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Cox-2 deletion in myeloid and endothelial cells, but not in epithelial cells, exacerbates murine colitis.

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Abbreviations: BrdU, bromodeoxyuridine; COX, cyclooxygenase; DSS, dextran sulfate sodium; EdU, 5-ethynyl-2′-deoxyuridine; IBD, inflammatory bowel disease; LPS, lipopolysaccharide; NSAID, non-steroidal anti-inflammatory drug; PGE2, prostaglandin E2; TUNEL, terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling.

The studies described above suggest that COX-2 is expressed in a number of cell types during the development of DSS-induced colitis. However, we do not know in which cell type(s) COX-2 expression is important for reduction of colitis symptoms. Only the effects of total systemic blockade of COX-2 function can be determined with NSAID or COX-2 inhibitors treatment (17,18). Similarly, only the effects of total systemic elimination of COX-2 expression can be determined in mice with a global Cox-2 gene deletion (4). To identify the cell type(s) in the colon in which COX-2 suppression exacerbates DSS-induced colitis, we used Cox-2flox/flox mice, in which Cox-2 exons 4 and 5 are flanked by loxP sites (19). In this study, we crossed Cox-2flox/flox mice with mice-expressing Cre recombinase in myeloid cells, endothelial cells and intestinal epithelial cells and examined the effect of cell type-specific Cox-2 deletion on DSS-induced colitis.

Materials and methods

Mice

Mice carrying a knock-in allele of the firefly luciferase-coding region in the Cox-2 gene (Cox-2loxmice) and mice in which Cox-2 exons 4 and 5 are flanked by loxP sites for conditional knockout (Cox-2fl/mice) were generated as described previously (19,20). Ly5.1cre mice (B6.129P2-Ly5.1mmtm1SorJ) and VillinCre transgenic mice (Tg(Fv-cre997Gum1F)) were purchased from Jackson Laboratory (Bar Harbor, MA). The VECadCreERT2 mice was provided by Dr Luisa Iruela-Arispe, University of California, Los Angeles (21). Animal experiments were carried out with the approval of the University of California, Los Angeles Animal Research Committee.
Mouse models of colitis

Twelve-week-old mice received 2.5% DSS (molecular weight, 36,000–50,000; MP Biomedicals, Solon, OH) in their drinking water for 8 days prior to killing. Body weight was measured each day during the DSS treatment; weight change was calculated as the percentage change compared with the weight prior to DSS treatment. Stool consistency was monitored and occult blood in the stool was tested daily using Hemocult cards (Beckman Coulter Inc., Fullerton, CA).

To assess the extent of colitis, body weight, stool consistency and Hemoccult results were scored as follows (22). Weight loss: 0, no weight loss; 1, 1–5%; 2, 5–10%; 3, 10–20% and 4, >20%. Stool consistency: 0, well-formed pellets; 2, pasty and semi-formed stools that did not stick to anus and 4, liquid stools that did stick to the anus. Hemoccult bleeding measurement: 0, no blood in hemoccult; 2, positive hemoccult and 4, gross bleeding. Scores for each category are added for each mouse and divided by 3 (from 0.0 for healthy to 4.0 for maximal activity of colitis) to obtain the final clinical score. After DSS treatment, the colons were isolated, rinsed with phosphate-buffered saline, filled with 4% paraformaldehyde and opened longitudinally for histological examination.

Detection of luciferase activity

For ex vivo colon imaging, mice were anesthetized by intraperitoneal administration of a ketamine (80 mg/kg; Phoenix Pharmaceutical, St Joseph, MO) and xylazine (4 mg/kg; Phoenix Pharmaceutical) mixture. Anesthetized mice were injected intraperitoneally with n-luciferin (125 mg/kg; Caliper Life Sciences, Hopkinton, MA) and placed in the light-tight box of the IVIS 100 imaging system (Caliper Life Sciences). Whole body 1 min images were acquired repeatedly. After the photon number during the 1 min scans reached a maximum, the mice were killed and the colons were rapidly excised. Isolated colons were placed on culture dishes and imaged with the IVIS system. Collected photon number and images were analyzed using LIVING IMAGE software (Caliper Life Sciences).

