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## Ulcerative colitis patients in remission have an altered secretory capacity in the proximal colon despite macroscopically normal mucosa

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### Abstract

**Background**—One of the hallmarks of acute colitis is loss of epithelial transport. For unknown reasons, many patients still suffer from GI symptoms during remission, indicating a sustained imbalance between absorption and secretion. We hypothesize that the colonic epithelium becomes more reactive to secretagogues to compensate for a failing barrier.

**Methods**—Biopsies from ascending colon and sigmoid colon of UC patients in remission and controls were mounted in Ussing chambers. Membrane current ( $I_m$ ) and epithelial capacitance ( $C_p$ ) were used as markers for anion secretion and mucus exocytosis. Carbachol ( $1 \text{ mmol L}^{-1}$ ) and forskolin ( $10 \text{ } \mu\text{mol L}^{-1}$ ) were used to study  $\text{Ca}^{2+}$  and cAMP-mediated secretion.

**Key Results**—Baseline values showed segmental patterns with higher  $I_m$  in ascending colon and higher  $C_p$  in sigmoid colon of both UC patients and controls, but the patterns did not differ between the groups. The  $I_m$  response to forskolin was increased (+35%) in the ascending colon of UC patients and the  $I_m$  response to carbachol was decreased (−40%) in the same segment. No group differences were seen in the distal colon for either the forskolin or carbachol-induced  $I_m$  responses. The  $C_p$  response to carbachol was instead up-regulated in the distal colon of UC patients, but remained unaffected in the proximal colon.

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#### DISCLOSURES

The authors have no conflicts of interest to disclose.

#### AUTHOR CONTRIBUTION

JKG collected the data. JKG and HS designed the study, analyzed and interpreted the data, and wrote the manuscript. GCH provided the Cfr F508 and Muc2<sup>-/-</sup> mice, and GCH revised the manuscript critically for intellectual content.

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**Conclusions & Inferences**—The proximal colonic mucosa of UC patients in remission seems to shift its reactivity to secretagogues, becoming more sensitive to cAMP-dependent secretion and less sensitive to  $\text{Ca}^{2+}$ -dependent secretion. This phenomenon may contribute to residual diarrhea in this patient group, despite resolution of inflammation.

### Keywords

carbachol; forskolin; mucus; ussing chamber

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## INTRODUCTION

Ulcerative colitis is a chronic relapsing disease characterized by a distal to proximal progression. During the acute phase of disease, the colonic mucosa is severely distorted with massive infiltration of immune cells, mucin depletion, and altered crypt architecture.<sup>1</sup> During remission most of the histological features of the epithelium are restored, but for unknown reasons, as many as 30% of patients still suffer from gastrointestinal symptoms, such as abdominal pain and diarrhea, despite no signs of ongoing inflammation.<sup>2,3</sup> Mechanisms behind these sustained symptoms are not known, but the altered bowel habits point to either motility defects and/or altered absorptive or secretory capacity. Similar symptoms are present in patient groups with low-grade inflammation such as collagenous and microscopic colitis and in patients with diarrhea-dominated irritable bowel syndrome.<sup>4,5</sup> Although the pathogenesis of these diseases varies, it is possible that low-grade inflammation is a common denominator.

In colon, both motility and secretion are regulated mainly by the enteric nervous system via cholinergic and VIPergic neurons. Activation of the cholinergic system induces muscle contractions and secretion of ions and mucus which increase transit, creates a driving force for fluid secretion, and ensures protection of the epithelial surface by forming the adherent mucus layer.<sup>6–8</sup> At the cellular level, acetylcholine binds muscarinic receptors on the epithelial cells and induces a rapid transient secretory response mediated via increased levels of intracellular  $\text{Ca}^{2+}$ .<sup>9</sup> In contrast, VIP-induced secretion acts via increased levels of intracellular cAMP and subsequent activation of the apical CFTR channel.<sup>10,11</sup>

Due to the implications of altered ion transport and mucus properties in the pathophysiology of UC and the importance of these systems in regulation of the colonic barrier, we studied the secretory capacity of the colonic epithelium in UC patients in remission. Due to the segmental pattern of disease and known regional differences in ion transport, we studied the response in both the proximal colon and the distal colon. Carbachol and forskolin were used to induce  $\text{Ca}^{2+}$  and cAMP-mediated secretion, respectively. Membrane current was used to quantify anion secretion and epithelial capacitance was used to monitor exocytosis e.g., mucus secretion.<sup>12,13</sup>

## MATERIALS AND METHODS

### Patient material

Study subjects were recruited among patients referred for colonoscopy at Sahlgrenska University Hospital, Gothenburg, Sweden. Three biopsies from the ascending colon and the sigmoid colon, respectively, were assessed from 24 patients with UC, all with previous pancolitis (7 women; mean age  $51 \pm 6$  years and 17 men; mean age  $47 \pm 4$  years). In the present study, we only included patients in endoscopic remission, for example, with an endoscopic Mayo score of 0.<sup>1</sup> Additional clinical information regarding the UC patients is given in Table 1. Biopsies from the ascending colon and the sigmoid colon were also assessed from 32 patients referred for colonoscopy for other reasons (i.e., anemia of unknown origin, previous diverticulitis, polyp surveillance, etc.), in which the colonic mucosa was macroscopically normal. This material was from 17 women (mean age  $61 \pm 5$  years) and 15 men; (mean age  $58 \pm 3$  years) who were used as a control group. Approval was granted by the Human Research Ethical Committee of the Medical Faculty, University of Gothenburg, and written informed consent was obtained from all study subjects.

### Colonoscopy

In the evening preceding colonoscopy, the bowel was cleaned by ingestion of 4 L of an osmotic laxative (Laxabon<sup>®</sup>, BioPhausia, Stockholm, Sweden). When the patient arrived at the endoscopy unit, he/she was given full information according to the ethical permit, and signed informed consent was taken. The patient was then given premedication consisting of Midazolam (1–2 mg) (Dormicum<sup>®</sup>, Roche, Basel, Switzerland) and Petidin (50 mg) (Petidin, Meda, Solna, Sweden) intravenously. Heart rate and peripheral oxygenation were monitored by pulse oximetry. If necessary, the patient received additional sedation during the procedure. In addition to biopsies for research purposes, up to 16 biopsies were taken segmentally from cecum to rectum according to our surveillance program. These biopsies were evaluated by a trained pathologist and the histological appearance of the tissue is presented in Table 1.

