

Original Article

Maresin 1 ameliorates iron-deficient anemia in IL-10^{-/-} mice with spontaneous colitis by the inhibition of hepcidin expression through the IL-6/STAT3 pathway

Honggang Wang¹, Peiliang Shi², Chuanjiang Huang¹, Qinghong Liu¹

¹Department of General Surgery, Taizhou People's Hospital Affiliated to Nantong University, No. 210 Yingchun Road, Taizhou, Jiangsu Province 225300, China; ²Model Animal Research Center of Nanjing University, No. 12 Xuefu Road, Nanjing, Jiangsu Province, China

Received March 18, 2016; Accepted May 8, 2016; Epub June 15, 2016; Published June 30, 2016

Abstract: Background: Approximately 50% of patients with inflammatory bowel disease (IBD) suffer from anemia, which is prevalently caused by iron deficiency. Maresin 1 (MaR1) is a novel docosahexaenoic acid-derived pro-resolving agent that promotes the resolution of inflammation. The aim of the present study was to investigate the therapeutic effects of MaR1 on iron-deficient anemia in IL-10 knockout (IL-10^{-/-}) mice with spontaneous chronic colitis. Methods: IL-10^{-/-} mice of 16 weeks of age with established colitis were used for the experiments with MaR1 treatment for 2 weeks. Histologic injury, CD4+ lymphocyte values in the lamina propria, blood hemoglobin, hematocrit, serum iron concentrations, transferrin saturation, splenic iron stores, levels of inflammatory cytokines, expression of liver hepcidin mRNA, and western blotting of STAT3 were analyzed in this study. Results: MaR1 treatment (0.3 ng/mouse) effectively attenuated histological colitis typically associated with decreased CD4+ lymphocytes in the lamina propria as well as the concentrations of MPO, TNF- α , IFN- γ , IL-6 and IL-17 ($P < 0.05$). Furthermore, reduced expression of liver hepcidin mRNA and p-STAT3 expression, as well as increased hemoglobin concentration, hematocrit, levels of serum iron, transferrin saturation and splenic iron stores were found in IL-10^{-/-} mice after MaR1 treatment ($P < 0.05$). Conclusions: These results indicate that MaR1 treatment ameliorates iron-deficient anemia by reducing colonic inflammation and inhibiting hepcidin expression through the IL-6/STAT3 pathway.

Keywords: Maresin 1, IL-10 knockout, anemia, hepcidin, IL-6/STAT3 pathway

Introduction

Inflammatory bowel disease (IBD) comprises a group of chronic relapsing inflammatory disorders of the intestine with 2 main subsets, Crohn's disease (CD) and ulcerative colitis, with symptoms including abdominal pain, diarrhea and an inability to digest food, and about half of the severe cases require surgery in order to remove the affected bowel segment [1, 2]. Recent studies have shown that the prevalence of anemia in patients with IBD is approximately 40% in adults and 70% in children, with iron-deficiency being the most common cause, followed by anemia associated with chronic disease [3, 4]. Iron-deficient anemia has been reported to be present in patients with IBD independent of active disease; however, anemia in chronic disease correlates better with

disease activity [5-7]. The deficiency of iron in IBD is due to a combination of poor intestinal absorption and blood loss [3]. This so-called anemia of chronic disease is seen in association with other inflammatory conditions, which is often refractory to oral iron supplementation and may require treatment with parenteral iron or erythropoietin (EPO) [8].

The recent elucidation of the mechanisms that regulate iron metabolism have shed light on anemia in IBD and have revealed an important role for hepcidin, an iron-regulating hormone secreted by hepatocytes [9]. Several pro-inflammatory cytokines have been shown to increase hepcidin expression, including IL-6, which stimulates hepcidin transcription through STAT3 signaling [10-12]. The inhibition of hepcidin may potentially serve as an attractive therapeutic

Maresin 1 ameliorates iron-deficient anemia

strategy in patients suffering from anemia or chronic inflammation. In IL-10-deficient (IL-10^{-/-}) mice, generated by gene targeting, most animals are growth retarded, anemic and suffer from chronic colitis under specific pathogen-free conditions [13]. Iron levels in the serum of IL-10^{-/-} mice have been shown to be reduced by 50% compared with normal mice [13]. Hence, iron deficiency is a probable contributing factor to anemia.

Maresin 1 (MaR1), a newly described docosahexaenoic acid-derived mediator, is biosynthesized in macrophages through the 14-lipoxygenation of docosahexaenoic acid and further conversion via 13(14)-epoxidation [14]. Recent studies have shown that MaR1 is a novel docosahexaenoic acid-derived pro-resolving agent that promotes the resolution of inflammation [14, 15]. However, its role in iron-deficient anemia in IL-10^{-/-} mice is unknown. Therefore, in the present study, we investigated the relationship between iron-deficient anemia and hepcidin expression in an IL-10^{-/-} model of colitis after MaR1 treatment.

Materials and methods

Animals

IL-10^{-/-} and wild-type mice (16 weeks old at the beginning of the study) on a C57BL/6 background were obtained from the Jackson Laboratory (Bar Harbor, Maine). Mice were bred and maintained under SPF conditions at the Model Animal Research Center of Nanjing University (Nanjing, China). Previous experiments have demonstrated that most IL-10^{-/-} mice on the C57BL/6 strain under SPF conditions develop spontaneous colitis at 12 weeks of age. All animal studies were carried out in accordance with the recommendations specified in the Guide for the Care and Use of Laboratory Animals of Nanjing University (Nanjing, China).