Histology and immunohistochemistry

Mouse colon tissues fixed in 4% paraformaldehyde were paraffin-embedded and sectioned on a 4 µm thickness. The sections were either stained with hematoxylin and eosin or processed for immunostaining.

The cryt damage was scored as follows for hematoxylin- and eosin-stained sections (Figure 3C): grade 0 = intact crypt; 1 = loss of the basal one-third of the crypt; 2 = loss of the basal two-third of the crypt; 3 = entire loss of crypt and 4 = loss of crypt and surface epithelium (22).

COX-2 was detected with polyclonal anti-COX-2 antibody (Thermo Scientific, Woodlands, TX). To detect macrophages, rat monoclonal antibody for F4/80 (Serotec, Oxford, UK) was used. To detect endothelial cells, rat anti-mouse CD34 (BD Biosciences, San Diego, CA) was used. Staining signals were visualized by using dually labeled Alexa Fluor 594- or Alexa Fluor 488-conjugated secondary antibodies (Molecular Probes, Eugene, OR). To detect epithelial cells, monoclonal antibody for pan-keratin conjugated with Alexa Fluor 488 (Cell Signaling Technology, Danvers, MA) was used.

Isolation of peritoneal macrophage

Mice were injected intraperitoneally with 3 ml of 3% thioglycolate medium. Media from peritoneal macrophage cultures were collected and assayed for prostaglandin E2 (PGE2) analysis; cells were lysed for western blotting.

To analyze COX-2 expression in the mouse (20). Consistent with the previous report (24), 3 days of DSS treatment (2.5% in drinking water) created ulcers in the descending colon and rectum of Cox-2−/− heterozygous knock-in mice (Figure 1A). These inflamed areas are clearly visualized by ex vivo imaging of Cox-2 promoter-driven luciferase activity (Figure 1B).

Strong COX-2 immunohistochemical staining has been shown in colonic epithelium and lamina propria cells of mice treated with DSS (16). To determine the cell types, which express COX-2 in our experiments, double immunohistochemical staining of COX-2 and cell type-specific markers was performed on inflamed colons from wild-type mice treated for 8 days with DSS (Figure 1C–E). Colon sections were stained for COX-2 along with F4/80, a representative macrophage marker (Figure 1C), CD34, an endothelial cell marker (Figure 1D) or pan-keratin, an epithelial cell marker (Figure 1E). Doubly stained cells were detected for all these marker-positive cells. These costaining experiments demonstrate that COX-2 expression is induced in multiple cell types in the colon, including (but not necessarily limited to) macrophages, endothelial cells and epithelial cells, after DSS administration.

Myeloid cell-specific Cox-2 deletion exacerbates DSS-induced colitis

It is clear from studies with DSS-treated Cox-2 knockout mice that eliminating COX-2 function in all cells of the body exacerbates inflammatory colitis (4). However, we cannot determine, by using either conventional knockout mice or pharmacological inhibitors, in which cells COX-2 is expressed at the start site of translation of the endogenous COX-2 allele, in which the firefly luciferase reporter enzyme is expressed at the start site of translation of the endogenous COX-2 gene, to analyze COX-2 transcriptional activity in the mouse (20). Consistent with the previous report (24), 3 days of DSS treatment (2.5% in drinking water) created ulcers in the descending colon and rectum of Cox-2−/− heterozygous knock-in mice (Figure 1A). These inflamed areas are clearly visualized by ex vivo imaging of Cox-2 promoter-driven luciferase activity (Figure 1B).
Cell type specific Cox-2 deletion in murine colitis

