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Segmental Regulation of Intestinal Motility by Colitis and the Adaptive Immune System in the Mouse Ileum and Colon

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Gastrointestinal motility disturbances are a hallmark of inflammatory bowel disease (IBD); however, their mechanisms remain unclear. This study used a dextran sulfate sodium—induced colitis mouse model, deficient in mature B and T lymphocytes, to assess intestinal motility and the role of the adaptive immune system in health and IBD. In healthy mice, the absence of adaptive lymphocytes reduced acetylcholine (ACh) sensitivity in the ileum. During colitis, it decreases motility by reducing the intensity and frequency of spontaneous contractions while increasing cholinergic responsiveness. In the proximal colon, adaptive immunity deficiency led to increased contractility and reduced ACh sensitivity in homeostasis, whereas colitis reduced contractile capacity. In the mid colon, immune-deficient mice had reduced ACh sensitivity in homeostasis and exacerbated contractile responses during colitis. In the distal colon, adaptive immunity loss reduced contractility in health and cholinergic responsiveness during colitis. These motility alterations were associated with altered acetylcholinesterase and M2/M3 muscarinic receptor expression. Notably, adaptive lymphocyte deficiency resulted in reduced tissue damage and lower tumor necrosis factor-α expression in the colon during colitis, paralleling intestinal motility changes. Overall, the adaptive immune system critically regulates motility and inflammation across different intestinal segments in IBD. *(Am J Pathol 2025, 195: 204—220; https://doi.org/10.1016/j.ajpath.2024.10.020)*

The gastrointestinal tract is divided into the small and large intestines, which contain a large population of commensal microbiota. This microbial community exhibits spatial heterogeneity along the proximal-distal axis, contributing to the organ's specialization and unique functions based on its position.[1—3] Although compartmentalization in the small intestine is well defined, the molecular regionalization in the colon is increasingly understood.[4]

The intestine relies on continuous epithelial regeneration and immune system regulation to maintain homeostasis. Disruption of these processes can lead to chronic intestinal conditions, such as inflammatory bowel disease (IBD). Following injury, the intestine must quickly adapt to minimize tissue damage, facilitate regeneration, and promote

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recovery. To achieve this, immune cells are recruited or expanded *in situ* to protect the host from invading pathogens and to coordinate the healing process by emitting resolving signals.[5]

IBD is a chronic inflammatory disorder of the intestine that manifests as ulcerative colitis and Crohn disease. It arises from an inappropriate host immune response to commensal bacteria in genetically susceptible individuals. The overall pathophysiology of the disease is well understood and comprises several interconnected processes: environmental influences can alter the composition of the gut microbiota, impacting mucosal barrier function and leading to epithelial alterations. This can ultimately result in inflammation in the lamina propria.[6–9] When the integrity of the intestinal epithelium is compromised, certain microbiota components can cross into the lamina propria, inducing the activation of dendritic cells and macrophages. This cascade of innate immune cell activation culminates in the recruitment of $CD4^+$ T cells to the intestinal tissue. Individuals with IBD often show increased proinflammatory helper T cells, particularly type 1 and type 17 helper T cells, along with reduced immunosuppressive regulatory T cells. The imbalance toward proinflammatory T cells guides the function of cells with an innate immune role, such as epithelial cells, fibroblasts, and phagocytes, leading to persistent hyperresponsiveness to microbial antigens, resulting in tissue injury and perpetuating chronic intestinal inflammation.[6–8]

Although most research has focused on investigating and treating the inflammation and histologic alterations within the digestive tract of individuals with IBD, there is a scarcity of studies examining the impact of IBD on gastrointestinal motility. The digestive tract exhibits diverse motility patterns that are regulated by complementary and overlapping control systems, including the enteric and autonomic nervous systems, smooth muscle cells (SMCs), and interstitial cells of Cajal (ICCs).[10,11] These patterns differ in intensity and rhythm across different parts of the gastrointestinal system.[12]

Interestingly, several factors implicated in the development of IBD are associated with gastrointestinal motor abnormalities.[13] Furthermore, certain motility disorders, previously labeled as idiopathic, are attributable to low-grade inflammation. Examples of such gastrointestinal disorders include functional dyspepsia, irritable bowel syndrome, and chronic constipation.[13] Moreover, alterations in the enteric nervous system are observed in patients with IBD, which affect the typical motor function and visceral reflexes of the gastrointestinal tract.[13] However, the precise impact of the immune system on intestinal motility remains poorly understood. Studies in various mouse models have produced conflicting results, indicating both increased and decreased motility based on the intestinal region or type of insult. Various models of inflammation have decreased motility.[12] Conversely, infection causes hypercontractility, which is associated with increased IL-9 and dysregulation of helper T cells and regulatory T cells.[14] The primary objective of this study was to evaluate the role of the adaptive immune system in the physiopathology of IBD, specifically in relation to intestinal motility. Additionally, the study aimed to identify any differences between the small and large intestine contractility in both homeostasis and colitis conditions.

Materials and Methods

Mice

Experiments were conducted on male mice, aged 8 to 14 weeks, including both wild-type (WT) C57BL/6J mice and C57BL/6J *Rag1*$^{-/-}$ mice lacking mature B and T lymphocytes.[15] The mice were bred under specific pathogen-free conditions at the Centro Nacional de Investigaciones Cardiovasculares in accordance with the sanitary recommendations of the Federation of European Laboratory Animal Science Associations. The experimental procedures involving animals were approved by the Fundación Centro Nacional de Investigaciones Cardiovasculares Carlos III, Universidad Autónoma de Madrid, and the Comunidad Autónoma de Madrid, in accordance with Spanish and European guidelines.

DSS Colitis Model

To induce colitis, as previously described,[16] mice were administered 2% (w/v) dextran sulfate sodium salt (DSS; Alfa Aesar; Thermo Fisher, Kandel, Germany) in their drinking water for 5 days. This was followed by a recovery period of 3 to 7 days during which they consumed only water. Mice from each genotype (WT and *Rag1*$^{-/-}$) were divided into three groups: healthy group (exposed solely to water), colitis group (sacrificed on day 8 post-DSS administration), and recovery group (sacrificed on day 12 post-DSS administration). Throughout the experimental period, daily monitoring of the mice's body weight and disease activity was diligently conducted. Disease severity was assigned a disease activity index as follows: stool consistency: 0 (normal), 1 (loose stool), and 2 (diarrhea); rectal prolapse: 0 (absent) and 1 (present); rectal bleeding: 0 (absent), 1 (low), and 2 (pronounced); and spine curvature: 0 (absent), 1 (low), and 2 (pronounced).

Solutions

The solutions used including the following: Krebs-Henseleit solution [KHS; concentration (mmol/L)]: NaCl 115, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, EDTA 0.01, and glucose 11.1, previously refrigerated and gassed with 5% CO_2 and 95% oxygen gas; calcium-free KHS [0 Ca^{2+}; concentration (mmol/L)]: NaCl 115.0, $NaHCO_3$ 25.0, KCl 4.7, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, glucose 11.1, and EGTA 10; and KCl solution [concentration (mmol/L)]: KCl 120, $MgSO_4 \cdot 7H_2O$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, $CaCl_2$ 2.5, EDTA 0.01, and glucose 5.55.

Organ Bath

After sacrifice, the entire intestine, from the ileum to the anus, was isolated and preserved in cold KHS. The ileum and colon underwent meticulous cleaning by gently flushing with KHS. The colon was divided into three regions: proximal, middle, and distal, relative to the ileum (Supplemental Figure S1). To account for variability in colon length due to inflammation[17] and differences among mice within the same group, the colon was sectioned as follows: the clean colon was separated from the cecum and rectum and divided into four equal segments. The segment closest to the cecum was designated as the proximal colon, the segment closest to the rectum was the distal colon, and the two middle sections were categorized as the mid colon. For the ileum, the last third of the small intestine was isolated and divided into four equally sized segments. The size of each segment was adjusted to fit the organ bath wells, ensuring they were all comparable in size. From each mouse, four segments were obtained from the ileum, one from the proximal colon, two from the middle colon, and one from the distal colon, each measuring approximately 5 to 6 mm. The segments were mounted longitudinally in individual organ bath chambers. A piece of suture thread was tied to each end of the intestinal segment; one thread was attached to a fixed metal support, whereas the other end of the intestine was connected to an isometric force transducer.[18] The chamber contained KHS gassed with a mixture of 95% oxygen and 5% CO_2 maintaining a pH of 7.3 to 7.4. The tissues were stretched to 0.5 g for the ileum and 1 g for the colon, then allowed to equilibrate. Equilibration lasted until the emergence of spontaneous contractions or for a minimum of 40 minutes. After recording spontaneous contractions, the tissue was exposed to a depolarization solution by replacing the KHS with a 120 mmol/L KCl solution. Following a washout period, dose-response curves were studied for muscarinic agonists, one for carbachol (Sigma-Aldrich, St. Louis, MO) and one for acetylcholine (ACh; Sigma-Aldrich), at concentrations ranging from 10^{-9} to 10^{-4} mol/L, with at least a 30-minute washout period between each agonist. ACh and carbachol stock solutions were prepared in saline ascorbic acid to prevent oxidation. At the end of the experiment, a basal measurement was obtained by using KHS without calcium. All parameters were measured in all mounted segments. The data were recorded and analyzed using LabChart 8 software (ADInstruments Ltd., Dunedin, New Zealand).

Histology

A clean piece from each segment (ileum, proximal, mid, and distal colon) from each group (WT and *Rag1*$^{-/-}$) was fixed in 4% paraformaldehyde for 24 hours. After fixation, the tissues were processed for paraffin embedding. Cross-sections of the colon (5 μm thick) were cut and mounted onto slides, then routinely stained with hematoxylin and eosin. Colitis severity was assessed by researchers (M.-J.F.-A) blinded to the sample identity, using criteria adapted from Valle-Noguera et al.[19] This evaluation involved calculating a combined score based on immune cell infiltration and epithelial damage, which included parameters such as crypt loss, hyperproliferation, and the presence of blood or ulcers. Each parameter was graded on a scale from 0 to 3, leading to a total possible score of 12.

