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Increased Expression and Cellular Localization of Spermine Oxidase in Ulcerative Colitis and Relationship to Disease Activity

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Abstract

Background—Polyamines are important in cell growth and wound repair, but have also been implicated in inflammation-induced carcinogenesis. Polyamine metabolism includes back-conversion of spermine to spermidine by the enzyme spermine oxidase (SMO), which produces hydrogen peroxide that causes oxidative stress. In ulcerative colitis (UC), levels of spermine are decreased compared to spermidine. Therefore, we sought to determine if SMO is involved in UC.

Methods—Colon biopsies and clinical information from subjects undergoing colonoscopy for evaluation of UC or colorectal cancer screening were utilized from 16 normal controls and 53 UC cases. Histopathologic disease severity was graded and the Mayo Disease Activity Index (DAI) and endoscopy subscore assessed. SMO mRNA expression was measured in frozen biopsies by Taq-Man-based real-time polymerase chain reaction (PCR). Formalin-fixed tissues were used for SMO immunohistochemistry.

Results—There was a 3.1-fold upregulation of SMO mRNA levels in UC patients compared to controls ($P = 0.044$), and a 3.7-fold increase in involved left colon versus paired uninvolved right colon ($P < 0.001$). With worsening histologic injury in UC there was a progressive increase in SMO staining of mononuclear inflammatory cells. There was a similar increase in SMO staining with worsening endoscopic disease severity and strong correlation with the DAI ($r = 0.653$, $P < 0.001$). Inflammatory cell SMO staining was increased in involved left colon versus uninvolved right colon.

Conclusions—SMO expression is upregulated in UC tissues, deriving from increased levels in mononuclear inflammatory cells. Dysregulated polyamine homeostasis may contribute to chronic UC by altering immune responses and increasing oxidative stress.

Keywords

ulcerative colitis; spermine oxidase; polyamines; gene expression; immunohistochemistry; disease activity index

Ulcerative colitis (UC) is a chronic, relapsing, and inflammatory condition of the gastrointestinal (GI) tract.¹ Abnormal regulation of the mucosal immune response toward commensal bacterial flora together with genetic and environmental factors appear to play an important role in the pathogenesis.² Patients with UC are at increased risk for development of colonic neoplasia, and the age at diagnosis and the extent of disease at diagnosis have been found to be strong and independent risk factors for colorectal cancer.³ Chronic inflammation is a well-recognized risk factor for the development of colorectal neoplasia and current medical therapy aims to target the immune cascade and resultant inflammatory response.⁴ Concomitant increases in reactive oxygen species and oxidative damage have been implicated as mechanisms by which chronic inflammation affects the colonic mucosa. However, knowledge about the cellular source and mechanisms of production of reactive oxygen species remain incompletely understood.

Polyamines are ubiquitous, polycationic alkylamines that are essential for eukaryotic cell growth and differentiation, and have also been shown to play an important role in inflammation-induced carcinogenesis.^{5,6} Polyamines are produced from L-ornithine by ornithine decarboxylase, and this ongoing synthesis has been strongly linked to the maintenance of epithelial integrity and wound repair in the GI mucosa.⁷ Cells have developed complex regulatory machinery to tightly control intracellular levels of polyamines, as dysregulation of polyamine metabolism can have serious effects on cell growth.⁸ Increased polyamine synthesis has been detected as a product of inflammation,⁹ and we have previously reported that *Helicobacter pylori* infection of the stomach can result in increased expression and activity of polyamine biosynthetic enzymes.^{10–14} Intracellular polyamines themselves are capable of regulating inflammation. The polyamine spermine has been shown to inhibit proinflammatory cytokine synthesis in human mononuclear cells,¹⁵ as well as nitric oxide (NO)-mediated intestinal damage.¹⁶

