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Immunomodulatory Effects of (n-3) Fatty Acids: Putative Link to Inflammation and Colon Cancer[1–3]

Robert S. Chapkin,[4,5]* Laurie A. Davidson,[4,5] Lan Ly,[4,7] Brad R. Weeks,[6] Joanne R. Lupton,[4,5] and David N. McMurray[4,5,7]

[4]Faculty of Nutrition, [5]Center for Environmental and Rural Health, and [6]Department of Veterinary Pathobiology, Texas A&M University, College Station, TX 77843 and [7]Department of Microbial and Molecular Pathogenesis, Texas A&M University System Health Science Center, College Station, TX 77843

Abstract

Chronic inflammation and colorectal cancer are closely linked. Although the overall mechanisms of inflammation-associated gastrointestinal carcinogenesis are complex, it is clear that antiinflammatory therapy is efficacious against neoplastic progression and malignant conversion. From a dietary perspective, fish oil containing (n-3) polyunsaturated fatty acids (PUFAs) has antiinflammatory properties, but for years the mechanism has remained obscure. Of relevance to the immune system in the intestine, we showed that (n-3) PUFA feeding alters the balance between $CD4^+$ T-helper (Th1 and Th2) subsets by directly suppressing Th1 cell development (i.e., clonal expansion). This is noteworthy because Th1 cells mediate inflammatory diseases and resistance to intracellular pathogens or allergic hypersensitivity, and Th2 cells mediate resistance to extracellular pathogens. Therefore, any changes induced by (n-3) PUFAs in T-cell subset balance and function are important because the outcome is expected to suppress the development of autoimmune diseases and possibly the occurrence of colon cancer. Precisely how the immunomodulatory effects of (n-3) PUFAs influence inflammation-associated colonic tumor development is the subject of an ongoing investigation. J. Nutr. 137: 200S–204S, 2007.

Human inflammatory bowel diseases (IBDs)[8] are chronic, relapsing inflammatory conditions of unknown etiology. Both genetic and environmental factors have been implicated (1). These diseases are clinically characterized by 2 overlapping phenotypes: ulcerative colitis and Crohn disease. Ulcerative colitis primarily involves the colon with inflammation restricted to the mucosa. The inflammation in Crohn disease often involves the small intestine along with the colon and other organs (2). Crohn disease affects >500,000 individuals in the United States and represents the second most common chronic inflammatory disorder after rheumatoid arthritis. In addition, patients with IBD are at a greatly increased risk of developing colon cancer (3). It is well accepted that inflammation facilitates the initiation and progression of normal cells to malignancy through production of proinflammatory cytokines (IL-1, tumor necrosis factor, and IL-6) and an array of reactive oxygen and nitrogen species (4,5). These mediators activate nuclear transcription factor-κB, inducible nitric oxide synthase, and cyclooxygenase-2–related signaling pathways, which generally delay or suppress apoptosis in intestinal epithelial cells and modulate angiogenesis and drug-metabolizing enzymes, especially phase II enzyme induction (5–7).

In both ulcerative colitis and Crohn disease, cytokine imbalance is believed to contribute to initiation and perpetuation. Mature $CD4^+$ effector T cells are divided into T-helper (Th)1 and Th2 subsets by the array of cytokines they produce (8). Th1 cytokines predominate in Crohn disease, whereas Th2 cytokines tend to predominate in ulcerative colitis (2). Specific Th1 cytokines include IL-2 and interferon-γ (IFN-γ) and are considered proinflammatory and important in cell-mediated immunity against intracellular microorganisms (9). Th2 cells produce IL-4, IL-5, and IL-10. Increasing evidence indicates that T-cell resistance against apoptosis is a major contributory factor to

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[8] Abbreviations used: DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; IFN-γ, interferon gamma; IBD, inflammatory bowel disease; IL, interleukin; NSAID, nonsteroidal antiinflammatory drug; PPAR, peroxisome proliferator-activated receptor; PUFA, polyunsaturated fatty acid; Th, T-helper cell.

