Dietary Fish Oil Increases Tumor Necrosis Factor Secretion but Decreases Interleukin-10 Secretion by Murine Peritoneal Macrophages

(Manuscript received 15 July 2002. Initial review completed 10 August 2002. Revision accepted 16 September 2002.)

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ABSTRACT Dietary fish oil has immunomodulatory effects that are mediated in part by its effects on cytokines. Secretion of the inflammatory and the anti-inflammatory cytokines tumor necrosis factor (TNF) and interleukin (IL)-10 by murine resident peritoneal macrophages was monitored after ex vivo stimulation with lipopolysaccharide. Macrophages were obtained from mice fed a corn oil diet containing 200 g/kg corn oil or a fish oil diet containing 180 g/kg fish oil and 20 g/kg corn oil. Dietary fish oil increased secretion of the proinflammatory cytokine, TNF, but decreased secretion of the anti-inflammatory cytokine, IL-10. The cytokines appeared in the medium after 1.5 h and peaked at 6–12 h. Neutralizing antibodies against TNF and IL-10 had little effect on secretion of the other cytokine, indicating that the effects of fish oil on TNF and IL-10 secretion by these cells are independent of one another. Furthermore, although inhibiting prostaglandin production enhanced TNF secretion by macrophages from mice fed the corn oil diet, it did not affect IL-10 secretion by macrophages in this group. Blocking leukotriene B4 production also did not affect IL-10 secretion in macrophages from mice fed a nonpurified diet. These results demonstrate that fish oil has an overall pro-inflammatory effect given its effects on secretion of both inflammatory and anti-inflammatory cytokines by resident peritoneal macrophages.


KEY WORDS: fish oil • cytokines • tumor necrosis factor • interleukin 10 • mice

Studies in humans and experimental animals have shown that dietary fish oil has immunomodulatory properties. Altered eicosanoid and cytokine production has been implicated in the immunomodulatory effects of fish oil. Cytokines are key mediators of immune function and can be either proinflammatory or anti-inflammatory.

Tumor necrosis factor-α (TNF-α) is a proinflammatory cytokine that is important in the primary immune response against bacterial and viral infections. It is produced by activated monocytes, macrophages and lymphocytes (1) and leads to proliferation of activated T- and B-cells (2), up-regulation of major histocompatibility complex expression (3) and expression of adhesion molecules on endothelial cells (4).

Interleukin (IL)-10, on the other hand, is an anti-inflammatory cytokine. It is synthesized by monocytes, macrophages and activated T- and B-cells (5). It was initially identified as a product of T-cells involved in T helper (Th) 2 type immune response and inhibits T-cell proliferation, development and function (6). It has now been shown to inhibit secretion of both Th1 and Th2 type cytokines (6,7). IL-10 protects mice from death during septic peritonitis (8) and down-regulates lipopolysaccharide (LPS)-induced mRNA expression of pro-inflammatory cytokines from monocytes/macrophages (9). It is thought to be produced later in the inflammatory response than TNF (7,8).

A number of studies have demonstrated enhanced TNF secretion by LPS-stimulated murine resident peritoneal macrophages from mice fed fish oil (10–13). The one study that has examined the effect of dietary fish oil on IL-10 secretion by resident peritoneal macrophages showed no effect of fish oil on IL-10 secretion but it also did not show a significant effect of fish oil on TNF secretion (14).

Fish oil is currently believed to have anti-inflammatory properties and this effect is now well established in murine T-cells. The increased TNF secretion by peritoneal macrophages from mice fed fish oil indicates a proinflammatory effect of fish oil on these cells. The aim of this study was to determine whether dietary fish oil also has a proinflammatory effect due to decreasing the secretion of the anti-inflammatory cytokine, IL-10, by murine peritoneal macrophages and to investigate whether the effects of fish oil on TNF and IL-10 are interrelated or mediated by eicosanoids.

MATERIALS AND METHODS

Animals and diet. Experimental procedures using laboratory animals complied with the NIH guidelines. Female BalbC mice (18–20g) (Bomholtgaard Copenhagen, Denmark) were assigned randomly to one of two experimental diets. Mice were housed five per cage at 25°C with a 12-h light:dark cycle.

Experimental diets were designed according to AIN-93 guidelines (15) with modification in fat content. They were based on a nutritionally complete diet made for the addition of 200 g/kg of fat (ICN Pharmaceuticals, Aurora, OH) containing (per kg): 230 g casein, 354.6 g cornstarch, 57.4 g fiber, 3.4 g L-cystine, 40.2 g mineral mix (AIN-93G), 11.5 g vitamin mix (AIN-93), 100 g sucrose and 2.9 g

1 Supported by a grant from the Icelandic Research Council, The University of Iceland Research Fund, Adstodarmannsband (I.O.) and Nyskuparmannsband (I.O.)