### Mouse tissue

All animal studies were approved by the animal ethics committee, University of Gothenburg. The experiments were performed using male and female wild type C57/Bl6 mice, *Muc2*<sup>-/-</sup> and *Cftr* F508 mice, both on C57/Bl6 background and 8–12 weeks old.<sup>14,15</sup> To increase the survival of the *Cftr* F508 mice, the mice were treated with an oral laxative (PEG4000 18 mmol L<sup>-1</sup>, KCl 10 mmol L<sup>-1</sup>, Na<sub>2</sub>SO<sub>4</sub> 40 mmol L<sup>-1</sup>, NaHCO<sub>3</sub> 84 mmol L<sup>-1</sup>, and NaCl 25 mmol L<sup>-1</sup>) in the drinking water. The mice were transferred to normal tap water 2 days prior to the experiments. All animals had free access to water and chow. The mice were anesthetized with isoflurane and euthanized by cervical dislocation. The proximal and distal colon (approximately 2 cm) were dissected and flushed with ice-cold oxygenized (95% O<sub>2</sub>, 5% CO<sub>2</sub>) Krebs buffer with the following composition (in mmol L<sup>-1</sup>) NaCl 115.8, CaCl<sub>2</sub> 1.3, KCl 3.6, KH<sub>2</sub>PO<sub>4</sub> 1.4, NaHCO<sub>3</sub> 23.1, and MgSO<sub>4</sub> 1.2 (Merck, Darmstadt, Germany) to remove colonic contents.

### Ussing chamber experiments

Colonic biopsies from the ascending colon and sigmoid colon were obtained one at a time using a single-use large capacity forceps (Olympus, Tokyo, Japan) and instantly put into oxygenated (95% O<sub>2</sub>, 5% CO<sub>2</sub>) ice-cold Krebs buffer with the following composition (in mmol L<sup>-1</sup>) NaCl 116, CaCl<sub>2</sub> 1.3, KCl 3.6, KH<sub>2</sub>PO<sub>4</sub> 1.4, NaHCO<sub>3</sub> 23, and MgSO<sub>4</sub> 1.2 (Merck). Biopsies were mounted in modified Ussing chambers (exposed area 0.03 cm<sup>2</sup>) within 30 min of collection. Both sides of the epithelium were bathed in 2 mL Krebs buffer that was constantly gassed with 95% O<sub>2</sub> and 5% CO<sub>2</sub> at a temperature of 37°C, pH 7.4 throughout the whole experiment. The mucosal solution also contained Na-Pyruvate (5.7 mmol L<sup>-1</sup>) (Sigma-Aldrich, Steinheim, Germany), Na-L-glutamate (5.13 mmol L<sup>-1</sup>) (Merck), and D-Mannitol (10 mmol L<sup>-1</sup>) (Sigma-Aldrich). In the serosal solution, D-Mannitol was replaced by D-Glucose (10 mmol L<sup>-1</sup>) (Sigma-Aldrich). In the mouse experiments, the dissected tissue was incubated on ice for 30 min followed by mounting in the Ussing chamber (exposed area:0.45 cm<sup>2</sup>). The chamber volume was 10 mL and the buffer composition was the same as described for the human experiments.

### Transepithelial electrophysiological measurements

Transepithelial potential difference (PD) was measured once every minute during the whole experiment with a pair of matched Ag/Ag calomel electrodes (Radiometer, Denmark) placed in saturated KCl and connected to the mucosal and serosal sides via a pair of agar bridges (0.9% NaCl/4% agar) (Marine Bioproducts, Canada). After mounting the tissue, PD was allowed to stabilize for 20 min to achieve steady-state conditions. Net membrane current (I<sub>m</sub>), epithelial resistance (R<sub>p</sub>), and epithelial capacitance (C<sub>p</sub>) were measured using square-pulse analysis.<sup>16</sup> The membrane model used in this analysis was one where the epithelium consists of a current generator (I<sub>m</sub>) in parallel with a resistor (R<sub>p</sub>) and a capacitor (C<sub>p</sub>), and all three are in series with a subepithelial resistance (R<sub>s</sub>). When a square-pulse is fed through this equivalent circuit, the resulting voltage change will be characterized by an instantaneous increase due to charging of R<sub>s</sub>, and a delayed phase that is due to charging of the R<sub>p</sub>-C<sub>p</sub> element. Likewise, on termination of the current pulse, the voltage reduction will be delayed with an approximately monoexponential decay that represents the discharge of the R<sub>p</sub>-C<sub>p</sub> element. By plotting the voltage reduction logarithmically vs time, one can generate a linear fit that can be extrapolated back to time zero (V<sub>0</sub>), i.e., the end of the current pulse. By following the linear part of the discharge event, one can selectively analyze the R<sub>p</sub>-C<sub>p</sub> element, i.e., the epithelium while excluding R<sub>s</sub> from the analysis. The epithelial resistance (R<sub>p</sub>) can be calculated from Ohm's law by dividing the voltage response at time zero (V<sub>0</sub>) by the applied current, and the epithelial capacitance can be calculated from the time constant of the R<sub>p</sub>-C<sub>p</sub> element. The formulas used were as follows:

$$V(t) = V_0 \times e^{-t/(R_p \times C_p)}$$

where V (t) is the voltage by time, V<sub>0</sub> the starting voltage, t is time after end of the current pulse, R<sub>p</sub> is the epithelial resistance, and C<sub>p</sub> is the epithelial capacitance. Plotting the voltage logarithmically vs time will generate a linear fit that can be extrapolated back to time

zero, i.e., the end of the current pulse. Epithelial resistance can then be calculated from the generated voltage and the applied current by using Ohm's law:

$$U = R \times I$$

at time zero  $t = 0$  and  $V(0) = V_0 \times e^{-0/(R \times C)}$  giving  $V(0) = V_0 \times 1$  and  $R = V_0/I$  where  $I$  is the current of the applied pulse.

The capacitance is also calculated from the time course of the voltage curve using the same formula:

$$V(t) = V_0 \times e^{-t/(R \times C)} \rightarrow C = (t_2 - t_1) / (R \times \ln(V_1/V_2))$$

where  $V_1$  and  $V_2$  are the voltages at two selected times  $t_1$  and  $t_2$  of the mono exponential discharge event.

