

Mast cell deficiency exacerbates inflammatory bowel symptoms in interleukin-10-deficient mice

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Abstract

AIM: To test the role of mast cells in gut inflammation and colitis using interleukin (IL)-10-deficient mice as an experimental model.

METHODS: Mast cell-deficient ($Kit^{W-sh/W-sh}$) mice were crossbred with IL-10-deficient mice to obtain double knockout (DKO) mice. The growth, mucosal damage and colitis status of DKO mice were compared with their IL-10-deficient littermates.

RESULTS: DKO mice exhibited exacerbated colitis compared with their IL-10-deficient littermates, as shown by increased pathological score, higher myeloperoxidase content, enhanced Th1 type pro-inflammatory cytokines and inflammatory signaling, elevated oxidative stress, as well as pronounced goblet cell loss. In addition, deficiency in mast cells resulted in enhanced mucosal dam-

age, increased gut permeability, and impaired epithelial tight junctions. Mast cell deficiency was also linked to systemic inflammation, as demonstrated by higher serum levels of tumor necrosis factor α and interferon γ in DKO mice than that in IL-10-deficient mice.

CONCLUSION: Mast cell deficiency in IL-10-deficient mice resulted in systematic and gut inflammation, impaired gut barrier function, and severer Th1-mediated colitis when compared to mice with only IL-10-deficiency. Inflammation and impaired gut epithelial barrier function likely form a vicious cycle to worsen colitis in the DKO mice.

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Key words: Colitis; Interleukin-10; Inflammation; Inflammatory bowel disease; Mast cells; Mice

Core tip: Colitis is characterized by chronic inflammation and mast cells accumulate at the pathological sites, implicating their mediating roles, but the exact roles of mast cells in colitis remain poorly defined and controversial. In this study, the authors cross-bred mast cell-deficient mice with interleukin-10-deficient mice to investigate the role of mast cells in gut inflammation and the onset of colitis. Data show that mast cells have protective roles in the development of colitis by suppressing Th1 type immune response and inflammation, altering gut microbiota composition, improving gut epithelial barrier function, and reducing epithelial damage.

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INTRODUCTION

Inflammatory bowel disease (IBD), primarily Crohn's disease and ulcerative colitis, is one of the most common gastrointestinal diseases. Our understanding of IBD etiology, however, is far from complete. Interleukin (IL)-10-deficient mice develop IBD spontaneously after 3 mo of age, which is a common model for studying the etiology of Crohn's disease. The onset of colitis in these mice is associated with enhanced CD4⁺ Th1/Th17 mediated inflammatory responses^[1].

Mast cells are generated from bone marrow derived hematopoietic progenitor cells that migrate into vascularized tissues, where they undergo final maturation^[2]. The resident mast cells comprise about 2%-3% of mucosal cells in the healthy gut^[3], but they can be recruited in large numbers in response to an array of stimuli. They regulate epithelial barrier function and inflammation through: (1) affecting the expression and distribution of tight junction proteins^[4,5]; (2) regulating enteric nervous system by secretion of neurotransmitters^[6]; and (3) recruiting and activating other immune cells by releasing cytokines^[7].

The density of mast cells increases in the gastrointestinal tract of IBD patients, indicating the likely involvement of mast cells in the etiology of IBD^[8-10]. It has been reported that mast cells potentiated inflammation in Dextran Sodium Sulfate (DSS) induced colitis, since mast cell deficiency dampened DSS-induced body weight loss and attenuated colonic hypersensitivity^[11]. In stress-induced gut inflammation, mast cells mediated epithelial barrier dysfunction in rats^[12,13], and mast cell-deficient mice had decreased basal jejunum permeability *ex vivo*^[14]. However, mast cells have also been documented to have a protective role in colonic colitis; deletion of mast cells in IL-10-deficient mice resulted in enhanced mucosal epithelial permeability^[15], while mast cell deficiency has no inhibitory role in helicobacter induced gut inflammatory response in IL-10-deficient mice^[15]. These data indicate a complex nature of the role of mast cells in gut inflammation and IBD pathogenesis, which possibly depends on the genotype and physiological status of mice as well as environmental factors. Here in this study, we cross-bred mast cell-deficient mice with IL-10-deficient mice to investigate the role of mast cells in gut inflammation and the onset of colitis, and further explored underlying mechanisms.

MATERIALS AND METHODS

Animal care and experimental design

All animal procedures were approved by the University of Wyoming Animal Care and Use Committee. IL-10-deficient mice (B6.129P2-IL-10^{tm1Cgn}/J; stock #002251) and mast cell-deficient mice (STOCK *Kit*^{W^{sh}}/HNIhrJaeB-smj; stock #005051) were obtained from the Jackson Laboratory (Bar Harbor, ME). Both strains are on the C57/BL6 background. IL-10-deficient mice and mast cell-deficient mice were cross-bred for two generations

to obtain mast cell heterozygous IL-10-deficient mice. At five weeks of age, mast cell heterozygous, IL-10-deficient female mice were fed either with a control diet (D12450B, 10% energy from fat, Research Diets Inc.) or a high energy diet (D12451, 45% energy from fat) for 3 mo and then bred with the same genotype male mice fed with the control diet. Offspring with both mast cell-deficient and IL-10-deficient (double deficient, for simplicity, we called double knockout, DKO) mice and only IL-10-deficient mice from the same litter were obtained and used for further studies. All mice were housed in sterile high-efficiency particulate air filter cages, with access to food and water *ad libitum*. However, for unknown reasons, very few or no viable neonatal DKO mice could be obtained from mothers fed the control diet. Therefore, only IL-10-deficient and DKO offspring from mothers fed the high energy diet were used for further studies. All mice were sacrificed at 10 wk of age.

