Role of EP4 receptor and prostaglandin transporter in prostaglandin E2-induced alteration in colonic epithelial barrier integrity

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Submitted 14 June 2010; accepted in final form 31 August 2010

Lejeune M, Leung P, Beck PL, Chadee K. Role of EP4 receptor and prostaglandin transporter in prostaglandin E2-induced alteration in colonic epithelial barrier integrity. Am J Physiol Gastrointest Liver Physiol 299: G1097–G1105, 2010. First published September 2, 2010; doi:10.1152/ajpgi.00280.2010.—Prostaglandin E 2 (PGE2) is a proinflammatory lipid mediator produced in excess in inflammatory bowel disease (IBD). PGE2 couples to and signals via four different E-prostanoid (EP) receptors, namely EP1, EP2, EP3, and EP4. In this study, we determined a role for PGE2 and EP4 receptors in altering colonic epithelial barrier integrity. In healthy colonic mucosa, EP4 receptors were localized on apical plasma membrane of epithelial cells at the tip of mucosal folds, whereas in patients with IBD and in rats with dextran sodium sulfate (DSS)-induced colitis, they were diffusely overexpressed throughout the mucosa. Similarly, expression of EP4 receptor was polarized in T84 colonic epithelial monolayer and mimics the normal epithelium. Apical exposure of T84 monolayer with high levels of PGE2 decreased barrier integrity, which was abrogated by an EP4 receptor antagonist. To reveal the mechanism of vectorial transport of basally produced PGE2 toward apical EP4 receptors, we identified prostaglandin transporters (PGT) in human colonic epithelia. PGT were least expressed on epithelial cells at the colonic mucosal folds of control subjects but overexpressed in epithelial cells of patients with IBD or animals with DSS-induced colitis. T84 monolayer also expressed PGT, which increased twofold following stimulation with TNF-α. Importantly, in T84 monolayer stimulated with TNF-α, there was a corresponding increase in the uptake of 3H-PGE2 to the apical surface. Knockdown of PGD2 decreased barrier integrity, which was also propagates a highly alternating and sometimes overlapping intracellular signaling. It is interesting to note that EP receptors exhibit distinct regional, temporal, and species differences in their expression and distribution across the length and breadth of the GI mucosa (11). Moreover, EP receptors are differentially expressed in the GI mucosa of disease compared with normal control subjects. Altered expression of various EP receptor subtypes has been reported in colon tumorigenesis, radiation-induced injury, and GI inflammation (18, 34, 36). Studies (10) have shown increased expression of EP4 receptor in the colonic mucosa of patients with UC. The functional significance of increased PGE2 production and the role EP receptor subtypes play in the pathogenesis of disease/inflammation are among the least studied aspects of PGE2-EP receptor biology in the gut.

One of the major advancements in prostaglandin biology was the identification of prostaglandin transporters (PGT) that act as a carrier in transporting various PGS across the plasma membrane (21). PGT belongs to a family of 12 transmembrane domain-containing proteins with functional characteristics similar to that of anion transporters in epithelial cells (32, 33). Because PGS are charged anions at physiological pH that poorly diffuse across biological membrane, PGT helps in the transport of PGS across the lipid bilayer. The three major functions of PGT are 1) efflux/release of PGS from cells, 2) influx/clearance from circulation, and 3) vectorial transport across the epithelia. Several studies demonstrate a role for PGT in vectorial transport of PGS across epithelia (4, 9, 13). Moreover, the presence of PGT was confirmed in human epithelia, especially those of urinary and reproductive organs (22, 29). However, there is a paucity of information available regarding its expression in the human GI tract. A recent study (17) highlights the presence of PGT in colonic epithelium, which was differentially downregulated during colorectal cancer. However, the role PGT plays in the pathogenesis of other enteric pathologies is not clearly known. It is essential to

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http://www.ajpgi.org

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understand the role of PGT in the pathogenesis of IBD because high-output PGE₂ is a hallmark of this disease. At present, it is not known whether a correlation exists between PGT, excess PGE₂, and increased EP4 receptor expression in IBD. In this study, we investigated the role that PGE₂-EP4 receptor and PGT play in alteration of colonic epithelial barrier function.

