Regional differences in colonic mucosa-associated microbiota determine the physiological expression of host heat shock proteins

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Hu S, Wang Y, Lichtenstein L, Tao Y, Musch MW, Jabri B, Antonopoulos D, Claud EC, Chang EB. Regional differences in colonic mucosa-associated microbiota determine the physiological expression of host heat shock proteins. Am J Physiol Gastrointest Liver Physiol 299: G1266–G1275, 2010. First published September 23, 2010; doi:10.1152/ajpgi.00357.2010.—Cytoprotective heat shock proteins (Hsps) are critical for intestinal homeostasis and are known to be decreased in inflammatory bowel diseases. Signals responsible for maintenance of Hsp expression are incompletely understood. In this study, we find that Hsp25/27 and Hsp70 protein expressions are differentially regulated along the longitudinal length of the large intestine, being highest in the proximal colon and decreasing to the distal colon. This longitudinal gradient was similar in both conventionally colonized mouse colon as well as biopsies of human proximal and distal colon but was abolished in the colon of germ-free mice, suggesting a role of intestinal microbiota in the Hsp regional expression. Correspondingly, analysis of 16S ribosomal RNA genes of bacteria from each colonic segment indicated increased bacterial richness and diversity in the proximal colon. The mechanism of regulation is transcriptional, as Hsp70 mRNA followed a similar pattern to Hsp70 protein expression. Lysates of mucosa-associated bacteria from the proximal colon stimulated greater Hsp25 and Hsp70 mRNA transcription and subsequent protein expression in intestinal epithelial cells than did lysates from distal colon. In addition, transrectal administration of cecal contents stimulated Hsp25 and Hsp70 expression in the distal colon. Thus host-microbial interactions resulting in differential Hsp expression may have significant implications for the maintenance of intestinal homeostasis and possibly for development of inflammatory diseases of the bowel.

16S ribosomal RNA gene; colonic bacteria; heat shock protein 25; heat shock protein 70

IN THE NORMAL GASTROINTESTINAL TRACT, the expression of inducible heat shock proteins (Hsps) is region and cell specific, found primarily in surface epithelial cells in regions continuously exposed to hostile environments such as the colon and stomach (1, 4, 8, 27). Their physiological expression in the colon is essential for maintenance of intestinal and immune homeostasis (17, 26), and, when decreased, the mucosa becomes highly susceptible to injury and inflammation (3, 7–10, 32, 33). Paradoxically, in both human and experimental inflammatory bowel diseases, the expression of mucosal Hsps is highly susceptible to injury and inflammation (3, 7–10, 27). Their physiological expression in the distal colon is essential for maintenance of intestinal and immune homeostasis and are known to be decreased in inflammatory bowel diseases. Signals responsible for maintenance of Hsp expression are incompletely understood. In this study, we find that Hsp25/27 and Hsp70 protein expressions are differentially regulated along the longitudinal length of the large intestine, being highest in the proximal colon and decreasing to the distal colon. This longitudinal gradient was similar in both conventionally colonized mouse colon as well as biopsies of human proximal and distal colon but was abolished in the colon of germ-free mice, suggesting a role of intestinal microbiota in the Hsp regional expression. Correspondingly, analysis of 16S ribosomal RNA genes of bacteria from each colonic segment indicated increased bacterial richness and diversity in the proximal colon. The mechanism of regulation is transcriptional, as Hsp70 mRNA followed a similar pattern to Hsp70 protein expression. Lysates of mucosa-associated bacteria from the proximal colon stimulated greater Hsp25 and Hsp70 mRNA transcription and subsequent protein expression in intestinal epithelial cells than did lysates from distal colon. In addition, transrectal administration of cecal contents stimulated Hsp25 and Hsp70 expression in the distal colon. Thus host-microbial interactions resulting in differential Hsp expression may have significant implications for the maintenance of intestinal homeostasis and possibly for development of inflammatory diseases of the bowel.

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sity of Chicago and were consistent with NIH guidelines for humane care of animals.