Tissue collection

After euthanasia, the colonic tissue was dissected from surrounding tissue. A 5 mm section from the colonic tissue at constant location was fixed in 4% (w/v) paraformaldehyde, processed and embedded into paraffin. The remaining gut segments were cut opened, rinsed in PBS, frozen in liquid nitrogen, and stored at -80 °C till analysis.

In vivo intestinal permeability

Seven-week-old mice were fasted for 5 h with water provided, and then gavaged with FITC-dextran (Sigma, St Louis, MO) at 120 µg/kg body weight. Blood was collected 4 hours after gavage and centrifuged for 5 min at 4000 × g. The resulting serum was 1:5 diluted in PBS (pH 7.4), and the fluorescence intensity was measured at excitation 485 nm and emission 520 nm by a SpectraMax M5 Spectrophotometer (Molecular Device, Sunnyvale, CA)^[16].

Glucose tolerance test

Mice at eight-week-old were subjected to intraperitoneal (*i.p.*) glucose tolerance test after overnight fasting with free access to water. D-glucose (2 mg/g body weight) was *i.p.* injected into mice. The blood glucose level was monitored at 0, 15, 30, 60 and 120 min after injection by tail tip bleeding using a Contour glucometer (Bayer Healthcare, Mishawaka, IN).

Measurement of GSH content

Glutathione *vs* glutathione disulfide ratio (GSH/GSSG) recycle assay was performed as previously described^[17]. Briefly, 10 mg colon tissues were homogenized in 200 µL of 1.3% picric acid solution (Sigma) and followed by sonication and centrifugation. The supernatant was assayed for total GSH and GSSG by incubation with 2-vinylpyridine (Sigma), which conjugates any GSH present in the sample so that only GSSG is recycled to GSH without interference by GSH. The GSSG (as GSH × 2) was then subtracted from the total GSH to calculate the level of GSH.

Table 1 Primer sets used for quantitative reverse transcriptase-polymerase chain reaction analysis of mouse colonic tissue

Gene name	Accession No.	Product size	Direction	Sequence (5' to 3')	Ref.
Claudin2	NM_016675.4	120 bp	Forward	GGCGTCCAACCTGGTGGCTAC	[42]
			Reverse	AACCGCCGTCACAATGCTGGC	
Claudin3	NM_009902.4	132 bp	Forward	CAGGGGCAGTCTCTGTGCGAG	[42]
			Reverse	GCCGCTGGACCTGGGAATCAAC	
GAPDH	NM_008084.2	132 bp	Forward	AACTTTGGCATTGTGGAAGG	[42]
			Reverse	GGATGCAGGGATGATGTTCT	
IL-1 β	NM_008361	73 bp	Forward	TCGCTCAGGGTCACAAGAAA	[43]
			Reverse	CATCAGAGGCCAAGGAGGAAAAC	
INF γ	NM_008337.3	93 bp	Forward	AGGTCCAGCGCCAAGCATTCAA	[42]
			Reverse	AGCAGCGACTCCTTTCCGCTT	
iNOS	U43428.1	76 bp	Forward	CAAAGTCTCAGACATGGCTTGC	This study
			Reverse	TTCCTCTGTCCAGGTCACATTGG	
NOX1	NM_172203.1	113 bp	Forward	CAGGCATCCTCATTITGCGG	This study
			Reverse	CCTCTGCTGGGAGCGATAA	
T-bet	NM_019507.2	138 bp	Forward	CCACTGGATGCCCCAGGAAGTT	[42]
			Reverse	TTCACCTCCACGATGTGCAGCC	
TNF- α	NM_013693.2	67 bp	Forward	TGGGACAGTGACCTGGACTGT	[43]
			Reverse	TTCCGAAAGCCCATTTGAGT	

Serum tumor necrosis factor α and interferon- γ level

Serum levels of tumor necrosis factor (TNF)- α and interferon (IFN)- γ were analyzed by ELISA (eBiosciences, San Diego, CA) according to the manufacturer's manual. The overall intra-assay and inter-assay coefficient of variation was < 5.0% and < 10%, respectively.

Histology

For pathobiological examination, embedded colonic tissue were cut into 5 μ m thickness and subjected to hematoxylin-eosin (HE) staining. HE stained slides were scored using a scale as previously described^[18]. Briefly, slides were scored for the presence of epithelial hyperplasia, the intensity and severity of inflammation. The maximum score of each colon section is 15. A higher number indicates more extensive/severe disease symptoms. To quantify the goblet cell density, the colonic tissue section was stained with alcian blue per published method^[19]. The quantification of goblet cells (goblet cell area *vs* the tissue section area) of alcian blue stained section was performed using the Image J software (split color channels).

Quantitative reverse transcriptase PCR

Total RNA was extracted from colonic tissue using Trizol reagent (Sigma) and treated with DNase I (Qiagen, Valencia, CA) followed by purification with RNeasy[®] Mini Kit (Qiagen). The cDNA was synthesized with the iScript[™] cDNA synthesis kit (Bio-Rad Laboratories, Hercules, CA). Reverse transcriptase PCR (qRT-PCR) was conducted on a Bio-Rad CFX96 thermocycler using glyceraldehyde 3-phosphate dehydrogenase as the housekeeping gene. The primers are listed in Table 1. SYBR Green Master Mix (Bio-Rad) was used in all PCR reactions. The amplification efficiency was 0.90 to 0.99. The qRT-PCR conditions were 95 $^{\circ}$ C, 3 min; 35 cycles of 95 $^{\circ}$ C for 10 s, 56 $^{\circ}$ C for 10 s and 72 $^{\circ}$ C for 20 s. At the end of each run, dissociation melt curves were obtained to confirm the purity of PCR products, and the PCR products were

electrophoresed to confirm the targeted sizes. Relative expression of mRNA was determined after normalization to GAPDH reference using $\Delta\Delta$ -Ct method.