It has been reported that mucosal spermidine levels were higher in active UC than in cases in remission or in normal controls, yet the activities of the polyamine synthetic enzymes were actually lower.¹⁷ The activity of the enzyme spermidine/spermine *N*¹-acetyl transferase (SSAT), which acetylates spermidine or spermine^{18,19} and allows for their back-conversion by polyamine oxidase,²⁰ was deemed not sufficient to explain the increase in spermidine in UC.^{17,21} Increased spermidine and decreased spermine levels compared to normal controls has also been reported in colonic epithelial cells isolated from UC patients.²² We have reported that in the murine *Citrobacter rodentium* model, polyamine levels are increased in colitis tissues, and this derived from spermidine rather than spermine.²³

SSAT is the rate-limiting enzyme in the 2-step catabolism of polyamines. Acetylation of spermine or spermidine by SSAT results in either excretion of the acetylated polyamines or oxidation by the constitutively active peroxisomal acetyl polyamine oxidase (PAO).²⁴ This PAO enzyme only oxidizes acetylated polyamines.^{24–26} A major role of SSAT is to facilitate the efflux of acetylated spermidine and spermine from cells,^{20,27} thus depleting their levels. In contrast, the enzyme spermine oxidase (SMO; previously termed polyamine oxidase 1),^{27,28} acts specifically on spermine to directly back-convert it to spermidine, thus increasing its intracellular levels, and this process produces hydrogen peroxide (H₂O₂) that can cause oxidative stress-related injury such as apoptosis and DNA damage.^{8,11,13} We have reported that SMO expression is upregulated in gastritis tissues from patients with *H. pylori* infection,¹¹ and that it is upregulated in both macrophages¹³ and epithelial cells,¹¹ indicating the potential involvement of SMO in GI mucosal inflammation. Moreover, SMO expression is increased in human prostate cancer and prostate intraepithelial neoplasia tissues,²⁹ suggesting a role in epithelial cell tumorigenesis. It should be noted that there are 5 splice variants from the *SMO* locus that have been identified by polymerase chain reaction (PCR) in human cancer cells^{26,30}; however, only 2 active splice variants have been observed in nontumor tissue, namely

SMO1 (PAOh1, 61.9 kDa) and SMO5 (65.0 kDa), and SMO1 is the predominant splice variant, constituting more than 90% of the protein.²⁶

We hypothesized that elevated SMO expression may represent an important pathway in the inflammatory pathogenesis of UC. Our aim was to determine if SMO levels are increased in the colonic biopsy samples of patients with UC when compared to biopsies from normal subjects, and whether SMO levels correlate with disease activity. We report that SMO mRNA expression is increased in UC when compared to normal mucosa from control subjects, and that it is increased in areas of active disease in the left colon when compared to paired uninvolved right colon tissues. We also demonstrate by immunohistochemistry that SMO protein expression localizes to both colonic epithelium and lamina propria mononuclear inflammatory cells, and that there is a progressive increase in inflammatory cell staining that correlates with disease activity, and is increased in the diseased left colon versus the uninvolved right colon.

PATIENTS AND METHODS

Patients

The study protocol was approved by the Institutional Review Board at Vanderbilt University. Written informed consent was obtained from all subjects for analyses of tissue biopsies from endoscopic procedures to be included in the Vanderbilt Gastroenterology Tissue Repository. All study information was deidentified prior to analysis by investigators.

Subjects were recruited prospectively prior to outpatient colonoscopy for either screening or surveillance purposes to undergo 2 additional biopsies per colonic segment. Study biopsies were snap-frozen with dry ice and then transferred for storage in the Tissue Repository at -80° C. Biopsies were reviewed by the Department of Pathology at Vanderbilt University and graded accordingly as: normal, quiescent, mild, moderate, or severe activity. Potential subjects excluded from the study were as follows: those younger than age 18, pregnant, having known coagulopathy or bleeding disorders, or unable to give informed consent.

Frozen tissues were utilized for RNA analysis and formalin-fixed, paraffin-embedded tissues were used for immunohistochemistry. A total of 69 cases were included. We used available frozen colonic biopsy samples from 14 normal subjects and 51 subjects with UC, based on a range of histopathologic disease severity, for analyses of gene expression. We utilized paraffin blocks from 16 normal cases and 53 UC cases for analyses of protein expression. Whenever possible, we used tissues from the same subjects for both RNA and immunohistochemistry analysis; in total, both assays were performed on 12 of the 16 normal cases and 41 of the 53 UC cases.

