

Reduced Colitis-Associated Colon Cancer in *Fat-1* (*n-3* Fatty Acid Desaturase) Transgenic Mice

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Abstract

Bioactive food components containing *n-3* polyunsaturated fatty acids (PUFA) modulate multiple determinants that link inflammation to cancer initiation and progression. Therefore, in this study, *fat-1* transgenic mice, which convert endogenous *n-6* PUFA to *n-3* PUFA in multiple tissues, were injected with azoxymethane followed by three cycles of dextran sodium sulfate (DSS) to induce colitis-associated cancer. *Fat-1* mice exhibited a reduced number of colonic adenocarcinomas per mouse (1.05 ± 0.29 versus 2.12 ± 0.51 , $P = 0.033$), elevated apoptosis ($P = 0.03$), and a decrease in *n-6* PUFA-derived eicosanoids, compared with wild-type (wt) mice. To determine whether the chemoprotective effects of *n-3* PUFA could be attributed to its pleiotropic anti-inflammatory properties, colonic inflammation and injury scores were evaluated 5 days after DSS exposure followed by either a 3-day or 2-week recovery period. There was no effect of *n-3* PUFA at 3 days. However, following a 2-week recovery period, colonic inflammation and ulceration scores returned to pretreatment levels compared with 3-day recovery only in *fat-1* mice. For the purpose of examining the specific reactivity of lymphoid elements in the intestine, CD3⁺ T cells, CD4⁺ T helper cells, and macrophages from colonic lamina propria were quantified. Comparison of 3-day versus 2-week recovery time points revealed that *fat-1* mice exhibited decreased ($P < 0.05$) CD3⁺, CD4⁺ T helper, and macrophage cell numbers per colon as compared with wt mice. These results suggest that the antitumor effect of *n-3* PUFA may be mediated, in part, via its anti-inflammatory properties. [Cancer Res 2008; 68(10):3985–91]

Introduction

Human inflammatory bowel diseases are chronic, relapsing inflammatory conditions of unknown etiology. Genetic, immunologic, and environmental factors have been implicated (1, 2). These diseases are characterized by two overlapping clinical phenotypes: ulcerative colitis and Crohn's disease. Ulcerative colitis primarily involves the colon with inflammation restricted to the mucosa. The

inflammation in Crohn's disease often involves the small intestine along with the colon and other organs (3). Crohn's disease affects more than 500,000 individuals in the United States and represents the second most common chronic inflammatory disorder after rheumatoid arthritis. In addition, the risk of developing colorectal cancer increases by ~0.5% to 1% each year after 7 years in patients with chronic intestinal inflammation (2, 4). However, despite compelling data indicating a functional link between inflammation and colon cancer, the pathways regulating initiation and maintenance of inflammation during cancer development remain poorly understood. Therefore, it is important to identify overlapping regulatory relationships among genes considered to drive inflammation-associated colonic tumor development. To date, the effects of *n-3* polyunsaturated fatty acid (PUFA) on susceptibility to colitis and colon cancer have not been determined.

Colorectal cancer continues to pose a serious health problem in the United States. Over a lifetime, a person has a 1 in 18 chance of developing invasive colorectal cancer (5). From a dietary perspective, a growing number of published reports support the contention that bioactive food components containing *n-3* PUFA modulate important determinants that link inflammation to cancer development and progression (6–10). In addition, clinical and experimental data indicate a protective effect of *n-3* PUFA on colon cancer (11–15). Eicosapentaenoic acid (EPA; 20:5^{Δ5,8,11,14,17}) and docosahexaenoic acid (DHA; 22:6^{Δ4,7,10,13,16,19}) are typical *n-3* PUFA (found in fish oil), defined according to the position of the first double bond from the methyl end of the molecule, which is designated "*n-3*." In contrast, dietary lipids rich in *n-6* PUFA [found in vegetable oils; e.g., linoleic acid (18:2^{Δ9,12}) and arachidonic acid (20:4^{Δ5,8,11,14})] enhance the development of colon tumors (14, 16). These effects are exerted at both the initiation and postinitiation stages of carcinogenesis (11, 14, 17). Consistent with human clinical trials (12, 13, 15, 18), we have shown that the balance between colonic epithelial cell proliferation and apoptosis can be favorably modulated by dietary *n-3* PUFA, conferring resistance to toxic carcinogenic agents (8, 14). This is significant because the typical Western diet contains 10 to 20 times more *n-6* than *n-3* PUFA (19). Unfortunately, to date, a unifying mechanistic hypothesis addressing how *n-3* PUFA selectively suppress colon cancer compared with *n-6* PUFA is lacking. Because mammals cannot produce *n-3* PUFA from the major *n-6* PUFA found in the diet due to the lack of ^Δ15-desaturase activity, it is necessary to enrich the diet with EPA and/or DHA to assess their biological properties *in vivo*. Recently, a *fat-1* gene encoding an *n-3* fatty acid desaturase has been cloned from *Caenorhabditis elegans* and expressed in mammalian cells (20). This enzyme can catalyze the conversion of *n-6* PUFA to *n-3* PUFA by introducing a double bond into fatty acyl chains. The generation

Note: Supplementary data for this article are available at Cancer Research Online (<http://cancerres.aacrjournals.org/>).