Drug administration protocol

Wild-type and IL-10^{-/-} mice were divided into a wild-type group (WT), a control group (IL-10-KO) and a treatment group (MaR1), with each group containing 8 mice, and 3-5 mice were housed in one cage. IL-10^{-/-} mice in the treatment group received 0.1, 0.3 or 1 ng MaR1 in 50 μ l intraperitoneally (i.p.) once a day for 14 days. The mice in the wild-type and control groups

received equal volumes of vehicle. The therapeutic effects of MaR1 were evaluated 2 weeks after the final drug administration.

Histology

Proximal colons were flushed with PBS and immediately fixed in neutral buffered formalin. The fixed samples were processed following standard paraffin-embedded histologic methods and hematoxylin and eosin staining [16]. Disease scoring was based on a standard scoring method for IL-10^{-/-} murine colitis progression that involves the measurement of epithelial hyperplasia, enterocyte injury, and the presence of lymphocytes and neutrophils in the lamina propria [17]. The total histologic score was calculated as the sum of the 4 individual variables and had a maximum score of 10.

Hematological and iron concentration analyses

At the endpoint of the experimental measurements, the mice were anesthetized with an intraperitoneal injection of 150 mg/kg ketamine and 8 mg/kg xylazine. Blood samples were collected from the cannulated postcava with a portion mixed with the anticoagulant EDTA to measure hemoglobin concentration and hematocrit, and the remaining untreated blood was processed to measure serum iron concentrations, which were measured as described previously [18]. Briefly, blood was collected in microtainer serum separator tubes (BD Bioscience), and serum was isolated according to the manufacturer's instructions. Serum iron and unsaturated iron-binding capacity (UIBC) were measured by a colorimetric assay using the Iron/UIBC kit (Thermo Electron Corporation). Total iron-binding capacity (TIBC) was calculated as the sum of serum iron and UIBC measurements, and the transferring saturation percentage was calculated as serum iron/TIBC \times 100. Immediately after harvesting, the spleens were sectioned and weighed. Quantitative measurements of splenic iron stores were performed as previously described [18]. Results are reported as micrograms iron/gram wet weight tissue.

Enzyme-linked immunosorbent assay

For cytokine determination in colonic mucosa, protein extracts were obtained by homogenization of colonic segments in homogenization

Maresin 1 ameliorates iron-deficient anemia

Table 1. Histologic variables in proximal colon obtained from mice in each group

Variable	WT	IL-10-KO	MaR1 (0.1 ng)	MaR1 (0.3 ng)	MaR1 (1 ng)
Enterocyte injury (0-3)	0	1.1±0.4	0.9±0.4	0.5±0.2	0.5±0.2
Epithelial hyperplasia (0-3)	0	1.2±0.5	1.0±0.4	0.7±0.3	0.6±0.3
Mononuclear infiltrate (0-2)	0	1.1±0.3	0.9±0.2	0.7±0.3	0.6±0.2
Neutrophil infiltrate (0-2)	0	1.1±0.3	0.9±0.3	0.7±0.2	0.8±0.3
Total score (0-10)	0	4.4±0.5	3.7±0.5	2.6±0.4*	2.5±0.3**

Data are expressed as mean ± SEM, n = 8 in each group. IL-10^{-/-} mice in the treatment group receiving 0.1, 0.3 or 1 ng MaR1 as a 50 µl intraperitoneal (i.p.) once a day for 14 days. *P<0.05 and **P<0.01 versus IL-10-KO group.

buffer containing a protease inhibitor. Cytokines were measured by enzyme-linked immunosorbent assay (ELISA) as described by the manufacturer's protocol. Mouse IL-6, TNF-α, IFN-γ and IL-17 were measured by ELISA using DuoSet ELISA development kits (R&D systems, Minneapolis, MN). The concentrations of the cytokines were established in triplicate supernatants by comparison with standard curves generated using the appropriate recombinant cytokines.

MPO determination was described previously [19, 20]. In brief, blood samples were collected by cardiac puncture under aseptic conditions using a 1-ml syringe and spun at 1500 g for 10 min at 4°C. Tissue myeloperoxidase (MPO) determination MPO was assessed as a marker for neutrophil leucocyte infiltration and accumulation in the inflamed colon tissue. MPO concentrations in colon tissues were evaluated.

Analysis of hepatic hepcidin gene expression

After the mice were sacrificed, pieces of liver were excised and total RNA was prepared using TRIzol reagent as directed by the manufacturer (Invitrogen). Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) was carried out as previously described [21], with primers specific for hepcidin. Relative expression was calculated using the 2^{-ΔΔCt} method after normalizing to GAPDH or actin. The primers used to amplify hepcidin mRNA were 5'CCATCTGCATCTTCTGCTGT3' and 5'AGAGAGGTCAGGATGTGGCT3'.

