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# Dietary n-3 Fatty Acids Increase Spleen Size and Postendotoxin Circulating TNF in Mice; Role of Macrophages, Macrophage Precursors, and Colony-Stimulating Factor-1<sup>1</sup>

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In experimental studies in mice, dietary supplementation with n-3 fatty acids (FA) alleviates inflammation and increases resistance to infection. Nevertheless, TNF production capacity was found to be increased in n-3 FA-fed mice. We previously found increased relative spleen weights in n-3 FA-fed mice. In this study, the nature of this increased spleen size was further investigated. Spleen cellularity was increased significantly in mice fed n-3 FA (fish oil 15% w/w), compared with controls fed corn oil (15%) or normal lab chow ( $p < 0.05$ ). Experiments with T cell-deficient nude mice and experiments using macrophage depletion through liposomal dichloromethylene-biphosphonate revealed that the increase in spleen cellularity is T cell independent and largely due to macrophage accumulation in the spleen. Accumulation of marginal zone and red pulp macrophages was histologically and immunohistochemically confirmed. n-3 FA induced peripheral blood monocytosis and an aspecific increase in bone marrow cellularity. Postendotoxin circulating TNF concentrations were increased significantly in n-3 FA-fed mice compared with controls. Splenectomy did not abolish this increase in circulating TNF. However, after macrophage depletion through liposomal dichloromethylene-biphosphonate, circulating TNF was not detectable after endotoxin challenge. Circulating concentrations of CSF-1 did not differ between the various experimental groups. It is suggested that the cellular changes observed relate to increased constitutive production of TNF. *The Journal of Immunology*, 1996, 157: 5569–5573.

Fatty acids (FA)<sup>3</sup> such as eicosapentaenoic acid and docosahexaenoic acid are polyunsaturated (n-3) FA, in which the last double bond is located between the third and fourth carbon atom from the methyl end of the FA chain. Dietary supplementation with n-3 FA results in modification of the FA composition of the cell membranes, thereby reducing the production and biologic activity of prostaglandins and leukotrienes (1). More recently, dietary supplementation with n-3 FA has been associated with modulations in the production of the proinflammatory cytokines IL-1 and TNF, the principal polypeptide mediators of inflammation (2, 3).

In experimental studies, dietary supplementation with n-3 FA has been shown to alleviate inflammation in various clinical disease states and in animal models (4–11). Preliminary evidence indicates that dietary n-3 FA supplementation may increase resistance to infection in multitrauma patients (12). n-3 FA increase survival following endotoxic shock or infections with live micro-

organisms in various animal models (13–18), and dietary n-3 FA increase IL-1 $\alpha$  and TNF production capacity of mouse peritoneal cells (15, 19, 20). In the course of our experiments in mice, we noticed a 1.5-fold increase in relative spleen weight in n-3 FA-fed mice compared with controls. Similar observations have been reported by other groups studying mice or rats (21–23).

To elucidate the mechanism of the enhanced resistance to infections and possibly relate this to the increased spleen weight in mice, we studied the effects of dietary n-3 FA on the cellular composition of spleens, peripheral blood, and bone marrow. In addition, the effects of dietary n-3 FA on circulating concentrations of endotoxin-induced TNF and steady state CSF-1 were investigated.

## Materials and Methods

### Mice

In most experiments, 6-wk-old female Swiss mice, approximate weight 25 g, were used. The animals were housed in plastic cages (type II and type III; Macrolon, Beyer en Eggelaar, The Netherlands) under specific pathogen-free conditions at  $23 \pm 2^\circ\text{C}$  and 7 ventilations/h. In some experiments, normal female BALB/c and T cell-deficient female BALB/c nude mice (nu/nu) were used. Nude mice were housed in cages covered with sterilized filter bonnets.

### Diet

Mice were fed a fat-free standard reference diet as a dry powder, supplemented with 14% fish oil (FO) concentrate and 1% corn oil (FO group), 15% corn oil (CO), or normal lab chow. The FO concentrate used was EPAX 3000 TG, a kind gift of Pronova (Bergen, Norway). The FO concentrate contained 37.1% n-3 FA, i.e., 17.7% eicosapentaenoic acid and 14.4% docosahexaenoic acid (w/w). D-alpha tocopherol (0.67 mg/g) and a natural mix of tocopherols (0.525 mg/g) were added to avoid auto-oxidation. CO was a commercially available preparation (Mazola). CO (1%) was added to the FO diet to avoid essential FA deficiency. The FO concentrate was kept under strict anaerobic conditions until preparation of the diet to avoid auto-oxidation. Diets were prepared at least twice per week, and kept at  $4^\circ\text{C}$  until administration.

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<sup>3</sup> Abbreviations used in this paper: FA, fatty acid; Cl<sub>2</sub>MDP, dichloromethylene-biphosphonate; CO, corn oil; FO, fish oil.

Table I. Effect of dietary n-3 FA on spleen, peripheral blood, and bone marrow cells

	Diet Group			p
	Fish oil	Corn oil	Normal	
<b>Spleen</b>				
Relative spleen weight (mg/g)	5.9 ± 0.5	4.8 ± 0.3	4.6 ± 0.2	0.01
Spleen cellularity (× 10 <sup>8</sup> )	2.7 ± 0.2	2.0 ± 0.2	1.4 ± 0.5	0.02
B 220 positive spleen cells (%)	41 ± 4	36 ± 2	31 ± 6	0.04
<b>Peripheral blood</b>				
Hematocrit	43 ± 2.6	42 ± 3.2	43 ± 1.6	NS
White blood cell count (× 10 <sup>6</sup> /ml)	12.2 ± 4.0	9.1 ± 1.9	7.9 ± 1.3	NS
Lymphocytes (%)	55 ± 8	69 ± 4	64 ± 5	NS
Granulocytes (%)	34 ± 8	25 ± 4	31 ± 6	NS
Monocytes (%)	11 ± 2	6 ± 1	5 ± 1	0.02
<b>Bone marrow</b>				
Cellularity (× 10 <sup>6</sup> )	24 ± 5	21 ± 7	13 ± 5	0.02
M-CFC (No. macrophage precursors/10 <sup>4</sup> NBMC)	58 ± 15	51 ± 17	77 ± 20	NS
M-clustFC (No. macrophage precursors/10 <sup>4</sup> NBMC)	94 ± 22	80 ± 18	98 ± 27	NS
ER-MP 21 high (erythroid cells) (%)	22 ± 5	25 ± 3	22 ± 5	NS
ER-MP 58 high (myeloid cells) (%)	50 ± 7	50 ± 2	54 ± 6	NS
ER-MP 12 high/20 neg (committed progenitors) (%)	1.2 ± 0.3	1.7 ± 0.2	1.6 ± 0.5	NS
ER-MP 12 pos/20 pos (immature myeloid precursors) (%)	5.4 ± 1.2	5.6 ± 0.5	5.0 ± 1.2	NS
ER-MP 12 neg/20 high (monocytes) (%)	5.7 ± 0.9	6.2 ± 0.8	5.7 ± 1.2	NS