Gut microflora analysis

Bacterial genomic DNA was extracted from fecal samples using QIAamp[®] DNA Stool Mini Kit (Qiagen) per the manufacturer's instruction. The abundance of specific intestinal bacterial groups was quantified by qPCR using Bio-Rad CFX96 thermocycler as stated above. Genus or species specific 16S rRNA gene primers were listed in Table 2. The 16S rRNA of Eubacteria was used as the internal control.

Immunoblotting

Immunoblot analysis was conducted according to the procedures previously described^[17]. Briefly, protein extracts from colonic tissues were separated by 5%-15% gradient SDS-PAGE gels and transferred to nitrocellulose membranes for immunoblotting analyses. Antibodies against myeloperoxidase, phospho-p65 and p65 were purchased from Cell Signaling Technology (Beverly, MA). Claudin2 and Claudin3 were purchased from Invitrogen (Camarillo, CA). GAPDH was purchased from GeneTex (Irvine, CA). Band density was quantified by Image J software and normalized according to the GAPDH content.

Serum total free fatty acid colorimetric assay

Plasma total free fatty acid (FFA) content was analyzed colorimetrically following the previous published methods^[20,21]. Total FFA concentration was calculated based on the standard curve. Each sample was analyzed in duplicate and mean values were reported.

Statistical analysis

Statistical analyses were conducted as previously described^[17,21]. Data were analyzed as a complete randomized design using GLM (General Linear Model of

Table 2 Primers for quantitative polymerase chain reaction analysis of selected fecal microbiota

Target organism	Primer set	Sequence (5' to 3')	Product size	Ref.
<i>Bacteroides</i>	BactF285	GGTCTGAGAGGAGGTCCC	53	[44]
	UniR338	GCTGCCTCCCCTAGGAGT		
<i>Ec-ssul</i>	Ec-ssu1F	GGATAACACTTGGAAACAGG	115	[45]
	Ec-ssu1R	TCCTTGTTCTCTCTAACA		
<i>Eubacteria</i>	UniF340	ACTCTACGGGAGGCAGCAGT	210	[46]
	UniR514	ATTACCGCGGCTGCTGGC		
<i>Lactobacillus</i>	LabF362	AGCAGTAGGGAATCTTCCA	315	[44]
	LabR677	CACCGCTACACATGGAG		
<i>Ruminococcus albus</i> (Ralb)	Ralb561F	CAGGTGTGAAATTAGGGGC	246	[47]
	Ralb807R	GTCAGTCCCCCACACCTAG		

Statistical Analysis System, SAS, 2000). Mean \pm SE are reported. Statistical significance is considered as $P < 0.05$.

RESULTS

DKO mice showed aggravated colitis compared to their IL-10-deficient littermates

The severity of colitis was evaluated by examining pathological changes, goblet cell density, pro-inflammatory cytokine expression and neutrophil content. As shown in Figure 1A, colon section of DKO mice had a much higher pathological score than that of IL-10-deficient littermates. Meanwhile, the colonic tissue of DKO mice exhibited an increased expression of Th1 type inflammatory cytokines such as IL-1 β and IFN- γ (Figure 1B, C), enhanced NF- κ B inflammatory signaling (Figure 1D), and elevated neutrophil infiltration, as indicated by increased myeloperoxidase (MPO) content (Figure 1E). Being the major source of secreted mucin in the gastrointestinal tract, goblet cells play a vital role in regulating intestinal homeostasis. The depletion of goblet cells in the large intestine is another characterized feature of IBD. Alcian blue staining revealed that mast cell deletion resulted in decreased goblet cell staining in the colon of IL-10-deficient mice (Figure 1F).

DKO mice experienced increased oxidative stress in the colon compared to their IL-10-deficient littermates

Oxidative stress has arisen to be another crucial etiological event in colitis progression^[22]. Consistent with aggravated colitis, GSH/GSSG recycle assay demonstrated that mast cell deficiency resulted in a marked decrease of GSH content (Figure 2A). Meanwhile, mRNA expression of NADPH oxidase 1 (NOX1) was increased in the colon of DKO mice compared to that of their IL-10-deficient littermates, but iNOS expression was unchanged (Figure 2B). These data indicated mast cell deletion resulted in a more severe oxidative stress in the colon of IL-10-deficient mice, which was consistent with the enhanced inflammatory responses observed in the colon of DKO mice.

DKO mice exhibited more mucosal damage than their IL-10-deficient littermates

Increased intestinal permeability is an important etiologi-

cal event in the development of colitis in IL-10-deficient mice^[23]. Consistent with aggravated colitis, the *in vivo* intestinal permeability of DKO mice was higher ($P < 0.01$) than that of their IL-10-deficient littermates (Figure 3A), indicating escalated mucosal barrier damage. In agreement with impaired intestinal permeability, mast cell deficiency decreased claudin-3 mRNA expression (Figure 3B) while increased “channel forming” claudin-2 protein content (Figure 3C). In addition, the myosin light chain 2 (MLC-2) phosphorylation and CK2 α protein content were enhanced in the colon of DKO mice (Figure 3C).