By measuring the transepithelial voltage (PD) after each pulse, the net membrane current ( $I_m$ ) can be calculated from the recorded PD (in the absence of current pulse) and the  $R_p$  value, also by using Ohm's law

$$PD = R_p \times I_m$$

### Experimental groups and interventions

Two to four biopsies from each patient were analyzed in the Ussing chamber. The experimental set up included a 20-min equilibration period followed by 40-min baseline recordings and 30-min exposure to either carbachol or forskolin. The same experimental set up was used in the mice experiments, but in these experiments, only two specimens per animal were tested, one proximal and one distal. Carbachol (Sigma-Aldrich) was dissolved in water and forskolin (Sigma-Aldrich) was dissolved in ethyl alcohol. Both substances were added to the serosal chamber at a dilution of 1: 1000.

### Data analysis

Due to the biphasic shape of the  $I_m$  response to carbachol, the response was divided into peak response and sustained response. The peak response was calculated by subtracting the baseline values from the maximum response. The baseline value represents the mean of the last 15 min of the 40-min baseline recording. In human colon, the sustained carbachol response was calculated by subtracting the baseline value from the mean of the response during the time points 20, 25, and 30 min after stimulation. In mouse colon, the sustained response was calculated by subtracting the baseline value from the mean of the response during the time points 10 and 15 min after carbachol stimulation. The  $C_p$  response to carbachol followed a transient pattern and is expressed as delta increase in  $\mu F \text{ cm}^{-2}$ . The baseline value (mean of the last 15 min of the 40-min baseline recording) was subtracted from the values obtained after stimulation. The  $I_m$  response to forskolin was calculated by subtracting the baseline values from the maximum  $I_m$  value. The  $C_p$  response to forskolin

followed a similar pattern as the  $I_m$  response with a gradual increase over time. The delta response was therefore calculated in the same way as for the  $I_m$  response, for example, the baseline values were subtracted from the peak response.

### Statistics

Data are presented as mean  $\pm$  standard error of the mean (SEM). For the human data, comparisons between groups were made using a two-tailed Students' *t*-test with Welch's corrections in case of differences in variation. Due to the small sample groups in the mouse experiments, data were analyzed using the Mann–Whitney *U*-test or a Kruskal–Wallis test followed by Dunnett's post-test. A *P*-value  $<0.05$  was considered statistically significant.

## RESULTS

### Basal electrical properties differed between proximal and distal colon, but not between controls and UC patients in remission

To determine whether baseline epithelial transport was altered in UC patients in remission, baseline values of  $I_m$ ,  $R_p$ , and  $C_p$  were recorded for 40 min. In both the controls and UC patients, basal  $I_m$  was significantly higher in the ascending (asc) colon than in sigmoid (sig) colon (Ctrl: asc:  $275 \pm 28 \mu\text{A cm}^{-2}$ , sig:  $154 \pm 16 \mu\text{A cm}^{-2}$ ,  $P < 0.01$ ; UC: asc:  $245 \pm 20 \mu\text{A cm}^{-2}$ , sig:  $175 \pm 20 \mu\text{A cm}^{-2}$ ,  $P < 0.05$ ). A similar difference was observed for epithelial resistance, which also was significantly higher in the ascending colon than in the sigmoid colon (Ctrl: asc:  $14.0 \pm 0.8 \text{ ohm cm}^2$ , sig:  $9.1 \pm 0.5 \text{ ohm cm}^2$ ,  $P < 0.001$ ; UC: asc:  $14.1 \pm 1.1 \text{ ohm cm}^2$ , sig:  $9.7 \pm 0.7 \text{ ohm cm}^2$ ,  $P < 0.001$ ). In contrast, epithelial capacitance was significantly higher in the sigmoid colon than in the ascending colon (Ctrl: asc:  $35.0 \pm 2.1 \mu\text{F cm}^{-2}$ , sig:  $54.1 \pm 2.6 \mu\text{F cm}^{-2}$ ,  $P < 0.001$ ; UC: asc:  $33.4 \pm 2.2 \mu\text{F cm}^{-2}$ , sig:  $54.0 \pm 4.4 \mu\text{F cm}^{-2}$ ,  $P < 0.001$ ). No significant differences were observed between the controls and UC patients for any of the three parameters in either segment (Fig. 1A–C).

### The forskolin-stimulated current response was up-regulated in the ascending colon of UC patients in remission

Forskolin has been widely used to study CFTR-mediated anion secretion in the colonic epithelium of various species.<sup>17,18</sup> To determine whether the reactivity of CFTR-mediated anion secretion was altered despite normal baseline values, forskolin ( $10 \mu\text{mol L}^{-1}$ ) was administered to the serosal side of the epithelium and the effect on  $I_m$  and  $C_p$  was recorded for 30 min. In both the ascending colon and sigmoid colon, forskolin induced a gradual and sustained increase in  $I_m$  and  $C_p$  in both groups. In the control patients, the  $I_m$  response was larger in the sigmoid colon than in the ascending colon (asc:  $150 \pm 10 \mu\text{A cm}^{-2}$ , sig:  $320 \pm 40 \mu\text{A cm}^{-2}$ ,  $P < 0.001$ ), but the  $C_p$  response was similar in the two segments (asc:  $26 \pm 4 \mu\text{F cm}^{-2}$ , sig:  $32 \pm 5 \mu\text{F cm}^{-2}$ ,  $P = \text{ns}$ ). In the UC patients, on the other hand, no segmental difference in the magnitude of the  $I_m$  response to forskolin was observed (asc:  $240 \pm 30 \mu\text{A cm}^{-2}$ , sig:  $230 \pm 30 \mu\text{A cm}^{-2}$ ,  $P = \text{ns}$ ). This loss of segmental differences was due to an up-regulation of the forskolin response in the ascending colon (Fig. 2A,B), while the response was not significantly altered in the sigmoid colon (Fig. 2C,D). The enhanced  $I_m$  response in the ascending colon was not accompanied by an increased  $C_p$  response (ctrl:  $26 \pm 4 \mu\text{F cm}^{-2}$ , UC:  $30 \pm 4 \mu\text{F cm}^{-2}$ ,  $P = \text{ns}$ ) and likewise, the response in

the sigmoid colon did not differ from the controls (ctrl:  $32 \pm 5 \mu\text{F cm}^{-2}$ , UC:  $25 \pm 5 \mu\text{F cm}^{-2}$ ,  $P = \text{ns}$ ). The  $I_m$  response to forskolin was thus consequently up-regulated in the proximal colon of UC patients in remission.

