Role of MutS homolog 2 (MSH2) in intestinal myofibroblast proliferation during Crohn’s disease stricture formation

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Submitted 29 April 2008; accepted in final form 16 July 2008

Floer M, Binion DG, Nelson VM, Manley S, Wellner M, Sadeghi S, Behmaram B, Sewell C, Otterson MF, Kucharzik T, Rafiee P. Role of MutS homolog 2 (MSH2) in intestinal myofibroblast proliferation during Crohn’s disease stricture formation. Am J Physiol Gastrointest Liver Physiol 295: G581–G590, 2008. First published July 17, 2008; doi:10.1152/ajpgi.90311.2008.—Tissue remodeling and mesenchymal cell accumulation accompanies chronic inflammatory disorders involving joints, lung, vasculature, and bowel. Chronic inflammation may alter DNA-mismatch repair (MMR) systems in mesenchymal cells, but is not defined in Crohn’s disease (CD) and its associated intestinal remodeling and stricture formation. We determined whether DNA-MMR alteration plays a role in the pathogenesis of CD tissue remodeling. Control and CD bowel tissues were used to generate primary cultures of muscularis mucosa myofibroblasts, which were assessed directly or following stimulation with TNF-α/LPS or H2O2. MutS homolog (MSH)2, MSH3, and MSH6 expression in tissues and myofibroblasts was determined. Immunohistochemical staining revealed an increased expression of MSH2 in CD muscularis mucosa and submucosal tissues compared with controls or uninvolved CD tissue, and MSH2 expression was increased in CD myofibroblasts compared with control cells. TNF-α/LPS and H2O2 further enhanced MSH2 expression in both control and CD cells, which were decreased by simvastatin. There were no significant changes in MSH3 and MSH6 expression. Proliferating cell nuclear antigen and Ki67 staining of CD tissue revealed increased proliferation in the muscularis mucosa and submucosa of chronically inflamed tissues, and enhanced proliferation was seen in CD myofibroblasts compared with controls. Simvastatin reversed the effects of inflammatory stress on the DNA-MMR and inhibited proliferation of control and CD myofibroblasts. Gene silencing with MSH2 siRNA selectively decreased CD myofibroblast proliferation. These data demonstrate a potential role for MSH2 in the pathogenesis of nonneoplastic mesenchymal cell accumulation and intestinal remodeling in CD chronic inflammation.

ulcerative colitis; simvastatin; MSH2 siRNA

CROHN’S DISEASE (CD) is a chronic inflammatory disorder characterized by destructive inflammation and tissue remodeling leading to stricture formation in the gastrointestinal tract (9). A majority of patients with CD with small intestinal involvement will require surgical intervention because of worsening episodes of intermittent partial obstruction from symptomatic small bowel strictures (25). One of the most severe forms of CD occurs in individuals who develop rapid symptomatic stricture recurrence following surgery, which will lead to repeated operative interventions with increasing morbidity and potentially mortality (4). Thus the most important and severe clinical manifestations of CD are directly linked to the uncontrolled cellular events underlying tissue remodeling and stricture formation.

Stricture formation in CD is linked to both acellular and cellular processes (7, 29). Excessive deposition of extracellular matrix in areas of chronic inflammation and stricture has been demonstrated in the remodeled CD intestine (13). In addition, the expansion of intestinal myofibroblasts within the muscularis mucosa has also been shown to play a role in the pathogenesis of stricture formation (22). However, the molecular mechanisms underlying enhanced accumulation of mesenchymal cells in CD tissue remodeling remain incompletely defined (29).

