Effects of Myeloperoxidase on Inflammatory Responses with Hypoxia in Citrobacter rodentium-Infectious Mice

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Abstract

Purpose: Myeloperoxidase(MPO) has been found to be the mediator in various inflammatory diseases. Bacterial infection of the intestine and hypoxia can both lead to inflammatory responses, but the effects of MPO in these phenomena are still unclear. Methods: By building the MPO-/- mice ,we examined relevant inflammatory factors and tissue damage in mice with intestinal Citrobacter rodentium infection and hypoxia. The body weight and excreted microorganisms were monitored. Intestinal tissues were collected 7 days after bacterial infection under hypoxia to undergo HE staining and assess the degree of pathological damage. The levels of TNF-α, IFN-γ, IL-6 and IL-1β inflammatory factors in the serum were quantified using ELISA. PCR, WB and IF assays to determine the expression of chemokines MCP1, MIP2 and KC in the colon and spleen. . Results: Citrobacter rodentium infection and hypoxia caused weight loss , intestinal colitis and splenic inflammatory cells active proliferation in wild type mice and this phenomena was alleviative in MPO-/- mice. Also,the capability of clearing bacteria in MPO-/- mice significantly declined. The level of TNF-α in the serum and spleen was both lower in MPO-/- hypoxia C. rodentium-infected mice than that in wild type mice. The chemokines expression levels of MIP2, KC and MCP1 in the spleen and colon of each bacterial infected group were significantly increased ($P<0.05$), while in hypoxia, the factors in the spleen and colon were decreased ($P<0.05$). Meanwhile, The levels of these chemokines in the MPO-/- mice were found to be lower than those observed in the wild type mice. Conclusion: MPO plays an important role of the inflammatory responses in infectious enteritis and hypoxia in mice. Loss of MPO may greatly reduces the body's inflammatory responses to fight deseases.

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Results: *Citrobacter rodentium* infection and hypoxia caused weight loss , intestinal colitis and splenic inflammatory cells active proliferation in wild type mice and this phenomena was alleviative in MPO$^{-/-}$ mice. Also,the capability of clearing bacteria in MPO$^{-/-}$ mice significantly declined. The level of TNF-α in the serum and spleen was both lower in MPO$^{-/-}$ hypoxia *C. rodentium* -infected mice than that in wild type mice. The chemokines expression levels of MIP2, KC and MCP1 in the spleen and colon of each bacterial infected group were significantly increased (P <0.05), while in hypoxia, the factors in the spleen and colon were decreased (P <0.05). Meanwhile, The levels of these chemokines in the MPO-/- mice were found to be lower than those observed in the wild type mice.

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Keywords: Hypoxia, Colitis, Inflammatory responses , MPO

1 Introduction

Myeloperoxidase (MPO) is an important member of the haem peroxidase superfamily [1], which was found in primary azurophilic granules of neutrophils. In the presence of H2O2 and a halide—chloride, bromide, or thiocyanate, MPO can catalyzes the formation of reactive oxygen intermediates including hypochlorous (HOCl), and hypothiocyanous acids, respectively. Upon activation of neutrophils in peripheral blood and tissues, MPO is released into both the phagolysosomal compartment and the extracellular environment and then affects the aggregation of inflammatory cells under antigen stimulation [2,3]. Studies have shown that MPO not only plays a role in the process of killing pathogenic bacteria but also promotes the intensification of the inflammatory response and causes tissue damage [4]. MPO is part of the innate immune system that helps phagocytes fight against invading microorganisms and can significantly increase its activities in the pathological process of neurological diseases, tumours, rheumatoid diseases, kidney damage, diabetes and other diseases which participating in the initiation and progression of inflammation [5,6]. Recently, studies on MPO-mediated inflammatory events has become a hot topic. To define the in vivo role of MPO in host defense, MPO-knockout (MPO$^{-/-}$) mice were created by two independent research groups and have been extensively studied for their susceptibility to infections. Mutant mice exhibit increased susceptibility to infection with C. albicans and Klebsiella pneumoniae compared to infected wild-type mice [7]. However, the role of MPO in regulating intestinal immune responses during infectious colitis and the mice response under hypoxia remains unclear. Here, we examined the inflammatory response of MPO$^{-/-}$ mice with intestinal bacterial infection merely, hypoxia merely, and bacterial infection accompanied with hypoxia than those in wild-type mice in order to explore the role of MPO in innate immunity.

2 Materials and methods

2.1 The Citrobacter rodentium infection and ***hypoxia mice model***

64 (32 WT and 32 MPO$^{-/-}$) C57BL/6 mice (SPF grade, 8 week olds, female, 18-20 g) in total were purchased from Hu'nan SJA Laboratory Animal Co., Ltd. (WT mice) and Cyagen Biosciences (Suzhou) Inc. (MPO$^{-/-}$ mice). The mice were kept in a specific-pathogen-free (SPF) facility with the Individual Ventilated Cage Animal Experiment System (H6, Su Hang Technology Equipment Co., Ltd., Suzhou) with autoclaved water and food. Animal care was provided under protocols endorsed by the Institutional Animal Care and Utilize Committee at the Medical College of Qinghai University. All animal experiments comply with the ARRIVE guidelines. *Citrobacter*colitis was induced by oral feeding with *C. rodentium* (strain DBS100, ATCC number

51459, 5×10^8 CFU/mouse).The hypoxia environment was made with a hypobaric chamber (Fukang Air Purification Equipment Engineering Co., Ltd., Shaanxi) at 5,000 m (atmospheric pressure was 405 mmHg, PO_2 was 84.7 mmHg) [8]. Mice were randomly divided into 8 groups with simple randomization method:WT normoxia control group (NC,n=8), $MPO^{-/-}$ normoxia control group (KC ,n=8), WT *C. rodentium* infection group (NI, n=8), $MPO^{-/-}$ *C. rodentium* infection group (KI,n=8), WT hypoxia control group (HC,n=8), $MPO^{-/-}$ hypoxia control group (HKC,n=8),WT hypoxia *C. rodentium* -infected group (HI,n=8) and $MPO^{-/-}$ hypoxia *C. rodentium* -infected group (HKI,n=8). 8 mice were kept in one cage. Only feeder was aware of the group allocation at the different stages of the experiment.