The Mayo Disease Activity Index (DAI) was determined at the time of colonoscopy by standard measures (0–12 scale), which included number of bowel movements per day, the presence or absence of blood, endoscopy scoring, and physician's global assessment.³¹ Endoscopic assessment was performed by a single gastroenterologist specializing in inflammatory bowel disease (D.A.S.) and graded on a scale of normal, mild disease (erythema, decreased vascular pattern, mild friability), moderate disease (marked erythema, lack of vascular pattern, friability, erosions), or severe disease (spontaneous bleeding, ulceration).

Gene Expression Analyses

RNA was extracted using a micro-isolation kit (RNeasy, Qiagen, Valencia, CA). RNA was reverse transcribed using the iScript cDNA synthesis kit (Bio-Rad, Hercules, CA) as described previously by our laboratory.³² Real-time PCR was performed using the iQ Multiplex Powermix reagent (Bio-Rad). SMO gene quantification was conducted by TaqMan-based

multiplex PCR with standardization of levels to the housekeeping gene GAPDH. The primers and probes utilized were as follows. For SMO: sense primer 3'-GGATGAGGATGAGCAGTGGTC-5', antisense primer 3'-CGACACGGTCACAATCACATG-5', and probe [6-FAM]CGGGATCAGCTCACAGTCCTCGCA[BHQ1a-6FAM]. For GAPDH: sense primer 3'-CAACAGCCTCAAGATCATCAGC-5', antisense primer 3'-TGAGTCCTTCCACGATACCAAAG-5', and probe [5HEX]TGCCTCCTGCACCACCAACTGCTT[BHQ2a-5HEX]. The PCR conditions were as follows: each cycle consisted of a denaturation step (94°C, for 30 sec) and an annealing step (58°C, for 30 sec). For SMO and GAPDH, the final concentrations of primers used were 300 nM and 100 nM, respectively. The final concentration of probes for SMO and GAPDH was 200 nM. Standard curves were generated using linearized SMO cDNA and GAPDH cDNA as templates, and copy number was calculated from cycle threshold values.

Immunohistochemistry for SMO

Biopsies were fixed in buffered formalin and embedded in paraffin. Four-micron-thick sections were cut and mounted on ProbeOn-Plus slides (Fisher Scientific, Pittsburgh, PA). Immunohistochemistry was then performed with a novel polyclonal antibody that was affinity-purified from antiserum to the human SMO peptide sequence CIHWDQASARPRGPEIPR raised in rabbits by standard methods as described.⁸ In Western blotting in nontumorigenic lung epithelial cells stimulated with tumor necrosis factor, this antibody detected only 1 band corresponding to SMO.¹⁸ The specificity of this antibody in immunohistochemistry has been verified by previous findings that an SMO blocking peptide abolishes immunostaining with this antibody.²⁹

After overnight heating at 37°C the sections were deparaffinized in xylene and rehydrated in graded alcohols. Endogenous peroxidase was blocked by adding H₂O₂ solution (3%) for 15 minutes at room temperature. Pretreatment was performed by boiling tissue sections at 100°C for 20 minutes in Reveal (Biocare Medical, Concord, CA). The slides were arranged in pairs and incubated with the primary antibody to SMO (1:10,000 dilution of a 4.09 mg/mL stock antibody concentration, for a working dilution of 409 ng/mL) using capillary action for 60 minutes in a humid chamber. After washing, incubation with biotinylated secondary antibody was performed for 30 minutes. Sections were rinsed and incubated with streptavidin-HRP (Biocare Medical) for 30 minutes. Diaminobenzidine (Sigma-Aldrich, St. Louis, MO) was used as a chromogen and the tissues were counterstained with hematoxylin. Negative controls were performed by omitting the primary antibody. All incubations with antibodies were performed at room temperature.

