Abnormal Motility in Patients With Ulcerative Colitis

The Role of Inflammatory Cytokines

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Hypothesis: Interleukin 1β (IL-1β) levels are elevated in the colonic mucosa of patients with ulcerative colitis (UC). We propose that IL-1β may also be elevated in the circular muscle layer of the colon and may be partially responsible for the motility dysfunction observed in patients with UC.

Design: Cohort analytic study.

Setting: Research laboratory in a tertiary academic medical center.

Participants: Normal smooth muscle was obtained from the disease-free margins of human sigmoid colon specimens resected from patients with cancer and compared with specimens from patients with UC.

Interventions: An enzyme-linked immunosorbent assay was used to measure IL-1β. Standard muscle chambers were used to measure force changes. Single muscle cells were isolated by enzymatic digestion, and cell shortening in response to neurokinin A (NKA) and thapsigargin was measured under a microscope. Cytosolic Ca2+ (calcium) concentrations were measured by standard techniques.

Main Outcome Measure: Effects of IL-1β on smooth muscle function in normal and UC colons.

Results: In patients with UC, IL-1β was elevated in the muscularis propria, and sigmoid circular smooth muscle contractions in response to NKA and thapsigargin were significantly reduced. In fura-2–loaded cells from patients with UC, the NKA-induced Ca2+ signal was also significantly reduced in Ca2+-free medium, indicating the reduced intracellular Ca2+ stores after UC. Exposure of normal cells to IL-1β mimicked the changes observed in patients with UC. An IL-1β–induced reduction in contraction and release of intracellular Ca2+ in response to NKA was partially restored by the hydrogen peroxide scavenger catalase.

Conclusion: In patients with UC, IL-1β was increased in colonic circular muscles and may contribute to motor dysfunction after UC through production of hydrogen peroxide.

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Abnormalities in colonic motor function in patients with ulcerative colitis (UC) were first documented in the 1950s.1,2 Inflammatory cytokines and free radicals present in the intestinal mucosa of patients with UC are thought to play an integral part in the inflammatory process.3–15 Numerous experimental models of UC are initiated by acute chemical insults to the intestinal mucosa. Although these models reproduce some histologic features of UC, they may not reflect the progression of the disease or accurately reproduce chronic inflammatory changes and motor function disturbances in the muscularis propria.

In this investigation, we compared contraction of normal sigmoid colon circular muscle with that of muscle from patients with UC and investigated the proinflammatory cytokine interleukin 1β (IL-1β) as a possible mediator of UC-induced changes.

RESULTS

CONCENTRATION OF IL-1β

In 6 patients with UC, IL-1β was measured from the sigmoid colon muscularis propria and compared with the concentrations of IL-1β from 6 normal sigmoid colon muscle specimens. The IL-1β concentration was 11.2±4.67 pg per milligram of protein in UC specimens and 0.705±0.10 pg per milligram of protein in normal muscle (P<.05) (Figure 1).

CONTRACTION OF MUSCLE STRIPS AND ISOLATED CELLS

Exposure to NKA caused dose-dependent contraction of normal colonic muscle...
PATIENTS AND METHODS

TISSUE COLLECTION

Experimental protocols were approved by the Institutional Review Board at Rhode Island Hospital, Providence. Full-thickness 1-cm to 2-cm strips of sigmoid colon were obtained from patients undergoing proctocolectomy for UC (n = 10) during the operations. In all patients, UC was diagnosed preoperatively and confirmed postoperatively. “Normal” specimens were taken from the grossly and histologically lesion-free margins of surgical resections from patients undergoing left colectomy for colon cancer (n = 20). These patients had no previous history of colonic motility disorder or evidence of diverticular disease. A full-thickness circumferential strip of sigmoid colon (measuring approximately 1 to 2 cm in length) was excised at the most distal portion of the specimen. The strip of fresh tissue was placed in a pre-oxygenated (95% oxygen and 5% carbon dioxide) physiologic Krebs solution (116.6mM sodium chloride; 21.9mM sodium bicarbonate; 1.2mM potassium dihydrogen phosphate; 5.4mM dextrose; 1.2mM magnesium chloride; 3.4mM potassium chloride; and 2.5mM calcium chloride) and transported on ice to the laboratory.