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of transgenic mice expressing *fat-1* will now allow us for the first time to investigate the biological properties of *n*-3 PUFA without having to incorporate DHA in the diet (21).

The dextran sodium sulfate (DSS) method of induced inflammation is an excellent preclinical model of colitis that exhibits many phenotypic characteristics relevant to the human disease (22, 23). When combined with azoxymethane, at least 50% of the animals (C57BL/6 mice) develop colonic adenocarcinomas (24, 25). Macroscopically, a dysplasia-invasive adenocarcinoma sequence is observed, resulting in both flat and polypoid tumors. This is analogous to the dysplasia-associated lesion or mass seen in patients with ulcerative colitis (4). Therefore, in this study we exposed *fat-1* mice to azoxymethane followed by three rounds of DSS to test the hypothesis that the endogenous production of *n*-3 PUFA affords protection against colitis-associated colon carcinogenesis. Specifically, we determined how *n*-3 PUFA and chronic inflammation influence colonic: (a) tumor formation, (b) inflammation and injury scores, (c) specific activity of lymphoid elements, and (d) eicosanoid production.

Materials and Methods

Animals. *Fat-1* transgenic mice were generated and backcrossed onto a C57BL/6 background as previously described (21). All procedures followed the guidelines approved by Public Health Service and the Institutional Animal Care and Use Committee at Texas A&M University. The colony of *fat-1* mice used for this study was generated by breeding heterozygous mice. The genotype and phenotype of offspring of each animal were characterized using isolated DNA and total lipids from mice tails (Supplementary Table S1). Specific pathogen-free animals were maintained under barrier conditions and fed a 10% safflower oil diet (Research Diets) *ad libitum* with a 12-h light/dark cycle. The diet contained (g/100 g diet) 40 sucrose, 20 casein, 15 corn starch, 0.3 DL-methionine, 3.5 AIN 76A salt mix, 1.0 AIN 76A mineral mix, 0.2 choline chloride, 5 fiber (cellulose), and 10 safflower oil.

Colon tumor induction. *Fat-1* and littermate wild-type (wt) (control) mice (10–20 wk old) were injected i.p. with 12.5 mg/kg body weight azoxymethane (Sigma-Aldrich) followed by three rounds of DSS treatment. After 1 wk, 2.5% DSS (molecular weight, 36,000–50,000; MP Biomedicals) was administered in the drinking water for 5 d, followed by 16 d of tap water. This cycle was repeated twice (5 d of 2.5% DSS followed by a 16-d recovery period and 4 d of 2% DSS), and mice were terminated 12 wk after completion of the final DSS cycle. Subsequently, each colon was resected proximally at the junction between the cecum and distally at the anus, flushed with PBS, and fixed with 4% paraformaldehyde (14). Tumors were sectioned and dysplasia, adenomas, and carcinomas were charted and evaluated by a board-certified pathologist in a blinded manner as we have previously described (14, 26).

In situ apoptosis measurement. Apoptosis was measured in paraformaldehyde-fixed, paraffin-embedded tissues using the terminal deoxynucleotidyl transferase-mediated dUTP biotin nick end labeling (Oncor) method (17, 27).

Colitis induction and histologic scoring. *Fat-1* and wt mice (8–14 wk) were provided 2.5% DSS in the drinking water for 5 d, after which animals were provided with water only and allowed to recover for either 3 d or 2 wk before sacrifice. At each necropsy interval, the entire colon was removed, measured, fixed in 4% paraformaldehyde, and paraffin embedded. Sections were stained with H&E. Histologic examination was done in a blinded manner by a board-certified pathologist, and the degrees of inflammation (score of 0–3) and epithelial injury (score of 0–3) on microscopic cross sections of the colon were graded. The presence of occasional inflammatory cells in the lamina propria was assigned a value of 0; increased numbers of inflammatory cells in the lamina propria as 1; confluence of inflammatory cells, extending into submucosa, as 2; and transmural extension of the infiltrate as 3. For epithelium injury, no

mucosal damage was scored as 0; discrete lymphoepithelial lesions were scored as 1; surface mucosal erosion or focal ulceration was scored as 2; and extensive mucosal damage and extension into deeper structures of the bowel wall was scored as 3.

Isolation of colonic lamina propria lymphocytes. Lamina propria lymphocytes were isolated from mouse colon as previously described with some modification (28, 29). Briefly, the colon was flushed clean and incubated in media containing Ca^{2+} - and Mg^{2+} -free HBSS (Sigma-Aldrich), 5 mmol/L DTT, and 30 mmol/L Na_4EDTA at 37°C, 100 rpm for 15 min. Subsequently, colons were placed on ice and gently scraped with a rubber policeman to remove the intact crypts. Following a washing step in Ca^{2+} - and Mg^{2+} -free HBSS, the remaining tissue was cut into small pieces and incubated in Ca^{2+} - and Mg^{2+} -free HBSS containing 1 mg/mL type II and type IV collagenase (Worthington) at 37°C, 100 rpm for 40 min. The liberated lamina propria cells were freely passed through a stainless steel grid (60-mesh) and further purified by density gradient centrifugation in 40% to 70% Percoll (Amersham) in PBS. Lymphocytes enriched at the 40% to 70% Percoll interface were collected.