Cell type loss of COX-2 function elicits exaggerated colitis. To identify the cell type(s) in which loss of COX-2 function enhances the colitis response to DSS, mice with a Cox-2 conditional knockout allele (Cox-2flox) in which Cox-2 exons 4 and 5 are flanked by loxP sequences (19) were crossed with appropriate Cre-expressing mice and offspring were treated with DSS. Because the macrophage is one of the cell types in which COX-2 expression is highly induced in a number of inflammatory conditions, we first crossed Cox-2flox mice with LysMCre knock-in mice. LysMCre mice express Cre recombinase in myeloid cell lineages, including macrophages (25). To confirm Cre-mediated recombination of loxP sites, peritoneal macrophages were isolated from Cox-2flox/flox;LysMCre/+ and littermate Cox-2flox/flox mice and recombination at the Cox-2flox locus was examined by genomic Southern hybridization (Figure 2A). Shift of the Cox-2 band to the lower (8 kb) position indicates nearly complete excision of the loxP-flanked sequence (19) in Cox-2flox/flox;LysMCre/+ macrophages.

COX-2 expression in peritoneal macrophages is highly induced in response to LPS administration; LPS treatment of littermate Cox-2flox/flox macrophages express substantially elevated COX-2 protein levels (Figure 2B). In contrast, COX-2 protein expression in response to LPS is eliminated in Cox-2flox/flox;LysMCre/+ macrophages. Production of PGE2 following LPS stimulation of macrophages requires COX-2 protein synthesis. However, in Cox-2flox/flox;LysMCre/+ macrophages, PGE2 accumulation induced by LPS treatment is eliminated (Figure 2C). To confirm knockout of the Cox-2 gene in colon macrophages of DSS-treated Cox-2flox/flox;LysMCre/+ mice, sections from the colons of mice receiving DSS for 8 days were costained with anti-COX-2 and anti-F4/80 (macrophage marker) antibodies (Figure 3D). Double stained cells are observed in the inflamed colons of Cox-2flox/flox control mice. In contrast, in the colons of Cox-2flox/flox;LysMCre/+ mice, F4/80:COX-2-positive cells are substantially reduced, indicating successful cell type-specific disruption of the Cox-2 gene.

Low-dose (2.5%) DSS treatment causes significant body weight loss and elevated clinical scores in Cox-2 global knockout mice when compared with wild-type control mice (4). To determine whether loss of COX-2 expression in myeloid cells contributes substantially to this phenotype, Cox-2flox/flox;LysMCre/+ myeloid Cox-2-knockout mice and Cox-2flox/flox littermate controls were treated with 2.5% DSS. Compared with littermate control, Cox-2flox/flox mice without Cox-2 deletion, Cox-2flox/flox;LysMCre/+ mice showed significantly greater

Fig. 1. Detection of Cox-2-expressing cells in colons of DSS-treated mice. (A and B) Ex vivo imaging of luciferase expression in the colon of a Cox-2luc/+ mouse after 8 days of DSS treatment. Photo (A) shows a fixed, opened, methylene blue stained inflamed colon. Arrowheads indicate areas exhibiting ulceration. The color overlay on image (B) illustrates the photons per second emitted from the colon, as shown in the pseudocolor scale. (C–E) Immunofluorescent detection of COX-2-expressing cells. Dual-color immunofluorescent analyses of inflamed colons from DSS-treated wild-type mice were performed with anti-COX-2 antibody (red) and antibody to (C) F4/80 for macrophage (green), (D) CD34 for endothelial cells (green) and (E) pan-keratin for epithelial cells (green). Examples of double stained cells (yellow) are indicated by arrowheads.
loss of weight (Figure 3A) and higher clinical scores (Figure 3B). The degree of crypt epithelial damage was determined by histological examination (Figure 3C and D). At day 8, increased areas of colon show ulceration (Figure 3C), and the crypt epithelial damage score is higher (Figure 3D) in Cox-2flox/flox;LysMCre/Cre mice. EdU-labeled proliferating cells is significantly lower in Cox-2flox/flox;LysMCre/Cre mice (Figure 3G and H). This difference in apoptotic cells is not seen at later time points following DSS treatment (data not shown), when a substantial difference is observed in colon epithelial cell proliferation.

