Novel anti-inflammatory functions for endothelial and myeloid cyclooxygenase-2 in a new mouse model of Crohn’s disease


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Inflammatory bowel disease (IBD) is a chronic inflammatory disease of the digestive tract that includes two major intestinal diseases, ulcerative colitis (UC) and Crohn’s disease (CD). CD and UC are distinguished by the location and regulation of inflammation. CD mostly develops at the ileo-ceco-colic junction, whereas UC is common to the colon. CD pathology includes mucosal leukocyte infiltration, ulceration, tissue necrosis, and intact epithelial layers. The molecular mechanisms that participate in the development of IBD pathology, especially in CD, remain unknown.

The two prostaglandin (PG) G/H synthases, commonly known as cyclooxygenases 1 and 2 (COX-1 and COX-2), are the targets for nonsteroidal anti-inflammatory drugs (NSAIDs) and aspirin (46). The loss of gastrointestinal protection resulting in adverse events in NSAID users is linked to the inhibition of COX-1-dependent thromboxane A2, PGE2, and PGI2 formation (39, 52). COX-2 selective inhibitors were therefore expected to reduce the incidence of gastrointestinal adverse events while retaining the anti-inflammatory and antipycletic effects of NSAIDs (34). Indeed, since their development, COX-2 inhibitors are preferred as anodyne and anti-inflammatory medications because of reduced (but not absent) gastrointestinal adverse events compared with NSAIDs, such as indomethacin (6, 28).

In contrast to the proinflammatory effects of COX-2 and its products, several studies have shown that COX-2-dependent PGs contribute to ulcer healing in the intestine (16, 29, 50) by promoting angiogenesis (21). COX-2 also protects against aspirin-induced gastric injury by producing lipoxins (14). In addition, COX-2 is known to participate in the development and maintenance of intestinal immune functions (2, 31, 35). These results suggest that COX-2-dependent PGs are anti-inflammatory in certain physiological contexts and bring the long-term beneficial effects of COX-2 inhibitors on intestinal inflammation into question.

It is now well established that long-term treatment with COX-2 inhibitors increases the risk of cardiovascular diseases (28, 32, 47). To understand the protective role of COX-2 in cardiovascular diseases, we previously attempted to study diet-induced atherosclerosis development in COX-2−/− mice.

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However, COX-2−/− mice fed a cholate-containing, high-fat (CCHF) diet uniformly had a high mortality attributable to severe intestinal inflammation localized at the ileo-ceco-colic junction (22). The ileo-ceco-colic inflammation is associated with CD-like pathology, leukocyte infiltration predominantly in the submucosal layers of the intestinal wall and in many cases perivascular inflammation around capillary vessels, coupled with tissue necrosis and destruction. The epithelial layer was relatively spared of inflammatory cells, and epithelial ulceration was absent except in the most severe cases. These observations suggested that COX-2 might be involved in the protection against cholate-mediated inflammation in the ileo-ceco-colic junction.

In this study, we examined the role of COX-2 in diet-mediated intestinal inflammation. We developed conditional COX-2−/− mice that are deficient for COX-2 expression specifically in vascular endothelial cells (COX-2−/−E), myeloid cells (COX-2−/−M), endothelial/myeloid cells (COX-2−/−ME), and intestinal epithelial cells (COX-2−/−IE, COX-2−/−IE50). However, COX-2−/−IE mice, but not COX-2−/−IE50 and wild-type mice, on a CCHF diet developed CD-like pathology localized at the ileo-ceco-colic junction with distinct pathological processes. Our results suggest that COX-2 products play previously unidentified protective functions at the ileo-ceco-colic junction in mice.