Alteration of gut microflora composition

We further evaluated whether gut microflora could be a factor contributing to the enhanced inflammation in the DKO gut. Using genus or species specific 16s rRNA primers, quantitative PCR indicated that DKO mice had decreased *Ruminococcus albus* ($P < 0.05$) but no change in *Bacteroides*, *Lactic acid bacteria*, *Clostridium perfringens*, *Enterococcus* and *Faecalibacterium prausnitzii* compared to their IL-10-deficient littermates (Figure 4).

Mast cell deficiency in IL-10-deficient mice led to systemic inflammation

Besides exasperated colitis, DKO mice exhibited lower wean (4-wk-old) body weight (Figure 5A) compared to that of their IL-10-deficient littermates. Upon necropsy (10-wk-old), DKO mice showed higher spleen and liver weight (Table 3), associated with enhanced serum TNF- α and IFN- γ levels (Figure 5B). In addition, mast cell deficiency dramatically impeded systemic glucose tolerance in IL-10-deficient mice at 15 min, 30 min and 60 min post injection of glucose (Figure 5C).

Interestingly, the subcutaneous fat weight of DKO mice at necropsy was 32.3% \pm 8.1% less than that of their IL-10-deficient littermates, while there was no difference in gonadal fat weight (Table 3). Subcutaneous fat is proposed to be the “sink” for free fatty acids (FFA)^[24]. Therefore, we further analyzed the serum FFA level, which, however, did not differ between DKO mice and their IL-10-deficient littermates (Figure 5D).

DISCUSSION

Mast cells play a crucial role in innate immune responses

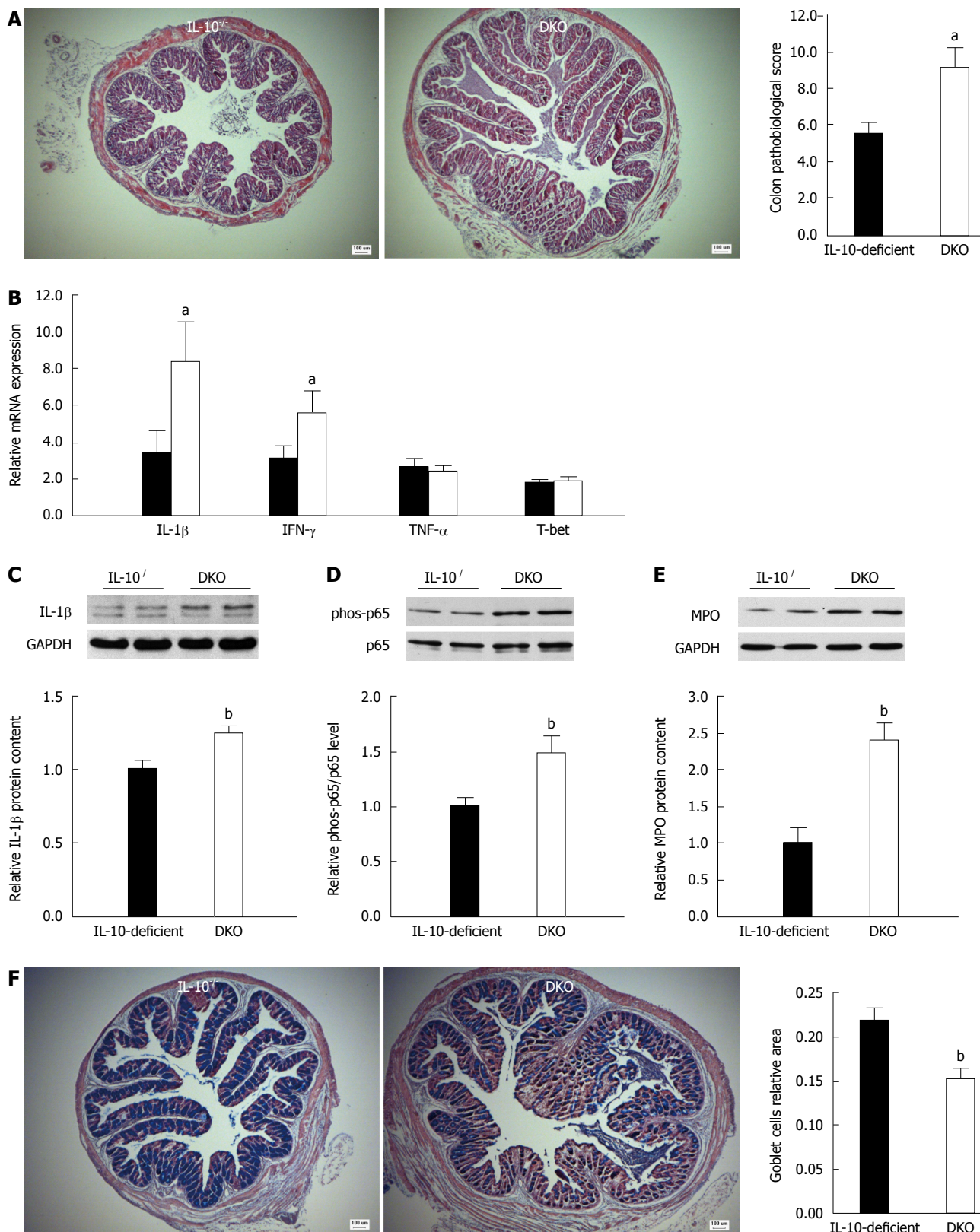


Figure 1 Mast cell deficiency aggravated colitis in the colon of interleukin-10-deficient mice. A: Pathological score; B: mRNA expression of inflammatory cytokines; C: Relative IL-1 β protein content; D: NF- κ B p65 inflammatory signaling; E: Relative myeloperoxidase content in colonic tissue; F: Goblet cell density. Mean \pm SE, ^a $P < 0.05$, DKO vs IL-10-deficient, ^b $P < 0.01$, DKO vs IL-10-deficient, $n = 8$ for mRNA analysis, $n = 10$ for others. IL: Interleukin; DKO: Double knockout; MPO: Myeloperoxidase; NF- κ B: Nuclear factor κ B.