2.2 Body weight and faecal bacterial output measurement

Body weight changes of the mice in each group were monitored every day from the first day of *C. rodentium* infection. The number of*C. rodentium* in each gram of faeces (CFU/g) was calculated and continuously recorded for 7 d. At the same time, the faeces of mice in the infected groups were collected and cultured on selective MacConkey agar (M8560, Solarbio). Bacterial colonies were then counted after culturing at 37 degC.

2.3 Haematoxylin-eosin (HE) staining

At necropsy, colonic tissues were collected, immersed in 4% paraformaldehyde (Solarbio, P110, USA), and stored for 48 h at 4 degC. Then, 5 μm sections were cut on a Leica RM2016 Microtome (Leica Biosystem) and stained with haematoxylin and eosin (H&E). The colon and spleen tissues were fixed at 40 g/L in polyformaldehyde for 12 h. After dehydration, the tissue was turned transparent with xylene and washed with PBS. The sample was embedded in paraffin, and the tissue slice was stained. Colon lesions and inflammatory infiltration of spleen tissue were observed under an optical microscope. Histological scoring was based on two parameters as indicated in Table 1 [9]. The total colon pathology score equalled the inflammatory cell score plus the tissue damage score.

2.4 ELISA

Mice were sacrificed after hypoxic exposure for 7 d. On the 8^{th} day, after blood collection from the mouse orbital vein, the mice were euthanized by cervical dislocation. The colon and spleen of the mice in each group were removed by surgery. The blood samples were allowed to stand at room temperature for 1 hour and processed by centrifugation at 3500 r/min for 15 min, and serum was collected and stored at -80 °C. Double antibody sandwich ELISA kits were used to determine the levels of TNF-α (JM-02415M1, Jing Mei Biotechnology), IL-1β (JM-02465M1, Jing Mei Biotechnology), IFN-γ (JM-02446M1, Jing Mei Biotechnology) and IL-6 (JM-02323M1, Jing Mei Biotechnology) in serum samples and spleen lymphoid specimens. A total of 100 μL of serum was added to the packaged microwell plate and incubated at 37 °C for 90 min. After discarding the supernatant and washing the plate, 100 μl of biotinylated cytokine-specific antibody was added and incubated at 37 °C for 60 min. Then, 100 μL of pro-subsidin HRP was added and incubated at 37 °C for 30 min. The substrate TMB was developed by placing the plate at room temperature for 15 to 20 min. Finally, the stop solution was added, the absorbance was measured at a wavelength of 450 nm by a microplate spectrophotometer.

2.5 Quantitative real-time PCR (qRT–PCR) analysis

Total RNA was extracted with a two-step isocyanate method. The total RNA concentration was measured by an ultraviolet spectrometer, and the OD260/280 value ranged from 1.8 to 2.0. 5 μg of total RNA was used for reverse transcription to synthesize cDNA, and 3 μg of cDNA was used for PCR amplification. β-Actin was regarded as an internal reference. Primers for β-actin, TNF-α, IFN-γ, IL-1β, MCP1, MIP2, and KC were synthesized by Primer Express. The PCR conditions included predenaturation at 94 °C for 2 min, denaturation at 94 °C for 30 s, annealing at 61 °C for 45 s, and extension at 72 °C for 1 min. The cycle was repeated 36 times, and each cycle included a reduction in annealing temperature by 0.3 °C, with a final extension of 6 min at 72 °C. The sequences of the primers used are listed in Table 2.

2.6 Western blot assay

The tissue was placed in a precooled homogenizer, and a lysis solution containing a protease and phosphatase inhibitor was added. After lysis for 15 min, the sample was centrifuged and stored at -80 °C. The protein concentration was determined according to BCA. SDS–PAGE electrophoresis (100 g/L) was carried out after protein denaturation. The proteins were electrophoresed to a PVDF membrane, which was blocked with BSA reagent for 1 h. Primary antibodies [anti-MCP1 (bs-1955R, Bioss Antibodies), anti-MIP2 (bs-1162R, Bioss Antibodies), and anti-KC (AB206411, Abcam), 1:1000] were added and incubated at 4 °C overnight, and the membranes were washed 5 times with TBST. Then, an HRP-labelled secondary antibody was added and incubated at room temperature for 1 h. The sample was developed with a Pierce ECL chemiluminescent kit.

2.7 Immunofluorescence assay

The colon and spleen tissue slices were blocked with goat serum for 30 min in each group. The goat serum was discarded, and the sample was washed twice with PBS and wiped with water-absorbing paper. 50 μL of diluted antibody (1:500) was added to the sample, and PBS was added to the negative control group instead. After washing 3 times, fluorescent secondary (1:2000) was added, and the sample was incubated in the dark at room temperature for 2 hours. Washing twice with PBS, adding an antiquenching agent dropwise, the sample was observed, and images were acquired under a fluorescence microscope.