MATERIALS AND METHODS

Animals. All animal protocols were approved by the Animal Research Committee at UCLA. Wild-type C57BL/6J mice were obtained from Jackson Laboratories (Bar Harbor, ME). COX-2+/+ and COX-2−/− mice (8–12 wk of age) on a mixed background (50% each of C57BL6/J and 129/Sv strains) were obtained from Taconic (Germantown, NY). COX-2 floxed C57BL/6J129Sv mice were created by one of our laboratories (19). C57BL/6J mice expressing Cre recombinase from the VE-cadherin promoter, lysozyme M promoter, and intestinal fatty acid-binding protein 1 (FABP1) were obtained from Dr. Luisa Iruela-Arispe (1), Dr. Yibin Wang (7), and the National Cancer Institute (Frederick, MD) (42), respectively. COX-2 floxed mice were crossed to either VE-cadherin Cre, lysozyme M Cre, or fatty acid-binding protein Cre mice to generate endothelial cell-specific COX-2−/−E, myeloid cell-specific COX-2−/−M, or intestinal epithelium cell-specific COX-2−/−IE mice, respectively. Endothelial/myeloid cell-specific COX-2−/−ME mice were generated by crossing COX-2−/−<sup>M</sup> with COX-2−/−<sup>E</sup> mice. All conditional COX-2−/− mice and control littermates were maintained through COX-2−/−ME intercrosses or alternatively COX-2−/−<sup>E</sup> and COX-2−/−<sup>M</sup> intercrosses. The primer sets (5‘-3’) and PCR conditions used to distinguish conditional COX-2−/− mice are provided in Supplemental Table S1, and an example genotyping PCR material for this article is available online at the American Journal of Physiology Gastrointestinal and Liver Physiology website). The background of all conditional COX-2−/− mice is ~62.5% C57BL/6J and ~37.5% 129Sv, except COX-2−/−<sup>IIE</sup>, which is 100% C57BL/6J. All mice were maintained on a 6% fat chow diet, and were given a CCHF (atherogenic) diet (15.8% fat, 1.25% cholesterol, and 0.5% cholate) (Harlan Teklad, Madison, WI) at the age of 16–20 wk and duration described in figure legends. For COX-2 inhibitor studies, female C57BL/6J mice at the age of 16–20 wk were fed a reformulated CCHF diet at 0.15% (wt/wt) celecoxib once a day for 3 wk.

Total cecum. A sample (0.5–1.0 cm) of terminal ileum, the entire cecum, and 0.5–1.0 cm of ascending colon were removed en bloc from euthanized mice. Each tissue was opened longitudinally from ileum through cecum and colon and washed in cold PBS to remove feces and debris. Histological scoring was performed before the tissues were frozen on the basis of size, thickness, and degree of ulceration of the ileo-ceco-colic junction on a scale of 0 (normal), 1 (ulceration and normal thickness), 2 (ulceration and mild thickening), 3 (softly thickened tissue), and 4 (firmly thickened tissue). The ileo-ceco-colic junction was resected, immediately frozen in liquid nitrogen, and stored in sterile tubes at −80°C until used. The most inflamed and thickened ileo-ceco-colic junction of each group was used for histological analysis and immunohistochemistry, whereas the remaining minor portions were used for PCR analysis.

Histological analysis. The frozen whole ceca from total COX-2+/+ and COX-2−/− mice and the severely inflamed ileo-ceco-colic junctions from conditional COX-2−/− mice were fixed in 10% formalin for 48 h. The fixed tissues were embedded in paraffin at the Translational Pathology Core Laboratory (TPCL) at UCLA. Four-micron sections were cut and stained with hematoxylin and eosin (H and E).

Quantitative RT-PCR. Total RNA was extracted by TRIzol (Invitrogen) and the cDNA was synthesized by High-Capacity cDNA Reverse Transcription Kit with RNase Inhibitor (Applied Biosystems, Foster City, CA) and further amplified by Bio-Rad iCycler iQ using the SYBR GreenER qPCR SuperMix (Bio-Rad, Hercules, CA). All PCR reactions were performed using standard cycling parameters: 95°C for 3 min, (95°C for 30 s, 60°C for 30 s, 72°C for 30 s) for 40 cycles except when primers with specific T<sub>m</sub> (Supplemental Table S1). Gene expression was normalized to the housekeeping gene, cyclophilin, and the relative expression was calculated using the 2<sup>−ΔΔC(t)</sup> method.

Primary macrophages. Primary macrophages were obtained by lavage 3 days after intraperitoneal injection of 3% thiglycolate into mice. Cells were plated on 12-well culture plates (Invitrogen) at ~1 × 10<sup>6</sup> cells per well. After incubation at 37°C for 2 h, adherent macrophages were washed and grown in DMEM (Invitrogen) with 10% FBS for 2 days at 37°C and 5% CO<sub>2</sub>. Fresh medium was added, and cells were treated with the indicated agonist for 72 h. Conditioned media were collected at the end of the experiment and kept at −80°C until use. Attached macrophages were washed with cold PBS, gently lifted using 5 mM EDTA, fixed in 3% paraformaldehyde, and stained with 10 µg/ml Nile Red (Sigma, St. Louis, MO) for 10 min at room temperature. Stained cells were then analyzed by flow cytometry to measure neutral and polar lipids as described previously (11, 15).