**Cell culture.** Young adult mouse colon cells (YAMC) were grown and maintained as previously described (37). Cells were switched to the nonpermissive temperature (37°C) 1 day before being treated for 24 h with lysates prepared from mucosa-associated bacteria harvested from the proximal and distal colons of C57BL/6 mice, LPS (1–10 mM), or butyrate (0.5–5.0 mM). Cells were rinsed twice and scraped into ice-cold PBS, pelleted (14,000 g for 20 s) and then lysed for RNA and protein extraction as described below.

**Immunohistochemical staining for Hsps.** Sections (4 μm) were cut from formalin-fixed human or mouse tissues and stained for human Hsp27, mouse Hsp25, or Hsp70 using the Dako immunohistochemistry kit (Dako, Glostrup, Denmark) according to the manufacturer’s instructions. Primary antibodies, human Hsp27 (SPA800; Stressgen, Victoria, BC, Canada), mouse Hsp25 (SPA801; Stressgen), and Hsp70 (SPA810; Stressgen) were used.

**Real-time PCR for Hsp mRNA.** YAMC total RNA was extracted by Trizol (Invitrogen, Grand Island, NY). Complementary DNA was synthesized using SuperScript II (Invitrogen) and random hexameric primers. The mouse Hsp25 (NM_013560.2, bases 385–493), mouse Hsp70 (NM_010478.2, bases 215–277), human Hsp27 (NM_001540.3, bases 288–358), human Hsp70 (NM_005345.5, bases 1231–1309), mouse GAPDH (NM_008084, bases 154–223), and human GAPDH (NM_002046.3, bases 160–229) were used. Real-time PCR was performed with an iCycler (Bio-Rad, Hercules, CA) using iQSYBR Green PCR Supermix (Bio-Rad). A two-step quantification cycling protocol was used. As a relative quantitation, fold changes were measured using the ΔΔCt method. For each sample, the Ct value of Hsp70 mRNA was measured and compared with the GAPDH endogenous control as ΔCt (ΔCt = Ct_Hsp - Ct_GAPDH). The fold change of Hsp70 mRNA in the unknown sample relative to control sample was determined by 2^(-ΔΔCt), where ΔΔCt = ΔCt_Uknown - ΔCt_Control (26).

**Western blot analysis.** Protein lysates were prepared from human or mouse intestinal tissue or culture YAMC cells, and Western blots were generated and developed as described previously (12, 19). Primary antibodies, human Hsp25 (SPA801; Stressgen), Hsp27 (SPA800; Stressgen), and heat shock cognate (Hsc)70 (SPA815; Stressgen), and villin (610359; BD Biosciences, San Jose, CA) were used. Quantification of images was done by scanning densitometry using NIH Image J 1.54 software (National Institutes of Health, Bethesda, MD).

**Terminal restriction fragment-length polymorphism analysis of bacteria 16S rRNA-encoding genes.** DNA was isolated from stool or intestinal mucosal scrapings, and 16S ribosomal RNA (rRNA) gene was amplified as previously described (36) using 8F and 1492R primers. Purified PCR products were digested with MspI, and fragment sizes were analyzed on an Applied Biosystems DNA sequencer 3130. Restriction-digest fragment abundance was determined using GeneMapper software (Applied Biosystems). On the basis of the normalized terminal restriction fragment-length polymorphism (TRFLP) profile, the number and height of peaks were treated as number and abundance of bacterial phylotypes represented in samples. Diversity indices for richness (distinctive peak numbers) and Shannon Diversity (peak distribution) were calculated using formulas as described previously (36). In addition to estimating species richness and diversity, the TRFLP patterns of bacterial community were viewed and community fingerprints and used to assess the similarity of different communities by analyzing the peak patterns of TRFLPs. Pairwise Bray-Curtis distances (18) were calculated to examine the relationship between communities using the software package MEGA (http://www.megasoftware.net).

