


The Deletion of IL-17A Enhances *Helicobacter hepaticus* Colonization and Triggers Colitis

Liqi Zhu^{1,2}, Zhihao Wu^{1,2}, Chen Zhu^{1,2}, Jun Yin^{1,2}, Yuzheng Huang^{3,4}, Jie Feng⁵, Quan Zhang^{1,2} 

¹Institute of Comparative Medicine, College of Veterinary Medicine, Yangzhou University, Yangzhou, Jiangsu, 225009, People's Republic of China; ²Jiangsu Co-Innovation Center for Prevention and Control of Important Animal Infectious Diseases and Zoonoses, Yangzhou University, Yangzhou, Jiangsu, 225009, People's Republic of China; ³National Health Commission Key Laboratory of Parasitic Disease Control and Prevention, Jiangsu Provincial Key Laboratory on Parasite and Vector Control Technology, Jiangsu Institute of Parasitic Diseases, Wuxi, Jiangsu Province, 214064, People's Republic of China; ⁴Public Health Research Center, Jiangnan University, Wuxi, Jiangsu Province, 214122, People's Republic of China; ⁵Shanghai Laboratory Animal Research Center, Shanghai Quality Monitoring Center of Laboratory Animals, Shanghai, 201203, People's Republic of China

Correspondence: Quan Zhang, Institute of Comparative Medicine, College of Veterinary Medicine, Yangzhou University, Yangzhou, People's Republic of China, Tel +86 138-1584-1244, Email zquan@yzu.edu.cn

Objective: IL-17 is a key regulator of the inflammatory response, and as such, it is involved in the constraint and clearance of pathogens. The mechanism of IL-17 in the pathogenesis of inflammatory bowel disease (IBD) caused by microbial infection is still unclear. *Helicobacter hepaticus* infection can induce colitis in many mouse strains, and thus, it has been widely used in the study of IBD pathogenesis.

Methods: In this study, male C57BL/6, BALB/c, IL-10^{-/-}, and IL-17a^{-/-} mice were infected with *H. hepaticus* for several weeks. Histopathology, *H. hepaticus* colonization and distribution, expression of inflammatory cytokines and lysozyme, and distribution of mucus in proximal colon were examined.

Results: The colonic colonization of *H. hepaticus* was abnormally high in IL-17a^{-/-} mice. *H. hepaticus* infection caused only mild to moderate colitis symptoms in IL-17a^{-/-} mice, including low levels of lymphocyte infiltration, epithelial cell defects, goblet cell reduction, and crypt atrophy without obvious hyperplasia in the later stage of infection. Furthermore, many inflammatory genes were significantly increased in the proximal colon of *H. hepaticus*-infected IL-17a^{-/-} mice compared with C57BL/6 mice. In addition, the reduction of colonic mucus and the down-regulation of ZO-1, Claudin-1, and IL-22 were observed in IL-17a^{-/-} mice compared with C57BL/6 mice post *H. hepaticus* infection.

Conclusion: These results demonstrated that the deletion of IL-17A impaired the integrity of the intestinal epithelium, weakened the secretion of mucus, attenuated colonic mucosal regeneration, reduced the ability to resist microbial infection, and finally led to colitis caused by *H. hepaticus*.

Keywords: *Helicobacter hepaticus*, IL-17A, colitis, crypt atrophy

Introduction

Inflammatory bowel disease (IBD), including Crohn's disease, and ulcerative colitis, seriously damages human health and creates a huge medical burden on society. IBD is a complex disease which caused by a variety of endogenous and exogenous factors including genetic mutation, microbiota dysbiosis, and stress.¹⁻³ *Helicobacter hepaticus* belongs to Proteobacteria, ϵ -proteobacteria, and Heliobacterium, bacteria that infect rodents such as mice and rats.^{4,5} In susceptible strains of mice such as nude, IL-10^{-/-}, Rag2^{-/-}, and A/JCr, *H. hepaticus* infection can induce colitis, hepatitis, and even liver and breast cancer.⁶ An *H. hepaticus* infection model has been widely used in the study of pathogenesis of IBD, especially for studying the inflammatory response and immune tolerance related to intestinal microorganisms.

In general, the abnormal continuous stimulation of intestinal flora can alter the intestinal immune homeostasis and lead to intestinal inflammation. For example, the flagellin of segmented filamentous bacteria can induce Th17 cell aggregation in the lamina propria of the intestine and promote IL-6 and IL-17 expression, leading to alterations of the intestinal immune environment.⁷

IL-17, usually referred to as IL-17A, was first isolated in 1993.⁸ There are six members of the IL-17 family, namely, IL-17A–IL-17F. IL-17 can robustly recruit and activate immune cells that cooperate with a variety of pro-inflammatory factors such as TNF and IFN- γ and finally mediate the inflammatory response.⁹ In the acute inflammatory response, IL-17 acts on non-myeloid cells to induce chemokines and further recruits concentrated granulocytes to act on damaged or infected tissues.¹⁰ Furthermore, IL-17 also plays an important role in maintaining immune barrier function. It has been reported that IL-17 can induce a variety of immune cells to express IL-22, an interleukin that promotes crypt proliferation and the production of antimicrobial peptides;^{11,12} IL-17 can also induce the expression of Claudin-1 and Claudin-2 in epithelial cells, thus promoting the formation of tight junctions of intestinal epithelial cells and accelerating the repair of the epithelial barrier.¹³ However, the function and mechanism of IL-17 in *H. hepaticus* infection have not been clearly characterized.

In this study, we investigated whether *H. hepaticus* infection in Il-17a^{-/-} mice caused typical colitis. By comparing the degree of enteritis and the expression of IL-17A in Il-10^{-/-}, BABL/c, and C57BL/6 mice infected with *H. hepaticus*, we found that the expression of IL-17A had a certain relationship with the severity of enteritis, and typical colitis was found in the *H. hepaticus*-infected Il-17a^{-/-} mice. Specifically, the expression of tight junction genes, secretion of mucus, and development of colon glands and crypts were all inhibited in the infected Il-17a^{-/-} mice. Particularly, the expression of IL-22 and the number of Paneth cells were significantly decreased in the infected Il-17a^{-/-} mice, factors that may be the main cause of severe colitis. Taken together, our findings imply that the upregulation of IL-17A induced by *H. hepaticus* may suppress colitis via promoting epithelial repair and crypt development.