Flow cytometry analysis of CD3⁺ and CD4⁺ T cells. Flow cytometry was done as previously described, using 1 million cells per mouse (30). Lamina propria lymphocytes collected from Percoll gradients were preincubated with an Fcγ receptor blocking monoclonal antibody (10 μg/mL; 2.4G2, BD PharMingen) for 5 min at 4°C. To measure the proportion of CD3⁺ and CD4⁺ T cells, sample contents were stained with both FITC-labeled (5 μg/mL) anti-CD3 (145-2C11, hamster IgG1, BD PharMingen) and phycoerythrin-labeled anti-CD4 (10 μg/mL; GK1.5, rat IgG2b, BD PharMingen). Flow cytometric analysis was conducted on a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry systems) and analyzed using the CellQuest analysis program.

Eicosanoid and phospholipid profiles. Colonic mucosal scrapings were collected and immediately snap frozen in liquid nitrogen. Samples were extracted using the method of Yang et al. (31). Briefly, aliquots of 1 N citric acid (20 mL) and 10% butylated hydroxytoluene (2.5 μL) were added to samples to prevent free radical peroxidation. Before extraction, an aliquot of deuterated eicosanoids [prostaglandin E_2 (PGE_2)- d_4 , 15-hydroxyeicosatetraenoic acid (HETE)- d_8 , 12-HETE- d_8 , and 13-hydroxyoctadecadienoic acid (13-HODE)- d_4 ; 100 ng/mL] was added to each sample as internal standard. Eicosanoids were subsequently extracted with 2 mL of hexane/ethyl acetate (1:1, v/v) and vortexed for 2 min. Samples were then centrifuged at $1,800 \times g$ for 10 min at 4°C. The upper organic layer was collected and the organic phases from three extractions were pooled and then evaporated to dryness under a stream of nitrogen at room temperature. All extraction procedures were done under low-light and low-temperature conditions to minimize potential photooxidation or thermal degradation of eicosanoid metabolites. Samples were then reconstituted in methanol/10 mmol/L ammonium acetate buffer (70:30, v/v; pH 8.5) before liquid chromatography-tandem mass spectrometry (LC/MS/MS) analysis. The extracted prostaglandins were quantified with a LC/MS/MS Quattro Ultima tandem mass spectrometer (Waters Corp.) equipped with an Agilent HP 1100 binary pump high-performance liquid chromatography inlet (Agilent Technologies). The prostaglandins were separated using a 2×150 -mm Luna 3 m phenyl-hexyl analytic column (Phenomenex). The mobile phase consisted of 10 mmol/L ammonium acetate (pH 8.5) and methanol. The column temperature was maintained at 50°C, and samples were kept at 4°C during the analysis. Individual analytes were detected by electrospray negative ionization and multiple reaction monitoring of the transitions m/z 351 → 271 for PGE_2 , m/z 349 → 269 for PGE_3 , and m/z 355 → 275 for PGE_2 - d_4 . Fragmentation of all compounds was done with argon as the collision gas at a collision cell pressure of 2.10×10^{-3} Torr. The identification of each prostaglandin was confirmed by comparison to authentic reference standards. Total phospholipids from scraped colonic mucosa and splenic CD4⁺ T cells were analyzed by gas chromatography as previously described (32).

Statistics. Data are expressed as mean ± SE. Differences between experimental groups were analyzed using one-way ANOVA (SPSS software package). $P < 0.05$ was accepted as significant.

Results

Weight gain. For azoxymethane/DSS-treated animals, neither carcinogen nor *fat-1* genotype significantly altered weight gain (starting weight: wt, 35.9 ± 3.0 g versus *fat-1*, 37.7 ± 2.9 ; final weight: wt, 35.2 ± 3.4 versus *fat-1*, 36.8 ± 3.2 ; $n = 19$; $P > 0.05$).

Fatty acid profiles in *fat-1* and wt mice. Both *fat-1* transgenic and wt offspring were fed a 10% safflower oil diet enriched in *n-6* PUFA throughout the duration of the study. Colonic mucosa and splenic CD4⁺ (systemic) T-cell total lipid fatty acid compositional analyses revealed an increase in EPA (20:5 *n-3*), docosapentaenoic acid (22:5 *n-3*), and DHA (22:6 *n-3*) in *fat-1* transgenic mice (Supplementary Tables S2 and S3). In addition, the ratio of *n-6* PUFA (20:4 *n-6*, 22:4 *n-6*, and 22:5 *n-6*) to the long-chain *n-3* PUFA (20:5 *n-3*, 22:5 *n-3*, and 22:6 *n-3*) was significantly ($P < 0.05$) suppressed in *fat-1* T cells and colonic mucosa (2.44 and 2.70), respectively, compared with wt mice (56.06 and 18.24). These data indicate that an appropriate activity of *n-3* fatty acid desaturase was present and that relevant cell types (e.g., T cells and colonocytes) were enriched in *n-3* PUFA.

