish oil–fed group was much greater than that of the corn oil–fed mice (Table VII). The fish oil–fed mice also had less incidence of CIA, which was also significantly less severe ($p < 0.05$) throughout the experiment (Fig. 2). The mean peak severity reached by the fish oil–fed mice was also significantly less than that reached by the corn oil–fed mice (Table VII). By all criteria used to assess CIA (incidence, severity, peak severity, and time of onset), the fish oil–fed female mice were markedly less susceptible to the disease than the fish oil–fed males (data not shown).

Discussion

In these experiments, we have shown that dietary fish oil has altered the fatty acid composition of macrophage phospholipids compared to those extracted from mice fed a corn oil diet or a commercial rodent chow. These fatty acid changes are reflected in significant alterations in the PG profile of the macrophage.

The fatty acid compositions of the macrophage phospholipids from the chow- and corn oil–fed mice are very similar. Both contain substantial amounts of the precursor of PG of the 2 series, AA, and little or none of the precursors of PG of the 1 or 3 series. Quantitatively however, in the corn oil–fed mice compared to the chow-fed mice, levels of AA and its metabolite DTA are increased, and the ω-3 fatty acid DHA is depleted. In this study, ~6–7 ng PGE_2 were found in the medium from incubated resident peritoneal macrophages prepared from corn oil–fed mice. This amount is similar to that reported by Kurland and Bockman (11) and Scott et al. (21). The 2:1 prostacyclin/PGE_2 ratio is also of the same order as that reported by others (22–24). Using a direct radioimmunoassay for TX, rather than the radiolabeled technique of Scott et al. (23) we found significant quantities of TXA_2 in the medium. Others have reported that unstimulated resident mouse peritoneal macrophages have a TX synthetase enzyme (23), and can produce TX (24, 25). TX has also been shown to be a major metabolite in human monocytes depleted of platelets (26), and in resident guinea pig peritoneal macrophages (27).

In mice on the fish oil diet, the fatty acid alterations, in comparison to the corn oil–fed and chow-fed mice, take place rapidly. After only 1 wk, AA levels are decreased and EPA and two of its ω-3 fatty acid metabolites, DHA and DPA, appeared in the fatty acid pool. Relative to EPA, both DHA and DPA seem to be either preferentially incorporated into the phospholipid fatty acid pool, or are less rapidly metabolized. In contrast, in mice fed a corn oil diet, AA seems to be selectively incorporated or less rapidly metabolized compared to its ω-6 metabolite, DTA. Although the in vitro conditions used to prepare the macrophage may have caused an underestimation of the size of the EPA pool in vivo,

