Peroxisome proliferator-activated receptor δ promotes colonic inflammation and tumor growth

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Abstract

Although epidemiologic and experimental evidence strongly implicates chronic inflammation and dietary fats as risk factors for cancer, the mechanisms underlying their contribution to carcinogenesis are poorly understood. Here we present genetic evidence demonstrating that deletion of peroxisome proliferator-activated receptor δ (PPARδ) attenuates colonic inflammation and colitis-associated adenoma formation/growth. Importantly, PPARδ is required for dextran sodium sulfate induction of proinflammatory mediators, including chemokines, cytokines, COX-2, and prostaglandin E2 (PGE2), in vivo. We further show that activation of PPARδ induces COX-2 expression in colonic epithelial cells. COX-2–derived PGE2 stimulates macrophages to produce proinflammatory chemokines and cytokines that are responsible for the recruitment of leukocytes from the circulation to local sites of inflammation. Our results suggest that PPARδ promotes colonic inflammation and colitis-associated tumor growth via the COX-2–derived PGE2 signaling axis that mediates cross-talk between tumor epithelial cells and macrophages.

colorectal cancer | COX-2/PGE2

Chronic inflammation is clearly associated with increased cancer risk for a number of malignancies, including esophageal, gastric, hepatic, pancreatic, and colorectal cancer (CRC). Indeed, ulcerative colitis (UC), a form of inflammatory bowel disease (IBD), is associated with an increased risk for the development of CRC (1). The common pathologic changes associated with IBD include a defect of the innate immune response to microbial agents, diminished epithelial barrier integrity, and increased infiltration of dysregulated immune cells. However, the underlying mechanism(s) responsible for the connection between inflammation and cancer remains of high interest, others have reported that NF-κB signaling and certain cytokines such as IL-6, -17, -22, and -23 are involved in mouse models of colitis-associated CRC (2–4).

Some of the evidence for the link between inflammation and cancer came from epidemiologic and clinical studies showing that use of nonsteroidal anti-inflammatory drugs (NSAIDs) reduced the relative risk for developing CRC by 40–50%. NSAIDs are known to exert one of their anti-inflammatory and anti-tumor effects by targeting an inducible enzyme cyclooxygenase 2 (COX-2). COX-2 expression is elevated in CRC and is associated with a lower survival of CRC patients (5–7). COX-2–derived prostaglandin E2 (PGE2) is the most abundant prostaglandin found in human CRC (8) and plays a predominant role in promoting tumor growth (9). Similarly, COX-2 and PGE2 levels are elevated in the gastrointestinal (GI) tract of patients with active IBD (10, 11). These results prompted us to ask whether the COX-2–derived PGE2 pathway could be involved in colitis-associated carcinogenesis.

Dietary fat intake is an environmental factor that is associated with some human diseases such as diabetes, obesity, dyslipidemias, and cancer (12, 13). Peroxisome proliferator-activated receptors (PPARs) have been shown to play a central role in regulating the storage and catabolism of dietary fats via complex metabolic pathways, including fatty acid oxidation and lipogenesis (14). PPARδ is a member of PPAR family that belongs to the nuclear hormone receptor superfamily and is also a ligand-dependent transcription factor. PPARδ is expressed in diverse tissues (15), and its expression level is very high in the GI tract compared with other tissues (16). Although PPARδ has been shown to be involved in chronic inflammation and in CRC progression, its role is still unclear and vigorously debated (17). Particularly, its role in colitis-induced carcinogenesis has never really been explored carefully.

Results

PPARδ Is Required for Dextran Sodium Sulfate-Induced Colonic Inflammation. To investigate the biological function of PPARδ in colonic inflammation, we first examined the phenotype of dextran sodium sulfate (DSS)-treated PPARδ-deficient mice generated by deletion of exons 4–5 (18). In this model, PPARδ was deleted in the whole organism. WT mice that repeatedly received DSS as described in Fig. 1A developed a shorter colonic length due to inflammation-induced changes (Fig. 1B) and histologic signs of severe colitis, characterized by infiltration of immune cells, extent (depth of inflammation), and crypt damage (Fig. 1C and D). In contrast, PPARδ-deficient mice exhibited marked resistance to DSS-induced colonic inflammation (Fig. 1 B and D). Water-treated WT or PPARδ-deficient mice showed no clinical and histologic signs of chronic inflammation. Moreover, the absence of PPARδ did not affect DSS-induced intestinal epithelial cell death or regeneration of epithelial cells (Fig. S1). In addition, we evaluated whether loss of PPARδ affected intestinal homeostasis, such as intestinal epithelial cell proliferation, survival, and total number of stem cells. Both WT and PPARδ-deficient mice exhibited the same rates of intestinal epithelial cell proliferation and survival as similar levels of Lgr5-expressing intestinal stem cells (Fig. S2).