### Spleen analysis

The mice were anesthetized with ether, and blood was taken from the retrobulbar vessels after eye extraction. The animals were killed by neck dislocation, and spleens were removed and weighed. Total body weight was determined immediately before bleeding, and relative spleen weight was calculated afterward. A suspension of spleen cells was made over a nylon filter, and cells were counted with a Coulter counter (Coulter Corp., Hialeah, FL). The effect of dietary n-3 FA on spleen weight was determined in normal Swiss mice, normal BALB/c mice, and T cell-deficient nude BALB/c mice (nu/nu).

Spleen cells were phenotypically characterized with flow cytometry using a set of mAbs specific for mouse cells: 59-AD 2.2 detecting Thy-1 (T lymphocytes), RA3 6B2 detecting B220 (B lymphocytes), H129.19 detecting MT4 (CD4-positive T lymphocytes), 53-6.72 detecting Ly-2 (CD8-positive T lymphocytes), KT3 (CD3-positive T lymphocytes), and F4/80 (macrophages). Details concerning these Abs can be found in Leenen et al. (24).

After immunohistochemical staining of spleen sections with RA3 6B2 (anti-B220), a mAb specific for mouse B cells, the relative surface area of white pulp was quantitated using a Leitz Diaplan light microscope (Leitz, Wetzlar, Germany) and a Videoplan image-processing system (Kontron, Munich, Germany).

To analyze the role of mature phagocytic macrophages in the increase in spleen size, mice were fed FO for 0, 2, or 4 wk. Subsequently the animals were injected i.v. in the tail vein with 1 mg of liposomal dichloromethylene-biphosphonate (Cl<sub>2</sub>MDP or clodronate), 24 h before LPS challenge, to deplete macrophages (25). Control animals receiving the same diet were injected simultaneously with saline. Liposomal Cl<sub>2</sub>MDP was prepared as described (25). Spleen histology was studied in treated and untreated animals to verify macrophage depletion.

### Peripheral blood cells

Peripheral blood cell count was performed by separately determining hematocrit and the total white blood cell count (using Coulter counter). Differential white blood cell count was performed by quantifying the light scatter profile of nucleated cells.

### Bone marrow analysis

Bone marrow was obtained from both femora of each mouse by flushing the femur shafts with 3 ml of culture medium (RPMI, Dutch modification; Flow Laboratories, Irvine, Scotland). Nucleated cells were counted with a Coulter counter. Soft agar cultures of bone marrow cells were stimulated with macrophage CSF for 2 wk, and the number of macrophage precursors in the original preparation was quantitated by counting the number of macrophage colonies and clusters (<50 cells); macrophage colony-forming cells and macrophage cluster-forming cells are given per 10<sup>4</sup> nucleated bone marrow cells (26). Nucleated bone marrow cells were phenotypically characterized with flow cytometry using mAbs detecting Ags of mouse macrophage and granulocyte precursors. The mAbs were produced at the

Department of Immunology (Erasmus University, Rotterdam, The Netherlands), as described (27). The following mAbs were used: ER-MP12 and ER-MP20, identifying subpopulations of macrophage precursors; ER-MP58, identifying myeloid cells; and ER-MP21, identifying transferrin receptor-expressing cells (28).

### Postendotoxin circulating TNF

At a time period of 1.5 h after i.p. administration of 10 µg of LPS (*Escherichia coli*, serotype O55:B5; Sigma Chemical Co., St. Louis, MO), mice were anesthetized with ether, and blood was taken from the retrobulbar vessels after eye extraction. Blood samples (approximately 1 ml) were mixed with 100 µl of EDTA solution (21.4 mg/ml of EDTA in H<sub>2</sub>O) and kept on ice until centrifugation at 1500 × g for 5 min. Resultant plasma was isolated and stored at -20°C until assay.

TNF-α was measured by ELISA using TN3, a hamster mAb specific for murine TNF-α, and lymphotoxin, as described (29, 30).

In separate experiments, the effect of splenectomy on postendotoxin circulating TNF was studied. Splenectomy was performed after ether anesthesia in 4-wk-old mice, 2 wk before the animals were started on the experimental diets.

### Colony-stimulating factor-1

CSF-1 was determined in unstimulated serum samples by specific RIA, as described in detail elsewhere (31). This assay is based on the competition by CSF-1 for the interaction between <sup>125</sup>I-labeled, purified mouse L cell CSF-1 glycoprotein and a rabbit polyclonal Ab to purified L cell CSF-1 (32), and is more sensitive than the conventional CSF-1 bioassay based on bone marrow colony formation. Assays were conducted in duplicate on 20-µl samples. The concentration of CSF-1 in U/ml (1 U = 12 pg) was determined with reference to a standard curve prepared using a stable, partially purified L cell CSF-1 preparation.

