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Original Article

Cholinergic regulation of macrophage polarization in ulcerative colitis: insights from a DSS-induced mice model

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Abstract: Objective: This study aimed to investigate the regulatory role of the cholinergic pathway on colonic macrophage polarization in ulcerative colitis. Methods: We selected 16 BALB/c mice, randomly divided into Model and Control groups. The Control group mice were fed normally, while the Model group mice were induced to develop colitis by drinking a 3.5% dextran sulfate sodium (DSS) solution for 7 days, followed by a return to normal drinking water for 7 days. During the experiment, we recorded the mice's body weight daily and assessed disease activity index (DAI) scores based on stool consistency, presence of blood in feces, and weight loss. The experiment concluded on day 14, with mice sacrificed to obtain colon samples for analysis. Results: During the DSS treatment period, Model group mice showed significant weight loss and developed soft stools and blood in feces. DAI scores started rising from day 5, peaking on day 8, with subsequent slight weight recovery and reduction in fecal blood. Analysis of colon samples on day 14 indicated that the colon length of mice in the Model group was significantly shorter than that of the Control group, with a markedly higher colon swelling rate (ratio of colon weight to length). Immunohistochemistry results revealed an increase in total macrophage count in the colons of Model group mice, with a higher count of pro-inflammatory M1 macrophages and a reduced count of anti-inflammatory M2 macrophages. Additionally, the expression of barrier proteins Occludin and ZO-1 was reduced in the colons of mice in the Model group. HE staining further demonstrated severe mucosal damage in the colons of the Model group mice. The study also found abnormal spleen enlargement in the Model group mice, associated with the development of colitis. Examination of the cholinergic pathway in the spleen revealed decreased choline acetyltransferase levels and increased acetylcholinesterase levels, indicating reduced acetylcholine levels. Additionally, reduced expression of α7 acetylcholine receptors (α7nAChRs) and increased nuclear translocation of nuclear factor κB (NF-κB) suggested damage to the spleen's cholinergic pathway. Elevated serum levels of pro-inflammatory cytokines were also observed in the Model group mice. Conclusion: Mice drinking 3.5% DSS solution exhibited damage to the spleen's cholinergic pathway, triggering an inflammatory response. Increased pro-inflammatory cytokines and decreased anti-inflammatory cytokines in the serum led to the migration of monocytes to the colon, where they differentiated into pro-inflammatory M1 macrophages, exacerbating inflammation. This excessive inflammatory activity damaged the colonic mucosal epithelium, resulting in soft stools, bloody feces, and weight loss, forming a vicious cycle that ultimately worsened the symptoms of ulcerative colitis.

Keywords: Ulcerative colitis, macrophage polarization, M1/M2 polarization, splenic cholinergic pathway, inflammation

Introduction

Ulcerative colitis (UC) is a chronic, non-specific inflammatory disease of the intestinal tract, the etiology of which is not completely clear, usually manifested by recurrent diarrhea, abdominal pain, bloody stools, and other symptoms, and is often accompanied by systemic symptoms such as weight loss and anemia [1, 2]. According to 2023 data, the global prevalence of UC is estimated to be 5 million cases, and the disease is on the rise [3]. In addition to the above symptoms, severe patients may also experience urinary urgency, urinary incontinence, bowel obstruction, nausea, vomiting, and even spasmodic pain in the left upper quadrant of the abdomen [4, 5]. Colorectal cancer is one of the most serious complications of UC, and studies have shown that the incidence of colorectal cancer can be as high as 18% in patients 30

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years after the diagnosis of UC [6], and this data highlights the urgency of further exploring the pathogenesis of UC and developing effective treatments.

The pathogenesis of UC is extremely complex, involving multiple factors such as abnormalities in the immune system, damage to the intestinal barrier function, and dysregulation of the intestinal microbiota [7]. Abnormal responses of the immune system are considered to be one of the central factors in the pathogenesis of UC, and macrophages, as an important component of the body's immune defense, play a crucial role in intestinal homeostasis [8, 9]. Under normal conditions, blood monocytes differentiate into F4/80+ macrophages and polarize into the M2 type, which exerts anti-inflammatory effects and promotes the proliferation and differentiation of intestinal stem cells, thereby maintaining the integrity of the intestinal barrier [10-12]. However, in the inflammatory environment of UC, the polarization of macrophages is altered, polarizing towards the M1 type and starting to secrete large amounts of pro-inflammatory cytokines, leading to over-activation of the immune response, which in turn damages the intestinal epithelial tissue [13-15]. Although macrophage polarization is known to be associated with the disease process in UC, the mechanism by which macrophage polarization is regulated remains unclear.

As an important immune organ, the spleen maintains the immune homeostasis of the body by regulating immune cells in the blood as well as secreting various immune factors. The cholinergic pathway in the spleen is considered to be one of the key pathways in its regulation of the immune response [16]. The cholinergic pathway releases acetylcholine (ACh) through the interaction of the nervous system with the immune system, which in turn regulates the function of immune cells. By binding to cholinergic receptors on immune cells, acetylcholine can regulate cell function, secretory behavior, and the production of inflammatory factors [17]. In response to inflammation or infection, the cholinergic pathway of the spleen can protect tissues from damage by suppressing excessive immune activation [18]. Although the splenic cholinergic pathway has been shown to play an important role in immunomodulation in other immune diseases [19], its specific role and mechanism in UC have not been fully investigated, especially its effects on the polarization process of colonic macrophages, which require further exploration.