### **Carbachol-stimulated current was downregulated in the ascending colon of UC patients in remission**

Carbachol is a well-characterized secretagogue known to induce anion secretion in various species.<sup>19,20</sup> Serosal administration of carbachol induced a biphasic increase in  $I_m$  with a rapid initial peak response followed by a sustained plateau phase. In the control patients, both the peak response and the plateau phase were significantly larger in the ascending colon than in sigmoid colon (peak response: asc:  $730 \pm 100 \mu\text{A cm}^{-2}$ , sig:  $440 \pm 60 \mu\text{A cm}^{-2}$ ,  $P < 0.05$ ; sustained response: asc:  $200 \pm 40 \mu\text{A cm}^{-2}$ , sig:  $70 \pm 10 \mu\text{A cm}^{-2}$ ,  $P < 0.01$ ). In the UC patients, the segmental difference in the  $I_m$  response was not observed either for the peak response or for the sustained response (peak: asc:  $460 \pm 70 \mu\text{A cm}^{-2}$ ; sig:  $480 \pm 80 \mu\text{A cm}^{-2}$ ,  $P = \text{ns}$ ; sustained: asc:  $70 \pm 20 \mu\text{A cm}^{-2}$ , sig:  $70 \pm 30 \mu\text{A cm}^{-2}$ ,  $P = \text{ns}$ ). Loss of segmental differentiation was mainly due to significantly reduced  $I_m$  response to carbachol in the ascending colon (Fig. 3A,B), while the response in the sigmoid colon did not differ from the controls (Fig. 3C,D).

### **The carbachol-stimulated increase in capacitance was up-regulated in the sigmoid colon of UC patients in remission**

Several aspects of mucosal function, like trafficking of membrane transporters and mucin exocytosis, are reflected by changes in membrane capacitance ( $C_p$ ).<sup>12,13</sup> Serosal administration of carbachol induced a transient increase in  $C_p$  in both the ascending colon and sigmoid colon. In the control patients, the time course, but not the total magnitude of the response, differed between the ascending colon and sigmoid colon. In the ascending colon, the response reached its maximum after  $6 \pm 1$  min compared to  $11 \pm 1$  min in the sigmoid colon,  $P < 0.001$ . Again, this segmental difference was not observed in the UC patients (asc:  $7 \pm 1$  min, sig:  $8 \pm 1$  min,  $P = \text{ns}$ ). The loss of segmental differences in UC patients was due to an up-regulated  $C_p$  response to carbachol in the sigmoid colon, whereas the response in the ascending colon remained unchanged (Fig. 4A,B).

No significant differences were observed for either the forskolin or carbachol responses when comparing UC patients who received medical treatment and those who did not.

### **The $I_m$ responses to both forskolin and carbachol were dependent on a functional CFTR channel**

To verify that the forskolin and carbachol-induced effects on membrane current reflect anion secretion through the CFTR channel, colonic specimens from Cfr F508 (CF) mice were stimulated with secretagogues and the membrane current was recorded. In WT mice, serosal addition of forskolin induced a sustained increase in  $I_m$  in both the proximal and distal colon (proximal:  $380 \pm 43 \mu\text{A cm}^{-2}$ , distal:  $323 \pm 28 \mu\text{A cm}^{-2}$ ,  $P = \text{ns}$ ). In the CF mice, the forskolin response was reduced by 90% in both the proximal (WT:  $380 \pm 43 \mu\text{A cm}^{-2}$ , CF:  $43 \pm 45 \mu\text{A cm}^{-2}$ ,  $P < 0.05$ ) and distal colon (WT:  $320 \pm 28 \mu\text{A cm}^{-2}$ , CF:  $31 \pm 3 \mu\text{A cm}^{-2}$ ,  $P < 0.001$ ) (Fig. S1A,B). In WT mice, stimulation with CCh induced a monophasic  $I_m$



response in the proximal colon and a biphasic response in the distal colon. The  $I_m$  response to CCh was significantly larger in the distal colon than in the proximal colon (proximal:  $131 \pm 23 \mu\text{A cm}^{-2}$ , distal:  $260 \pm 26 \mu\text{A cm}^{-2}$ ,  $P < 0.05$ ). In the CF mice, the peak response to CCh was reduced by 70% in the proximal colon (WT:  $131 \pm 23 \mu\text{A cm}^{-2}$ , CF:  $49 \pm 7 \mu\text{A cm}^{-2}$ ,  $P < 0.05$ ) and 95% in the distal colon (WT:  $260 \pm 26 \mu\text{A cm}^{-2}$ , CF:  $10 \pm 9 \mu\text{A cm}^{-2}$ ,  $P < 0.001$ ). In addition, the sustained response in the distal colon was also reduced in the CF mice (WT:  $92 \pm 27 \mu\text{A cm}^{-2}$ , CF:  $17 \pm 9 \mu\text{A cm}^{-2}$ ,  $P < 0.05$ ) (Fig. S2A,B). Accordingly, both the forskolin and carbachol effect on  $I_m$  seems to require a functioning CFTR channel.

### The carbachol-induced effect on capacitance was not observed in mice that lack the main component of the mucus layer

The two most likely mechanisms behind carbachol-induced changes in  $C_p$  are insertion of CFTR into the plasma membrane and mucin exocytosis. To test the involvement of these mechanisms, we studied the  $C_p$  response in mice that lack intestinal mucus the  $\text{Muc2}^{-/-}$  mice and CF mice lacking a functional CFTR channel in the plasma membrane. Stimulation with CCh induced a transient increase in  $C_p$  in both the proximal and distal colon of WT mice. Similar to the human tissue, the response was larger in the proximal colon than in distal colon, but the time frame of the response did not differ between segments (Fig. 5A–D). The CF mice responded to CCh with an increase in  $C_p$  that was similar in magnitude to that seen in the WT mice, but the time course of the response was delayed in the distal colon compared with WT mice (Fig. 5A–D). In contrast, CCh stimulation did not induce an increase in  $C_p$  in  $\text{Muc2}^{-/-}$  mice either in the proximal or in the distal colon (Fig. 5A–D). Thus,  $\text{Muc2}$  production is essential for a normal  $C_p$  response, whereas a dysfunctional CFTR only affects the time course of the  $C_p$  response.