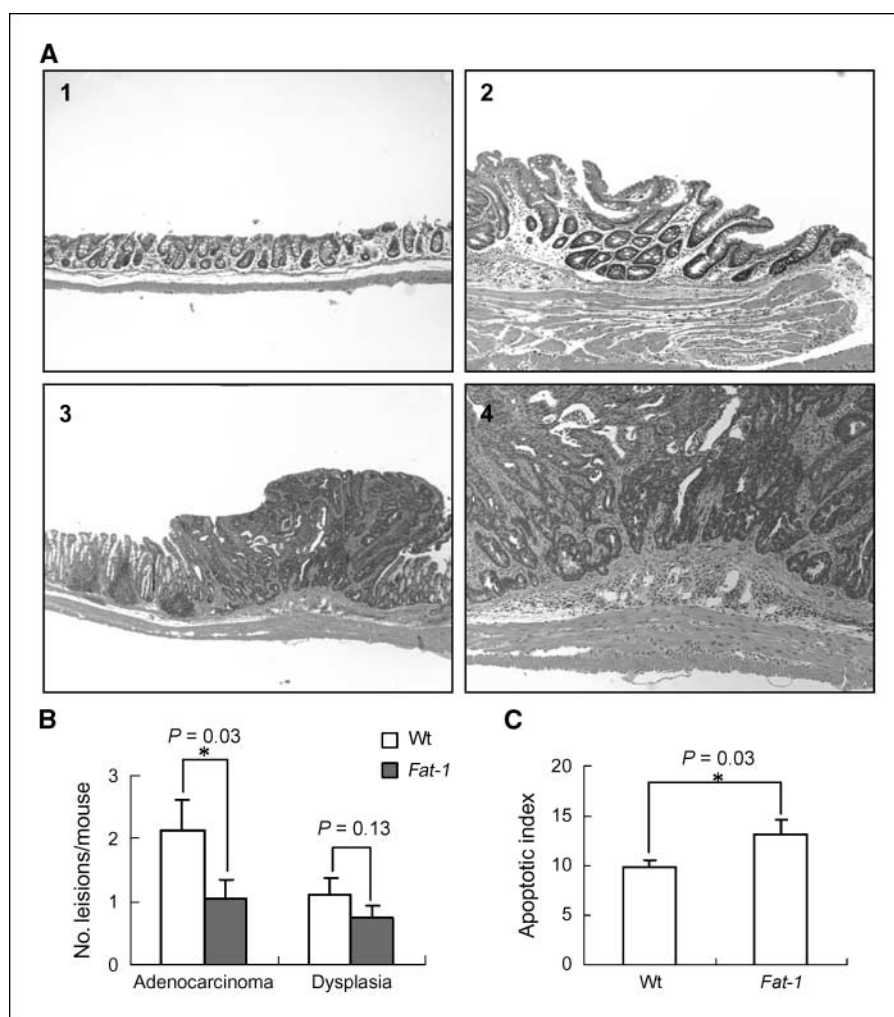
Suppression of colorectal tumorigenesis in *fat-1* mice following azoxymethane and DSS treatment. Colitis-associated colonic tumors were induced by a single injection of azoxymethane followed by repeated cycles of DSS ingestion using a well-

established protocol (25). Mice were terminated 12 weeks after completion of the final DSS cycle, and grossly visible masses or lesions were typed as adenomas, adenocarcinomas, or dysplasia (Fig. 1A, 1-4). The incidence of colonic tumors (adenocarcinomas) was lower in *fat-1* relative to wt mice: 15 of 19 (79%) for *fat-1* versus 17 of 17 (100%; $P = 0.001$) for wt mice. *Fat-1* mouse tumors on average tended to be smaller (12.53 ± 1.31 versus 14.11 ± 2.02 mm²; $P = 0.09$) compared with wt mice. In addition, *fat-1* mice ($n = 19$) exhibited a reduced average number of adenocarcinomas (1.05 ± 0.29 versus 2.12 ± 0.51 ; $P = 0.033$) and dysplasia (0.75 ± 0.19 versus 1.12 ± 0.26 ; $P = 0.13$) per mouse compared with wt mice (Fig. 1B).

ω -3 desaturase expression enhances apoptosis in colonic epithelial cells. Measurements of apoptosis have greater prognostic value to detect dietary effects on colon tumor incidence than do measurements of cell proliferation (14, 17). Therefore, apoptosis was assessed in the colonic epithelium (Fig. 1C). The apoptotic index was significantly ($P = 0.03$) higher in *fat-1* compared with wt mice, suggesting that the observed reduction in tumor incidence (described above) may, in part, be explained by an increase in apoptosis.