Therefore, in this study, we investigated the mechanisms underlying the polarization of colonic macrophages towards the pro-inflammatory M1 type by constructing a UC mice model, and focused on analyzing the role of the splenic cholinergic pathway in this observation of the UC mice model. It revealed that colonic macrophages were significantly polarized toward the M1 type, and the enlargement of the spleen indicated that the spleen might play an important role in the immune response. Further investigation revealed that aberrant modulation of the cholinergic pathway in the spleen may regulate the integrity of the intestinal barrier by influencing the polarization state of macrophages and, in turn, the integrity of the intestinal barrier. The present study further revealed the molecular mechanism of macrophage polarization, identified the potential regulatory role of the splenic cholinergic pathway in UC, and explored its potential as a therapeutic target. This study not only reveals the immunoregulatory process of UC pathogenesis but also provides a new theoretical basis and reference for early diagnosis and targeted therapy of UC.

Materials and methods

Animals and materials

Sixteen male BALB/c mice (SPF grade, 7-8 weeks old, initial body weight 20-22 g) were purchased from Shanghai Sipulpikai (SLH-07r-BK) Co., Ltd. Dextran sulfate sodium (DSS, MW 36000-50000, item no. 60316ES60) was purchased from Next Sage Bio-technology Co., Ltd. Antibodies against Occludin (27260-1-AP), CD86 (13395-1-AP), CD163 (16646-1-AP), F4/80 (28463-1-AP), and iNOS (18985-1-AP) were purchased from Proteintech. Antibodies against ZO-1 (ab307799) and α7nAChR (ab216485) were purchased from Abcam. Enzyme-linked immunosorbent assay (ELISA) kits for interleukin 6 (IL-6) (CB10187-Mu), interleukin 10 (IL-10) (CB10161-Mu), CHAc (CB10251-Mu), and AChE (CB10796-Mu) were purchased from Cohiba Biologics. The experimental protocol was approved by the Bioethics Committee of the East China University of Science and Tech-nology

(Ethics Approval: ECUST-2024-022). We conducted all the animal-related experiments based on the Guide for the Care and Use of Laboratory Animals (National Institutes of Health).

Animal grouping and model construction

The mice were randomly divided into Control and Model groups according to their body weight, with 8 mice per group. The acute UC model was induced by DSS free-drinking, and freshly prepared 3.5% DSS solution was given to the Model group every day from day 0 to 7, while mice in the Control group drank sterilized water as a control, and the Model group was replaced with normal drinking water from day 8 to 14. Survival was recorded during this period, and mice were scored DAI according to fecal looseness, fecal occult blood, and survival [20]. DAI score = body weight loss (%) + fecal consistency score + gross rectal bleeding score. During the experimental period, the animal housing facility was maintained under tightly regulated environmental conditions: ambient temperature was strictly controlled at 24±2°C, relative humidity was maintained within 40-60%, and a 12-hour light/12-hour dark cycle was implemented. Animals were provided ad libitum access to standard rodent chow and sterile drinking water throughout the study. After the experimental period, or upon reaching a humane endpoint, animals were euthanized by overdose of isoflurane.

Hematoxylin-eosin staining

Paraffin sections were deparaffinized by immersion in xylene twice for 10 minutes each. Sequentially, they were rehydrated by immersion in 100%, 95%, 80%, and 75% ethanol for 5 minutes each, and washed with PBS with shaking. The nuclei were stained with drops of hematoxylin staining solution for 5 minutes, rinsed, and then differentiated with hydrochloric acid-ethanol differentiation solution for 10 seconds, and counterstained with running water for 10 minutes. Sections were then counterstained with eosin staining solution for 10 seconds, and washed with PBS with shaking. Paraffin sections were sequentially soaked in distilled water, 75% ethanol, 80% ethanol, 95% ethanol, 100% ethanol, and xylene for 5 minutes each for dehydration and transparency, and the sections were sealed with drops of neutral resin.

Splenic tissue homogenization preparation

Mice spleens were rinsed using pre-cooled PBS to remove residual blood, weighed, and the tissue was sheared. A small amount of the cut tissue was placed in a grinding tube, and PBS containing protease inhibitor and grinding beads were added at a weight-to-volume ratio of 1:9. The tissue was thoroughly ground into a homogenate using a tissue grinder, the homogenate was centrifuged at 5,000 g for 5-10 min, and the supernatant was extracted for assay.

Enzyme-linked immunosorbent assays

All the experiments in this study were performed in strict compliance with the experimental protocols specified in the manufacturer's instructions. Add 50 µl of standard to the standard wells, add 50 µl of serum or spleen homogenate to the sample wells, add 100 µl of HRP-labeled detection antibody to each well, and incubate for 60 minutes at 37°C away from light. After incubation, wash five times with washing solution, add 50 µl of A and B substrate to each well, incubate at 37°C for 15 minutes, then add 50 µl of termination solution, and measure the OD value at 450 nm within 15 minutes.