**Endothelial cell-specific Cox-2 deletion exacerbates murine DSS-induced colitis**

Immunofluorescent staining demonstrated that endothelial cells also express Cox-2 after DSS treatment (Figure 1D). To examine the role of endothelial cell COX-2 in DSS-induced colitis, Cox-2flox/flox mice were crossed with VECadCreERT2Tg mice. These mice express a tamoxifen-inducible Cre recombinase (CreERT2) transgene under the regulation of the vascular endothelial cadherin (VECad) promoter (21). CreERT2 was activated by tamoxifen injection in adult animals, to avoid the Cre recombination of Cox-2flox alleles in cells of the hematopoietic lineage (21), including myeloid cells. Recombination of the Cox-2flox allele was examined in endothelial cells isolated from livers of tamoxifen-treated Cox-2flox/flox;VECadCreERT2Tg mice and littermate Cox-2flox/flox mice. Because the numbers of recovered cells were not sufficient for genomic Southern hybridization, recombination was examined by polymerase chain reaction. The Cre-excised product was detected in the endothelial cell-enriched fraction from tamoxifen-treated Cox-2flox/flox;VECadCreERT2Tg mice (Figure 4A). To exclude the possibility of recombination of the Cox-2flox allele in myeloid cells in Cox-2flox/flox;VECadCreERT2Tg mice, peritoneal macrophages were analyzed by genomic Southern hybridization (Figure 4B). In contrast to the macrophage from Cox-2flox/flox;LysMCre/Cre mice (Figure 2A), little or no recombination was detected in Cox-2flox/flox;VECadCreERT2Tg macrophage (Figure
Fig. 3. DSS-induced colitis in myeloid cell-specific Cox-2-knockout mice. (A) Body weight change during 8 days of 2.5% DSS administration. Cox-2\textsuperscript{flox/flox;LysM\textsuperscript{Cre/+}} mice (grey line, n = 7) loose significantly more weight than do littermate Cox-2\textsuperscript{flox/flox} mice (black line, n = 7, P < 0.01 by two-way analysis of variance analysis). Asterisks indicate the time points at which weights are significantly different by the Wilcoxon test. (B) Clinical scores during 8 days of 2.5% DSS administration. Cox-2\textsuperscript{flox/flox;LysM\textsuperscript{Cre/+}} mice (grey line, n = 7) showed significantly higher clinical scores when compared with littermate Cox-2\textsuperscript{flox/flox} mice (black line, n = 7). P < 0.01 by two-way analysis of variance analysis. (C) Representative hematoxylin- and eosin-stained colon sections of each crypt damage grade, as described in Materials and Methods, following treatment for 8 days with DSS. The percentage distribution of damaged area in each grade is plotted for littermate Cox-2\textsuperscript{flox/flox} and Cox-2\textsuperscript{flox/flox;LysM\textsuperscript{Cre/+}} mice. (D) Total crypt damage is calculated from each score and the distribution percentage. Averages ± SE are shown. Cox-2\textsuperscript{flox/flox;LysM\textsuperscript{Cre/+}} mice show significantly higher scores by t-test (P < 0.01) compared with littermate Cox-2\textsuperscript{flox/flox} mice. (E) Representative images of EdU-labeled epithelial cells in colon sections from Cox-2\textsuperscript{flox/flox;LysM\textsuperscript{Cre/+}} and littermate Cox-2\textsuperscript{flox/flox} mice treated for 5 days with 2.5% DSS. (F) Number of EdU-positive cells (10 fields per mouse) in colon sections. Averages ± SE are shown. The number of proliferating cells is significantly lower in colon sections from Cox-2\textsuperscript{flox/flox;LysM\textsuperscript{Cre/+}} mice than in colon sections from littermate Cox-2\textsuperscript{flox/flox} mice (P < 0.01 by t-test). (G) Apoptotic epithelial cells in the colon crypts were determined by TUNEL assay. Representative images are shown for colon sections from mice treated for 3 days with 2.5% DSS. TUNEL-positive cells are observed only sparsely in the Cox-2\textsuperscript{flox/flox} colon (arrowhead). In contrast, clusters of apoptotic cells are detected in the Cox-2\textsuperscript{flox/flox;LysM\textsuperscript{Cre/+}} colon (bracket). (H) Number of TUNEL-positive cells (10 fields per mouse) in colon sections. Averages ± SE are shown. The number of apoptotic cells is significantly greater in colon sections from Cox-2\textsuperscript{flox/flox;LysM\textsuperscript{Cre/+}} mice than in colon sections from littermate Cox-2\textsuperscript{flox/flox} mice (P < 0.01 by t-test).
indicate endothelial cell-specific Cox-2 knockout, with normal COX-2 expression in myeloid cells, in Cox-2\textsuperscript{2lox/lox;VECadCreERT2\textsuperscript{+}} mice.