We further quantified the inflammatory response by profiling the type and density of immune cells in the colonic mucosa using

Significance

Our study not only reveals a novel role of peroxisome proliferator-activated receptor δ (PPARδ) in colonic inflammation and colitis-associated tumorigenesis, but also provides some rationale for development of PPARδ antagonists as new therapeutic agents in the treatment of inflammatory bowel disease and colitis-associated colorectal cancer. Moreover, our findings indicate that prostaglandin E2 generated by chronic inflammation is a crucial mediator connecting chronic inflammation and colorectal carcinogenesis.

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colonic mucosa. We found that loss of PPARδ dramatically re-
duced DSS induction of certain chemokines and cytokines in the colonic mucosa of mice compared with water-treated WT mice (Fig. 2 A and B). We found that the severity of chronic inflammation directly correlated with the level of colonic tumor burden. Histological analysis showed that a massive infiltration of immune cells was observed in all adenomas taken from DSS-treated Ppard+/ApcMin/+ mice, but not in all tumors taken from DSS-treated Ppard−/−/ApcMin/+ mice (Fig. 3C). To further confirm the role of PPARδ in promoting colonic inflammation and colitis-associated carcinogenesis, another mouse model of colitis-associated tumorigenesis was examined. Deletion of Ppard attenuated chronic inflammation in azoxymethane (AOM)-treated Il-10−/− mice compared with their control littermates (Ppard+/−/ Il-10−/−) (Fig. 4A). Similarly, Il-10−/− mice contained a much more massive infiltration of immune cells in colonic mucosa compared with WT or PPARδ-deficient mice (Fig. 4B). In contrast, PPARδ-deficient IL-10−/− null mice had significantly less infiltration of immune cells within the colonic mucosa compared with water-treated mice (Fig. S3). In contrast, loss of PPARδ attenuated the ability of DSS to induce these genes and markedly reduced the infiltration of immune cells in the colonic mucosa of ApcMin/+ mice (Fig. S3). In particular, deletion of PPARδ impaired DSS induction of chemokines that are involved in promotion of colitis-associated tumorigenesis, such as IL-6, -17A, and -22 (Fig. S3C).

PPARδ Is Required for Colitis-Associated Tumorigenesis. We first investigated the role of PPARδ in DSS-treated ApcMin/+ mice. Mice were treated with DSS as described in Fig. 1 A. Consistent with the above results, DSS-treated Ppard+/−/ApcMin/+ mice exhibited higher levels of these genes in colonic mucosa with a massive infiltration of the immune cells compared with water-

Fig. 1. Loss of PPARδ inhibits DSS-induced chronic colonic inflammation. (A) Schematic of mice treated with 2% (wt/vol) DSS. (B) The average length of mouse colon was measured after completion of the experiments. (C) The histopathologic alterations of the colon were examined on H&E-stained sections, and blinded histological scoring of inflammation in colonic mucosa of mice was performed as described (44). For B and C, data represent mean ± SE. *P < 0.05. (D) Representative H&E-stained sections from WT (Left) and Ppard−/− (Right) treated with DSS as described in A are shown. (Scale bars, 250 μm.)

Fig. 2. Loss of PPARδ attenuates DSS-induced massive infiltration of immune cells and proinflammatory gene expression in the colonic mucosa. (A) Cells isolated from the colonic mucosa of indicated genotypic mice treated with either DSS or water as described in SI Materials and Methods were incubated with antibodies against indicated cell-surface markers to characterize the subpopulations by flow cytometry. Values are reported as the number of Gr-1, CD3, CD4, F4/80, and CD11c positive cells per gram of each colon tissue, respectively. *P < 0.05. (B and C) The mRNA (B) and protein (C) levels of indicated genes in colonic mucosa were analyzed by q-PCR and ELISA from a DSS-treated cohort of 12 mice for each genotype and a water-treated cohort of seven mice for each genotype. For qPCR, equal total proteins from each sample were subjected to ELISA. Data represent the mean ± SE of protein concentration (picograms per milligram of tissue weight). *P < 0.05.