PREPARATION OF MUSCLE STRIPS

Specimens were transferred to a preoxygenated Krebs solution in a dissection bath. The mesenteric fat and serosa were sharply dissected from the outer surface of the specimen, and the mucosa and submucosa were excised, leaving a clean muscle square. When the tissues were used to measure in vitro force development, consecutive circular muscle strips (10 mm long × 2 mm wide) of sigmoid colon were cut with razor blades held in a metal block 2 mm apart. When the tissues were used to obtain smooth muscle cells by enzymatic digestion or to measure levels of IL-1/β, circular muscle squares were cut into very thin muscle strips (around 1 mm wide) under the microscope. For cell isolation, circular muscle squares were cut into very thin muscle strips (around 1 mm wide) under the microscope.

MEASUREMENTS OF IL-1/β

Sigmoid circular smooth muscles (100 mg) were homogenized in phosphate-buffered saline (0.1M; pH, 7.4). Homogenization consisted of a 20-second burst with a Tissue Tearer (Biospec Products, Inc, Bartlesville, Okla), followed by 30 strokes with a Dounce tissue grinder (Wheaton Science Products, Millville, NJ). An aliquot of homogenate was taken for protein measurement. The homogenate was centrifuged at 15000 rpm for 15 minutes at 4°C in a Beckman J-21 centrifuge with a fixed-angle JA-20 rotor (Beckman Coulter, Inc, Palo Alto, Calif), and the supernatant was collected.

The IL-1/β concentration was quantified using an IL-1/β enzyme immunoassay kit (Cayman Chemical Co, Ann Arbor, Mich). This assay is based on a double antibody “sandwich” technique. Each well of the microtiter plate supplied with the kit is coated with a monoclonal antibody specific for human IL-1/β (IL-1/β capture antibody). This antibody will bind any human IL-1/β introduced into the well. An acetylcholinesterase Fab conjugate, which binds selectively to a different epitope on the IL-1/β molecule, is added to the well. This allows the 2 antibodies to form a sandwich by binding on opposite sides of the IL-1/β molecule. The sandwiches are immobilized on the plate so that the excess reagent may be washed away. The IL-1/β concentration is determined by measuring the enzymatic activity of acetylcholinesterase. An acetylcholinesterase substrate is added to the well, and the product of this reaction absorbs strongly at 412 nm. Measurement of IL-1/β is made spectrophotometrically. The intensity of the color is directly proportional to the amount of bound conjugate, which, in turn, is proportional to the concentration of IL-1/β. All measurements were standardized to protein content.

MUSCLE STRIP CONTRACTION

Muscle strips were mounted in separate 1-mL muscle chambers, as was previously described in detail.15 The strips were initially stretched to 2.5 g to bring them to a condition of optimum force development, then equilibrated for an additional 30 minutes while being continuously perfused in an oxygenated Krebs solution that was equilibrated with a gas mixture containing 9% oxygen and 5% carbon dioxide and maintained at a pH of 7.4 and 37°C. During the perfusion period, spontaneous phasic contractions developed gradually and stabilized over the equilibration period.

Muscle strips were assigned to a control group or an experimental group. The experimental group was exposed to 200 U/mL (20 ng/mL) of IL-1/β (Endogen, Woburn, Mass) for 2 hours, whereas the controls remained in Krebs solution for 2 hours. Each muscle strip was then exposed to the neurotransmitter neurokinin A (NKA) 10−6 mol/L. The response to NKA was compared between the 2 groups.