Both body weight loss (Figure 5A) and clinical scores (Figure 5B) were significantly exacerbated in response to DSS-induced colitis in endothelial cell-specific Cox-2-knockout Cox-2\textsuperscript{2lox/lox;VECadCreERT2\textsuperscript{+}} mice when compared with littermate Cox-2\textsuperscript{2lox/lox} mice. In addition, histological crypt damage is significantly higher in Cox-2\textsuperscript{2lox/lox;VECadCreERT2\textsuperscript{+}} mice than in littermate Cox-2\textsuperscript{2lox/lox} mice (Figure 5C and D).

To examine the possibility that an endothelial cell-specific Cox-2 knockout, like the myeloid-specific Cox-2 knockout, affects epithelial cell proliferation following DSS insult, Cox-2\textsuperscript{2lox/lox;VECadCreERT2\textsuperscript{+}} and littermate Cox-2\textsuperscript{2lox/lox} mice were treated with DSS for 5 days and then injected with BrdU 2 h before killing. In DSS-treated Cox-2\textsuperscript{2lox/lox} littermate mice, areas with clusters of BrdU-positive epithelial cells are observed (Figure 5E). In contrast, similar areas are not observed in colon sections of DSS-treated Cox-2\textsuperscript{2lox/lox;VECadCreERT2\textsuperscript{+}} mice, although a small number of BrdU-positive cells are present (Figure 5E). Overall, the labeling index through mid to distal colon is significantly lower in Cox-2\textsuperscript{2lox/lox;VECadCreERT2\textsuperscript{+}} mice when compared with littermate Cox-2\textsuperscript{2lox/lox} mice (Figure 5F).

We also examined the number of apoptotic cells at 3 days and 5 days after DSS treatment. In contrast to the results with Cox-2\textsuperscript{2lox/lox;VillinCreTg} mice, no statistically significant difference in TUNEL-positive cells was observed between Cox-2\textsuperscript{2lox/lox;VECadCreERT2\textsuperscript{+}} mice and littermate Cox-2\textsuperscript{2lox/lox} mice at these two time points (Figure 5G).

Epithelial cell-specific Cox-2 deletion does not exacerbate murine DSS-induced colitis

COX-2 expression is also induced in epithelial cells by DSS [Figure 1E and ref. (16)]. To examine the role of epithelial cell COX-2 in DSS-induced colitis, Cox-2\textsuperscript{2lox/lox} mice were crossed with VillinCre transgenic mice. VillinCre mice express Cre recombinase throughout the entire intestinal epithelium (27). Recombination of the Cox-2\textsuperscript{2lox} allele in Cox-2\textsuperscript{2lox/lox;VillinCreTg} mice was confirmed in epithelial cells dissociated from colon by ethylenediaminetetraacetic acid (Figure 6A). In contrast to the results for Cox-2\textsuperscript{2lox/lox;LysMCre/+} mice and Cox-2\textsuperscript{2lox/lox;VECadCreERT2\textsuperscript{+}} mice, Cox-2\textsuperscript{2lox/lox;VillinCreTg} mice and control Cox-2\textsuperscript{2lox/lox} mice were treated with 2.5% DSS displayed the same levels of body weight loss (Figure 6B) and clinical scores (Figure 6C), indicating that Cox-2 expressed in epithelial cells is not involved in development of DSS-induced colitis.