Western blot

Total protein from colon tissue was extracted with RIPA lysate (RIPA:PMSF:Cocktail = 970:10:20), and the concentration was measured by the BCA method. Proteins were separated by 10% SDS-PAGE (80 V for 15 min, electrophoresis continued at 120 V) and transferred to PVDF membrane (70 min). The membrane was blocked with 0.05 g/mL skimmed milk for 2 h. The primary antibody was incubated at 4°C overnight, the membrane was washed with TBST on the next day, and the HRP-secondary antibody was incubated at room temperature for 2 h. The membrane was washed and then developed by ECL, and the grayscale values were analyzed by ImageJ.

Immunohistochemical staining

Paraffin sections were deparaffinized with xylene and dehydrated with a gradient of alco-

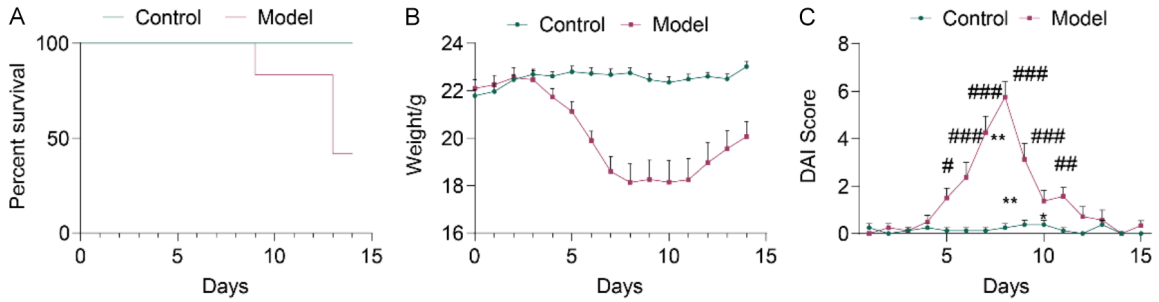

Figure 1. Percent survival (A), weight (B), and DAI score (C) of mice induced with 3.5% DSS. Data are presented as mean ± SEM (n = 8), #P < 0.05; ##P < 0.01; ###P < 0.001 (Student's t-tests).

hol as in Hematoxylin-eosin staining. Sections were placed in citric acid antigen repair solution, microwaved at 95°C, and cooled. 3% H_2O_2 was incubated at room temperature and protected from light for 20 min to inactivate the peroxidase. Non-specific binding was blocked by incubating sections with 5% bovine serum albumin for 30 min, the primary antibody was incubated at 4°C for 16 h, and the secondary antibody was incubated at room temperature for 1 h. The color was developed by DAB, and the nuclei were stained, dehydrated, and sealed as in Hematoxylin-eosin staining.

Data analysis and processing

All experimental data were statistically analyzed using GraphPad Prism 9.0 with Student's t-tests. All results are expressed as the mean ± standard error of the mean (SEM). "n" represents the number of samples in each group. "P < 0.05" represents a significant difference. #P < 0.05, ##P < 0.01, and ###P < 0.001. Western blot and immunohistochemistry images were statistically processed using ImageJ, and the data were analyzed using GraphPad Prism 9.0.

Results

Changes in body weight and DAI score in the UC mice model

In this study, an acute UC model was induced by free drinking of DSS. On days 0 to 7, a freshly prepared 3.5% DSS solution was given to the Model group every day, and mice in the Control group drank sterilized water as a control. Both the Control and Model groups were replaced with sterilized water on days 8 to 14. During this period, the mice were weighed daily, DAI scores were measured, and mice mortality was recorded.

No mortality was observed in the Control group. In contrast, the Model group exhibited a 25% mortality rate, with one mouse dying on day 9 and another on day 13 (**Figure 1A**). Control group mice maintained stable body weight. Model group mice began to lose body weight from day 5 post-DSS administration. Although body weight started to recover after DSS withdrawal, it did not return to baseline levels by day 14 (**Figure 1B**). The DAI scores of mice remained at a low level, increased significantly in the model group from the fifth day, peaked on the eighth day, and began to decline after the cessation of DSS, returning to near the baseline on the thirteenth day (**Figure 1C**). After stopping the drinking of DSS solution, the body weight of mice in Model group started to rebound, but the body weight of Model group still did not return to the normal level until the end of the experiment on day 14. Control group mice maintained stable body weight. Model group mice began to lose body weight from day 5 post-DSS administration. Although body weight started to recover, DAI scores in the model group significantly increased from day 4, peaked on day 8, and compared with the Control group, the DAI scores of the Model group increased from day 4 and reached a peak on day 8, and began to decrease after stopping the DSS free-drinking, with significant differences from days 5 to 9 and 11 in the Model group (P < 0.05). DSS administration induced typical UC symptoms, including weight loss, loose/soft stools, and bloody stools, which were partially alleviated upon DSS withdrawal. The above results were consistent with the disease development process in the acute UC mice model, and thus, the mice were preliminarily judged to be successful in the modeling.