Chronic inflammation stresses tissues with inflammatory cytokines, nitrogen radicals, and reactive oxygen species, which can lead to low level damage, repeated attempts at repair, and ultimately remodeling of the bowel wall. These tissue changes are accompanied by alterations at a molecular level as cells respond to and adapt to chronic stress. One of the molecular changes associated with exposure to oxidative stress is genetic damage, including mutations to DNA single base pairs. This has been best studied in the context of chronic inflammation in the epithelium leading to the development of cancer (8). Investigation into chronic inflammation-associated DNA alteration has been focused on microsatellite regions in the human genome, the noncoding areas of mononuclear or dinuclear acid repeats, which are chemically unstable and prone to mutations. Eukaryotic organisms normally have intrinsic mechanisms to monitor and repair DNA damage, exemplified by the classic DNA-mismatch repair (MMR) system, which includes the MutS homolog and MutL homolog enzymes. MutS has two forms: MutS-α and MutS-β. MutS-α is a dimer, formed from the proteins MutS homolog 2 and 3 (MSH2 and 3) (12). MutS-β components are MSH2 and 6. The former is important for the repair of single base mismatches, whereas the latter is felt to play a role in loop MMR. Failure of the MMR system can result in a hypermutable state and DNA microsatellite instability (MSI), which has been implicated in uncontrolled epithelial proliferation and neoplasia, e.g., hereditary nonpolyposis colon cancer (24).

Although these mechanisms are implicated in the molecular pathogenesis of epithelial cell carcinogenesis, new data suggest

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that alterations in the DNA-MMR system are also found in mesenchymal cells undergoing tissue remodeling in chronic inflammatory disorders (34). It is now known that nonmalignant DNA alterations occur in atherosclerosis, asthma, chronic obstructive pulmonary disease, and rheumatoid arthritis (23, 31, 36). These studies hypothesized that alterations in mesenchymal cells and their behavior is linked to an accumulation in DNA damage, which may be directly related to a loss of DNA-MMR function. This hypothesis suggests that mesenchymal cells, that exhibit an aggressive “invasive” phenotype with uncontrolled proliferation may in fact result from DNA alterations implicated in the transformation of epithelial populations into adenocarcinoma.

The tissue remodeling and stricture formation found in CD intestinal inflammation is associated with an uncontrolled cellular accumulation of mesenchymal cells, which may in part originate from the muscularis mucosa (7). It is unknown whether altered expression of the DNA-MMR mechanisms, found in other forms of chronic inflammation (20, 23), may also play a role in the tissue remodeling that characterized CD chronic inflammation.

The aim of this study was to determine the expression patterns of DNA-MMR system components MSH2, MSH3, and MSH6 and potential changes in MSI in myofibroblasts derived from the muscularis mucosa of human CD tissues compared with non-inflammatory bowel disease (IBD) control bowel. In addition, we sought to determine whether alterations in the expression of DNA-MMR system enzymes are functionally linked with the proliferative capacity of mesenchymal cells, which could contribute to altered cellular accumulation and the formation of strictures in the setting of CD chronic inflammation.

MATERIALS AND METHODS

Reagents. Cell growth supplement was obtained from Upstate Cell Signaling Solutions (Temecula, CA); RPMI 1640 medium, FBS, MCDB-131 medium, and penicillin/streptomycin/fungizone were from Invitrogen (Carlsbad, CA); human plasma fibronectin was from Chemicon International (Temecula, CA); porcine heparin and anti-human vimentin (Clone VIM 13.2) were from Sigma Chemical (St. Louis, MO); TNF-α, anti-human-α smooth muscle actin (Clone 1A4) was from R&D Systems (Minneapolis, MN). Anti-human MSH2 (Clone FE11) was obtained from Calbiochem (La Jolla, CA); anti-human MSH3 (Clone H-300), anti-human proliferating cell nuclear antigen (PCNA) (Clone PC-11), Ki67 monoclonal antibody, and secondary antibodies conjugated to horseradish peroxidase were from Santa Cruz Biotech (Santa Cruz, CA); anti-human MSH6 (Clone 44) was from BD Transduction Laboratory (Franklin Lakes, NJ). Unless otherwise indicated, all other chemicals were from Sigma.