2.8 Statistical analysis

Statistical analysis was performed using SPSS 27.0 software. The measurement data are expressed as $x \pm S$, and multigroup comparisons were undertaken by single-factor variance analysis or nonparametric statistics (rank sum test). The validation test was performed by the Levene test. When the variance was quarriable, the SNK test was used, and the multigroup comparison was chosen when it was different. P <0.05 indicated that the difference was statistically significant.

3 Results

3.1 Weight loss of mice was alleviative in $MPO^{-/-}$ mice under bacterial infection and hypoxia exposure.

After 2 days of the experiment, the weight of mice in the infection groups decreased significantly (Figure 1). On the 4th day, the weight of the mice in the NI group showed an upwards trend, and that of the mice in the KI group decreased significantly. Compared with the control group, the infection groups showed a significant change in the body weight of the mice, and the body weight decreased significantly (P<0.05), indicating that the infection caused the mice to lose weight; the HI group was compared with the NI and KI groups. Compared with that of mice in the HKI group, there was a difference in body weight change (P<0.05), and the weight loss of mice in the HI and HKI groups was more obvious, indicating that the hypoxic environment aggravated the weight loss of mice after infection. Comparing the $MPO^{-/-}$ and WT mice, the weight of the WT mice decreased significantly (P<0.05).

3.2 The lack of MPO expression inecreased greatly bacterial output and aggravated the shortening of the colon in mice with bacterial infection and hypoxia exposure.

The bacterial colonies on the faces of the mice were enumerated on the following day. (Figure 2a). An increase in excretion indicated an increase in the severity of inflammation (Figure 2b). Compared with that in the NI, HKI and KI groups, the number of bacteria excreted in the HI groups was significantly increased. Comparing the WT and $MPO^{-/-}$ groups, the number of bacteria excreted in the $MPO^{-/-}$ groups iecreased significantly. The total number of bacteria excreted in the HKI groups was the largest.We found that the colon lengths of the mice in the infected groups were significantly shorter (Figure 3a-b) after the mice were infected with *C. rodentium* , indicating that after the mice were infected, the colonic inflammation in the mice was aggravated as a result of colon shortening. There was a difference between the HI and NI groups (P= P <0.05), which indicated that the colon shortening of mice was more obvious under hypoxic conditions. Compared with that of the HKI group, the colon shortening of the KI group was more obvious, and there was a significant difference (P <0.05), indicating that the colon shortening of the $MPO^{-/-}$mice in the hypoxic environment was not as obvious as that under normoxia. Comparing the NC with HC groups and the KC and HKC groups, we found that hypoxic exposure could significantly increase the colon length in mice (P <0.05). The

average length of each group is NC 7.01cm,NI 6.05cm,HC 8.75cm,HI 6.43cm,KC 7.00cm,KI 5.78cm,HKC 8.35cm,HKI 6.45cm. There were significant differences between the KI and NI groups and the HKI and HI groups (P <0.05), which indicated that the deletion of the MPO gene could aggravate the shortening of the colon in mice.

3.3 Colitis and inflammatory cell infiltration in spleen was alleviative in MPO$^{-/-}$ mice.

The results of H&E staining of colon tissue showed (Figure 4a) that the structure of the small intestinal mucosa of the mice in each control group was clear and complete, the villus crypts were deep, and the villi were neatly arranged. We found that the pathological damage to colon tissue was more severe in the HI and KI groups as a result of colon oedema, thinning, disorganized and variable villi, inflammatory cell infiltration around crypts, and shallow crypts.

We evaluated the pathological changes in colon tissue in each group of mice. The results (Figure 4b) showed that the pathological changes of the mice in the HI group were the most serious,and the normal tissue structure of part of the mucosa was significantly damaged. Compared with the control group, the HI group had significant differences in colon pathological tissue scores after infection. However, comparing the pathological scores of the HKI group with the KI group and the HI group with the NI group, the scores were significantly lower (P <0.05), indicating that MPO could increase the inflammatory response in mice. Figure 4c shows that the spleens of mice in the NI and KI groups had a large amount of inflammatory cell infiltration.

3.4 The effect of MPO in inflammatory response of C. rodentium-infected mice under hypoxia.

Compared with the control mice (Figure 5a-d), the infected mice had significantly increased expression levels of the inflammatory factors TNF-α, IFN-γ and IL-1β in the serum, spleen and colon, and the IL-1β levels in the serum were also significantly increased. The expression of IL-6 was significantly increased in the serum (P<0.05), while the expression of the above factors decreased under hypoxia exposure, which suggests that the immune response was downregulated under hypoxia (P <0.05). Comparing WT mice and MPO$^{-/-}$ mice, the content of TNF-α in the KI and HKI groups was different from that in the NI and HI groups. Comparing the IL-1β, IFN-γ and IL-6 levels between the KI and HKI groups with those in the NI and HI groups, there was no significant difference, which indicated that the MPO gene has no effect on the production of inflammatory factors in the body.

3.5 The effect of MPO in the chemotaxis of neutrophils and macrophages of C. rodentium-infected mice under hypoxia

The results showed that compared with those of the control groups (Figure 6 a-d), the expression levels of MIP2, KC and MCP1 in the spleen and colon of each infected group were significantly increased (P <0.05), while in hypoxia, the expression levels of MIP2, KC and MCP1 in the spleen and colon were decreased compared with those in the control condition (P <0.05), which suggested that the neutrophil and macrophage chemotaxis capacity was reduced in hypoxia. The results of fluorescence (Figure 7) quantitative analysis showed that compared with that in the normal groups, the expression of chemokines in the infected groups was significantly increased, and the content of chemokines in the MPO$^{-/-}$groups was even greater.