ELISA. IL-6 and IL-10 levels in supernatants from treated mouse peritoneal macrophages were quantified by direct ELISA, as described previously (53). Briefly, 100 µl of supernatant was coated on 96-well PVC microfilter plates (BD Biosciences, Franklin Lakes, NJ) at 4°C overnight. Primary antibodies against mouse IL-6 and IL-10 (eBioscience, San Diego, CA) were used at 1:1,000. The primary antibodies were detected by horseradish peroxidase (HRP)-conjugated secondary anti-rat antibodies (used at a 1:5,000 dilution). Following incubation with tetramethylbenzidine solution (KPL, Gaithersburg, MD), HRP activity was measured at A450. HRP-conjugated detection antibody was used as an internal standard to convert A450 of each sample to the concentration of detection antibody. The value for each protein from a given sample was calculated relative to the average of that protein concentration in mice on a chow diet, which was set to 1.

Flow analysis on intestinal lamina propria leukocytes. Intestinal lamina propria leukocytes from the ileo-ceco-colic junction and colon were isolated as described previously (51, 54, 55). Cell viability was estimated with trypan blue. The cell suspensions were filtered and incubated for 30 min on ice with the following PE-, APC-, PerCP-Cy5.5-, and PE-Cy7-conjugated antibodies at 1:200 dilution: B220, CD11b, Gr-1, and F4/80 (eBioscience); Siglec-F and 7-AAD (BD Bioscience). Stained cell suspensions were washed twice and subjected to
BD LSR-II flow cytometer (Beckman Coulter, Fullerton, CA). Data were analyzed with FlowJo software (TreeStar, Ashland, OR).

Statistical analysis. All data were statistically analyzed by t-test. Significance was determined as \( P < 0.05 \).

RESULTS

Localized ileo-ceco-colic inflammation in COX-2\(^{-/-}\) mice on a cholate-containing diet. We previously reported that a CCHF diet was lethal to COX-2\(^{-/-}\) mice, but not COX-1\(^{-/-}\) and wild-type littermate controls (22), due to severe intestinal inflammation that consistently localized to the ileo-ceco-colic junction. The inflamed ileo-ceco-colic junctions were associated with ulceration and cellular infiltration in the submucosa and mucosa (Fig. 1), whereas the epithelial layers were otherwise intact (22).

Ileo-ceco-colic inflammation by COX-2 inhibitors in mice on a CCHF diet. To rule out the possibility that the diet-dependent intestinal inflammatory abnormalities observed in COX-2\(^{-/-}\) mice are due to ‘passenger gene effect’ (23) or compensatory mechanisms associated with total knockout mice, we examined the effect of the COX-2 inhibitor, celecoxib, in wild-type C57BL6/J mice fed a CCHF diet. After 3 wk of treatment the ileo-ceco-colic junction tissue from the C57BL6/J mice treated with celecoxib and CCHF diet was associated with cellular infiltration, tissue necrosis, ulceration, and intact epithelium (Fig. 1) reminiscent of CD-like pathology in the COX-2\(^{-/-}\) mice. C57BL6/J mice treated with celecoxib and CCHF diet showed thickening of ileo-ceco-colic junctions (Fig. 2C) and increased spleen size (78\%, \( P < 0.002 \), data not shown), compared with C57BL6/J mice on a CCHF diet alone. Furthermore, although there are no significant differences between the two groups in average body weight (Fig. 2A) and the percent of cecal weight/body weight (Fig. 2B), there is a significant correlation between decreased body weight and increased cecal weight among individual mice treated with celecoxib and CCHF diet, but not CCHF diet alone (Fig. 2, D and E).

To understand the cell and molecular mechanisms involved in inflammation at the ileo-ceco-colic junction mediated by COX-2 inhibition in C57BL6/J mice on the CCHF diet, gene expression analysis was carried out on the inflamed ileo-ceco-colic junction tissues by qRT-PCR. Compared with control mice, there was increased expression of adherent molecules (intercellular adhesion molecule), leukocytes (SelL), granulocytes, including granulocyte and macrophages, into the ileo-ceco-colic junction. Moreover, there was significantly increased expression of IL-6, TNF-α, IFN-γ, and IL-5 (Fig. 3, E–H), suggesting that both innate and adaptive immune responses might be participating in this inflammatory setting.