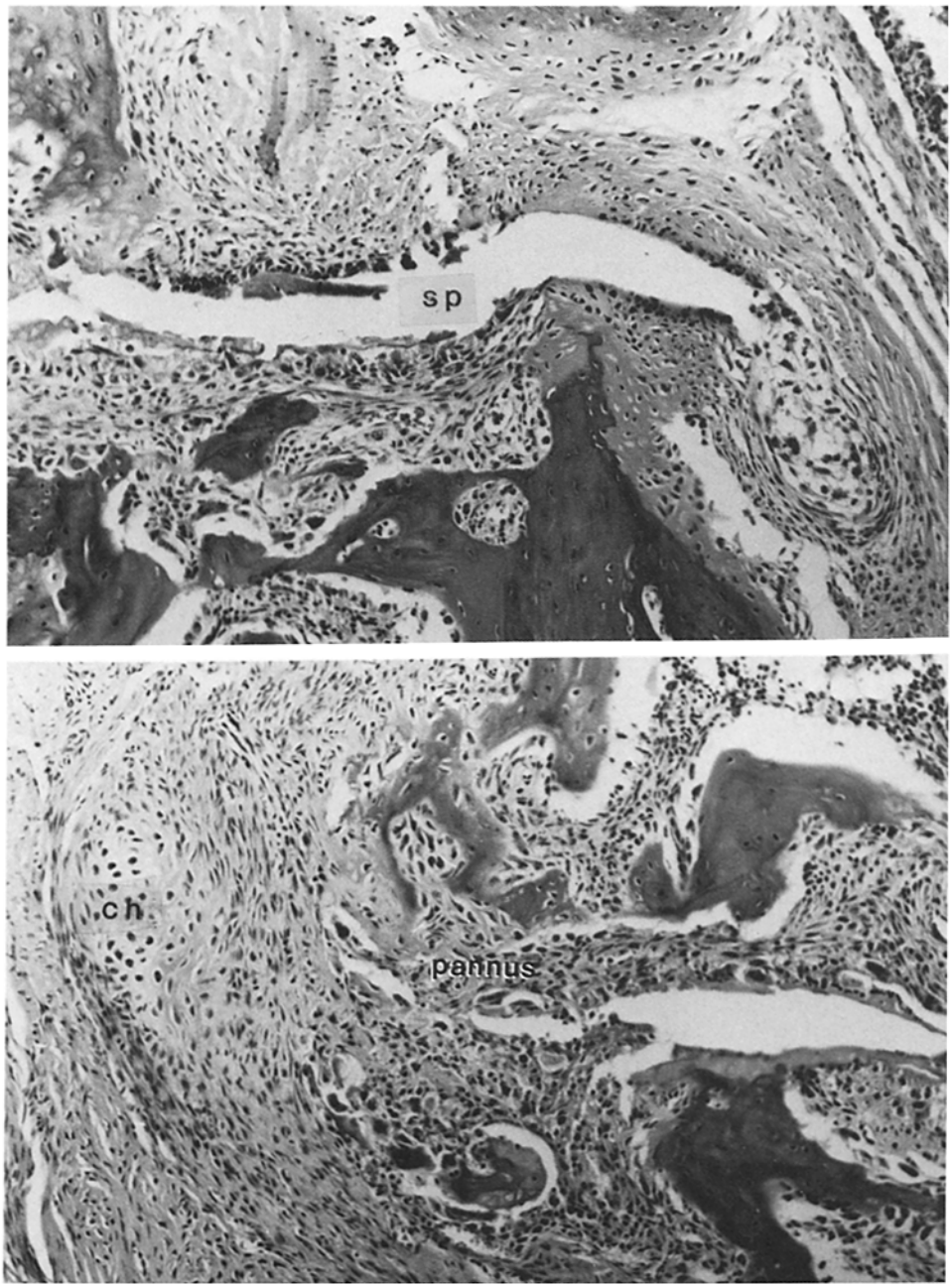

FIGURE 1. Sections (× 300) through the tarsal joint of the mouse 80 d after immunization with type II collagen, 48 d after the time of onset of clinically observed arthritis, and 23 d from the time of peak severity. (*Top*) Opposing articular surfaces across the synovial space (*sp*) reveal fragmented articular cartilage and extensive resorption and loss of supporting bone structure. (*Bottom*) Extensive pannus extends from the lateral synovium into the joint space. Islands of resorbing bone are evident in areas of former articular bony plate, and a focus of chondroplasia (*ch*) is organizing in the periarticular connective tissue.

TABLE VII

Effect of Diet on Incidence, Severity, and Time of Onset of Collagen-induced Arthritis in B10R.III Mice

Arthritis	Fish oil	Corn oil
Mice	74	59
Incidence	69%	93%
Peak severity (score ± SE)	6.7 ± 0.4	9.8 ± 0.3
Time of onset (days ± SE)	33.7 ± 1.6	25.1 ± 0.8

8-wk-old B10R.III mice were fed either a fish or corn oil diet. After 26 d, all mice were immunized intradermally with 100 μg type II collagen, and examined for signs of arthritis every 4 or 5 d. Incidence was calculated from the total number of animals developing any arthritis from time of immunization until 66 d later. Severity of arthritis was assessed by scoring each of four paws as described in Materials and Methods. The highest score attained by each arthritic mouse was used to calculate the mean peak severity. Time of onset was the time (from immunization) at which a positive score was first observed. The corn oil–fed mice (41% female) had a significantly higher peak severity ($p < 0.001$) and shorter time of onset ($p < 0.001$) compared to fish oil–fed mice (43% female).

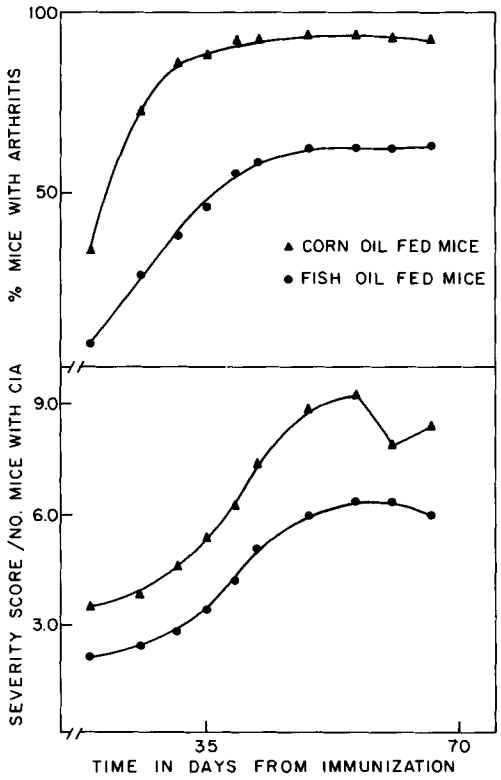

FIGURE 2. Incidence and severity of arthritis was assessed as described in Materials and Methods. The mean severity score at each time was calculated from the sum of the severity scores in each group divided by the number of mice with CIA. The corn oil–fed mice compared to the fish oil–fed mice had a greater incidence of arthritis which was significantly ($p < 0.05$) more severe throughout the experiment.

EPA does not reach the size of the AA pool. Further, EPA is rapidly replaced by AA whenever possible.