flow cytometry. A massive infiltration of neutrophils, T cells, T helper cells, macrophages/monocytes, and dendritic cells (DCs) into the colonic mucosa was observed in the DSS-treated WT mice compared with water-treated WT mice (Fig. 2A). In contrast, the infiltration of immune cells in the colonic mucosa was greatly attenuated in DSS-treated Ppard−/− null mice (Fig. 2A). Because certain chemokines are responsible for the recruitment of leukocytes from the circulation to local inflammatory sites and are regulated by proinflammatory cytokines, we measured an array of proinflammatory chemokines and cytokines in the colonic mucosa. We found that loss of PPARδ dramatically reduced DSS induction of certain chemokines and cytokines in colonic mucosa, including CXC ligand 1 (CXCL1), CC ligand 2 (CCL2), CCL3, CCL4, and IL-1β (Fig. 2 B and C). DSS treatment also significantly induced expression of other PPARδ-independent proinflammatory chemokines and cytokines, including IFN-γ, IL-23, and CXCL10. We focused our next studies on the evaluation of PPARδ-dependent proinflammatory mediators. Consistent with the results of massive immune cell infiltration, CXCL1 is a neutrophil chemokine, whereas CCL2, CCL3, and CCL4 are potent chemoattractants for monocytes/macrophages, T cells, and DCs. Together, these results indicate that PPARδ promotes chronic inflammation via induction of proinflammatory chemokines that attract immune cells into the colonic mucosa.
COX-2 is a downstream target of PPARδ. Because the levels of COX-2 and PGE2 are elevated in inflamed mucosa of IBD patients, we examined whether COX-2-derived PGE2 signaling was affected during colonic inflammation. Indeed, DSS treatment led to increased COX-2 expression in colonic mucosa taken from WT mice, but not in the samples taken from PPARδ-null mice (Fig. 5A). Interestingly, COX-2 was expressed in both epithelial and stromal cells in the colonic ulcerative areas of DSS-treated WT mice (Fig. 5B). Moreover, the results from immunofluorescent staining of COX-2, EpCAM (epithelial cell marker), and CD45 (immune cell marker) further confirmed that COX-2 is expressed in both epithelial and immune cells (Fig. S4). In contrast, even in the markedly reduced ulcerative areas of PPARδ-deficient mice, no COX-2 staining was observed (Fig. 5C). These results reveal that the COX-2-derived PGE2 signaling is one of the downstream pathways of PPARδ in the context of these experiments.

Because COX-2 is mainly expressed in colonic epithelial cells and macrophages of inflamed mucosa and colorectal carcinoma tissues, we examined whether activation of PPARδ induces COX-2 expression in these cells. As expected, activation of PPARδ by its agonist (GW501516) induced COX-2 expression in colonic tumor epithelial cells isolated from ApcMin+/- mice (Fig. 6A) and HCT-116 colorectal carcinoma cells (Fig. 6B), but not in PPARδ-deficient mouse colonic tumor epithelial cells or PPARδ-deficient HCT-116 cells (Fig. 6A and B). Similarly, GW501516 induced PGE2 production in HCT-116 cells, but not in PPARδ-deficient HCT-116 cells (Fig. 6C). In addition, overexpression of PPARδ alone resulted in elevation of COX-2 expression compared with vector control cells, but treatment of PPARδ-overexpressing HCT-116 cells with GW501516 did not further induce COX-2 expression (Fig. S5A). These results demonstrate that the effect of GW501516 on induction of COX-2 and PGE2 is most likely due to specific activation of PPARδ nuclear receptor. Moreover, activation of PPARδ also induced COX-2 expression in other colorectal carcinoma cell lines and young adult mouse colonic epithelial cells (Fig. S5B).

Next, we examined whether Wnt and PPARδ signaling cooperatively induced COX-2 expression. Treatment of HCT-116 cells with Wnt3a did not affect COX-2 expression or further enhance PPARδ induction of COX-2 (Fig. S5C). In contrast, GW501516 treatment had no effect on COX-2 expression in the mouse bone marrow-derived macrophages (BMMs) (Fig. S5D) or other macrophages such as RAW264.7 and THP-1-derived macrophages. These results demonstrate that activation of PPARδ induces COX-2 expression in colonic epithelial cells, but not in the macrophages we evaluated.