Tissues. Surgical tissue from patients with IBD [CD and ulcerative colitis (UC)] as well as controls (normal resection margins for diverticulitis disease, bariatric surgery, etc.) were obtained. Patients with CD requiring surgical intervention due to recurrent obstruction were specifically evaluated for studies on tissue remodeling and stricture formation. Tissues were used from 10 patients with CD (4 men, 6 women, age 38 ± 16 yr), as well as UC and normal controls (n = 10 each group). Myofibroblast cultures were generated from these samples; involved and uninvolved tissue from the same patient served as an internal control. All experiments were approved by the Institutional Review Board of the Medical College of Wisconsin.

Myofibroblast culture. Myofibroblasts were isolated according to previously published reports (11) and used between passages 4 and 8.

Activation and pharmacologic modulation of myofibroblasts. Myofibroblasts were stimulated with 1 μM H2O2 or with TNF-α (100 U)/LPS (1 μg) for specified time periods. In some experiments, 10 μM simvastatin combined with activator(s) was used.

Immunohistochemical analysis. Tissues were prepared as described previously (16) using antibodies to MSH2, MSH3, MSH6, Ki67, and PCNA. For control staining IgG-isotype-matched nonimmune serum and only the secondary antibody were performed.

Immunofluorescence staining. Myofibroblasts were grown on cover slips to 80% confluency. Immunofluorescence and hydroethidine staining was performed as previously described (16).

RNA extraction and semiquantitative RT-PCR. Myofibroblast RNA extraction was performed as previously described (16). The PCR reactions were done in 25 cycles (both MSH2 and β-actin, 95°C (denaturation), 55°C (annealing), and 72°C (extension) each 1 min and 72°C for an additional 5 min. The PCR products were analyzed as previously described (16). The primer sequences and PCR product sizes are as follows: β-actin: forward, 5'-TGA CGG GTG CAC CCA TAC GTC GC-3' and reverse, 5'-CTA GAA GCA TTG CAG TGG AAC AGG GAG G-3'; MSH2: forward, 5'-GTC GCC TTC GTG GCC TTC TTT-3' and reverse, 5'-TCT CTG GCC ATC AAC TGC GGA-3' (350 bp).

Western blot analysis. Western blot analysis was performed as previously described (16) using MSH2, PCNA, and Ki67 antibodies. Each band was quantified with the use of the NIH ImageJ 1.31v analysis software. The values are from three individual experiments and expressed as means ± SD.

MSI analysis. Microsatellite status was determined after DNA extraction (DNeasy kit; Qiagen, Valencia, CA) from 10 CD cultures (passages 2–4). The D2S123, BAT25, BAT26, D17S250, and DSS346 markers were from the National Cancer Institute MSI reference panel for colorectal cancer.

Electrophoretic mobility changes or loss of bands of the amplified PCR products defined microsatellite status from myofibroblast cells. PCR was performed with 1 μg of DNA, and PCR products were separated on 10% Tris-buffered saline polyacrylamide gels. MSI was detected by the presence of visible altered allelic shifts or loss of bands in the PCR products of involved vs. uninvolved sample. High MSI showed more than two allelic shifts, whereas low MSI showed no more than one change in allelic shifts.


MSH2 siRNA transfection and real-time PCR. All siRNAs were obtained from Dharmacon (La Fayette, CO). Primary human smooth muscle cells were transfected with 2 μg green fluorescent protein (GFP) DNA as a qualitative indicator of successful transfection. Nontargeting siRNAs (NT1, NT2; catalog no. D-001810-01-05 and D-001810-02-05) were used as negative controls to demonstrate the specificity of MSH2 siRNA. The efficiency of the transfection was demonstrated by GFP.

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**RESULTS**

**Immunohistological analysis.** Expansion of the myofibroblasts in the muscularis mucosa is frequently seen as a component of tissue remodeling in areas affected by CD chronic inflammation, which may lead to stricture formation (22). Initial experiments were performed to screen for the expression of DNA-MMR components in the nonepithelial, structural elements of the human small and large intestine, specifically focusing on myofibroblasts and endothelial cells in the mucosa and submucosa. Experiments were also focused on disease-specific differences between control, as well as involved and uninvolved specimens from IBD (CD and UC).