As the AA pool decreases and the EPA pool increases with the continued feeding of the fish oil diet, PG of the 2 series and leukotrienes (LT) of the 4 series should be reduced, with the potential for an increase in PG of the 3 series and LT of the 5 series. The observed reduction in PGE_2 and PGI_2 produced from the macrophages of mice fed fish oil for 26 d is consistent with these altered precursor pools. Alternately, EPA may competitively inhibit the cyclooxygenase enzyme (28, 29). The unexpected finding that TX production, unlike PG production, was not reduced indicates that fish oil has an initial effect other than depletion of the AA pool or inhibition of the cyclooxygenase. On the other hand, the TX antibody used may not discriminate between TX of the 2 and 3 series. One reason we dismiss this possibility is the observation that after 80 d on the fish oil diet, a decrease in TX from the macrophage is observed (our unpublished observation). Tashijan et al. (30) noted a differential effect of the fish oil diet on AA products in that cylooxygenase products were reduced but lipoxygenase products were unchanged. Socini et al. (31) has shown in the vascular system that a fish oil diet will reduce prostacyclin but not TX.

Prickett et al. (6) noted that a diet containing 25% (by weight) of fat as menhaden oil augmented the incidence of arthritis in rats. In contrast, we found that when B10.RIII mice were immunized with collagen after 26 d of dietary fish oil, there was a reduced incidence of arthritis, which was much less severe and later in onset compared to similarly treated mice on a corn oil diet. The female mice, especially those on the fish oil diet, tended to have less arthritis than the male mice. Although an immunological component is suspected, neither the mechanism by which collagen induces arthritis in rats or mice, nor the means by which fish oil may ameliorate or exacerbate the disease are fully understood. One possible explanation for the effect of fish oil is that, as noted for the macrophage, the altered fatty acid composition has produced changes in the PG and possibly the LT profile of the cell. It has recently (32) been shown in humans that fish oil diets decrease LTB_4 generation from neutrophils and monocytes, as well as changing neutrophil function in a direction that would theoretically diminish the inflammatory response. Alternatively, changes in fatty acid composition may influence cell function through altered membrane fluidity (33). Although the EPA and AA pools have nearly equilibrated after 26 d on the diet, other fatty acids that may influence subsequent EPA metabolism (31, 34, 35) were still subject to change. Thus, the exact fatty acid composition of the diet, its fatty acid content by weight, and the timing of the collagen immunization to induce arthritis, could all influence the fatty acid composition of the macrophage and a subsequent immune or inflammatory response.

In summary, an EPA metabolite produced by the macrophage may partially protect the fish oil–fed mouse from CIA. Since the females also produce greater amounts of AA and possibly EPA metabolites than the males on the same diet, this may account for an additional degree of protection from the disease. Our findings support the hypothesis that the macrophage, with its large PG and LT

precursor pools that can be altered by diet, plays a key role in the pathogenesis of CIA and its subsequent modulation by a fish oil diet.

Summary

B10.RIII and B10.G mice were transferred from a diet of laboratory rodent chow to a standard diet in which all the fat (5% by weight) was supplied as either fish oil (17% eicosapentaenoic acid [EPA], 12% docosahexaenoic acid [DHA], 0% arachidonic acid [AA], and 2% linoleic acid) or corn oil (0% EPA, 0% DHA, 0% AA, and 65% linoleic acid). The fatty acid composition of the macrophage phospholipids from mice on the chow diet was similar to that of mice on a corn oil diet. Mice fed the fish oil diet for only 1 wk showed substantial increases in macrophage phospholipid levels of the ω-3 fatty acids (of total fatty acid 4% was EPA, 10% docosapentaenoic acid [DPA], and 10% DHA), and decreases in ω-6 fatty acids (12% was AA, 2% docosatetraenoic acid [DTA], and 4% linoleic acid) compared to corn oil–fed mice (0% EPA, 0% DPA, 6% DHA, 20% AA, 9% DTA, and 8% linoleic acid). After 5 wk this difference between the fish oil–fed and corn oil–fed mice was even more pronounced. Further small changes occurred at 5–9 wk.

We studied the prostaglandin (PG) and thromboxane (TX) profile of macrophages prepared from mice fed the two diets just before being immunized with collagen. Irrespective of diet, macrophages prepared from female mice and incubated for 24 h had significantly more PG and TX in the medium than similarly prepared macrophages from male mice. The increased percentage of EPA and decreased percentage of AA in the phospholipids of the macrophages prepared from the fish oil–fed mice was reflected in a reduction in the amount of PGE_2 and PGI_2 in the medium relative to identically incubated macrophages prepared from corn oil–fed mice.

When this same fish oil diet was fed to B10.RIII mice for 26 d before immunization with type II collagen, the time of onset of arthritis was increased, and the incidence and severity of arthritis was reduced compared to arthritis induced in corn oil–fed mice. The females, especially those on the fish oil diet, tended to have less arthritis than the males. These alterations in the fatty acid pool available for PG and leukotriene synthesis suggest a pivotal role for the macrophage and PG in the immune and/or inflammatory response to type II collagen.

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