SMOOTH MUSCLE CELL ISOLATION

Sigmoid circular muscle strips (1 mm wide) were digested in N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES)–buffered solution, containing 0.5 mg/mL of collagenase Sigma-type F (Sigma-Aldrich Fine Chemicals, St Louis, Mo), 1 mg/mL of papain, 1 mg/mL of bovine serum albumin, 1 mM DL-dithiothreitol, 1 mM calcium chloride, 0.25 mM EDTA, 10 mM glucose, 10 mM sodium HEPES, 4 mM potassium chloride, 1.25 mM sodium chloride, 1 mM magnesium chloride, and 10 mM taurine. The solution was oxygenated (100% oxygen) at 31°C for 10 minutes, and the pH was adjusted to 7.2 before the solution was placed in a refrigerator overnight. The following morning, the tissue was warmed at room temperature for 30 minutes and incubated at 31°C in a water bath for an additional 30 minutes with constant 100% oxygen infused at a low flow rate to avoid agitating the tissue. The tissue was then poured out over a 200-µm Nitex mesh (Tetko Inc, Briarcliff Manor, NY) and rinsed in collagenase-free HEPES-buffered strips.17 After a 2-hour exposure to IL-1/β (200 U/mL), NKA-induced contraction (1 µmol/L) was significantly reduced (P < .001) to 56% of the contraction of the untreated strips. In addition, NKA caused dose-dependent contraction of colonic circular smooth muscle cells, with maximal shortening at 10−6 mol/L. Maximum shortening of normal cells was 24.9% ± 0.8%. In cells from patients with...
solution to eliminate any trace of collagenase. The tissue was placed in 5 mL of collagenase-free HEPES buffer (pH 7.4), 112.5 mM sodium chloride, 5.3 mM potassium chloride, 2.0 mM potassium dihydrogen phosphate, 10.8 mM glucose, 24.0 mM sodium HEPES, 1.9 mM calcium chloride, 0.6 mM magnesium chloride, 0.3 mg/mL basal medium Eagle amino acid supplement, and 0.08 mg/mL soybean trypsin inhibitor. The cell suspension was filtered over a 450-µm Nitex mesh, yielding isolated single cells. Care was taken to avoid agitation of the solution to avoid cell contraction in response to mechanical stress. All glassware used in this procedure was siliconized with a 0.05% silicon solution (Sigma-Aldrich Fine Chemicals) to prevent the cells’ adherence to the glass.

TREATMENT WITH IL-1β
To test the effect of IL-1β on muscle cell contraction, very thin circular muscle strips were incubated in an oxygenated Krebs solution or in Krebs solution containing IL-1β (100 U/mL) before the muscle strips were put into the collagenase solution. To assess the role of reactive oxygen species in IL-1β–induced effects, catalase (78 U/mL) or superoxide dismutase (300 U/mL) was added 15 minutes before the IL-1β. The total incubation period was 2 hours, 15 minutes, for each group.

CONTRACTION OF ISOLATED MUSCLE CELLS
Receptor-mediated contraction of single cells was induced by NKA (10^{-13}-10^{-9} mol/L). The Ca^{2+} adenosine triphosphatase inhibitor thapsigargin (3 µmol/L) was used to deplete intracellular Ca^{2+} stores. Potassium chloride (20 mmol/L) was used to depolarize the cell membrane, opening voltage sensitive Ca^{2+} channels and allowing the influx of extracellular Ca^{2+} into the cytoplasm. After a 30-second exposure to these agents, the cells were fixed in acrolein at a final concentration of 1%. A drop of the fixed solution was placed on a glass slide and covered by a coverslip, and the edges were sealed with clear nail enamel to prevent evaporation. Thirty consecutive cells from each slide were observed through a phase-contrast microscope (Carl Zeiss, Oberkochen, Germany) and a closed-circuit television camera (model WV-CD51; Panasonic, Secaucus, NJ) connected to a Macintosh computer (Apple Inc, Cupertino, Calif). The Image software program (National Institute of Health, Bethesda, Md) was used to acquire images and to measure cell length and data accumulation. The average length of 30 cells, measured in the absence of an agonist, was taken as the age length of 30 cells, measured in the absence of an agonist. The age is then thresholded, and values below a selected level are considered outside the cell and called zero. For each ratio metric image, the outline of the cell is determined, and the isosbestic image (ie, an image insensitive to Ca^{2+} changes) is determined from the ratios of fluorescence elicited by 340-nm and 380-nm excitation. This image is then thresholded, and values below a selected level are considered outside the cell and called zero. For each ratio metric image, the outline of the cell is determined, and the generated mask is applied to the ratiometric image. This method allows the simultaneous imaging of both the rapid changes in Ca^{2+} and changes in cell length. Our algorithm has been incorporated into the IonOptix software.