MATERIALS AND METHODS

Materials. Sulfobromophthalein sodium hydride (BS) was purchased from Acros Organics (Fair Lawn, NJ). Geneticin (G-418) was purchased from Invitrogen (Carlsbad, CA). The antibodies for human PGT and EP4 receptor (COOH terminal) were purchased from Cayman Chemical [catalog no. 160200 and 101775, respectively; Ann Arbor, MI]. These antibodies cross react with their respective rat counterparts. Biotinylated anti-rabbit IgGs were purchased from R&D Systems (Minneapolis, MN). Peroxidase-conjugated avidin biotin complex was purchased from eBiosciences (San Diego, CA). Diaminobenzidine/3-amin, 9 ethyl-carbazole (DAB/AEC) peroxidase substrate were purchased from Vector Laboratories (Burlingame, CA). PGT (solute carrier organic anion transporter family, member 2A1) shRNA was obtained from Super Array Bioscience (Frederick, MD). PGE₂, butaprost, PGE₁OH, and AH23848 were purchased from Cayman Chemical (catalog no. 14010, 13740, 13020 and 19023, respectively). [³H-PGE₂ and ¹⁴C-ethanolamine were purchased from Amersham, GE Healthcare (Piscataway, NJ). Twelve-well Transwell plates were purchased (catalog no. 3460; Corning, Costar, Corning NY). All other chemicals were purchased from Sigma-Aldrich (St. Louis, MO) or otherwise mentioned.

T84/Caco-2 cell culture and shRNA transfection. T84 human colonic cells (ATCC, Manassas, VA) were maintained in DMEM with Ham’s F-12 supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin sulfate, and 20 mM HEPES. They were incubated at 37°C in 5% CO₂ and passaged once the monolayer reached 90% confluency. Cells between passages 64 and 68 were used for plating on culture plates for Transwell studies. To passage cells, monolayer in a T75 flask was rinsed with 5 ml of sterile PBS and incubated with 1 ml of Transwell studies. To passage cells, monolayer in a T75 flask was rinsed with 5 ml of sterile PBS and incubated with 1 ml of Transwell studies. To passage cells, monolayer in a T75 flask was rinsed with 5 ml of sterile PBS and incubated with 1 ml of Transwell studies. To passage cells, monolayer in a T75 flask was rinsed with 5 ml of sterile PBS and incubated with 1 ml of Transwell studies. 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macological inhibition of PGT, 6 μM BSP was added together with 3H-PGE2 to the basal media.

Real-time PCR. Intron spanning primers were designed from the known sequence of human PGT. The sequences were as follows: forward, 5′-GCCACACGAGTAGAAGCAAG-3′; reverse, 5′-TG-GAGAGCCAGCAATTGAC-3′. GAPDH was used as internal control. Real-time quantitative PCR was performed using SYBR green according to the manufacturer’s instruction. The reaction mixture was denatured for 5 min at 95°C and subjected to 50 cycles of three-step PCR consisting of denaturation (95°C for 10 s), annealing (60°C for 15 s), and extension (72°C for 20 s). The amplified products were verified by agarose gel electrophoresis for predicted size. PGT mRNA levels were expressed as a ratio of PGT to GAPDH.

Estimation of PGE2 in cell culture supernatant. PGE2 in culture supernatant was assayed using Assay Designs Correlate-CLIA kit (catalog no. 910–001; Ann Arbor, MI). This high-sensitivity PGE2 chemiluminescence enzyme immunoassay is designed for quantitative sensitive determination of human PGE2 in biological fluids. The assay was carried out as per manufacturer’s protocol.

Statistical analysis. TER data were analyzed by two-way ANOVA and 3H-PGE2 transport data by one-way ANOVA followed by a Bonferroni posttest for comparison between groups using GraphPad Prism version 4.0 (GraphPad Software, San Diego, CA). All values are means ± SD of three independent experiments unless otherwise indicated. Significance was indicated at *P < 0.05, **P < 0.01, and ***P < 0.001, respectively.