4 Discussion

Previous studies have shown that hypoxia leads to abnormal systemic immune function, which may be closely related to the development of various chronic diseases [10,11]. TLRs [12], NOD-like receptors [13] and HIN200 [14,15] family members expressed by intestinal epithelial cells are widely involved in limiting the invasion of intestinal pathogenic microorganisms and preventing the deterioration of infectious diseases.The oxygen-sensing mechanism in the body can regulate the expression of genes under exposure to different oxygen concentrations [16]. An important cellular metabolic feature of hypoxia is the elevated expression of hypoxia-inducible factor (HIF-1), which participates in human physiological and pathological processes by regulating a variety of related genes [17].Inflammatory bowel disease (IBD) caused by *C. rodentium* can produce a large amount of proinflammatory cytokines and severely damage the layer of epithelial cells [18,19].

The levels of the proinflammatory cytokines TNF-α, IL-6 and IL-1β in colon tissues are significantly increased with the occurrence of IBD [20,21]. MPO plays an important role in bacterial infections [22].

Based on the experimental results, we found that in each group, the colon length of mice infected with *C. rodentium* in a normoxic environment was shortened, which was more pronounced under hypoxic exposure. The stained colon tissues of mice exposed to hypoxia showed obvious damage to or loss of colonic epithelial mucosa and erosion. The arrangement of intrinsic glands on the intestine was more disordered. A large amount of inflammatory cell infiltration could be seen in the mucosa and submucosa. According to pathological observation and scoring, it was clear that hypoxia exposure could aggravate the occurrence and development of the colitis inflammatory response.This study proved for the first time that a hypoxic environment can reduce the innate immune function of mice by affecting the chemotaxis level of phagocytes, resulting in an increase in bacterial excretion in *C. rodentium* -infected mice, weight loss, diarrhoea, blood in the stool, and shortened colon length, which confirmed that hypoxic exposure can exacerbate the inflammatory response in colitis. Neutrophils mainly rely on respiratory oxidative bursts [23] and the production of reactive oxygen species [24] to destroy pathogens. However, hypoxia causes insufficient oxygen pressure in the environment, resulting in insufficient recruitment of NOX2 in neutrophils and insufficient production of reactive oxygen species, which can lead to a decrease in the ability to clear pathogens.

Macrophages are the main phagocytic cells in the intestine and are closely related to the pathogenesis of IBD [25].In the process of maturation, phagosomes need to further fuse with lysosomes to generate phagolysosomes [26]. In the acute phase, the number of macrophages significantly increased in the intestinal mucosa in IBD. The expression of lymphocyte costimulatory molecules and TLR was upregulated, and the receptor for myeloid cell triggering-1 (TREM-1) was coexpressed [27,28]. TREM-1 can activate the synthesis and secretion of TNF, IL-1β, IL-6 and other inflammatory factors, which can cause damage to local tissues.

In our study, it was found that although a hypoxic environment can increase the chemotaxis of macrophages, macrophage activation is reduced due to the lack of oxygen. It has been reported in the literature that glycolytic capacity is significantly increased after the induction of macrophages by LPS and IFN-γ [29]. HIF-1, a heterodimeric transcription factor, played a key role in the adaptation of macrophages to changes in oxygen tension, and HIF-1α, as the active subunit of HIF-1, was the main functional factor [30]. HIF-1α has a very wide range of target genes, including GLUT1, GAPDH, LDHA, PKM and a variety of glycolytic enzymes [31]. After macrophages were activated, it adapted to the stimulation of inflammatory factors through the transformation of metabolic pathways. The hypoxic environment in our experiment significantly upregulated HIF-1α expression, which resulted in an increase in the glycolysis pathway [32], but the massive production of inflammatory factors can cause a more severe inflammatory response at the same time.

Previous studies have found that MPO can participate in the regulation of various inflammatory responses and pathological effects in the body [22], so we focused on the changes in MPO levels on the pathological response of *C. rodentium* -induced colitis in mice in a hypoxic environment.

MPO activity is an indicator commonly used to monitor IBD activity [33]. MPO in epithelial cells can release a variety of proinflammatory substances, such as HOCl and H_2O_2 [34]. After infecting WT mice and $MPO^{-/-}$ mice with *C. rodentium* , we found that $MPO^{-/-}$ mice excreted more bacteria and lost more weight. This is mainly because neutrophils in $MPO^{-/-}$mice cannot function to clear bacteria normally in the absence of MPO. Subsequently, we further analysed the results and found that the pathological changes of *C. rodentium* -induced enteritis in $MPO^{-/-}$ mice in a normoxic environment were more severe, mainly manifested as obvious submucosal oedema and a large amount of neutrophil and macrophage infiltration. Goblet cell numbers decreased significantly, and intestinal epithelial integrity was significantly damaged. In addition, we found that a large number of inflammatory factors and chemokines were produced in large quantities with the occurrence and development of infection, but although the secretion of inflammatory factors and chemokines in $MPO^{-/-}$ mice increased, the intestinal inflammatory pathological damage was relatively mild in hypoxic mice compared with that in WT mice.In addition, changes in cytokine levels in IBD patients deserve attention. IL-1β, TNF-α and IFN-γ participate in and aggravate the progression of IBD. In the NF-κB pathway, the lack of MPO inhibits the activation of NF-κB after inhibiting ROS production, resulting in

a decrease in the mRNA transcription of IL-1β, TNF-α and IFN-γ [35].

In conclusion, in the *C. rodentium* -induced colitis mouse model, MPO can not only affect bacterial clearance but also participate in pathological damage to tissues. On the one hand, MPO may have a direct effect itself, and on the other hand, MPO may modulate histopathological damage through the ROS pathway.