Materials and Methods

Mice

Il-10^{-/-} mice (B6.129P2-IL10tm1Cgn/J) were purchased from Jackson Laboratory. Il-17a^{-/-} mice (B6.129 (SJL)-Il17atm1.1 (icre) Stck/RthsnJ) were gifted by prof. Wang Chengming of Yangzhou University. Wild type C57BL/6 and BABL/c mice were purchased from GemPharmatech Co., Ltd (Nanjing, China). All mice were bred and maintained in an accredited specific pathogen-free facility, and in vivo experiments were conducted in accordance with the China laboratory Act (2017) under a Project License (SYXK (su) 2017–0044) authorized by Jiangsu Provincial Science and Technology Department and approved by Institutional Animal Care and Use Committee (IACUC) of Yangzhou University. The mice were free of *Helicobacter* species as assessed by PCR as previously described.¹⁴

Bacterial Culture and Collection

H. hepaticus 3B1 (ATCC 51449) were purchased from ATCC (Maryland, USA), and were cultured on Brucella agar plates (BD, USA) supplemented with 5% defibrinated sheep blood and antibiotics for 4–5 days under microaerobic conditions (85% N₂, 10% CO₂, 5% O₂) at 37°C. Bacteria were harvested in PBS and used for oral infection when OD₆₀₀ reading was 1.¹⁵

Animal Experiment 1

Thirty 4-week-old *H. hepaticus* negative BALB/c, Il10^{-/-} and wild type C57BL/6 male mice were used, respectively. Then, each strain mice were randomly divided into 2 groups. All mice were given gentamicin orally once. On the second day, 0.1 mL (OD₆₀₀≈1) *H. hepaticus* or Brucella broth were administration by gavage. Three days later, intragastric administration of *H. hepaticus* or Brucella broth was performed again. Then, after 4 days, the feces of each mouse were collected to detect *H. hepaticus* colonization. Five mice in each group were scarified at 2, 4 and 8 weeks after confirmation of *H. hepaticus* colonization, and colon samples were collected to detect the amount of *H. hepaticus* colonization and mouse enteritis.

Animal Experiment 2

Fifty 4-week-old *H. hepaticus* negative Il-17a^{-/-} and wild type C57BL/6 male mice were randomly divided into 2 groups, respectively. The method of infecting mice with *H. hepaticus* was the same as that in animal experiment 1. Five mice in

each group were scarified at 2, 4, 8, 12 and 16 weeks after *H. hepaticus* colonization, and the colon length, the amount of *H. hepaticus* colonization, and the degree of enteritis were detected respectively.

DNA Extraction and PCR Analysis

Bacterial DNA was extracted from colon tissue according to manufacturer's instructions using the TIANamp Bacteria DNA kit (Tiangen, China). Abundance of *H. hepaticus* in colon was determined according to *HH1450* gene primers, Hs-16s-F1 and Hs-16s-R1 (see as in Table 1), by qPCR using AceQ Universal SYBR qPCR Master Mix (Vazyme, China) in the Applied Biosystems StepOne Real-Time PCR System (ABI, US)¹⁶. Briefly, to quantify the DNA copy number of *H. hepaticus*, the colon tissue DNA (diluted to 10 ng/μL) was used as a template for Real-time PCR assay in the Applied Biosystems StepOne Real Time PCR System using AceQ Universal SYBR qPCR Master Mix. Serial dilutions of *H. hepaticus* DNA, including 2×10^7 , 2×10^6 , 2×10^4 , 2×10^2 , 2×10^1 , and 2 fg, were used to generate a standard curve.

Histopathology

During necropsy, colon tissues of each animal were fixed in 4% paraformaldehyde for 24h, and proximal, middle and distal part embedded in paraffin for microscopy examination respectively. Tissue sections (4 μm) were stained with H&E and Alcian blue. Histological scores were assessed as previously described based on the degree of inflammatory infiltrate in the mucosa, submucosa and muscularis/serosa, epithelial damage, crypt atrophy and submucosal hyperplasia.¹⁷

Immunohistochemistry Analysis

The methods of immunohistochemistry test and analysis were graded as described previously.¹⁸ In brief, the paraffin sections of intestinal tissues in each group were soaked in xylene, ethanol and PBS in turn. Then, antigen repair was performed with EDTA-citrate solution. Then, Rabbit anti-HMGB1 antibody (Abcam, China), Rabbit anti-Lysozyme

Table 1 Primer sequences used for RT-qPCR

| Gene Symbol | Sequence (5'-3') | Product Size (bp) |
|-------------|--------------------------|-------------------|
| GAPDH-F | AGGTCGGTGTGAACGGATTTG | 123 |
| GAPDH-R | TGTAGACCATGTAGTTGAGGTCA | |
| IL6-F | CCAAGAGGTGAGTGCTTCCC | 76 |
| IL6-R | CTGTTGTTCACTCTCTCCCT | |
| NOS2-F | GTTCTCAGCCCAACAATAACAAGA | 127 |
| NOS2-R | GTGGACGGGTCGATGTCAC | |
| TNF-F | GACGTGGAAGTGGCAGAAGAG | 228 |
| TNF-R | TTGGTGGTTTGTGAGTGTGAG | |
| IL17A-F | TTTAACTCCCTTGGCGCAAAA | 165 |
| IL17A-R | CTTCCCTCCGCATTGACAC | |
| IL23A-F | ATGCTGGATTGCAGAGCAGTA | 213 |
| IL23A-R | ACGGGGCACATTATTTTAGTCT | |
| MMP9-F | CTGGACAGCCAGACACTAAAG | 145 |
| MMP9-R | CTCGCGGCAAGTCTTCAGAG | |
| IL22-F | ATGAGTTTTTCCCTTATGGGGAC | 124 |
| IL22-R | GCTGGAAGTTGGACACCTCAA | |
| IL10-F | GCTCTTACTGACTGGCATGAG | 105 |
| IL10-R | CGCAGCTCTAGGAGCATGTG | |
| MUC1-F | GGCATTCTGGGCTCCTTTCTT | 132 |
| MUC1-R | TGGAGTGGTAGTCGATGCTAAG | |
| ZO1-F | GCCGCTAAGAGCACAGCAA | 134 |
| ZO1-R | TCCCCACTCTGAAAATGAGGA | |
| CLDN1-F | GGGGACAACATCGTGACCG | 100 |
| CLDN1-R | AGGAGTCGAAGACTTTGCACT | |

antibody (Abcam) or Rabbit anti-*H. hepaticus* polyclonal antibody was added and incubated at 4 °C overnight. After treatment with biotin labeled secondary antibody (Boster, China), HRP labeled streptavidin and DAB solution (Boster), the sections were observed with Leica DM1000 microscope (Leica, Germany), and the area and gray value of positive cells were analyzed with Image Pro Plus software. The anti-*H. hepaticus* polyclonal antibody was prepared by ourself. Briefly, *H. hepaticus* was cultured and collected; Then, sheep blood cells were removed by low-speed centrifugation and soluble proteins were washed by high-speed centrifugation; After that, formaldehyde was added to inactivate the bacteria; Lately, the inactivated *H. hepaticus* was used to immunize rabbits; Finally, the anti- *H. hepaticus* antibody was purified from serum by protein A/G beads.

Detection of SOD Concentration

The SOD concentration in colon tissue of mice was measured by SOD activity detection kit (Beyotime, China). At the same time, the protein concentration was determined using BCA protein concentration assay kit (Beyotime) and used to calibrate SOD concentration.

Cytokines Quantification by Real-Time RT-PCR

Total RNA was extracted from proximal colon tissue trituration in liquid nitrogen using Trizol (Invitrogen, US). cDNA was synthesized from 1 µg total RNA using Prime Script RT reagent Kit with gDNA Eraser (Takara, China). The mRNA transcription levels were amplified by qPCR using the Universal SYBR Green master and mRNA level of the GAPDH gene in each cDNA sample was measured and used for normalization. The qPCR primers (see as in Table 1) were synthesized by Shanghai Shenggong Biotech Co., Ltd. All samples were run in triplicate. Relative expression levels were calculated using $2^{-\Delta\Delta CT}$ method, as previously described.¹⁹

Statistical Analysis

Statistical analysis was performed using SPSS 18.0 software. Pathological scores were analyzed using a Mann–Whitney nonparametric *U*-test. *H. hepaticus* colonization, colon length, gene expression, SOD value, and IHC positive area were analyzed using one-way ANOVA and Tukey HSD test. Figures were drawn using GraphPad 8.0 software.