Discussion

COX-2 levels are elevated in a variety of pathophysiological conditions, including (but not limited to) acute and chronic inflammation, neurodegenerative diseases, cancer, ischemia and pain (28,29). The causal role of COXs in general and COX-2 in particular in these conditions is demonstrated by their responses to NSAIDs and COX-2 inhibitors, respectively. The use of mice with a global Cox-2 gene deletion has given researchers an additional investigative tool that permits analysis of the consequences of unequivocal removal of the function of the COX-2 protein, eliminating problems of drug accessibility, pharmacokinetics and off-target effects.

In many—if not most—of the disorders cited above, COX-2 levels are elevated either simultaneously or sequentially in a variety of cell types within the affected tissues. The role of COX-2 and its products produced by distinct cell types—e.g. stromal cells, immune cells, epithelial cells and endothelial cells—in the progression and/or resolution of these conditions cannot be determined either by pharmacological means or by global Cox-2 gene deletion; both cause indiscriminant (with respect to cell type) inhibition/elimination of COX-2, excluding any information on the distinct role(s) of COX-2-dependent prostanoids produced by alternative cell types. To investigate cell-specific roles of COX-2, we created mice carrying the Cox-2\textsuperscript{2lox} allele (19). COX-2 production can be eliminated in specific cell types in Cox-2\textsuperscript{2lox/lox} mice by crossing with mice in which Cre

4B). To confirm the cell type specificity of Cox-2 deletion in the colon of DSS-treated Cox-2\textsuperscript{2lox/lox;VECadCreERT2\textsuperscript{+}} mice, colon sections from mice treated with DSS for 8 days were double stained with anti-COX-2 and either anti-CD34 or anti-F4/80. As expected, blood vessel cells were stained with both COX-2 and CD34 antibodies in the colon of Cox-2\textsuperscript{2lox/lox} littermates. However, CD34-positive endothelial cells are negative for COX-2 in Cox-2\textsuperscript{2lox/lox;VECadCreERT2\textsuperscript{+}} colon (Figure 4C). In contrast, F4/80\textsuperscript{+} macrophages are observed in both Cox-2\textsuperscript{2lox/lox;VECadCreERT2\textsuperscript{+}} and Cox-2\textsuperscript{2lox/lox} littermate colons, as expected (Figure 4D). These staining patterns, like genomic analyses from isolated cells (Figure 4A and B),
recombinase is expressed from appropriate promoters, and the consequences of cell type-specific COX-2 inactivation can be analyzed.

Clinical colitis and its animal model, DSS-induced colitis, are unusual in that pharmacological and genetic data suggest that COX-2 is protective against an inflammatory insult (15,16). COX-2 expression is elevated in DSS-induced colitis in a variety of cell types, including myeloid cells, endothelial cells and epithelial cells. For example, DSS-induced COX-2 elevation in rats is observed in smooth muscle, blood vessels, infiltrating cells in the submucosa and in macrophages (5). In mice, the number of lamina propria mononuclear cells, epithelial cells and macrophages expressing COX-2 increases during DSS-induced inflammation (15,16). In IBD patients, elevated COX-2 expression is also observed in epithelial cells and inflammatory cells (30,31), and a relationship between endoscopic activity and the level of COX-2 messenger RNA has been demonstrated (32). Distinctions in the cell types in which COX-2 elevation is observed during colon inflammation may result from differences in the nature of the model system, the extent of injury, the time of analysis and/or in