Flow cytometry analysis

The colons were opened longitudinally and then cut into strips 1 cm in length and stirred in

Hanks Balanced Salt Solution (HBSS, Gibco-Invitrogen, Grand Island, NY) containing 2 mM EDTA and 1 mM DTT at 37°C for 30 min. The cells from intestinal LP were isolated as described previously [16, 21]. Cell suspensions from the LP were washed twice in RPMI-1640, and isolated cells were thoroughly suspended in each tube in 100 µl RPMI-1640. For cell surface antigen staining, ce-

lls were counted and approximately one million cells transferred to each flow test-tube. These cells were stained with FITC-conjugated anti-CD4 (RM4-5; BD Biosciences, San Diego, CA), PE-conjugated anti-CD45 (30-F11; BD Biosciences) or an appropriate negative control. Then, the stained cells were incubated at room temperature for 30 min in the dark. The cells were washed twice with 2 ml RPMI-1640 at room temperature and suspended in 500 µl RPMI-1640, and the cells were evaluated on a FACSCalibur flow cytometer (BD Biosciences). All flow data were analyzed using FlowJo software.

Western blot analysis

For analysis of the effects of MaR1 on STAT3 signaling, lymphocytes were isolated from livers homogenized in 2-3 ml of lysis buffer supplemented with a protease inhibitor cocktail (Sigma). Debris was eliminated by centrifugation at 10,000 g at 4°C for 15 min. Immunoblotting was performed as described previously [11]. The primary antibodies against STAT3 and p-STAT3 were purchased from Cell Signaling Technology (Danvers, MA, USA). Antibodies were detected by incubating the membranes with ECL Plus (Amresco, Solon, OH, USA) following exposure to X-ray film. The blots were quantified by densitometrically using QUANTITY ONE 1-D analysis software (Bio-Rad, Hercules, CA).

Statistical analysis

SPSS version 17.0 software (SPSS, Inc., Chicago, IL) was used to perform the statistical analyses. The data were expressed as means with their standard errors (SEM). Single-factor

Maresin 1 ameliorates iron-deficient anemia

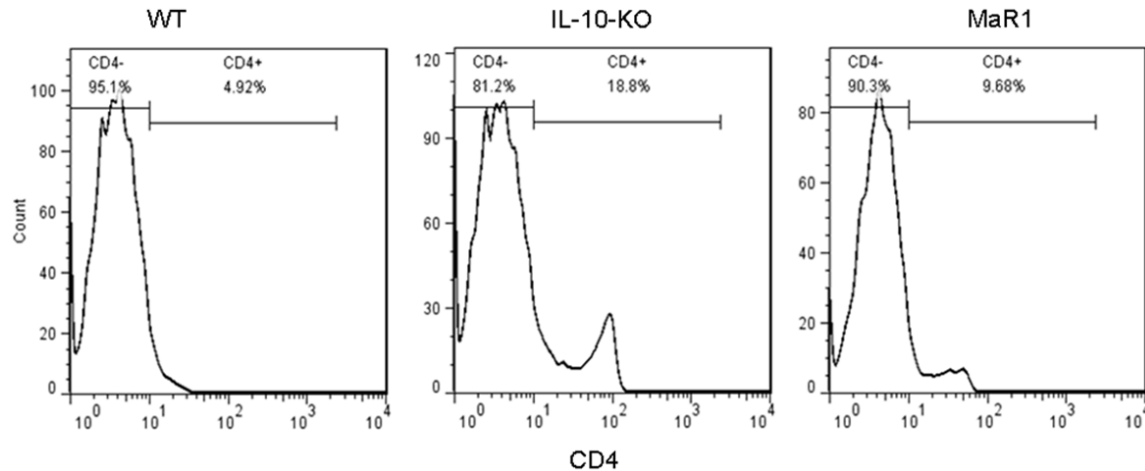


Figure 1. Representative images of CD4⁺ lymphocyte values in wild-type, IL-10^{-/-} and MaR1 treatment mice in colon lamina propria (LP) (n = 6 in each group).

variance analyses of variance were used to evaluate changes in groups. Results were considered statistically significant if *P*-values were <0.05.

Results

MaR1 treatment ameliorated histological colitis associated with decreased CD4⁺ lymphocytes in IL-10^{-/-} mice

We first quantified the protective effects of MaR1 treatment on histologic injury. Compared with wild-type mice, IL-10^{-/-} mice that received vehicle treatment showed a higher total histologic score (*P*<0.01). IL-10^{-/-} mice that received MaR1 treatment (0.3 ng and 1 ng/mouse) showed a significant reduction in total histologic score when compared with mice in the control group (**Table 1**). Interestingly, doses of 0.3 and 1 ng/mouse showed similar effect (**Table 1**) and, for this reason, MaR1 was used at 0.3 ng/mouse for subsequent experiments.

It has been shown that CD4 mediates chronic inflammation in the colon of IL-10^{-/-} mice. Hence, we evaluated the effect of MaR1 (0.3 ng/mouse) on LP CD4⁺ lymphocytes. We found that MaR1 treatment significantly reduced the CD4⁺ values in IL-10^{-/-} mice (*P*<0.01, **Figure 1**).

MaR1 ameliorates anemia by improving the iron status in IL-10^{-/-} mice

We found that the development of colonic inflammation resulted in significant anemia in

IL-10^{-/-} models of IBD (**Figure 2**). Compared with the WT group, hemoglobin concentrations and hematocrit percentages were significantly decreased in IL-10^{-/-} mice (*P*<0.01). After MaR1 treatment, blood hemoglobin values and hematocrit were significantly increased in the group of IL-10-KO mice (*P*<0.05).