Quantitative PCR

RNA was extracted from a piece of each segment (ileum, proximal, mid, and distal colon) from two to four animals per group (WT and *Rag1*$^{-/-}$). The tissues were weighed (<30 mg), mechanically disrupted in lysis buffer from the RNeasy Mini Kit (Qiagen, Hilden, Germany) using a TissueRuptor II (Qiagen), and subjected to RNA extraction following the RNeasy Mini Kit instructions. Subsequently, the RNA yield from each sample was measured using a NanoDrop spectrophotometer (Thermo Fisher). For cDNA synthesis, 1.5 μg of total RNA per reaction was used, and the RT High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Thermo Fisher, Waltham, MA) with random primers was used, following the manufacturer's protocol. The resulting cDNA was diluted 1:100, and quantitative PCR was performed using the QuantStudio 5 Dx Real-Time PCR System (Thermo Fisher) with technical duplicates. The PowerUp SYBR Green PCR Master Mix (Applied Biosystems, Thermo Fisher) was used for the reaction. To determine gene relative expression, normalization was performed using the housekeeping gene hypoxanthine phosphoribosyltransferase 1, and the experimental group WT control was used as the reference sample. Relative expression values were calculated using the $2^{-\Delta\Delta C_T}$ formula based on the cycle threshold (C_T) values obtained. Quantitative RT-PCR primer sequences were as follows: hypoxanthine phosphoribosyltransferase 1 forward (FW), 5′-AGCCTAAGATGAGCGCAAGT-3′; hypoxanthine phosphoribosyltransferase 1 reverse (RV), 5′-TTACTAGGCAGATGGCCACA-3′; claudin 7 (Cldn7) FW, 5′-CAGGCCACTCGAGCCTTAAT-3′; Cldn7 RV, 5′-GCAAGACCTGCCACAATGAAA-3′; tumor necrosis factor (Tnf) FW, 5′-CATCTTCTCAAAATTCGAGTGACAA-3′; Tnf RV, 5′-TGGGAGTAGACAAGGTACAACCC-3′; acetylcholinesterase FW, 5′-ACCTGTGGGCTCACGTAGATT-3′; acetylcholinesterase RV, 5′-CCACGTACTGGTAGCAGACATT-3′; muscarinic receptor M2 FW, 5′-TGGTTTGGCTATTACCAGTCCT-3′; muscarinic receptor M2 RV, 5′-CTGAAGGTGGCGGTTGACTT-3′; muscarinic receptor M3 FW, 5′-CCTCGCCTTTGTTTCCCAAC-3′; and muscarinic receptor M3 RV, 5′-TTGAGGAGAAATTCCCAGAGGT-3′.

Statistical Analysis

Statistical analysis and graphics were performed using GraphPad Prism 9 (GraphPad Software, Boston, MA). Weight evolution and disease activity index were analyzed using two-way analysis of variance with Tukey *post hoc*

tests. One-way analysis of variance with Tukey *post hoc* tests was used for multiple comparisons among all groups. The *t*-test was used to compare healthy WT and *Rag1*$^{-/-}$ groups. Maximal spontaneous contraction and KCl contraction were corrected with the length of the segment. For the analysis of the carbachol and ACh curves, the contraction of each agonist was related to the maximal KCl contraction and corrected to start at 0. Then, the area under the curve (AUC) and maximal responses and the pharmacology parameter pD2 ($-\log EC_{50}$, with EC_{50} being the half maximal effective concentration, the concentration of a drug that induces a response halfway between the baseline and maximum) to assess the efficacy and sensitivity of the muscarinic receptors were calculated. It was considered that $P < 0.05$ was significant and $P < 0.1$ was a tendency.

Results

Mice Administered DSS Develop Colitis, and the Impact of Adaptive Immunity on Inflammation and Tissue Damage Varies Across Different Regions of the Intestine

Six experimental groups were defined in the study. These groups included non-treated healthy WT mice (healthy WT), WT mice treated with DSS for 5 days and euthanized on day 8 (WT colitis) or day 12 (WT recovery), non-treated healthy *Rag1*$^{-/-}$ mice (healthy *Rag1*$^{-/-}$), and *Rag1*$^{-/-}$ mice treated with DSS for 5 days and euthanized on day 8 (*Rag1*$^{-/-}$ colitis) or day 12 (*Rag1*$^{-/-}$ recovery). Non-treated WT and *Rag1*$^{-/-}$ mice exhibited a slight, nonsignificant increase in weight from the beginning of the experiment until day 12, with no significant differences between the two groups (Figure 1A). Both DSS-treated WT and *Rag1*$^{-/-}$ mice experienced gradual weight loss until 7 to 8 days after the start of treatment, indicating the development of colitis. They then regained weight until the end of the experiment on day 12. By day 8, both the DSS-treated WT and *Rag1*$^{-/-}$ mice experienced significant body weight loss compared with the non-treated WT and *Rag1*$^{-/-}$ mice, respectively. By day 12, the body weights of both the DSS-treated WT and *Rag1*$^{-/-}$ mice were similar to those of the non-treated WT and *Rag1*$^{-/-}$ mice. The DSS-treated *Rag1*$^{-/-}$ mice exhibited less weight loss on days 8 and 9 and reached similar weight levels by day 12 compared with the WT mice treated with DSS (Figure 1A). Regarding the disease activity index, there was no significant difference between non-treated WT and *Rag1*$^{-/-}$ mice throughout the experiment. Both DSS-treated WT and *Rag1*$^{-/-}$ mice experienced gradual increase in the disease activity index from day 2 to day 5 after treatment initiation, indicating the progression of colitis. However, the DSS-treated *Rag1*$^{-/-}$ mice exhibited a lower disease activity index compared with the DSS-treated WT mice (Figure 1B). The length of the colon was measured after isolation (Figure 1C). WT and *Rag1*$^{-/-}$ colitis mice had shorter colons than healthy WT and *Rag1*$^{-/-}$ mice on day 8 (colitis groups), suggesting colon inflammation. By day 12 (recovery groups), the colon length of recovery WT and *Rag1*$^{-/-}$ mice was significantly different from that of the WT and *Rag1*$^{-/-}$ colitis mice, but not from that of the healthy WT and *Rag1*$^{-/-}$ mice, respectively, indicative of complete recovery. No differences in colon length were observed between the healthy WT and *Rag1*$^{-/-}$ groups. However, the colon length of the colitis and recovery *Rag1*$^{-/-}$ mice was greater than that of colitis and recovery WT mice, respectively (Figure 1C). To analyze local damage in different intestinal segments, histologic analysis was performed on intestinal sections stained with hematoxylin and eosin to determine the disease score (Figure 1, D and E). Barrier integrity (Figure 1F) and inflammation levels (Figure 1G) were evaluated by quantifying mRNA levels of claudin 7 (*Cldn7*) and TNF-α (*Tnf*) in the various sections of the intestine. An increased histology score, which encompasses the presence of inflammatory infiltrates, transmural lesions, goblet cell depletion, and epithelial or crypt hyperplasia, along with elevated TNF-α levels, indicated disease exacerbation. In contrast, claudin7, a tight junction protein, plays a protective role in IBD by maintaining epithelial barrier function.

Overall, the histology score remained low in healthy mice and increased significantly during colitis. This increase was absent in the ileum and progressively more pronounced in the three regions of the colon. On recovery, the histology score decreased, returning to levels comparable to healthy controls in most groups, except in the mid and distal colon of WT mice. Similarly, TNF-α levels remained low in the ileum but increased significantly in the colon during colitis, with a return to lower levels during recovery. Claudin7 levels were lower in the ileum than in the colon and tended to increase during recovery, with variable changes during colitis depending on the segment.

In the ileum, no significant changes were observed in the histology score, TNF-α, or claudin7 mRNA levels, regardless of colitis induction or the absence of an adaptive immune system, suggesting a lack of local inflammation. In the proximal colon, a similar increase in the histology score was observed in both WT and *Rag1*$^{-/-}$ mice during colitis, followed by a reduction to healthy levels during recovery (Figure 1E). TNF-α levels mirrored this pattern, with an increase during colitis and a reduction during recovery. However, TNF-α levels in *Rag1*$^{-/-}$ mice during colitis were significantly lower than in WT mice, indicating milder inflammation in the absence of an adaptive immune system (Figure 1G). Claudin7 levels in the proximal colon decreased during colitis and increased during recovery in both WT and *Rag1*$^{-/-}$ mice (Figure 1F). However, the differences between healthy and colitis conditions were only statistically significant in *Rag1*$^{-/-}$ mice, and the increase during recovery was more pronounced in *Rag1*$^{-/-}$ mice compared with WT mice, suggesting enhanced epithelial barrier repair in the absence of adaptive immunity (Figure 1F). In the mid colon, the histology score and