Fig. 5. DSS-induced colitis in endothelial cell-specific Cox-2floxflox;VECadCreERT2Tg-knockout mice. (A) Weight change during 2.5% DSS administration. Cox-2floxflox;VECadCreERT2Tg mice (n = 10) lose significantly more weight than littermate Cox-2floxflox mice (n = 11, P < 0.01, two-way analysis of variance analysis). Asterisks indicate points with significantly different weights (Wilcoxon test, P < 0.01). (B) Clinical scores during 2.5% DSS administration. Cox-2floxflox;VECadCreERT2Tg mice (n = 10) showed significantly higher scores compared with littermate Cox-2floxflox mice (n = 11, P < 0.01, two-way analysis of variance analysis). Asterisks indicate times with significantly different scores (Wilcoxon test, P < 0.01, respectively). (C) Percentage distribution of damaged area of each grade, determined from hematoxylin- and eosin-stained sections, are plotted for Cox-2floxflox; VECadCreERT2Tg and littermate Cox-2floxflox mice. (D) Total crypt damage scores. Averages ± SE are shown. Cox-2floxflox;VECadCreERT2Tg mice show significantly higher scores (t-test, P < 0.01) compared with littermate Cox-2floxflox mice. (E) Representative images of BrdU-labeled epithelial cells in colon sections from mice treated for 5 days with 2.5% DSS. Clusters of BrdU-positive cells are observed at the bottom half of crypts in the colon of littermate Cox-2floxflox mice (asterisk). In contrast, positive cells are sparsely distributed in the colon of Cox-2floxflox; VECadCreERT2Tg mice (arrowheads). (F) Number of BrdU-positive cells (10 fields per mouse) in colon sections. Averages ± SE are shown. The number of proliferating cells is significantly lower in colon sections from Cox-2floxflox; VECadCreERT2Tg mice than in colon sections from littermate Cox-2floxflox mice (P < 0.01 by t-test). (G) Number of TUNEL-positive cells (10 fields per mouse) in colon sections of mice treated with 2.5% DSS for 3 days and for 5 days. Averages ± SE are shown. No statistically significant difference is observed between colon sections from Cox-2floxflox; VECadCreERT2Tg mice and littermate Cox-2floxflox mice (P > 0.05 by t-test).
immunohistochemical procedures. We also found that elevation of COX-2 expression occurs in epithelial cells, endothelial cells and macrophages in response to DSS-induced colitis in mice, leading to our initial choices for targeted COX-2 deletion studies.

Epithelial cells destroyed in the intestinal epithelium following DSS treatment are replaced by proliferation and differentiation of precursor cells. Our results demonstrate that the signals for this proliferative response depend on COX-2 production by myeloid/macrophage cells and by endothelial cells. In contrast, signals for proliferation of epithelial cell precursors in the damaged colon do not depend on COX-2 production in the remaining epithelial cells.

Macrophages play a required role in the epithelial repair process following DSS-induced colon damage (33). This repair process also requires recognition of commensal microflora by the innate immune system (34). Compensatory proliferation of epithelial cells after DSS-induced injury, following recognition of microflora, is mediated by bacterially derived endotoxin stimulation of toll-like receptors and is thought to be mediated by toll-like receptor- and MyD88-dependent nuclear factor-kappaB activation (16,33–35). COX-2 expression induced by DSS injury is also dependent on commensal bacteria (33) and the presence of toll-like receptor 4 (16). Greten et al. (36) report that myeloid-specific inhibition of nuclear factor-kappaB activation reduces DSS-induced COX-2 expression. The results presented here also suggest that macrophage COX-2 plays a critical role both in macrophage-dependent protection of epithelial cells from apoptosis following DSS-induced colon damage and in macrophage promoted epithelial cell precursor proliferation to repair the damaged colon.

Exogenous PGE2 protects against DSS-induced colitis (15). Global deletion of the EP4 PGE2 receptor exacerbates DSS-induced colitis (17) also suggesting that PGE2 protects the epithelium against this inflammatory insult. DSS-induced epithelial injury permits exposure of both epithelial cells of the intestine and macrophages of the lamina propria to commensal bacteria, leading to stimulation of COX-2 production and elevated PGE2 production (37). Although COX-2 is increased both in colon epithelial cells and in macrophages in response to DSS treatment (Figure 1), the biological consequences of COX-2 expression/PGE2 production in macrophages versus epithelial cells has not previously been determined. Our data suggest that DSS-induced COX-2 production in epithelial cells does not play a role in ameliorating intestinal damage. In contrast, macrophage COX-2 induced by the same insult is required both to protect against colitis damage and to enhance recovery.