***Fat-1* mice are less susceptible to DSS-induced chronic inflammation.** In complementary experiments, we also determined the ability of *n-3* PUFA to modulate susceptibility to DSS-induced

Figure 1. Effect of *fat-1* genotype on colonic adenocarcinomas and apoptosis levels. Animals were injected with azoxymethane followed by three cycles of DSS to induce colitis-associated colon tumors. Animals were terminated 12 wk after completion of the final DSS cycle. **A**, representative histopathologic features of H&E-stained colorectal adenocarcinomas. The lesions were counted on high-resolution photographs of the colons; 1, normal colon, $\times 100$; 2, dysplasia, $\times 100$; 3 and 4, adenocarcinoma, $\times 40$ and $\times 100$. **B**, columns, mean ($n = 17$ – 20 mice per treatment); bars, SE. **C**, apoptosis levels in *fat-1* versus wt mouse colon 38 d after the final DSS treatment. Data are expressed as an apoptotic index (i.e., the total number of apoptotic cells per 100 crypts). Columns, mean ($n = 17$ – 20 mice per treatment); bars, SE.



colitis. Both acute and chronic colitis were assessed by administering 2.5% DSS in the drinking water for 5 days, followed by a recovery period of 3 days (acute) or 2 weeks (chronic). DSS administration was associated with a significant ($P < 0.05$) loss of body weight and a reduced colon length (data not shown). Histologic evaluation was subsequently done to assess immune cell infiltration and epithelial injury (Fig. 2A). After 3 days of recovery, there was no effect of *n-3* PUFA with respect to the acute phase inflammatory score or the severity of injury (Fig. 2B and C). In contrast, following a 2-week recovery period, colonic inflammation scores returned to pretreatment levels relative to 3-day recovery only in *fat-1* mice [2.29 ± 0.29 to 1.67 ± 0.17 ($P = 0.03$), compared with wt mice, 2.17 ± 0.17 to 2.50 ± 0.29 ($P = 0.21$)]. Similar trends were observed with regard to injury scores: 2.43 ± 0.30 to 1.33 ± 0.33 in *fat-1* mice ($P = 0.003$) versus 2.67 ± 0.21 to 2.25 ± 0.25 in wt mice ($P = 0.19$). Because DSS treatment followed by a 2-week recovery period represents a chronic inflammation model in C57BL/6 mice (33), these data suggest that *fat-1* mice exhibit an enhanced long-term resolution of inflammatory processes.

Eicosanoid profiles following azoxymethane/DSS exposure. Because arachidonic acid (20:4 *n-6*)-derived two-series prostaglan-

dins seem to promote cancer development in the colon (34), we also examined key eicosanoids in colonic mucosa following azoxymethane/DSS exposure. Both *n-3* PUFA- and *n-6* PUFA-derived metabolites were assayed, including PGE₂, PGD₂, leukotriene B₄ (LTB₄), 15-HETE, 12-HETE, 5-HETE, PGF_{2 α} , PGE₁, PGE₃, 13-HODE, 13,14-dihydro-15-keto-PGE₂, 13,14-dihydro-15-keto-PGD₂, 12-hydroxyheptadecatrienoic acid (12-HHTre), LTB₅, 5-hydroxyeicosapentaenoic acid (HEPE), 12-HEPE, and 15-HEPE. In general, *n-6* PUFA-derived eicosanoids (PGE₂, PGD₂, PGE₁, and 12-HETE) were significantly reduced in *fat-1* mice (Fig. 3). In contrast, PGE₃, an EPA-derived prostaglandin, was elevated in *fat-1* mice. No changes were observed with respect to LTB₄, 15-HETE, 5-HETE, PGF_{2 α} , PGE₃, 13-HODE, 13,14-dihydro-15-keto-PGE₂, 13,14-dihydro-15-keto-PGD₂, 12-HHTre, LTB₅, 5-HEPE, 12-HEPE, and 15-HEPE (data not shown). These results indicate that the enhanced incorporation of *n-3* PUFA into *fat-1* mouse colonic mucosa suppressed *n-6* PUFA-derived cyclooxygenase and lipoxygenase metabolism.

Altered infiltration of CD3⁺ and CD4⁺ T cells in *fat-1* mice after DSS exposure. A pathogenic role for CD4⁺ T cells has been shown with respect to the DSS induced inflammatory bowel disease (35, 36). Therefore, to contrast and compare local