Results

H. hepaticus Colonization Correlated with Colitis

The difference in the degree of inflammatory response caused by *H. hepaticus* infection in different strains of mice has not been fully explained. Our previous report⁶ confirmed that hepatitis, liver fibrosis and mild chronic enteritis were induced by *H. hepaticus* infection in BALB/c mice. However, it was difficult to observe the inflammation in liver or colon from C57BL/6 mice infected with *H. hepaticus*. We suspect that the content of *H. hepaticus* in colon may be an important reason for the great difference in colon pathological changes among different mice strains. Therefore, we examined inflammation, *H. hepaticus* colonization, IL-17 levels, and expression of other inflammatory factors in the colon from IL-10^{-/-}, BALB/c, and C57BL/6 mice with or without *H. hepaticus* infection. It is not surprising that IL-10^{-/-} mice infected with *H. hepaticus* showed typical symptoms of colitis. Only mild inflammation and crypt atrophy were found in BALB/c mice infected with *H. hepaticus*. In contrast, there were no pathological changes in the colon of C57BL/6 mice infected with *H. hepaticus* or in uninfected controls (Figure 1A and B). Similar to the pathological analysis, colon length was significantly shortened in IL-10^{-/-} mice, slightly shortened in BALB/c mice, but unaffected in C57BL/6 mice at 8 weeks post infection (WPI) (Figure 1C). The weight gain was also significantly inhibited from 4 WPI in IL-10^{-/-} mice and slowed slightly after 7 WPI in BALB/c mice (Figure 1D). Corresponding to the pathological analysis, the expression levels of IL-6, TNF, iNOS, and IL-17 also showed a similar trend (Figure 1E–H). In addition, the colonization of *H. hepaticus* in the colon was the highest in IL-10^{-/-} mice, followed by that in BALB/c mice, and was the lowest in C57BL/6 mice (Figure 1I). These results suggested that *H. hepaticus* infection could promote the expression of a variety of cytokines, including IL-17, that were related to the symptoms of colitis in different strains of mice.

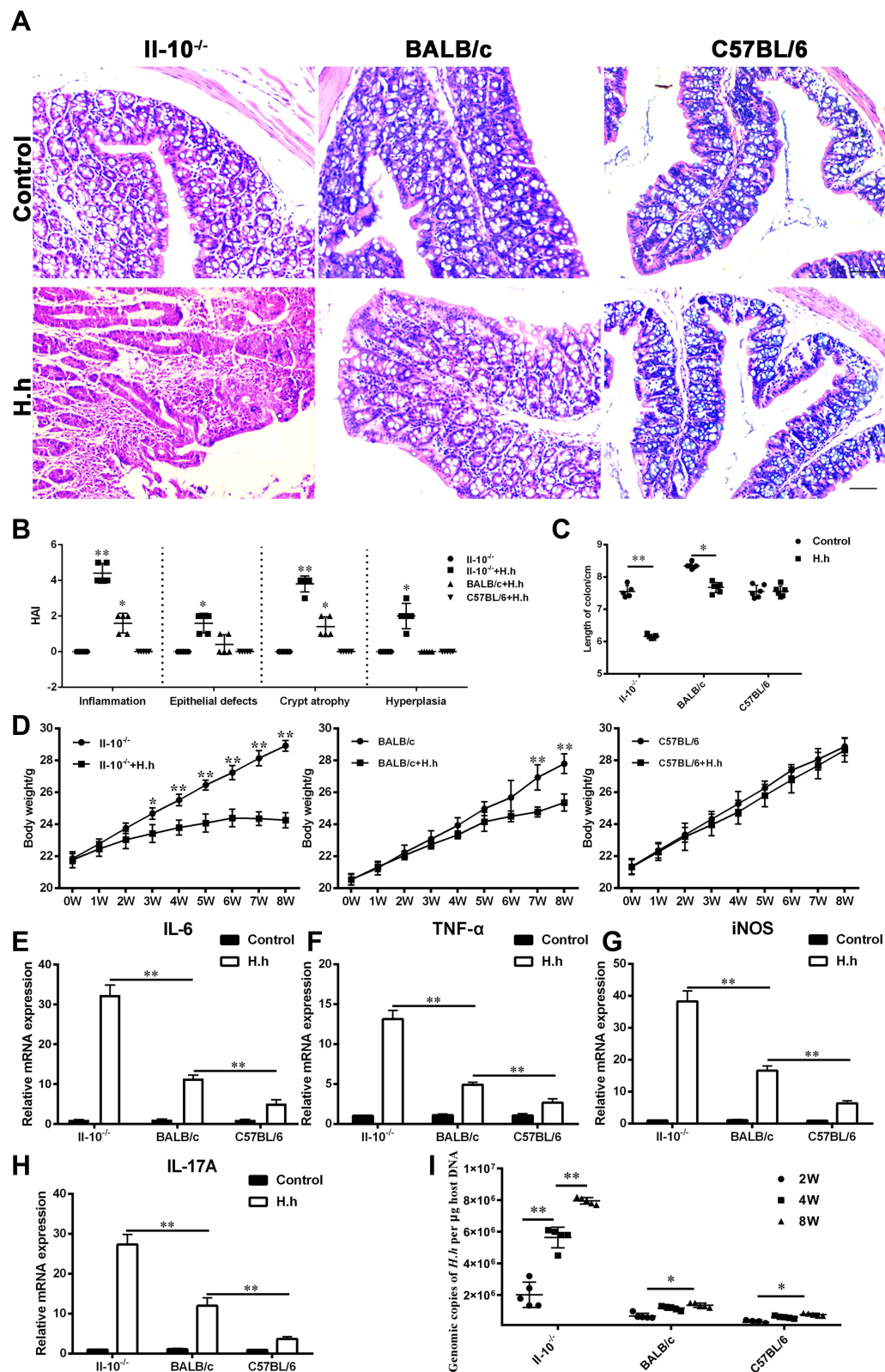


Figure 1 *H. hepaticus* colonization correlated with colitis. **(A)** Pathological analysis of H&E staining in the proximal colon of II10^{-/-}, BALB/c and C57BL/6 mice infected with or without *H. hepaticus* at 8 weeks, bar = 20 μm. **(B)** The statistics of pathological according to **(A)**. **(C)** The colon length of II10^{-/-}, BALB/c and C57BL/6 mice infected with or without *H. hepaticus* at 8 weeks. The difference analysis was carried out between the two groups of the same strain at the same time. **(D)** The body weight of II10^{-/-}, BALB/c and C57BL/6 mice infected with or without *H. hepaticus* from 0 to 8 weeks. **(E–G)** and **(I)** The mRNA expression of IL-6, TNF-α, iNOS and IL-17A in colon tissue of II10^{-/-}, BALB/c and C57BL/6 mice infected with or without *H. hepaticus* at 8 weeks. **(H)** The quantitative PCR of *H. hepaticus* 16s rDNA in II10^{-/-}, BALB/c and C57BL/6 mice infected with *H. hepaticus* at 2, 4 and 8 weeks. Data are expressed as the means ± SEM (n = 5/group), ** P < 0.01; * P < 0.05.