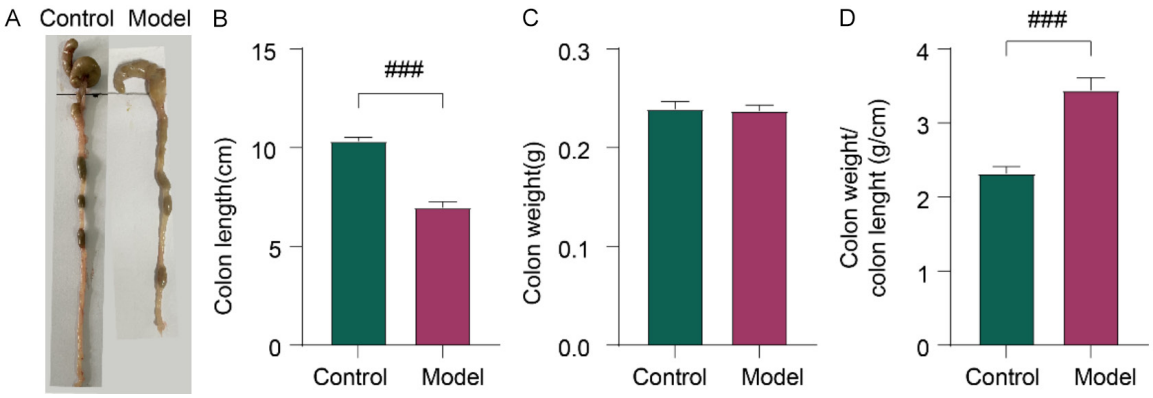

Figure 2. Colonic changes in mice after DSS induction. (A) Representative pictures of the colon. (B) Colon length, (C) Colon weight and (D) Colon weight/colon length of mice. Data are presented as mean ± SEM (Control group n = 8, Model group n = 6), ###P < 0.001 (Student's t-tests).

Elevated colonic swelling in UC mice models

Acute UC patients and acute UC mice models often showed colonic contracture and swelling, and these pathological changes significantly affected the absorption function of the colon. The mice were executed on day 14, and the colon was removed for examination. Model group mice exhibited significantly shorter colon lengths compared to control group mice (**Figure 2A**, **2B**), indicating that the colon was significantly damaged and contracted in the acute UC model. However, colon weight did not show a significant difference between the two groups (**Figure 2C**), suggesting that the overall mass of the colon tissue did not change significantly, possibly reflecting the fact that the swelling was mainly a morphological change of the colon lumen. To quantitatively assess the extent of colonic swelling, the ratio of colonic length to colonic weight was utilized as an indicator of the swelling rate. The colon weight-to-length ratio was significantly higher in the model group (**Figure 2D**). These preliminary results suggest that in the acute UC mice model, the significant shortening of the colon length and the significant increase in the swelling rate suggest that the colon is significantly damaged.

Polarization of colonic macrophages to pro-inflammatory M1 type in the UC mice model

Macrophages are involved in many inflammatory and autoimmune diseases [21] and are the first line of defense for nonspecific immunity, playing an important role in the disease process of UC. Macrophages regulate the inflammatory response mainly through activation into two distinct phenotypes; M1-type macrophages highly express CD86, secrete pro-inflammatory cytokines such as TNF-α and IL-6 [22], and enhance the inflammatory response [8], whereas M2-type macrophages highly express CD-163, secrete the anti-inflammatory cytokine IL-10, and suppress the inflammatory response [14, 23].

To assess the total number of macrophages and the expression of M1-type and M2-type macrophages in mice colon tissues, immunohistochemical analysis was performed in this study. Immunohistochemical analysis (**Figure 3A**) revealed significantly higher total macrophage infiltration (F4/80-positive cells) in the colonic tissues of model group mice compared to controls. Further quantitative analysis of the immunohistochemical results of M1-type and M2-type macrophages revealed that the expression of pro-inflammatory M1-type macrophage marker CD86 was significantly increased in the mice of the Model group, whereas the expression of the anti-inflammatory M2-type macrophage marker CD163 showed a decreasing trend (**Figure 3B**, **3C**). Consequently, the M1/M2 ratio (CD86/CD163) was significantly elevated in the model group, indicating a shift towards M1 polarization of intestinal macrophages (**Figure 3D**). This may have promoted the inflammatory response and tissue damage in the intestine.

Reduced expression of colonic mucosal barrier proteins in UC mice models

Under normal physiological conditions, macrophages maintain immune homeostasis in the gut by regulating immune responses. Dys-

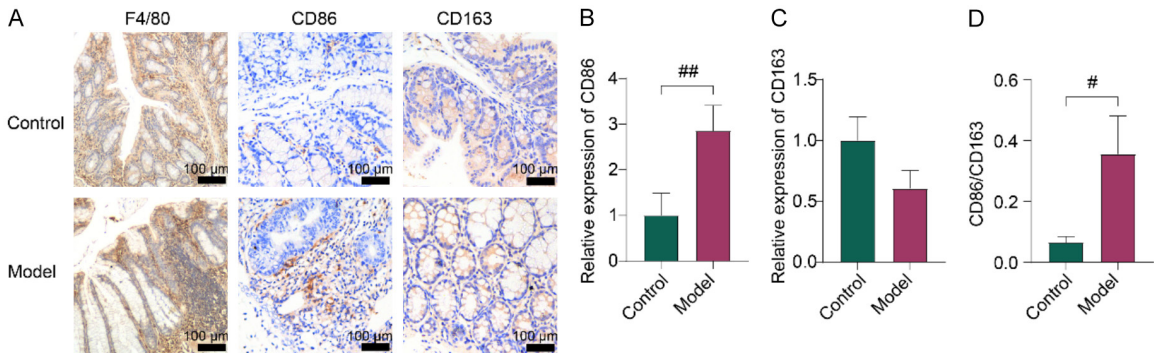

Figure 3. Changes in mice colonic macrophages after DSS induction. A. Representative immunohistochemical plots of mice colon F4/80, CD86, and CD163. B-D. Quantitative statistical plots of IHC results for CD86, CD163, and CD86/CD163. Data are presented as mean ± SEM (Control group n = 8, Model group n = 6), #P < 0.05; ##P < 0.01 (Student's t-tests).

regulation of macrophage function, especially conversion to pro-inflammatory M1 type, is one of the key factors leading to local inflammation and tissue damage.