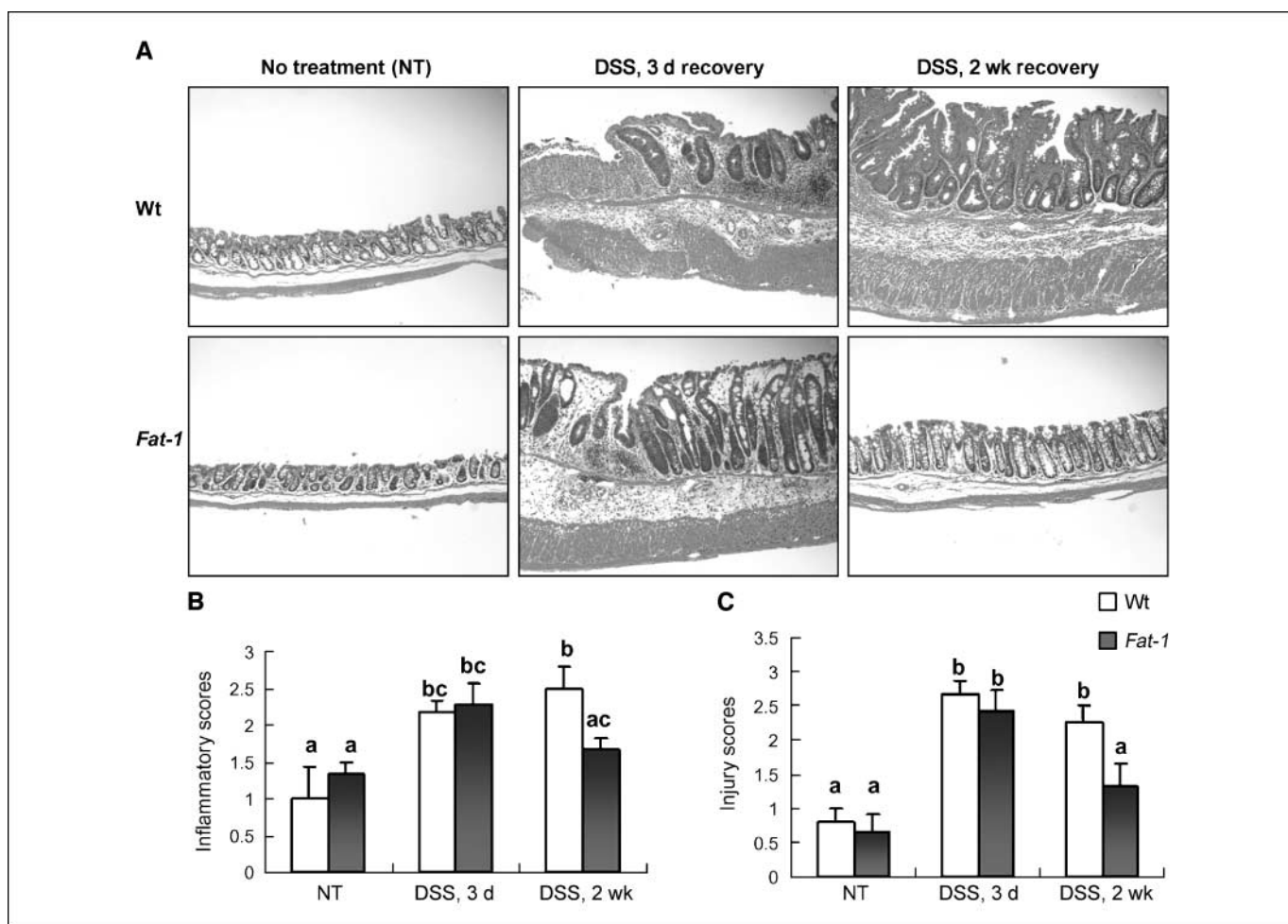
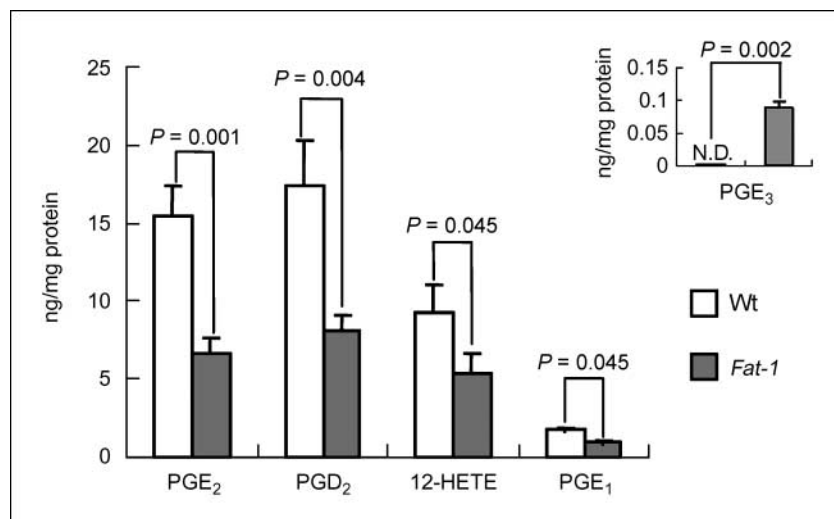


Figure 2. Histologic features of colonic inflammation and mucosal injury. To explore the effect of *n-3* PUFA on DSS-induced colonic inflammation and mucosal injury, mice were treated with a single 5-d cycle of DSS followed by either a 3-d or 2-wk recovery period. A, representative H&E-stained colonic tissues from mice without or with 2.5% DSS treatment based on degrees of inflammatory cell infiltration and epithelial injury ($\times 40$). B and C, columns, mean inflammatory scores (B) and injury scores (C; $n = 5-8$ mice per treatment); bars, SE. Data not sharing common letters are significantly different, $P < 0.05$. NT, no treatment.

