Gastrointestinal, Hepatobiliary and Pancreatic Pathology

Predisposition to Colorectal Cancer in Rats with Resolved Colitis

Role of Cyclooxygenase-2-Derived Prostaglandin D₂

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Colitis markedly increases the risk of developing colon cancer, but the underlying mechanisms are not fully understood. In a rat model of colitis, alterations in epithelial secretion, proliferation, and barrier function persist long after healing has occurred. In the present study, we examined whether rats that have recovered from a bout of colitis are more susceptible to preneoplastic lesions and whether this susceptibility is mediated by cyclooxygenase (COX)-2-derived prostaglandin (PG) D₂. Colitis was induced by intracolonic administration of trinitrobenzenesulfonic acid. Six weeks later, weekly treatment with the carcinogen azoxymethane was initiated. Postcolitis rats exhibited significantly more aberrant crypt foci after azoxymethane treatment than controls. The postcolitis rats also exhibited markedly increased colonic PGD₂ synthesis and elevated COX-2, H-PGD synthase, and β -catenin expression. Treatment for 1 week with a selective COX-2 inhibitor or with a selective PGD₂ receptor (DP1) antagonist significantly reduced susceptibility of postcolitis rats to aberrant crypt foci development, β -catenin expression, and mucosal thickness. The results from this animal model suggest that prolonged elevation of COX-2-derived PGD₂ synthesis after resolution of colitis may contribute significantly to colitis-associated increases in colon cancer incidence. PGD₂ may therefore represent a rational target for therapies directed at reducing the incidence of colitis-associated colorectal cancer. (*Am J Pathol* 2005, 167:1293–1300)

Ulcerative colitis is a chronic inflammatory condition of the digestive tract. Patients with colitis are at increased risk of developing colorectal cancer, with the incidence increasing with the duration of colonic inflammation.¹ The mechanisms through which inflammation of the colonic mucosa predisposes an individual to neoplastic changes are not clear. A genetic basis to explain colorectal cancer predisposition in these patients has not been identified.¹ However, the high levels of inflammatory mediators being produced in a setting of colitis may in some way elevate the occurrence and progression of colon cancer.² Mechanisms that could underlie these effects might include induction of genetic mutations, increased crypt cell proliferation, changes in crypt cell metabolism, and alterations in epithelial barrier function.³

The intestinal epithelium serves as a protective barrier separating luminal contents from the underlying tissue compartments. Numerous studies have documented impairment of epithelial secretion and epithelial barrier function during acute intestinal inflammation,^{4–7} and we have demonstrated that such dysfunction persists long after resolution of the inflammatory response.^{8–10} In addition to epithelial hyposecretion in response to a number of secretagogues, we observed significant increases in bacterial translocation, despite a lack of detectable change in epithelial permeability to small molecular weight markers.^{9,10} Moreover, we observed a marked increase in expression of cyclooxygenase (COX)-2 in the colon after resolution of colitis, along with a substantial increase in prostaglandin (PG) D₂, but not E₂, synthe-

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sis.¹⁰ COX-2 and PGD₂ have been implicated as important mediators of the resolution of inflammation in the colon^{11,12} and in other tissues.¹³ Interestingly, we observed that COX-2-derived PGD₂ synthesis contributed to the altered epithelial secretion and bacterial translocation that persisted after resolution of colitis.¹⁰ Indeed, treatment with a selective COX-2 inhibitor reduced PGD₂ synthesis to normal levels and reversed the alterations in epithelial secretion and bacterial translocation.¹⁰

Selective COX-2 inhibitors and nonselective nonsteroidal anti-inflammatory drugs have been suggested to reduce the incidence of colon cancer in humans and in experimental models.^{14,15} The increased COX-2 expression (and PGD₂ synthesis) that persists after resolution of colitis in the rat may contribute to a predisposition to neoplastic changes in the colon. We tested this hypothesis in the present study, exploiting the ability of azoxymethane (AOM) to induce precancerous lesion formation (ie, aberrant crypt foci; ACF) in the colon. We first determined if rats that previously had colitis (induced by intracolonic trinitrobenzene sulfonic acid; TNBS)¹⁶ exhibited an increased susceptibility to such lesion formation relative to healthy controls. ACF are a well-established marker of the early stages of colon cancer development in rodents¹⁷ and humans.¹⁸ They are characterized by dysplastic or hyperplastic crypts, and subsequent expansion generates larger adenomas, which in turn may proceed to carcinoma.¹⁹ We then evaluated the potential contribution of COX-2 and PGD₂ to the predisposition of postcolitis rats to colon cancer.