H. hepaticus Infection Induced Moderate Colitis in Il-17a^{-/-} Mice

It has been reported that the colitis induced by *H. hepaticus* infection is also associated with the abnormality of IL-17 or Th17 cells,^{20–22} and the colitis caused by *H. hepaticus* infection could be alleviated by blocking IL-17A/F.²³ Therefore, it was necessary to explore whether Il-17a^{-/-} mice infected with *H. hepaticus* would develop colitis. Pathological examination of the colon and statistical analysis of the results (Figure 2A and B) showed that in Il-17a^{-/-} mice, the loss of goblet cells and lymphocytic infiltration in the lamina propria of the colon were not observed precisely until 8 WPI. The mild lamina propria hyperplasia has appeared from 4 to 16 WPI; crypt atrophy and gland reduction were found during 8–16 WPI in Il-17a^{-/-} mice. In addition, the weight gain of Il-17a^{-/-} mice decreased significantly from 5 WPI (Figure 2C); the length of colon also decreased from 4 WPI (Figure 2D). Surprisingly, the colonization of *H. hepaticus* in Il-17a^{-/-} mice was much higher than that in C57BL/6 mice from 2 to 16 WPI (Figure 2E). In addition, the concentration of SOD representing antioxidant capacity in the colon also decreased from 4 WPI (Figure 2F). In general, Il-17a deletion was conducive to the colonization of *H. hepaticus* and promoted colitis in C57BL/6 mice.

H. hepaticus Infection Promoted Inflammatory Factors but Inhibited Lysozyme Expression in the Colon of Il-17a^{-/-} Mice

It is well known that IL17 has a strong ability to recruit inflammatory cells and thereby promote inflammation. Whether IL17 deficiency changes the degree of inflammatory response caused by *H. hepaticus* should be explored. Our results showed that the proinflammatory genes IL-6, IL-23, and TNF- α were sustained at much higher levels in Il-17a^{-/-} mice compared with C57BL/6 mice after *H. hepaticus* infection (Figure 3A–C). The expression levels of iNOS and MMP9 in activated macrophages were also strongly upregulated in Il-17a^{-/-} mice compared with C57BL/6 mice during *H. hepaticus* infection (Figure 3D and E). Unlike pro-inflammatory genes, the expression of IL-10 was only slightly upregulated in Il-17a^{-/-} mice at each point in time (Figure 3F). Moreover, the expression of HMGB1, a late pro-inflammatory protein, gradually increased in the epithelium, lamina propria, and submucosa in Il-17a^{-/-} mice during *H. hepaticus* infection (Figure 3G and I). Lysozyme is expressed in Paneth cells, monocytes, and granulocytes, and it plays an important role in resisting bacterial invasion and defining the composition of mucolytic microbiota.^{24,25} In our results, the lysozyme-positive cells in the proximal colon were mainly located in the lamina propria, and these cells may be monocyte/macrophages^{26,27} and neutrophils²⁸ (Figure 3H and J). Surprisingly, the number of lysozyme-positive cells of the proximal colon was significantly decreased during *H. hepaticus* infection in Il-17a^{-/-} mice, especially at 12 and 16 WPI (Figure 3H and J). Conversely, there were significantly more lysozyme-positive cells in the C57BL/6 mice. These results suggested that *H. hepaticus* promoted the expression of inflammation genes, but inhibited the expression of anti-microbial gene, which may further aggravate the pathological injury to the colon in Il-17a^{-/-} mice.

H. hepaticus Infection Damages the Colon Barrier and Destroys the Function of the Colorectal Gland in Il-17a^{-/-} Mice

Mucus is an important part of the intestinal barrier, a structure that can prevent most bacteria from infecting mucosal epithelial cells. Based on the results of Alcian blue staining, the mucus content and the number of mucus secretory cells were decreased with the prolongation of *H. hepaticus* infection in Il-17a^{-/-} mice; however, these factors were increased in C57BL/6 mice after *H. hepaticus* infection (Figure 4A and B). Similarly, the expression of Mucin-1, a type I transmembrane mucin, was decreased in Il-17a^{-/-} compared with C57BL/6 mice after *H. hepaticus* infection (Figure 4C). The tight junctions and the extracellular matrix are also important components of the intestinal barrier. According to our results, the mRNAs of Claudin-1 and Zo-1, two major tight junction genes, were significantly suppressed in Il-17a^{-/-} mice from 4 weeks after *H. hepaticus* infection, while there was no significant difference between the other groups (Figure 4D and E). In addition, the upregulation of IL-22, a key cytokine regulating crypt growth, induced by *H. hepaticus* was significantly suppressed in Il-17a^{-/-} mice compared with C57BL/6 mice from 2 to 16 WPI (Figure 4F). The colon crypt is not only the basis of maintaining the ability of epithelial regeneration but also the origin of goblet cells and colorectal glands. Moreover, the colonization of *H. hepaticus* was clearly observed in colon crypts of Il-17a^{-/-} mice but was nearly absent in C57BL/6 mice at 16 WPI (Figure 4G). In general, the deletion of IL-17A