HYDROGEN PEROXIDE ASSAY
Normal sigmoid circular muscle squares were incubated in Krebs solution at 37°C without (control group) or with IL-1β (200 U/mL) for 2 hours, then frozen and kept in liquid nitrogen. Frozen tissues were thawed and homogenized by a 20-second burst with a tissue tearer, followed by 50 strokes with a Dounce tissue grinder. An aliquot of homogenate was taken for protein measurement. The homogenate was centrifuged at 15000 rpm for 15 minutes at 4°C in a Beckman J2-21 centrifuge with a fixed-angle JA-20 rotor, and the supernatant was collected. Hydrogen peroxide content was measured with the use of a quantitative hydrogen peroxide assay kit (Bioxytech H_{2}O_{2}-560; Oxis International, Inc, Portland, Ore).

CYTOSOLIC CALCIUM MEASUREMENTS
Freshly isolated cells were loaded with fura-2/AM (1-[2-(5-carboxyoxazol-2-yl)-6-aminobenzofuran-5-oxyl]-2-(2'-aminophenox)-ethane-N,N,N',N'-tetraacetic acid pentacetoxyethyl; 1-2.5 µmol/L) and placed in a 3-mL chamber mounted on the stage of an inverted microscope (Carl Zeiss). The chamber contained HEPES-buffered solution as described in the previous section. The cells were allowed to settle onto a cover slip at the bottom of the chamber. The agonist was applied directly to the cells using a pressure-ejection micro pipette system. When Ca^{2+}-free medium was needed to eliminate influx of extracellular calcium, the HEPES-buffered solution (pH 7.4) contained 112.5 mM sodium chloride, 5.5 mM potassium chloride, 2.0 mM potassium dihydrogen phosphate, 10.8 mM glucose, 24.0 mM sodium HEPES, 0.6 mM magnesium chloride, 200 µM BAPTA (1,2-bis(2-aminophenox)-ethane-N,N',N'-tetraacetic acid), 0.3 mg/mL basal medium Eagle amino acid supplement, and 0.08 mg/mL soybean trypsin inhibitor. Calcium measurements were obtained using a dual excitation wavelength imaging system (IonOptix Corp, Milion, Mass). The Ca^{2+} concentrations were determined from the ratios of fluorescence elicited by 340-nm to 380-nm excitation using standard techniques. Ratiometric images were masked in the region outside the borders of the cell because low photon counts would give unreliable ratios near the edges of the cells. We developed a method for generating an adaptive mask, which follows the borders of the cell as Ca^{2+} changes and as the cell contracts. A pseudo-isosbestic image (ie, an image insensitive to Ca^{2+} changes) is formed in computer memory from the weighted sum of the images generated by 340-nm and 380-nm excitation. This image is then thresholded, and values below a selected level are considered outside the cell and called zero. For each ratiometric image, the outline of the cell is determined, and the generated mask is applied to the ratiometric image. This method allows the simultaneous imaging of both the rapid changes in Ca^{2+} and changes in cell length. Our algorithm has been incorporated into the IonOptix software.

STATISTICAL ANALYSIS
Data are expressed as mean±SEM. Statistical differences between means were determined by a t test. Differences between multiple groups were tested using analysis of variance (ANOVA) for repeated measures and checked for significance using a Macintosh StatView program (SAS Institute Inc, Cary, NC).

UC, maximal shortening was reduced to 14.8%±1.1%. The difference in maximal contraction between normal and UC cells was statistically significant (P<.001; ANOVA) (Figure 2).

Normal smooth muscle cells shortened in a time-dependent manner in response to the Ca^{2+} adenosine triphosphatase inhibitor thapsigargin (3 µmol/L). Maximal shortening (22.3%±1.1%) occurred at 1 minute after
addition of thapsigargin. Shortening decreased slowly after 1 minute and returned to the fully relaxed state by 20 minutes, presumably reflecting depletion of releasable Ca²⁺. In cells from patients with UC, maximum shortening occurred earlier than in normal cells (30 seconds) and shortening was reduced to 11.5%±1.3% (P<.001; ANOVA) (Figure 3).