Figure 3. Colonic mucosa eicosanoid profiles of *fat-1* and wt mice. Animals were injected with azoxymethane followed by three cycles of DSS and terminated 12 wk after completion of the final DSS cycle. Eicosanoids from scraped colonic mucosa were immediately extracted and quantified by LC/MS/MS. *Inset*, elevated EPA-derived PGE₃ in *fat-1* mice; *N.D.*, not detectable. *Columns*, mean ($n = 8$ mice per treatment); *bars*, SE. Levels of *n*-6-derived eicosanoids were significantly decreased in *fat-1* mice.



immunologic features in *fat-1* and wt mice, colonic lamina propria lymphocytes were isolated from mice before and after a single dose of DSS (3 days and 2 weeks of recovery period). Notably, there was a dramatic increase in the number of lamina propria lymphocytes and macrophages in both *fat-1* and wt animals following DSS treatment (Supplementary Figs. S1 and S2). Interestingly, only *fat-1* mice exhibited a return to pretreatment levels after 2 weeks of recovery. This suggests an inflammation-prone status in intestines from wt mice. For the purpose of examining the specific reactivity of lymphoid elements in the intestine, CD3⁺ T cells and CD4⁺ T-helper cells from colonic lamina propria were quantified by flow cytometry (Fig. 4). Comparison of 3-day versus 2-week recovery time points revealed that *fat-1* mice exhibited decreased CD3⁺ T cell numbers ($\times 10^5$) per colon [3.08 ± 0.53 to 1.84 ± 0.43 ($P = 0.03$) in *fat-1* mice and 2.95 ± 0.61 to 2.27 ± 0.61 ($P = 0.18$) in wt mice] and CD4⁺ T helper cell numbers [1.80 ± 0.35 to 1.12 ± 0.25 ($P = 0.048$) in *fat-1* mice and 1.78 ± 0.37 to 1.30 ± 0.32 ($P = 0.13$) in wt mice]. These results suggest that the antitumorigenic effect of *n*-3 PUFA may be mediated, in part, via its immunosuppressive/anti-inflammatory properties.

Discussion

Dietary *n*-3 PUFA are well known for both their anti-inflammatory and tumor-suppressing properties (11, 37, 38). With respect to colon cancer development, we have shown that the chemoprotective effect of fish oil is due to the direct action of *n*-3 PUFA and not to a reduction in the content of *n*-6 PUFA (8). Despite compelling data indicating a functional link between inflammation and colon cancer, the effects of *n*-3 PUFA on the pathways regulating initiation and maintenance of inflammation during colon cancer development remain poorly understood. Therefore, we examined the phenotype of *fat-1* mice, which synthesize high tissue levels of *n*-3 PUFA (21), to test the hypothesis that the endogenous production of *n*-3 PUFA affords protection against colitis-associated colon carcinogenesis. Our data show that *n*-3 PUFA effectively alter colonic membrane phospholipid composition in the *fat-1* mouse (Supplementary Table S3), resulting in the suppression of inflammation-driven tumor formation (Fig. 1). These findings support our postulate that *n*-3 PUFA have

chemoprotective properties. In addition, colonocyte apoptosis was elevated in *fat-1* mice. This is noteworthy because apoptosis is progressively inhibited during colon cancer development (39). It is possible, therefore, that the observed protective effect of *n*-3 PUFA is due, in part, to the enhanced deletion of cells through the activation of targeted apoptosis (8, 17).

It is well established that microbially driven chronic inflammation can lead to colon cancer (38). Conditions that reduce mucosal barrier integrity (e.g., DSS) promote the production of proinflammatory cytokines, which act in a paracrine fashion to promote angiogenesis and tumor growth (40). These mediators can in turn promote cyclooxygenase-2-related signaling pathways, which are capable of enhancing cell proliferation, angiogenesis, cell migration, and invasion, while inhibiting apoptosis (34). Therefore, it is noteworthy that eicosanoid levels in colonic mucosa were significantly suppressed in *fat-1* relative to wt mice (Fig. 3). This finding is consistent with the well-documented ability of *n*-3 PUFA (EPA and DHA) to supplant arachidonic acid and subsequently antagonize prostaglandin (PGE₂ and PGD₂) and hydroxy fatty acid (12-HETE) biosynthesis (41).

Although the subject of much debate, there is growing evidence that *n*-3 PUFA suppress inflammatory bowel disease in humans (7, 42). Because an inability to maintain an appropriate balance of T-cell subsets is a critical component contributing to the development of inflammatory bowel disease (1, 43), and anti-inflammatory therapy is efficacious against neoplastic progression and malignant conversion, we specifically determined the susceptibility of *fat-1* mice to DSS-induced chronic inflammation. Our studies reported herein have shown that *fat-1* mice exhibit an enhanced ability to resolve chronic colitis. Similar effects of *n*-3 PUFA were observed in previous acute inflammation experiments using this model (10, 44) as well as the interleukin-10 null mouse colitis model (38), although a previous report has described contrasting data (45). Of relevance to the immune system in the intestine, we have shown that *n*-3 PUFA alter the balance between CD4⁺ T-helper (Th1 and Th2) subsets by directly suppressing Th1 cell development (30). This is noteworthy because Th1 cells, in part, mediate inflammatory bowel disease onset and progression (38, 43). Collectively, these observations suggest that *n*-3 PUFA dampen the persistent inflammation and immune activation that