* To whom correspondence should be addressed. E-mail: r-chapkin@tamu.edu.

the inappropriate T-cell accumulation and perpetuation of chronic mucosal inflammation in IBD (1,10). Therefore, our research efforts have focused on identifying bioactive dietary agents capable of enhancing apoptosis, suppressing the clonal expansion of Th1 cells, or both. These, in turn, may ameliorate intestinal inflammation and colon tumor development.

Immunosuppressive effects of (n-3) PUFAs

From a dietary perspective, data are insufficient to draw conclusions regarding the ability of (n-3) PUFAs to suppress IBD (11,12) and colon cancer risk in humans (13–15). Interestingly, aspects of immune function that have an intimate involvement of the cell membrane, such as oxidative burst and proliferation, appear to be more strongly influenced by fatty acid composition (16). In addition, the complex effects of (n-3) PUFAs on cytokine biology can be explained in part by polymorphisms and genotypes of the responsive subjects (17–19). Collectively, these studies emphasize the need to elucidate the precise genetic and epigenetic determinants that influence the effects of foods enriched with (n-3) PUFAs on immune function.

Regarding the immunomodulatory properties of (n-3) PUFAs, we demonstrated that a short-term feeding paradigm in mice with diets enriched with fish oil containing eicosapentaenoic acid (EPA, 20:5Δ5,8,11,14,17) and docosahexaenoic acid (DHA, 22:6Δ4,7,10,13,16,19) or DHA (97% pure) ethyl ester results in suppressed antigen-specific delayed hypersensitivity reactions and mitogen-induced proliferation of T cells (20). The loss of proliferative activity was accompanied by reduction in IL-2 secretion and IL-2 receptor α-chain mRNA transcription, suggesting that dietary DHA acts in part by interrupting the autocrine IL-2 activation pathway (21). In addition, dietary DHA blunted the production of intracellular second messengers, including diacylglycerol and ceramide, after mitogen stimulation ex vivo (21–23). These data conclusively demonstrate that dietary (n-3) PUFAs modulate components of intracellular signaling pathways regulating T-cell activation.

In theory, these alterations in T-cell function could reflect the direct effects of dietary (n-3) PUFAs in impairing the ability of the target T-cell population to respond to activating stimuli; the indirect effects of (n-3) PUFAs on the activity of other cells (accessory cells or other T-cell populations) to suppress the response of the target cells; or a combination of these 2 distinct mechanisms. Most previous studies addressing these issues were carried out in unseparated populations of T cells and accessory cells stimulated with mitogenic agents that act indiscriminately on generic T-cell surface ligands. We demonstrated that (n-3) PUFAs affect T-cell receptor–mediated activation by both direct and indirect (accessory cell) mechanisms (24). Other studies affirmed these observations (25,26).

Dietary (n-3) PUFA suppresses antigen-driven proliferation in Th1-polarized cells from DO11.10 mice

Data from our laboratory demonstrate that dietary (n-3) PUFAs present in fish oil affect both mitogen- and antigen-induced $CD4^+$ T-cell proliferation and apoptosis (24,27–29). To further elucidate the effect of (n-3) PUFAs on Th1 polarization, DO11.10 mice were fed a control diet [5% corn oil, no (n-3) PUFAs] or a fish oil diet (4% fish oil + 1% corn oil) for 2 wk. For Th1 polarization, naïve splenic and lymph node–derived $CD4^+$ naïve T cells were cultured in the presence of anti-IL-4 antibody, IL-2, IL-12, ovalbumin peptide, and homologous mouse serum for 5 d. Diet did not affect percentage of Th1-positive (IFN-γ^+, IL-4^-) cells harvested after 5 d in culture (corn oil = 98.8 ± 0.7%, fish oil = 97.4 ± 0.8%). In contrast, the Th1 effector cell proliferative response to antigen was decreased by ~50% in the fish oil group (30). These data suggest that the antiinflammatory effects of fish oil may be explained in part by a shift in the effector cell balance as a result of the direct suppression of Th1 clonal expansion in vitro. These results together with in vivo evidence of direct suppression of Th1 development after polarization (30–32) support our hypothesis that DHA may ameliorate Th1-mediated inflammation and colon tumorigenesis.