The colonic mucosal barrier is capable of organizing the entry of intestinal contents into or damage to the colon [24]. Tight Junctions (TJs) are the main connections of the mucosal barrier, which are mainly constructed by transmembrane proteins (Occludin) and peripheral membrane proteins (ZOs). Occludin proteins are involved in the regulation of intercellular permeability and polarity [25]. Immunohistochemistry is shown in **Figure 4A**, **4C**. In the Control group, Occludin was localized to the intercellular junctions of epithelial cells throughout the mucosal depth, particularly at the apical regions, but the localization of Occludin in the Model group was only visible on the surface of the colonic mucosa, with a significant decrease in the expression. ZO-1, as an important peripheral membrane protein in TJs, can bind to transmembrane proteins, and co-link to cytoskeletal actin, affecting the colonic permeability, and the Model group showed a significant decrease in ZO-1 expression, which was significantly reduced (**Figure 4A**, **4D**). The results of Western blot experiments in mice colon tissue also showed that Occludin and ZO-1 protein expressions were reduced in the Model group (**Figure 4B**, **4E**, **4F**). The above results could confirm the reduced expression of colonic mucosal barrier proteins in mice in the Model group, suggesting the impairment of the colonic barrier function.

UC mice model colon showed tissue damage

The HE staining results were shown in **Figure 5A**. Control group mice had a clear colonic structure. The mucosal layer, submucosal layer, muscularis propria and plasma membrane layer were structurally intact. Mucosal cells were neatly arranged and no ulcers were seen. However, the Model group had severe damage to the colonic mucosa, large ulcers were visible, intestinal glands around the ulcers were irregularly arranged, a large amount of hyperplastic connective tissue was observed, and the depth of infiltration of lymphocytes and neutrophils reached all layers. The necrotic part penetrated deep into the intrinsic muscular layer and submucosal layer, and the pathologic tissue score [26] was significantly higher (**Figure 5E**).

Inflammatory mediators TNF-α and iNOS play an important role in the pathogenesis of UC. TNF-α enhances M1-type macrophage polarization and expands inflammation by activating neutrophils and platelets. At the same time, M1-type macrophages produce large amounts of iNOS, synthesize high concentrations of NO, and consume arginine, leading to metabolic hypoxia and damage to colonic tissues [27]. Western blot detected the expression of TNF-α and iNOS in the mice colon, and the results, as shown in **Figure 5B-D**, showed that the protein content of TNF-α and iNOS in the colon of the Model group was significantly elevated, indicating that the level of colonic inflammation was elevated in the Model group.

Taken together, after DSS free-drinking induction, the mice in the Model group exhibited