Next, we investigated whether MaR1 treatment regulates serum iron levels. Serum iron and transferrin saturation were significantly decreased in the IL-10^{-/-} mice compared with WT mice (*P*<0.01, **Figure 3**). After MaR1 treatment, serum iron and transferrin saturation considerably increased (*P*<0.05). We also measured splenic iron stores. As expected, mice in the IL-10-KO group showed lower spleen iron content compared with mice in the WT group (*P*<0.01). However, treatment with MaR1 in the IL-10^{-/-} mice significantly increased iron concentrations (*P*<0.05) (**Figure 3**).

MaR1 suppressed colonic MPO and pro-inflammatory cytokines in IL-10^{-/-} mice

MPO concentration is widely accepted as a marker to quantify the degree of the accumulation of inflammatory cells, especially neutrophils in colon tissues. As shown in **Figure 4A**, MPO concentration was reduced significantly by MaR1 treatment in IL-10^{-/-} mice (*P*<0.05).

It has been shown that Th1/Th17 cells differentiated from CD4 mainly mediate chronic inflammation in the colon of IL-10^{-/-} mice. We next analyzed whether MaR1 treatment was associ-

Maresin 1 ameliorates iron-deficient anemia

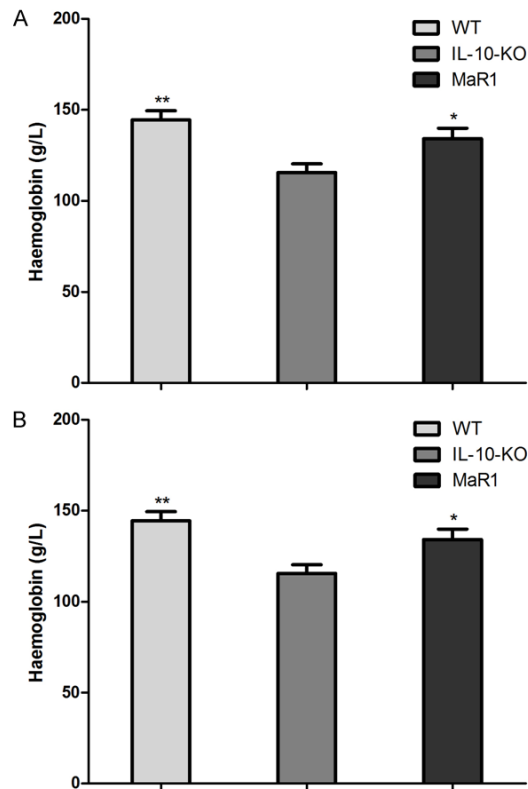


Figure 2. Effect of MaR1 on hemoglobin concentration and hematocrit in IL-10^{-/-} mice. Hemoglobin concentration (A) and percentage of hematocrit (B) in mice of each group (n = 8). Data are presented as means ± SEM. *P<0.05 and **P<0.01 versus IL-10-KO group.

ated with changes in the production of cytokines in colon tissues. As shown in **Figure 4**, all of the markers of Th1 and Th17 cells were increased significantly in IL-10^{-/-} mice when compared with WT mice. TNF- α , IFN- γ , IL-6 and IL-17 levels were significantly lower in mice treated with MaR1 than mice in the IL-10-KO group (P<0.05).

MaR1 treatment reduced the levels of liver hepcidin and STAT-3 activation in IL-10^{-/-} mice

As shown in **Figure 5A**, IL-10^{-/-} mice in the IL-10-KO group expressed significantly higher levels of hepcidin in their livers than mice in WT group (P<0.01), while the relative expression of liver hepcidin mRNA was successfully reduced after MaR1 treatment (P<0.05). Previous studies have shown that TNF, pathogens and IL-6 stimulate hepcidin synthesis via STAT-3 activation [9]. To further determine the therapeutic effects of MaR1, Western blotting of STAT 3 and p-STAT