Scoring of SMO Immunostaining

Immunostained slides were reviewed and scored by a single GI pathologist (M.B.P.) who was blinded to the clinical status of the subjects. SMO immunostaining was evaluated semiquantitatively in epithelium and inflammatory cells in colonic mucosa biopsies. Epithelial cells were graded for intensity of staining on a scale of 0 (absent), 1 (weak), 2 (moderate), and 3 (strong). The percentages of epithelial cells staining at each intensity level was multiplied by the intensity score, divided by 100, and totaled, resulting in a scoring range of 0–3. For inflammatory cells the percentage of cells staining positive for SMO was assessed in every specimen. Only cells with moderate to strong immunostaining were considered positive. We also quantified the inflammation score, which was graded as 0 (none), 1 (mild), 2 (moderate), and 3 (severe). Then the percentage of inflammatory cells staining positive was multiplied by the inflammation score, divided by 100, resulting in a score of 0–3. Average scores were obtained for each subject with multiple biopsy samples.

Statistics

Results are expressed as means $\pm$ SEM. Statistical analysis was performed with SPSS v. 15 (Chicago, IL) and GraphPad Prism 5.0 (San Diego, CA). D'Agostino and Pearson omnibus normality testing was performed to assess for normality in the groups. If the data did not meet criteria for normal distribution, log transformation was performed to normalize the data. Where 2 groups were compared, unpaired data was evaluated by Student's *t*-test, while paired data was evaluated by paired *t*-test. Data with more than 2 groups were analyzed by analysis of variance (ANOVA) and Tukey post-hoc multiple comparisons test. $P < 0.05$ was considered statistically significant.

Ethical Considerations

The study protocol was approved by the Institutional Review Board at Vanderbilt University. Written informed consent was obtained from all subjects before entry into this study.

RESULTS

Patient Characteristics

To test the hypothesis that UC is associated with dysregulation of polyamine homeostasis and potential down-stream effects of SMO, we analyzed gene expression and cellular localization of this enzyme in human subjects with histologically proven UC of varying disease severity. The demographic information for the 69 cases used in this study, categorized by histologic disease activity, is shown in Table 1. Notably, there were no significant differences in age, gender distribution, smoking, or alcohol consumption between groups. There was no decrease in body mass index in UC, and in fact there was an increase in the quiescent group when compared to the normal and mild group.

The majority of UC patients were on 5-aminosalicylate therapy and there were no significant differences between the quiescent, mild, moderate, and severe UC groups. There was more corticosteroid use only in the moderate colitis group. It should be noted that there were 3 subjects in our study presenting for evaluation of possible UC who were on drug therapies and were found to have normal histologic and endoscopic findings and were classified into the normal group. One of these subjects was on 5-aminosalicylate only, 1 on corticosteroid only, and 1 was on each of these agents plus biologic and immunodulator therapy.

SMO Gene Expression by TaqMan-based PCR

For SMO mRNA expression a total of 65 cases were included in the analysis and tissues from left colon were utilized. When all patients with UC were considered there was a significant increase in SMO mRNA levels (Fig. 1) from $0.95 \pm 0.29 \times 10^{19}$ copies of SMO/ng of GAPDH in normal tissues ($n = 14$) to $2.98 \pm 0.83 \times 10^{19}$ copies in UC tissues ($n = 51$), which represented a 3.1-fold increase ($P = 0.044$).

We also compared SMO levels in actively involved left-sided colonic tissues with paired histologically uninvolved right colon from the same subjects in a group of the patients ($n = 22$) with left-sided colitis only (Fig. 2). There was an increase in SMO levels from uninvolved right colon to the involved left colon of $0.46 \pm 0.11 \times 10^{19}$ to $1.71 \pm 0.54 \times 10^{19}$ copies of SMO/ng GAPDH, a 3.7-fold increase ($P < 0.001$).

Immunohistochemical Staining for SMO in Epithelial and Mononuclear Inflammatory Cells and Relationship to UC Histologic Disease Activity

Because we found that SMO mRNA expression was increased in UC and SMO can be regulated at the posttranscriptional level,³³ we sought to examine SMO expression further, which was

addressed by analyzing protein expression in left colon tissues. We utilized immunohistochemistry, which also allowed us to evaluate the cellular localization of SMO protein (Fig. 3A). Because we used immunoperoxidase staining with hematoxylin counterstain, we were able to simultaneously grade the inflammatory cell infiltration and SMO staining in the epithelium (Fig. 3B) and inflammatory cells (Fig. 3C), followed by comparing SMO levels to the histologic disease activity in the tissue sections.