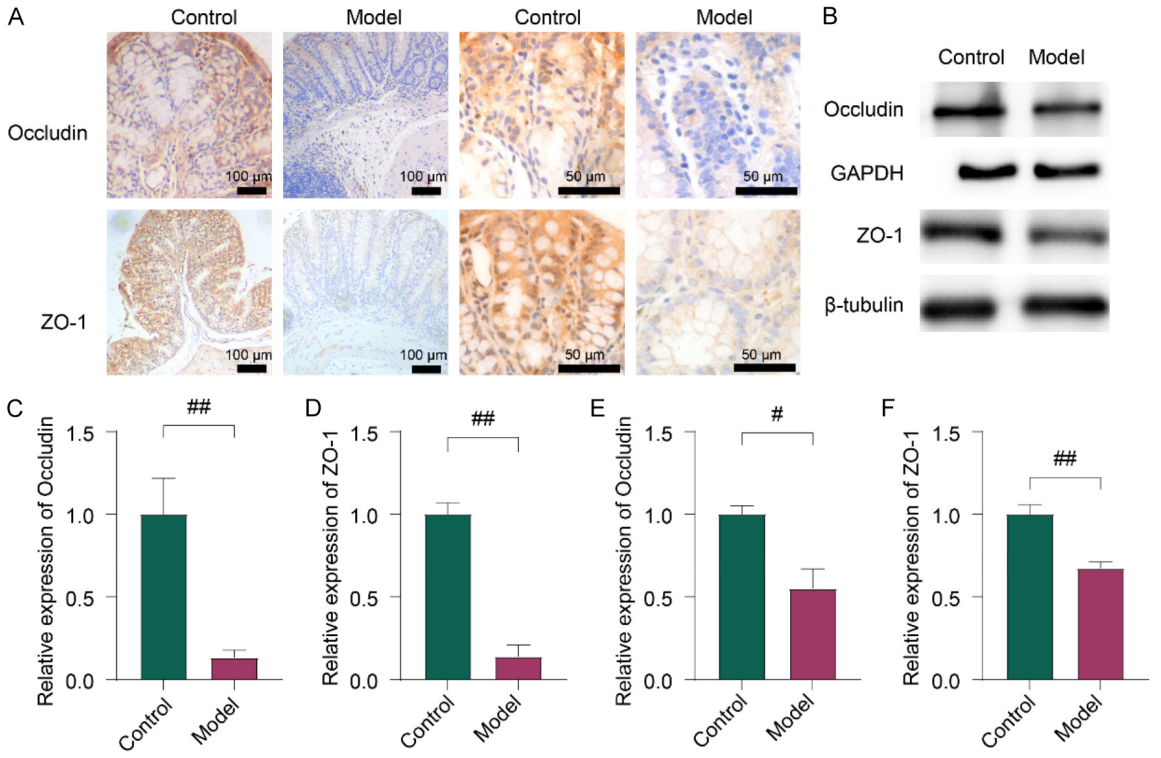

Figure 4. Changes in colonic barrier proteins in mice after DSS induction. A. Representative images of 100× (left) and 400× (right) immunohistochemistry of mice colon Occuldin and ZO-1. B. Protein expression of mice colon Occuldin and ZO-1. C, D. Quantitative statistic graphs of IHC results of Occludin and ZO-1. E, F. Western blot quantitative statistics of Occludin and ZO-1 results. Data are presented as mean ± SEM (Control group n = 8, Model group n = 6), #P < 0.05; ##P < 0.01 (Student's t-tests).

weight loss, elevated DAI scores, and an increased rate of colonic swelling, and were initially diagnosed with UC symptoms. Immunohistochemical analysis showed that the colon of mice in the Model group had a significant increase in the polarization of pro-inflammatory M1-type macrophages and a significant decrease in the polarization of anti-inflammatory M2-type macrophages. The M1-type macrophages further promoted inflammatory responses through the secretion of pro-inflammatory cytokines TNF-α and iNOS, which led to a decrease in the expression levels of the colonic mucosal barrier proteins Occludin and ZO-1, and ultimately caused severe damage to the colonic mucosa. These results suggest that polarization of M1-type macrophages and their pro-inflammatory effects play an important driving role in the occurrence and development of UC.

Impaired cholinergic pathway in the spleen of the UC mice model

Macrophages in the intestine are mainly differentiated from circulating monocytes, which are continuously recruited to the intestine in a chemokine receptor-dependent process and differentiate into mature macrophages in a gradual process of differentiation [28, 29]. The immunomodulatory cytokines transforming growth factor-β (TGF-β) and IL-10 play a key role in controlling the polarization of intestinal macrophages [30]. During inflammation, this process is disrupted, leading to the accumulation of large numbers of stimulated monocytes, which have highly pro-inflammatory characteristics and eventually differentiate into pro-inflammatory M1-type macrophages [8]. The spleen is one of the important immune organs in the human body, which mainly maintains systemic immune homeostasis by regulating immune cells in the blood and secreting a variety of immune factors, among which, the cholinergic pathway is one of the key mechanisms by which the spleen regulates immune responses. In this study, we observed that the spleens of mice in the Model group were abnormally enlarged, and the statistical spleen index showed that it was significantly higher compared with that of the Control group (**Figure 6A**). This suggests