We find that specific loss of COX-2 expression in endothelial cells also exacerbates DSS-induced colitis. Although several reports suggest endothelial cell involvement in DSS-induced colitis (38–43), a role for endothelial cell COX-2 expression has only recently been considered (44,45). These reports discuss COX-2 only in the context of suppression of a number of factors that modulate inflammatory responses. Prostacyclin (PGI2) is the major prostanoid generated in endothelial cells (46). However, global deletion of the PGI2 (IP) receptor does not reduce DSS-induced colitis (17); suggesting less involvement of PGI2 and, perhaps, a role for another prostanoid product from endothelial cells, in protection against colitis.

Although we found that COX-2 elimination in both myeloid cells and endothelial cells reduced the proliferative response of epithelial cells in DSS-treated mice, we observed a difference in the apoptotic responses of these two cell-specific Cox-2-knockout mice. At present, this difference remains unexplained. It is possible that this difference is due to minor differences in genetic background resulting from the introduction of the Cre recombinase gene into the Cox-2flox/flox recipients; the phenotype of DSS colitis is affected by genetic background (14). For this reason, we have used littermate Cre-negative Cox-2flox/flox mice as controls. Although this makes it difficult to compare quantitative values among groups of mice with different tissue-specific Cox-2 deletions, it seems clear that the role of induced endothelial cell COX-2 expression and its prostanoid products in colitis deserves additional attention. For similar reasons, we have not tried to draw comparisons in the extent of colitis between mice with cell type-specific Cox-2 targeted gene deletions and mice with global systemic Cox-2 gene deletions.

Pharmacological data and studies with global Cox-2-knockout mice demonstrate that COX-2 plays a causal role both in spontaneous human colon cancer and in the most commonly used murine model of spontaneous colon cancer induced in mice by repeated intraperitoneal azoxymethane injection (11,47). Similarly, both pharmacological data and Cox-2-knockout studies demonstrate a causal role for COX-2 both in Familial Adenomatous Polyposis (one form of hereditary colon cancer) and its murine analogue (48,49). In contrast, studies with Cox-2-knockout mice demonstrate that the murine model of colon cancer most closely associated with inflammation, azoxymethane-initiated/DSS-promoted colon cancer, does not require COX-2 expression (11). Thus, the mechanism(s) of tumor promotion for spontaneous and hereditary colon cancers differ(s) from that of colitis-associated colon cancer.

COX-2 protein is constitutively expressed in a variety of human epithelial carcinomas. However, during the beginning of tumor development, COX-2 expression is observed mainly in stromal cells, including fibroblasts, myofibroblasts, endothelial cells and myeloid cells, both in adenomas and in adenocarcinomas of human cancers and in murine epithelial cancer models (47). Conditional deletion of the Cox-2 gene in alternative cell types, using the Cox-2flox/flox mouse, will provide a means to determine in which of these various stromal cells the COX-2-dependent promotion event(s) in epithelial cancer progression necessary. In particular, cell type-specific COX-2 deletions, using the Cox-2flox/flox mouse, in spontaneous versus hereditary murine colon cancer models should permit us to determine if the COX-2-driven promotion/progression event is provided by the same cell type, or by different cell types, in these two routes to murine colon cancer.
In many human pathologies and murine disease models, induced COX-2 expression is observed in more than one cell type. In this study, we showed a requirement for COX-2 expression in myeloid and endothelial cells, but not in epithelial cells, for protection against colitis. In a mouse hindlimb model of vascular insufficiency, ischae mia-induced revascularization is impaired in mice with a Tie2Cre endothelial cell-specific COX-2 knockout (50). Similar approaches using COX-2 conditional knockout mice will be valuable in analyzing the cell-specific roles of COX-2 expression in murine models of inflammatory diseases, neurodegenerative diseases and cancer progression in a variety or organs.

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References

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