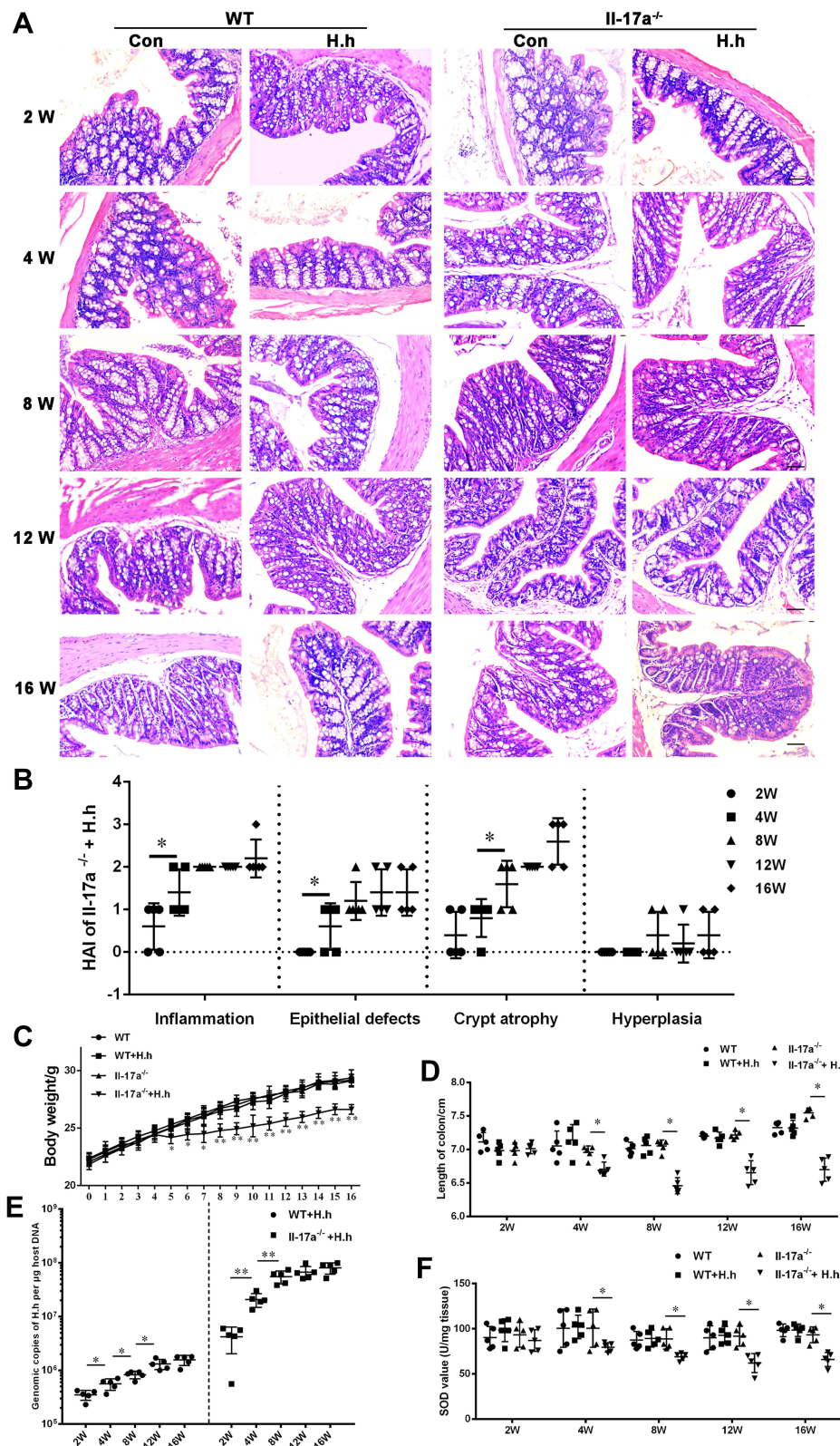


Figure 2 *H. hepaticus* infection induced moderate colitis in IL-17a^{-/-} mice. **(A)** Pathological analysis of H&E staining in the proximal colon of IL-17a^{-/-} and WT mice infected with or without *H. hepaticus* at 2, 4, 8, 12 and 16 weeks, bar = 20 μm. **(B)** The statistics of pathological according to IL-17a^{-/-} mice infected *H. hepaticus* in A. **(C)** The body weight of IL-17a^{-/-} and WT mice infected with or without *H. hepaticus* from 0 to 16 weeks. The difference analysis was carried out between the two groups of the same strain at the same time. **(D)** The colon length of IL-17a^{-/-} and WT mice infected with or without *H. hepaticus* at 2, 4, 8, 12 and 16 weeks. **(E)** The quantitative PCR of *H. hepaticus* 16S rDNA in IL-17a^{-/-} and WT mice infected with *H. hepaticus* at 2, 4, 8, 12 and 16 weeks. **(F)** The SOD value of colon tissue from IL-17a^{-/-} and WT mice infected with *H. hepaticus* or uninfected control at 2, 4, 8, 12 and 16 weeks. Data are expressed as the means ± SEM (n = 5/group), **P < 0.01; *P < 0.05.

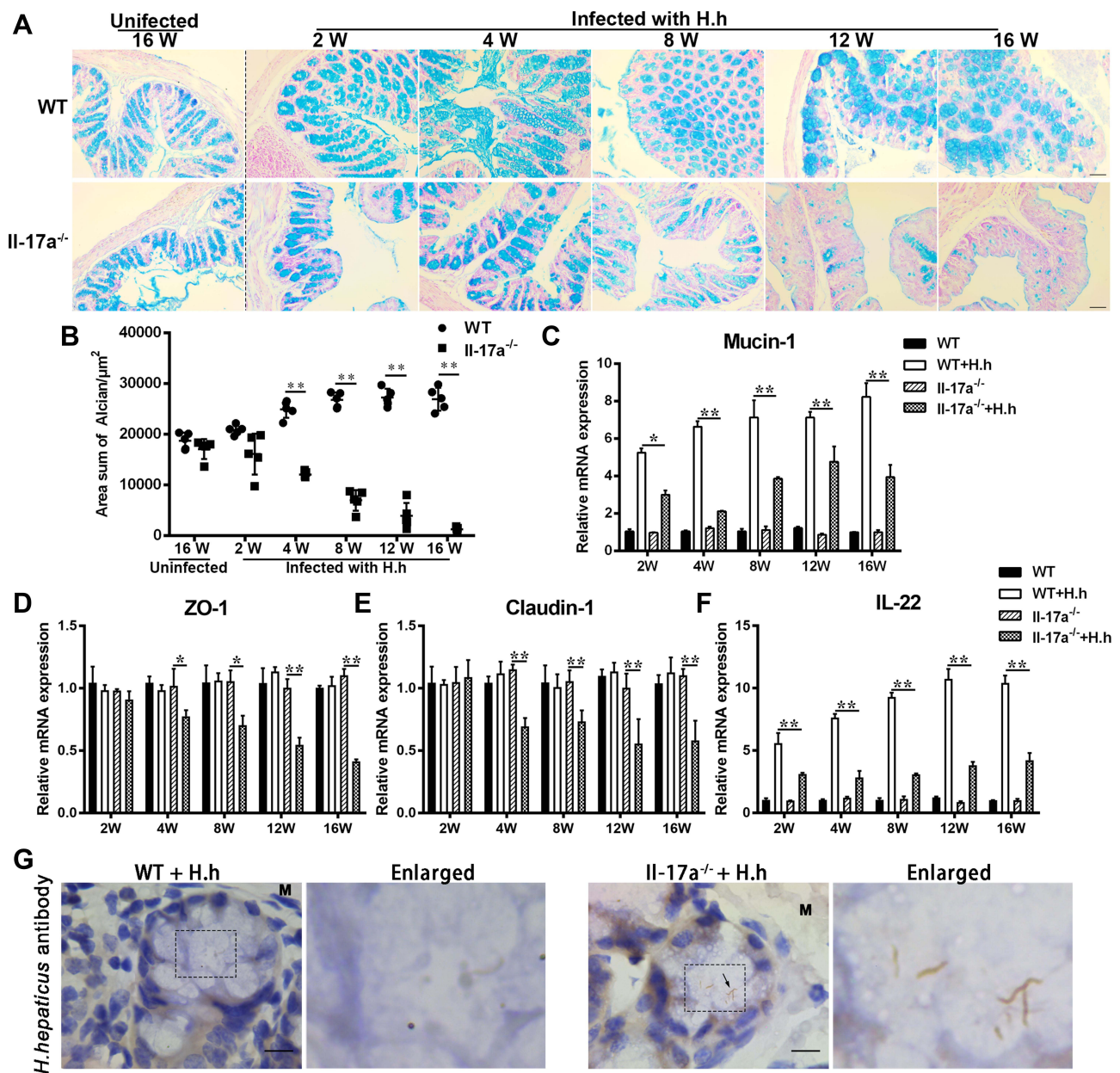


Figure 4 *H. hepaticus* infection damages the colon barrier and destroys the function of the colorectal gland in IL-17a^{-/-} mice. **(A)** Mucus analysis by Alcian blue staining in the proximal colon of IL-17a^{-/-} and WT mice infected *H. hepaticus* at 2, 4, 8, 12 and 16 weeks, bar = 20 μm . **(B)** The statistics of Alcian blue positive area according to **(A)**. **(C–F)** The mRNA expression of Mucin-1, ZO-1, Claudin-1, and IL-22 in colon tissue of IL-17a^{-/-} and WT mice infected with or without *H. hepaticus* at 2, 4, 8, 12 and 16 weeks. **(G)** The IHC analysis of *H. hepaticus* antibody in the proximal colon of IL-17a^{-/-} and WT mice infected *H. hepaticus* at 16 weeks. The black dashed box area was enlarged to 4 times and displayed on the right. M represented muscularis, and the arrow indicated *H. hepaticus*, bar = 10 μm . Data are expressed as the means \pm SEM ($n = 5/\text{group}$), ** indicates significance at the 0.01 level, and * indicates significance at the 0.05 level.