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WZ contributed to the research design. XG performed most of the experimental work, acquired and analysed the results, and wrote the first draft of the manuscript. QZ, YH, RJ, YZ, and XG performed the experimental work and analysed the results. WZ and YZ designed, supervised, and interpreted the experimental data. WZ critically revised the manuscript. XG and WZ wrote the final version of the manuscript. All listed authors agreed to be an author.

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Disclosure of interest

The authors declare that they have no competing interests.

Ethics approval and consent to participate

This study was approved by the Ethics Committee of the Medical College of Qinghai University.

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Tables

Table 1 Scores of intestinal tract inflammations.

Inflammatory cells	Score 1	damage	S
Normal	0	normal	0
Scattered	1	minimal inflammation and colonic crypt hyperplasia	1
Increased	2	mild colonic crypt hyperplasia with or without focal invasion of the epithelium	2
Confluence	3	obvious colonic crypt hyperplasia, invasion of the epithelium, and goblet cell depletion	3
Transmural extension	4	extensive mucosal damage and extension to deeper structures of the bowel wall	4

Table 2. Primers used in this study

Primer	Sequence (5'–3')
TNF-α	Sense primer: GTGGAACTGGCAGAAGAGGC
	Antisense primer: CACAAGCAGGAATGAGAAGAGG
IL-1β	Sense primer: TTGACCTGGGCTGTCCTGAT
	Antisense primer: TGAGTGATACTGCCTGCCTGAA
IFN-γ	Sense primer: AAGTGGCATAGATGTGGAAGAAAA
	Antisense primer: GTTGCTGATGGCCTGATTGTC
MCP1/CCL2	Sense primer: TGCATCTGCCCTAAGGTCTTC
	Antisense primer: AGTGCTTGAGGTGGTTGTGGA
MIP2/CXCL2	Sense primer: CACCAACCACCAGGCTACAG
	Antisense primer: GCTTCAGGGTCAAGGCAAACT
KC/CXCL1	Sense primer: ACCCAAACCGAAGTCATAGC
	Antisense primer: ACAGGTGCCATCAGAGCAGT
GAPDH	Sense primer: CTCCACTCACGGCAAATTCA
	Antisense primer: ATACTCAGCACCGGCCTCAC

Figures

Figure 1 . Changes in the body weight of mice in each group.

The WT normoxia control group (NC), MPO-/- normoxia control group (KC), WT C. rodentium infection group (NI), MPO-/- C. rodentium infection group (KI)**,** WT hypoxia control group (HC), MPO-/- hypoxia control group (HKC), WT hypoxia C. rodentium-infected group (HI) and MPO-/- hypoxia C. rodentium-infected group (HKI).

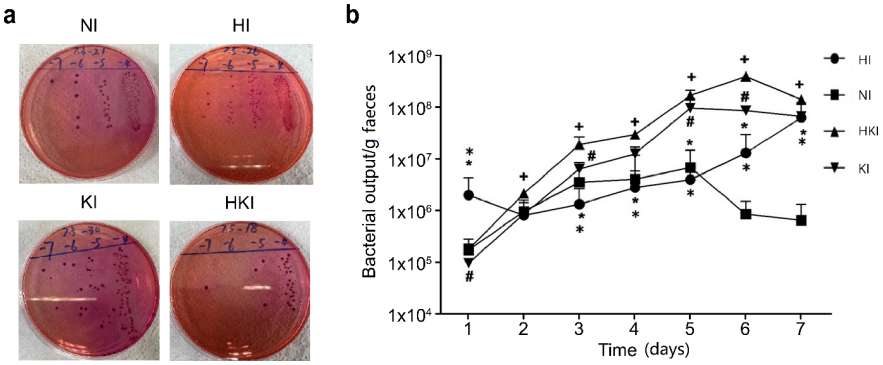

Figure 2 a: 24 h results of mouse faecal smear culture. b: The number of bacteria excreted by mice in each group . The WT normoxia control group (NC), MPO-/- normoxia control group (KC), WT C. rodentium infection group (NI), MPO-/- C. rodentium infection group **(KI),**WT hypoxia control group (HC), MPO-/- hypoxia control group (HKC), WT hypoxia C. rodentium-infected group (HI) and MPO-/- hypoxia C. rodentium-infected group (**HKI**).

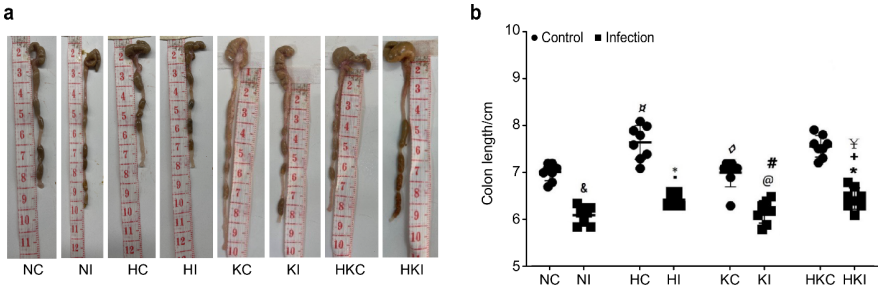

Figure 3 . a: Photographs of the colons of mice in each group. b: Changes in the colon length of mice in each group. The WT normoxia control group (NC), MPO-/- normoxia control group (KC), WT C. rodentium infection group (NI), MPO-/- C. rodentium infection group (KI), WT hypoxia control group (HC), MPO-/- hypoxia control group (HKC), WT hypoxia C. rodentium-infected group (HI) and MPO-/- hypoxia C. rodentium-infected group (HKI).