**Harvest and preparation of colonic bacterial lysates.** Mouse colon was opened longitudinally, and luminal contents were removed with gentle manual manipulation. The mucosa from the proximal one-third of the colon and the distal colon were then lightly scraped to collect mucosa-associated bacteria that were then prepared as lysates according to a previously described protocol (3). Briefly, colonic contents were solubilized, digested with DNase, and then homogenized using 0.1-mm glass beads in a mini-bead beater (Biospec Products, Bartlesville, OK). Beads were removed by centrifugation (17,000 g for 20 s), and the supernatant was collected and filter sterilized through a 0.45-μm filter. Sterility was confirmed by aerobic and anaerobic culturing of the lysates. The protein concentration was determined using a Coomassie dye-binding assay (Bio-Rad). Optimal concentrations of lysates for cell activation varied between 50 and 200 μg/ml of lyse protein in different batches.

**Quantitative PCR of bacterial 16S rRNA encoding genes.** Total DNA was extracted from colonic luminal content as described above and amplified with 16S rRNA genes used to generate amplicons, including the following: 5'-ACT CCT ACG GGA GGC AGC AG-3' and 5'-ATT ACC GCG GCT GCT GG-3'. Quantitative PCR (qPCR) was performed with an iCycler (Bio-Rad) using IQSYBR Green PCR Supermix (Bio-Rad). Each run contained a standard curve using DNA extracted from unrelated bacteria from the proximal and distal colons, run at different dilutions. As a relative quantitation, the Ct value of samples were measured and compared with the standard curve for fold-change calculation.

**Rectal enemas with cecal contents.** Littermate conventional C57BL/6J mice were randomly divided into a control or treatment group (n = 3 each). One additional mouse from the same litter was terminated at day 0 as the donor and the cecal content were collected, resuspended in 4 ml of sterile saline, and stored in −20°C. Each morning and afternoon for 3 consecutive days, mice were anesthetized by an intraperitoneal injection of 200 μl of a mixture of 10 mg/ml of ketamine (Lloyd, Shandnoah, IA) and 1 mg/ml of xylazine (Abbott Laboratories, Chicago, IL). Mice then received a rectal instillation of 200 μl of saline or 200 μl of a cecal slurry in saline by 2.4-cm steel cannula for control mice or treatment mice, respectively. Mice were kept in a head-down vertical position for 20 min before returning to their cages to optimize retention of the enema solution. Following the last treatment on day 3, all mice were euthanized, and mucosal scrapings from the distal colon were collected for analysis.

**Statistical analysis.** Results are presented as the means ± SE for the indicated number of experiments. The results of multiple experiments were analyzed using paired Student’s t-test or ANOVA using Bonferroni correction for multiple comparisons.

**RESULTS**

Differential Hsp27 and Hsp70 expression between human proximal and distal colonic mucosa. To determine differences in Hsp expression, immunohistochemical staining of colonoscopic biopsies from the proximal and distal colon of normal patients was performed. Significant differences in Hsp27 and Hsp70 expression were noted in the surface epithelial cells in direct contact with the luminal intestinal contents, but not in crypt epithelial cells (Fig. 1A). The ascending colon had high levels of both Hsp27 and Hsp70 staining, but biopsies from the descending colonic mucosa showed lower level Hsp27 and Hsp70 staining in the surface epithelial cells (Fig. 1A).

Western blot analyses were performed to quantify differences. As shown in Fig. 1B, significantly higher levels of both Hsp27 and Hsp70 were detected in the proximal colonic mucosa compared with the mucosa of the distal colon, whereas constitutively expressed Hsc70 was the same in different colonic areas. Hsp mRNA abundance in intestinal mucosal biopsies was measured by real-time PCR (Fig. 1C). Both Hsp27 and Hsp70 mRNA levels were significantly higher in the ascending colon compared with the sigmoid colon, suggesting that the differences in Hsp expression may be regulated at the
level of gene transcription. It should be noted that Hsp expression is known to be regulated posttranscriptionally, e.g., by mRNA binding proteins and regulation of mRNA translation (10) or by localization of Hsp mRNA in stress granules (9); therefore multiple mechanisms may be involved.