Vascular conditional COX-2\(^{-/-}\) mice on a normal chow. To further elucidate the role of COX-2 in the ileo-ceco-colic inflammation mediated by cholate, we established conditional COX-2\(^{-/-}\) mice in myeloid cells (COX-2\(^{-/-}\)ME–ME), endothelial cells (COX-2\(^{-/-}\)ME–ME), and myeloid/endothelial cells (COX-2\(^{-/-}\)ME–ME) (Supplemental Fig. S1). On the chow diet, none of the cell-specific conditional COX-2\(^{-/-}\) mice showed any signs of developmental abnormalities or physical disabilities and presented normal body, spleen, and liver weights compared with wild-type mice (Supplemental Fig. S2, A–C). Although ceca from COX-2\(^{-/-}\)ME–ME were sig-

Fig. 1. Submucosal inflammation at the ileo-ceco-colic junction in mice on a cholate-containing, high-fat (CCHF) diet due to lack of cyclooxygenase-2 (COX-2). Female COX-2\(^{-/-}\) and wild-type (WT) mice (10–11 wk of age) were fed normal chow or a CCHF diet for 3 wk. Female C57BL6/J mice (BL6WT, 16–20 wk old) were fed a fresh reformulated CCHF diet once a day for 3 wk with either PBS (N, n = 8) or 0.15% (wt/wt) celecoxib (CX, n = 24). Body weight (A) (g), whole cecum/body weight (%) (B), and thickness of ileo-ceco-colic junction tissues (ICJ) (C) were measured during the dissection procedure. Circles and bars represent individual datum, and the average of relative expression of the various target genes, respectively. **\( P < 0.0005 \) compared with no treatment. D and E: body (g) and whole cecal weights (g) of individual mice in grams were correlated from untreated (D) and CX-treated mice (E). The linear regression was performed for statistical analysis. Slope, \( R^2 \) and \( P \) value for the significance of slope to the background are shown. NS, not significant.
performed using the expression of the various target genes, respectively. Statistical analysis was conducted using the t-test. *P < 0.05 compared with no treatment; **P < 0.0005 compared with wild-type.

Leukocyte infiltration was observed in submucosa and mucosa layers in the ileocecal junctions of all conditional COX-2−/− mice on CCHF diet. To determine the effect of COX-2 deficiency in myeloid cells and endothelial cells on cellular infiltration, RNA from the junction tissue was analyzed by qRT-PCR for leukocytes (L-selection) and granulocytes (MPO). The ileocecal-colonic inflammation in all three conditional COX-2−/− mice was associated with increased expression of L-selectin (fold induction of 1.7 ± 1.2, 2.1 ± 1.2, and 5.3 ± 3.3 for M, E and M/E, respectively) (Fig. 5A) and MPO (Fig. 5B), suggesting the infiltration of leukocytes, including granulocytes. Furthermore, lamina propria leukocytes isolated from the ileocecal-colonic junction tissues of COX-2−/-E, COX-2−/-M, and wild-type mice on the CCHF diet for 10 wk were analyzed by flow cytometry. The ileocecal-colonic junctions from COX-2−/-M mice had more leukocytes (200% increase compared with wild-type, P < 0.0002), which were identified as mostly eosinophils (Fig. 5C) and macrophages (Fig. 5D). In contrast, the ileocecal-colonic junctions in COX-2−/-E mice contained only ~40% more leukocytes (P < 0.3) than wild-type mice, with significantly elevated levels of B-lymphocytes (Fig. 5E). In addition, T-lymphocytes (CD4+, CD8+, and Treg) were constant among all

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Fig. 4. Submucosal inflammation at the ileocecal-colonic junction in vascular conditional COX-2−/− mice on CCHF diet. 16–20-wk-old female WT, myeloid cell-specific COX-2−/-M, endothelial cell-specific COX-2−/-E, and myeloid endothelial cell-specific COX-2−/-ME mice were fed a CCHF diet for 10 wk. Whole cecum/body weight (%) (A) and thickness of ICJ (B) were measured during dissecting procedure. Circles and bars represent individual datum and the average, respectively. *P < 0.05 and **P < 0.0005 compared with wild-type. C: most severely inflamed ileocecal-colonic junction tissue of each group (score = 4, rigid, thickened tissue as described in MATERIALS AND METHODS) were fixed and stained with hematoxylin and eosin. The images are at ×5 (top) and ×20 (bottom) magnifications.
groups (data not shown). These results suggest that distinct intestinal inflammatory processes might exist in the ileo-ceco-colic junctions in COX-2−/−M and COX-2−/−E mice on the CCHF diet.

