Effects of Dietary n-3 Fatty Acids on T Cell Activation and T Cell Receptor-Mediated Signaling in a Murine Model

David N. McMurray,1,2 Christopher A. Jolly,2,a and Robert S. Chapkin2  

A short-term feeding paradigm in mice, with diets enriched with eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), was used to study the modulation of T cell activation via the T cell receptor (TcR) and the downstream pathways of intracellular signaling. Diets enriched in EPA and DHA suppressed antigen-specific delayed hypersensitivity reactions and mitogen-induced proliferation of T cells. Cocultures of accessory cells and T cells from mice given different diets revealed that purified fatty acid ethyl esters acted directly on the T cell, rather than through the accessory cell. The loss of proliferative capacity was accompanied by reductions in interleukin (IL)-2 secretion and IL-2 receptor α chain mRNA transcription, suggesting that dietary EPA and DHA act, in part, by interrupting the autocrine IL-2 activation pathway. Dietary EPA and DHA blunted the production of intracellular second messengers, including diacylglycerol and ceramide, following mitogen stimulation in vitro. Dietary effects appear to vary with the agonist employed (i.e., anti-CD3 [TcR], anti-CD28, exogenous IL-2, or phorbol myristate acetate and ionomycin).

Several placebo-controlled studies involving dietary fish oil supplementation of patients with rheumatoid arthritis established conclusively that constituents of fish oil, when consumed at different levels and for various time periods, result in clinical improvement and a reduction in the dependence on traditional nonsteroidal anti-inflammatory drug therapy [1–6]. Table 1 summarizes the essential features of these successful supplementation regimens. These studies suggest that constituents in fish oil are anti-inflammatory, and the most likely candidates for this beneficial effect are the two n-3 fatty acids found in highest abundance in fish oil, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). The supplemental regimens used in the studies in table 1 are shown as the calculated intakes of EPA and DHA in grams per day based upon the total fish oil consumption and the concentrations of these two fatty acids in the fish oil supplements used [1–6].

The prevailing dogma is that a major mechanism by which dietary n-3 fatty acids exert their anti-inflammatory effects is by down-modulation of T lymphocyte activation. Table 2 summarizes several studies in humans, rats, mice, and primates, in which dietary supplementation with fish oil was examined for its effects on various T cell functions. Two clinical studies reported significant reductions in mitogen-induced T cell proliferation and interleukin (IL)-2 production in human peripheral blood cells [7, 8]. A third clinical study showed similar T cell suppressive effects of a diet supplemented with fish, instead of purified fish oil. The subjects had reduced delayed hypersensitivity reactions in the skin and impaired lymphoproliferative capacity in vitro [9]. Mitogen-induced lymphoproliferation was also suppressed in the cells of rats [10] and mice [11] given diets enriched with 20% or 10% fish oil by weight, respectively. Of interest, in a similar study in nonhuman primates fed fish oil containing EPA and DHA equivalent to about 3.3% of total calories for 14 weeks, the animals showed enhancement of proliferation and IL-2 production [12].

If dietary supplementation with fish oil or its purified constituents is to become an effective and reproducible means of altering T cell–mediated inflammatory reactions, it is essential that the precise mechanisms by which the anti-inflammatory effects of n-3 fatty acids are mediated be understood completely. Such an understanding will allow the identification of the most biologic active components of fish oil, elucidate the impact of dose and timing on therapeutic outcome, and delineate the specific immune cells, cytokines, and the functions affected, thus resulting in a more focused approach to supplementation. In addition, knowledge of the mechanisms of T cell suppression by dietary n-3 fatty acids will reveal the potential detrimental effects that might result from loss of antimicrobial resistance due to the diet-induced loss of protective components of the T cell–mediated inflammatory response.

There are several hypothetical mechanisms by which dietary
n-3 fatty acids might alter T cell functions, and some of the most likely are listed in table 3. The role of essential accessory cells (e.g., dendritic cells, macrophages) in the process of T cell activation might be altered by dietary n-3 fatty acids, resulting in reduced antigen processing and presentation, altered costimulation, or changes in the production of soluble mediators such as IL-1 or prostaglandin E2 [13, 14]. Alternatively, diets might alter the balance of regulatory and effector subsets of T cells (e.g., CD4 vs. CD8, Th1 vs. Th2, naive vs. memory) in the host, resulting in loss of some functions and gain of others [15]. Since dietary lipids are incorporated quickly and easily into lymphocyte membrane lipids, another likely mechanism would be the disruption of normal membrane architecture and function. In this latter regard, it is useful to point out very recent conceptual advances in the fundamental biology of T cell activation, such as the concepts of the “immunologic synapse” [16], functional “rafts” of receptors and transmembrane signaling molecules moving in the lipid bilayer [17], and membrane “scaffolds” by which effective signal transduction occurs [18]. Surely, changes in membrane lipid composition might affect significantly all of these events at the T cell surface.