Inducible Hsp25 and Hsp70 expression are high in proximal and low in distal colon. To further investigate differences in Hsp expression, we used a mouse model. Hsp25, the homolog of human Hsp27, and Hsp70 expression in murine intestine from wild-type C57BL/6 mice were evaluated. Immunohistochemical examination of proximal colon tissue again revealed significant Hsp25 and Hsp70 expression in surface epithelial cells and no detectable levels in the crypt epithelial cells (Fig. 2A). Tissues from distal colon of mice showed reduced Hsp25 and Hsp70 staining in the surface epithelial cells.

Hsp expression was also assessed in the mucosa from small intestine (jejunum) and multiple locations of the colon by Western blot analysis. Consistent with previous studies, Hsp25 and Hsp70 levels were low or undetectable in the small intestine (1, 27). Mucosal Hsp25 and Hsp70 demonstrated the highest expression levels in the proximal colon with a trend from high to low Hsp25 and Hsp70 expression from proximal to distal colon.

When assessed by real-time PCR analysis, Hsp25 and Hsp70 mRNAs in colonic mucosa were again significantly higher in the proximal colon compared with the distal colon (Fig. 2C), suggesting that differences in expression were attributable to regulation of gene transcription but may also involve additional posttranscriptional mechanisms, as discussed earlier.

Low expression level of Hsp25 and Hsp70 in proximal colon mucosa of germ-free mice. To determine the effect of colonic bacteria on Hsps, Hsp25 and Hsp70 levels were measured in germ-free C57BL/6 mice. Minimal Hsp25 and Hsp70 expression was noted in the surface epithelial cells of both proximal and distal colon tissue from germ-free mice (Fig. 3A). In germ-free mice, Hsp25 and Hsp70 levels were significantly lower by both Western blot and PCR compared with control with conventional bacterial colonization in the proximal colon (Fig. 3, B and C). Hsp25 and Hsp70 proteins and mRNA were expressed at similar levels in the distal colon of the germ-free mice compared with control (Fig. 3, B and C). These data

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Fig. 1. Expression of inducible heat shock protein 27 (Hsp27) and Hsp70 is higher in human ascending (proximal) colon compared with descending (distal) colon. A: immunohistochemical staining for Hsp27 and Hsp70. B: Western blots of Hsp27, Hsp70, Hsc70, and villin. C: real-time PCR analysis of mRNA abundance for Hsp27 and Hsp70 in colonic mucosa. Changes in Hsp mRNA relative to GAPDH were determined as fold change over right colon. Images shown are representative of 4 experiments. Results are means ± SE, n = 4. *P < 0.05 comparing distal and proximal colon by Student’s t-test for protein (B) or mRNA (C).
Differential colonic heat shock protein expression

support the notion that Hsp expression in the colon is dependent on microbial signals and mediators, particularly in the proximal colon.

Mucosa-associated bacteria of the proximal colon are distinct from those of the distal colon. To determine whether differential expression of Hsp between the proximal and distal colon could be explained by differences in bacterial colonization, we analyzed the mucosa-associated bacteria in the proximal and distal colon of regular C57BL/6 mice by PCR amplification of 16S rRNA gene and T-RFLP as previously described (36). As shown in Fig. 4A, representative T-RFLP profiles from mouse proximal and distal colonic mucosa illustrated the differential colonization of bacterial population in these two places. To quantify the differences, T-RFLP peak patterns were viewed as community fingerprints and calculated by pairwise Bray-Curtis distances to examine the similarity between communities (18). Although different bacterial populations were found between mice from different litters (data not shown), mucosal samples from proximal colon always clustered together and were separated from those collected from distal colon in littermate mice (Fig. 4B). The clustering analysis does not preclude the possibility that many bacterial species are shared between proximal and distal colon. It only serves as a tool to assess the degree of similarity between sample sets.

To further quantify the differences of bacterial populations between proximal and distal colon, the absolute richness (the number of distinct terminal restriction fragments in each sample) and Shannon diversity of the bacterial community were calculated. Notably, both indices were significantly higher ($P < 0.05$) in the proximal colon than in the distal colon, indicating that more species of intestinal bacteria at higher diversity were detected in proximal colon.