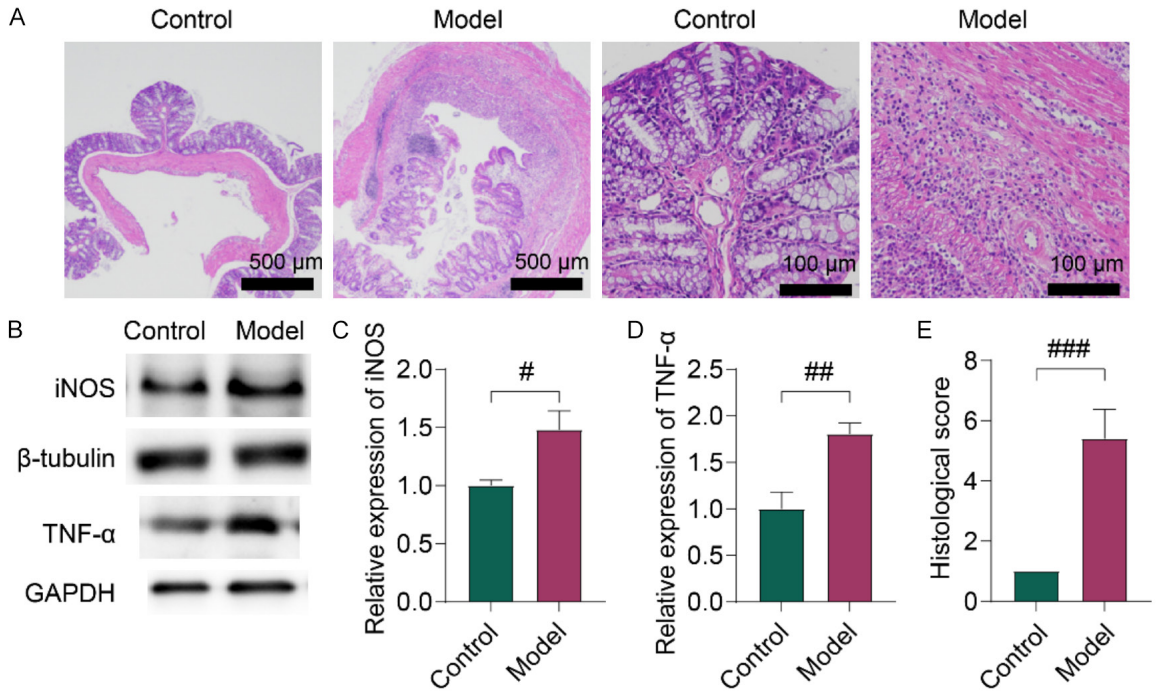

Figure 5. Pathological changes in the mice colon after DSS induction. A. Representative images of HE staining of mice colon at 40× (left) and 200× (right). B. iNOS and TNF-α protein expression in mice colon. C, D. Quantitative statistical plots of Western blot results for iNOS, TNF-α. E. HE staining for pathological tissue scores. Data are presented as mean ± SEM (Control group n = 8, Model group n = 6), #P < 0.05; ##P < 0.01; ###P < 0.001 (Student's t-tests).

that polarization of colonic macrophages toward pro-inflammatory M1 type in mice in the Model group may be associated with impaired cholinergic pathway.

The mechanism of the cholinergic pathway in anti-inflammation is that inflammatory response signals stimulate the excitatory vagus nerve to release acetylcholine (Ach), which binds to the α7 nicotinic acetylcholine receptor (α7nAChR) and then inhibits pro-inflammatory factor production by regulating nuclear factor-κB (NF-κB) signaling, thus exerting anti-inflammatory effects [16]. Choline acetyltransferase (ChAT) is a transferase that synthesizes acetylcholine from acetyl coenzyme A and choline, and acetylcholinesterase (AChE) exists in the choline synaptic gap and can degrade acetylcholine; the balance of the two can determine the amount of acetylcholine. As shown in **Figure 6B-D**, compared with the Control group, the ChAT content in the Model group was significantly reduced, the acetylcholinesterase content was significantly increased, and the ratio of ChAT to acetylcholinesterase was significantly reduced, indicating that the amount of acetylcholine was reduced in the spleens of the mice in the Model group. The results of **Figure 6E** and **6G** showed reduced α7 acetylcholine receptor in the spleen. NF-κB, as a key inflammatory regulator, often in the form of dimers, is an important transcription factor that can enter the nucleus of the cell and bind to specific nucleic acid sequences to modulate intrinsic and adaptive immune responses [31]. The results of the Western Blot assay (**Figure 6F**, **6H**, **6I**) showed that compared with the Control group, the cytoplasmic NF-κB content in the Model group tended to decrease, but was not statistically significant, but the nuclear NF-κB content in the Model group was significantly higher, indicating that more NF-κB entered the nucleus, bound to DNA, and promoted inflammatory responses. Serum inflammatory factor levels were detected, and the results, as shown in **Figure 6J** and **6K**, showed that the serum levels of pro-inflammatory cytokine IL-6 were significantly increased and the levels of anti-inflammatory cytokine IL-10 were significantly decreased in the Model group. This may be the reason for the polarization of intestinal macrophages into pro-inflammatory M1-type macrophages.