and IBD pathogenesis. Deletion of mast cells markedly attenuated multiple organ injury and damped systematic inflammation in response to trauma^[25]. In chemical

induced colitis, mast cells act as an initiator of innate immune response and likely aggravate disease indices^[10]. However, our data showed that lack of mast cells exac-

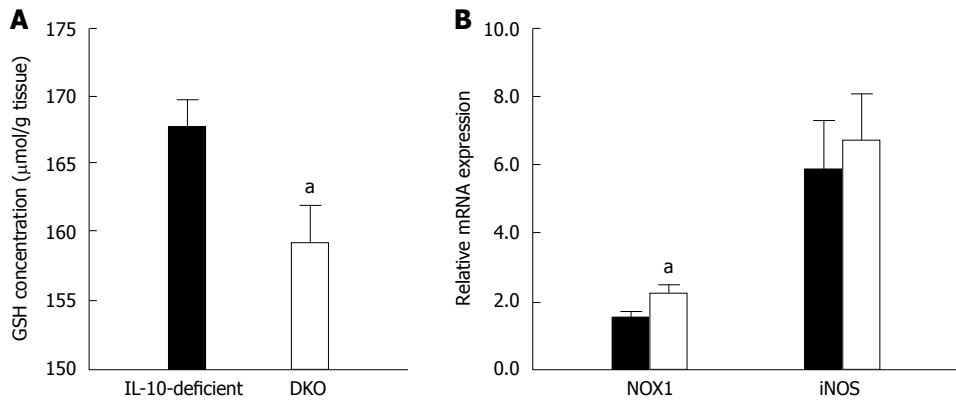


Figure 2 Oxidative stress in the colon tissue of interleukin-10-deficient and double knockout mice. **A:** Colonic glutathione concentration was measured by GSH/GSSG recycling assay; **B:** mRNA expression of NADPH oxidase 1 (NOX1) and inducible NO synthase (iNOS). Mean \pm SE, ^a $P < 0.05$, DKO vs IL-10-deficient, $n = 8$. IL: Interleukin; DKO: Double knockout.