(n-3) PUFA effects in IL-10 null mice: a model for Th1-mediated inflammatory bowel disease

Based on observations that dietary (n-3) PUFAs suppress Th1 proliferation and enhance activation-induced cell death (29,30,32,33), we hypothesized that (n-3) PUFAs play a role in protecting the colonic epithelium against Th1-mediated inflammation. Therefore, in a preliminary study we tested the antiinflammatory potential of fish oil in a well-established Th1-mediated experimental model for IBD, the IL-10 knockout mouse (34). These animals spontaneously develop IBD and are considered an excellent model for identifying the complex Th1-dependent immunologic mechanisms involved in initiating and perpetuating different types of human IBD (35,36). To initially test our hypothesis, 6- to 10-wk-old IL-$10^{-/-}$ mice on a 129SvEv background were placed on complete balanced diets containing either 5% corn oil by weight [control diet, contains no (n-3) PUFAs] or 4% fish oil (contains DHA) + 1% corn oil (32). A group of mice were exposed to piroxicam for 1 wk before diet treatment, which resulted in the development of severe, chronic IBD (37). Animals were killed after 3, 5, and 10 wk on the diets with and without prior nonsteroidal antiinflammatory drug (NSAID) treatment. Entire colons (Swiss rolls) and intestinal lymphocytes from Peyer's patches were subsequently isolated. Peyer's patch cultures were incubated with αCD3/αCD28 for 72 h. Mice fed (n-3) PUFAs had significantly reduced ($P < 0.05$) levels of proliferating T cells before disease development (38) (**Fig. 1**). After NSAID exposure, the fish oil diet (3 wk) was associated with a trend toward reduced T-cell proliferation (Fig. 1). To further evaluate the effect of diet on colonic inflammation, we examined the pathology of spontaneous and NSAID-induced colitis (**Figs. 2 and 3**). Dietary (n-3) PUFAs reduced the clinical score (severity and character of the colitis, as indicated by a reduction in crypt length and the transmural infiltrate of mononuclear cells). In addition, the production of tumor necrosis factor-α by splenic $CD4^+$ T cells was suppressed. Tumor necrosis factor-α appears to play a pivotal role in the pathogenesis of chronic IBD (39), and this result is consistent with previous reports documenting the prophylactic effect of (n-3) PUFAs on colitis (40–42).

Mechanisms

Dietary (n-3) PUFAs displace signaling proteins from membrane rafts by altering raft lipid composition. To elucidate the molecular mechanisms by which (n-3) PUFAs inhibit T-cell activation, we recently demonstrated that dietary EPA and DHA alter the phospholipid and signaling protein composition of lipid rafts (28,43). These specialized microdomains within the plasma membrane are important in the maintenance and amplification of T-cell receptor signaling pathways in part by acting as platforms to compartmentalize and facilitate protein-protein interactions (44–46). Because fundamental differences in the organization of signaling complexes in nonpolarized and polarized T cells exist (47), we hypothesized that dietary EPA and DHA selectively modulate membrane microdomains containing the T-cell receptor signaling complex in Th1