impaired the integrity of the intestinal epithelium, weakened the secretion of mucus, attenuated the regeneration ability of the intestine, reduced the ability to resist microbial infection, and finally led to colitis after infection by *H. hepaticus*.

Discussion

The mouse model of chronic colitis caused by *H. hepaticus* infection has been widely studied and applied. Although *H. hepaticus* can infect a variety of rodents, not all strains of mice present the disease. C57BL/6 mice infected with *H. hepaticus* usually have no obvious symptoms, which may be related to the significant increase of *H. hepaticus*-specific iTreg cells.¹⁵ In addition, the large polysaccharides secreted by *H. hepaticus* could induce anti-inflammatory cytokines in

macrophages via the TLR2/MSK/CREB signaling pathway and accelerate the proliferation of intestinal stem cells in crypts to maintain the homeostasis of the colonic barrier.²⁹ The *H. hepaticus* infected Il-10^{-/-}, Rag1^{-/-}, or Rag2^{-/-} and other immunodeficient or genetically engineered mice could present different degrees of chronic colitis symptoms similar to those of human IBD. In most cases, the colonic symptoms in the early stages of *H. hepaticus* infection were mild. With the extension of infection time, the colonization of *H. hepaticus* in the colon increased significantly, and the symptoms of colitis gradually became serious. Our previous study³⁰ has also confirmed that the colonization of *H. hepaticus* is correlated with the severity of colitis in Il-10^{-/-} mice. Our results showed that various inflammatory cytokines in the colon were upregulated in the three strains of mice infected with *H. hepaticus* compared with mock infection mice (Figure 1A). Interestingly, most inflammatory cytokines, including IL-17A, showed similar trends in *H. hepaticus* infection groups. These results suggest that *H. hepaticus* can promote the basic expression levels of inflammatory cytokines in the colon, and this may increase the risk of colitis.

It is believed that an abnormal colorectal expression level of IL-17 is associated with chronic IBD and colorectal cancer.^{5,31,32} Abnormal IL-17 expression and signal transduction are closely associated with anomalous pathological response during microbial infection.³³ IL-17A is the most representative cytokine in the IL-17 family. IL-17 can induce the expression of chemokines, inflammatory cytokines, acute phase reactive proteins, and antibacterial proteins after binding to target cells and thereby indirectly regulate the intestinal microbiota.^{34,35} In our results, the symptoms of colitis caused by *H. hepaticus* were mild, with a low degree of lymphocyte infiltration in the submucosa, goblet cell reduction in the mucosal epithelium, and crypt atrophy in Il-17a^{-/-} mice. Notably, the colonization level of *H. hepaticus* was much higher (about 6×10⁷ copies/μg host DNA) in Il-17a^{-/-} mice than the level (about 8×10⁶ copies/μg host DNA) in Il-10^{-/-} mice at 8 WPI (Figures 1H and 2F). The higher colonization level of *H. hepaticus* in Il-17a^{-/-} mice may be related to the impairment of antibacterial ability on account of the absence of IL-17A. Although the intestinal flora was destroyed before *H. hepaticus* infection by the one-time oral high-dose gentamicin, some drug-resistant bacteria could still exist in the colonic mucosa, which may have an uncertain impact on the colonization of *H. hepaticus*. Therefore, the difference of colonic flora should be considered when analyzing the colonic colonization ability of *H. hepaticus*. Ideally, cohabitation with three strains of mice is the most rigorous approach to eliminating the interference of intestinal microbiota differences on foreign microbial colonization.

IL-17 plays a vital role in host defense against microbial infection through inducing the production of a variety of antimicrobial proteins, including lysozyme, to promote microbial homeostasis.^{36,37} Studies have shown that deletion of IL-17 resulted in decreases in the expression of various antimicrobial peptides,³⁸ and this may be conducive to the colonization and growth of *H. hepaticus*. Lysozyme, one of the most important anti-bacterial proteins in the innate immune system, is expressed in monocytes, granulocytes, and Paneth cells of the mouse intestine.³⁹ Although the release of substantial amounts of lysozyme by Paneth cells into the colonic cavity could change the composition of mucolytic microbiota and promote inflammation in the colon,²⁵ lysozyme expressed by macrophages and granulocytes is also important for the removal of invading bacteria in the submucosa and lamina propria of the colon. Surprisingly, *H. hepaticus* infection caused the decrease of lysozyme-positive cells in the colonic submucosa of Il-7a^{-/-} mice, especially at 12 and 16 WPI (Figure 3H). It has been reported that LPS and other bacterial metabolites can inhibit the expression of lysozyme in macrophages.^{40–42} The decrease of lysozyme-positive cells in Il-7a^{-/-} mice may be caused by the LPS and other bacterial components entering the submucosa after the epithelial barrier is destroyed.

It is reported that *H. hepaticus* can express a variety of virulence proteins such as CDT. This expression can affect the proliferation of intestinal stem cells and has been linked to colitis and colorectal tumorigenesis.^{43,44} The excessive *H. hepaticus* colonization in crypts may lead to the decrease of colonic goblet cells and atrophy of colonic glands, which could be the reason for the decrease of mucus secretion in Il-17a^{-/-} mice (Figure 4A and C). Although, C57BL/6 mice infected with *H. hepaticus* did not show obvious colonic pathological symptoms, the immunoregulatory factors, such as IL-10, IL-17A, IL-22 and IL-23 were still up-regulated (Figures 1H, 3F and 4F). It has reported that *H. hepaticus* could induces an IL-23-driven colitis in the absence of an intact IL-10 signaling in C57BL/6 mice.²⁹ And IL-23 is the main stimulator of IL-17A and IL-22⁴⁵ production. We speculate that the “asymptomatic” in colon of C57BL/6 mice once post *H. hepaticus* infection is closely related to the mutual regulation and balance of these cytokines.