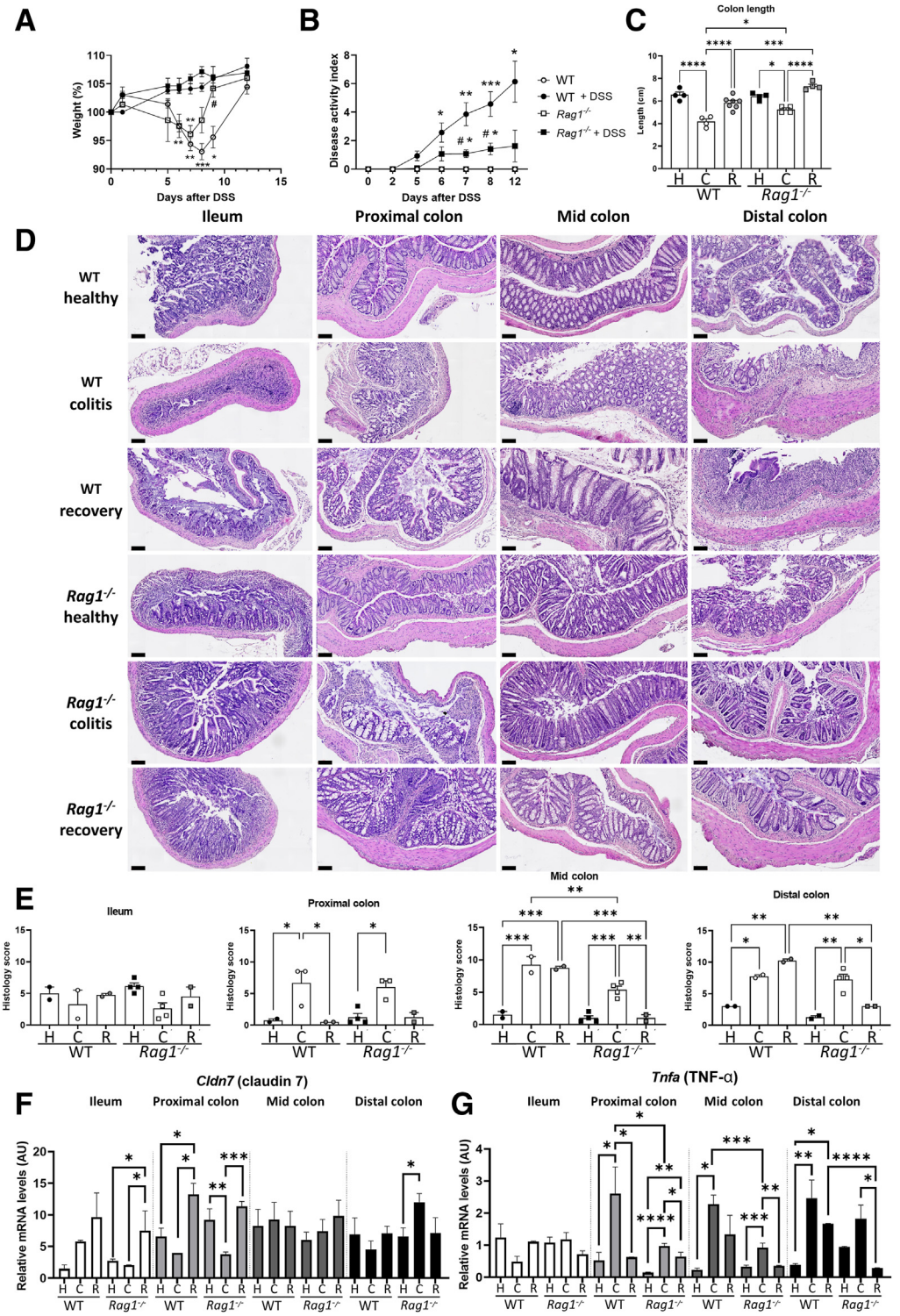

Figure 1 Dextran sulfate sodium (DSS)—induced colitis in wild-type (WT) and *Rag1*$^{-/-}$ mice. **A:** WT and *Rag1*$^{-/-}$ mice were administered either normal water or 2% (w/v) DSS for 5 days, followed by water alone. Body weight and disease activity were monitored throughout the experiment, with day 0 marking the start of DSS treatment. Data were analyzed using two-way analysis of variance, followed by Tukey *post hoc* test. **B:** Disease activity index, represented as the sum of scores. Data were analyzed using two-way analysis of variance, followed by Tukey *post hoc* test. **C:** Colon length at the end point for the healthy (H; water only), colitis (C; 5 days of DSS followed by 3 days of water), and recovery (R; 5 days of DSS followed by 7 days of water) groups. Data were analyzed using one-way analysis of variance, followed by Tukey *post hoc* test. **D:** Representative histologic images (hematoxylin and eosin staining) from the four intestinal segments. **E:** Histologic damage scores for the four intestinal segments. Data were analyzed using one-way analysis of variance, followed by Tukey *post hoc* test. **F** and **G:** mRNA expression of *Cldn7* (claudin 7) and *Tnfa* [tumor necrosis factor-α (TNF-α)], separated by intestinal segment. Data were analyzed using one-way analysis of variance, followed by Tukey *post hoc* test. $n = 4$ to 7 (**A**—**C**); $n = 2$ to 4 (**E**—**G**). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, and $^{****}P < 0.0001$ within the same genotype; $^{\#}P < 0.05$ compared with the same treatment group. Scale bars = 100 μm (**D**). AU, arbitrary unit.

TNF-α levels were lower in *Rag1*$^{-/-}$ mice during both colitis and recovery (not significant) compared with WT mice, with a marked decrease to healthy levels in *Rag1*$^{-/-}$ mice during recovery, which was only partially observed in WT mice. Similarly, in the distal colon, *Rag1*$^{-/-}$ mice showed lower histology scores and reduced TNF-α levels during recovery compared with WT mice. Once again, recovery in *Rag1*$^{-/-}$ mice led to a reduction in the histology score and TNF-α levels to values comparable to healthy controls, which was not fully achieved in WT mice. In both the mid and distal colon, no significant differences in claudin7 levels were observed due to colitis or the absence of adaptive immunity, except for a significant increase in claudin7 during colitis in *Rag1*$^{-/-}$ mice, which was not observed in WT mice, probably due to early recovery.

In summary, no evidence of local damage or inflammation was found in the ileum due to colitis or the absence of adaptive immunity. In contrast, local damage and inflammation of varying degrees across the different segments of the colon was observed, with global reductions in inflammation and damage observed in the absence of adaptive immunity. These findings suggest that DSS treatment induced colitis in mice, with the notable difference that *Rag1*$^{-/-}$ mice developed a milder pathology and recovered more effectively, indicating a role for the adaptive immune system in the pathology. The absence of mature adaptive lymphocytes led to a less severe phenotype. Importantly, these effects occurred in a segment-specific manner, indicating that the impact of adaptive immunity on inflammation and tissue damage varies across different regions of the intestine.

Ileal Spontaneous Contractions Are Influenced by the Presence of Mature Adaptive Lymphocytes in Acute Colitis

In the organ bath experiments, spontaneous contractions were assessed by measuring their maximal contraction and frequency (Figure 2A). Two states of colitis were studied: acute colitis, characterized by a period of weight loss during which mice were examined at their minimum weight by day 8; and recovery, when mice completed the process of weight regain, reaching either the same percentage as healthy groups or at least 100% of the weight from day 0.

In healthy conditions, no differences in maximal contractions were detected between WT and *Rag1*$^{-/-}$ mice. In WT ileum, no disparities were observed among the healthy, colitis, and recovery groups. However, in *Rag1*$^{-/-}$ mice, the colitis group exhibited lower maximal spontaneous contractions compared with healthy and recovery ($P = 0.0868$ versus colitis) *Rag1*$^{-/-}$ ileum (Figure 2C). Regarding the frequency of contractions, there were no differences between genotypes in healthy conditions (Figure 2D). In the WT ileum, acute colitis increased the spontaneous contraction frequency, with this effect returning to similar levels as the healthy conditions in the recovery WT group. Conversely, in *Rag1*$^{-/-}$ ileums, acute colitis resulted in the opposite trend, with a lower frequency of spontaneous contractions observed in colitis *Rag1*$^{-/-}$ samples when compared with healthy *Rag1*$^{-/-}$ mice. Additionally, incomplete recovery of the frequency of spontaneous contractions was observed in the *Rag1*$^{-/-}$ recovery group ($P = 0.0774$ versus healthy).

Maximum spontaneous contractions and their frequency are induced by acute colitis and reduced in the absence of mature B and T lymphocytes, giving the primary distinction between genotypes during the acute colitis phase (Figure 2).

The Contractile Response of the Ileum to Stimuli Is Influenced by Colitis and the Presence of Mature Adaptive Lymphocytes

Ileum contractions in response to stimulus were examined by studying the maximal response to a depolarizing potassium solution (Figure 2B), and the contractile response to the muscarinic agonists carbachol and ACh, which were studied using dose-response curves (Figure 2F).

In healthy conditions, no significant differences were observed between WT and *Rag1*$^{-/-}$ mice in response to KCl. In WT mice (Figure 2E), there were no significant differences in the contractile capacity between healthy individuals and different stages of colitis disease. However, in *Rag1*$^{-/-}$ mice, ileums from those with active colitis displayed a tendency ($P = 0.0888$ versus healthy) to decrease in their maximal contractile capacity, which returned to similar levels as healthy *Rag1*$^{-/-}$ samples in the recovery group ($P = 0.0605$ recovery versus colitis).

Subsequently, the contractile response to muscarinic agonists, carbachol (Figure 2, G–I) and ACh (Figure 2, J–L), was studied. This experimental design allowed the authors to compare the response to the physiological agonist, ACh (including its action on muscarinic receptors and its degradation by acetylcholinesterase), with that of carbachol, an analog of ACh that exhibits resistance to degradation by acetylcholinesterase.

In the WT ileum under healthy conditions, the AUC was larger for carbachol than for ACh, indicating active acetylcholinesterase in ileal preparations under these experimental conditions. This effect was also observed in healthy *Rag1*$^{-/-}$ samples (Figure 2, H and K). Moreover, carbachol and ACh intensity of responses did not differ between genotypes under healthy conditions. Colitis did not significantly affect responses to both agonists in WT mice, whereas *Rag1*$^{-/-}$ presented a reduced response to ACh due to colitis ($P = 0.0976$ versus healthy). A significant reduction in AUC of both agonists was observed in WT ileum on recovery when compared with healthy samples, and the effect was abolished in *Rag1*$^{-/-}$ ileum (Figure 2, H and K).

Regarding the PD2 (negative logarithm of the EC_{50}), in healthy conditions, no significant differences were found in carbachol responses between WT and *Rag1*$^{-/-}$ mice. However, *Rag1*$^{-/-}$ mice showed a lower PD2 value to ACh