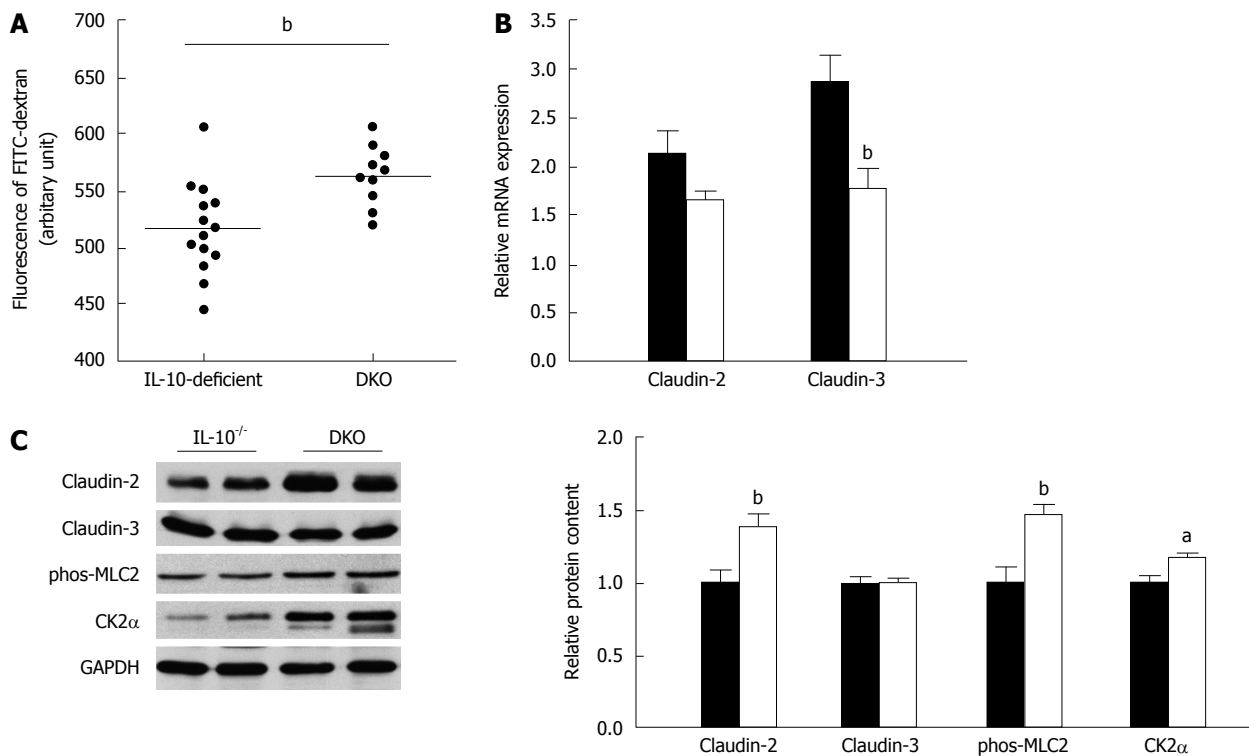


Figure 3 Mast cell deficiency exaggerated mucosal damage in interleukin-10-deficient mice. **A:** *In vivo* intestinal paracellular permeability; **B:** mRNA expression of Claudin-2 and Claudin-3; **C:** Relative protein content of Claudin-2, Claudin-3, phosphorylation of MLC-2 and CK2 α in colonic tissues. Mean \pm SE, ^a $P < 0.05$, ^b $P < 0.01$, DKO vs IL-10-deficient, $n = 8$ for mRNA analysis, $n = 10$ for western blotting analysis. IL: Interleukin; DKO: Double knockout.

erhabated colitis in IL-10-deficient mice, associated with impaired mucosal barrier function, which was consistent with a previous study, where deletion of mast cell resulted in earlier onset of spontaneous colitis and associated with increased intestinal permeability in IL-10-deficient mice^[15]. We speculate that mast cells might have bilateral roles under different circumstances. Mast cells may act as inflammatory mediators in intact immune system, but serve as sentinels under immune compromised conditions, which is currently underappreciated. As an anti-inflammatory cytokine, IL-10 plays a substantial role in intestinal immune regulation and homeostasis. The level

of IL-10 was negatively correlated with the mucosal infiltration of inflammatory cells and the severity of IBD in the colon^[26]. Loss of IL-10 signaling by itself is sufficient to drive changes in pro-inflammatory gene expression, but other existing endogenous compensatory mechanisms may be able to prevent robust inflammation. Indeed, inflammatory TLR4 signaling functions to maintain Treg cell populations and intestinal epithelial homeostasis in IL-10-deficient mice^[27]. In this regard, mice lacking mast cells in addition to IL-10 deficiency would lose their immune regulation ability thus resulted in exasperated immune deregulation and aggravated colitis. In agreement,

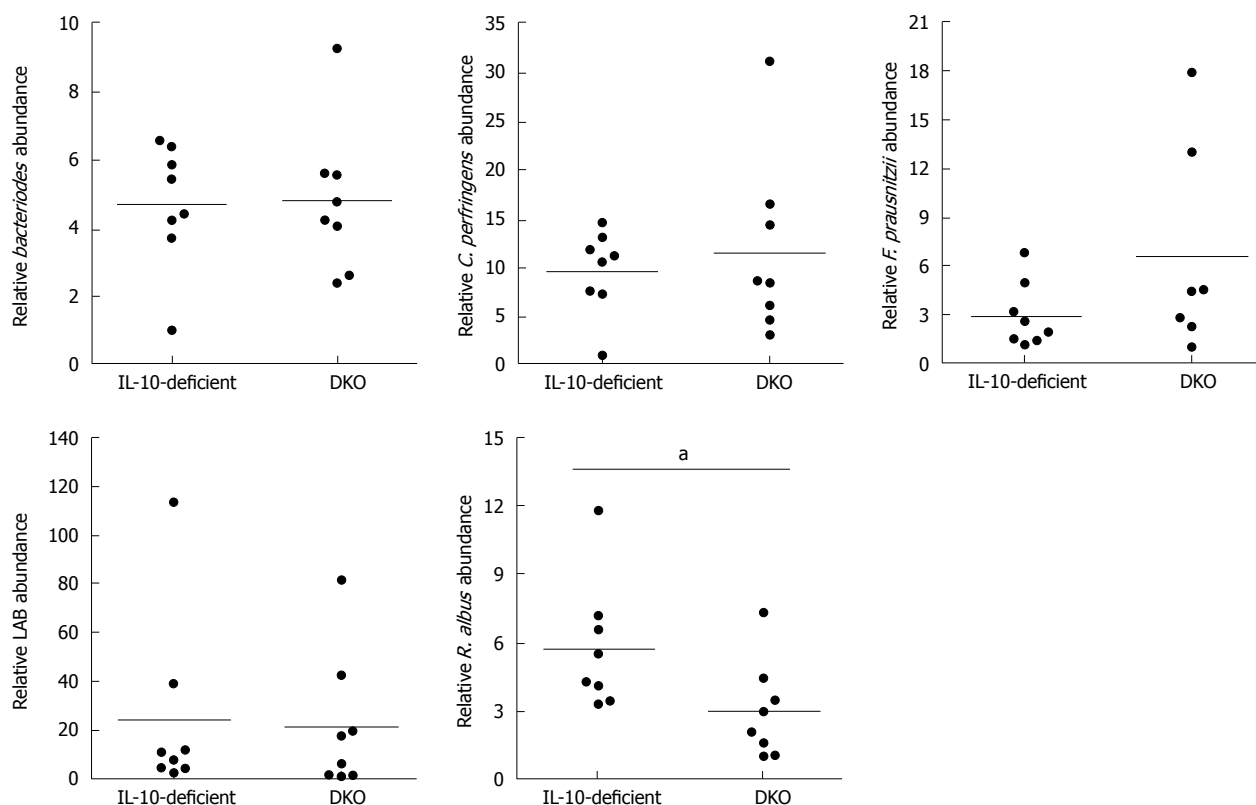


Figure 4 Abundance of selected fecal microflora in interleukin-10-deficient and double knockout mice. Mean \pm SE, ^a*P* < 0.05, DKO vs IL-10-deficient, *n* = 8. IL: Interleukin; DKO: Double knockout.