Mucus and mucins are also important for intestinal barrier and immune homeostasis. It has reported that IL-10 could promote the production of intestinal mucus;⁴⁶ and IL-17A, along with IL-22, could stimulates colonic epithelial cells to produce Mucin-1.⁴⁷ These may be the part of reason why *H. hepaticus* increase both Alcian blue positive cells and Mucin-1 expression during the infection in C57BL/6 mice (Figure 4A and C). On the other hand, Mucin-1 can also be strongly induced by proinflammatory cytokines such as TNF- α and IFN- γ through the NF- κ B or STAT1 signal.⁴⁸ The higher levels of proinflammatory factors, like TNF- α and IL-6 (Figure 3A and C), may also promote Mucin-1 transcription in IL-17a^{-/-} mice with incomplete Th17 signal. Moreover, IL-22 is a key cytokine that functions to maintain the integrity of the intestinal barrier and to boost stem cell proliferation in crypts.⁴⁹ In our results, the upregulation of IL-22 was not significant in IL-17a^{-/-} mice compared with that in WT C57BL/6 mice after *H. hepaticus* infection (Figure 4F), which may be an important reason for the crypt atrophy caused by *H. hepaticus*. The insufficient upregulation of IL-22 expression in the colon of IL-17a^{-/-} mice may be related to the inefficient recruitment of lymphocytes such as Th22 in the absence of IL-17A.

Conclusion

Taken together, these data suggest that the deletion of IL-17A is beneficial to *H. hepaticus* colonization and predisposes mice to chronic colitis. In view of the inhibitory effect of IL-17A on colitis induced by *H. hepaticus*, the role of IL-17A in regulating intestinal microbiota, maintaining barrier integrity, and immune homeostasis is worthy of further study.

Data Sharing Statement

The data used to support the findings of this study are available from the corresponding authors upon request.

Acknowledgments

We thank Prof. Wang Chengming for kind gift of IL-17a^{-/-} mice (B6.129 (SJL)-Il17atm1.1 (icre) Stck/RthsnJ) for this study.

Funding

This work was supported by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD), Key R & D plan of Jiangsu Province (Social Development) [BE2020674]; High-end Talent Support Program of Yangzhou University and the Young and Middle-aged Academic Leaders Plan of Yangzhou University.

Disclosure

The authors declare that they have no competing interest.

References

1. Ananthakrishnan AN, Bernstein CN, Iliopoulos D, et al. Environmental triggers in IBD: a review of progress and evidence. *Nat Rev Gastroenterol Hepatol*. 2018;15(1):39–49. doi:10.1038/nrgastro.2017.136
2. de Souza HS, Fiocchi C. Immunopathogenesis of IBD: current state of the art. *Nat Rev Gastroenterol Hepatol*. 2016;13(1):13–27. doi:10.1038/nrgastro.2015.186
3. Lavelle A, Sokol H. Gut microbiota-derived metabolites as key actors in inflammatory bowel disease. *Nat Rev Gastroenterol Hepatol*. 2020;17(4):223–237. doi:10.1038/s41575-019-0258-z
4. Fox JG, Ge Z, Whary MT, Erdman SE, Horwitz BH. *Helicobacter hepaticus* infection in mice: models for understanding lower bowel inflammation and cancer. *Mucosal Immunol*. 2011;4(1):22–30. doi:10.1038/mi.2010.61
5. Fujino S, Andoh A, Bamba S, et al. Increased expression of interleukin 17 in inflammatory bowel disease. *Gut*. 2003;52(1):65–70. doi:10.1136/gut.52.1.65
6. Cao S, Zhu C, Feng J, et al. *Helicobacter hepaticus* infection induces chronic hepatitis and fibrosis in male BALB/c mice via the activation of NF- κ B, Stat3, and MAPK signaling pathways. *Helicobacter*. 2020;25(2):e12677. doi:10.1111/hel.12677
7. Ivanov II, Atarashi K, Manel N, et al. Induction of intestinal Th17 cells by segmented filamentous bacteria. *Cell*. 2009;139(3):485–498. doi:10.1016/j.cell.2009.09.033
8. Rouvier E, Luciani MF, Mattéi MG, Denizot F, Golstein P. CTLA-8, cloned from an activated T cell, bearing AU-rich messenger RNA instability sequences, and homologous to a herpesvirus saimiri gene. *J Immunol*. 1993;150(12):5445–5456.
9. Amatya N, Garg AV, Gaffen SL. IL-17 Signaling: the Yin and the Yang. *Trends Immunol*. 2017;38(5):310–322. doi:10.1016/j.it.2017.01.006
10. Onishi RM, Gaffen SL. Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. *Immunology*. 2010;129(3):311–321. doi:10.1111/j.1365-2567.2009.03240.x
11. Kim K, Kim G, Kim J-Y, Yun HJ, Lim S-C, Choi HS. Interleukin-22 promotes epithelial cell transformation and breast tumorigenesis via MAP3K8 activation. *Carcinogenesis*. 2014;35(6):1352–1361. doi:10.1093/carcin/bgu044