## DISCUSSION

The key observation in the present study is that despite a macroscopically normal mucosa with normal basal electrical parameters, UC patients in remission showed an altered responsiveness to induced secretion. The  $I_m$  response to forskolin was up-regulated in the proximal colon and the  $I_m$  response to carbachol was down-regulated in the same segment. In the distal colon, the  $I_m$  responses to both forskolin and carbachol remained unaffected, whereas the  $C_p$  response to carbachol was up-regulated. The ascending colon of UC patients behaved more like the normal sigmoid colon when it comes to ion secretion, and the distal colon of UC patients behaved like the normal proximal colon in regard to exocytosis, resulting in loss of segmental differentiation.

To verify that the  $I_m$  response to forskolin and carbachol reflects CFTR-mediated secretion, we tested the responses in mice which lack a functional  $\text{Cftr}$  channel. In these mice, the  $I_m$  response to both forskolin and carbachol was significantly reduced in both the proximal and distal colon, making it reasonable to conclude that we measured anion secretion directly or indirectly via the  $\text{Cftr}$  channel. The CFTR conducts both  $\text{Cl}^-$  and  $\text{HCO}_3^-$ , but as the  $\text{Cl}^-$  conductance is higher than the  $\text{HCO}_3^-$  conductance, chloride will be preferentially transported.<sup>21</sup> However, this method does not allow us to differentiate between the contribution of  $\text{Cl}^-$  and  $\text{HCO}_3^-$  to the  $I_m$  response.



The mechanism behind the Cp response to carbachol is more difficult to delineate. However, studies in cultured epithelial cells have shown that the presence of mucus producing goblet cells was required for a Cp response to carbachol and the time frame and magnitude of the response correlated with mucus secretion.<sup>22</sup> To confirm that changes in epithelial capacitance can be used to monitor mucus secretion also in our system, we measured the carbachol-induced effect on Cp in mice that lack the intestinal Muc2 mucin. Our results showed that the Cp response was absent in both the proximal and distal colon of the Muc2-deficient mice. Furthermore, the response was not related to CFTR trafficking, which is known to occur in response to carbachol.<sup>23</sup> The magnitude of the carbachol-induced increase in capacitance in human colon suggests that the surface area is increased by 55% in the proximal colon and by 35% in the distal colon following carbachol treatment. These values are compatible with previous studies in human colonic cell lines where the capacitance increased by 35% following carbachol stimulation.<sup>24</sup> Studies in rabbit colon have shown that the exposed membrane area of a goblet cell can increase as much as five times during acetylcholine-induced compound exocytosis.<sup>6</sup> In the human colonic crypts, 25% of the cells are goblet cells and if all these cells increase their surface area five times, the total tissue area could theoretically increase by 100%.<sup>25</sup> As it is not likely that every single goblet cell responds to carbachol stimulation and that the degree of emptying likely differs between cells, an increase in surface area in the range of 35–55% seems realistic.

Surprisingly, little is known regarding the secretory capacity of the colonic epithelium during UC in remission. Studies in animal models of colitis show that acute inflammation is associated with an impaired secretory response that can be attributed to down-regulation of the  $\text{Na}^+/\text{K}^+$ -ATPase, resulting in a loss of the electrochemical gradient required for anion secretion.<sup>26</sup> Although not studied in detail, a severe reduction in the responsiveness to induced secretion can be expected also in the human colon during acute inflammation, as a 70% reduction in the  $\text{Na}^+/\text{K}^+$ -ATPase activity has been observed in patients with acute inflammation.<sup>27,28</sup> Our observation of normal baseline electrical parameters during remission implies that this down-regulation is reversed during remission, as also baseline membrane current depends on a functional  $\text{Na}^+/\text{K}^+$ -ATPase.<sup>29</sup> Normal absorptive functions have also been observed in patients in remission and a single dose of steroids normalizes sodium absorption during acute colitis.<sup>30</sup> Thus, the impaired absorption appears to be more related to active inflammation than remission.

Clinicians and researcher have been puzzled for a long time why up to 30% of patients still suffer from GI symptoms despite no signs of inflammation.<sup>2,3</sup> Our results show that despite normal macroscopical morphology, the responsiveness of the colonic mucosa to induced secretion was different from that in control patients. In proximal colon, there was an up-regulated response to forskolin and a down-regulation of the carbachol response. Our results from the Cftr F508 mice and other studies in patients with cystic fibrosis show that loss of a functional CFTR reduces both the forskolin and the carbachol response, suggesting that the altered responsiveness observed in the UC patients is not directly linked to the activity of the CFTR channel, as this should have similar effects on both the forskolin and CCh responses.<sup>31,32</sup> Furthermore, as the altered responsiveness was restricted to the proximal colon, this cannot be general consequence of previous inflammation. On the contrary, the capacitance response to carbachol was up-regulated, suggesting that the epithelium is

hyperresponsive to induced mucus secretion. This means that despite morphological recovery, the mucosa of UC patients in remission retains a “functional scar” making the proximal colon hyperreactive to agonists of the cAMP system and hyporeactive to agonist of the Ca<sup>2+</sup> system, and the goblet cells of the distal colon hyperreactive to muscarinic mucus secretion.

The finding of an up-regulated electrogenic secretion is not unique to our study. Increased anion secretion had been reported to occur in microscopic and collagenous colitis, conditions associated with a low-grade inflammation.<sup>4,5</sup> Also, chronic exposure to mast cells has been shown to induce a hyperreactive response to secretagogues in cultured epithelial cells.<sup>33</sup>

The most likely functional consequence of the hyperreactivity of the CFTR system in the proximal colon is an increased fluid load in the distal colon. Depending on the magnitude of this increased fluid load, this might in fact account for the observation that a substantial proportion of UC patients in remission still have diarrheal problems. It has been shown that the colonic epithelium has the ability to absorb up to 4 L of fluid before normal subjects notice diarrhea symptoms, but for predisposed persons with a mild chronic inflammation in the distal colon, increased secretion from the proximal colon will cause additional problems.<sup>2</sup> One of the pathophysiological models for UC is that there is an underlying increased reactivity of the mucosal immune system toward the commensal flora. If this is true, an up-regulation of the secretory system could be functional by flushing the epithelium and reducing the bacterial–epithelial interaction.