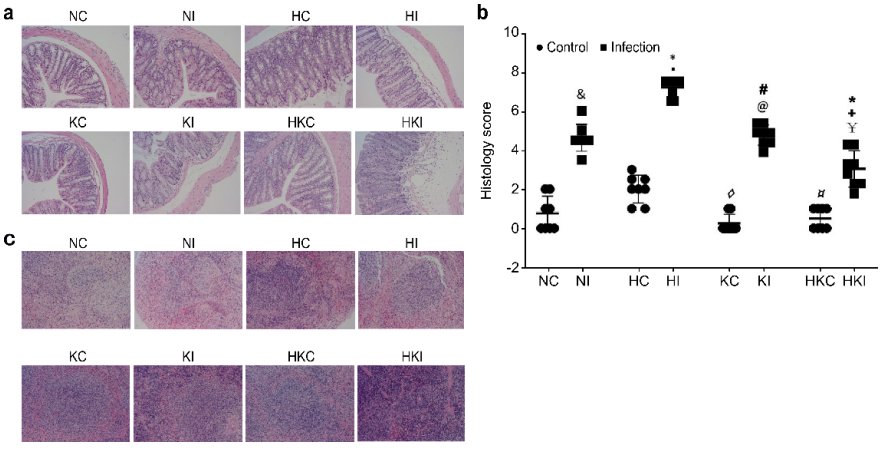

Figure 4 a: Pathological sections of the colons of mice in each group.(20×) b: Results of pathological scoring

of mice in each group. c: Histopathological sections of the spleens of mice in each group. (20×)

The WT normoxia control group (NC), MPO-/- normoxia control group (KC), WT C. rodentium infection group (NI), MPO-/- C. rodentium infection group (KI), WT hypoxia control group (HC), MPO-/- hypoxia control group (HKC), WT hypoxia C. rodentium-infected group (HI) and MPO-/- hypoxia C. rodentium-infected group (HKI).

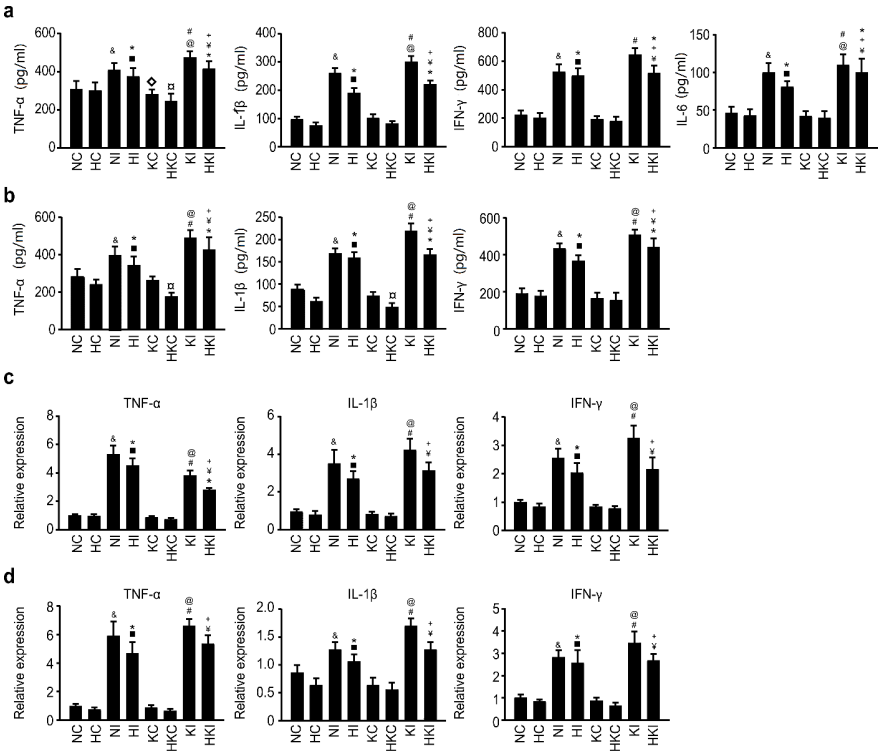

Figure 5 a: Detection of related inflammatory factors in serum by ELISA. b: Detection of related inflammatory factors in the spleen by ELISA.c: Detection of inflammatory factors in the spleen by PCR. d: Detection of inflammatory factors in the colon by PCR. The WT normoxia control group (NC), MPO-/- normoxia control group (KC), WT C. rodentium infection group (NI), MPO-/- C. rodentium infection group (KI), WT hypoxia control group (HC), MPO-/- hypoxia control group (HKC), WT hypoxia C. rodentium-infected group (HI) and MPO-/- hypoxia C. rodentium-infected group (HKI).