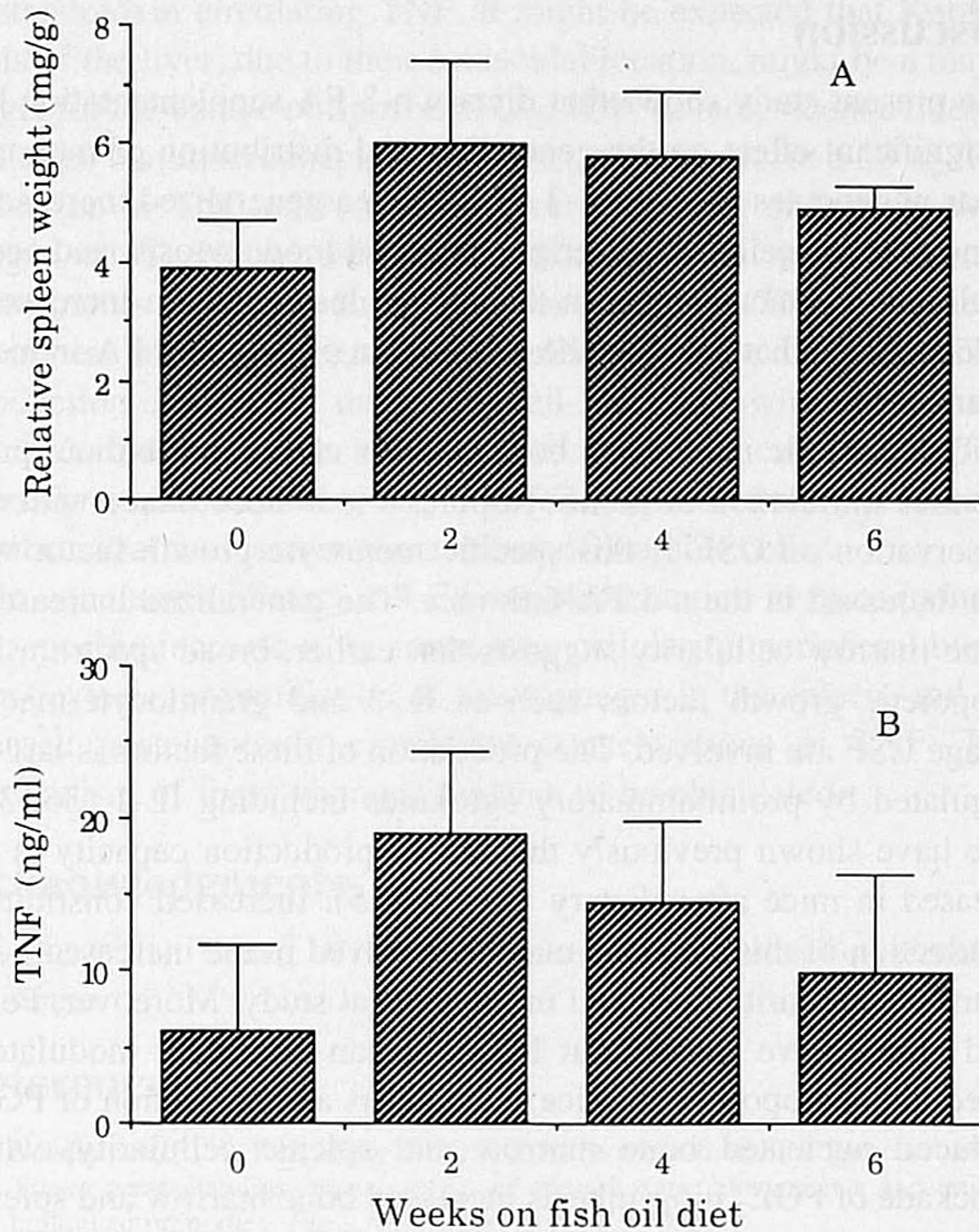
### Statistical analysis

Differences between groups were analyzed using the Kruskal-Wallis non-parametric ANOVA test, corrected for ties. Results were considered statistically significant at p < 0.05.

## Results

### Effects of FO supplementation on spleen cellularity

The effects of dietary n-3 FA on spleen, peripheral blood, and bone marrow cells are summarized in Table I. The following experiments were done to elucidate the mechanisms of the increased spleen weight in FO-fed mice. After 4 wk of dietary supplementation with FO or CO, experimental Swiss mice were compared with control animals on normal lab chow. FO-fed mice had significantly increased relative spleen weights (p = 0.01) and spleen