After incubation in IL-1β (50 U/mL) for 2 hours, normal human sigmoid smooth muscle cells shortened normally in response to 10⁻⁹ mol/L of NKA, with a 21.7%±1.2% maximum shortening. The difference in shortening between untreated cells and cells incubated with IL-1β was not statistically significant (P<.3; ANOVA). Increasing the IL-1β dose to 100 U/mL for 2 hours reduced maximum shortening to 12.9%±1.3%. Similarly, treatment with IL-1β (100 U/mL) for 2 hours reduced thapsigargin-induced shortening to 14.4%±0.9%.

The reduction in the NKA- and thapsigargin-induced contractions was statistically significant when compared with untreated cells (P<.001; ANOVA). Shortening in response to NKA and thapsigargin was partially restored by the hydrogen peroxide scavenger catalase, increasing to 18.4%±1.2% and 17.2%±1.3%, respectively, in the presence of catalase (P<.001; ANOVA). The superoxide radical anion scavenger superoxide dismutase had no effect on the reduction in shortening induced by 100 U/mL of IL-1β, and shortening remained low (13.1%±1.1% and 11.9%±1.4%, respectively) (Figure 4 and Figure 5).

**HYDROGEN PEROXIDE MEASUREMENT**

Hydrogen peroxide was measured in the untreated circular muscles of normal sigmoid colons and after incubation with IL-1β (200 U/mL). Hydrogen peroxide concentrations increased from 0.069±0.025 nmol per milligram of protein in untreated tissue to 0.222±0.046 nmol per milligram of protein in the IL-1β–treated muscle. (P<.01) (Figure 6).

**Ca²⁺ SIGNALING**

In normal cells, NKA caused a 323±26 nmol/L increase in cytosolic Ca²⁺ levels from 84±6 nmol/L to 407±29 nmol/L (n=12). When the cells were in a calcium-free medium, NKA caused a 320±22 nmol/L increase in cyto-
tosolic Ca\(^{2+}\) levels, from 48±8 nmol/L to 368±24 nmol/L (n=14) (Figure 7).

In sigmoid circular smooth muscle cells from patients with UC, the NKA-induced calcium signal was reduced to 186±37 nmol/L (24 cells of 3 patients) in Ca\(^{2+}\)-free medium, when compared with the normal cells (320 nmol/L). The difference was statistically significant (P<.05; unpaired t test).

In normal cells exposed to IL-1\(\beta\), NKA caused a 40±11 nmol/L increase in cytosolic Ca\(^{2+}\) levels from 54±3 nmol/L to 94±12 nmol/L (n=7) in Ca\(^{2+}\)-free medium. When muscle cells exposed to IL-1\(\beta\) had been pretreated with catalase, NKA caused a 156±41 nmol/L increase in cytosolic Ca\(^{2+}\) from 95±5 nmol/L to 251±43 nmol/L (n=10; P<.05) (Figure 8).

**COMMENT**

Ulcerative colitis is an idiopathic inflammatory disease of the colon with an incidence of 10.9 per 100000 live births in the United States.\(^1^8\) It is thought that a combination of multiple risk factors in the presence of a genetic component may predispose individuals to an abnormal immunologic response to luminal antigens and/or environmental factors.\(^1^9^–2^3\)

In some patients, UC can present acutely with unrelenting symptoms, requiring immediate surgery; the majority of patients, however, experience chronic intermittent symptoms. In this group of patients, colonic motility dysfunction has been demonstrated in vivo and in vitro. In 1951, Kern et al and Almy\(^2^4\) demonstrated that colonic wave patterns were different in patients with UC when compared with normal volunteers. Many investigators have since demonstrated abnormalities of colonic circular smooth muscle function in patients with UC.\(^2^4^–2^7\) Experimental studies have also identified abnormalities in animals with chemically induced colitis when compared with controls.\(^2^8^–3^0\) Links to inflammatory cytokines have been established. Cytokines found locally and systemically during active UC are produced by circulating and resident inflammatory cells and by the epithelial cells of the colonic mucosa.\(^3^1^–4^9\) Little is
known, however, about the presence of cytokines in the abnormally functioning muscularis propria of patients with UC.