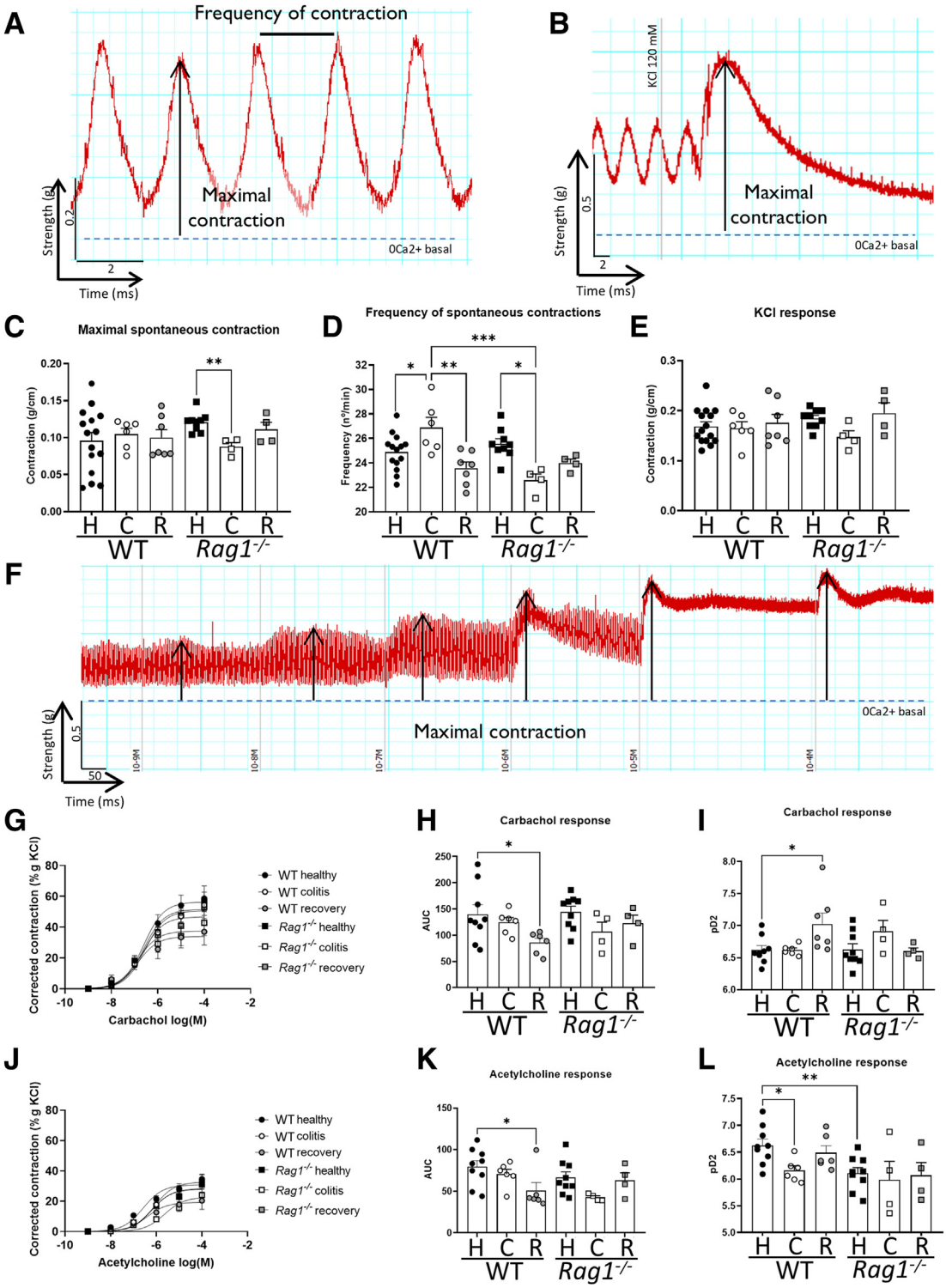

Figure 2 Analysis of ileum motility in wild-type (WT) and *Rag1*$^{-/-}$ mice across three conditions: healthy (H), acute colitis (C), and recovery (R). **A:** Example showing how maximal contraction and the frequency of spontaneous contractions are measured from the recorded data. Maximal contraction is indicated by the **vertical arrow** extending from the baseline, marked by the 0 Ca^{2+} solution, to the peak of the recorded contraction, whereas frequency is defined as the interval between the peak of one contraction and the peak of the next. **B:** Representative example of the characteristic contraction pattern in response to KCl stimulation, with the method for measuring maximal contraction outlined, as described in **A**. **C**–**E:** Results for maximal spontaneous contractions (**C**), frequency of spontaneous contractions (**D**), and maximal contraction in response to KCl (**E**). **F:** Example showing contraction curves in response to muscarinic agonists [carbachol and acetylcholine (ACh)] at increasing concentrations, with an indication of how maximal contraction (indicated by **vertical arrows**) is measured for each concentration, as described in **A**. **G**–**L:** Results for carbachol (**G**–**I**) and ACh (**J**–**L**), represented as contraction curves (**G** and **J**), area under the curve (AUC; **H** and **K**), and agonist potency as PD2 (negative logarithm of the EC_{50}; **I** and **L**). Data were analyzed using one-way analysis of variance, followed by Tukey *post hoc* test. $n = 4$ to 15 (**C**–**E** and **G**–**L**). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

compared with WT, suggesting a lower sensitivity of muscarinic receptors (or more active acetylcholinesterase) in mice lacking an acquired immune system. In WT mice with colitis, PD2 values for ACh, but not for carbachol, were also significantly lower compared with healthy conditions, becoming normal on recovery. This was not observed in *Rag1*$^{-/-}$ mice, probably because they already had a lower basal sensitivity to ACh (or more active basal acetylcholinesterase). However, in mice with colitis, the PD2 value to carbachol tended to be higher in *Rag1*$^{-/-}$ mice compared to WT ($P = 0.0688$), suggesting increased muscarinic receptor sensitivity in mice lacking the adaptive immune system, and in the WT recovery group in comparison with healthy and colitis ($P = 0.0567$).

In summary, in the ileum, adaptive immunity did not affect the maximal contractile response to stimuli but enhanced sensitivity to ACh. Colitis also reduced sensitivity to ACh but only in WT mice; and recovery reduced the maximal response while it increased sensitivity to muscarinic agonists.

Colon Intestinal Motility Exhibits Geographic Heterogeneity

Furthermore, the response in the organ bath system of the mouse colon was investigated. To perform a comprehensive study, considering the diverse physiological roles of the different segments of the colon, the entire colon was divided into four parts based on their distance to the cecum: proximal, two middle sections, and distal. A pattern of large spontaneous contractions like those found in the ileum was observed in the colon segments. Additionally, small contractions within these large contractions were detected, and the frequency of both was measured (Figure 3).

Distinct patterns were observed in the segments from the colon of WT mice (Figure 4). Although the distal colon exhibited a greater contractile response of spontaneous contractions compared with the mid and proximal sections (Figure 4A), the proximal colon presented the highest frequency of these spontaneous contractions, particularly small contractions (Figure 4, B and C). Moreover, the distal segment was also characterized by a more substantial contractile response to KCl and carbachol, but not ACh, compared with mid and proximal segments (Figure 4, D–F, H, and I). PD2 to ACh, but not carbachol, is lower compared with the other segments (Figure 4, G and J), indicative of larger acetylcholinesterase activity. The mid colon segments showed an intermediate contractile response, similar to the proximal segment but with lower spontaneous frequencies. Because of these variations, an analysis of differences between groups was conducted within each segment.

In summary, maximal spontaneous contraction and response to stimuli increased from the ileum to the distal colon, whereas the frequency of spontaneous contractions was reduced, associated with the different physiological functions during digestion.

The Absence of Mature Adaptive Lymphocytes Increases Contractile Response in the Proximal Colon, whereas Colitis Reduces It

In the proximal segment of the colon, closest to the ileum, two phenomena were primarily observed. Under healthy conditions, *Rag1*$^{-/-}$ mice exhibited higher maximum and frequency in spontaneous contractions (Figure 5, A and B), as well as in response to KCl ($P = 0.0813$), compared with WT (Figure 5D), with no differences in the AUC to carbachol or ACh between genotypes (Figure 5, E, F, H, and I). However, ACh PD2 was lower in *Rag1*$^{-/-}$ mice compared with WT, suggesting that WT animals have higher sensitivity to ACh in their proximal colon (Figure 5J). Because this phenomenon did not occur with carbachol (Figure 5G), it may be due to differences in acetylcholinesterase activity, which may be increased in *Rag1*$^{-/-}$ mice.

When assessing the effects of colitis on the spontaneous maximal contraction of the colon (Figure 5A), colitis WT mice did not exhibit a significant difference compared with healthy WT mice. However, colitis reduced spontaneous maximal contraction in colitis *Rag1*$^{-/-}$ mice compared with healthy *Rag1*$^{-/-}$ ($P = 0.0916$ healthy versus colitis, and $P = 0.0603$ versus recovery) mice to similar levels as in colitis WT mice. Regarding the KCl response (Figure 5D), a similar trend was observed, with a tendency or a significant reduction in KCl response in colitis WT ($P = 0.0884$) and colitis *Rag1*$^{-/-}$ mice compared with their respective healthy groups. Carbachol and ACh responses were not affected by colitis in both WT and *Rag1*$^{-/-}$ mice.

On recovery from colitis, no significant differences were observed in the recovery groups compared with colitis groups in either WT or *Rag1*$^{-/-}$ samples in all the quantified parameters more than increased sensitivity to ACh in *Rag1*$^{-/-}$ mice not observed in WT (Figure 5).

Under healthy conditions, the frequency of large contractions was higher in *Rag1*$^{-/-}$ compared with WT. However, in mice with colitis, no differences were found between genotypes and when comparing colitis with healthy conditions. There were also no differences observed in the frequency of spontaneous small contractions between genotypes and between healthy and colitis conditions (Figure 5C). On recovery, a significant increase in large contractions was found in WT mice, an effect that was not observed in *Rag1*$^{-/-}$.

In summary, in the proximal colon, under healthy conditions, the adaptive immune system appeared to negatively modulate frequency and contractile response, and thus the degree of excitability. Colitis in this region did not significantly alter the frequency of contractions but did reduce contractile capacity, whereas recovery may have increased the frequency of spontaneous contractions. The adaptive immune system did not seem to play a role under colitis conditions.