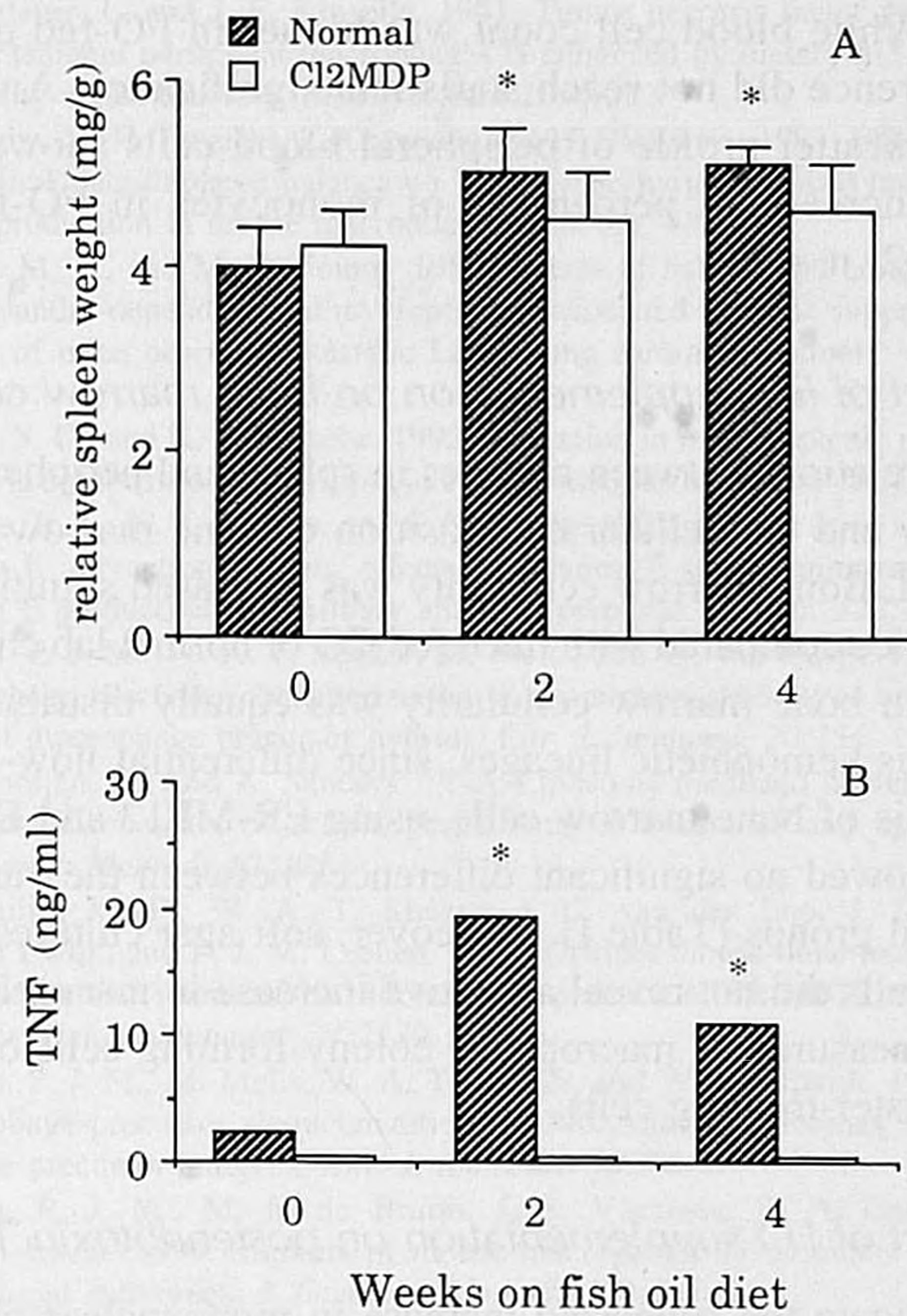
**FIGURE 1.** Time-effect relationships of dietary n-3 FA on relative spleen weight (A) and on postendotoxin circulating TNF (B). Relative spleen weight was increased significantly from 2 wk on FO diet, as was postendotoxin circulating TNF ( $n = 12$ ).

cellularity ( $p = 0.02$ ). In separate experiments, time-effect relationships in these responses were investigated. Mice were studied simultaneously after 0, 2, 4, and 6 wk of dietary FO supplementation. Increases in spleen weight were most prominent after 2 wk, and appeared somewhat blunted after 6 wk (Fig. 1A). Similar results were obtained in normal BALB/c mice. To investigate whether the increase in spleen size was accounted for or mediated by T cells, T cell-deficient female BALB/c mice were fed FO for 0, 2, or 4 wk. Relative spleen weight was increased significantly from 2 wk on, indicating that the increase in spleen weight was independent of the presence of T cells (results not shown).

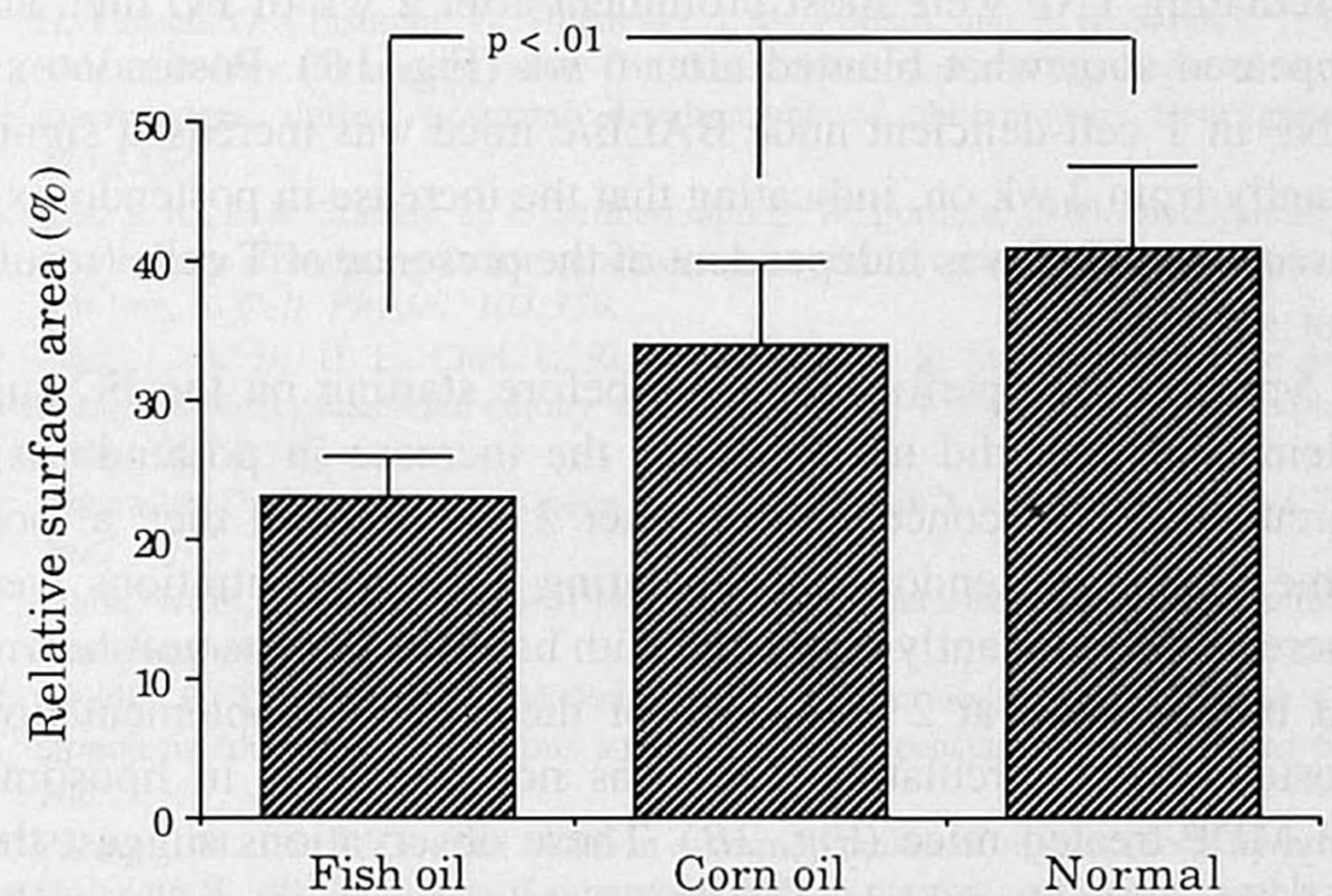
Phenotypic characterization of spleen cells using a set of mAbs revealed no percentual differences in T lymphocytes or macrophages between the various dietary groups. However, FO-fed mice had a small but significant increase in B220-positive B lymphocytes (Table I).