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TABLE 1

Selected fatty acid composition of the dietary oils

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Corn oil diet1</th>
<th>Fish oil diet2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/100 g total fatty acids</td>
<td></td>
</tr>
<tr>
<td>16:0</td>
<td>11.05</td>
<td>20.06</td>
</tr>
<tr>
<td>18:0</td>
<td>2.25</td>
<td>4.00</td>
</tr>
<tr>
<td>18:1 (n-9)</td>
<td>27.77</td>
<td>12.48</td>
</tr>
<tr>
<td>18:2 (n-6)</td>
<td>57.26</td>
<td>8.25</td>
</tr>
<tr>
<td>18:3 (n-3)</td>
<td>1.63</td>
<td></td>
</tr>
<tr>
<td>18:4 (n-3)</td>
<td>—</td>
<td>3.05</td>
</tr>
<tr>
<td>20:5 (n-3)</td>
<td>—</td>
<td>10.14</td>
</tr>
<tr>
<td>22:5 (n-3)</td>
<td>—</td>
<td>2.19</td>
</tr>
<tr>
<td>22:6 (n-3)</td>
<td>—</td>
<td>12.47</td>
</tr>
<tr>
<td>(n-3)/(n-6)3</td>
<td>0.02</td>
<td>3.51</td>
</tr>
</tbody>
</table>

1 Corn oil diet contained 200 g/kg corn oil.
2 Fish oil diet contained 180 g/kg menhaden fish oil and 20 g/kg corn oil.

TABLE 2

Selected fatty acid composition of liver phospholipids from mice fed fish oil or corn oil diets

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Corn oil</th>
<th>Fish oil</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mol/100 mol</td>
<td>mol/100 mol</td>
</tr>
<tr>
<td>16:0</td>
<td>21.62 ± 0.26a</td>
<td>26.21 ± 0.41b</td>
</tr>
<tr>
<td>18:0</td>
<td>20.14 ± 0.35b</td>
<td>16.79 ± 0.42a</td>
</tr>
<tr>
<td>18:1 (n-9)</td>
<td>5.43 ± 0.15</td>
<td>5.21 ± 0.23</td>
</tr>
<tr>
<td>18:2 (n-6)</td>
<td>14.86 ± 0.42b</td>
<td>7.26 ± 0.39a</td>
</tr>
<tr>
<td>18:4 (n-3)</td>
<td>25.79 ± 0.36b</td>
<td>7.86 ± 0.18a</td>
</tr>
<tr>
<td>20:5 (n-3)</td>
<td>0.03 ± 0.03a</td>
<td>8.14 ± 0.33b</td>
</tr>
<tr>
<td>22:5 (n-3)</td>
<td>0.3 ± 0.13b</td>
<td>0.93 ± 0.13b</td>
</tr>
<tr>
<td>22:6 (n-3)</td>
<td>8.17 ± 0.39a</td>
<td>23.51 ± 0.45b</td>
</tr>
<tr>
<td>(n-3)/(n-6)3</td>
<td>0.3 ± 0.02a</td>
<td>4.18 ± 0.17b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEM, n = 12. Means in a row with different letters differ, P < 0.05.
2 (n-3)/(n-6): 20.5; 22.5; 22.6/20.3; 20.4; 22.4.

MK886 (Affinity Research Products, Exeter, UK), a 5-lipoxygenase activating protein (FLAP) inhibitor. A concentration of 20 nmol/L has been shown to be effective in inhibiting leukotriene (LT)B4 synthesis by rat neutrophils after stimulation with the Ca2+ ionophore, A23187 (20).

Cytokine measurements. After incubation, the culture plates were centrifuged and cell culture supernatants collected and stored at −70°C. TNF-α and IL-10 were measured in the supernatants using Duo Set ELISA kits (R&D Systems).

Statistical analysis. Differences between dietary groups were analyzed using an unpaired t test. Differences between treatments within dietary groups were analyzed using two-way ANOVA, followed by Bonferroni’s post-hoc test. Differences were determined to be significant when P < 0.01.

RESULTS

Mouse growth and cell count. There were no differences in the body weights or relative weight gains of mice fed the different diets. Cell numbers from the peritoneum did not differ between groups.

Liver phospholipid fatty acid composition. Hepatic phospholipids from mice consuming the fish oil diet had a significantly higher (n-3) polyunsaturated fatty acid (PUFA) content and significantly lower (n-6) PUFA content, than hepatic phospholipids from mice fed the corn oil diet (Table 2). These differences in fatty acid composition of hepatic phospholipids are similar to what has been shown previously (19) and demonstrate the effectiveness of the diets in changing tissue fatty acid composition.

LPS-induced TNF and IL-10 secretion. When stimulated with LPS (1 mg/L), macrophages from mice fed the fish oil diet secreted twice the amount of TNF (178 ± 25 ng/L) as macrophages from mice fed the corn oil diet (86 ± 10 ng/L), confirming previous results by others (10–13). However, macrophages from mice fed the fish oil diet secreted only one third of the IL-10 (93 ± 10 ng/L) that macrophages from mice fed the corn oil diet (262 ± 30 ng/L) secreted.

Time courses for TNF and IL-10 secretion. Because IL-10 has been shown to suppress TNF secretion by LPS-stimulated human monocytes (7), the increase in IL-10 secretion by macrophages from mice fed the fish oil diet could be responsible for the increase in TNF secretion. We therefore determined the time courses for LPS-induced TNF and IL-10 secretion by macrophages from mice fed corn oil and fish oil diets (Fig. 1). TNF was significantly increased in the medium...
peritoneal macrophages are independent of the effect on the other cytokine.