Materials and Methods

Animals

Male, Wistar rats (175 to 200 g) were obtained from Charles River Breeding Farms (Montreal, PQ, Canada). The rats were allowed free access to standard laboratory rodent chow and tap water. All experiments were approved by the Animal Care Committee of the University of Calgary and were performed in accordance with the guidelines of the Canadian Council on Animal Care.

Induction of Colitis

Colitis was induced as previously described.^{9,10} Briefly, rats were lightly anesthetized with halothane and an infant feeding tube fitted onto a blunt 18-gauge needle was inserted rectally. The tip of the tube was placed ~8 cm into the colon and 30 mg of 2,4,6-trinitrobenzene sulfonic acid (TNBS) in 0.5 ml of 50% ethanol was instilled. Age-matched rats given an equivalent volume of 0.9% saline, or in some cases 50% ethanol, served as controls. At 6 weeks after induction of colitis, the rats were anesthetized with halothane and then euthanized by cervical dislocation. In previous studies we found that colitis had resolved by 6 weeks after TNBS administration; that is, the macroscopic appearance of the colon, colonic myeloperoxidase activity (a marker of granulocyte infiltration), and colonic prostaglandin E₂ synthesis were no longer differ-

ent from those in healthy controls.^{9,10} In the present study, the severity of colonic damage was blindly scored on a 0 to 10 scale using criteria that have been previously reported in detail.²⁰ Briefly, a score of 0 represents normal appearance, a score of 1 is given for focal hyperemia but no ulcers, a score of 2 is given for ulceration without associated inflammation, and a score of 3 or greater is given when ulceration and inflammation are both evident (the score increasing further with the extent of ulceration). In the vast majority of cases, the colonic damage score in rats sacrificed 6 weeks after TNBS administration was 0 or 1. In rare cases, ulceration of the colon was observed. However, because this study was focused on resolved colitis, any rat with a colonic damage score of greater than 1 was excluded from further analysis. After scoring, a sample of the distal colon was frozen for subsequent Western blot analysis of expression of COX-2 and hematopoietic PGD (H-PGD) synthase, two of the key enzymes involved in PGD₂ synthesis. There are two known forms of PGD synthase: lipocalin-type and hematopoietic. Although the former is expressed in the central nervous system and male genital organs of various mammals, H-PGD synthase is widely expressed in peripheral tissues, as well as in antigen-presenting cells, mast cells, and megakaryocytes.

It has been reported that β -catenin is a key participant in a signaling cascade critical in the initiation and progression of colon cancer.^{21,22} We therefore examined the expression of β -catenin in the colon of healthy and postcolitis rats by Western blot. For all Western blots, expression of β -actin was used as a housekeeping control. Additional colonic tissue from each rat was fixed in neutral buffered formalin and processed by routine techniques for light microscopy. The slides were stained with hematoxylin and eosin and were examined by an observer unaware of the treatments the rats had received. In addition to making general observations on the tissue structure, the observer made measurements, using an ocular micrometer, of mucosal thickness of each specimen. At least four measurements were made on each section, at randomly selected regions. The mucosal thickness for each sample was taken as the mean of these measurements.

Prostaglandin Synthesis

Six weeks after intracolonic administration of TNBS or saline, groups of rats ($n = 10$ to 12 each) were treated with a selective COX-2 inhibitor (rofecoxib; 3 mg/kg p.o.) or vehicle (1% carboxymethylcellulose). One hour later, the rats were given the colon-specific carcinogen, AOM (15 mg/kg i.p.) or vehicle. The rats were anesthetized with halothane 3 hours later and euthanized by cervical dislocation. Colonic tissue was collected and processed for measurement of PGD₂, as described previously.¹⁰ The levels of the prostaglandin D₂ generated by the tissue samples were measured using a specific, commercially available enzyme-linked immunosorbent assay kit.¹⁰