Pretreatment with liposomal  $Cl_2MDP$  24 h before endotoxin challenge did not significantly affect spleen weight. However, in mice that had been on FO diet for 2 or 4 wk, liposomal  $Cl_2MDP$  resulted in a decrease in spleen weight (Fig. 2A). In these FO-fed mice, treatment with liposomal  $Cl_2MDP$  resulted in spleen weights that were not significantly different from mice that had been on normal diet only. Depletion of marginal zone and red pulp macrophages was histologically confirmed, but accurate quantitation of F4/80-positive macrophages was not possible in cell suspensions, in which their numbers were equal in control and FO-fed mice. However, for other reasons (see below), the results indicate that the increase in spleen weight in FO-fed mice is mainly attributable to an increase in the splenic macrophage population that is depleted by pretreatment with liposomal  $Cl_2MDP$ .

To a lesser extent, B lymphocytes seem to contribute to the increased spleen cellularity in FO-fed mice. Quantification of the relative surface area of red and white pulp in spleen sections indicated a decrease in T and B cell-containing white pulp (Fig. 3).



**FIGURE 2.** BALB/c mice were fed FO for 0, 2, or 4 wk. At the end of the diet period, subsets of mice were injected i.v. with liposomal  $Cl_2MDP$  24 h before LPS challenge. In contrast to mice injected with saline, animals injected with liposomal  $Cl_2MDP$  did not show a significant increase in relative spleen weight following 2 or 4 wk of FO supplementation (A). Postendotoxin circulating TNF was not detectable in the liposomal  $Cl_2MDP$ -treated mice (B). \*, Indicates a statistically significant difference compared with mice that had no FO diet.



**FIGURE 3.** Spleen sections were stained for B220, an Ag specifically expressed by mouse B cells. The relative surface area of white pulp was quantitated using a Leitz Diaplan light microscope and a Videoplan image-processing system. The boundaries of white pulp areas could be established easily since B220 staining is confluent in the peripheral B cell zones of the white pulp, but only scattered in the red pulp. Relative surface areas are expressed as mean  $\pm$  SD.

A significant number of B220-positive B cells, however, is also present in the red pulp. We therefore conclude that FO-fed mice had a significantly increased content of red pulp area, containing mainly macrophages. F4/80 staining of spleen sections, identifying the red pulp areas, confirmed the relative increase of the red pulp area in the FO-fed mice.

#### *The effect of FO supplementation on peripheral blood cells*

To investigate a possible relation between circulating blood cells and changes in spleen cellularity, peripheral blood cells were quantitated. Hematocrit did not differ between the various treatment

groups. White blood cell count was higher in FO-fed mice, but this difference did not reach statistical significance. Analysis of the light-scatter profile of peripheral blood cells showed a significant increase in percentage of monocytes in FO-fed mice ( $p = 0.02$ ,  $n = 6$ ).

#### *The effect of FO supplementation on bone marrow cells*

Possible relations between changes in spleen and peripheral blood cellularity and the cellular composition of bone marrow were investigated. Bone marrow cellularity was increased significantly in FO-fed mice compared with mice fed CO or normal lab chow. This increase in bone marrow cellularity was equally distributed over the various hemopoietic lineages, since differential flow-cytometric analysis of bone marrow cells, using ER-MP12 and ER-MP20 mAbs, showed no significant differences between the various experimental groups (Table I). Moreover, soft agar cultures of bone marrow cells did not reveal a relative increase in macrophage precursors, measured as macrophage colony-forming cells or macrophage cluster-forming cells.

#### *The effect of FO supplementation on postendotoxin TNF*

To investigate the suggested increase in mononuclear phagocyte functionality, we measured circulating TNF concentrations after endotoxin challenge. Postendotoxin concentrations of circulating TNF were significantly higher in FO-fed mice than in control mice. Postendotoxin TNF was  $10.4 \pm 6.6$  ng/ml in FO-fed mice,  $2.2 \pm 1.4$  in CO-fed mice, and  $2.7 \pm 1.3$  in mice fed normal diet (mean  $\pm$  SD,  $p < 0.05$ ). In separate experiments, time-effect relationships in TNF production and FO diet were investigated. Similar to the increase in spleen weight, increases in postendotoxin circulating TNF were most prominent after 2 wk of FO diet, and appeared somewhat blunted after 6 wk (Fig. 1B). Postendotoxin TNF in T cell-deficient nude BALB/c mice was increased significantly from 2 wk on, indicating that the increase in postendotoxin circulating TNF was independent of the presence of T cells (results not shown).