**Common and distinct cytokine responses in COX-2−/−E and COX-2−/−M mice on CCHF diet.** To characterize the inflammatory process in the ileo-ceco-colic junction, RNA from the inflamed junction tissue was analyzed by qRT-PCR for cytokines. The ileo-ceco-colic junctions in COX-2−/−E, COX-2−/−M, and COX-2−/−ME mice on the CCHF diet showed decreased expression of the anti-inflammatory cytokine IL-10 but increased expression of IL-5 (Fig. 6, A, B, and E). Moreover, the ileo-ceco-colic inflammation in COX-2−/−ME mice on the CCHF diet associated with leukocyte infiltration (Figs. 4C and 5, A and B), similar to COX-2−/−M mice. Furthermore, the molecular and cellular pathology in COX-2−/−ME was similar to those of celecoxib-treated C57BL6/J mice on the CCHF diet (Figs. 1–3).

**Macrophages in COX-2−/−M mice are smaller, less inflammatory, and more susceptible to inflammatory stimuli.** The ileo-ceco-colic inflammation in COX-2−/−M mice on CCHF diet developed CD-like pathology associated with the infiltration of leukocytes, including macrophage (Fig. 5). However, the expression of cytokines known to be involved in CD

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**Fig. 5.** Lamina propria leukocytes in the ileo-ceco-colic junction from conditional COX-2−/− mice on CCHF diet. 16–20-wk-old, female WT, COX-2−/−M (M), COX-2−/−E (E), and COX-2−/−ME−/−ME (ME) mice were fed a CCHF diet (WT, n = 5; M, n = 7; E, n = 9; ME, n = 9) for 10 wk. RNA was extracted from the individual ileo-ceco-colic junction tissue and analyzed by qRT-PCR for L-selection (SelL) (A) and MPO expression (B). Circles and bars represent individual datum and the average relative expression of target genes, respectively. Statistical analysis was performed using the t-test. *P < 0.05 compared with wild-type. **Fig. 6.** Inflammation at the ileo-ceco-colic junction from conditional COX-2−/− mice on the CCHF diet. 16–20-wk-old, female WT, COX-2−/−M (M), COX-2−/−E (E), and COX-2−/−ME−/−ME (ME) mice were fed a normal chow (WT, n = 6; M, n = 4; E, n = 5; V, n = 6) or CCHF diet (WT, n = 5; M, n = 7; E, n = 9; ME, n = 9) for 10 wk. RNA was extracted from the individual ileo-ceco-colic junction tissue and analyzed by qRT-PCR for indicated target genes. Circles and bars represent individual datum and the average relative expression of target genes, respectively. Statistical analysis was performed using the t-test. W and C represent P < 0.05 compared with wild-type and chow, respectively. #P < 0.07. *P < 0.05 compared with wild-type.
pathogenesis was unaffected in COX-2−/− mice compared with wild-type mice on the CCHF diet (Fig. 6, A, B, and E). Previous studies demonstrated that COX-2 inhibitors attenuate cellular responses of intestinal macrophages to inflammatory stimuli (17, 43). To characterize the cellular responses of macrophages from COX-2−/− mice to inflammatory stimuli, peritoneal macrophages from wild-type and COX-2−/− mice were treated with cholate, deoxycholate (DCA), acetylated LDL, and TNF-α for 3 days. Cells were stained with Nile Red to quantify cellular lipid content and size by flow cytometry. The gates were set as shown in Supplemental Fig. S3. The size of macrophages from COX-2−/− mice was significantly smaller than macrophages from wild-type mice (Fig. 7A). COX-2-deficient macrophages had significantly lower lipid contents than wild-type macrophages, particularly after treatment with DCA (Fig. 7B), and produced significantly lower levels of IL-6 and IL-10 (Fig. 7, C and D). Macrophages from COX-2−/− mice contained higher IL-6 levels after incubation with TNF-α for 3 days, whereas the IL-6 levels were at background in macrophages from wild-type mice (Fig. 7C).