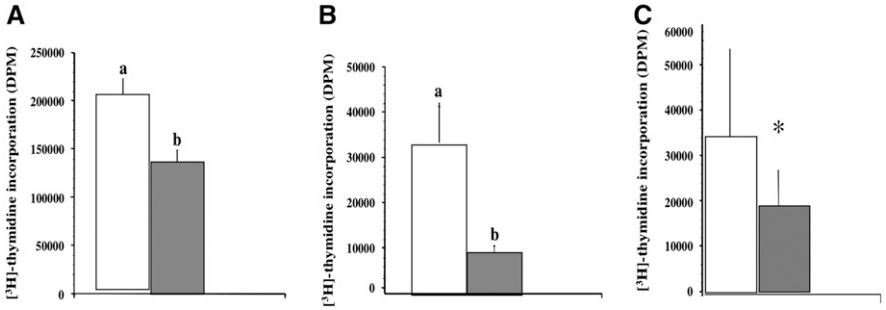

Figure 1 Dietary (n-3) PUFAs reduced gut lymphocyte proliferation. IL-10$^{-/-}$ lymphocytes from Peyer's patches were stimulated with αCD3/αCD28 for 72 h and pulsed with ^{3}H-thymidine: (*A*) spontaneous, 5 wk on diet; (*B*) spontaneous, 10 wk on diet; (*C*) piroxicam treated, 3 wk on diet. Data represent mean ± SEM thymidine uptake (dpm) from 5–7 mice per diet (*open bars* = corn oil, (n-6) PUFA diet; *solid bars* = fish oil, (n-3) PUFA diet) per time. Different letters denote significant differences between diet groups ($P < 0.01$). *Denotes $P = 0.12$.

polarized cells. This would alter the T-cell receptor macromolecular complex signaling, creating an environment for suppression of Th1 cell activation. Additionally, it is also possible that long-chain (n-3) PUFAs perturb signaling protein coposttranslational lipidation (i.e., palmitoylation, myristoylation, and/or prenylation), which subsequently may alter protein targeting to lipid rafts and therefore influence protein function (48).

Cyclooxygenase, lipid oxidation, and T-cell nuclear receptor activity are unlikely targets of (n-3) PUFAs. It is now generally accepted that the inhibitory effects of (n-3) PUFAs on T-cell polarization and proliferation are not mediated by eicosanoids or lipid peroxidation (26,49). Antioxidant cosupplementation in humans was shown not to alter the suppressive effect of fish oil feeding on T-cell proliferation (50). Consistent with this observation, we showed that there is no change in systemic oxidative stress after the ingestion of experimental diets containing (n-3) PUFAs in a murine model system (27). This is not surprising because the experimental diets used by our laboratory typically contain vitamin E at 120 IU/kg to help protect against peroxidation. This exceeds the estimated minimal vitamin E requirement of 75 IU/kg diet. With respect to cell culture conditions that can affect murine CD4$^+$ cell responses, Pompos and Fritsche (51) demonstrated that alterations in lipid peroxidation are not important with respect to (n-3) PUFA-mediated suppression of T-cell proliferation. Collectively, these data suggest that the immunosuppressive effects of (n-3) PUFAs are not mediated by oxidative stress. Another mechanism by which (n-3) PUFAs could alter T-cell function might involve peroxisome proliferator-activated receptors (PPARs). In addition to their ability to alter membrane function and dynamics, recent data indicate that dietary PUFAs are also ligands for select nuclear receptors. Although some ligands for PPARs (γ, α) are known to modulate T-cell function (52,53), this class of nuclear receptor binds (n-3) and (n-6) PUFAs equally and lacks fatty acid