There was moderate to strong staining of the colonic epithelium in each tissue category. It should be noted that in general the epithelial staining was stronger in the deeper glands in the colitis tissues than in the normal mucosa, although overall SMO epithelial staining scores were not significantly different between normal subjects and patients with UC (Fig. 3B).

In contrast, there was minimal staining of the lamina propria immune cells in the normal control tissues and a progressive increase in inflammatory cell staining in the UC tissues in a disease activity-dependent manner (Fig. 3A,C). Review of our cases by a blinded GI pathologist (M.B.P.) led to the conclusion that the staining of the immune cells was restricted to mononuclear cells and that polymorphonuclear cells were negative for SMO staining. In the higher-power views there is both nuclear and cytoplasmic staining for SMO in the epithelium and the mononuclear cells. Furthermore, the specificity of the immunohistochemistry for SMO is demonstrated by the complete absence of staining in the negative control tissues from severe colitis in which the primary antibody was omitted (Fig. 3A); similar results were observed for each level of disease activity (data not shown).

When SMO staining scores were specifically assessed for the mononuclear inflammatory cells, there were increases from 0.30 ± 0.08 in the normal group ($n = 16$), to 0.53 ± 0.13 in the quiescent ($n = 13$), 1.06 ± 0.19 in the mild ($n = 14$), 1.60 ± 0.21 in the moderate ($n = 14$), and 1.85 ± 0.22 in the severe ($n = 12$) groups (Fig. 3C). Accordingly, the mild ($P = 0.01$), moderate ($P < 0.001$), and severe ($P < 0.001$) groups each exhibited a significant increase when compared to normal control tissues. Additionally, the staining in the severe group was significantly increased from the mild ($P = 0.016$) and quiescent ($P < 0.001$) groups and the moderate group was different from the quiescent group ($P < 0.001$).

Correlation of SMO Staining with UC Clinical Parameters

Because we had identified an association between mononuclear inflammatory cell SMO staining and histologic disease severity, we next sought to determine if there was a similar correlation with endoscopy score (Fig. 4A) and the DAI (Fig. 4B). As shown in Figure 4A, the SMO inflammatory cell protein expression level was increased in a step-wise progressive manner with increasing endoscopic disease activity. Staining scores were increased from the level in subjects with normal appearing colonic mucosa of 0.39 ± 0.09 ($n = 23$) to the levels of 1.05 ± 0.16 in the mild ($n = 23$; $P = 0.007$), 1.47 ± 0.20 in the moderate ($n = 12$, $P < 0.001$), and 1.86 ± 0.27 in the severe ($n = 11$, $P < 0.001$) UC groups. Additionally, SMO inflammatory cell staining in tissues from subjects with endoscopically severe UC were significantly increased from those with mild UC ($P = 0.009$). In contrast, the epithelial staining intensity was not statistically different between any of the endoscopic groups (data not shown). When the inflammatory cell protein expression was plotted against the composite DAI (Fig. 4B) there was a strong correlation when assessed by Pearson's correlation test ($r = 0.653$, $P < 0.001$).

Further, in 22 UC patient subjects we were able to use each patient's own uninvolved right colon biopsies as controls for comparison to their diseased left colon biopsies. There was no significant difference in epithelial staining scores between diseased left colon and uninvolved right colon tissues (2.36 ± 0.30 versus 2.45 ± 0.51 , respectively, $P = 0.49$; Fig. 5A). However, when inflammatory cell scores were considered there was a 2-fold increase in SMO

immunohistochemical staining intensity in the diseased left colon in comparison to the normal right colon (1.32 ± 0.19 versus 0.66 ± 0.12 , $P = 0.01$; Fig. 5B).