Immunohistochemistry using antibodies against MSH2, MSH3, and MSH6 revealed a significantly higher expression of MSH2 in the muscularis mucosa of CD compared with control samples (Fig. 1, A, B, and C, arrows). However, MSH3 expression did not differ between CD and controls (Fig. 1, E and F). Similarly, there was no significant change detected in the patterns of MSH6 staining between CD and controls (Fig. 1, G and H). MSH2 staining was restricted to spindle-shaped cells, which were identified morphometrically by anatomic pathologists as smooth muscle/myofibroblasts in remodeled areas of intestine. No endothelial cells were found to stain positive.

Next we used antibody targeting PCNA, also known as cyclin, which is a nuclear protein involved in DNA replication and interacts during replication with several DNA polymerases such as cyclin D, DNA ligase I, MSH2, MSH3, and MSH6 (19). Therefore, PCNA is an established proliferation marker in immunohistochemical studies (17). Figure 1 (J and K) demonstrates strong staining with PCNA antibody in the submucosa of CD sections compared with controls, and lymph node was used for the PCNA-positive staining (Fig. 1K). Nonimmunoserum IgG staining was performed on all samples to ensure the antibody specificity. These data suggest that mesenchymal cell populations from areas of tissue remodeling in CD muscularis mucosa demonstrate increased proliferative capacity. Interestingly, these areas of PCNA staining also corresponded to the areas of increased MSH2 expression.

**Immunofluorescence staining of myofibroblasts.** To confirm that MSH2 staining was specific to myofibroblasts, we used primary cultures of these mesenchymal cells for immunofluorescence staining.

Fig. 1. Immunohistochemical staining of normal and Crohn’s disease (CD) sections using MutS homolog (MSH)2, MSH3, MSH6, and proliferating cell nuclear antigen (PCNA) antibodies (n = 10 for all samples). MSH2 staining of control tissue demonstrates a very weak signal in the submucosa (A, arrow). In contrast, MSH2 staining of CD tissue shows a strong MSH2 signal in the submucosa (B, arrow). Magnification ×400. C–D: MSH2 staining of CD tissue shows a strong MSH2 signal in the submucosa. Magnification ×100. E–H: extremely weak MSH3 and MSH6 signal in both control and CD submucosa (arrow). Magnification ×400. PCNA staining was weak in control submucosa (I, arrow), whereas PCNA staining of CD demonstrates stronger proliferative activity in the submucosal compartment compared with control samples (J, arrow). Magnification ×100. PCNA staining of lymph node served as a positive control (K). All sections were counterstained with Mayers hematoxylin. Images are representative of 10 independent experiments.
were stained with antibodies to vimentin and α-smooth muscle actin to confirm that these cells demonstrated characteristic markers of myofibroblasts (Fig. 2, A and B, respectively). Immunofluorescence staining of myofibroblasts with anti-MSH2 antibody showed a higher expression in cells derived from CD tissues compared with controls (Fig. 2, C and D). In contrast to the myofibroblasts, human intestinal microvascular endothelial cells from both CD and controls failed to show expression for MSH2 (data not shown).

Next we examined whether MSH2 expression in myofibroblasts could be modulated by various mediators associated with chronic intestinal inflammation in CD, specifically oxyradical stress, inflammatory cytokines, and bacterial LPS. When myofibroblasts were exposed to 1 μM H2O2, MSH2 expression was significantly upregulated in both control cells as well as in CD cells (Fig. 2, E and F). MSH2 expression increased in TNF-α/LPS treated cells but to a lesser degree than that seen in the H2O2-treated cells (data not shown).