Western Blotting

Colonic tissue was taken from the rats killed 6 weeks after intracolonic TNBS or saline administration [with or without administration of AOM (15 mg/kg) i.p. 3 hours before euthanasia] for examination of expression of various proteins by Western blotting. The samples were homogenized in lysis buffer (0.1% Triton X-100, 50 μ mol/L pepstatin-A, 0.2 mmol/L leupeptin, 1 μ g/ml aprotinin, 10 mg/ml phenylmethyl sulfonyl fluoride, 50 mmol/L Tris, 10 mmol/L ethylenediamine tetraacetic acid), then centrifuged (9000 $\times$ g). The protein concentration of the supernatant was determined by colorimetric assay (Bio-Rad, Hercules, CA). Thirty μ g of protein were separated on a 10% polyacrylamide gel and then transferred to a nitrocellulose membrane. The membrane was incubated for 1 hour with blocking buffer (20 mmol/L Tris, 100 mmol/L NaCl, 0.5% Tween 20, and 5% nonfat dried milk) and then probed overnight with antibodies against COX-2, PGD synthase (1:500 and 1:1000, respectively; Cayman Chemical, Ann Arbor, MI), β -catenin, (1:3000; BD Biosciences, Ontario, Canada), or β -actin (1:500; Santa Cruz Biotechnology, Santa Cruz, CA). The membrane was then incubated with an appropriate peroxidase-conjugated secondary antibody for 1 hour at room temperature. A chemiluminescence reagent (Amersham Life Sciences, Oakville, ON, Canada) was added to visualize the labeling according to the manufacturer's instructions. Densitometry was performed using a GS-710 calibrated imaging densitometer (Bio-Rad) and analyzed with Quantity One software (Bio-Rad).

Immunohistochemistry

Samples fixed in neutral buffered formalin were processed by routine techniques and then embedded in paraffin. The antibodies used were the same as those used for Western blotting (see above). After deparaffinization and rehydration, sections were placed in blocking solution (3% hydrogen peroxide) for 20 minutes. To reduce nonspecific binding of antibody, sections were incubated with normal goat serum for 30 minutes (Invitrogen Corp., Grand Island, NY). Slides were then incubated overnight with anti-COX-2 or anti-PGD synthase antibodies at dilutions of 1:200 and 1:300, respectively. After three washes in phosphate-buffered saline, the sections were incubated with a biotinylated second antibody for 60 minutes. The immunoreaction was visualized with diaminobenzidine peroxidase substrate kit (Vector Laboratories, Burlingame, CA). Negative controls were obtained by omitting the primary antibody. The sections, counterstained in Mayer's hematoxylin, were mounted and observed by light microscopy.

Induction of ACF

Beginning 6 weeks after TNBS administration, the rats were given four injections of AOM (15 mg/kg) at weekly intervals.²³ The rats were euthanized (pentobarbital overdose) 3 weeks after the final injection of AOM for

blind assessment of numbers of ACF, as described below. Additional groups of rats were treated only with 0.9% saline or 50% ethanol in place of TNBS and then received treatment with AOM as above. A laparotomy was performed and the entire colon was excised. After gentle flushing with 0.9% saline, the colon was tied at both ends with silk sutures and insufflated with 10% phosphate-buffered formalin (pH 7.4). After 1 to 2 hours the colons were opened along the mesenteric border and pinned flat, mucosal side up, then submerged in formalin for a further 24 hours. The tissues were then stained with 0.2% methylene blue. Using a dissecting microscope at $\times 40$ magnification, the number of ACF in the entire colon was determined by an observer unaware of the treatments the rats had received.²³ ACF are clearly discernable as abnormally dilated crypts, with multiple, adjacent crypts often appearing to be contiguous. The number of ACF per colon and the location of each focus were recorded.

Effects of Selective COX-2 Inhibition

To determine whether suppression of COX-2 activity throughout the course of a week would reduce the susceptibility of postcolitis rats to AOM-induced precancerous lesion formation, the following study was performed. Five weeks after TNBS administration, rats ($n \geq 10$ per group) were treated orally with rofecoxib (3 mg/kg) or vehicle (1% carboxymethylcellulose) at 12-hour intervals for 1 week. This dose of rofecoxib was previously found to selectively inhibit COX-2 in the rat.²⁴ Subgroups of five to six rats from each group were euthanized at the end of the treatment period for determination of colonic expression of COX-2, PGD synthase, and β -catenin, as well as for histological examination. The remaining rats were treated with AOM or vehicle ($n = 5$ to 8 per group), as described above, to determine the effects of rofecoxib on colitis-associated ACF formation.