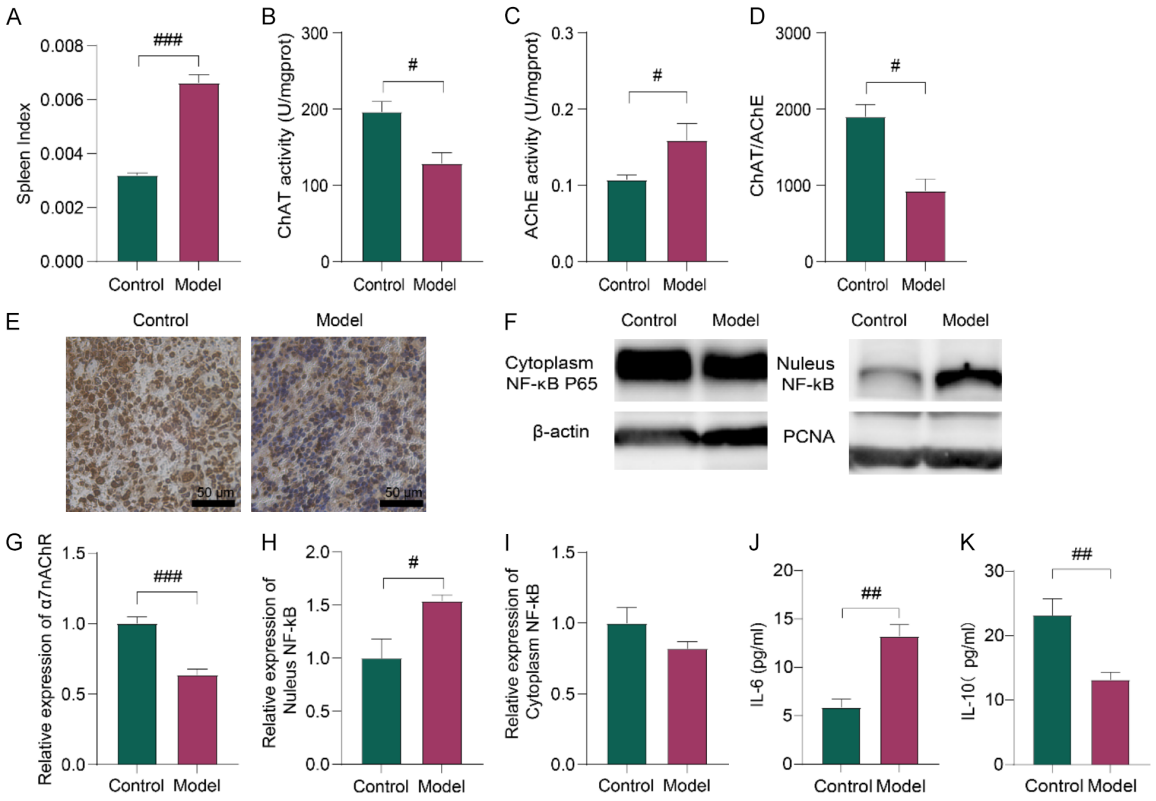

Figure 6. Changes in cholinergic pathways in mice spleen after DSS induction. A. Statistics of mice spleen indices. B-D. Detection of mice spleen ChAT, AChE, and ChAT/AChE content. E. Representative immunohistochemical pictures of mice spleen α7nAChR. F. Protein expression of mice spleen intracytoplasmic and nuclear NF-κB p65. G. Quantitative statistical graphs of IHC results of α7nAChR. H, I. Quantitative statistical graphs of Western blot results of intracytoplasmic and intranuclear NF-κB p65. J, K. Serum levels of inflammatory factors IL-6 and IL-10. Data are presented as mean ± SEM (Control group n = 8, Model group n = 6), #P < 0.05; ##P < 0.01; ###P < 0.001 (Student's t-tests).

Discussion

In this study, we demonstrate that impairment of the splenic cholinergic pathway leads to macrophage M1 polarisation and exacerbates UC in a mice model. Our findings highlight the central role of M1-type pro-inflammatory macrophages in the pathogenesis of UC. These cells secrete large amounts of pro-inflammatory cytokines, which are markedly elevated during the inflammatory process, thereby contributing to the persistence and worsening of inflammation.

Moreover, during the active phase of UC, inflammatory cytokines disrupt the expression of tight junction proteins in intestinal epithelial cells, significantly increasing epithelial permeability. This increased permeability allows bacteria and endotoxins to penetrate the mucosal barrier and enter the bloodstream, further triggering severe inflammatory responses and exacerbating the pathological progression of UC. The pathogenesis of ulcerative colitis also involves an imbalance in immune response regulation, where the ratio of pro-inflammatory to anti-inflammatory cytokines is disrupted. This imbalance results in a diminished tolerance of the intestinal immune system to normal microbiota and dietary antigens, leading to an exaggerated immune response.

In recent years, the splenic cholinergic pathway has gained considerable attention in the field of immune regulation research. This pathway primarily influences splenic immune function through cholinergic signaling transmitted via the vagus nerve, thereby modulating systemic inflammatory responses. As part of the autonomic nervous system, the vagus nerve is responsible for transmitting cholinergic signals. These signals release acetylcholine, which acti-

vates cholinergic receptors in the spleen, particularly the α7nAChR. Studies have shown that when acetylcholine binds to α7nAChR, it can inhibit the release of pro-inflammatory cytokines such as TNF-α and IL-6 by splenic macrophages, a mechanism known as the "cholinergic anti-inflammatory pathway" [32]. The α7nAChR, a key component of this anti-inflammatory pathway, has demonstrated protective effects in various inflammatory diseases.

The spleen, as a crucial immune organ, plays a significant role in the pathogenesis of UC. Through the "gut-spleen-brain axis" [33], the spleen interacts directly with the gut, participating in the regulation of immune homeostasis. Our results showed that in DSS-induced UC mice models, the spleens of Model group mice were significantly enlarged, with a markedly increased spleen index compared to Control groups, indicating the spleen's important role in UC immune responses. Concurrently, the cholinergic pathway in the spleens of Model group mice is impaired, leading to reduced acetylcholine levels and decreased expression of α7nAChR, thereby affecting the spleen's ability to regulate immune responses.

The impairment of splenic function may result in an imbalance in macrophage polarization in the gut, skewing towards the pro-inflammatory M1 type, which exacerbates intestinal inflammation and disrupts the integrity of the intestinal barrier. Furthermore, our results indicated elevated serum IL-6 levels and decreased IL-10 levels in Model group mice, consistent with the decreased anti-inflammatory capacity due to the impaired splenic cholinergic pathway.

In conclusion, the splenic cholinergic pathway and the spleen itself play crucial roles in the pathogenesis of UC. The impairment of the cholinergic anti-inflammatory pathway leads to a significant reduction in anti-inflammatory effects, with a corresponding increase in the secretion of pro-inflammatory cytokines. Simultaneously, impaired splenic function may further exacerbate UC progression by affecting macrophage polarization states. These experimental findings not only elucidate the pathogenesis of UC but also provide theoretical foundations for developing new therapeutic targets.

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Disclosure of conflict of interest

None.

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