DISCUSSION

In this report we demonstrate for the first time the involvement of SMO in human UC. We show that SMO mRNA expression is increased in colitis tissues when compared to normal controls and, importantly, there was an increase in the involved left colon when compared to the uninvolved right colon from UC subjects. SMO protein expression was present in the colonic epithelium in normal control tissues, and this did not increase significantly in colitis tissues, whereas lamina propria immune/inflammatory cell staining increased progressively in colitis tissues of increasing disease activity. As with the mRNA data, the staining was increased in the inflammatory cells of the involved left colon versus uninvolved right colon in the subjects with left-sided colitis. The inflammatory cell SMO staining was strongly correlated with the DAI and with the endoscopic subscore, further supporting the likelihood that SMO is involved in the pathogenesis of colitis.

In this study there was not a disease activity-specific increase in SMO in the colonic epithelium. Metabolism of spermine is required for polyamine homeostasis in order to prevent toxic accumulation of spermine,²⁷ and our data indicating a high level of colonic epithelial SMO staining suggest that this enzyme is involved in the homeostatic regulation of polyamines in the colonic epithelium. This is in contrast to the human stomach, where we have found that SMO expression is very low in gastric epithelium of normal mucosa but increased in these cells in *H. pylori* gastritis tissues.¹¹

It should be noted that while SMO mRNA levels were increased in UC tissues in our 51 combined cases, there was not a statistically significant increase in the individual groups when these data were separated by histologic disease activity (data not shown). When these findings are interpreted in light of the characteristics of UC that as disease activity increases there is marked expansion of inflammatory and immune cells and a relative loss of colonic glands, we speculate that the high level of SMO expression in the epithelium in the normal tissue, coupled with the loss of epithelium due to the tissue injury in the moderate and severe colitis tissues, reduced the ability to detect a disease activity-specific increase in SMO by mRNA analysis. Along these lines, when mRNA and mononuclear cell SMO protein staining were compared for individual patients, there was no correlation (data not shown). SMO is transcriptionally regulated, as evidenced by our findings of induction of SMO promoter activity in gastric epithelial cells exposed to *H. pylori*¹¹ or lung epithelial cells exposed to a spermine analog.³³ However, SMO can also be regulated at the posttranscriptional level, including the level of protein translation,³³ which could also contribute to differences in expression of mRNA and protein in UC tissues. While our immunohistochemistry data clearly demonstrated a quantifiable increase in SMO expression in the mononuclear immune/inflammatory cells, we were not able to confirm these data by isolation of epithelial cells and mononuclear cells followed by SMO detection due to the limited availability of tissue from the endoscopic biopsies in our tissue repository. To this end, we intend to pursue such studies in mouse colitis models where availability of tissue is not an issue.

A key issue raised by our studies is the biological importance of the upregulation of SMO that we detected in the mononuclear inflammatory cells in UC. Dysregulation of SMO may have profound deleterious consequences. H_2O_2 is generated in a stoichiometrically equivalent manner for each molecule of spermidine produced.^{11,13,20,24,27,34} This H_2O_2 may be relevant to GI carcinogenesis, since we have reported that *H. pylori*-induced oxidative DNA damage in gastric epithelial cells is SMO-dependent.¹¹ A similar association between SMO induction and oxidative DNA damage has also been demonstrated in cytokine-activated lung epithelial

cells.⁸ Thus, the increased SMO in the immune/inflammatory cells in the colitis tissues could be increasing the overall oxidative stress to which the colonic epithelium is exposed. In the current study it was not feasible to measure oxidative stress markers in mononuclear cells because we did not have sufficient tissue quantities to isolate these cells and perform flow cytometry for signatures of oxidative DNA damage or protein modifications. This could be a fruitful area of investigation in studies where more tissue is available, such as mouse models. We did not examine SSAT in the present study because recent work has convincingly demonstrated that SMO, and not the SSAT/PAO pathway, is the primary source of polyamine-derived oxidative stress.^{8,11,13,25,26}