Effects of DP Receptor Antagonism

Experiments were performed in exactly the same manner as the studies involving rofecoxib, except that rats ($n = 12$ per group) were treated with a DP1 receptor antagonist (BWA868c; 100 μ g/kg i.p.) or vehicle twice daily for 1 week. The selection of this dose was based on published studies demonstrating effective DP1 receptor blockade.²⁵

Statistical Analysis

All data are reported as mean $\pm$ SEM. Comparisons among groups of data were made using a one-way analysis of variance followed by the Student-Newman-Keuls test. An associated probability (P value) of less than 5% was considered significant.

Materials

TNBS was obtained from Fluka Chimika (Buchs, Switzerland). AOM was obtained from ACR Laboratories, Inc.

Figure 1. Histological appearance of colonic mucosa from control (A, C, and E) and postcolitis (B, D, and F) rats 6 weeks after intracolonic saline or TNBS administration, respectively. A and B show the morphological appearance, with an increase in mucosal thickness being evident in the postcolitis sample. The mucosa from the postcolitis rat is not inflamed and the epithelium is intact. C and D show immunostaining for COX-2. There is a clear increase in staining in the sample from a postcolitis rat. The staining is most evident in the surface epithelial cells. E and F show immunostaining for H-PGD synthase. There is increased expression of this enzyme in the postcolitis sample, both in the epithelium and in cells within the lamina propria (arrows). Scale bars, 100 μ m.

(San Antonio, TX). Enzyme-linked immunosorbent assay kits for PGD₂ were obtained from Cayman Chemical Co. (St. Louis, MO). All other reagents were obtained from VWR Scientific (Edmonton, AB, Canada).

Results

Macroscopic and Histological Appearance of Tissue after Colitis

As described previously,^{8–10} samples of colon from rats treated 6 weeks earlier with TNBS did not exhibit overt signs of inflammation or injury. The epithelium was intact (Figure 1A) and there did not appear to be any increase in leukocyte infiltration as compared to healthy controls. Indeed, colonic myeloperoxidase activity (a marker of granulocyte numbers) in postcolitis samples did not differ significantly from that in samples from healthy controls (4.0 ± 0.3 U/mg versus 3.8 ± 0.2 U/mg, respectively; $n = 12$ per group).

We previously reported an increase (~85%) in epithelial cell proliferation in the postcolitis colonic mucosa, as measured by BrdU staining.⁹ Consistent with that finding, we observed a significant increase in mucosal thickness

Figure 2. Mucosal thickness in samples of colon from age-matched, healthy controls and in postcolitis rats (6 weeks after intracolonic TNBS administration), and the effects of twice daily treatment with a selective COX-2 inhibitor (rofecoxib; 3 mg/kg) or a DP1 receptor antagonist (BWA868c; 100 μ g/kg i.p.), for the previous week. The colonic mucosa of postcolitis rats was significantly greater than that of controls ($***P < 0.001$). Treatment with rofecoxib significantly reduced mucosal thickness in the postcolitis rats ($*P < 0.05$), but it remained significantly greater than rofecoxib-treated controls. Treatment with BWA868c resulted in a reduction of mucosal thickness in postcolitis rats to levels not significantly different from healthy controls treated with vehicle. Each bar represents the mean $\pm$ SEM for at least five rats per group.

in the postcolitis rats as compared to healthy controls (Figure 1A and Figure 2). Twice daily treatment with rofecoxib for 1 week significantly reduced mucosal thickness in postcolitis rats, although the mucosa in these rats remained significantly increased in thickness as compared to healthy controls (Figure 2). Twice daily treatment with the DP1 receptor antagonist (BWA868c) for 1 week significantly reduced mucosal thickness in postcolitis rats, to levels not significantly different from healthy controls (Figure 2).