Only lysates of mucosa-associated microbiota of the proximal colon induce intestinal epithelial Hsp25 and Hsp70 expression in vitro. Mucosa-associated microbiota that reside in close proximity to the intestinal epithelial cells are more likely to be stable than transient microbiota of the colonic lumen (5). Moreover, their selection by local host factors can greatly influence the structure and function of the microbial community, resulting in region-specific differences in colonic mucosa-associated microbiota (see Fig. 4). Although the proximal mucosa-associated colon contained greater numbers of bacteria than the distal (Fig. 5A) by quantitative PCR analysis of bacterial 16S rRNA-encoding DNA, it does not mean that the induction of Hsp expression is dependent on the dosage of bacterial signal. The effects of lysates of mucosa-associated microbiota from the proximal and distal colon on YAMC Hsp expression were therefore examined. YAMC cells are a non-transformed colon epithelial cell line (37), which maintain many of the cellular characteristics of their in vivo counterparts and demonstrate a significant induction of Hsp expression to thermal stress (13). Both Hsp25 and Hsp70 proteins were induced by proximal colonic lysates to a greater degree than lysates from distal colonic mucosa-associated microbiota (Fig. 5B) when YAMC cells were treated with the same amount of bacterial lysates from proximal and distal colons, respectively. The results shown are the greatest increases observed using these lysates to treat YAMC cells. However, increasing the amount of lysate from mucosa-associated bacteria of the distal colon did not result in equivalent induction to the proximal colon lysates (data not shown). Real-time, quantitative PCR also showed that both Hsp25 and Hsp70 mRNAs were significantly induced by proximal colonic mucosa-associated bacterial lysates, whereas lysates of distal colonic mucosa-associated microbiota have a small effect, similar to Hsp induction by these lysates (Fig. 5C).
Previous studies have demonstrated that butyrate and LPS are major bacterial components involved in inducing Hsp expression. YAMC cells were treated with butyrate or LPS for 24 h. At physiological concentrations, butyrate (0.5, 1.5, and 5.0 mM) induced Hsp25 in a dose-dependent manner but had much less effect on Hsp70 expression (Fig. 5D). LPS (1, 3, and 10 mM) induced both Hsp25 and Hsp70 in a dose-dependent manner.

Fig. 3. Hsp25 and Hsp70 expression levels are low in colonic mucosa of germ-free mice, and differential Hsp25 and Hsp70 expression between proximal and distal colon are not present in germ-free mice. A: immunohistochemical staining for Hsp25 and Hsp70 in proximal and distal colon sections from germ-free mice on C57BL/6 background. B: Western blots of Hsp25, Hsp70, Hsc70, and villin in mucosa of jejunum (J) and proximal (P) and distal colon (D) from germ-free mouse. Image shown is representative of 4 individual experiments. C: real-time PCR analysis of mRNA abundance of Hsp25 (top) and Hsp70 (bottom) in intestinal mucosa. Changes in Hsp mRNA relative to GAPDH were determined as fold change over control proximal colon. Results are means ± SE, n = 5. +P < 0.05 comparing Hsp (B) or mRNA (C) from control and germ-free mice by paired Student’s t-test.
Induction of Hsp expression in distal colon upon exposure to cecal microbiota. To test whether the differential expression of Hsp25 and Hsp70 in the colonic mucosa is determined by the regional distribution of microbiota, rectal enemas were performed using cecal microbiota, which had been shown to be very similar in bacterial composition to the mucosa-associated microbiota in the proximal colon using T-RFLP analysis (data not shown). After twice-daily treatments for 3 consecutive days, we observed the induction of Hsp expression in the distal colon by exposure of the mucosal surface with the cecal slurry, but not with sterile saline (Fig. 6). A major difference between the cecal slurry and luminal pellets of stool in the distal colon is that the former is dispersed and more likely to directly interact with the mucosa. The expression of distal mucosal Hsp25 and Hsp70 induced by the cecal microbiota was significantly increased compared with controls. In contrast, we did not detect changes in actin protein expression in either group (Fig. 6). To confirm whether nonbacterial (e.g., epithelial) components of the cecal lysate might be responsible for the Hsp induction, cecal contents from germ-free mice were administered transrectally as described above. This treatment did not induce Hsps, suggesting that the microbiota of the proximal colon are the primary determinant of Hsp70 gradients along the proximal-distal colonic axis. Thus the distal colonic mucosa is not restricted in its ability to mount a Hsp response, provided that the appropriate signals from mucosa-associated microbiota (in this case from cecal microbiota) are present.