Functional implications for an up-regulated mucin secretory response are more difficult to understand as less is known about the connection between colonic anion and mucus secretion. It is well established that CFTR-mediated secretion is important for formation of a normal mucus layer in the small intestine and that loss of CFTR-mediated secretion results in mucus accumulation in the airways, thus the balance between anion and mucus secretion can affect mucus properties.<sup>34,35</sup> However, there are no results supporting that an excessive chloride or bicarbonate secretion further improves the quality of the formed mucus layer. The down-regulated secretory response to carbachol in the proximal colon would suggest that the coordinated secretion of fluid ion and mucus is shifted toward reduced ion secretion in relation to mucus secretion. This could possibly affect mucus properties, but the role of carbachol-mediated secretion in regulating colonic mucus properties remains to be explored.

The observation of an up-regulation of the capacitance response to carbachol points to a similar shift toward more mucus secretion. In colon, the mucus layer has a clear protective effect by limiting contact between the luminal bacteria and the epithelium.<sup>36</sup> Therefore, a faster response could have a protective role by preventing harmful agents to come in contact with the epithelial surface. However, if the system is pushed to secrete more than the synthesis machinery allows, this might result in mucus depletion and compromise the protective functions of the mucus layer.

In conclusion, our data imply that despite normal basal electrical parameters, the colonic mucosa from UC patients in remission appears to have an increased reactivity of the CFTR

system in the proximal colon and increased goblet cells exocytosis in distal colon. Both these phenomena may represent adaptive changes to minimize the risk for bacterial – epithelial interactions, but, if driven too far, might cause diarrhea.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## Acknowledgments

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## Abbreviations

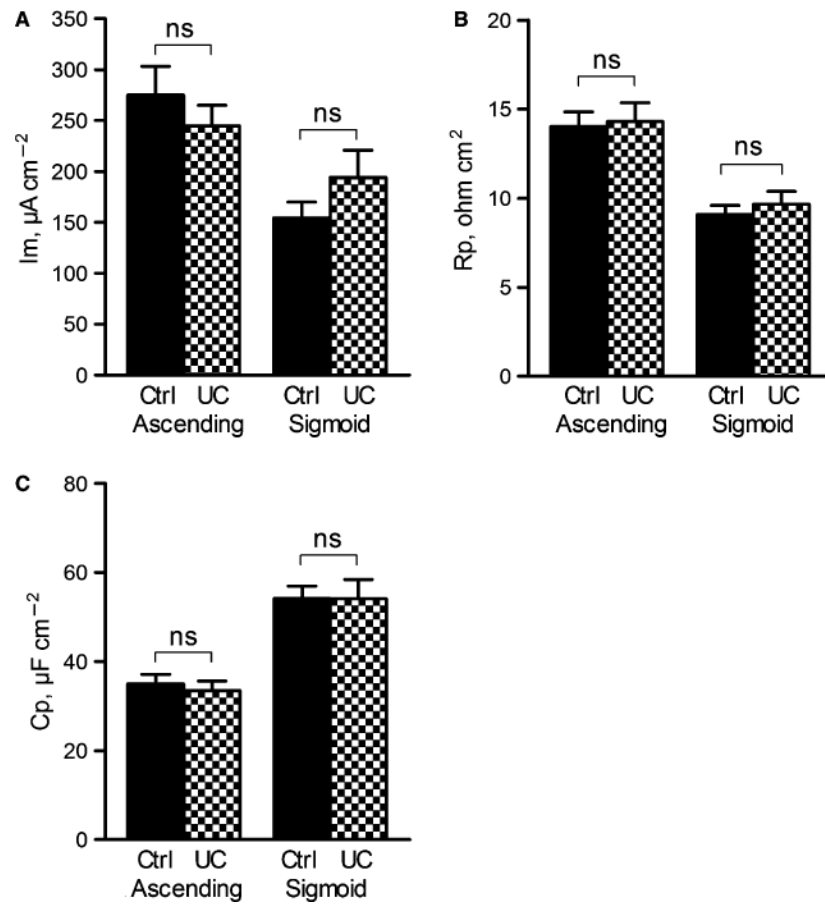
<b>CCh</b>	carbachol
<b>CF</b>	<i>Cftr</i> F508
<b>CFTR</b>	cystic fibrosis transmembrane conductance regulator
<b>Cp</b>	epithelial capacitance
<b>Im</b>	net membrane current
<b>Muc2</b>	mucin 2
<b>Rp</b>	epithelial resistance
<b>UC</b>	ulcerative colitis