Effect of blocking PG production on TNF and IL-10 secretion by macrophages. PGE$_2$ decreases TNF secretion by elicited peritoneal macrophages (23) but enhances IL-10 secretion (24). Dietary fish oil decreases PGE$_2$ production by murine peritoneal macrophages (10) and the decreased PGE$_2$ production is responsible in part for the effect of fish oil on TNF secretion (19). We therefore determined whether decreased IL-10 secretion by macrophages from mice fed the fish oil diet could also be mediated by the decrease in PGE$_2$ production. As expected, blocking PG production with IM increased LPS-induced TNF secretion by macrophages from mice fed the corn oil diet, but did not affect TNF secretion by macrophages from mice fed the fish oil diet (Fig. 2A), eliminating the difference in TNF secretion by macrophages from mice fed the two experimental diets. In contrast, IM had no effect on IL-10 secretion by macrophages from mice fed the corn oil and slightly decreased IL-10 secretion by macrophages from mice fed the fish oil diet (Fig. 2B); thus, the difference in IL-10 secretion by macrophages from mice fed the different diets remained after inhibition of PG production. Thus, decreased IL-10 secretion by macrophages from mice fed the fish oil diet is not likely to be mediated by decreased PGE$_2$ production.

Effect of blocking LT production on IL-10 secretion. LTB$_4$ enhances IL-10 secretion by spleen cells after concanavalin A stimulation (25). Resident peritoneal macrophages do not produce much LTB$_4$ after LPS stimulation but they do produce some (26). We therefore investigated whether blocking LTB$_4$ production affected IL-10 secretion by the resident peritoneal macrophages. Blocking 5-lipoxygenase (LO) with the FLAP inhibitor, MK886, had little effect on IL-10 secretion (172 ± 41 ng/L without MK886, 152 ± 21 ng/L with MK886) compared with 24 h in the previous study (22).

FIGURE 2 The effects of neutralizing antibody against interleukin (IL)-10 and tumor necrosis factor (TNF) and of indomethacin (IM) on lipopolysaccharide (LPS)-induced TNF (A) and IL-10 (B) secretion by resident peritoneal macrophages from mice fed fish oil (FO) or corn oil (CO) diets. Values are means ± SEM, n = 10. Bars with different letters differ, P < 0.01.
with 10 nmol/L and 146 ± 12 with 20 nmol/L) by macrophages from mice fed a nonpurified diet, rendering it unlikely that the effect of fish oil on IL-10 secretion is mediated by its effect on LTB production. Using a very high concentration (10 μmol/L) of MK886 reduced IL-10 secretion to 117 ± 13 ng/L, but at this concentration it may be affecting cellular mechanisms in addition to the 5-LO (27).

DISCUSSION

These results demonstrate that fish oil feeding increases TNF secretion but decreases IL-10 secretion by murine resident peritoneal macrophages, resulting in a net proinflammatory effect of fish oil on these cells.

One other study has investigated the effect of dietary fish oil on IL-10 secretion by resident peritoneal macrophages and showed no effect compared with safflower oil (14). Neither was there a significant effect of dietary fish oil on TNF secretion (14), although results from the present study and other similar studies show an increase in TNF production by macrophages from mice fed a fish oil diet (10–13). The conditions used in the study by Wallace et al. (14) are similar to the conditions used in the present study except for the strain of mice (C57BL/6) and the use of fetal calf serum (FCS) in the cell cultures. The BalbC mouse strain is thought to be more Th2 prone than the C57BL/6 mouse strain (28). However, studies using both C57BL/6 (11) and BalbC mice (10) showed increased TNF production by macrophages from mice fed fish oil. Similarly, although the effects of dietary lipid manipulation on fatty acid composition are reversed when lymphocytes are cultured in FCS (29), increased TNF production by resident peritoneal macrophages from mice fed a fish oil diet has been shown in studies using FCS (13). It is therefore not likely that the strain of mice or the use of FCS in the study by Wallace et al. (14) is responsible for the lack of effect of fish oil on IL-10 secretion.

The results from the present study show similar time courses for TNF and IL-10 secretion after LPS stimulation and are consistent with other studies (21,22). The effect of fish oil on IL-10 secretion was evident at an earlier time point than its effect on TNF secretion. However, the effects of fish oil on IL-10 and TNF secretion seem to be independent of the effect on the other, as demonstrated using neutralizing antibodies against each cytokine. Furthermore, the effect of fish oil on IL-10 secretion is probably mediated by some mechanism other than decreasing PG or LT production, although the effect of fish oil on TNF secretion is mediated in part by decreased PG production.

Regulation of LPS-induced IL-10 production by macrophages is not fully understood but is currently under rigorous investigation. The effect of fish oil on IL-10 secretion could be mediated through the different signaling molecules or pathways that are involved in regulation of IL-10 production. Further studies are required to elucidate the mechanism by which dietary fish oil affects secretion of IL-10 by murine resident peritoneal macrophages.

LITERATURE CITED