RESULTS

EP4 receptor expression patterns in control and inflamed colonic mucosa. Colonic tissues derived from patients with UC and from control subjects were examined for EP4 receptor expression by immunohistochemistry. As shown in Fig. 1A, in controls, EP4 receptors were localized exclusively to the apical plasma membrane of epithelial cells (Fig. 1A, inset, with arrow) especially at the tip of mucosal folds. However, in patients with UC, EP4 receptors were strongly expressed throughout the mucosa and not localized on the plasma membrane of epithelial cells. Notably, EP4 receptors were diffusely expressed throughout the cytoplasm in epithelial cells lining the entire mucosal folds (Fig. 1A, inset). EP4 receptors were also moderately expressed in the underlying lamina propria inflammatory cells. To determine whether a similar EP4 expression profile occurred during experimental colitis, rats were given DSS for 7 days. As shown in Fig. 1B, EP4 receptors were overexpressed in rat colonic mucosa on induction of colitis. Remarkably the pattern of expression was similar to that observed in human colonic mucosa where EP4 receptors were localized to apical plasma membrane of epithelial cells at the tip of mucosal folds (arrow) in control rats. However, in DDS-induced colitis, EP4 receptors were strongly expressed in epithelial cells lining the entire mucosal folds. Moreover, EP4

A

EP4 receptor expression – Human colonic mucosa

Control

Ulcerative colitis

B

PGT expression – Rat colonic mucosa

Control

DSS Colitis

Fig. 1. Immunohistochemical analysis of E-prostanoid (EP4) receptor expression in the colonic mucosa of patients with ulcerative colitis (UC) and rats with dextran sodium sulfate (DSS) colitis. A: representative photomicrograph (×200) showing EP4 receptor expression in human colonic tissues from control patients and patients with UC. Inset: higher magnification (×400) of a selected region from the same slide. Red staining indicates EP4 receptor expression, and blue is counterstain for the nucleus. Arrow points at EP4 receptor localization on apical plasma membrane of normal epithelium. Five separate biopsy specimens were examined from controls and patients with inflammatory bowel disease (IBD). B: representative photomicrograph (×400) showing EP4 receptor expression in colonic mucosa of control and DSS-induced colitis in rats. Arrows point to red staining for EP4 receptor expression. Five rats were used per group, and all DSS-treated animals exhibited low-grade colitis on the basis of histopathology and weight loss. PGT, prostaglandin transporter.
receptors were diffusely expressed in the cytoplasm of epithelial cells and in inflammatory cells of the lamina propria (Fig. 1B, arrows).

EP4 receptors are localized on the apical pole of T84 monolayer. To determine the cellular localization of the EP4 receptors, T84 colonic epithelial cells grown as monolayer were examined for the polarity of EP4 receptor expression by confocal microscopy (Fig. 2). Tight junctions that act as a fence and demarcate apical and basolateral plasma membrane of polarized epithelial monolayer (25, 30) were probed by immunotagging for one of its representative protein, claudin-4. Immunofluorescence signal from the tags for respective proteins were determined in individual en face (xy-axes) planes throughout the cellular z-axis at 0.35-μm intervals. A total of 107 slices from the basal-to-apical side were Z stacked and 3D reconstructed. As shown in Fig. 2, a high degree of fluorescence for claudin-4 was found within sections 59 to 66 that approximately mark the boundary of cellular tight junctions. However, weak signals for claudin-4 were generally observed throughout the stacks, indicating another cytoplasmic location for this protein. A strong fluorescence for EP4 receptor was observed between stacks 59 and 99; however, no signal was detected below stack 59. The merge signal between EP4 receptors and claudin-4 clearly shows that EP4 receptors were localized on the apical side of polarized T84 monolayer.

High-output PGE2 signals via EP4 receptor to alter colonic epithelial integrity. TER is the gold standard to measure the integrity of contiguous epithelial monolayer (25, 37). To test whether activation of EP4 receptor altered the integrity of colonic epithelium, T84 monolayer grown on Transwell plates were stimulated on the apical side with PGE2 and EP4 receptor agonist, and TER was measured (Fig. 3). Because the diverse biological functions of PGE2 in the gut are dependent on the amount released in the microenvironment around target cells (11), we tested the effect of low-output (10 nM) and high-output (1 μM) PGE2 (Fig. 3A). In general, 10 nM PGE2 did not alter TER. However, after 5 min, it caused a slight (9%) decrease in TER compared with control, but at subsequent time points TER was maintained well above control. In marked contrast, 1 μM PGE2 caused a precipitous decrease in TER; after 5 min there was a 68% decrease in TER compared with control and remained significantly decreased at all time points measured (P < 0.001). TER began to recover around 720 min but stayed 26% lower than that of control (P < 0.01). To discern which EP receptor PGE was coupling through to cause a decrease in TER, we used well-characterized EP2/4 receptor-specific agonists or antagonists (12). Stimulation with 0.5 μM PGE1OH, an EP2/4 receptor agonist, decreased TER similar to
that of 1 μM PGE2 (Fig. 3B). The differences in TER between control and 0.5 μM PGE2 were significant at all time points (P < 0.001, P < 0.01). Likewise, in response to the EP4 receptor-specific agonist, ONO-AE1 329, there is a rapid decrease in TER (60% at 5 min) that was sustained over 60 min (data not shown). Thus, to show specificity for PGE2 coupling and signaling via the EP4 receptor, we used the specific EP2 receptor agonist butaprost (10 μM). Butaprost modestly decreased TER at 5 min but had no significant effects at any other time points. We also tested the effect of EP1/3 agonist, 17 phenyl trinor PGE2, and EP3-specific agonist, sulprostone (3 μM), and observed no alterations in TER at any time points measured (data not shown). Moreover, T84 monolayer pre-treated with an EP4 receptor antagonist, AH23848 (30 μM), for 45 min and then exposed to 1 μM PGE2 inhibited PGE2-induced decrease in TER to control levels (Fig. 3B). Interestingly, EP4 antagonist pretreatment completely prevented the decrease in TER caused by 1 μM PGE2 at all time points except at 720 min, clearly demonstrating a role for EP4 receptors in mediating high-output PGE2-induced alteration in TER.