Colonic PGD₂ Synthesis

As reported previously, colonic PGD₂ synthesis was markedly elevated in postcolitis rats as compared to healthy controls (Figure 3). Rofecoxib reduced PGD₂ synthesis in postcolitis samples to control levels, indicating that COX-2 was its primary enzymatic source. Acute administration of the carcinogen, AOM, resulted in an increase in colonic PGD₂ synthesis in both control and postcolitis rats, but the increase was substantially greater in the latter group. The stimulation of PGD₂ synthesis by AOM could be substantially suppressed by pretreatment with rofecoxib (Figure 3).

PGD Synthase, COX-2, and β -Catenin Expression

The primary enzymes involved in the conversion of arachidonic acid to PGD₂ are COX (particularly COX-2, as

Figure 3. Effects of selective inhibition of COX-2 (rofecoxib; 3 mg/kg i.p.) on colonic synthesis of prostaglandin D₂ in postcolitis rats (6 weeks after intracolonic administration of TNBS) and in age-matched, healthy controls. Colonic PGD₂ synthesis was significantly greater in postcolitis rats than in controls (**P* < 0.05 versus vehicle-treated controls). Acute administration of AOM (15 mg/kg i.p.) significantly increased colonic PGD₂ synthesis in controls and in postcolitis rats, with the effect being much greater in the latter group. Pretreatment with rofecoxib 1 hour before taking tissue samples resulted in a significant decrease in colonic PGD₂ synthesis in every condition in which it was elevated above control levels (^ψ*P* < 0.05 versus corresponding vehicle-treated group). Results are expressed as the mean ± SEM of five to six rats per group.

the results above indicate) and PGD synthase. We examined the expression of COX-2 and H-PGD synthase in postcolitis rats versus healthy controls, and in the groups of rats also given the carcinogen, AOM, 3 hours before euthanasia. In the postcolitis rats, COX-2 expression, which was primarily observed in epithelial cells, was el-

Figure 4. Colonic expression of COX-2 in postcolitis rats (6 weeks after TNBS administration) and in age-matched, healthy controls. Subgroups of rats were treated intraperitoneally with AOM (15 mg/kg) 3 hours before the samples being taken. Representative Western blots are shown at the top. Densitometry data for five to nine rats per group are shown below the blots (mean ± SEM). **P* < 0.05 versus the corresponding control group. ^ψ*P* < 0.05 versus the corresponding vehicle-treated group.

Figure 5. Colonic expression of H-PGD synthase in postcolitis rats (6 weeks after TNBS administration) and in age-matched, healthy controls. Subgroups of rats were treated intraperitoneally with AOM (15 mg/kg) 3 hours before the samples being taken. Representative Western blots are shown at the top. Densitometry data for five to nine rats per group are shown below the blots (mean ± SEM). **P* < 0.05 versus the corresponding control group.

evated to 340% of control levels (*P* < 0.01; Figures 1B and 4). When control rats were given AOM, it did not cause a significant increase in COX-2 expression in the colonic tissue. Conversely, acute administration of AOM to postcolitis rats elicited an increase in COX-2 expression to 520% of control levels (Figure 4). Treatment with rofecoxib or BWA868c did not affect colonic expression of COX-2 in healthy controls or in postcolitis rats (data not shown).

Colonic expression of H-PGD synthase, seen in epithelial and some lamina propria cells, was also elevated in postcolitis rats versus controls (~2.6-fold; Figure 5). Unlike the case for COX-2, however, acute administration of AOM did not result in any further increase in H-PGD synthase expression. Treatment with rofecoxib or BWA868c did not affect colonic expression of PGD synthase in healthy controls or in postcolitis rats (data not shown).

β-Catenin expression in postcolitis rats was approximately double that in controls (Figure 6). Rofecoxib treatment resulted in a significant decrease in β-catenin expression in the colon of postcolitis rats, while not significantly affecting the expression in healthy controls. BWA868c treatment did not affect colonic expression of β-catenin in healthy controls or in postcolitis rats (data not shown).