Fig. 4. Difference of mucosa-associated bacteria profile in mouse proximal and distal colon. A: terminal restriction fragment-length polymorphism (T-RFLP) profiles of 16S ribosomal RNA (rRNA) genes from mucosa-associated bacteria from murine proximal and distal colon. Images shown are representative of 4 experiments. B: representative phylogenetic tree was built up from 4 mice on the basis of T-RFLP analysis. Similarities between proximal colon and distal colon were compared by Bray-Curtis distance calculations. The scale bar shows the distance of similarity. C: calculated richness and Shannon diversity for different bacteria profiles. Results are means ± SE, n = 4. *P < 0.05 comparing distal and proximal colon by Student’s t-test.
DISCUSSION

Mutualistic interactions between host and enteric microbes are critical for maintaining intestinal health and prevention of disease (7, 36). Previous studies have demonstrated that, compared with mice with conventional microbiota (specific pathogen-free mice), germ-free mice have an immature intestinal phenotype with blunted villi and heightened inflammatory responses. Germ-free mice also have higher caloric intake, decreased epithelial cell turnover, disordered gut associated lymphoid tissue, and abnormal gut motility (7, 36). Here we demonstrate another important benefit derived from the enteric microbiota, the regional differential expression of cytoprotective inducible Hsps in the colon.

Hsps play an important role in colonic homeostasis and in protecting cells against physiological and pathogenic stressors (11, 32). In intestinal epithelial cells, Hsp70 confers protection against a variety of stressors (19). Hsp25 and Hsp70 are physiologically expressed in regions of the gastrointestinal tract with the most hostile environments, e.g., the acidic environment of the stomach and the colon, which is continuously in contact with potentially disease-causing bacteria. However, they are minimally expressed in other areas of the gastrointestinal tract such as the small intestine. Hsps are known to mitigate injury caused by inflammatory cell-derived oxidants by preventing aggregation of denatured or damaged cell proteins and in some cases refolding partially denatured proteins to a functional configuration (11, 23, 31, 38). Hsp25 stabilizes the actin cytoskeleton, thus preserving barrier function under hostile conditions (21, 28, 34, 35). Inducible Hsps have also been shown to antagonize proinflammatory and proapoptotic signaling pathways (2, 14–16, 39). Absence of Hsp70 results in increased susceptibility to experimentally induced colitis and, in dextran sodium sulfate (DSS)-treated Hsp70 gene-deficient mice, can promote a self-sustained chronic colitis that is remarkably similar to human ulcerative colitis (33).

The maintenance of the physiological expression of colonic Hsps is highly dependent on microbial signals and metabolites (1, 13, 14, 22, 27). However, what was not previously appre-
compared with saline enema-treated mice by paired Student’s t-test to analyze induction in cecal content in enema-treated mice. Averages for mice with saline enema in each group, and this average was used to analyze induction in cecal content in enema-treated mice. Densitometric values were obtained using Image J and averaged for mice with saline enema in each group, and this average was used to analyze induction in cecal content in enema-treated mice. *P < 0.01 compared with saline enema-treated mice by paired Student’s t-test.

Fig. 6. Treatment of distal colon with cecal lysate by enema induces Hsps. Mice were treated daily by enema for 3 days with saline or cecal contents from conventional or germ-free mice suspended in saline. After treatment, mucosa was washed with saline and scraped, and protein lysates were prepared and analyzed by Western blots for Hsp25 and 70 and Hsc70 as described in MATERIALS AND METHODS. Image shown is from 3 individual mice with saline or cecal lysate enema. Densitometric values were obtained using Image J and averaged for mice with saline enema in each group, and this average was used to analyze induction in cecal content in enema-treated mice. *P < 0.01 compared with saline enema-treated mice by paired Student’s t-test.