Once signals are transduced, n-3 fatty acids could exert their immunomodulatory effects intracellularly by altering the levels of important phospholipid-derived second messengers, such as diacylglycerol (DAG) and ceramide [19, 20]. Disruption of normal signaling within the T cell could result in failure to produce the transcriptional activators (e.g., nuclear factor [NF]-κB, NF-AT [activated T cells]), which are required for the production of IL-2 and other important cytokines [19]. Finally, even if all of the events occurred normally in the T cells of n-3 fatty acid–supplemented persons, there could be effects at the posttranslational level (e.g., failure to secret active IL-2 or failure of the IL-2 to bind effectively to its receptor [IL-2R]) [21]. These issues were recently reviewed in some detail [22].

For several years, we have used a short-term feeding paradigm in the mouse to attempt to elucidate the mechanisms by which diets enriched in EPA and DHA exert their effects on T cell activation. These diets contain either 3% safflower oil ethyl esters (control) or 1% safflower oil plus either 2% arachidonic acid, 2% EPA, or 2% DHA ethyl esters fed for 14 days [23, 24]. In some experiments, mice are immunized with the human tuberculosis vaccine, bacille Calmette-Guérin (BCG) several weeks before being placed on the experimental diets. Total splenocytes, adherent cells only, or column-purified T cells are harvested and cultured separately or together. In some experiments, the relative proportions of CD4 and CD8 T cells in the spleen are determined by flow cytometry. We use various agonists individually or in combination to stimulate T cell activation, including polyclonal T cell mitogens (e.g., concanavalin A [ConA]), anti-CD3, anti-CD28, phorbol myristate acetate (PMA), and ionomycin. The readouts are lymphoproliferation, bioactive IL-2 production in culture supernatant fluids, quantitation of IL-2 and IL-2Rα mRNA, or intracellular DAG and ceramide levels.

With this model, we have shown that mice fed diets enriched with EPA or DHA for only 14 days undergo significant remodelling of their cell membranes in a manner consistent with the fatty acid composition of their diets [24]. Mice vaccinated with viable BCG and then fed either EPA or DHA are suppressed in their ability to mount a delayed hypersensitivity reaction in the footpad after injection of purified protein derivative [23]. When freshly isolated splenocytes were placed immediately in culture with a T cell mitogen, ConA, cells from EPA- and DHA-fed mice proliferated significantly less than cells from control diet animals [25]. These mitogen-activated T cells secreted less detectable IL-2 protein and expressed significantly less mRNA for the α chain of the IL-2 receptor (IL-2Rα) [25, 26]. In contrast, the level of IL-2 mRNA expression in the splenocytes of EPA- and DHA-fed mice was not affected, suggesting that the apparent inability of these cells to secrete mature IL-2 protein was not due to a defect in IL-2 gene transcription [26]. This somewhat paradoxical result may point to an effect of diet on posttranslational modifications of IL-2, which are required for secretion, or on the ability of these T cells from EPA- and DHA-fed mice to actively export IL-2 protein from the cytoplasm [21].

One issue that was important to resolve before proceeding to generate hypothetical mechanisms to explain these effects of dietary EPA and DHA on T cell activation and the IL-2 autocrine stimulation pathway was the question of whether the dietary effect was exerted principally on the T cells themselves, on accessory cells, or on both. To shed light on this issue, a series of experiments was conducted in which cocultures of purified accessory cells and purified T cells, each population derived from mice maintained on different diets, were stimulated with ConA. These studies revealed that the effect of dietary EPA and DHA was observed only when the T cells came from those treatment groups and not when the accessory cells came from those groups [23]. Although one might question the potential effect of a very small population of contaminating cells of the other type in these cocultures, even a conservative analysis of these results strongly suggests that EPA and DHA mainly exert their effects on the T cells themselves in our model system. For that reason, we have focused on events in the T lymphocyte.

It has been suggested that dietary n-3 fatty acids alter the relative proportions of T cell subsets and that this might explain...
We also observed a decrease in phospholipase C (PLC) early spike of DAG and ceramide did not occur in ConA-containing safflower oil or arachidonic acid. In contrast, this within the first 2 min in activated T cells from mice fed diets enriched in EPA or DHA. As expected, a sharp increase in intracellular DAG and ceramide was seen from mice fed diets for the first 3 h of activation in vitro in ConA-stimulated splenocytes. We examined the levels of intracellular DAG and ceramide at frequent intervals during the first 3 h of activation in vitro in ConA-stimulated splenocytes from mice fed diets enriched in EPA or DHA. As expected, a sharp increase in intracellular DAG and ceramide was seen within the first 2 min in activated T cells from mice fed diets containing safflower oil or arachidonic acid. In contrast, this early spike of DAG and ceramide did not occur in ConA-activated T cells from mice fed diets enriched with EPA or DHA. We also observed a decrease in phospholipase C γ (PLCγ) in lymphocytes from EPA- and DHA-fed mice, suggesting that the diet effect may actually occur upstream of DAG and ceramide in the intracellular signaling cascade and involve membrane-proximal events as well.