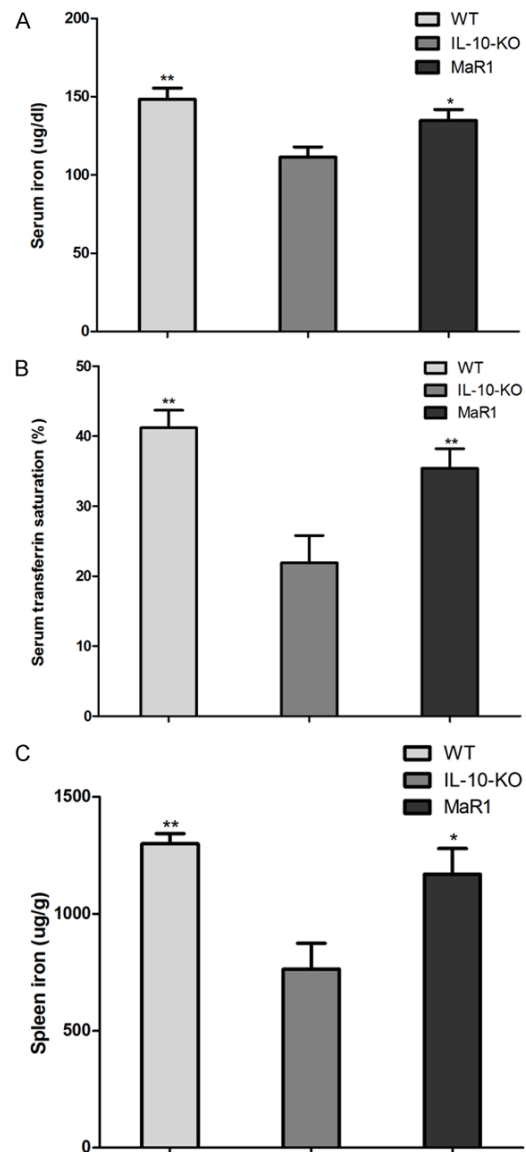


Figure 3. Effect of MaR1 on the iron status in IL-10^{-/-} mice. Levels of serum iron (A), transferrin saturation (B) and splenic iron stores (C) in each group (n = 8). Data are presented as means ± SEM. *P<0.05 and **P<0.01 versus IL-10-KO group.

3 expression in lymphocytes isolated from mouse liver was performed. As shown in **Figure 5B** and **5C**, treatment with MaR1 reduced p-STAT 3 expression significantly, while the levels of total STAT 3 proteins were not affected (P>0.05).

Discussion

Although the exact pathogenesis of IBD has not yet been fully elucidated, it is suspected to involve a resistance of T-cell apoptosis in the

Maresin 1 ameliorates iron-deficient anemia

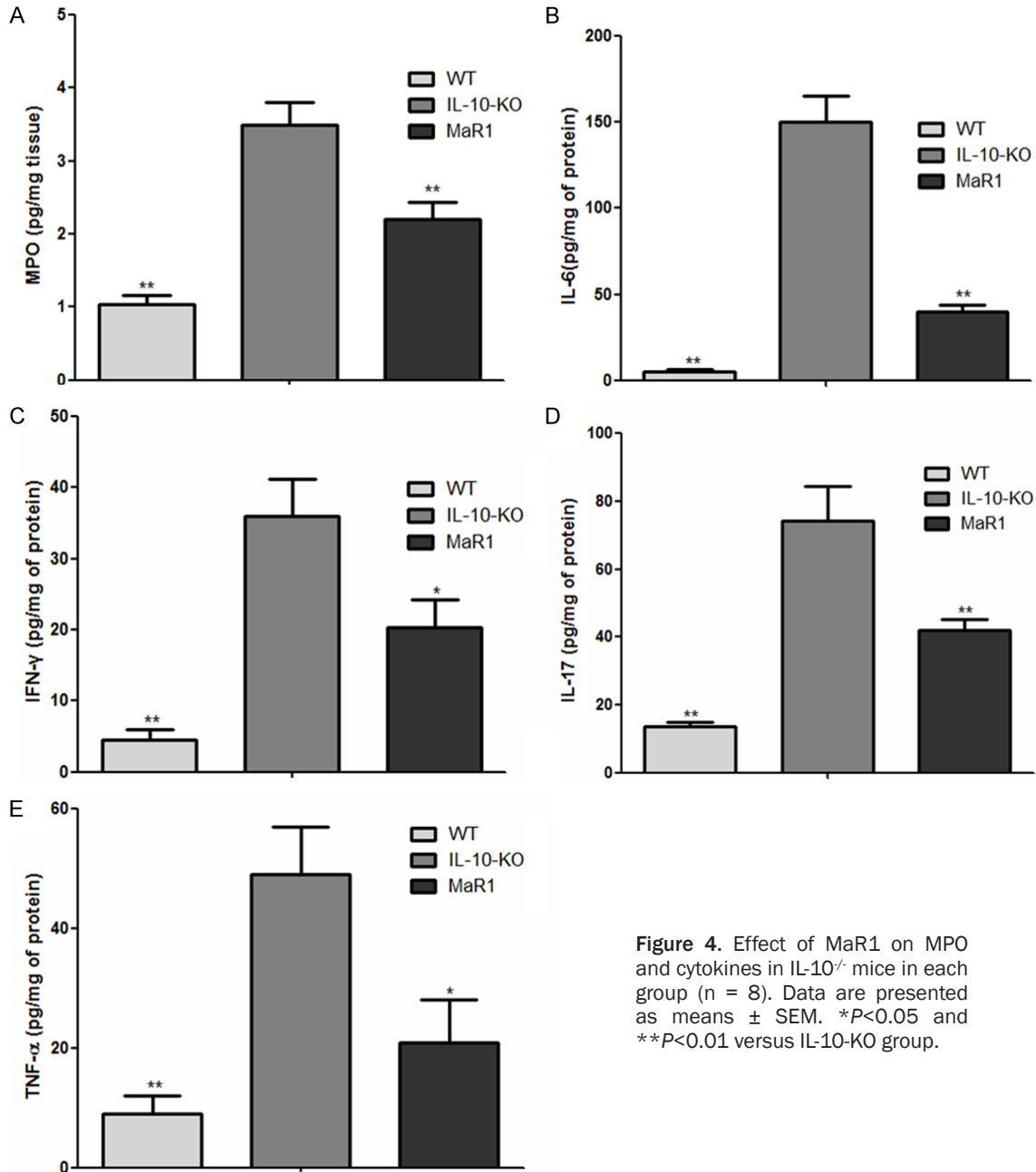


Figure 4. Effect of MaR1 on MPO and cytokines in IL-10^{-/-} mice in each group (n = 8). Data are presented as means \pm SEM. **P*<0.05 and ***P*<0.01 versus IL-10-KO group.