In previous studies, cytokines were measured in fecal contents or in mucosal biopsy specimens taken at the time of endoscopic evaluation. In the present study, we demonstrate that IL-1β is significantly elevated in the human sigmoid circular smooth muscle layer of patients with UC and that circular smooth muscle from UC specimens does not contract normally. The reduced shortening observed in our single-cell experiments is consistent with data reported by other investigators.

We used NKA and thapsigargin to induce contraction. A member of the tachykinin family, which includes substance P and neurokinin B, NKA acts on the NK-2 receptor that exists on colonic smooth muscle cells and causes contraction of colonic smooth muscle by inducing the release of intracellular Ca2+ from the sarcoplasmic reticulum. In addition, NKA is 100 times more potent than NK-1 agonists and perhaps is the dominant neurotransmitter in the human sigmoid colon.

Thapsigargin is a high-affinity inhibitor of sarcoplasmic reticulum adenosine triphosphatase, and it is useful in testing the role of intracellular Ca2+ release. Calcium levels in the sarcoplasmic reticulum depend on the balance of Ca2+ uptake and Ca2+ release. When uptake is inhibited by blocking the Ca2+ adenosine triphosphatase, Ca2+ is released into the cytoplasm, causing contraction until the stores are depleted. The findings that in patients with UC shortenings in response to NKA and thapsigargin were reduced suggest that depletion of intracellular Ca2+ stores may contribute to the reduction of circular muscle contractility. These data are consistent with some previous reports and do not support the view that the observed changes may be caused by a damaged contractile mechanism.

Our data demonstrate that IL-1β has a significant effect on muscle strip force development and cell shortening and are consistent with previously reported data in colonic muscle strips in the rat. Two-hour exposure of normal human sigmoid circular muscle cells to 100 U/mL of IL-1β reproduced the abnormal contractile response observed in UC tissue specimens. The effect of IL-1β was inhibited by the hydrogen peroxide scavenger catalase, suggesting that the effect of IL-1β may be partly mediated by production of hydrogen peroxide. This view is further supported by our finding that hydrogen peroxide concentration was significantly increased after IL-1β treatment. It is known that the levels of hydrogen peroxide are elevated in the mucosa of patients with inflammatory bowel disease, as well as in experimental models of inflammation. Hydrogen peroxide content was also increased in the colon muscle of dextran sodium sulfate–treated rats, suggesting that production of hydrogen peroxide may play a role in the inflammatory cascade. To directly examine the changes in Ca2+ signaling, we used a calcium-imaging system utilizing fura-2/AM. Cells from patients with UC and normal cells exposed to IL-1β had reduced Ca2+ signals, when compared with normal controls.

In conclusion, our data demonstrate that elevated levels of IL-1β are present in the circular muscle layer of sigmoid specimens from patients with UC. In addition, exposure of normal cells to IL-1β causes increased formation of hydrogen peroxide and a decrease in muscle contraction similar to those observed in patients with UC. The reduction in contraction induced by IL-1β is reversed in part by the hydrogen peroxide scavenger catalase.

Taken together, the data support the view that the presence of IL-1β in colonic muscle in patients with UC contributes to impaired contraction through production of hydrogen peroxide and depletion of releasable Ca2+ stores.

This paper was presented at the 82nd Annual Meeting of the New England Surgical Society, Providence, RI, September 21, 2001.

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REFERENCES


Herbert Hechtman, MD, Boston, Mass; Dr Pricolo, members and guests of the New England Surgical Society, it is a pleasure for me to discuss this very nice piece of science. The chair- man is to be congratulated, together with his fellow, Dr Vrees, for designing and carrying out this protocol.

In contrast to the vast majority of studies on inflammatory bowel disease, this investigation focuses on muscle contraction. Indeed, these data may provide insight into the mechanisms underlying in vivo motility problems as suggested in the title, but motility itself was really not the subject of the study. The techniques used to examine muscle shortening and its calcium dependence are elegant and convincing. The data are straightforward, save for the problems requiring assay of IL-1β, which is reported in terms of milligrams of protein resident in tissue, whereas the added IL-1β is described in units. This brings me to the first question. Do the authors believe that IL-1β concentration used to bathe normal muscle is in the range of that measured in tissue from patients with UC?