Expression of PGT in normal and inflamed colonic mucosa.

During the onset of intestinal inflammation, inflammatory cells of the lamina propria produce high-output PGE2. Under these conditions we speculate a role for PGT in vectorial transport of PGE2 across the colonic epithelium toward the apical EP4 receptors. Because PGT presence or distribution in the gut is not known, we first checked for PGT expression in colonic tissues isolated from control subjects and from patients with UC by immunohistochemistry (Fig. 4A). In healthy control colonic mucosa, PGT expression was least expressed on epithelial cells of mucosal folds. In marked contrast, PGT was differentially overexpressed in the colonic epithelium of patients with UC. PGT expression was strongly present in epithelial cells at the tip and lateral sides of mucosal folds as well as the crypts. Notably, PGT was diffusely expressed throughout the cytoplasm and strongly present on the basolateral sides of mucosal folds as well as the crypts. Notably, PGT was diffusely expressed throughout the cytoplasm and strongly present on the basolateral sides (Fig. 4A, inset). To determine whether PGT was differentially altered during colitis, mucosal expression of PGT was examined by immunohistochemistry in the rat model of DSS-induced colitis (Fig. 4B). In control rat mucosa, PGT was moderately expressed in the cytoplasm of epithelial cells, and its expression was restricted to cells at the tip of mucosal folds (arrow). However, during the induction of colitis, PGT was strongly expressed in the cytoplasm of epithelial cells at the tip of mucosal folds and moderately expressed in epithelial cells lining the lateral sides and the base of crypts (arrows). Interestingly, nuclear localization of PGT was also noticed in rat colonic epithelial cells, specifically in those cells that express PGT in the cytoplasm. Overall, PGT expression was upregulated in colonic epithelial cells during colonic inflammation.