Splenectomy, performed 2 wk before starting on the FO-supplemented diet, did not influence the increase in postendotoxin circulating TNF concentrations after 2 and 4 wk of diet: at both time points, postendotoxin circulating TNF concentrations were increased significantly compared with baseline (results not shown). At baseline and at 2 and 4 wk of dietary FO supplementation, postendotoxin circulating TNF was not detectable in liposomal  $\text{Cl}_2\text{MDP}$ -treated mice (Fig. 2B). These observations suggest that splenic macrophages, despite their accumulation after dietary FO supplementation, are not the most important contributors to the production of circulating TNF. On the other hand, the macrophage population that is depleted by liposomal  $\text{Cl}_2\text{MDP}$  and that does not reside in the spleen appears to be the most important producer of postendotoxin circulating TNF.

#### *The effect of FO supplementation on circulating concentrations of CSF-1*

The mononuclear phagocyte growth factor, CSF-1, is required for the development of the majority of mouse macrophages (33). To accurately measure circulating concentrations of CSF-1, serum samples were subjected to a mouse CSF-1-specific RIA. In biologic samples, this RIA detects only biologically active CSF-1 (32, 34), including both glycoprotein and proteoglycan forms (35). Circulating concentrations of immunoreactive CSF-1 did not differ among the various diet groups:  $702 \pm 89$  U/ml in FO-fed mice,  $753 \pm 96$  in CO-fed mice, and  $802 \pm 158$  in mice fed normal diet (mean  $\pm$  SD,  $p = 0.40$ ).

## Discussion

The present study shows that dietary n-3 FA supplementation has a significant effect on the generation and distribution of mononuclear phagocytes in mice: n-3 FA induce a generalized increase in bone marrow cellularity, peripheral blood monocytosis, and accumulation of macrophages in the spleen, leading to an increase in spleen size. What could be the mechanism of these n-3 FA-induced changes?

The aspecific increase in bone marrow cellularity without preferential stimulation of monocytopoiesis is in accordance with our observation on CSF-1: this specific monocyte growth factor was not increased in the n-3 FA-fed mice. The generalized increase in bone marrow cellularity suggests that earlier, broad spectrum hemopoietic growth factors such as IL-3 and granulocyte-macrophage CSF are involved. The production of these factors is largely regulated by proinflammatory cytokines including IL-1 (36, 37). We have shown previously that IL-1 $\alpha$  production capacity is increased in mice after dietary n-3 FA (15). Increased constitutive production of this cytokine may be involved in the increased bone marrow cellularity observed in the present study. Moreover, Pelus and others have shown that PGE<sub>2</sub> has an important modulatory effect on hemopoiesis in mice: exogenous administration of PGE<sub>2</sub> reduced nucleated bone marrow and splenic cellularity, while blockade of PGE<sub>2</sub> biosynthesis increased bone marrow and splenic cellularity, especially in the presence of IL-1 $\alpha$  (38–42). These observations may partly explain the results obtained in the present study, since dietary supplementation with n-3 FA in mice basically leads to increased IL-1 $\alpha$  and a decreased PGE<sub>2</sub> production capacity.

Peripheral blood monocytosis induced by dietary n-3 FA has not been described before, but may be induced through a feedback mechanism following the accumulation of macrophages in the red pulp of the spleen. It is apparently not due to increased concentrations of CSF-1. This is in accordance with the observation that the number of red pulp macrophages is reduced only slightly in CSF-1-deficient op/op mice (43). Other factors, including IL-3, granulocyte-macrophage CSF, and the proinflammatory cytokines, may be considered. The results of our experiments with macrophage depletion using liposomal  $\text{Cl}_2\text{MDP}$  suggest that the observed increase in spleen size following dietary FO supplementation is largely due to accumulation of macrophages in the spleen. The immunohistochemical staining of spleen sections with B220 showed a decrease in the relative surface area of white pulp in the FO-fed mice, thereby supporting the concept of accumulation of red pulp macrophages in FO-fed mice. As regulator cells, macrophages are responsible for the growth of lymphoid organs such as the spleen. Since TNF is an important growth factor for lymphoid organs, increased endogenous TNF production in FO-fed mice may contribute to this effect (44). The phenotypic characterization of spleen cell suspensions using a set of mAbs is not suitable for the assessment of the number of macrophages in the spleen, since especially tissue-fixed macrophages are lost in the process of making a cell suspension over a nylon filter. We therefore regard the concept that dietary FO supplementation leads to macrophage accumulation in the spleen as valid.

The increase in spleen size after dietary n-3 FA appeared to be accompanied by increased circulating concentrations of postendotoxin TNF. We and others have shown previously that peritoneal macrophages of mice fed n-3 FA have increased TNF production capacity at the level of the single cell (15, 20, 45). Since splenectomy did not affect the increased TNF concentrations following dietary n-3 FA, macrophage accumulation in the spleen is not responsible for the increased TNF concentrations. Interestingly, macrophage depletion by liposomal  $\text{Cl}_2\text{MDP}$  completely abolished

postendotoxin circulating TNF. It might be expected that Kupffer cells of the liver, due to their sinusoidal location, might be a major target for the uptake of liposomal  $Cl_2$ MDP. In fact, recent evidence indicates that macrophage depletion in mice results in a 50 to 70% reduction in TNF mRNA in the liver following endotoxin challenge (46). Therefore, the Kupffer cells may be the most important contributors to the production of postendotoxin TNF. Similar to peritoneal macrophages, Kupffer cells may have increased TNF production capacity at the single cell level following dietary n-3 FA. We did not assess the number of Kupffer cells. The small increase in relative liver weight in FO-fed mice does not rule out a substantial increase in the number of Kupffer cells.

In conclusion, dietary n-3 FA supplementation in mice induces an aspecific increase in bone marrow cellularity, peripheral blood monocytes, accumulation of macrophages in the spleen, and increased postendotoxin circulating concentrations of TNF. The mechanism of these changes remains to be elucidated.