Table 3 Organ weights of 10-week-old interleukin-10-deficient and double knockout mice at necropsy

Organ weight (g)	IL-10-deficient	DKO	<i>P</i> value
Liver	1.12 \pm 0.041	1.26 \pm 0.03	0.0098
Heart	0.13 \pm 0.01	0.13 \pm 0.01	NS
Spleen	0.09 \pm 0.01	0.16 \pm 0.02	0.0006
<i>Vastus</i> muscle	0.24 \pm 0.01	0.23 \pm 0.02	NS
<i>Gastrocnemius</i> muscle	0.27 \pm 0.01	0.25 \pm 0.01	NS
<i>Tibialis</i> muscle	0.081 \pm 0.003	0.076 \pm 0.003	NS
Subcutaneous fat	0.31 \pm 0.03	0.21 \pm 0.02	0.0052
Gonadal fat	0.33 \pm 0.04	0.27 \pm 0.03	NS

¹Mean \pm SE, *n* = 10. IL: Interleukin; DKO: Double knockout.

we found that mast cell deficiency induced exaggerated inflammatory responses in the gut as indicated by increased expression of inflammatory cytokines, enhanced NF- κ B inflammatory signaling and elevated neutrophil infiltration. Such inflammation might directly contribute to the far severe colitis pathological changes observed in the colon of DKO mice. Aligned with enhanced gut inflammation, we also detected more severe oxidative stress in colonic tissues, which is another possible etiological factor in the initiation or progression of IBD^[28].

Impairment of the epithelial barrier function allows the transmission of antigens, viruses and bacteria, which aggravates inflammation and forms a vicious circle to induce colitis. In IL-10-deficient mice, high intestinal permeability preceded the development of colitis, whereas

improved epithelial barrier function alleviated colitis^[23], clearly indicating that intestinal permeability is an important etiological factor in the development of colitis in IL-10-deficient mice. In alignment with enhanced gut inflammation, mast cell deficiency markedly enhanced the gut permeability in IL-10-deficient mice, which might propel the progress of colitis. Consistent with the enhanced gut permeability, we observed that IL-10-deficiency increased the “pore forming” Claudin-2 protein content, while decreased mRNA expression of barrier sealing protein, Claudin-3 in colonic tissue of DKO. Myosin light-chain kinase (MLCK) phosphorylates the regulatory light chain of myosin 2 (MLC2) and regulates actin-myosin contraction and further impairs tight junction formation to enhance paracellular permeability^[29]. The dramatic increased phosphorylation of MLC2 in our study might serve as one of mechanisms for the impaired epithelial barrier function in DKO mice. Casein kinase 2 (CK2) is a key regulator of intestinal epithelial homeostasis in chronic intestinal inflammation, and enhanced intestinal epithelial cell CK2 protein content was observed in chronic experimental induced colitis^[30]. Consistent with the previous report^[30] and enhanced colitis, DKO mice showed increased CK2 α protein content in the colon tissue. The impairment of gut epithelial barrier function and exaggerated gut inflammatory responses might reinforce each other to deteriorate colitis symptoms.

Besides local gut inflammation, we also observed systemic inflammation in DKO mice, as evidenced by