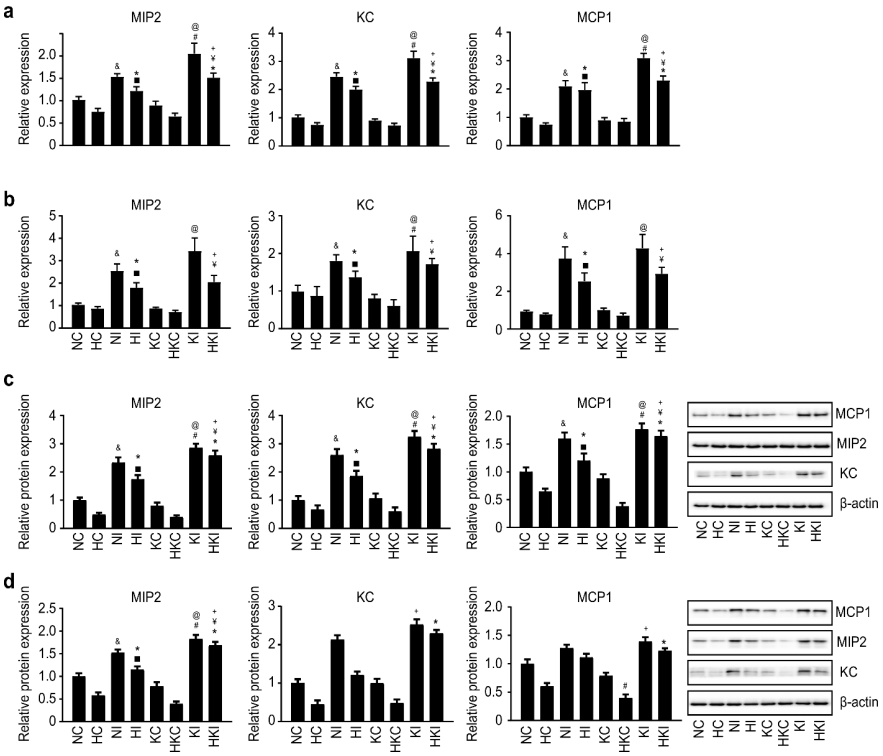

Figure 6 a: Detection of phagocytic chemokines in the spleen by PCR. b: Detection of phagocytic chemokines in the colon.c: Detection of related chemokines in the spleen by WB.

d: Detection of related chemokines in the colon by WB. The WT normoxia control group (NC), MPO-/- normoxia control group (KC), WT C. rodentium infection group (NI), MPO-/- C. rodentium infection group (KI), WT hypoxia control group (HC), MPO-/- hypoxia control group (HKC), WT hypoxia C. rodentium-infected group (HI) and MPO-/- hypoxia C. rodentium-infected group (HKI).

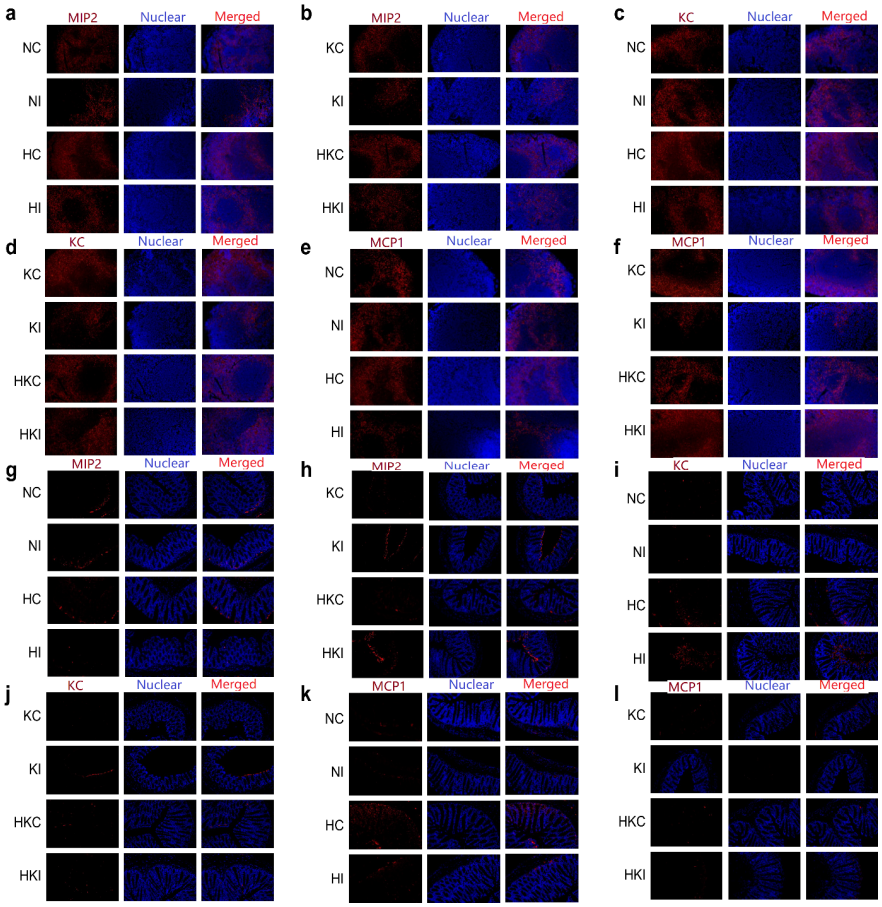

Figure 7 Results of immunofluorescence staining for chemokines in the spleen and colon of mice in each group.(200×). The WT normoxia control group (NC), MPO-/- normoxia control group (KC), WT C. rodentium infection group (NI), MPO-/- C. rodentium infection group (KI), WT hypoxia control group (HC), MPO-/- hypoxia control group (HKC), WT hypoxia C. rodentium-infected group (HI) and MPO-/- hypoxia C. rodentium-infected group (HKI).

Figure captions

Figure 1 . Changes in the body weight of mice in each group (mean±SD, n= 8/group; &P_i 0.05 NI vs. NC; @P<0.05 KI vs. KC; ¥P<0.05 HKI vs. HKC; +P<0.05 KI vs. NI; #P<0.05 HI vs. HC; *P<0.05 HKI vs. HI).

Figure 2 . a: Twenty-four-hour results of mouse faecal smear culture. *C. rodentium* grew well on MacConkey medium.

b: The number of bacteria excreted by mice in each group (mean ± SD, n= 8/group; *P¡0.05 vs. NI; #P$_i$ 0.05 vs. KC; +P$_i$ 0.05 vs. KI; *P$_i$ 0.05 vs. HKI).