The pathophysiological effect of MSH2 expression was reversed in vitro by treatment with the 3-hydroxy-3-methylglutaryl-coenzyme-A reductase (HMG-CoA reductase) inhibitor, simvastatin. Simvastatin was selected because of its pharmacological effect as an antioxidant on mesenchymal cells (10). Oxidative stress has been shown to play a significant role in DNA disruption either directly or through a downregulatory effect on the expression of DNA-MMR in a human erythroleukemia cell line (8). Using our nontransformed primary cultures of intestinal myofibroblasts, we have demonstrated the opposite finding. Figure 2 (G and H) has shown that simvastatin treatment of myofibroblasts resulted in inhibition of MSH2 expression in both control and CD cells, which had been activated either by H2O2 or TNF-α/LPS.

To confirm the antioxidant effect of simvastatin on intestinal myofibroblasts, experiments were performed using fluorescence microscopy of live myofibroblast monolayers treated with and without simvastatin, which were then stained with hydroethidine and photographed. Figure 2J demonstrates the constitutive superoxide generation in CD myofibroblasts and the effect of simvastatin in decreasing intracellular superoxide generation (Fig. 2J).

**Enhanced MSH2 mRNA expression in CD myofibroblasts.**

Next, we determined whether there was differential expression of MSH2 mRNA between control and CD myofibroblasts using semiquantitative RT-PCR and the respective primers. Levels of MSH2 and β-actin mRNA were examined in control and CD cells, and densitometric analysis was performed after

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**Immunofluorescence Staining of Myofibroblasts**

![Immunofluorescence Staining of Myofibroblasts](http://ajpgi.physiology.org/)

Fig. 2. Immunofluorescence staining of myofibroblasts. A: anti-α smooth muscle actin. B: anti-vimentin. Nuclear staining with DAPI. C: MSH2 staining of control myofibroblast. D: MSH2 staining of CD myofibroblasts. Note; enhanced staining of MSH2 in CD cells compared with control cells. E and F: enhanced MSH2 expression in control and CD cells following H2O2 stimulation. G and H: simvastatin inhibited MSH2 expression in both control and CD cells. Magnification ×400. Image is representative of 5 independent experiments. Constitutive intravital superoxide generation in CD myofibroblasts detected with hydroethidine nuclear fluorescence is demonstrated in I, which was decreased by simvastatin treatment (J).
normalization to β-actin (436 bp), which served as an internal control. MSH2 mRNA (350 bp) expression was significantly higher in CD cells compared with control cells (Fig. 3, A and B). TNF-α/LPS and H2O2 stimulation of the cells resulted in significantly higher MSH2 mRNA expression in both control and CD cells (Fig. 3, A and B). MSH2 mRNA expression was inhibited in myofibroblasts by simvastatin treatment (Fig. 3, A and B). Neither MSH3 nor MSH6 mRNA expression was altered in CD cells compared with control cells (data not shown).

**Enhanced MSH2 protein expression in CD myofibroblasts.** The next series of experiments were performed to determine whether MSH2 protein was differentially expressed between myofibroblast cultures derived from control and CD bowel using Western blotting. As demonstrated in Fig. 3C, the level of MSH2 protein expression was significantly higher in myofibroblasts generated from CD compared with controls. H2O2 stimulation of the cells for 48 h resulted in significantly higher MSH2 protein expression in both control and CD cells (Fig. 3C). Simvastatin treatment of the cells inhibited MSH2 protein expression.
expression (Fig. 3D). Again, MSH3 and MSH6 protein expression was unchanged in control and CD myofibroblasts after H2O2 stimulation (data not shown).

**MSI and intestinal myofibroblasts.** DNA was obtained from 10 different CD myofibroblast cell cultures isolated from 10 patients. Individually matched samples were examined for the five standard colorectal cancer MSI markers described in MATERIALS AND METHODS. Only one of ten samples demonstrated a pattern of MSI in the D2S123 locus in cells generated from involved CD. This MSI was classified as MSI low. No patterns of instabilities in other loci were found. Figure 4 demonstrates the rare occurrence of MSI in a single myofibroblast cultures derived from resected surgical CD tissue. These data suggest that MSI is a rare phenomenon in CD mesenchymal cells exposed to chronic inflammation during intestinal remodeling, and these findings are in agreement with the patterns of increased MSH2 expression in myofibroblasts described earlier.