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## References

- Needleman, P., A. Raz, M. S. Minkes, J. A. Ferrendelli, and H. Sprecher. 1979. Triene prostaglandins: prostacyclin and thromboxane biosynthesis and unique biological properties. *Proc. Natl. Acad. Sci. USA* 76:944.
- Endres, S., R. Ghorbani, V. E. Kelley, K. Georgilis, G. Lonnemann, J. W. van der Meer, J. G. Cannon, T. S. Rogers, M. S. Klempner, P. C. Weber, E. J. Schaeffer, S. M. Wolff, and C. A. Dinarello. 1989. The effect of dietary supplementation with n-3 polyunsaturated fatty acids on the synthesis of interleukin-1 and tumor necrosis factor by mononuclear cells. *N. Engl. J. Med.* 320:265.
- Meydani, S. N., S. Endres, M. M. Woods, B. R. Goldin, C. Soo, A. Morrill-Labrode, C. A. Dinarello, and S. L. Gorbach. 1991. Oral (n-3) fatty acid supplementation suppresses cytokine production and lymphocyte proliferation: comparison between young and older women. *J. Nutr.* 121:547.
- Kremer, J. M., A. Michalek, and L. Lininger. 1985. Effects of manipulation of dietary fatty acids on clinical manifestations of rheumatoid arthritis. *Lancet* 1:184.
- Kremer, J. M., D. A. Lawrence, W. Jubiz, R. DiGiacomo, R. Rynes, L. E. Bartholomew, and M. Sherman. 1990. Dietary fish oil and olive oil supplementation in patients with rheumatoid arthritis: clinical and immunologic effects. *Arthritis Rheum.* 33:810.
- Ziboh, V. A., K. A. Cohen, C. N. Ellis, C. Miller, T. A. Hamilton, K. Kragballe, C. R. Hydrick, and J. J. Voorhees. 1986. Effects of dietary supplementation of fish oil on neutrophil and epidermal fatty acids: modulation of clinical course of psoriatic subjects. *Arch. Dermatol.* 122:1277.
- Aslan, A., and G. Triadafilopoulos. 1992. Fish oil fatty acid supplementation in active ulcerative colitis: a double-blind, placebo-controlled, crossover study. *Am. J. Gastroenterol.* 87:432.
- Prickett, J. D., D. R. Robinson, and A. D. Steinberg. 1981. Dietary enrichment with the polyunsaturated fatty acid eicosapentaenoic prevents proteinuria and prolongs survival in NZB  $\times$  NZW F<sub>1</sub> mice. *J. Clin. Invest.* 68:556.
- Prickett, J. D., D. R. Robinson, and A. D. Steinberg. 1983. Effects of dietary enrichment with eicosapentaenoic acid upon autoimmune nephritis in female NZB  $\times$  NZW/F<sub>1</sub> mice. *Arthritis Rheum.* 26:133.
- Bloch, K. J., B. Ho, L. L. Xu, M. Bloch, and D. R. Robinson. 1989. Effect of fish-fat or beef-fat supplemented diet on immune complex-induced enteropathy in the rat. *Prostaglandins* 38:385.
- Endres, S., R. De Caterina, E. B. Schmidt, and S. D. Kristensen. 1995. n-3 polyunsaturated fatty acids: update 1995. *Eur. J. Clin. Invest.* 25:629.
- Moore, F. A., E. E. Moore, K. A. Kudsk, R. O. Brown, R. H. Bower, M. J. Koruda, C. C. Baker, and A. Barbul. 1994. Clinical benefits of an immune-enhancing diet for early postinjury enteral feeding. *J. Trauma* 37:607.
- Mascioli, E. A., Y. Iwasa, S. Trimbo, L. Leader, B. R. Bistrain, and G. L. Blackburn. 1989. Endotoxin challenge after menhaden oil diet: effects on survival of guinea pigs. *Am. J. Clin. Nutr.* 49:277.
- Pomposelli, J. J., E. Flores, Y. Hirschberg, T. C. Teo, G. L. Blackburn, S. H. Zeisel, and B. R. Bistrain. 1990. Short-term TPN containing n-3 fatty acids ameliorate lactic acidosis induced by endotoxin in guinea pigs. *Am. J. Clin. Nutr.* 52:548.
- Blok, W. L., M. T. Vogels, J. H. Curfs, W. M. Eling, W. A. Buurman, and J. W. van der Meer. 1992. Dietary fish-oil supplementation in experimental Gram-negative infection and in cerebral malaria in mice. *J. Infect. Dis.* 165:898.
- Fernandes, G., V. Tomar, M. N. Venkatraman, and J. T. Venkatraman. 1992. Potential of diet therapy on murine AIDS. *J. Nutr.* 122:716.
- Barton, R. G., C. L. Wells, A. Carlson, R. Singh, J. J. Sullivan, and F. B. Cerra. 1991. Dietary omega-3 fatty acids decrease mortality and Kupffer cell prostaglandin E<sub>2</sub> production in a rat model of chronic sepsis. *J. Trauma* 31:768.
- Kumaratilake, L. M., B. S. Robinson, A. Ferrante, and A. Poulos. 1992. Antimalarial properties of n-3 and n-6 polyunsaturated fatty acids: in vitro effects on *Plasmodium falciparum* and in vivo effects on *P. berghei*. *J. Clin. Invest.* 89:961.
- Hardardottir, I., and J. E. Kinsella. 1991. Tumor necrosis factor production by murine resident peritoneal macrophages is enhanced by dietary n-3 polyunsaturated fatty acids. *Biochim. Biophys. Acta* 1095:187.
- Watanabe, S., H. Hayashi, K. Onozaki, and H. Okuyama. 1991. Effect of dietary alpha-linolenate/linoleate balance on lipopolysaccharide-induced tumor necrosis factor production in mouse macrophages. *Life Sci.* 48:2013.
- Young, M. R., and M. E. Young. 1989. Effects of fish oil and corn oil diets on prostaglandin-dependent and myelopoiesis-associated immune suppressor mechanisms of mice bearing metastatic Lewis lung carcinoma tumors. *Cancer Res.* 49:1931.
- Huang, S. C., and K. L. Fritsche. 1992. Alteration in mouse splenic phospholipid fatty acid composition and lymphoid cell populations by dietary fat. *Lipids* 27:25.