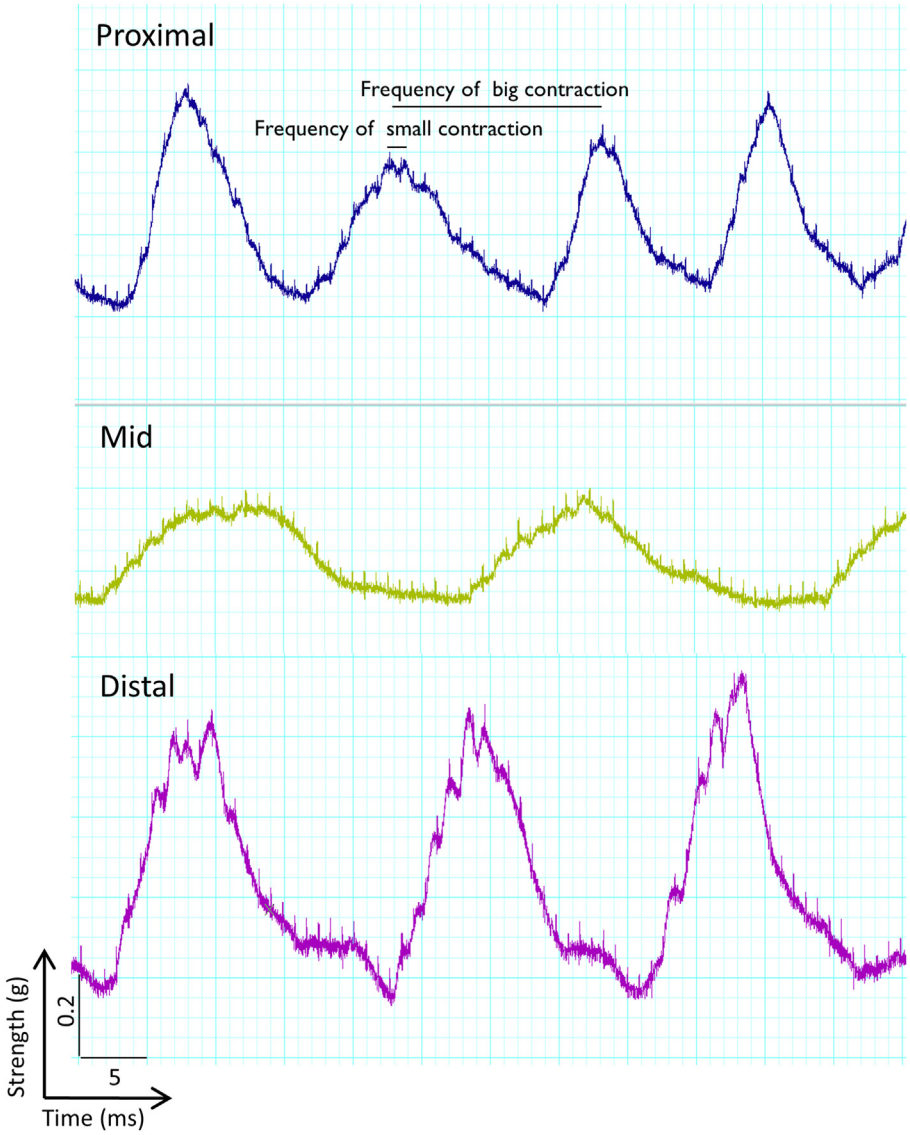

Figure 3 Example of organ bath system recordings for proximal, mid, and distal colon segments of a wild-type mouse. This figure illustrates how the frequency of large and small contractions is measured from the recorded data in the organ bath system.

The Frequency of Spontaneous Contractions and the Contractile Response of Mid Colon Are Affected by the Absence of Mature Adaptive Lymphocytes During Colitis but Not in a Healthy State

The mid colon was defined as comprising the two middle segments of the four that were dissected. In healthy animals, there were no significant differences due to genotype in spontaneous contraction maximum or frequency (Figure 6, A–C), nor in the maximal response to KCl or muscarinic agonists (Figure 6, D–F, H, and I). The only difference was a tendency of less sensitivity to ACh in *Rag1*$^{-/-}$ healthy mice ($P = 0.0600$) (Figure 6J), as observed in the ileum and proximal colon.

In WT animals, no changes due to colitis were observed in any parameter, except for a tendency for less contractile response to KCl of the colitis group compared with recovery ($P = 0.0503$ colitis versus recovery). However, *Rag1*$^{-/-}$ animals in colitis state, compared with WT colitis, exhibited higher contractile capacity in both spontaneous contractions ($P = 0.0677$) and in response to KCl ($P = 0.0747$) and carbachol (Figure 6, A, D, and F), and higher sensitivity to carbachol (Figure 6G).

Distinct differences emerged in the frequency of spontaneous contractions. In WT mice, no differences were observed between healthy, colitis, and recovery samples. However, in *Rag1*$^{-/-}$ mice, the frequency of large contractions was higher in the recovery group (Figure 6B), whereas the frequency of small contractions was higher in the colitis group (Figure 6C).

In summary, in the mid colon, the absence of the adaptive immune system in healthy conditions enhanced the sensitivity to ACh, whereas colitis had no notable impact on the frequency of contractions or contractile capacity. However,

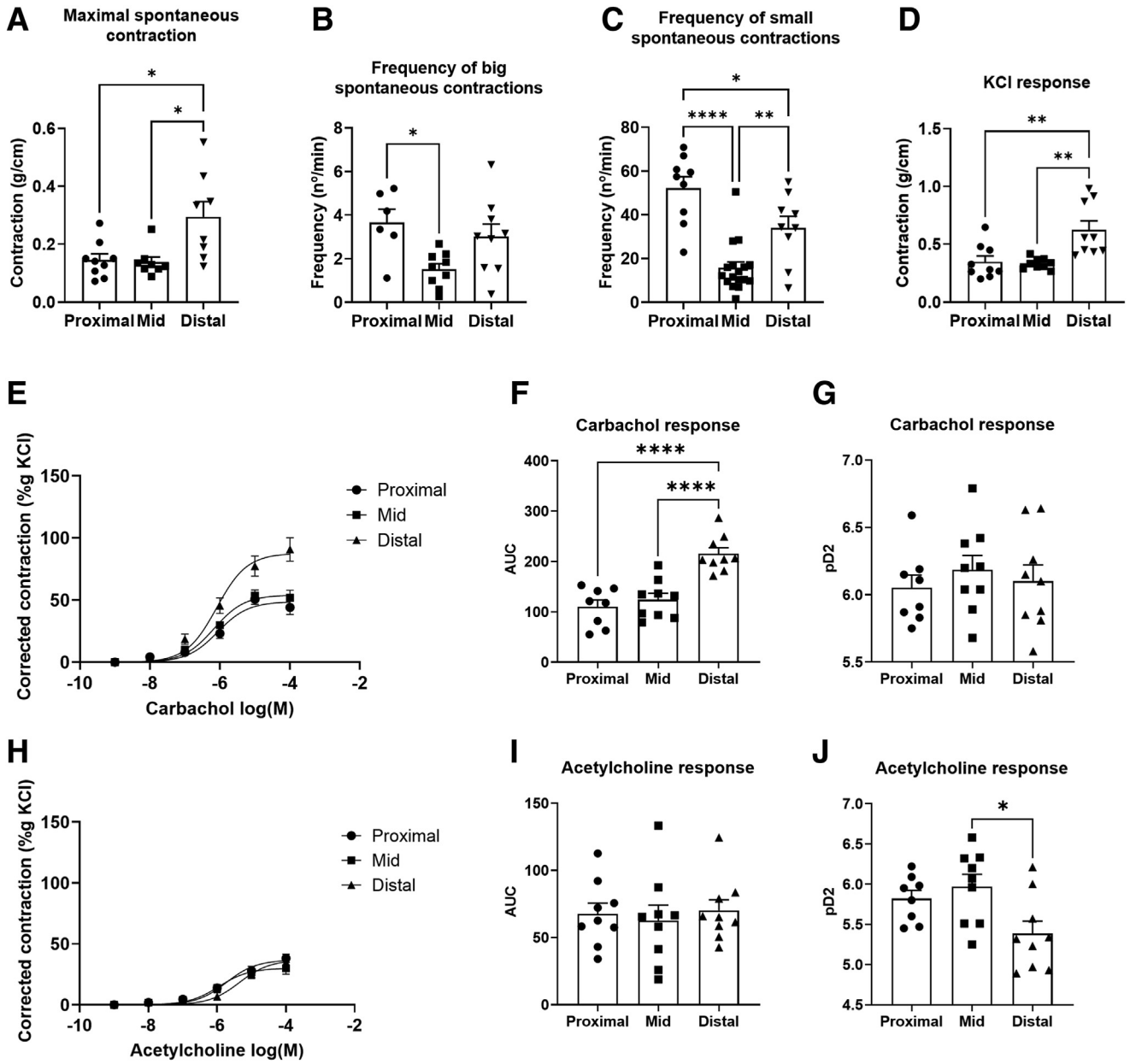

Figure 4 Comparison of colon segment behavior (proximal, mid, and distal) in wild-type mice, evaluated in the organ bath system. **A:** Maximum spontaneous contractions. **B:** Frequency of large spontaneous contractions. **C:** Frequency of small spontaneous contractions. **D:** Maximum contractile response to KCl. **E**–**J:** Contraction response to increasing concentrations of muscarinic agonists carbachol (**E**–**G**) and acetylcholine (**H**–**J**), represented as contraction curves (**E** and **H**), area under the curve (AUC; **F** and **I**), and agonist potency as PD2 (negative logarithm of the EC_{50}; **G** and **J**). Data were analyzed using one-way analysis of variance, followed by Tukey *post hoc* test. $n = 9$ (**A**–**J**). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{****}P < 0.0001$.

under an inflammatory state of colitis, the presence of mature B and T lymphocytes appeared to negatively modulate the frequency and maximum of spontaneous and KCl contractions, thus affecting the excitability degree of SMC and/or ICC, and decreasing SMC sensitivity to muscarinic agonists.

Distal Colon Contraction Is Influenced by Both Colitis and the Presence of Mature Adaptive Lymphocytes

In the distal segment of the colon, in healthy animals, no differences were observed in the maximum or frequency of spontaneous contractions between $Rag1^{-/-}$ and WT mice (Figure 7, A–C). However, KCl contraction was significantly lower in $Rag1^{-/-}$ without any change in the response to muscarinic agonists (Figure 7, D, F, and I).

In WT animals, colitis induced a decrease in contractile capacity, as seen in both spontaneous maximal contractions (Figure 7A) and responses to KCl (Figure 7D) compared with healthy WT. Interestingly, the colitis WT group exhibited a heightened contractile response to ACh (Figure 7 H–J), whereas the response to carbachol remained unchanged by colitis (Figure 7, E–G). Conversely, $Rag1^{-/-}$