[^3H]Thymidine proliferation in myofibroblasts. Proliferation rates of myofibroblasts, assessed by [^3H]thymidine uptake, were significantly higher in CD cells compared with control cells (Fig. 5). Simvastatin treatment significantly reduced the proliferation rate in CD cells (Fig. 5). Stimulation with TNF-α/LPS increased the [^3H]thymidine uptake and proliferation rate in both control and CD cells, which was effectively inhibited by simvastatin treatment (Fig. 5). All conditions were assessed in triplicate, and results are expressed as the mean of three separate experiments ± SD. **P < 0.05 CD vs. control and P < 0.05 statin vs. CD/TNF/LPS.**

**PCNA staining in myofibroblasts.** Immunofluorescence staining of control and CD cells using anti-PCNA demonstrated a strong signal in CD myofibroblasts isolated from areas of chronic inflammation and a weak staining signal in control cells (Fig. 6, B and E). H2O2 stimulation increased the PCNA signal in both control and CD cells (Fig. 6, C and F). Nonimmunoserum IgG served as a negative control (Fig. 6, A and D). Ki67 staining of control and CD myofibroblasts revealed a similar pattern of staining to PCNA, again suggesting an increased proliferative capacity in chronic inflammation derived myofibroblasts (data not shown). Additional data confirming these findings regarding PCNA and Ki67 expression in myofibroblasts using Western analysis are presented in Fig. 8 (A and B).

**Gene silencing of MSH2 in CD myofibroblasts.** To confirm whether a functional effect on cell proliferative capacity was linked to enhanced MSH2 expression in involved CD myofibroblasts, we performed gene-silencing experiments using transfection of siRNA specific for MSH2 (35).

Using real-time PCR, we confirmed significant loss of MSH2 gene product in CD myofibroblasts following transfection of siRNA for MSH2, which was sustained from 24 to 48 h (Fig. 7). We performed a series of control experiments to demonstrate the specificity of this effect. Primary human myofibroblasts cells were transfected with 2 μg of GFP DNA as a qualitative indicator of successful transfection, and in a second series of control experiments 100 nM of nontargeting siRNA (NT1, NT2) were used to confirm that MSH2 gene expression was not affected by the transfection process alone (Fig. 7). Myofibroblast MSH2 protein expression was inhibited by MSH2 siRNA as assessed by Western blotting (data not shown). Once the parameters for MSH2 gene silencing in myofibroblasts were established, subsequent experiments were performed to examine the functional significance of the alteration in gene expression. We compared the effect of siRNA against MSH2 in control and CD myofibroblasts by assaying the proliferation markers PCNA and Ki67 using Western blotting.
Both PCNA and Ki67 expression were significantly higher in CD myofibroblasts compared with control cells as assessed by Western blotting (Fig. 8, A and B). Both simvastatin treatment and MSH2 siRNA transfection of CD myofibroblasts resulted in decreased PCNA and Ki67 expression (Fig. 8, A and B). These data demonstrate that the proliferative capacity of both control and CD myofibroblasts was linked to MSH2 expression and also suggest that the increased proliferative capacity of the CD myofibroblasts was associated with increased basal expression of MSH2.