Fig. 4. Immunohistochemical analysis of PGT expression in the colonic mucosa of patients with UC and rats with DSS colitis. A: representative photomicrograph (×200) showing PGT expression in human colonic tissues from control and patients with UC. Inset: higher magnification (×400) of a selected region from the same slide. Bold arrow points to PGT expression in the epithelium. Broken arrow indicates absence of PGT expression. Five separate biopsy specimens were examined from controls and patients with IBD. B: representative photomicrograph (×400) showing PGT expression in the colonic mucosa of control and DSS-induced colitis in rats. Arrows indicate PGT expression. Five rats were used per group, and all DSS-treated animals exhibited low-grade colitis on the basis of histopathology and weight loss.
TNF-α upregulates PGT expression and increases vectorial transport of PGE₂ across T84 monolayer. As shown above, PGT expression was significantly upregulated in the epithelium of inflamed colonic mucosa. However, the factors responsible for modulating PGT during inflammation are not known. Because proinflammatory cytokines and, in particular, TNF-α are produced in high quantities during colitis, we determined whether TNF-α can upregulate PGT expression in colonic epithelial cells in vitro. T84 monolayer was stimulated with TNF-α (5 ng/ml) for 12 h, and, as shown in Fig. 5A, there was a four- and twofold increase in PGT mRNA and protein expression as revealed by real-time PCR and Western blotting, respectively. To ascribe a functional role for PGT, we checked for vectorial transport of the tracer ³⁵S-PGE₂ across the T84 monolayer (Fig. 5B). The monolayer was pretreated or not with TNF-α and exposed on the basal side with ³⁵S-PGE₂. Uptake and transport of ³⁵S-PGE₂ in the apical chamber was measured at 12-h intervals. As shown in Fig. 5B, baseline transport of ³⁵S-PGE₂ in nonstimulated control was negligible. However, in T84 monolayer pretreated with TNF-α, there was a significant increase in the uptake and transport of ³⁵S-PGE₂ (P < 0.001) on the apical side. Specificity for PGT uptake and transport of ³⁵S-PGE₂ to the apical side was shown in cells pretreated with the PGE₂ transport inhibitor, BSP (6 μM), which abrogated transport with value identical to that of the untreated control (P < 0.001). To rule out passive transport of ³⁵S-PGE₂ across the paracellular space of contiguous monolayer, we determined the vectorial translocation of an inert tracer, ¹⁴C-ethanolamine. As predicted (Fig. 5B), there was no significant translocation of ¹⁴C-ethanolamine in control or TNF-α-pretreated monolayer. Additionally, to ascertain the effect of TNF-α on T84 monolayer integrity, we determined whether 12-h treatment with TNF-α could stimulate PGE₂ production. As shown in Supplemental Fig. 1A, the mean PGE₂ level in control supernatant was 29 pg/ml that increased 91-fold (2,650 pg/ml) in response to TNF-α; supplemental material is available for this article online at the American Journal of Physiology Gastrointestinal and Liver Physiology website. However, the molar equivalence of the mean PGE₂ produced in the TNF-α-treated group was only 7.5 nM. This concentration of PGE₂ does not decrease TER as shown in Fig. 3A, where 10 nM PGE₂ had no effect. Moreover, EP4 receptor expression remained unchanged in T84 monolayer treated for 12 h with TNF-α (Supplemental Fig. 1B). Taken together, these results demonstrate that TNF-α can upregulate PGT expression and increase vectorial transport of PGE₂ across a colonic epithelial monolayer without affecting epithelial barrier integrity.

ShRNA knockdown of PGT decreases PGE₂ transport across colonic epithelium. Having established a role for PGT for vectorial transport of PGE₂ across the colonic epithelium, we confirmed its specificity by shRNA-based knockdown studies.
Because T84 cells are difficult to develop as stable transfectants, we used Caco-2 colonic epithelial cells for this study. Caco-2 are similar to T84 cells in expressing polarized EP4 receptors and reach peak resistance of ~1,100 Ω/cm² (data not shown). Caco-2 transfectants that stably express low PGT (shRNA-PGT) or not (shRNA-Control) were analyzed for PGT expression by Western blot (Fig. 6A, i). As shown, PGT expression was significantly decreased in the shRNA knockdown cells, and the transcripts for PGT was decreased ninefold as quantified by Q-PCR (Fig. 6A, ii). As predicted, vectorial transport of ³H-PGE₂ in shRNA-PGT monolayer was significantly decreased (Fig. 6B, P < 0.001), whereas the basal-to-apical translocation of ¹⁴C-ethanolamine was unaltered.

**DISCUSSION**

Onset of intestinal inflammation involves a variety of host mediators, such as cytokines, growth factors, reactive oxygen intermediates, and arachidonic acid metabolites (12, 39). Excessive biosynthesis of arachidonic acid metabolites including PGE₂ is highly implicated in the pathogenesis of IBD (1, 8, 31). However, the role PGE₂ play in onset or exacerbating inflammation is not clearly known. In this study, we identified a role for high-output PGE₂ in altering colonic epithelial barrier integrity. We also revealed a role for EP4 receptors and PGT in mediating PGE₂-induced epithelial barrier alteration.

PGE₂ is known for versatile biological functions in the GI tract (6, 11). Its versatility is attributed to its differential local production and its selectivity for EP receptor subtypes (EP1, EP2, EP3, and EP4). Evidently, the physiological concentration of PGE₂ is around 10 nM (15), and in inflamed intestinal mucosa it increases 10- to 100-fold (31, 35). Therefore, we marked 1 μM PGE₂ as high output in our study. In fact, 1 μM PGE₂ has been used in studies involving dog colon to see a maximal effect on smooth muscle relaxation (5). Thus the concentration of PGE₂ used in our study has biological relevance. Recently, a genome-wide association study reported an association of EP4 receptor (PTGER4) with IBD (19). However, the roles EP4 receptor plays in early onset/progression/resolution of colitis are not clearly understood. Moreover, nothing is known of the pattern of EP4 receptor expression in the normal and inflamed colon. Therefore, we analyzed the pattern of EP4 receptor expression in normal and inflamed mucosa.