These data differ from those reported in human subjects, where few differences were found along the length of the colon (5). We attribute this difference to the possible confounding effects of colonic lavage used to prepare patients for colonoscopy, a procedure that can dramatically perturb mucosa-associated and luminal microbiota (Y. Wang, D. Antonopoulos, and E. B. Chang, unpublished observations). In our human subjects, the gradient of Hsp expression appears to be preserved despite the colonic lavage taken by patients 12 h before the procedure. We attribute the retained expression of mucosal Hsps to their relative stability and long half-lives (6).

The functional profiles of the regional mucosa-associated microbiota in the murine colon also appear different. Here a reductionistic system was used where intestinal epithelial cells were exposed to lysates prepared from mucosa-associated bacteria from the proximal and distal colon. Hsp25 and Hsp70 were only induced by lysates from mucosa-associated bacteria of the proximal colon. One plausible mechanism accounting for these results is the greater numbers of mucosa-associated bacteria of the proximal colon compared with distal colon (as assessed by qPCR). This could result in greater LPS concentrations that are well known to stimulate intestinal expression of Hsps (13, 26) and were shown to have a dose-effect stimulation of YAMC Hsp expression. We also believe that these results reflect differences in community structure and, accordingly, functional profiles of microbiota in proximal and distal colon. Consistent with this possibility is the observation that exposure of distal colonic mucosa to cecal microbiota administered transrectally induced Hsp protein expression. These findings therefore suggested a functional profile of cecal microbiota that is distinct from that found in distal colon under normal circumstances. The results also suggest that the decreased Hsp expression in distal colon is not attributable to inherent restrictions of host Hsp tissue response. Another possible mechanism that could explain the functional differences of these two populations is their ability to generate short-chain fatty acids like butyrate. Butyrate, a major byproduct of bacterial metabolism of unabsorbed dietary carbohydrates, clearly stimulates Hsps in YAMC cells in a dose-dependent fashion. Because most short-chain fatty acids are produced in the proximal colon (40), this could be one factor that accounts for the proximal-distal gradient of Hsp expression.

Inflammatory bowel diseases are thought to result from an aberrant host immune response to commensal bacteria in a genetically susceptible host (24). Ulcerative colitis specifically is known to affect the surface epithelium and progress from the rectum proximally in an uninterrupted manner (20). Our present finding of a proximal-to-distal gradient in Hsp expression could potentially be a factor in explaining this regional phenotype of ulcerative colitis. Previous experiments by us and others have shown that DSS-induced colitis is typically most severe in the distal colon (9, 32). Conversely, forced expression of Hsp70 in villin promoter Hsp70 transgenic mice reduces the extent of mucosal injury. In Hsp70-null mice, decreased Hsp70 is associated with increased mucosal injury, chronic colitis, dysplastic transformation, and invasive carcinoma in models of inflammatory bowel disease. Dysplastic lesions were associated with increased proinflammatory cytokines, COX-2 expression, and inducible nitric oxide synthase expression, suggesting that limitation of inflammation by inducible Hsp proteins
contributes to protection against carcinoma by modulating production of reactive oxygen species that may contribute to tumor formation (33).

Our present findings of the role of bacteria in differences in Hsp between the proximal and distal colon may offer clues as to means of regulating these cytoprotective factors and potentially modulating disease expression. The finding that exposure of intestinal epithelial cells to lysates of mucosa-associated bacteria of the proximal, but not distal, colon induces Hsp expression suggests that this is a modulatable system. Coupled with the previously published finding that differences in Hsp expression correlate with disease phenotype and severity, it is suggestive that this is a strategy potentially worth exploiting (8, 9). Studies to further investigate the differences between proximal and distal mucosal-associated bacteria and luminal contents, along with the host factors that maintain these differences, are key to understanding regional differences in inflammatory bowel disease and intestinal carcinoma susceptibility and may form the basis of preventative therapy.

GRANTS

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DISCLOSURES

None of the authors have any conflict of interest to disclose.

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