ACF Formation in Healthy versus Postcolitis Rats

Repeated administration of AOM to saline-treated (healthy) rats resulted in the formation of an average of

Figure 6. Effects of selective inhibition of COX-2 on colonic expression of β -catenin in postcolitis rats (6 weeks after TNBS administration) and in age-matched, healthy controls. Representative Western blots are shown at the top. Densitometry data for five to nine rats per group are shown below the blots (mean $\pm$ SEM). Rats were treated with rofecoxib (3 mg/kg i.p.) or vehicle at 12-hour intervals during the 6th week after intracolonic TNBS or saline administration. * $P < 0.05$ versus the corresponding control group. $^{\psi}P < 0.05$ versus the corresponding vehicle-treated group.

~500 ACF in the colon when examined 3 weeks after the final injection (Figure 7). These lesions were fairly evenly distributed throughout the colon. In rats that had received the vehicle for TNBS (50% ethanol) intracolonic 6 weeks before the start of AOM administration, the number

Figure 7. Effects of a previous bout of colitis on susceptibility to AOM-induced formation of ACF. Rats received 0.5 ml of one of the following intracolonic: TNBS (30 mg in 50% ethanol), 0.9% saline, or 50% ethanol. Six weeks later, weekly intraperitoneal treatment with AOM (15 mg/kg) was started (total of four injections). Three weeks after the final administration of AOM, the number of ACF in the colon was quantified in a blind manner. Significant numbers of ACF were only seen in rats that were treated with AOM. However, postcolitis rats (ie, treated with TNBS intracolonic 6 weeks before the first injection of AOM) exhibited nearly double the number of ACF as the rats treated with saline or with 50% ethanol. * $P < 0.05$ versus the group treated with TNBS but not with AOM. $^{\psi}P < 0.05$ versus the groups treated with saline or 50% ethanol 6 weeks before treatment with AOM. Each bar represents the mean $\pm$ SEM of at least six rats.

Figure 8. Effects of twice daily treatment for 1 week with a selective COX-2 inhibitor (rofecoxib; 3 mg/kg i.p.) or a DP1 receptor antagonist (BWA868c; 100 μ g/kg i.p.) on the number of ACF in the colon of postcolitis rats (6 weeks after intracolonic treatment with TNBS) and age-matched controls. Treatment with the test drugs was performed during the 6th week after intracolonic administration of TNBS or saline. At the end of this week, the rats received the carcinogen, AOM (15 mg/kg i.p.) once weekly (total of four injections). The number of ACF in the colon was blindly assessed 3 weeks after the final administration of AOM. Postcolitis rats exhibited a significantly greater number of ACF than controls. Treatment with rofecoxib or with BWA868c significantly reduced the number of ACF in postcolitis rats ($^{\psi}P < 0.05$), while having no effect in control rats. Each bar represents the mean $\pm$ SEM for at least five rats.

and distribution of ACF was similar to that in saline-treated rats. Intracolonic administration of TNBS without subsequent administration of AOM did not result in significant formation of ACF. However, when AOM was administered to rats that had recovered from TNBS-induced colitis, the number of ACF was almost double that seen in the other treatment groups (Figure 8). Although ACF were observed throughout the colon, there was clearly a much higher incidence of them in the region that had been inflamed (distal 8 cm) than in the more proximal parts of the colon. Exposure to TNBS per se did not increase the incidence of ACF formation after administration of AOM. We observed that intraperitoneal administration of the same dose of TNBS 6 weeks before the start of AOM administration did not significantly change the number of ACF (485 ± 78 ; $n = 6$) as compared to saline-treated rats treated with AOM (578 ± 54 ; $n = 8$).

Treatment with rofecoxib during the 6th week after induction of colitis significantly reduced the susceptibility to ACF formation in response to repeated administration of AOM (Figure 8). Rofecoxib treatment did not significantly alter the number of ACF in healthy control rats. The levels of ACF observed in postcolitis rats treated for 1 week with BWA868c did not differ significantly from the levels observed in healthy controls treated with vehicle. BWA868c treatment did not significantly alter the number of ACF in healthy control rats.

Discussion

The risk for colorectal cancer is increased in association with the extent and duration of inflammatory bowel dis-

ease^{26,27} Studies of animal models of colitis have demonstrated a similar increase in the risk of experimental colon cancer.^{28,29} In the present study, we focused on susceptibility to carcinogen-induced preneoplastic lesion formation in rats that had recovered from a bout of colitis. Colitis in the rat induced by TNBS gradually resolves throughout the course of several weeks. Despite the absence of discernable inflammation, the colonic epithelium is markedly altered after a bout of colitis in terms of its secretory function, its function as a barrier to bacterial translocation, and its rate of proliferation.^{8–10} The results of the present study demonstrate that postcolitis rats also exhibit an increased susceptibility to formation of preneoplastic lesions (ACF) after administration of a carcinogen (AOM). Moreover, the susceptibility of postcolitis rats to ACF formation can be returned to the levels of healthy rats via inhibition of COX-2 or via blockade of the DP1 receptor for PGD₂.