In the present study, we revealed a striking localization of EP4 receptor on the apical plasma membrane of epithelial cells in control human subjects and a marked upregulation in the epithelial and lamina propria cells in patients with UC. A similar pattern was observed in the rat model of DSS-induced colitis. Previous studies (10, 28) highlighted the importance of EP4 receptor on subepithelial lamina propria cells in augmenting IL-10 (anti-inflammatory cytokine) production during the progression of colitis. However, nothing is known on the role epithelial EP4 receptors play in initiation/progression of colitis. Because the expression of EP4 receptors in the control colonic mucosa is restricted to epithelial surface apical plasma membranes, we studied PGE₂-EP4 receptor-coupling events in altering epithelial barrier function. T84 colonic epithelial cells were used as an in vitro model for these studies. Interestingly, we observed polarized expression of EP4 receptor in T84 monolayer, mimicking the normal pattern of distribution in the colonic epithelium. Accordingly, T84 monolayer was exposed on the apical side with either PGE₂ or EP4 receptor agonist and antagonist and checked for epithelial barrier integrity (TER).

Because the epithelium lining the lumen of colon is an important host barrier that primarily regulates the bilateral movement of solutes or ions from/into the host, we were interested in analyzing the barrier integrity in response to PGE₂. First, we proved that PGE₂ alters TER in a dose-dependent manner. Low/physiological concentration did not decrease epithelial monolayer integrity, whereas high/pathological concentration caused a precipitous decrease as early as 5 min and was sustained even after 1 h. However, after 12 h, TER recovered significantly from the initial decrease that indicated that cell death did not occur and showed that the initial loss of barrier integrity is specifically caused by PGE₂. This confirms a deleterious role for PGE₂ that is usually produced in high output during inflammation. Second, using a pharmacological approach, we show that high-output PGE₂ selectively and specifically signaled via EP4 but not EP2 receptors to alter...
barrier function. Moreover, 17 phenyl trinor PGE₂ (EP1/3 agonist) and sulprostone (EP3-specific agonist) did not decrease T84 monolayer integrity. Our results corroborate previous studies that indicate a major role for EP1, EP2, and EP3 receptors in intestinal epithelial cytoprotection, and we recently (11) discussed in detail how each EP receptor does this.

Apart from PGE₁ OH (EP4/2 agonist), we also used a specific EP4 receptor agonist, ONO-AE1 329, which caused a similar decrease in TER. More importantly, AH23848 (EP4 antagonist) prevented high-dose PGE₂ induced decrease in TER. These results confirm a pathological role for colonic epithelial EP4 receptors during the onset of inflammation. EP4 receptors have been shown to play an important role in suppressing colitis and downregulating immune responses in DSS-induced colitis (20). However, the study did not look at the role of EP4 signaling in either epithelial cells or the submucosal cells that induced the anti-inflammatory effects, so it is difficult to ascribe a mechanism for this effect. We have recently discussed this issue (11) and emphasized that EP4 receptors on mucosal epithelial cells or immune cells can play a differential role in colitis. For example, it appears that EP4 receptor functions differ between early onset (proinflammatory on colonic epithelial cells) and late progressive stages of colitis (anti-inflammatory on immune cells in the lamina propria).

Nonetheless, in the present study, we show that excessive PGE₂ that is produced during early inflammatory conditions preferentially and specifically signals via colonic epithelial EP4 receptors to decrease epithelial barrier integrity. We are presently studying the nature of high-output PGE₂-induced, EP4 receptor-mediated dynamic changes at paracellular tight junctions that alter TER.