COX-2-Produced PGE2 Is a Downstream Target of PPARδ. Because the levels of COX-2 and PGE2 are elevated in inflamed mucosa of IBD patients, we examined whether COX-2-derived PGE2 signaling was affected during colonic inflammation. Indeed, DSS treatment led to increased COX-2 expression in colonic mucosa taken from WT mice, but not in the samples taken from PPARδ-null mice (Fig. 5A). Interestingly, COX-2 was expressed in both epithelial and stromal cells in the colonic ulcerative areas of DSS-treated WT mice (Fig. 5B). Moreover, the results from immunofluorescent staining of COX-2, EpCAM (epithelial cell marker), and CD45 (immune cell marker) further confirmed that COX-2 is expressed in both epithelial and immune cells (Fig. S4). In contrast, even in the markedly reduced ulcerative areas of PPARδ-deficient mice, no COX-2 staining was observed (Fig. 5C). These results reveal that the COX-2-derived PGE2 signaling is one of the downstream pathways of PPARδ in the context of these experiments.

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cells and macrophages. Because COX-2-derived PGE₂ signaling is downstream of PPARδ (Figs. 5 and 6), we postulated that PGE₂ mediates the effects of PPARδ on induction of these genes. Indeed, PGE₂ induced the expression of CXCL1, CCL2, CCL3, CCL4, and IL-1β in THP-1-derived macrophages (Fig. 6D), in WT mouse BMMs, and PPARδ-deficient BMMs (Fig. 6E). These results indicate that PGE₂ is a downstream effector of PPARδ in vivo. Moreover, PGE₂ stimulates WT BMMs to secrete IL-6 that promotes colitis-associated tumorigenesis (Fig. 6E, Right). However, we did not detect IL-22 and -17 proteins in the supernatants from BMMs in the absence or presence of PGE₂ treatment. Analysis of quantitative PCR (q-PCR) revealed that all four prostaglandin E receptors (EP) were expressed in BMMs (Fig. S5).

Discussion

Despite emerging evidence showing that PPARδ is involved in the pathogenesis of IBD and CRC, its roles in pathobiology are still hotly debated. Administration of a PPARδ agonist exacerbated colitis in IL-10-deficient mice and accelerated intestinal tumor growth in Apc<sup>Min/+</sup> mice (19–21). Studies from two independent groups revealed that loss of PPARδ by deletion of its exons 4–5 or exon 4 reduced intestinal adenoma burden in both Apc<sup>Min/+</sup> and AOM-treated mice without exposure to DSS (22, 23). A recent report described a role of PPARδ in Helicobacter pylori-associated gastric carcinogenesis, which represents another example of its effects in a proinflammatory pathway (24). These results suggest that PPARδ has proinflammatory and protumor effects. However, one group reported conflicting results showing that deletion of PPARδ (at exon 8) significantly aggravated colitis in the DSS-treated mice and enhanced adenoma growth in Apc<sup>Min/+</sup> and AOM-treated mice in the absence of DSS treatment (25, 26). Their results suggest that PPARδ exerts anti-inflammatory and antitumor effects. The reason for this discrepancy may be due to the use of different deletion strategies to remove PPARδ. The deletion of PPARδ exon 4–5, which encodes an essential portion of the DNA binding domain, is thought to totally disrupt PPARδ function as a nuclear transcriptional factor, whereas deletion of exon 8, the last exon of the PPARδ gene, is postulated to generate a hypomorphic allele, which retains some aporeceptor function. Here, to our knowledge, we provide the first evidence demonstrating that deletion of PPARδ at exons 4–5 attenuated chronic colonic inflammation and colitis-associated tumor growth in two different mouse models (Figs. 1–4). These results strongly support the notion that PPARδ promotes chronic colonic inflammation and colitis-associated tumorigenesis.

A classic paradigm in IBD is the concept that PPARδ in the gut is involved in the gut microbiota-driven inflammation and remodeling of gut epithelial functions, such as tight junctions and homeostatic ileum. This is likely related to the regulation of proinflammatory and anti-inflammatory factors in the gut. PPARδ has been shown to regulate the expression of inflammatory cytokines and chemokines such as IL-6, -17, and -22 in the colonic macrophages isolated from the DSS-treated mice (27). More recently, genetic and pharmacologic studies have provided evidence that PPARδ is involved in the regulation of these chemokines and cytokines, as well as leukocyte infiltration during colonic inflammation and colitis-associated tumorigenesis (Figs. 2 and 4 as well as Fig. S3). These results indicate that these PPARδ-dependent chemokines attract immune cells into colonic mucosa.