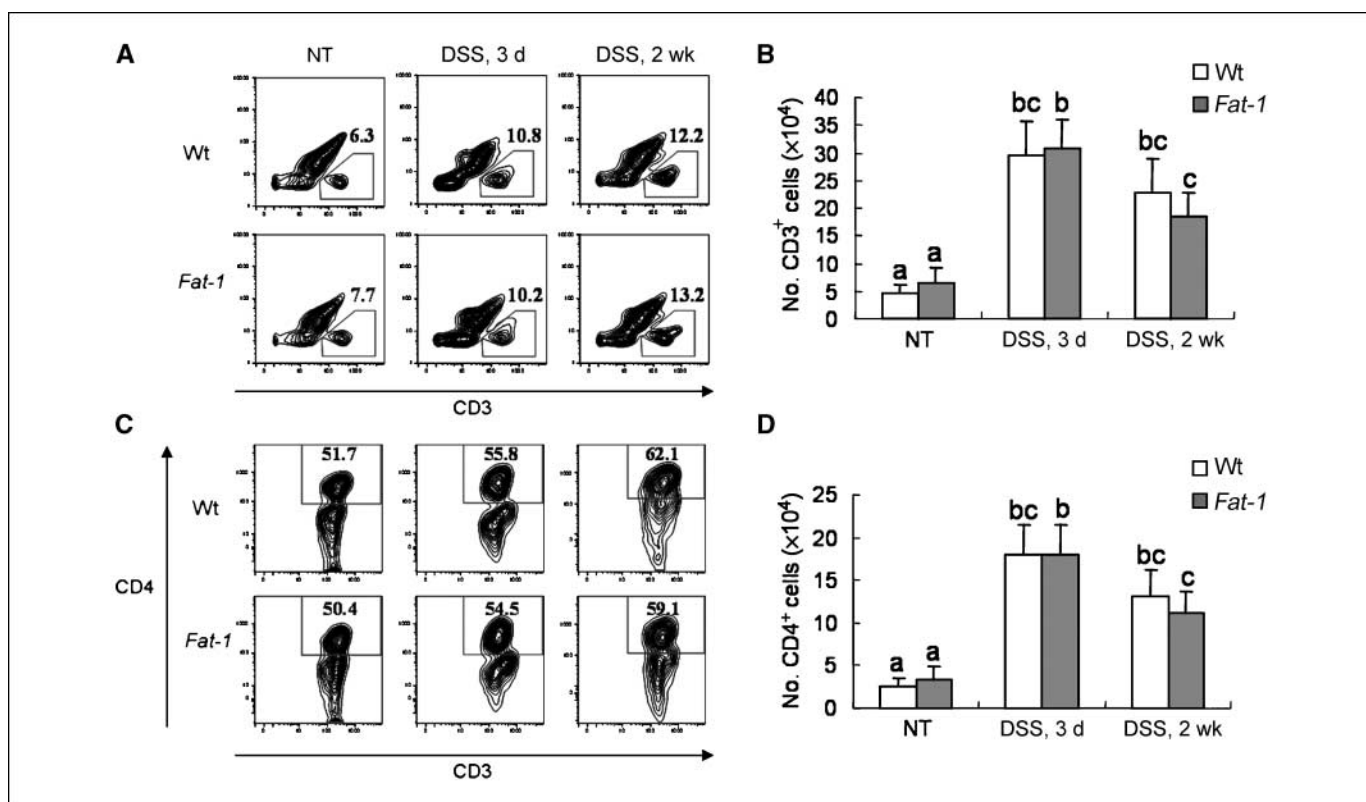


Figure 4. Flow cytometric analysis of CD3⁺ and CD4⁺ colonic lamina propria lymphocytes. Mice were exposed to a single 5-d cycle of DSS followed by either a 3-d or 2-wk recovery period, and lamina propria lymphocytes were isolated from the colon. *A*, plots were gated on CD3⁺ expressing cells. The number in each image indicates the percentage of CD3⁺ cells. *B*, columns, mean ($n = 5$ mice per treatment); bars, SE. *C*, plots were gated on CD4⁺ cells within the CD3⁺ population. The number in each image indicates the percentage of CD4⁺ cells. *D*, *fat-1* mice exhibited a decreased number of CD3⁺ and CD4⁺ cells in lamina propria after 2 wk of recovery compared with 3 d. Columns, mean of two experiments ($n = 14-18$ per treatment per experiment); bars, SE. Data not sharing common letters are significantly different, $P < 0.05$.

are associated with DSS-induced mucosal ulceration, thereby suppressing epithelial carcinogenesis.

In conclusion, we have shown that endogenously synthesized *n-3* PUFA suppress colonic (*a*) chronic inflammation and tissue injury, (*b*) specific activity of lymphoid and macrophage elements in the intestine, and (*c*) tumor formation. To our knowledge, this is the first study to show that *n-3* PUFA, which are incorporated into both colonocytes and T cells, suppress inflammation-driven tumor progression. Further understanding of the effects of fatty acids on the bidirectional interactions between colonocytes and T cells in the lamina propria will provide insight into the ability of *n-3* PUFA to favorably modulate the inflammation-dysplasia-carcinoma axis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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