Having identified a definite role for EP4 receptor in altering colonic barrier integrity, we then answered the question how PGE₂ that is usually produced in high levels by lamina propria cells (24) during the onset of mucosal inflammation reaches the site of EP4 receptor expressed on apical membranes of the epithelium. We hypothesized that PGT helps in vectorial transport of PGE₂ across the colonic epithelium. To quantify this, we first visualized the pattern of PGT expression in control and inflamed human colonic mucosa. PGT is least expressed in the cytoplasm of epithelial cells of the colonic mucosal fold of control subjects. However, in inflamed (UC) mucosa, it is abundantly expressed in most of the epithelial cells lining the mucosal folds. Although a pattern similar to human PGT expression is observed in the rat model of DSS-induced colitis, the differential expression is not that noticeable. However, what is more striking is the nuclear localization of PGT in rat epithelial cells. It is exclusively noticed in those cells that express PGT in the cytoplasm, thus ruling out cross-reactivity of the PGF antibody with that of a DNA-binding nuclear matrix protein, matrin F/G, with rat PGT that share 37% protein homology (21, 16). Mouse colonic epithelium shows a similar nuclear localization (data not shown) but is apparently absent in the human colonic epithelium. At present, the role of PGT in nuclear function cannot be ruled out.

It is clear that PGT is differentially upregulated during UC. However, the mediators that regulate colonic epithelial PGT expression are not clearly known. PGT is an inducible gene. For example, PGT is upregulated in response to shear and stress (38). However, many reports rule out a role for proinflammatory mediators such as LPS, IL-1β, and TNF-α in upregulating PGT (38, 23). Surprisingly, in our studies prolonged exposure to TNF-α increased PGT expression twofold in T84 cells. Moreover, TNF-α pretreatment significantly increased the basal-to-apical transport of the tracer PGE₂, thus implicating TNF-α in PGT function. Because TNF-α is a proinflammatory cytokine, we ruled out its deleterious effects on epithelial integrity that could negate the role of PGT in vectorial transport of PGE₂ by the following findings. First, there was no alteration in EP4 receptor expression in T84 monolayer on prolonged exposure with TNF-α; second, PGE₂ produced by T84 cells in response to TNF-α was not sufficient to decrease TER; third, TNF-α treatments by themselves did not compromise TER. Previous studies (14) have also ruled out a direct role for TNF-α in altering TER in T84 cells. Because TNF-α did not alter epithelial integrity, it is clear that the observed apical translocation of tracer PGE₂ is the effect of PGT-mediated vectorial transport. Furthermore, BSP, a specific inhibitor of PGT (21), abrogated the vectorial transport of tracer PGE₂ during conditional upregulation of PGT. As a result of inherent difficulty, we were unable to knockdown PGT and develop a stable T84 cell line. Therefore, we used shRNA-based PGT knockdown studies in Caco-2 cells. Caco-2 is similar to T84 cells in having polarized EP4 receptor expression as confirmed by confocal microscopy (data not shown). They exhibit a baseline TER of around 1,100 Ω/cm². The stable Caco-2 cell line (shRNA control and PGT) exhibited similar TER to that of wild-type cells and showed no leaky paracellular barrier as revealed by ¹⁴C-ethanolamine translocation study. Therefore, the observed decrease in vectorial transport of tracer PGE₂ in shRNA-PGT cell monolayer is the effect of knockdown. However, PGT knockdown did not completely prevent the apical appearance of the tracer PGE₂, indicating another anion transporter could play a role in vectorial transport of PGE₂. Moreover, we examined whether silencing PGT can protect against high-dose PGE₂-induced alteration in TER. As shown in Supplemental Fig. 2, knockdown of PGT did not prevent PGE₂-induced barrier alteration. This clearly indicates that PGT was responsible for vectorial transport of PGE₂ across the epithelium toward lumen and that alteration in TER is dependent on the signaling outcome of apically expressed EP4 receptors following PGE₂ coupling.

In summary, our studies reveal that the colonic epithelium can differentially express PGT under resting/inflamed condition and helps in vectorial transport of PGE₂ across the epithelium to be delivered to the site of EP4 receptor. High-output luminal PGE₂ in turn activates EP4 receptor of normal epithelial monolayer that leads to loss of barrier integrity. This study also emphasizes the need to study cell type-specific roles of EP4 receptor for a comprehensive knowledge of its function in the colonic mucosa.

ACKNOWLEDGMENTS

We thank Tehmeena Malik and Dr. Pina Colarussi from the live cell imaging facility for technical service and Dr. Derek McKay for use of microscopy facility.

GRANTS

This research was supported by grants from the Crohn’s and Colitis Foundation of Canada and the Canadian Institute for Health Research. Dr. Chadee is a Tier 1 Canada Research Chair in Gastrointestinal Inflammation.
DISCLOSURES

The authors have no conflicts of interest to disclose.

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