12. Iwakura Y, Ishigame H, Saijo S, Nakae S. Functional specialization of interleukin-17 family members. *Immunity*. 2011;34(2):149–162. doi:10.1016/j.immuni.2011.02.012
13. Kinugasa T, Sakaguchi T, Gu X, Reinecker HC. Claudins regulate the intestinal barrier in response to immune mediators. *Gastroenterology*. 2000;118(6):1001–1011. doi:10.1016/S0016-5085(00)70351-9
14. Qin H, Tang G, Yi P, et al. Diagnosis of Genus *Helicobacter* through a hemi-nested PCR assay of 16S rRNA. *Saudi Pharm J*. 2016;24(3):265–272. doi:10.1016/j.jsps.2016.04.015
15. Xu M, Pokrovskii M, Ding Y, et al. c-MAF-dependent regulatory T cells mediate immunological tolerance to a gut pathobiont. *Nature*. 2018;554(7692):373–377. doi:10.1038/nature25500
16. Ge Z, Feng Y, Whary MT, et al. Cytolethal distending toxin is essential for *Helicobacter hepaticus* colonization in outbred Swiss Webster mice. *Infect Immun*. 2005;73(6):3559–3567. doi:10.1128/IAI.73.6.3559-3567.2005
17. Kitajima S, Takuma S, Morimoto M. Changes in colonic mucosal permeability in mouse colitis induced with dextran sulfate sodium. *Exp Anim*. 1999;48(3):137–143. doi:10.1538/expanim.48.137
18. Cao S, Zhu L, Zhu C, et al. *Helicobacter hepaticus* infection-induced IL-33 promotes hepatic inflammation and fibrosis through ST2 signaling pathways in BALB/c mice. *Biochem Biophys Res Commun*. 2020;525(3):654–661. doi:10.1016/j.bbrc.2020.02.139
19. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods*. 2001;25(4):402–408. doi:10.1006/meth.2001.1262
20. Buoncore S, Ahern PP, Uhlig HH, et al. Innate lymphoid cells drive interleukin-23-dependent innate intestinal pathology. *Nature*. 2010;464(7293):1371–1375. doi:10.1038/nature08949
21. Friedrich V, Forné I, Matzek D, et al. *Helicobacter hepaticus* is required for immune targeting of bacterial heat shock protein 60 and fatal colitis in mice. *Gut Microbes*. 2021;13(1):1–20. doi:10.1080/19490976.2021.1882928
22. Han X, Huang T, Han J. Cytokines derived from innate lymphoid cells assist *Helicobacter hepaticus* to aggravate hepatocellular tumorigenesis in viral transgenic mice. *Gut Pathog*. 2019;11:23. doi:10.1186/s13099-019-0302-0
23. Morrison PJ, Ballantyne SJ, Macdonald SJ, et al. Differential requirements for IL-17A and IL-22 in cecal versus colonic inflammation induced by *Helicobacter hepaticus*. *Am J Pathol*. 2015;185(12):3290–3303. doi:10.1016/j.ajpath.2015.08.015
24. Bevins CL, Salzman NH. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. *Nat Rev Microbiol*. 2011;9(5):356–368. doi:10.1038/nrmicro2546
25. Yu S, Balasubramanian I, Laubitz D, et al. Paneth cell-derived lysozyme defines the composition of mucolytic microbiota and the inflammatory tone of the intestine. *Immunity*. 2020;53(2):398–416 e398. doi:10.1016/j.immuni.2020.07.010
26. Gordon S, Todd J, Cohn ZA. In vitro synthesis and secretion of lysozyme by mononuclear phagocytes. *J Exp Med*. 1974;139(5):1228–1248. doi:10.1084/jem.139.5.1228
27. Ralph P, Moore MA, Nilsson K. Lysozyme synthesis by established human and murine histiocytic lymphoma cell lines. *J Exp Med*. 1976;143(6):1528–1533. doi:10.1084/jem.143.6.1528
28. Gao X, Guo M, Zhang Z, Shen P, Yang Z, Zhang N. Baicalin promotes the bacteriostatic activity of lysozyme on *S. aureus* in mammary glands and neutrophilic granulocytes in mice. *Oncotarget*. 2017;8(12):19894–19901. doi:10.18632/oncotarget.15193
29. Danne C, Ryzhakov G, Martínez-López M, et al. A large polysaccharide produced by *Helicobacter hepaticus* induces an anti-inflammatory gene signature in macrophages. *Cell Host Microbe*. 2017;22(6):733–745.e5. doi:10.1016/j.chom.2017.11.002
30. Zhu L, Zhu C, Cao S, Zhang Q. *Helicobacter hepaticus* induce colitis in male IL-10(-/-) mice dependent by cytolethal distending toxin B and via the activation of jak/stat signaling pathway. *Front Cell Infect Microbiol*. 2021;11:616218. doi:10.3389/fcimb.2021.616218
31. Fauny M, Moulin D, D'Amico F, et al. Paradoxical gastrointestinal effects of interleukin-17 blockers. *Ann Rheum Dis*. 2020;79(9):1132–1138. doi:10.1136/annrheumdis-2020-217927
32. Li J, Huang L, Zhao H, Yan Y, Lu J. The role of interleukins in colorectal cancer. *Int J Biol Sci*. 2020;16(13):2323–2339. doi:10.7150/ijbs.46651
33. Marks BR, Craft J. Barrier immunity and IL-17. *Semin Immunol*. 2009;21(3):164–171. doi:10.1016/j.smim.2009.03.001
34. Brevi A, Cogrossi LL, Grazia G, et al. Much more than IL-17A: cytokines of the IL-17 family between microbiota and cancer. *Front Immunol*. 2020;11:565470. doi:10.3389/fimmu.2020.565470
35. Benakis C, Brea D, Caballero S, et al. Commensal microbiota affects ischemic stroke outcome by regulating intestinal gammadelta T cells. *Nat Med*. 2016;22(5):516–523. doi:10.1038/nm.4068
36. Stockinger B, Omenetti S. The dichotomous nature of T helper 17 cells. *Nat Rev Immunol*. 2017;17(9):535–544. doi:10.1038/nri.2017.50
37. Liang SC, Tan XY, Luxenberg DP, et al. Interleukin (IL)-22 and IL-17 are coexpressed by Th17 cells and cooperatively enhance expression of antimicrobial peptides. *J Exp Med*. 2006;203(10):2271–2279. doi:10.1084/jem.20061308
38. Kuwabara T, Ishikawa F, Kondo M, Kakiuchi T. The role of IL-17 and related cytokines in inflammatory autoimmune diseases. *Mediators Inflamm*. 2017;2017:3908061. doi:10.1155/2017/3908061
39. Bergamo A, Gerdol M, Pallavicini A, et al. Lysozyme-induced transcriptional regulation of TNF-alpha pathway genes in cells of the monocyte lineage. *Int J Mol Sci*. 2019;20:21. doi:10.3390/ijms20215502
40. Warfel AH, Zucker-Franklin D. Down-regulation of macrophage lysozyme by lipopolysaccharide and interferon. *J Immunol*. 1986;137(2):651–655.
41. Schoeniger A, Adolph S, Fuhrmann H, Schumann J. The impact of membrane lipid composition on macrophage activation in the immune defense against *Rhodococcus equi* and *Pseudomonas aeruginosa*. *Int J Mol Sci*. 2011;12(11):7510–7528. doi:10.3390/ijms12117510
42. Singh SB, Lin HC. Autophagy counters LPS-mediated suppression of lysozyme. *Innate Immun*. 2017;23(6):537–545. doi:10.1177/1753425917721630
43. He Z, Gharaibeh RZ, Newsome RC, et al. *Campylobacter jejuni* promotes colorectal tumorigenesis through the action of cytolethal distending toxin. *Gut*. 2019;68(2):289–300. doi:10.1136/gutjnl-2018-317200
44. Ge Z, Feng Y, Ge L, Parry N, Muthupalani S, Fox JG. *Helicobacter hepaticus* cytolethal distending toxin promotes intestinal carcinogenesis in 129 Rag2 -deficient mice. *Cell Microbiol*. 2017;19(7):e12728. doi:10.1111/cmi.12728
45. Dudakov JA, Hanash AM, van den Brink MR. Interleukin-22: immunobiology and pathology. *Annu Rev Immunol*. 2015;33:747–785. doi:10.1146/annurev-immunol-032414-112123
46. Hasnain SZ, Tauro S, Das I, et al. IL-10 promotes production of intestinal mucus by suppressing protein misfolding and endoplasmic reticulum stress in goblet cells. *Gastroenterology*. 2013;144(2):357–368 e359. doi:10.1053/j.gastro.2012.10.043

47. Nishida A, Lau CW, Zhang M, et al. The membrane-bound mucin Muc1 regulates T helper 17-cell responses and colitis in mice. *Gastroenterology*. 2012;142(4):865–874 e862. doi:10.1053/j.gastro.2011.12.036
48. Lagow EL, Carson DD. Synergistic stimulation of MUC1 expression in normal breast epithelia and breast cancer cells by interferon-gamma and tumor necrosis factor-alpha. *J Cell Biochem*. 2002;86(4):759–772. doi:10.1002/jcb.10261
49. Lindemans CA, Calafiore M, Mertelsmann AM, et al. Interleukin-22 promotes intestinal-stem-cell-mediated epithelial regeneration. *Nature*. 2015;528(7583):560–564. doi:10.1038/nature16460

Journal of Inflammation Research

Dovepress

Publish your work in this journal

The Journal of Inflammation Research is an international, peer-reviewed open-access journal that welcomes laboratory and clinical findings on the molecular basis, cell biology and pharmacology of inflammation including original research, reviews, symposium reports, hypothesis formation and commentaries on: acute/chronic inflammation; mediators of inflammation; cellular processes; molecular mechanisms; pharmacology and novel anti-inflammatory drugs; clinical conditions involving inflammation. The manuscript management system is completely online and includes a very quick and fair peer-review system. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/journal-of-inflammation-research-journal>