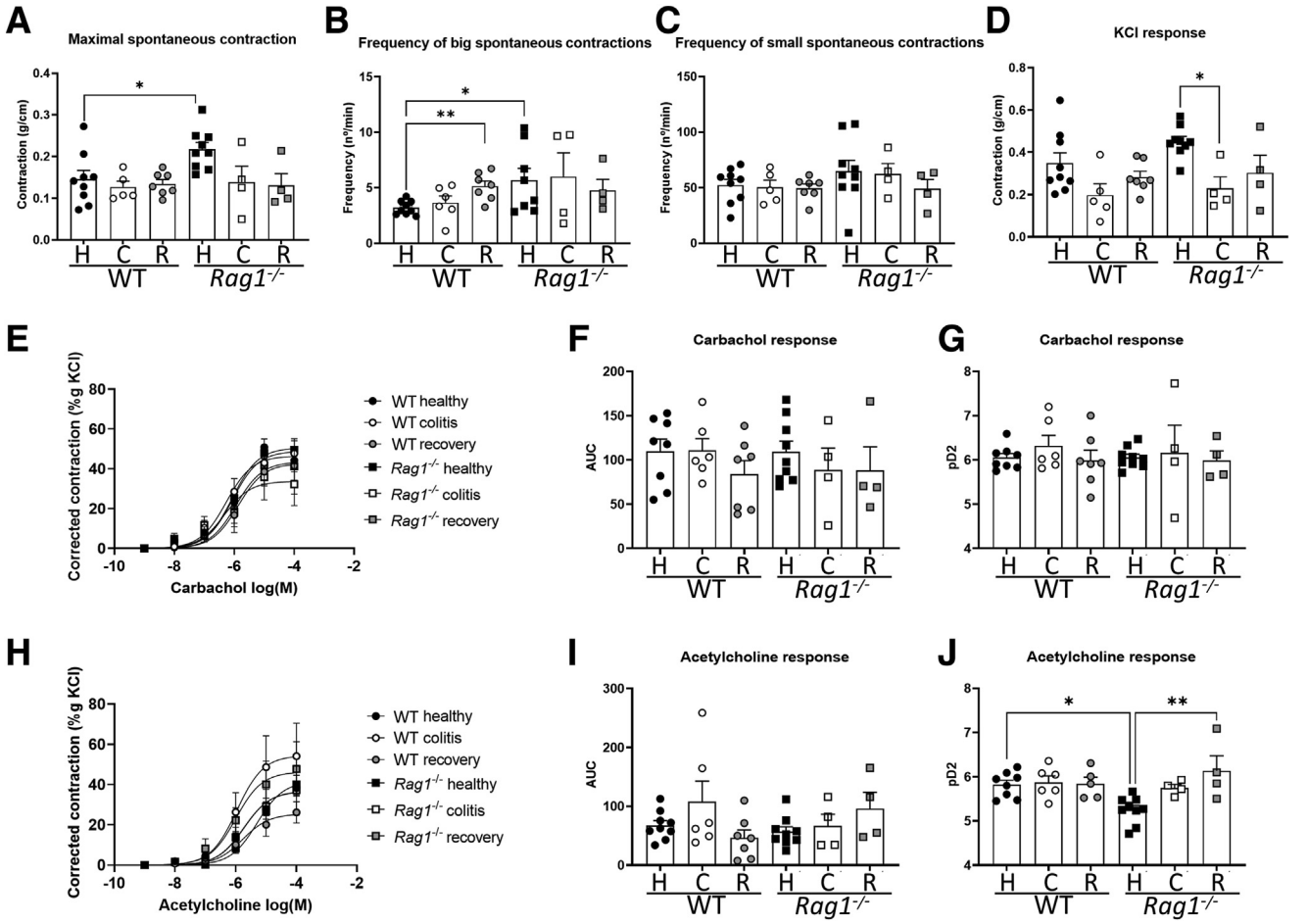

Figure 5 Analysis of proximal colon motility in wild-type (WT) and *Rag1*$^{-/-}$ mice across three conditions: healthy (H), acute colitis (C), and recovery (R). **A:** Maximum spontaneous contractions. **B:** Frequency of large spontaneous contractions. **C:** Frequency of small spontaneous contractions. **D:** Maximum contractile response to KCl. **E**–**J:** Contraction response to increasing concentrations of muscarinic agonists carbachol (**E**–**G**) and acetylcholine (**H**–**J**), represented as contraction curves (**E** and **H**), area under the curve (AUC; **F** and **I**), and agonist potency as PD2 (negative logarithm of the EC_{50}; **G** and **J**). Data were analyzed using one-way analysis of variance, followed by Tukey *post hoc* test. $n = 4$ to 9 (**A**–**J**). $^{*}P < 0.05$, $^{**}P < 0.01$.

mice showed the opposite behavior, with a trend toward increased contractile capacity in the colitis group and a significant increase in the recovery group in spontaneous contractions and in response to KCl (Figure 7, A and D), and a decreased response to carbachol that is maintained during recovery (Figure 7F), presenting a reduced response to both muscarinic agonists in colitis compared with WT colitis.

Regarding the frequency of spontaneous contractions, no changes were observed in either large or small contractions among groups (Figure 7, B and C).

In summary, in the distal colon under healthy conditions, the adaptive immune system enhanced KCl response without modification of muscarinic contractions, suggesting a positive modulation of SMC excitability. Under colitis conditions, the presence of mature adaptive lymphocytes enhanced ACh and carbachol responses, suggesting, that under an inflammatory environment, they increase the response of muscarinic receptors, which may contribute to enhanced motility.

Altered Expression of Acetylcholinesterase and Muscarinic Receptors in Response to Colitis and the Absence of Adaptive Immune Cells

To determine whether the observed changes in response to muscarinic agonists were due to alterations in the expression of their receptors or acetylcholinesterase, mRNA levels were analyzed by quantitative PCR in the four intestinal segments studied.

In healthy animals, no significant differences in the mRNA levels of acetylcholinesterase or the M3 muscarinic receptor were observed when comparing the ileum with various segments of the colon (Figure 8, A and C). However, under homeostatic conditions, the ileum exhibited significantly higher levels of M2 receptor mRNA compared with any colon segment, in both genotypes (Figure 8B).

The absence of lymphocytes did not affect acetylcholinesterase or M2 and M3 receptor levels in any segment under

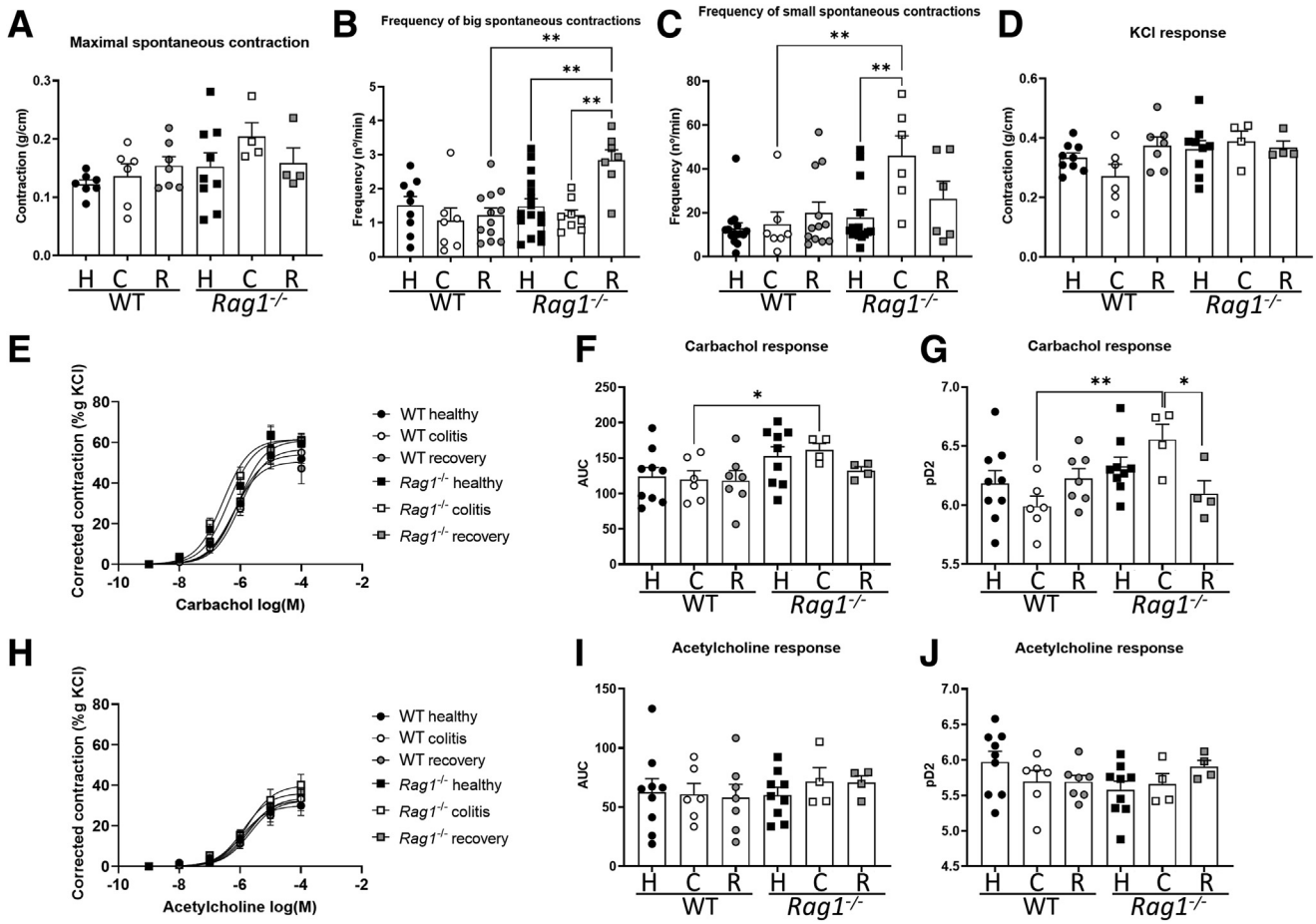

Figure 6 Analysis of mid colon motility in wild-type (WT) and *Rag1*$^{-/-}$ mice across three conditions: healthy (H), acute colitis (C), and recovery (R). **A:** Maximum spontaneous contractions. **B:** Frequency of large spontaneous contractions. **C:** Frequency of small spontaneous contractions. **D:** Maximum contractile response to KCl. **E**–**J:** Contraction response to increasing concentrations of muscarinic agonists carbachol (**E**–**G**) and acetylcholine (**H**–**J**), represented as contraction curves (**E** and **H**), area under the curve (AUC; **F** and **I**), and agonist potency as PD2 (negative logarithm of the EC_{50}; **G** and **J**). Data were analyzed using one-way analysis of variance, followed by Tukey *post hoc* test. $n = 4$ to 18 (**A**–**J**). $^{*}P < 0.05$, $^{**}P < 0.01$.

healthy conditions (Figure 8, B and C). Colitis significantly increased acetylcholinesterase mRNA levels in the proximal colon, with no changes in the ileum, mid colon, or distal colon in WT mice. Additionally, colitis significantly elevated M3 receptor mRNA levels in the ileum and proximal colon in WT mice, but a decrease was observed in the mid colon ($P = 0.0867$ colitis versus recovery), with no significant changes in the distal colon. M3 receptor levels returned to baseline during recovery. A similar trend was noted for M2 receptor mRNA levels, with increased expression in the proximal and distal colon during colitis compared with healthy or recovered mice ($P = 0.0994$ colitis versus recovery), and reduced expression in the mid colon (Figure 8B). In *Rag1*$^{-/-}$ mice, no significant differences were found in acetylcholinesterase or M3 receptor levels in any intestinal segment when comparing the homeostatic state with colitis. However, their expression increased during recovery in the proximal ($P = 0.0887$ healthy versus recovery) and distal colon (Figure 8, A and C). Conversely, colitis led to a reduction in M2 receptor levels in the ileum (not significant) and proximal colon (Figure 8B).