## References

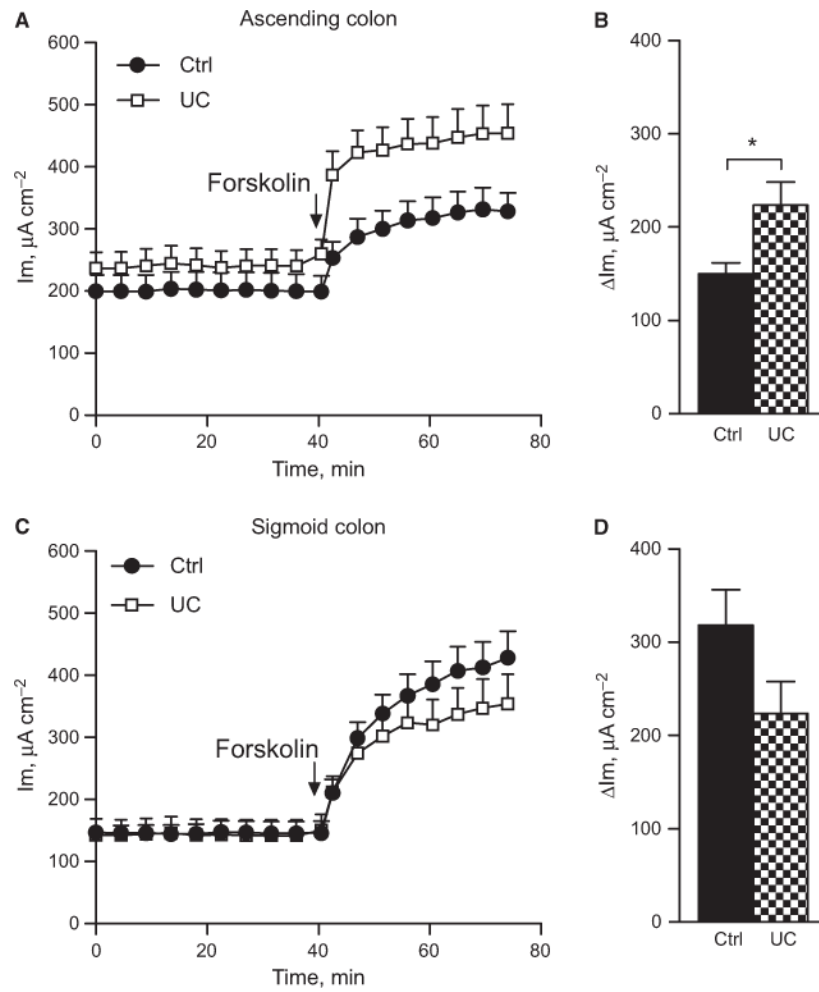
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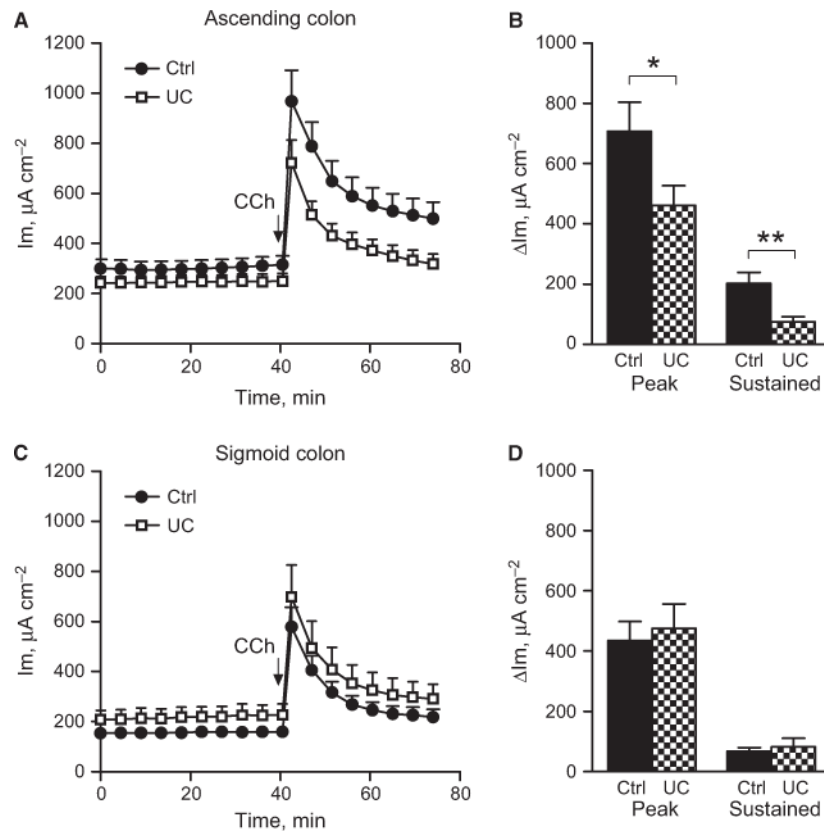


**Figure 1.** Basal electrophysiological parameters in the ascending colon and sigmoid colon of controls ( $n = 32$ ) and UC patients ( $n = 24$ ). (A) Membrane current ( $I_m$ ), (B) Epithelial resistance ( $R_p$ ) (C) Epithelial capacitance ( $C_p$ ). Data are presented as mean  $\pm$  SEM, ns = non significant.

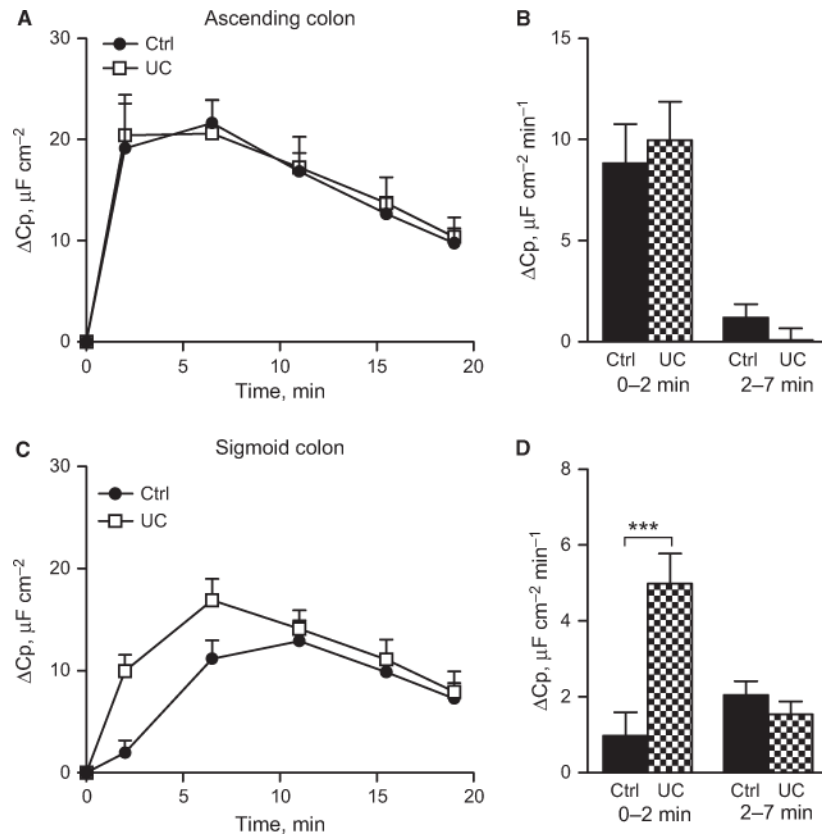


**Figure 2.** Forskolin effect on membrane current in human colon. (A) Total response over time in the ascending colon of control  $n = 12$  and UC ( $n = 15$ ) patients. (B) Delta max response in the ascending colon during 30 min incubation. (C) Total response over time in the sigmoid colon of control ( $n = 10$ ) and UC ( $n = 13$ ) patients. (D) Delta max response in the sigmoid colon during 30-min incubation. Data are presented as mean  $\pm$  SEM, \* $P < 0.05$ .