COX-2 is an immediate–early response gene normally absent from most cells, but it is found in high levels at sites of inflammation in response to inflammatory stimuli (31, 32). To our knowledge, here we provide the first in vivo evidence showing that COX-2 is a downstream target of PPARδ (Fig. 5), although PPARδ has previously been shown to induce COX-2 expression in liver and lung carcinoma cells in vitro (33, 34). Although no peroxisome-proliferator response element has been identified in the COX-2 promoter, PPARδ is known to mediate its transcriptional activity via interaction with other transcriptional factors, including NF-κB and C/EBP (35, 36). It is well established that COX-2 expression is regulated by various transcription factors such as NF-κB, C/EBP, CREB, NFAT, and AP-1. Thus, PPARδ could up-regulate COX-2 expression via NF-κB and C/EBP. Because PGE₂ promotes tumor growth in vivo (9), our results indicate that PGE₂, at least in part, mediates the effect of PPARδ on promotion of colitis-associated tumorigenesis in the animal models we studied. In addition to COX-2–derived PGE₂ signaling, it is possible that other pathways may also mediate the effects of PPARδ on promotion of inflammation and colitis-associated tumorigenesis. Further studies are needed to investigate whether other PPARδ downstream targets mediate the proinflammatory and protumor effects of PPARδ.

In experimental IBD models, COX-2–deficient mice suffer increased sensitivity to DSS-induced colitis (37), suggesting that COX-2 may be critical for healing of colonic injury by stimulation of epithelial cell proliferation and other wound-healing pathways. Conversely, dietary administration of nimesulide (a somewhat selective COX-2 inhibitor) effectively suppressed the development of colonic tumors induced by AOM/DSS (38),
suggesting that elevation of COX-2 resulting from chronic inflammation contributes to tumorigenesis. Similarly, basal physiological levels of PGE2 are required for protection against DSS-induced or inflammation-associated epithelial barrier injury by enhancement of epithelial cell survival and regeneration of epithelial barrier (39), whereas high levels of PGE2 exacerbate the inflammatory process (40). However, our results demonstrate that loss of PPARδ only reduced inflammation-elevated COX-2 expression and PGE2 production to the physiologic levels (water-treated WT mice) but did not totally block COX-2 expression and PGE2 levels, respectively. COX-2 protein expression and PGE2 levels were measured as described in Fig. 5. (D and E) THP-1-derived macrophages (D) and BMMs (E) were treated with the indicated dose of PGE2 for 24 h for mRNA expression (Left) and 48 h for secreted proteins (Right) after serum starvation for 24 h, respectively. (D) The levels of induced genes at mRNA levels (Left) and secreted protein levels (Right) were quantified by q-PCR and ELISA or Bio-Plex assays. (E) Left panel represents the gene mRNA levels and the rest of panels represents protein levels. Data are represented as the mean ± SE of relative expression for mRNA or protein concentration from three independent experiments. (F) The colonic macrophages were isolated from a cohort of five mice for each genotype treated with either 2% DSS or water as described in Fig. 1 and pooled together. A total of 1 × 10^{5} pooled colonic macrophages from each indicated group was subjected to q-PCR. Data represent the mean ± SD of relative expression for mRNA. (G) COX-2 expression at the mRNA (Left) and protein (Right) levels was quantified by q-PCR and Western blotting. **P < 0.05.

In conclusion, this study not only reveals novel functions of PPARδ in colonic inflammation and colitis-associated tumorigenesis, but also provides a rationale for development of PPARδ antagonists as potential new therapeutic agents in treatment of IBD and colitis-associated CRC. Moreover, we found a novel function of COX-2-derived PGE2 signaling in mediating cross-talk between colonic tumor epithelial cells and macrophages. Our results indicate that both PPARδ and COX-2 signaling coordinately promote colonic inflammation and colitis-associated tumorigenesis and is likely to be clinically relevant because the elevation of both PPARδ and COX-2 in tumor tissues correlates with a poor prognosis in CRC patients (43).

Materials and Methods

Animals. PPARδ-null mice and their littermate control mice as well as PPARδ-deficient {Apc<sup>Min/+</sup>} mice and their littermate controls were generated as described (22) and fed with standard mouse diet in the Animal Care Facility according to National Institutes of Health and institutional guidelines. Information describing the animal experiments is presented in SI Materials and Methods.