When comparing WT and *Rag1*$^{-/-}$ mice during colitis, WT mice showed significantly higher levels of acetylcholinesterase, M2, and M3 receptors in the ileum and proximal colon, with no changes in the mid or distal colon (Figure 8).

In summary, acetylcholinesterase and muscarinic M3 receptor levels significantly increased in the proximal colon and ileum during colitis in WT mice, whereas M2 receptor levels also increased in the proximal and distal colon, although less prominently. In contrast, *Rag1*$^{-/-}$ mice had reduced levels in acetylcholinesterase and M3 in the proximal and distal colon during colitis compared with recovery, and reduced levels of M2 receptors in the ileum and proximal colon. When comparing WT and *Rag1*$^{-/-}$ mice with colitis, *Rag1*$^{-/-}$ mice exhibited lower levels of acetylcholinesterase, M2, and M3 receptors levels in the ileum and proximal colon, highlighting the influence of adaptive immune cells in modulating these responses during inflammation.

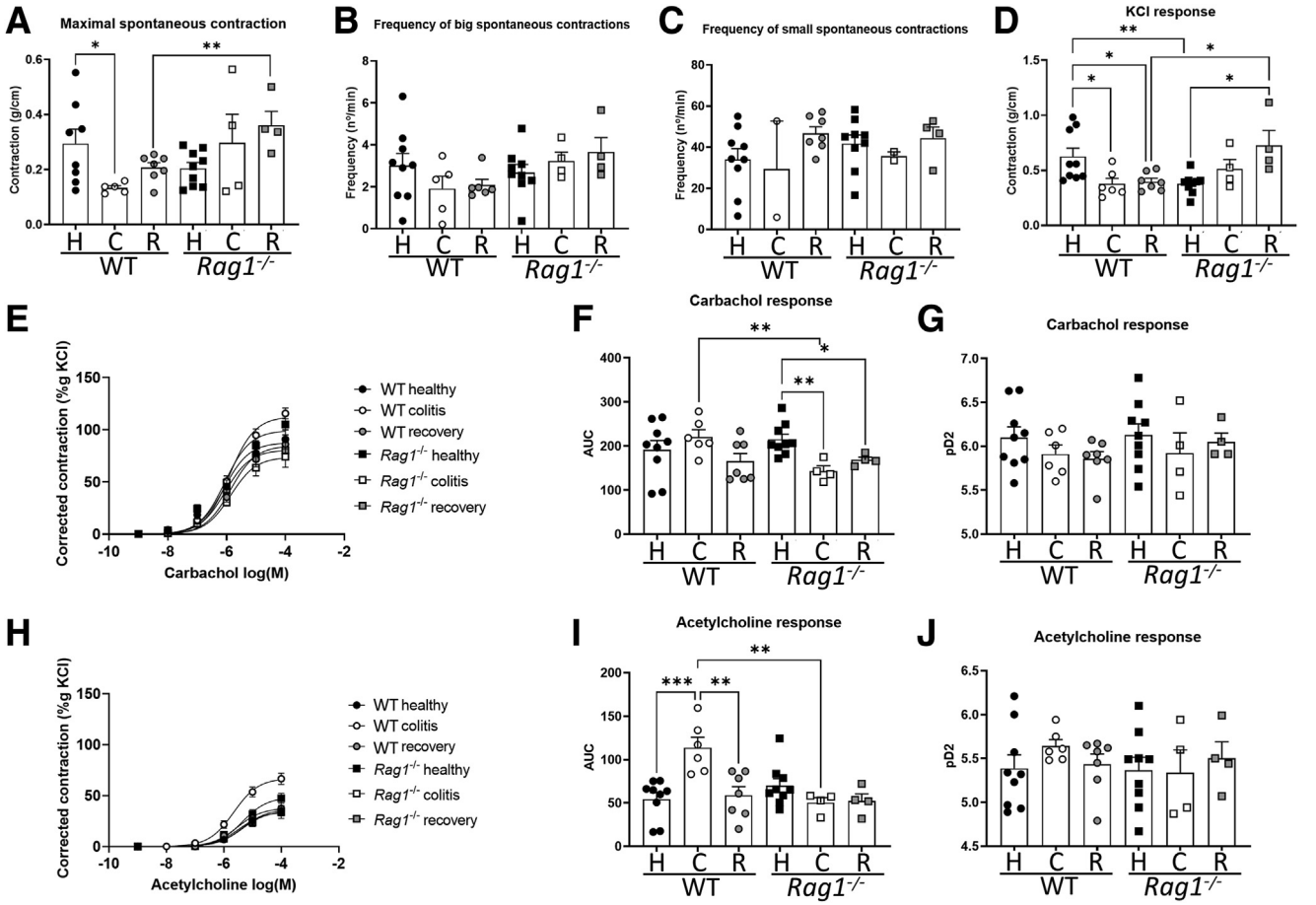

Figure 7 Analysis of distal colon motility in wild-type (WT) and *Rag1*$^{-/-}$ mice across three conditions: healthy (H), acute colitis (C), and recovery (R). **A:** Maximum spontaneous contractions. **B:** Frequency of large spontaneous contractions. **C:** Frequency of small spontaneous contractions. **D:** Maximum contractile response to KCl. **E**–**J:** Contraction response to increasing concentrations of muscarinic agonists carbachol (**E**–**G**) and acetylcholine (**H**–**J**), represented as contraction curves (**E** and **H**), area under the curve (AUC; **F** and **I**), and agonist potency as PD2 (negative logarithm of the EC_{50}; **G** and **J**). Data were analyzed using one-way analysis of variance, followed by Tukey *post hoc* test. $n = 4$ to 9 (**A**–**J**). $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$.

Discussion

Although IBD is primarily viewed as an innate immune-mediated inflammation, T[6–8] and B[20,21] cells play a critical role in its pathogenesis. Therefore, in this study, the impact of adaptive immune cells and inflammation on the characteristics of ileal and colonic motility was investigated using *Rag1*$^{-/-}$ mice lacking mature adaptive immune cells (T and B cells).[15] The findings in this article suggest that the adaptive immune system regulates intestinal motility in both healthy conditions and during colitis.

Intestinal motility is regulated mainly by slow waves produced by ICCs, muscle distension, and neurotransmitters, such as ACh, coming from myenteric plexus.[22] Muscle distension and neurotransmitters depolarize the SMC and make them more excitable.[22] Different aspects of motility were evaluated, including the frequency of spontaneous contractions, which depend on ICC activity, contractility to KCl, which is regulated by the depolarizing state of the SMC, and the responses to ACh and carbachol. ACh acts through M2 and M3 muscarinic receptors, and its bioavailability is regulated by acetylcholinesterase, an enzyme responsible for ACh degradation.[12,22] The study of both agonists and the levels of acetylcholinesterase and M2 and M3 muscarinic receptors allowed us to examine possible modifications in the function of muscarinic receptor sensitivity and acetylcholinesterase activity.

Gastrointestinal motor functions depend on its two layers of smooth muscle: circular and longitudinal, which function in a coordinated manner. Although previous studies have mainly focused on circular smooth muscle contractions,[10–12] this system allows for the investigation of longitudinal smooth muscle contractions due to the preparation's assembly. Different regions were analyzed, including the ileum and three regions of the colon (proximal, medium, and distal colon), with the mid colon being more difficult to precisely define. Each of these regions performs distinct physiological roles. The main function of the ileum is to absorb nutrients derived from the digestion of food that have not been absorbed in the previous regions of