Figure 3 . a: Photographs of the colons of mice in each group. Compared with that in the normal group, the colon morphology of the mice in the infected group changed to varying degrees; the main symptoms were shortening, and individual mice had colon thinning and ulceration.

b: Changes in the colon length of mice in each group (mean±SD, n= 8/group; &P_i 0.05 vs. NC; P_i 0.05 vs. HC; *P<0.05 vs. NI; @P_i 0.05 vs. KI; ¥P<0.05 vs. HKC;+P<0.05 vs. KI; ***P$_i$** 0.05 vs. NC;¤P_i 0.05 vs. HC; #P<0.05 vs. NI; *P<0.05 vs. HI).

Figure 4 . a: Pathological sections of the colons of mice in each group. According to the comparison, the intestinal mucosal tissue damage in the HI and KI groups was more serious, and the main symptoms were thinning of the intestinal wall, becoming almost transparent, with more severe oedema, disordered villi with different lengths, and not only shallower but also a reduced number of crypts. These alterations were accompanied by a large amount of inflammatory cell infiltration.(20×)

b: Results of pathological scoring of mice in each group. Compared with those of the NI and HI groups, the pathological results of the infection group were more significant with hypoxia ($P < 0.05$), indicating that hypoxia can aggravate the mouse pathological lesions of the colon (mean±SD, n= 8/group; & P_j 0.05 vs. NC; P<0.05 vs. HC; *P<0.05 vs. NI; @P ¡0.05 vs. KC; ¥P<0.05 vs. HKC;+P<0.05 vs. KI; ***P_j** 0.05 vs. NC; ¤P_j 0.05 vs. HC; #P<0.05 vs. NI; *P<0.05 vs. HI).

C: Histopathological sections of the spleens of mice in each group.

Figure 5 . a: Detection of related inflammatory factors in serum by ELISA (mean±SD, n= 9/group; &P_j 0.05 vs. NC; P_j 0.05 vs. HC; *P<0.05 vs. NI; @P_j 0.05 vs. KI; ¥P<0.05 vs. HKC; +P<0.05 vs. KI;***P_j** 0.05 vs. NC; ¤P_j 0.05 vs. HC; #P<0.05 vs. NI; *P<0.05 vs. HI).

b: Detection of related inflammatory factors in the spleen by ELISA (mean±SD, n=3/group; &P_j 0.05 vs. NC; P_j 0.05 vs. HC; *P<0.05 vs. NI; @P_j 0.05 vs. KI; ¥P<0.05 vs. HKC; +P<0.05 vs. KI; ¤P_j 0.05 vs. HC; #P<0.05 vs. NI; *P<0.05 vs. HI).

c: Detection of inflammatory factors in the spleen by PCR (mean±SD, n=3/group; &P_j 0.05 vs. NC; P_j 0.05 vs. HC; *P<0.05 vs. NI; @P_j 0.05 vs. KI; ¥P<0.05 vs. HKC; +P<0.05 vs. KI; #P<0.05 vs. NI; *P<0.05 vs. HI).

d: Detection of inflammatory factors in the colon by PCR (mean±SD, n=3/group; &P_j 0.05 vs. NC; P_j 0.05 vs. HC; *P<0.05 vs. NI; @P_j 0.05 vs. KI; ¥P<0.05 vs. HKC; +P<0.05 vs. KI; #P<0.05 vs. NI; *P<0.05 vs. HI).

Figure 6 . a: Detection of phagocytic chemokines in the spleen by PCR (mean±SD, n=3/group; &P_j 0.05 vs. NC; P_j 0.05 vs. HC; *P<0.05 vs. NI; @P_j 0.05 vs. KI; ¥P<0.05 vs. HKC; +P<0.05 vs. KI; #P<0.05 vs. NI; *P<0.05 vs. HI).

b: Detection of phagocytic chemokines in the colon (mean±SD, n=3/group; &P_j 0.05 vs. NC; P_j 0.05 vs. HC; *P<0.05 vs. NI; @P_j 0.05 vs. KI; ¥P<0.05 vs. HKC; +P<0.05 vs. KI; #P<0.05 vs. NI; *P<0.05 vs. HI).

c: Detection of related chemokines in the spleen by WB (mean±SD, n=3/group; &P_j 0.05 vs. NC; P_j 0.05 vs. HC; *P<0.05 vs. NI; @P_j 0.05 vs. KI; ¥P<0.05 vs. HKC; +P<0.05 vs. KI; #P<0.05 vs. NI;*P<0.05 vs. HI).

d: Detection of related chemokines in the colon by WB (mean±SD, n=3/group; &P_j 0.05 vs. NC; P_j 0.05 vs. HC; *P<0.05 vs. NI; @P_j 0.05 vs. KI; ¥P<0.05 vs. HKC; +P<0.05 vs. KI; #P<0.05 vs. NI; *P<0.05 vs. HI).

Figure 7 .: Results of immunofluorescence staining for chemokines in the spleen and colon of mice in each group (protein fluorescence, nuclear fluorescence, and merged coincidence signals,200x). A: MIP2 for WT-spleen. B: MIP2 for $MPO^{-/-}$-spleen. C: KC for WT-spleen. D: KC for $MPO^{-/-}$-spleen. E: MCP1 for WT-spleen. F: MCP1 for $MPO^{-/-}$-spleen. G: MIP2 for WT-colon. H: MIP2 for $MPO^{-/-}$-colon. I: KC for WT-colon. J: KC for $MPO^{-/-}$-colon. K: MCP1 for WT-colon. L: MCP1 for $MPO^{-/-}$-colon.