Cell Culture and Reagents. Human CRC cell lines and a monocytic cell line (THP-1) were obtained from the ATCC, and HCA-7 cells were a gift from Susan Kirkland (University of London, London). Additional information on culture of all cancer cells, THP-derived macrophages, BMMs, and primary colonic
tumor epithelial cells as well as isolation of colonic tumor epithelial cells, macrophages, and reagents is provided in SI Materials and Methods.

Analysis of Flow Cytometry. For multicolor flow-cytometry immunotypic analysis, cells were stained with the indicated monoclonal antibodies and analyzed on BD LSRII system (BD Biosciences) to determine the percentage of positive cells. Information on antibodies and a description of experimental procedures are presented in SI Materials and Methods.

q-PCR. The procedure describing the q-PCR assay is included in SI Materials and Methods.

ELISA and Bio-Plex Assays. Information on extraction of total proteins from colon tissues and ELISA kits as well as Bio-Plex assay is presented in SI Materials and Methods.

Western Blot Analysis. Detailed information about Western blotting assay and treatment of indicated cells with indicated reagents is provided in SI Materials and Methods.

Supporting Information

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SI Materials and Methods

Animals. For dextran sodium sulfate (DSS)-treatment experiments, male mice for each genotype at age 7 wk were randomly divided into two groups. DSS-treated mice received 2% DSS (40–50 kD; USB; catalog no. 14489) in their drinking water for four cycles as shown in Fig. 1A. Control mice were given water without DSS. For azoxymethane (AOM)-treatment experiments, Ppard<sup>−/−</sup>/Il-10<sup>−/−</sup> and Ppard<sup>+/+</sup>/Il-10<sup>−/−</sup> were generated by conventionally crossing Ppard<sup>−/−</sup> mice with Il-10<sup>−/−</sup> mice. Genotypes of offspring were verified by PCR analysis with allele-specific primers. Eight-week-old male mice of each genotype were injected intraperitoneally with AOM (10 mg/kg) once weekly for 4 wk and then killed at 24 wk of age. At the end of the experiment, colon length was measured, and some of colons from each group were used for counting tumors and inflammation scores, and the rest were selected to examine immune cell profiles, proinflammatory genes, and prostaglandin E<sub>2</sub> (PGE<sub>2</sub>) levels. After tumor burden was determined, colon tissues were embedded in paraffin. For histologic analysis, 5-μm-thick sections from all groups were stained with H&E to determine colonic inflammation scores and polyph morphology. The colonic inflammation score was quantified by using a described approach (1). The unstained sections were subjected to immunohistochemical staining.

Cell Culture and Reagents. All cancer cells were maintained in McCoy’s 5A medium with 10% FBS. Colonic tumor epithelial cells and colonic macrophages were isolated from Ppard<sup>+/+</sup>/Apc<sup>Min/+</sup> and Ppard<sup>−/−</sup>/Apc<sup>Min/+</sup> mice treated with 2% DSS or water. To prepare THP-1–derived macrophages, monocytes were treated with 50 ng/mL phorbol 12-myristate 13-acetate in RPMI medium 1640 with 10% FBS overnight.

For bone marrow–derived macrophages (BMMs), bone marrow cells were flushed aseptically from the femurs of mice and cultured in Falcon Petri dishes (BD Biosciences) with DMEM supplemented with 10% FBS and 10 ng/mL macrophage colony-stimulating factor (M-CSF) for 5 d. These cells were continuously cultured in DMEM with 10% FBS, 10 ng/mL M-CSF, and 20 ng/mL IL-4 for 2 d before the experiments.

The conditionally immortalized Young Adult Mouse Colonic (YAMC) cell line was a gift from Robert Whitehead (Vanderbilt University). The YAMC cells were cultured as described (2). Briefly, cells were maintained under permissive conditions at 33 °C and 5% CO<sub>2</sub> with IFN-γ. Confluent cells were incubated in 10% FBS medium without IFN-γ under normal conditions, at 37 °C and 5% CO<sub>2</sub>, for 48 h before all experiments.

All cell culture media and supplements were purchased from Gibco Life Technologies. Murine rIL-4, rM-CSF, and rIFN-γ were obtained from PeproTech. Human rWnt-3a was obtained from R&D Systems.

Establishment of Stable Cell Line. pBMN-I-GFP and pBMN-I-GFP-PPARδ retroviral vectors were transduced into Phoenix cells in 60-mm dishes by using LipoLamine reagent (Invitrogen) according to the manufacturer’s protocol. Culture medium containing virus particles was collected 48 h later and was added to HCT-116 cells. Infected cells were sorted by GFP positivity to eliminate uninfected cells.