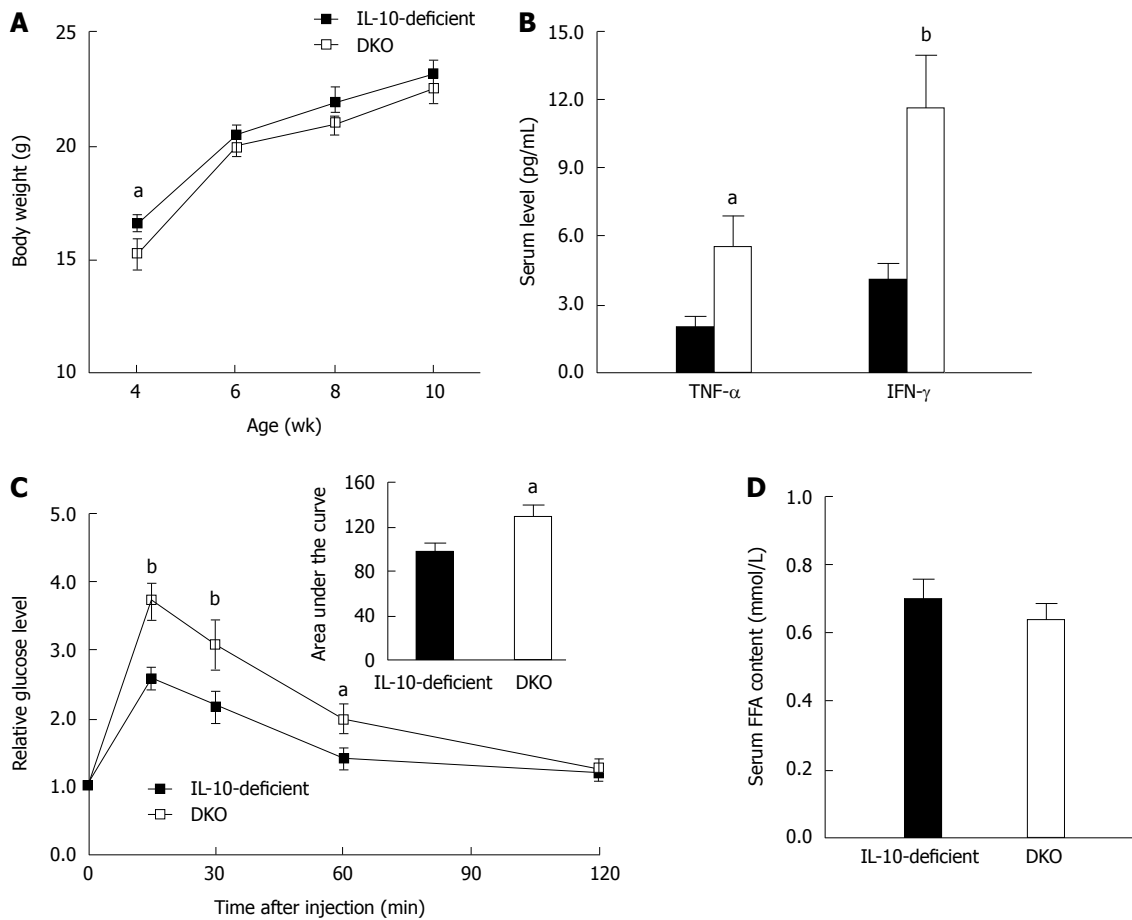


Figure 5 Systemic inflammation in interleukin-10-deficient and double knockout mice. A: Body weight; B: Serum tumor necrosis factor (TNF)- α and interferon (IFN)- γ level; C: Glucose tolerance test; D: Circulatory free fatty acids. Mean \pm SE, ^a $P < 0.05$, ^b $P < 0.01$, $n = 8$. IL: Interleukin; DKO: Double knockout; FFA: Free fatty acid.

splenomegaly and hepatomegaly, as well as elevated serum TNF- α and IFN- γ levels. We speculated that splenomegaly and hepatomegaly were mediated by increased serum pro-inflammatory cytokines, which accompanied colitis as previously reported^[31]. Increased serum pro-inflammatory cytokines could enhance glucose metabolism disorder^[32]. Consistently, DKO mice showed glucose intolerance compared with their IL-10-deficient littermates, which could be attributed to the increased serum TNF- α and IFN- γ levels in DKO^[32-34], but the exact mechanism remains to be determined.

It has been reported that mast cells are necessary for adipogenesis, while deficiency in mast cells reduces fat mass^[33,36]. In the lean mice, mast cells are more prevalent in subcutaneous fat than visceral fat^[37]. Indeed, we observed decreased subcutaneous fat in DKO mice while no change in gonadal fat. Because subcutaneous fat functions as FFA buffer^[24], insufficient subcutaneous fat might limit the ability of absorbing circulating FFA, leading to elevated serum FFA level and systemic inflammation^[24,38]. However, we did not observe significant difference in the serum FFA level between mice with/without mast cells, ruling out FFA as a source of observed exacerbated inflammatory response in DKO mice.

Gut microbiota is increasingly recognized as an important player in gut inflammation and IBD^[39,40]. The

current results showed increased *Ruminococcus albus* in DKO compared to their IL-10-deficient littermates. In support to our result, a previous report indicated that IBD patients had decreased *Ruminococcus albus* content in the gut^[41]. The significance of *Ruminococcus albus* in colitis development needs to be further defined.

In conclusion, mast cell deficiency resulted in exaggerated colitis in IL-10-deficient mice, which was associated with enhanced gut and systematic inflammation, oxidative stress, altered gut microbiota and impaired gut barrier function. Enhanced inflammation and gut permeability likely form a vicious cycle to propel the aggravation of colitis in DKO mice. Our data suggest a protective role of mast cells in the development of colitis in IL-10-deficient mice through a balance of multiple factors.

COMMENTS

Background

Colitis is characterized by chronic inflammation and mast cells accumulate at the pathological sites, implicating their mediating roles, but the exact roles of mast cells in colitis remain poorly defined and controversial. The interleukin-10-deficient mice (IL-10^{-/-}) are one of the most frequently used models for studying inflammatory bowel diseases, which will be used to assess the role of mast cells in gut inflammation and colitis.

Research frontiers

In this study, the authors cross-bred mast cell-deficient mice with IL-10-deficient

mice to investigate the role of mast cells in gut inflammation and the onset of colitis. Data show that mast cells have protective roles in the development of colitis by suppressing Th1 type immune response and inflammation, altering gut microbiota composition, improving gut epithelial barrier function, and reducing epithelial damage in IL-10-deficient mice.

Innovations and breakthroughs

Up to now, the roles of mast cells in the development of colitis remain poorly defined and controversial. This study shows that mast cells protect gut epithelium from the development of colitis. Mast cell deficiency in IL-10-deficient mice resulted in systematic and gut inflammation, impaired gut barrier function, and severer Th1-mediated colitis when compared to mice with only IL-10 deficiency. Inflammation and impaired gut epithelial barrier function likely form a vicious cycle to worsen colitis in the double knockout mice. Therefore, both excess and deficiency of mast cells appear to be detrimental for the incidence of colitis.

Applications

The data suggest a protective role of mast cells in the development of colitis in IL-10-deficient mice through a balance of multiple factors. Thus mast cells likely provide a clinical target to mitigate the symptoms of inflammatory bowel disease.

Peer review

The authors are describing interesting results about the effects of mast cell deficiency on colitis in a double knockout mouse model obtained by cross-breeding mast cell-deficient mice with IL-10-deficient mice. The paper is well-written, the methods used are sound, and results established background for future research in this area.

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