In light of our observation that early ceramide production was blunted by dietary EPA and DHA and the implication of ceramide in the pathway leading to cell death by apoptosis, we examined the effect of exogenous ceramide on proliferation in our system. We observed that the addition of ceramide to ConA-stimulated murine splenocytes at a concentration of 10 μM resulted in significant enhancement of proliferation [28]. Thus, the biologic significance of diet-induced changes in ceramide levels remains to be determined. However, activation-induced apoptosis is not an unreasonable hypothetical mechanism to explain loss of proliferative capacity in T cells from mice fed EPA or DHA [30]. In preliminary studies to address this mechanism, we observed a significant increase in the level of apoptosis in ConA-activated T cells from EPA- and DHA-fed mice and a statistically significant inverse correlation between the degree of apoptosis and proliferative capacity in cells from diet-fed mice (data not shown). This line of investigation will be pursued in our model.

All of the results discussed were derived from T cells activated by a single pathway, polyclonal activation by a mitogen (ConA). On the basis of current knowledge of the pathways for transmembrane and intracellular signaling in the T cell following a variety of receptor-mediated events [22, 31], it is likely that dietary EPA and DHA would affect each of these pathways differently. For that reason, we have begun to examine different agonists for their ability to reveal unique features of the influence of dietary n-3 ethyl esters on T cell function. We can stimulate directly the TcR by using antibodies to CD3; the essential costimulatory molecule, CD28, with anti-CD28 antibodies; the IL-2R by using exogenous recombinant IL-2; or bypass the surface receptors by activating the T cells with PMA and ionomycin. We have developed T cell activation protocols involving these agonists, alone or in combination, and have begun to examine the differential effect of dietary EPA and DHA on activation of T cells by these discrete pathways. In preliminary experiments, T cell activation, as measured by proliferative capacity, in splenic T cells from fish oil–fed mice was impaired to different degrees following stimulation in vitro with ConA, anti-CD3/anti-CD28, or PMA/anti-CD3 (data not shown). We will pursue this approach to elucidate the specific steps in these different T cell activation pathways that are affected by dietary n-3 fatty acids.

The other major issue that we are beginning to address is the question of which T cell subpopulation(s) is most affected

<table>
<thead>
<tr>
<th>Dietary intake/duration</th>
<th>Species</th>
<th>Outcome</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 g/day fish oil; 6 weeks</td>
<td>Human</td>
<td>(–) Proliferation (–) IL-2</td>
<td>[7]</td>
</tr>
<tr>
<td>3 g/day fish oil;12 weeks</td>
<td>Human</td>
<td>(–) Proliferation (–) IL-2</td>
<td>[8]</td>
</tr>
<tr>
<td>180 g/day fish; 24 weeks</td>
<td>Human</td>
<td>(–) Proliferation, (–) delayed type hypersensitivity</td>
<td>[9]</td>
</tr>
<tr>
<td>20% fish oil; 8 weeks</td>
<td>Rat</td>
<td>(–) Proliferation</td>
<td>[10]</td>
</tr>
<tr>
<td>10% fish oil; 8 weeks</td>
<td>Mouse</td>
<td>(–) Proliferation</td>
<td>[11]</td>
</tr>
<tr>
<td>3.3% energy EPA/DHA; 14 weeks</td>
<td>Primate</td>
<td>(++) Proliferation (+) IL-2</td>
<td>[12]</td>
</tr>
</tbody>
</table>

NOTE: EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; IL, interleukin.

Table 3. Hypothetical mechanisms of altered T cell function by n-3 fatty acids.

1. Accessory cell functions are altered (antigen presentation, costimulation, monokine or prostaglandin E2 production)
2. Imbalance between T cell subsets (e.g., CD4 vs. CD8; Th1 vs. Th2; naïve vs. memory)
3. Changes in T cell membrane structure (concepts of immunologic synapse, functional rafts, membrane scaffolds)
4. Modification of intracellular signaling (e.g., second messengers, transcriptional activators)
5. Posttranslational events
by dietary n-3 fatty acids. Although we have conducted some of the experiments described above in column-purified T cells, we have just begun similar studies in purified CD4 and CD8 T cell subsets using magnetic bead and affinity column separation techniques. Obviously, the functional consequences of suppressed T cell activation by dietary n-3 ethyl esters will differ significantly depending upon the principal cell type affected.

Table 4 summarizes the major observations that we have made on the effects of short-term feeding of EPA- and DHA-enriched diets on T cell activation in a murine model system. Taken together, our results indicate that EPA and DHA suppress the activation of T cells by mechanisms that include disruption of the IL-2 autocrine stimulation pathway. The effect of dietary n-3 fatty acids appears to involve alterations in intracellular signaling by important second messengers (e.g., PLC, DAG, ceramide) and may depend upon the precise surface receptors involved in the activation process.

References