TUNEL Assays. The fragmented DNA of apoptotic cells in tissue sections was end-labeled by using the DeadEnd colorimetric TUNEL system according to the manufacturer’s instructions (Promega). Sections were deparaffinized, rehydrated, incubated with 20 μg/mL proteinase K, washed with PBS, and then immersed in a terminal deoxynucleotidyl transferase reaction mixture for 60 min at 37 °C. The slides were then washed in 2× SSC for 15 min to stop the reaction. Following another wash and blocking cycle, the slides were incubated with Streptavidin peroxidase for 30 min at room temperature and then stained with 3,3′-diaminobenzidine.

Analysis of Flow Cytometry. The whole colon was weighed and cut into 2-mm pieces. Colon pieces were digested with 1 mg/mL dispase, 0.25 mg/mL collagenase A, and 25 U/mL DNase (Roche) at 37 °C for 20 min. The suspension was then passed through a 70-μm cell strainer (BD Biosciences). After cells were washed with PBS containing 4% FBS, 1 × 10<sup>7</sup> viable cells were suspended in the labeling buffer (0.05% BSA and 2 mM EDTA in PBS, pH 7.4) and labeled with the following mouse polyclonal antibodies: anti–Gr-1 conjugated with phycoerythrin (PE) (dilution 1:100), anti–CD3 conjugated with FITC (dilution 1:250), anti-F4/80 conjugated with alkaline phosphocyanin (APC) (dilution 1:200), anti–CD11c conjugated with APC–cyanine-7 (dilution 1:100), Alexa 700-conjugated anti-Ly6G (1:50), and FITC-conjugated anti–CD11b (1:50). After the cells were washed with 1 mL of the labeling buffer, they were analyzed by using a multicolor flow cytometry. All antibodies were purchased from BD Biosciences.

Quantitative PCR. RNA was extracted from mouse tissues and cells by using an RNeasy Mini Kit (Qiagen), and 5 μg of total RNA from each sample was reverse-transcribed with iScript Reverse Transcription Supermix (Qiagen). The mRNA levels of target genes were quantified by real-time PCR with an iCycler and SYBR Green Supermix (both from Bio-Rad) as described (3). Primers for these genes and mGapdh were chosen by using the Beacon Designer 5 program (Premier BioSoft International). The relative expression of target gene is the average of triplicates that were normalized against the transcription levels of mGapdh.

Western Blot Analysis. After serum starvation for 24 h, colonic epithelial cells (1.5 × 10<sup>6</sup>) were treated with the indicated concentration of GW501516 for 24 h, and THP-1–derived macrophages (1.5 × 10<sup>6</sup>) were treated with the indicated dose of PGE<sub>2</sub> for 1 d. Antibody to COX-2 (Santa Cruz Biotechnology) was used in 1:200 dilutions. The blots were stripped and then re-probed with β-actin antibody (Sigma).

ELISA and Bio-Plex Assay. Total proteins were extracted from colonic tissues by homogenizing and subsequently sonicating in antiprotease buffer (50 mM Hepes, 150 mM NaCl, and 1 mM EDTA, pH 7.4) containing protease inhibitor mixture tablets (Boehringer Mannheim). THP-1–derived macrophages and BMMs (7 × 10<sup>5</sup>) were cultured in serum-free medium for 24 h and then were treated with the indicated concentrations of PGE<sub>2</sub> for 48 h, respectively. The levels of CXCL1, CCL2, CCL3, CCL4, and IL-1β in mouse colonic tissues and cell-free supernatants were measured by using the Quantikine ELISA kits (R&D) according to the manufacturer’s instructions. IL-6, -17, -22, and -23 protein levels were measured by using Bio-Plex Pro Mouse cytokine, chemokine, and growth factor assays (Bio-Rad) following a protocol established by the manufacturer.

Immunohistochemical and Immunofluorescent Staining. Tissue sections (5-μm thick; n = 5 per animal) were stained with anti-mCOX-2 rabbit antibody (1:250) (Cayman) and rabbit anti-Ki67
monoclonal antibody (SP6; 1:200) (Abcam). The immunohistochemical staining was completed by using a Zymed-Histostain-SP Kit (Zymed) as described (4).