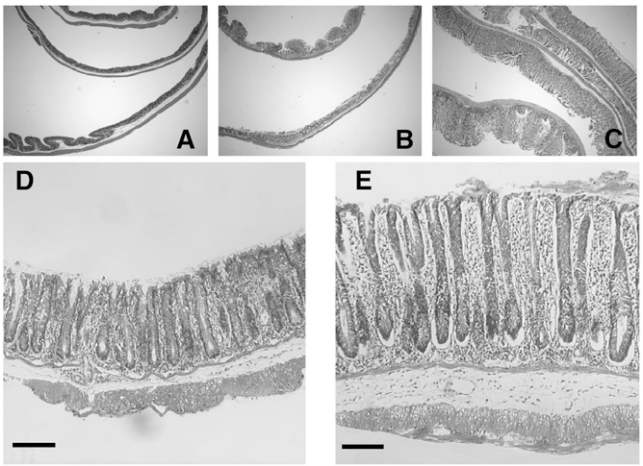

Figure 2 Hematoxylin and eosin (H&E)-stained sections of Swiss rolled colon from (*A*) SVE29 mouse (wild type genetic background control); (*B*) IL-10 knockout mouse (untreated); (*C*) IL-10 knockout mouse treated with piroxicam; (*D*) IL-10 knockout mouse treated with piroxicam and fed an (n-3) PUFA diet; and (*E*) IL-10 knockout mouse treated with piroxicam and fed an (n-6) PUFA diet. There is markedly enhanced cellular infiltration in the lamina propria along with mucosal thickening. *A–C* sections at 20× magnification. For *D* and *E* sections the *scale bar* indicates 100 μm; note the elongated crypts and prominent lamina propia infiltration in the (n-6) PUFA mucosa.

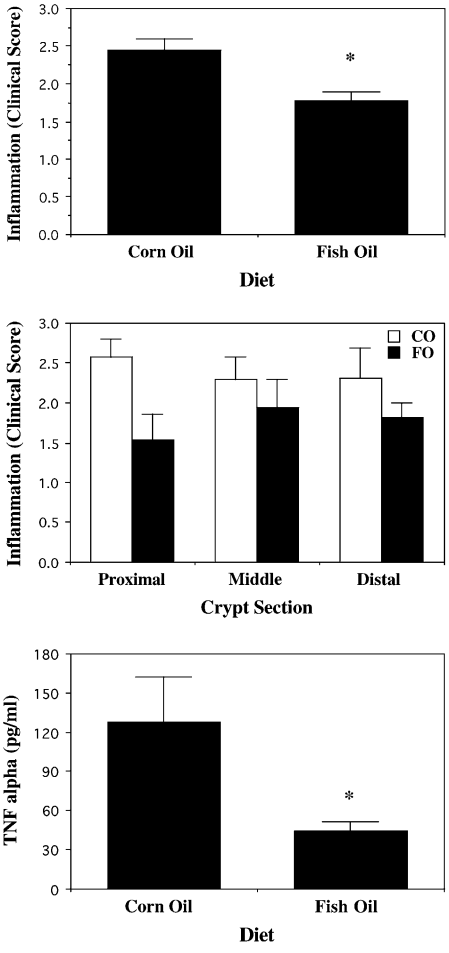

Figure 3 Effect of diet on IL-10 null colitic mice. Clinical score averaged over the entire colon, clinical score in different regions of the colon, and tumor necrosis factor (TNF) levels in splenic CD4$^+$ cultures stimulated with αCD3 and CD28. Data represent 5 mice per diet at 10 wk. *Asterisk* denotes $P < 0.05$. CO = corn oil; FO = fish oil.

class [(n-3) vs. (n-6)] specificity (54–57). Therefore, the unique effects of (n-3) PUFA are likely not mediated via PPARs.

Significance and conclusions

Some forms of IBD are characterized by overactive Th1-mediated responses toward resident bacterial flora in genetically susceptible individuals. However, how the indigenous microflora modulate the mucosal immune system and how this response is regulated are currently unknown. Considering the new insights into the molecular link between chronic inflammation and colon tumorigenesis (9,58), the Th1 immune pathway appears to be an important therapeutic target. A growing number of published reports support the contention that bioactive food components containing (n-3) PUFAs modulate important determinants that link inflammation to cancer development and progression. Therefore, it is essential to understand precisely how specific (n-3) PUFAs modulate immune function so that recommendations regarding the health benefits to be derived from dietary manipulation can be based on a firm scientific foundation. With regard to new experimental tools to determine the molecular mechanisms of inflammation protection afforded by (n-3) PUFAs, Kang et al. (59) cloned (n-3) fatty acid desaturase into mice in an attempt to endogenously produce high tissue levels of (n-3) fatty acids. Interestingly, these mice are protected from colitis (60). As for future directions, the biggest challenges will be to determine the precise mechanisms by which dietary PUFAs influence the maintenance of appropriate T-cell subset balance for a healthy immune system and to translate these basic findings into clinical practice.

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