**DISCUSSION**

Products of COX-2 have been directly implicated in both inflammatory and thrombotic disease. However, predominant end products of the COX pathways vary between cells and have contrasting biology. In seven placebo-controlled trials, COX-2 selective inhibitors conferred an increased risk of stroke and myocardial infarction (4, 20, 36, 38, 40, 47, 48), providing strong evidence for anti-inflammatory roles for COX-2 and its products in cardiovascular biology. The incidence of gastrointestinal adverse events in randomized trials of two COX-2 selective inhibitors, rofecoxib (3) and lumiracoxib (13), is half that compared with control NSAIDs but is not completely eliminated. On the basis of these and other studies (3, 13, 16, 26, 28, 29, 50), it is now well established that COX-2 plays a protective role in vascular and intestinal physiology. However, the cellular and molecular mechanisms that regulate the COX-2-dependent pathophysiology are not known.

We recently reported that COX-2 protects against diet-mediated intestinal inflammation in mice (22). COX-2−/− mice fed a CCHF diet or diet containing cholate alone, but not chow or high fat alone, exhibited high mortality, whereas COX-1−/− mice and wild-type mice on the cholate diet were unaffected. Histological analysis identified the cause of death in COX-2−/− mice, as severe intestinal inflammation that was limited to the ileo-ceco-colic junction (22). In the present study, we examined the cell types that are responsible for the initiation of inflammation at the ileo-ceco-colic junction using cell-specific conditional
COX-2<sup>−/−</sup> mice. Pathological, cellular, and molecular characterization of the inflammation in total COX-2<sup>−/−</sup> (22), myeloid cell COX-2<sup>−/−</sup>, endothelial cell COX-2<sup>−/−</sup> mice (Fig. 4), and wild-type mice treated with COX-2 selective inhibitors (Figs. 1 and 2) revealed that inhibition of COX-2 in cholate-fed mice results in CD-like pathology. Thus combination of COX-2 inhibition and cholate feeding offers a new animal model to understand CD pathology and also to test intervention modalities.

To our surprise, conditional COX-2<sup>−/−</sup> mice in colonic epithelial cell (COX-2<sup>−/−M−/E</sup>) had no effect induction of cholate-mediated inflammation (data not shown). It is important to note that in all of our studies using COX-2 knockout mice the intestinal epithelium was intact despite severe inflammation in the submucosa and mucosa regions (Figs. 1 and 4). Previous reports demonstrated that COX-2 protein is induced in intestinal epithelial cells in CD patients (41, 44). Our results suggest that COX-2 products from intestinal epithelial cells are not involved in cholate-mediated inflammation at the ileo-ceco-colic junction.

Recent studies in patients have identified a causative link between IL-10 and CD (9, 10, 24). Reduced IL-10 expression in lamina propria and in vascular leukocytes is proposed to be involved in the development of CD (8, 9). Furthermore, IL-10-deficient mice, a well-characterized experimental model of IBD, spontaneously develop colitis with many similarities to CD (18, 25, 45, 56, 58). Our results show that IL-10 is reduced in both COX-2<sup>−/−M−/M</sup> and COX-2<sup>−/−E−/E</sup> mice (Fig. 6c). It is interesting to note that, following a 10-wk cholate-containing diet, myeloid cells in the ileo-ceco-colic junctions are increased in COX-2<sup>−/−M−/M</sup> and COX-2<sup>−/−E−/E</sup> mice (Fig. 4) compared with wild-type control mice. Thus myeloid cells, endothelial cells, and other cells such as lymphocytes (Fig. 5) may mediate the IL-10 reduction in these conditional COX-2<sup>−/−</sup> mice. Future studies are needed to determine the cause and role of reduced IL-10 in the COX-2 conditional knockout mice.

Laminar shear stress upregulates the expression of PGI<sub>2</sub> in endothelial cells in vitro (12, 37, 49). Fitzgerald and colleagues (5, 27) were the first to report that COX-2 was the dominant source of PGI<sub>2</sub> biosynthesis in humans. Given that endothelial cell components of this inflammatory process are necessary and sufficient to elicit this inflammation, in response to cholate feeding, that is reminiscent of CD like pathology. Finally, we have demonstrated that COX-2 has anti-inflammatory functions in the ileo-ceco-colic junction tissue. Future studies will determine whether the myeloid and endothelial cell components of this inflammatory process are inherent to the ileo-ceco-colic junction or whether they are a secondary response to a pathology initiated by cholate feeding and absence of COX-2 in another cellular compartment.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

REFERENCES