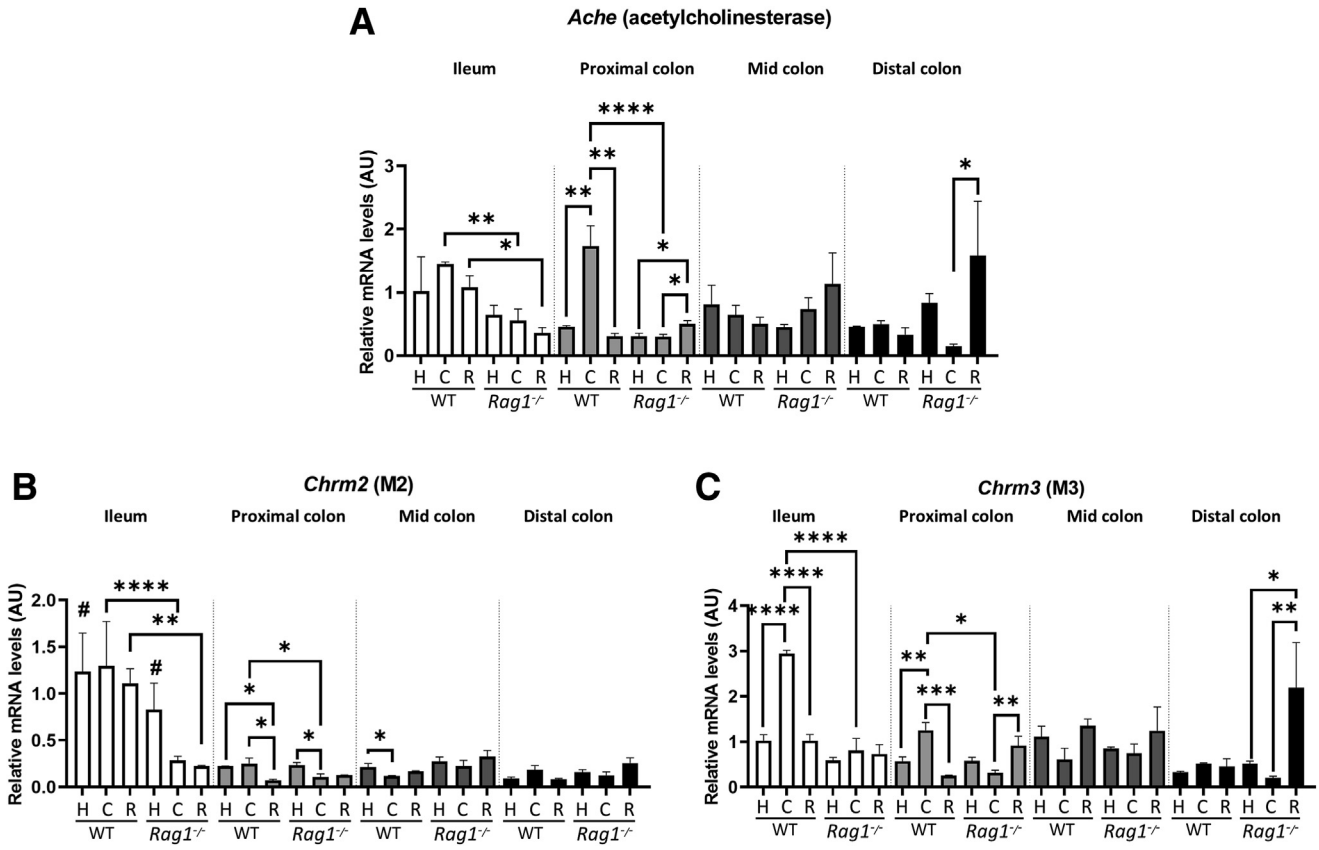

Figure 8 Analysis of mRNA expression of acetylcholinesterase (*Ache;* **A**) and muscarinic receptors M2 (*Chrm2;* **B**) and M3 (*Chrm3;* **C**) in the ileum, proximal, mid, and distal colon of wild-type (WT) and *Rag1*$^{-/-}$ mice across three conditions: healthy (H), acute colitis (C), and recovery (R). Data were analyzed using one-way analysis of variance, followed by Tukey *post hoc* test. $n = 2$ to 4 (**A**–**C**). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$ compared with different groups within the same segment; #$P < 0.05$ between the same group in different segments. AU, arbitrary unit.

the small intestine, whereas the colon has two main functions: absorbing water, electrolytes, and metabolites derived from the microbiota (mainly proximal colon), and storing fecal matter until its expulsion (distal colon).[22]

In healthy mice under physiological conditions, mature adaptive immune cells help to maintain a low contractile response and frequency in the proximal colon, where water, electrolytes, and nutrients derived from the microbiota are primarily absorbed.[22] This allows for sufficient time for the absorption process.[23,24] However, in an inflammatory environment, such as acute colitis, significant changes in contractile response are observed. In colitis, mature adaptive immune cells promote a high frequency of spontaneous contractions in the ileum and proximal colon, an effect that is reverted in the mid and distal colon. Additionally, the strength of spontaneous contractions in the ileum is enhanced by the presence of these cells during colitis. However, in the colon, the inflammation coupled with adaptive immune activity reduces the contractile capacity of colon walls, particularly in the mid and distal colon. A possible explanation for our observations is that the DSS model induces damage specifically in the colon, with minimal impact on the small intestine,[25–27] a finding that was corroborated by our experiments. This damage appears to be more pronounced in WT compared with *Rag1*$^{-/-}$ animals, which exhibit milder colitis. Consequently, the increased frequency and contractility of the ileum may be attributed to a compensatory mechanism in the relatively healthy or less damaged tissue, which attempts to eliminate the damaging agent (DSS). This compensatory response, however, does not function effectively in the more severely damaged segments of the colon.

Other findings in this article suggest that inflammation and the presence of mature adaptive immune cells can alter the response to muscarinic agonists, whether through modulating the expression or activity of muscarinic receptors or acetylcholinesterase. In the ileum, proximal, and mid colon, the data suggested that the acquired immune system in healthy conditions participates in the contractile effect of ACh increasing PD2 values. These data indicate that mature adaptive lymphocytes either increase muscarinic receptor sensitivity or increase ACh bioavailability by reducing acetylcholinesterase activity. Because the effect was not observed with carbachol, it can be suggested that, under healthy conditions, the adaptive immune system may reduce acetylcholinesterase activity and, thus, prolong the action of ACh, rather than affecting the receptors themselves. The measurement of M2 and M3 receptors, as well

as acetylcholinesterase mRNA in the ileum, proximal colon, and mid colon, did not reveal any significant differences that could clarify this possibility. This may be due to the absence of differences at the mRNA level, or it may suggest that variations exist at the protein level or in functional activity. It is also possible that adaptive lymphocytes contribute to ACh production, as T and B cells can produce ACh, influencing hematopoiesis, immunity, and vascular biology.[28,29]

On the other hand, in the ileum, inflammation associated with DSS treatment led to a reduction in PD2 values in WT mice compared with healthy controls in response to ACh. Similarly, in the proximal colon, the overall contractile capacity of SMCs was diminished by colitis. These findings suggest that under noxious stimuli, the adaptive immune system or associated inflammation reduces the sensitivity of muscarinic receptors.

Analysis of M2 and M3 receptor mRNA, along with acetylcholinesterase mRNA, in the ileum and proximal colon of DSS-treated mice revealed a significant increase in their expression in WT mice compared with *Rag1*$^{-/-}$ mice. This supports decreased PD2 values in ileum. The presence of adaptive immune cells and increased acetylcholinesterase expression could reduce the bioavailability of ACh, thereby diminishing the muscarinic response during colitis. The upregulation of muscarinic receptor expression may represent a compensatory mechanism resulting in the absence of differences in PD2 values in proximal colon. The increased expression of acetylcholinesterase and M2 muscarinic receptor persists through recovery in ileum of WT mice, potentially explaining the increased PD2 values, although AUC is reduced.

Last, in the distal colon, adaptive immunity in colitis reduced spontaneous contractions and KCl-induced contraction through depolarization but increased the contractile response to muscarinic agonists without affecting the PD2, which again suggests a reduction in the activity of acetylcholinesterase or changes in the number of muscarinic receptors. The measurement of M2 and M3 receptors and acetylcholinesterase mRNA in the distal colon did not reveal any significant differences that could clarify this possibility. However, in recovery, the absence of mature adaptive immunity impedes the recovery of normal muscarinic response, which can be explained by an increase in acetylcholinesterase expression that only appears in *Rag1*$^{-/-}$ mice.

Several studies indicate that high levels of ACh act as an anti-inflammatory substance in colitis, pointing to acetylcholinesterase inhibition as a possible treatment.[30,31] Here, it was shown that the adaptive immune system in healthy conditions is augmenting ACh bioavailability, probably reducing acetylcholinesterase activity without affecting its expression, from the ileum to mid colon, protecting from inflammation. In the context of intestinal inflammation, studies with different irritating agents have shown changes in muscarinic receptor activity in circular smooth muscle[32] and at least in longitudinal smooth muscle.[12,33,34] The experimental approach using an organ bath allows for a detailed examination of the contractile response of the longitudinal smooth muscle. The results suggest that colitis alters agonist-mediated contractions modulating the expression of acetylcholinesterase and M2 and M3 muscarinic receptors in a segment-dependent manner.

In the ileum and proximal colon, the presence of mature T and B lymphocytes reduces muscarinic response by enhancing ACh degradation through the stimulation of acetylcholinesterase activity. However, the increased muscarinic response observed in the distal colon cannot be explained by changes in acetylcholinesterase or muscarinic receptor expression, suggesting a potential role for inflammatory mediators in regulating their activity or sensitivity.

Changes in the strength of spontaneous contractions and the response to KCl can be explained by possible changes in the depolarization of the cells, as well as the frequency of spontaneous contractions, which also can be affected by the functioning of ICC. For example, in the proximal colon of healthy mice, the absence of an adaptive immune system increases spontaneous contractions and KCl contractions, with no effect on maximal muscarinic agonist—induced contractions, suggesting possible changes in the depolarization level of SMCs. Previous research has shown that inflammation has a significant impact on gastrointestinal motility patterns,[12,35] resulting in decreased excitability of SMCs.[35] This effect is particularly evident in the distal colon, as observed in this study.

This study reveals a distinct, segment-specific modulation of proinflammatory cytokines, particularly TNF-α, which closely correlates with changes in contractile responses within the same intestinal regions. It is suggested that variations in the cytokine microenvironment may explain the observed differences in intestinal contractile responses, as demonstrated in other experimental models of inflammation, including nematode infection, trinitrobenzene sulfonic acid (TNBS), surgical manipulation, experimental obstruction, hemorrhagic shock, or peritonitis.[12,34,36–43] Under homeostatic conditions, the tissue microenvironment is characterized by the presence of regulatory molecules, such as IL-10, IL-35, and transforming growth factor-β. In the acute phase of colitis, there is an increase in type 1 cytokines, followed by an increase in regulatory molecules during the recovery phase, although immune cell infiltration persists.[30,31] The correlation between proinflammatory TNF-α and contractility underlines the complex interplay between localized cytokine expression and tissue-specific physiological responses, providing deeper insights into the mechanisms regulating intestinal function during inflammation.

Conclusions

This study revealed alterations in intestinal motility during IBD, emphasizing the role of adaptive immunity. The organ bath system was valuable in segment-specific analyses, shedding light on distinct responses in the ileum and various

colon segments. Understanding these motility changes contributes to the broader understanding of IBD pathology and highlights the need for comprehensive investigations beyond inflammation. This study provides a foundation for future studies aiming to address the intricacies of gastrointestinal motility in health and disease.

Author Contributions

S.-M.A. and J.-M.G.-G. conceptualized the study; R.G.-B. and S.-M.A. curated, analyzed, validated, and visualized data; P.R.-R. and R.C.-G. curated and analyzed data; M.-J.F.-A., M.O.-Z., S.R., A.S., and J.-M.G.-G. analyzed data; R.G.-B., P.R.-R., R.C.-G., M.-J.F.-A., M.O.-Z., S.R., A.S., S.-M.A., and J.-M.G.-G. performed experiments; R.G.-B., P.R.-R., R.C.-G., M.O.-Z., S.R., A.S., and S.-M.A. developed methods; R.G.-B., S.-M.A., J.-M.G.-G. wrote the manuscript; R.G.-B., S.-M.A., J.-M.G.-G., P.R.-R., M.O.-Z., S.R., A.S., and A.C.-A. edited the manuscript; R.G.-B., S.-M.A., and J.-M.G.-G. provided software; A.C.-A., S.-M.A., and J.-M.G.-G. acquired funding and provided resources; A.C.-A., S.-M.A., and J.-M.G.-G. supervised the study; and S.-M.A., and J.-M.G.-G. administered the project. S.-M.A. and J.-M.G.-G. are the guarantors of this work and, as such, had full access to all of the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis.

Disclosure Statement

None declared.

Supplemental Data

Supplemental material for this article can be found at *http://doi.org/10.1016/j.ajpath.2024.10.020*.

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