lamina propria, resulting in inappropriate cell activation and proliferation accompanied with an exaggerated immune response and the release of pro-inflammatory cytokines such as TNF- α , Th1 (IFN- γ) and Th17 (IFN- γ) cytokines [1, 2]. The production of Th1 and Th17 is predominantly attributed to CD4⁺ T cells [22]. In this study, we utilized an IL-10^{-/-} mouse model of spontaneous experimental colitis similar to CD to investigate the therapeutic effects of MaR1 on chronic inflammation. Our results demonstrated that IL-10^{-/-} mice MaR1 treat-

ment promoted a significant reduction in total histologic score when compared with IL-10-KO mice. In addition, MaR1 effectively reduced the infiltration of CD4⁺ T cells in the LP and the levels of MPO, IL-6, IL-17, IFN- γ and TNF- α in the colon tissue compared with vehicle treatment in IL-10^{-/-} mice, which could, at least in part, explain the reduction in histologic score.

IL-10 affects the growth and differentiation of many hematopoietic cell types in vitro, and most IL-10^{-/-} mice show growth retardation and

Maresin 1 ameliorates iron-deficient anemia

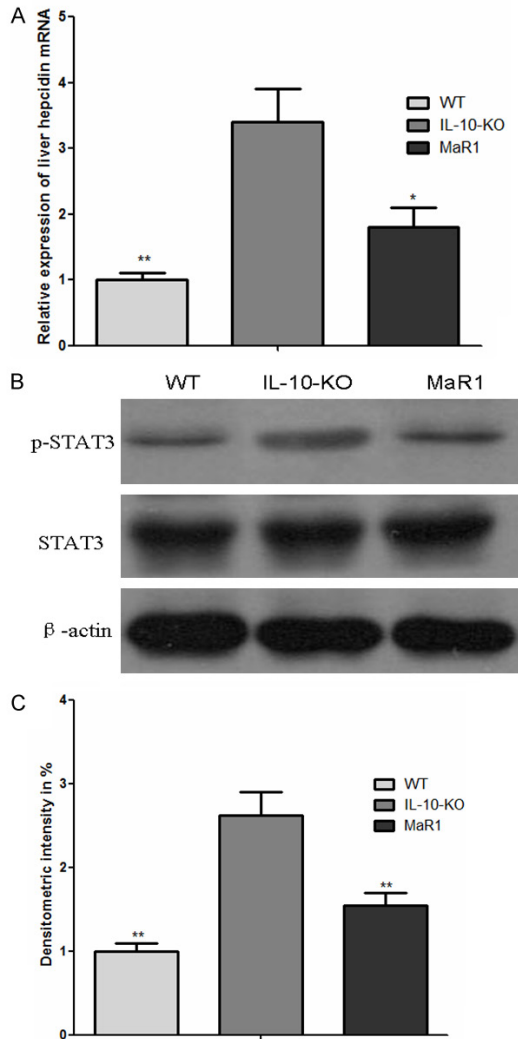


Figure 5. Effect of MaR1 on liver hepcidin mRNA and STAT3 activation in IL-10^{-/-} mice in each group (n = 8). Data are presented as means \pm SEM. *P<0.05 and **P<0.01 versus IL-10-KO group.

anemia as a result of chronic inflammation in the gut [13, 18]. Kuhn *et al.* [13] demonstrated that iron levels in the sera of IL-10^{-/-} mice were reduced by 50% compared with normal animals and iron stores were found to be depleted in the spleen, and alterations of hematopoietic tissues of IL-10^{-/-} mice under SPF conditions were similar to those from conventional breeding. Several parameters such as serum iron, iron-binding capacity and transferrin saturation were used to evaluate the anemia [18, 23, 24]. Both iron-deficient anemia and anemia of chronic disease, the 2 main types of anemia found in patients with IBD, are characterized by decreases in serum iron concentrations and

transferrin saturation [25]. In the present study, hematocrit and blood hemoglobin were decreased in IL-10^{-/-} mice, suggesting significant anemia, and we found that levels of serum iron, transferrin saturation and spleen iron stores were also significantly decreased. The anemia observed in IL-10^{-/-} mice is similar to that in human patients with IBD, where iron-deficient anemia is the most prevalent diagnosis [3]. Our findings are in line with Carter *et al.* [4], demonstrating that transferrin saturation and spleen iron concentration were significantly decreased in a T-cell transfer model of chronic colitis. Furthermore, serum iron and transferrin saturation were significantly lower in anemic patients with Crohn's disease [26]. However, MaR1 treatment significantly improved the iron status and anemia. These hematology and iron concentration modifications may result, at least in part, from reduced colon inflammation following MaR1 treatment.