DISCUSSION

We report increased expression of the DNA-MMR enzyme MSH2 in CD intestinal remodeling during chronic inflammation as well as increased expression in CD myofibroblasts isolated from chronically inflamed bowel. Increased expression of MSH2 was linked to an increased proliferative capacity of CD myofibroblasts compared with control cells. However, MSI was rarely encountered in CD myofibroblasts exposed to chronic inflammation in vitro. Increased MSH2 expression was not identified in either resected tissue or myofibroblasts generated from UC, where inflammation is limited to the mucosa and which typically does not result in extensive tissue remodeling and fibrosis. Using gene knockdown with siRNA targeted against MSH2, we demonstrated a decrease in proliferative capacity of CD myofibroblasts, suggesting that overexpression of this molecule is linked to a novel function, namely enhanced accumulation of these mesenchymal cells during tissue remodeling in chronic inflammation. Finally, treatment of myofibroblasts with simvastatin decreased MSH2 expression and proliferative capacity in these mesenchymal cells.

Changes to the DNA-MMR mechanisms and alterations in the microsatellite status are established mechanisms involved in the development of cancer and have also been observed in a variety of nonmalignant chronic inflammatory diseases including arteriosclerosis (14), chronic obstructive pulmonary disease (31), and rheumatoid arthritis (20, 36). Tissue remodeling and an expansion of mesenchymal cell populations occur in various forms of chronic inflammatory injury. Investigators have previously hypothesized that alterations in the DNA-MMR apparatus may result in increased MSI and DNA mutations, which may in turn produce an aggressive mesenchymal cell phenotype with potential for tissue destruction. Although this has been identified in some studies of rheumatoid arthritis (6), the loss of MMR expression and function with resultant increase in MSI has not been uniformly identified. Additional studies in both cancer and chronic inflammation suggest that increased expression of the MMR enzymes may be integrally linked to an alternative pathophysiological mechanism, namely increased cell proliferation (34).

Fig. 6. Proliferative capacity of myofibroblast cultures isolated from control and CD intestine (B and E) was assessed using immunofluorescence staining for PCNA. Control myofibroblasts demonstrated increased PCNA staining following H2O2 stimulation (C). In contrast, CD myofibroblasts demonstrated increased PCNA expression at baseline, which remained elevated following H2O2 exposure (F). These data suggest increased proliferative capacity of CD myofibroblasts compared with control cells. A and D: control isotype staining of myofibroblast. Representative data from 1 of 6 individual experiments using unique myofibroblast cell lines.
MSI is uncommon in the intestinal epithelium of patients with CD (27). This lack of MSI has been suggested to underlie the overall lower rates of adenocarcinoma found to complicate patients with CD compared with UC (18). Given the fact that UC is characterized by superficial inflammation at the level of the epithelium, and CD is more classically found to have a transmural distribution, one can hypothesize that the epithelium is at higher risk for inflammatory damage in UC, whereas mesenchymal populations in the bowel wall, including the muscularis mucosa, are exposed to chronic inflammatory stress in CD. Our findings of altered DNA-MMR expression in the mesenchymal cells isolated from CD and not UC support this hypothesis. Although additional factors including a higher surgical intervention rate (and hence resection of “at-risk” tissue) will differentiate patients with CD and UC regarding cancer risk, the differential finding of MSI may represent a contributing mechanism to increased cancer potential in UC.

The mechanisms underlying CD intestinal fibrogenesis have become an area of intense research interest (7). Proliferation of myofibroblasts in the muscularis mucosa and the thickening of the muscularis propria is a well-established part of CD intestinal remodeling, and TGF-β appears to play a pivotal role in this process. Interestingly, mesenchymal cells derived from CD appear to maintain altered patterns of protein expression in vitro despite multiple passages over several generations, which may represent an epigenetic modification in DNA expression (21). The aberrant behavior of myofibroblasts in CD strictures, which is characterized by uncontrolled proliferation, led us to the hypothesis that molecular mechanisms contributing to uncontrolled proliferation in neoplasia might also be involved in the altered behavior of mesenchymal cells. Hence the exploration of the DNA-MMR process, which can also be influenced by TGF-β, provided the rationale for this study (26).