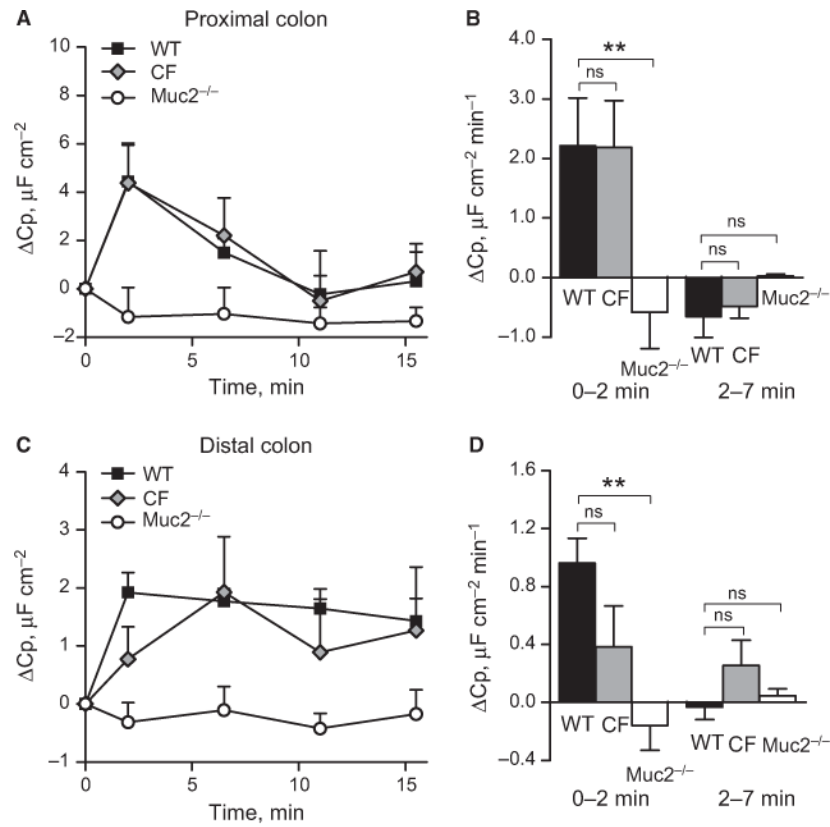




**Figure 3.** Carbachol effect on membrane current in human colon. (A) Total response over time in the ascending colon of control ( $n = 21$ ) and UC patients ( $n = 18$ ). (B) Delta response to carbachol divided into the peak response (max value - baseline) and sustained response (average of three last recordings) baseline). (C) Total response over time in the sigmoid colon of control ( $n = 17$ ) and UC patients ( $n = 19$ ). (D) Delta response to carbachol divided into peak response (max value - baseline) and sustained response (average of three last recordings) baseline). Data are presented as mean  $\pm$  SEM, \* $P < 0.05$ , \*\* $P < 0.01$ .



**Figure 4.** Carbachol effect on epithelial capacitance in human colon. (A) Delta increase in capacitance in the ascending colon of control ( $n = 21$ ) and UC patients ( $n = 18$ ). (B) Secretion rate during the first (0–2 min) and second (2–7 min) time points in the ascending colon. (C) Delta increase in capacitance in the sigmoid colon of control ( $n = 17$ ) and UC patients ( $n = 19$ ). (D) Secretion rate during the first (0–2 min) and second (2–7 min) time points in the sigmoid colon. Data are presented as mean  $\pm$  SEM, \*\*\* $P < 0.001$ .



**Figure 5.** Carbachol-induced effect on epithelial capacitance in mouse colon. (A) Delta increase in capacitance in the proximal colon of WT ( $n = 6$ ), CF ( $n = 9$ ) and Muc2<sup>-/-</sup> ( $n = 3$ ) mice. (B) Secretion rate during the first (0–2 min) and second (2–7 min) time points in the proximal colon. (C) Delta increase in capacitance in the distal colon of WT ( $n = 9$ ), CF ( $n = 9$ ) and Muc2<sup>-/-</sup> mice ( $n = 6$ ). (D) Secretion rate during the first (0–2 min) and second (2–7 min) time points in distal colon. Data are presented as mean  $\pm$  SEM, \*\* $P < 0.01$ , ns = non significant.

Table 1

Clinical information of the analyzed UC patients

Gender*	Age	Mayo Score		Histological assessment <sup>†</sup>			Medication <sup>‡</sup>	Relapse/year
		Ascending	Sigmoid	Ascending	Sigmoid			
F	45	0	0	NAD	NAD		5-ASA	<1
F	69	0	0	NAD	NAD		5-ASA	<1
M	46	0	0	A	A		5-ASA	<1
M	58	0	0	A, L	L		5-ASA	<1
M	45	0	0	NAD	NAD		5-ASA	<1
F	46	0	0	NAD	NAD		5-ASA	<1
M	56	0	0	NAD	NAD		5-ASA	<1
M	54	0	0	A	A		5-ASA	<1
F	44	0	0	A	A, P		None	<1
M	21	0	0	L	NAD		5-ASA, Azathioprine	<1
M	75	0	0	NAD	E, L, P		5-ASA, Azathioprine	<1
M	26	0	0	NAD	NAD		None	<1
M	61	0	0	NAD	NAD		None	<1
F	33	0	0	NAD	NAD		5-ASA	<1
M	51	0	0	A, E, L, P	A, E, L, P		5-ASA	<1
M	48	0	0	NAD	NAD		5-ASA	1
F	77	0	0	A	A		5-ASA	<1
M	37	0	0	NAD	NAD		5-ASA, Azathioprine	<1
M	54	0	0	NAD	NAD		5-ASA	<1
F	64	0	0	N/A	N/A		None	<1
M	22	0	0	A, E, L, P	A, E, L, P		5-ASA	<1
M	53	0	0	NAD	NAD		5-ASA	>1
M	29	0	0	NAD	A, E, L, P		5-ASA	<1
M	33	0	0	NAD	NAD		None	<1

\* M, male; F, female.

<sup>†</sup> A, alterations in crypt architecture; E, eosinophils; L, lymphocytes; P, plasma cells; NAD, nothing abnormal detected; N/A, not available.

\*5-ASA: 5-aminosalicylic acid.

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