Paraffin-embedded specimens were treated with xylene and ethanol to remove the paraffin. The slides (5-μm thick; n = 5 per animal) were immersed in Borg decloaker solution (Biocare Medical) and boiled in a pressure cooker at 125 °C for 5 min for antigen retrieval. The slides were blocked with 5% normal rabbit serum and incubated with rabbit anti–COX-2 (1:200), rabbit anti-CD45 (1:200), or rabbit anti-EpCAM (1:200) antibody at 4 °C overnight. After washing with PBS, the slides were incubated with 1:200 Alexa Fluor 488- or 564-conjugated secondary antibody. The nuclei were stained with DAPI.

**In Situ Hybridization.** In situ hybridization was performed as described (5). In brief, frozen sections (10 μm) were mounted onto poly(L-lysine)-coated slides and fixed in cold 4% paraformaldehyde in PBS. The sections were prehybridized and hybridized at 45 °C for 4 h in 50% formamide hybridization buffer containing the 35S-labeled antisense or sense cRNA probes. Sections hybridized with sense probes served as negative controls.


**Fig. S1.** TUNEL staining of colon tissues taken from WT and peroxisome proliferator-activated receptor δ (PPARδ)-deficient mice treated with water or DSS. 

**Upper** A set of representative images from WT and PPARδ-deficient mice treated with water as control (Left), 2% DSS for 3 d (Center), or 2% DSS for 1 cycle (Right) shows that apoptotic nuclei are stained as dark brown by the DeadEnd colorimetric TUNEL system as described in SI Materials and Methods. The red arrows denote a group of TUNEL-positive stained epithelial cells undergoing apoptosis. (Scale bars, 100 μm.) **Lower** The bar graph represents mean ± SEM of apoptotic cells in five fields per section of four sections per mouse from five mice for each group. *P < 0.05.
Fig. S2. Loss of PPARδ does not affect intestinal epithelial cell proliferation, survival, and Lgr5-positive cell numbers. Three mice for each genotype were used to examine rates of colonic epithelial cell proliferation and survival as well as Lgr5-expression levels in the intestine. (Top) Left represents immunoreactive staining (brown) for Ki-67. (Scale bars, 50 μm.) Right represents the average numbers of Ki67+ cells in five fields per section of four sections per mouse from three mice for each group. (Middle) Left represents TUNEL-positive stained epithelial cells undergoing apoptosis. (Scale bars, 50 μm.) Right represents the average numbers of apoptotic cells in five fields per section of four sections per mouse from three mice for each group. (Bottom) In situ hybridization for Lrg5 mRNAs in the small intestine taken from WT and PPARδ-deficient mice. (Scale bar, 100 μm.)
Fig. S3. Loss of PPARδ attenuated DSS-induced proinflammatory mediators and massive infiltration of immune cells in the colon mucosa of Apc\(^{Min/+}\) mice. A cohort of 12 mice for each genotype was treated with DSS, and a cohort of 7 mice for each genotype was fed with water as control as described in Fig. 1A. (A–C) The levels of indicated genes at mRNA (A) and protein (B and C) in mouse colonic tissue samples were analyzed as described in Fig. 2B and C. (D) The profiles of immune cells in the colon mucosa of indicated genotypic mice treated with either DSS or were determined as described in Fig. 2A.

Fig. S4. Immunofluorescent staining of colon tissues taken from WT and PPARδ-deficient mice treated with water or DSS. A set of representative images from colons taken from WT mice treated with water and 2% DSS for four cycles shows that COX-2 (red), CD45 (green), and EpCAM (green) immunofluorescence was detected by a fluorescence microscope (Nikon ECLIPSE TE300, 10×). (Scale bar, 100 μm.)
The effect of PPARδ agonist or PGE₂ on induction of COX-2 or chemokines and cytokines as well as the profiles of prostaglandin E receptors (EP) in BMMs. (A) PPARδ-overexpressing HCT-116 (HCT-116/PPARδ) and control cells (HCT-116/vector) were treated with the indicated dose of GW501516 for 24 h following serum starvation for 24 h. (B–D) LS-174T, HCA-7, YAMC, and HCT-116 cells (C) as well as mouse BMM cells (D) were treated with the indicated dose of GW501516 and/or Wnt3a for 24 h after serum starvation for 24 h. COX-2 protein expression was analyzed by Western blotting. (E) The mRNA levels of indicated genes in HCT-116/PPARδ and HCT-116/vector cells treated with vehicle or the indicated dose of GW501516 were determined by q-PCR. (F) The mRNA levels of indicated genes in BMM cells were quantified by quantitative PCR as mentioned above.