Increased H₂O₂ in the immune cell compartment is also likely to directly disrupt the homeostasis of these cells. For example, we have reported that SMO induction in *H. pylori*-stimulated macrophages leads to mitochondrial membrane depolarization and apoptosis, which can be attenuated by either scavenging of H₂O₂ or siRNA knockdown of SMO.¹³ SMO-mediated apoptosis of monocytes and macrophages could be an important contributor to the inflammatory process in colitis. It has been reported that macrophage apoptosis contributes to the pathogenesis of enteric infections by the release of preformed cytokines, as has been described with IL-1 in *Shigella flexneri* infection.^{35,36} Additionally, loss of macrophages via apoptosis likely results in impairment of innate immunity, and an altered balance between the human host and the surrounding microbiota, which has been implicated in the pathogenesis of colitis.² Spermine has been shown to inhibit proinflammatory cytokine production¹⁵ and to decrease inflammatory cell locomotion,³⁷ so depletion of spermine by SMO may exacerbate inflammatory cell responses. Additionally, we have shown that spermine inhibits the translation of inducible NO synthase (iNOS) in macrophages,¹⁰ suggesting that spermine depletion in macrophages by SMO upregulation could lead to increased generation of NO by these cells. This is relevant because iNOS-derived NO has been suggested to have a causal role in colitis in many animal studies, although its effects in human colitis may vary in a cell- and disease-dependent manner.³⁸ Consistent with an antiinflammatory role for spermine in colitis, we have reported that treatment of mice with an inhibitor of polyamine synthesis, α -difluoromethylornithine (DFMO), results in severe exacerbation of *Citrobacter rodentium*-induced colitis, while treatment with L-arginine that enhanced colonic polyamine synthesis by providing substrate for generation of L-ornithine ameliorated colitis.²³

Another interesting finding in our immunohistochemistry studies was that SMO staining occurred in both the cytoplasm and the nucleus of the epithelium and the mononuclear cells. These findings are consistent with the recent description that SMO can be localized to both regions of the cell.²⁶ The nuclear localization increases the likelihood that oxidative stress is occurring in the nucleus, with implications for risk for accumulated DNA damage.^{11,26} Additionally, spermine functions as a free radical scavenger,³⁹ so depletion of spermine in the nucleus by the action of SMO could also contribute to oxidative DNA damage by loss of this beneficial function.²⁶

In summary, our studies indicate that SMO may be involved in the pathogenesis of colitis by potentially impairing innate immunity, causing oxidative stress, and exacerbating risk for oxidative DNA damage. This raises the question as to whether there are opportunities to intervene in this pathway to alter the course of disease. DFMO has recently been shown to be beneficial in chemoprevention of colonic polyps in humans,⁴⁰ which has been attributed to the anti-proliferative effect of blocking polyamine synthesis. However, DFMO would not be expected to be beneficial in human colitis, since polyamines are essential in epithelial restitution⁴¹ and regulation of immune responses,^{10,15} and this treatment exacerbated colitis in our mouse studies.²³ In contrast, MDL 72527 is an inhibitor of polyamine oxidases, including SMO,^{11,13} and it has recently been shown to reduce tumor development in a mouse skin cancer model.⁴² We have found that SMO levels are significantly upregulated in the colitis

tissues in the dextran sulfate sodium mouse model that mimics UC (unpubl. obs.); therefore, we plan to test MDL 72527 in that model. However, MDL 72527 is not commercially available, and may not be practical for human use, since it blocks the constitutively expressed acetyl polyamine oxidase in addition to SMO.^{24,27,34,42,43} Nonetheless, SMO is a potential target for development of inhibitory compounds that could serve as a potential novel treatment strategy in UC.

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Figure 1.

SMO mRNA expression in colon tissues from normal controls and patients with UC. Gene expression was assessed by real-time PCR using TaqMan-based multiplex PCR with standardization of levels to the housekeeping gene GAPDH. When all patients with UC were considered, there was a significant increase in SMO mRNA levels ($P = 0.044$ by unpaired Student's *t*-test).

Figure 2.

Comparison of SMO mRNA levels in UC-involved left-sided colon tissues with uninvolved right colon from the same patients determined by real-time PCR using Taq-Man-based multiplex PCR. There was a significant increase in SMO mRNA levels from uninvolved right colon to involved left colon ($P < 0.001$ by paired Student's *t*-test).