- Fritsche, K. L., N. A. Cassity, and S. C. Huang. 1992. Dietary (n-3) fatty acid and vitamin E interactions in rats: effects on vitamin E status, immune cell prostaglandin E production and primary antibody response. *J. Nutr.* 122:1009.
- Leenen, P. J. M., W. A. T. Sliker, M. Melis, and W. van Ewijk. 1990. Murine macrophage precursor characterization. I. Production, phenotype and differentiation of macrophage precursor hybrids. *Eur. J. Immunol.* 20:15.
- Van Rooijen, N., and A. Sanders. 1994. Liposome mediated depletion of macrophages: mechanism of action, preparation of liposomes and applicabilities. *J. Immunol. Methods* 174:83.
- De Bruijn, M. F., W. A. T. Sliker, J. C. van der Loo, J. S. Voerman, W. van Ewijk, and P. J. M. Leenen. 1994. Distinct mouse bone marrow macrophage precursors identified by differential expression of ER-MP12 and ER-MP20 antigens. *Eur. J. Immunol.* 24:2279.
- Leenen, P. J. M., M. Melis, W. A. T. Sliker, and W. van Ewijk. 1990. Murine macrophage precursor characterization. II. Monoclonal antibodies against macrophage precursor antigens. *Eur. J. Immunol.* 20:27.
- Leenen, P. J. M., M. F. de Bruijn, J. S. Voerman, P. A. Campbell, and W. van Ewijk. 1994. Markers of mouse macrophage development detected by monoclonal antibodies. *J. Immunol. Methods* 174:5.
- Sheehan, K. C., N. H. Ruddle, and R. D. Schreiber. 1989. Generation and characterization of hamster monoclonal antibodies that neutralize murine tumor necrosis factors. *J. Immunol.* 142:3884.
- Von Asmuth, E. J., J. G. Maessen, C. J. van der Linden, and W. A. Buurman. 1990. Tumor necrosis factor alpha (TNF-alpha) and interleukin 6 in a zymosan-induced shock model. *Scand. J. Immunol.* 32:313.
- Stanley, E. R. 1985. The macrophage colony-stimulating factor, CSF-1. *Methods Enzymol.* 116:564.
- Stanley, E. R. 1979. Colony-stimulating factor (CSF) radioimmunoassay: detection of a CSF subclass stimulating macrophage production. *Proc. Natl. Acad. Sci. USA* 76:2969.
- Cecchini, M. G., M. G. Dominguez, S. Mocchi, A. Wetterwald, R. Felix, H. Fleisch, O. Chisholm, W. Hofstetter, J. W. Pollard, and E. R. Stanley. 1994. Role of colony stimulating factor-1 in the establishment and regulation of tissue macrophages during postnatal development of the mouse. *Development* 120:1357.
- Das, S. K., E. R. Stanley, L. J. Guilbert, and L. W. Forman. 1980. Discrimination of a colony stimulating factor subclass by a specific receptor on a macrophage cell line. *J. Cell. Physiol.* 104:359.
- Price, L. K. H., H. U. Chol, L. Rosenberg, and E. R. Stanley. 1992. The predominant form of secreted colony stimulating factor-1 is a proteoglycan. *J. Biol. Chem.* 267:2190.
- Dinarello, C. A. 1991. Interleukin-1 and interleukin-1 antagonism. *Blood* 77:1627.
- Fibbe, W. E., and R. Willemze. 1991. The role of interleukin-1 in hematopoiesis. *Acta Haematol.* 86:148.
- Gentile, P., D. Byer, and L. M. Pelus. 1983. In vivo modulation of murine myelopoiesis following intravenous administration of prostaglandin E<sub>2</sub>. *Blood* 62:100.
- Pelus, L. M., and P. Gentile. 1988. In vivo modulation of myelopoiesis by prostaglandin E<sub>2</sub>. III. Induction of suppressor cells in marrow and spleen capable of mediating inhibition of CFU-GM proliferation. *Blood* 71:1633.
- Nikcevic, D. A., M. R. Young, N. K. Ellis, M. Newby, and H. T. Wepsic. 1986. Stimulation of hematopoiesis in untreated and cyclophosphamide treated mice by the inhibition of prostaglandin synthesis. *J. Immunopharmacol.* 8:299.
- Pelus, L. M. 1989. Blockade of prostaglandin biosynthesis in intact mice dramatically augments the expansion of committed myeloid progenitor cells (colony-forming units-granulocyte, macrophage) after acute administration of recombinant human IL-1 $\alpha$ . *J. Immunol.* 143:4171.
- O'Reilly, M., and R. L. Gamelli. 1990. Indomethacin augments granulocyte-macrophage colony-stimulating factor-induced hematopoiesis following 5-FU treatment. *Exp. Hematol.* 18:974.
- Takahashi, K., S. Umeda, L. D. Shultz, S. Hayashi, and S. Nishikawa. 1994. Effects of macrophage colony-stimulating factor (M-CSF) on the development, differentiation, and maturation of marginal metallophilic macrophages and marginal zone macrophages in the spleen of osteopetrosis (op) mutant mice lacking functional M-CSF activity. *J. Leukocyte Biol.* 55:581.
- De Kossodo, S., G. E. Grau, T. Daneva, P. Pointaire, L. Fossati, C. Ody, J. Zapf, P. F. Piguet, R. C. Gaillard, and P. Vassalli. 1992. Tumor necrosis factor  $\alpha$  is involved in mouse growth and lymphoid tissue development. *J. Exp. Med.* 176:1259.
- Lokesh, B. R., T. J. Sayers, and J. E. Kinsella. 1990. Interleukin-1 and tumor necrosis factor synthesis by mouse peritoneal macrophages is enhanced by dietary n-3 polyunsaturated fatty acids. *Immunol. Lett.* 23:281.
- Salkowski, C. A., R. Neta, T. A. Wynn, G. Strassmann, N. van Rooijen, and S. N. Vogel. 1995. Effect of liposome-mediated macrophage depletion on LPS-induced cytokine gene expression and radioprotection. *J. Immunol.* 155:3168.