The discovery of the iron-regulating role of the hormone hepcidin and the elucidation of its mechanism of action have led to a better understanding of the physiopathology of human iron disorders [24, 27]. Although several pro-inflammatory cytokines have been shown to increase hepcidin expression, IL-6 has been the best studied. It stimulates hepcidin transcription through STAT3 signaling [9]. STAT3 belongs to the signal transducer and activator of transcription (STAT) family of signal responsive transcription factors. In non-stimulated cells, STAT3 is kept in an inactive cytoplasmic form [28]. The JAK2/STAT3 pathway is a stress-response mechanism that transduces signals from the cell surface to the nucleus, thereby modulating gene expression. Previous studies have shown that STAT3 inhibitors or neutralization of IL-6 showed potential therapeutic effects in anemia [9, 29, 30]. In this study, we found that significantly increased levels of hepcidin and p-STAT3 were observed in IL-10^{-/-} mice, while MaR1 treatment significantly reduced their expression. These findings indicated that increased levels of hepcidin regulated by the IL-6/STAT3 pathway may contribute to the dysregulation of iron metabolism and the inflammation of the colon in IL-10^{-/-} mice with spontaneous colitis.

In conclusion, this study shows for the first time that MaR1 treatment ameliorates anemia by

Maresin 1 ameliorates iron-deficient anemia

reducing colonic inflammation and inhibiting hepcidin expression. These findings may help to develop novel horizons in the treatment of IBD patients with iron-deficient anemia. However, further studies are required to determine the exact role that MaR1 plays in attenuating the activation of mucosal immunity.

Acknowledgements

This work was supported in part by funding from the Clinical Young Science and Technology Talent Foundation of Taizhou People's Hospital (grants 20150830).

Disclosure of conflict of interest

None.

Address correspondence to: Qinghong Liu, Department of General Surgery, Taizhou People's Hospital Affiliated to Nantong University, No. 210 Yingchun Road, Taizhou, Jiangsu Province 225300, China. E-mail: drliuqinghong@126.com

References

- [1] Baumgart DC, Sandborn WJ. Crohn's disease. *Lancet* 2012; 380: 1590-1605.
- [2] Ordas I, Eckmann L, Talamini M, Baumgart DC, Sandborn WJ. Ulcerative colitis. *Lancet* 2012; 380: 1606-1619.
- [3] Goodhand JR, Kamperidis N, Rao A, Laskaratos F, McDermott A, Wahed M, Naik S, Croft NM, Lindsay JO, Sanderson IR, Rampton DS. Prevalence and management of anemia in children, adolescents, and adults with inflammatory bowel disease. *Inflamm Bowel Dis* 2012; 18: 513-519.
- [4] Carter PR, Watts MN, Kosloski-Davidson M, Prasai K, Grisham MB, Harris NR. Iron status, anemia, and plasma erythropoietin levels in acute and chronic mouse models of colitis. *Inflamm Bowel Dis* 2013; 19: 1260-1265.
- [5] Lomer MC, Cook WB, Jan-Mohamed HJ, Hutchinson C, Liu DY, Hider RC, Powell JJ. Iron requirements based upon iron absorption tests are poorly predicted by haematological indices in patients with inactive inflammatory bowel disease. *Br J Nutr* 2012; 107: 1806-1811.
- [6] Filmann N, Rey J, Schneeweiss S, Ardizzone S, Bager P, Bergamaschi G, Koutroubakis I, Lindgren S, Morena FL, Moum B, Vavricka SR, Schroder O, Herrmann E, Blumenstein I. Prevalence of anemia in inflammatory bowel diseases in European countries: a systematic review and individual patient data meta-analysis. *Inflamm Bowel Dis* 2014; 20: 936-945.
- [7] Goldenberg BA, Graff LA, Clara I, Zarychanski R, Walker JR, Carr R, Rogala L, Miller N, Bernstein CN. Is iron deficiency in the absence of anemia associated with fatigue in inflammatory bowel disease. *Am J Gastroenterol* 2013; 108: 1392-1397.
- [8] Stein J, Hartmann F, Dignass AU. Diagnosis and management of iron deficiency anemia in patients with IBD. *Nat Rev Gastroenterol Hepatol* 2010; 7: 599-610.
- [9] Rochette L, Gudjoncik A, Guenancia C, Zeller M, Cottin Y, Vergely C. The iron-regulatory hormone hepcidin: a possible therapeutic target. *Pharmacol Ther* 2015; 146: 35-52.
- [10] Poli M, Asperti M, Naggi A, Camprostrini N, Girelli D, Corbella M, Benzi M, Besson-Fournier C, Coppin H, Maccarinelli F, Finazzi D, Arosio P. Glycol-split nonanticoagulant heparins are inhibitors of hepcidin expression in vitro and in vivo. *Blood* 2014; 123: 1564-1573.
- [11] Millonig G, Ganzleben I, Peccerella T, Casanovas G, Brodziak-Jarosz L, Breikopf-Heinlein K, Dick TP, Seitz HK, Muckenthaler MU, Mueller S. Sustained submicromolar H₂O₂ levels induce hepcidin via signal transducer and activator of transcription 3 (STAT3). *J Biol Chem* 2012; 287: 37472-482.
- [12] Alkhateeb AA, Buckett PD, Gardeck AM, Kim J, Byrne SL, Fraenkel PG, Wessling-Resnick M. The small molecule ferritin II induces hepatic hepcidin expression in vivo and in vitro. *Am J Physiol Gastrointest Liver Physiol* 2015; 308: G1019-1026.
- [13] Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. *Cell* 1993; 75: 263-274.
- [14] Nordgren TM, Bauer CD, Heires AJ, Poole JA, Wyatt TA, West WW, Romberger DJ. Maresin-1 reduces airway inflammation associated with acute and repetitive exposures to organic dust. *Transl Res* 2015; 166: 57-69.
- [15] Gong J, Liu H, Wu J, Qi H, Wu ZY, Shu HQ, Li HB, Chen L, Wang YX, Li B, Tang M, Ji YD, Yuan SY, Yao SL, Shang Y. Maresin 1 prevents lipopolysaccharide-induced neutrophil survival and accelerates resolution of acute lung injury. *Shock* 2015; 44: 371-380.
- [16] Wang H, Dong J, Shi P, Liu J, Zuo L, Li Y, Gong J, Gu L, Zhao J, Zhang L, Zhang W, Zhu W, Li N, Li J. Anti-mouse CD52 monoclonal antibody ameliorates intestinal epithelial barrier function in interleukin-10 knockout mice with spontaneous chronic colitis. *Immunology* 2015; 144: 254-262.
- [17] Madsen K, Cornish A, Soper P, McKaigney C, Jijon H, Yachimec C, Doyle J, Jewell L, De Simone C. Probiotic bacteria enhance murine and human intestinal epithelial barrier function. *Gastroenterology* 2001; 121: 580-591.