Figure 3.

SMO protein expression by immunohistochemistry in subjects with normal histology and increasing grades of colitis as determined by histopathology. (A) Representative SMO immunoperoxidase staining observed in normal mucosa and in quiescent, mild, moderate, and severe UC tissues. The histopathologic disease status and microscopic magnification are shown. Note the progressively increased staining intensity of the inflammatory cells in the lamina propria of the UC samples with increasing disease activity, and that this staining is in mononuclear cells, with absence of staining of polymorphonuclear cells infiltrating the epithelial surface. (B) Quantification of immunohistochemistry staining score for SMO in colonic epithelium for the 5 groups of subjects (normal, and quiescent, mild, moderate, or severe UC). Overall P -value was 0.07 by ANOVA. (C) SMO expression in the inflammatory cells demonstrating that there was a significant increase in immunohistochemistry staining score with increasing disease severity by histopathology (overall P -value < 0.001 by ANOVA).

Figure 4.

Comparison of SMO inflammatory cell protein expression levels as assessed by immunohistochemistry, with clinical parameters. (A) SMO protein levels by endoscopic disease category. Overall P -value < 0.001 by ANOVA. SMO levels were higher in the mild, moderate, and severe groups when compared to the cases with normal appearing mucosa. (B) Spline graph of SMO protein expression versus the DAI. Protein levels were strongly correlated with DAI, as determined by Pearson's correlation test ($r = 0.653$, $P < 0.0001$).

Figure 5.

Quantification of immunohistochemistry staining scores in 22 UC patient subjects, in which the right colon biopsies uninvolved with disease were compared to diseased left colon biopsies from the same patient, using a paired Student's *t*-test. (A) There was no significant difference ($P = 0.49$) in SMO staining score in the comparison of the diseased left colon to the normal right colon in the epithelial cell component. (B) Increased SMO staining score in the lamina propria mononuclear cells ($P = 0.01$) in the diseased left colon in comparison to the normal right colon.

TABLE 1

Demographic Features of the Study Subjects

Histopathology	Normal (n = 16)	Quiescent (n = 13)	Mild (n = 14)	Moderate (n = 14)	Severe (n = 12)	P-value (overall)
Age, mean $\pm$ SD (range)	49.7 $\pm$ 12.3 (25 – 67)	47.1 $\pm$ 15.5 (25 – 78)	41.5 $\pm$ 11.3 (21 – 61)	43.7 $\pm$ 14.1 (26 – 67)	47.6 $\pm$ 14.9 (30 – 68)	0.510 ^a
Gender	5M:11F	6M:7F	5M:9F	10M:4F	5M:9F	0.201 ^b
Body mass index, mean $\pm$ SD (range)	25.2 $\pm$ 3.2 (20.9 – 31.4)	30.6 $\pm$ 4.6* (22.5 – 40.3)	24.4 $\pm$ 2.9 (21.0 – 30.7)	25.5 $\pm$ 3.9 (20.2 – 34.2)	27.6 $\pm$ 6.0 (20.2 – 36.4)	0.003 ^a
Smoking	0/16	0/13	0/14	1/14	0/12	0.768 ^b
Alcohol consumption ($>$ 5 drinks/wk)	3/16	0/13	3/14	2/14	1/12	0.506 ^b
5-ASA	2/16**	11/13	11/14	13/14	8/12	$<$ 0.001 ^b
Corticosteroids	2/16 [†]	3/13	0/14	7/14	5/12	0.008 ^b
Biologics	1/16	2/13	1/14	1/14	3/12	0.627 ^b
Immunomodulators	1/16 [‡]	7/13	6/14	4/14	6/12	0.032 ^b

^aOne-way ANOVA.

^bExact $r \times c$ contingency table.

* $P = 0.01$ versus normal and mild UC samples by post-hoc comparisons test.

** $P < 0.001$ versus quiescent, mild, moderate, and severe UC.

[†] $P = 0.046$ versus moderate.

[‡] $P = 0.006$ versus quiescent, mild, moderate, and severe UC.