The nonneoplastic contribution of DNA-MMR in uncontrolled, destructive tissue remodeling was first shown in rheumatoid arthritis (23). Fibroblasts generated from resected rheumatoid pannus tissues showed features remarkably similar to those found in colorectal cancers, which included a disrupted DNA-MMR mechanism as well as MSI (23). In the present study, immunohistochemical analysis of tissues from patients with CD revealed an increased expression of MSH2 in the submucosa of chronically inflamed segments but not uninvolved or control bowel specimens. Further investigation showed that spindle-shaped cells in the areas of profound tissue remodeling were myofibroblasts.

One of the more novel findings that arose from our study was that increased MSH2 expression in CD myofibroblasts can...
be reversed by simvastatin, an HMG-CoA reductase inhibitor that is widely used as a cholesterol-lowering medication in the primary prevention and treatment of atherosclerosis and coronary artery disease. Simvastatin was chosen because of the known antioxidant effect of this drug group (10), and the effect of statins on muscle cell metabolism (within the vascular system) has been well characterized (2). It has been shown that simvastatin increases the expression of two DNA repair proteins, MLH1 and XRCC1; thus MSH2 suppression by simvastatin is likely to be a very specific and not a general phenomenon in the repair system (37). In addition, it has been previously suggested that the MSH2 expression can be altered pharmacologically (30).

A physiologically relevant concentration of simvastatin was used in these studies to reduce the TNF-α/LPS- or H₂O₂-induced MSH2 overexpression at the RNA and protein levels in myofibroblasts. In addition, cell cultures treated with simvastatin showed significantly reduced cell proliferation. The effect was even more pronounced in CD-derived myofibroblasts treated with simvastatin. The increased proliferation effect of TNF-α and LPS treatment on myofibroblasts was eliminated by simvastatin treatment.

Evidence for the use of statins as therapeutic agents with action beyond their lipid-lowering effect is growing. The antioxidant and anti-inflammatory capacity of statins have been demonstrated in vitro in neurology and cardiology fields. It has been demonstrated that statins may protect neurons against ethanol-mediated injury as well as human immunodeficiency virus type 1-induced oxidative stress (1). Mechanistically, statins have been shown to downregulate expression of Bcl-2, which was linked with increased apoptosis in mesenchymal cells (5, 38). Moreover it has been shown that statins may increase calpastase expression and induce endothelial nitric oxide synthase activity (3, 28, 32, 33). As our data suggest, similar to the pleiotropic effect of statins in other cell lines, statins might be able to reduce inflammatory oxidative stress in myofibroblasts and might possibly reduce pathologic proliferation. In addition, statins might restore the disrupted formation of MutS-β in CD and prevent further damage to the DNA.

In conclusion, our results demonstrate a novel mechanism of the MSH2 enzyme in mesenchymal cell proliferation in CD. Our data support the concept that alterations in the DNA-MMR system have novel function in the nonneoplastic cell compartment of the bowel wall and are not limited to repair of acquired genetic defects. Increased expression of MSH2 in CD myofibroblasts appears to be linked to their increased proliferative capacity and may be a pathophysiological mechanism underlying stricture formation and bowel wall remodeling in CD. In contrast to the findings in CD, MSH2 overexpression was not seen in UC mesenchymal cells. Finally, the increased expression of MSH2 in CD myofibroblasts was blocked by treatment with simvastatin. This demonstrates a novel use of a statin agent to inhibit proliferative capacity of human intestinal myofibroblasts, which play an integral role in CD fibrogenesis and stricture formation. These preliminary studies suggest a potential role for statins in the treatment and prevention of CD bowel wall remodeling and stricture formation, which warrants further investigation.

GRANTS

This work was supported by a scholarship from the Deutsche Gesellschaft fuer Verdauungs- und Stoffwechselkrankheiten (DGVS) (M. Floer) and a postdoctoral fellowship from the Medical College of Wisconsin (M. Floer) and NIH Grant DK065948 (D. Binion) and support from the Digestive Disease Center of the Medical College of Wisconsin (P. Rafiee, D. Binion).

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