Maresin 1 ameliorates iron-deficient anemia

- [18] Babitt JL, Huang FW, Xia Y, Sidis Y, Andrews NC, Lin HY. Modulation of bone morphogenetic protein signaling in vivo regulates systemic iron balance. *J Clin Invest* 2007; 117: 1933-1939.
- [19] Dong J, Wang H, Wu G, Zhao J, Zhang L, Zuo L, Zhu W, Gong J, Li Y, Gu L, Li J. Oral treatment with SEW2871, a sphingosine-1-phosphate type 1 receptor agonist, ameliorates experimental colitis in interleukin-10 gene deficient mice. *Clin Exp Immunol* 2014; 177: 94-101.
- [20] Zhao J, Sun Y, Shi P, Dong JN, Zuo LG, Wang HG, Gong JF, Li Y, Gu LL, Li N, Li JS, Zhu WM. Celastrol ameliorates experimental colitis in IL-10 deficient mice via the up-regulation of autophagy. *Int Immunopharmacol* 2015; 26: 221-228.
- [21] Wang H, Dong J, Zuo L, Liu J, Zhu W, Li Y, Gu L, Zhao J, Zhang L, Gong J, Zhang W, Li N, Li J. Anti-mouse CD52 monoclonal antibody ameliorates iron-deficient anaemia in IL-10 knockout mice. *Br J Nutr* 2014; 111: 987-95.
- [22] Annunziato F, Romagnani C, Romagnani S. The 3 major types of innate and adaptive cell-mediated effector immunity. *J Allergy Clin Immunol* 2015; 135: 626-635.
- [23] Hentze MW, Muckenthaler MU, Galy B, Camaschella C. Two to tango: regulation of mammalian iron metabolism. *Cell* 2010; 142: 24-38.
- [24] Nemeth E, Tuttle MS, Powelson J, Vaughn MB, Donovan A, Ward DM, Ganz T, Kaplan J. Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. *Science* 2004; 306: 2090-2093.
- [25] Katsanos KH, Tatsioni A, Natsi D, Sigounas D, Christodoulou DK, Tsianos EV. Recombinant human erythropoietin in patients with inflammatory bowel disease and refractory anemia: a 15-year single center experience. *J Crohns Colitis* 2012; 6: 56-61.
- [26] Gasche C, Reinisch W, Lochs H, Parsaei B, Bakos S, Wyatt J, Fueger GF, Gangl A. Anemia in Crohn's disease. Importance of inadequate erythropoietin production and iron deficiency. *Dig Dis Sci* 1994; 39: 1930-1934.
- [27] Qiao B, Sugianto P, Fung E, Del-Castillo-Rueda A, Moran-Jimenez MJ, Ganz T, Nemeth E. Hepcidin-induced endocytosis of ferroportin is dependent on ferroportin ubiquitination. *Cell Metab* 2012; 15: 918-924.
- [28] Meli R, Mattace RG, Irace C, Simeoli R, Di PA, Paciello O, Pagano TB, Calignano A, Colonna A, Santamaria R. High Fat Diet Induces Liver Steatosis and Early Dysregulation of Iron Metabolism in Rats. *PLoS One* 2013; 8: e66570.
- [29] Fatih N, Camberlein E, Island ML, Corlu A, Abgueguen E, Detivaud L, Leroyer P, Brissot P, Loreal O. Natural and synthetic STAT3 inhibitors reduce hepcidin expression in differentiated mouse hepatocytes expressing the active phosphorylated STAT3 form. *J Mol Med (Berl)* 2010; 88: 477-846.
- [30] Aggarwal BB, Shishodia S. Molecular targets of dietary agents for prevention and therapy of cancer. *Biochem Pharmacol* 2006; 71: 1397-1421.