Data interpretation can always be an issue. Even here, there might be alternatives and questions.
for the study. What about the role of others, for example, tumor necrosis factor? Or do the authors believe that this agent or others are simply inflammatory and not motility mediators?

To fulfill the Koch postulates demonstrating conclusively that the central role of an agonist is present, one must show, first, that the agonist, the agent, is present in a temporal and geographically relevant manner, with regard to the pathophysiologic effect in question; second, that addition of the agonist will reproduce the effect; and finally, that blockade of the agonist will prevent the effect. The latter was not reported. Do the authors have data on the effect of the antagonist to IL-1β?

The hydrogen peroxide data are interesting. It would appear that IL-1β triggers hydrogen peroxide production. Could there be neutrophil contamination as a cell source for this peroxide? If not, what cell do the authors believe is the source?

Finally, could colon ischemia during the surgical procedure have influenced IL-1β levels in the bowel wall?

I believe these data are important and may help in providing an understanding about the pathophysiology of motility changes in inflammatory bowel disease.

Peter Cataldo, MD, South Burlington, Vt: Just one question about your use of controls: I wonder about considering other colitides for controls, such as pseudomembranous colitis, infectious colitis, and even diversion proctitis. Perhaps you could also answer the question as to whether the changes in calcium and IL-1β are the cause or the effect of colonic colitis.

Dr Vrees: In regard to the first question, which was phrased in 2 parts, I wonder about considering other colitides for controls, such as pseudomembranous colitis, infectious colitis, and even diversion proctitis. Perhaps you could also answer the question as to whether the changes in calcium and IL-1β are the cause or the effect of colonic colitis.

Dr Vrees: In regard to the first question, which was phrased in 2 parts, is the IL-1β that we used in a range equal to that which is found in UC? and to why in one instance we discuss IL-1β in units per milliliter and in other instances in milligrams of protein. I cannot state with certainty that the dose of IL-1β used in our experiments is equal to the concentrations found in inflammatory tissue. We used a dose that was able to replicate changes seen in inflammatory tissue, and those doses were similar to those used by other investigators as reported in the literature. One must keep in mind that during our digestion process in a HEPES solution, there is certainly an aperiod of time in which proteins can be lost from the samples. This process results in a decrease in recoverable IL-1β. In response to the second part of the question, IL-1β is distributed in units per milliliter because not all IL-1β produced has equal potency, milligram per milligram. The companies control for this in units. In contrast, assays used to measure IL-1β are milligram-based. We control for differences in tissue sample size by measuring total tissue protein. Unfortunately, in our lab, we cannot convert these measurements into units.

In response to the second question, What about other cytokines? we certainly plan on looking at other cytokines. If you dissect the literature, IL-1β is described in the majority of assays performed either in the mucosa or from fecal contents. This is why we chose to investigate IL-1β initially. We do hypothesize that other cytokines play a role in inflammatory bowel disease, but we have not yet investigated this hypothesis.

In response to the question, Do we have any data in the presence of the IL-1β antagonist? the answer is yes. We did muscle bath experiments in the presence of the IL-1β antagonist, which demonstrated a blockade of its effects. We did not, however, perform these experiments at the single-cell level.

In response to Dr Hechtman’s final question, could the ischemia of surgery affect IL-1β levels? is an interesting question. I think this is certainly a theoretical possibility. An important factor in our experiments is that the tissue samples were removed immediately following ligation of the inferior mesenteric artery, regardless of the sample assignments. So all ischemic time was minimized and all tissue, whether from inflammatory bowel disease or from control patients, should have been exposed to similar ischemic times.

In regard to Dr Cataldo’s question, Did we ever use other types of controls? the answer to this question is no. We felt the best way to set up a control was with what we felt was completely normal tissue. It would be interesting but difficult to study other forms of colitis because most are not operative and obtaining adequate full-thickness tissue samples would be difficult.