Also consistent with human colon cancer, we observed a marked increase in β -catenin expression in the colon of the rats that had recovered from colitis. Positive staining for β -catenin was mainly localized to the epithelium. β -Catenin is an important protein for cell-cell adherence and is a transcriptional activator mediating Wnt signal transduction.³⁰ It also participates in a large cytoplasmic protein complex, which includes the tumor suppressor gene product of adenomatous polyposis coli (*APC*).³¹ *APC* gene mutations, which are common in colorectal neoplasms,^{32,33} are known to result in reduced degradation of β -catenin. The accumulation of β -catenin leads to dysregulated transcription of several genes that can promote neoplastic changes.³⁴ In the AOM model of colon cancer used in the present study, mutations in *APC* are rare, but altered expression of β -catenin has been reported to occur in ~75% of AOM-induced adenocarcinomas.³⁵ The normalization of β -catenin expression in the colon of postcolitis rats after treatment with a selective COX-2 inhibitor (rofecoxib) or a DP1 receptor antagonist (BWA868c) suggests that COX-2 and PGD₂ are involved in the up-regulation of β -catenin in the colonic epithelium. This also raises the possibility that suppression of COX-2 and blockade of the DP1 receptor may have therapeutic benefit in other situations in which there is dysregulation of the β -catenin pathway.

An increased development of ACF was only observed in the rats that had previously had TNBS-induced colitis. It was not observed in rats that had acute colonic injury induced by intracolonic administration of 50% ethanol (the vehicle for TNBS) or in rats given TNBS systemically, rather than intracolonic. Others have shown that giving carcinogens such as AOM to rodents with active colitis results in greater ACF formation than AOM alone.^{28,29} The present study demonstrates that a predisposition to carcinogen-induced neoplastic changes persists after resolution of the tissue injury and granulocyte infiltration that characterizes active colitis.

The elevated production of PGD₂ was detected in samples of the entire wall of the colon, so we cannot be sure of the cellular source(s) of this mediator. However, immunohistochemistry suggested that the cells in which COX-2 and H-PGDS were primarily up-regulated were

the enterocytes, and in the case of the latter, some lamina propria cells. Enterocytes produce PGD₂ and studies with cultured enterocytes have shown that in some circumstances it is derived from COX-2.³⁶ In terms of cells in the lamina propria, mucosal mast cells are a likely source of PGD₂. PGD₂ is the major product of arachidonic acid produced by the mucosal mast cell, and like the enterocyte, it is produced selectively via COX-2 in some circumstances.^{37,38}

Although the experiments with the DP1 receptor antagonist suggest a key role for that receptor in mediating the effects of PGD₂ in the context of predisposing postcolitis rats to preneoplastic lesions, we cannot rule out the possibility that other receptors for PGD₂ (ie, DP2) and its metabolites play some role. PGD₂ can be metabolized to 15-deoxy- $\Delta(12,14)$ PGJ₂, which is a ligand for the PPAR- γ receptor.¹³ PPAR- γ has been implicated as being important in the context of inflammation, cancer, and inflammation-associated cancer.^{39,40}

In summary, the present results demonstrate that COX-2-mediated PGD₂ plays an important role in the predisposition of postcolitis rats to colorectal cancer. Thus, PGD₂ can be viewed as a potential target for chemopreventative therapies. Given the evidence that COX-2 (in part through synthesis of PGD₂), exerts important anti-inflammatory and prohealing roles in the context of experimental colitis,^{11,12} and evidence that this may also be the case in humans,⁴¹ inhibiting COX-2 may not be a viable approach to reduce the risk of colorectal cancer in patients with colitis. However, downstream targets such as H-PGDS, the DP1 receptor, and possibly other receptors for PGD₂ and its metabolites may be possible in this population.

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