Brief Report

DOI: 10.5582/bst.2014.01088

Local immune compartments are related to the severity of dextran sodium sulphate induced colitis

Luman Wang, Xuechao Jiang, Xiaoming Liu, Tingting Qian, Yiwei Chu*

Department of Immunology and Key Laboratory of Medical Molecular Virology of MOE/MOH, School of Basic Medical Sciences, Biotherapy Research Center of Fudan University, Shanghai, China.

Summary **Dextran sodium sulphate (DSS) induced colitis is commonly used to simulate human ulcerative colitis (UC). However, the mucosal immune responses related to the severity of disease have not been comprehensively documented. We used different concentration of DSS, induced various severities of colitis, and simultaneously examined the frequency of immune cells, antibodies and cytokine production. We found that T regulatory cells (Tregs), B cells, and IgA secretion increased on the recovery phase of mild colitis, accompanied by $CD11b^+$ cells, interleukin (IL)-6 and tumor necrosis factor (TNF)-α accumulated mildly. While during severe and irreversible colitis, the $CD11b^+$ cells, IL-6, and TNF-α infiltrated severely with Tregs, B cells, and IgA increased inconspicuously. These results demonstrate that Tregs, B cells, and IgA may play a significant role in maintaining the homeostasis of gut, by suppressing $CD11b^+$ cells and the pro-inflammatory cytokines.**

Keywords: DSS induced colitis, Tregs, B cells, IgA, $CD11b^+$ cells

1. Introduction

Ulcerative colitis (UC) is an inflammatory bowel disease characterized by intestinal inflammation and mucosal damage (*1*). A widely used experimental model of colitis involves oral administration of dextran sulphate sodium (DSS) dissolved in drinking water. The exact mechanism through which DSS initiates colitis is unknown. One of the possible mechanisms is that DSS activates gut-associated lymphoid tissues (GALT) immune cells (*2*).

GALT are important for the maintenance of intestinal homeostasis, which consist of Peyer's patches, intraepithelial lymphocytes (iEL), lamina propria (LP) and mesenteric lymph nodes (MLN). The GALT that contain lymphocytes can be divided into two main immune-function-based compartments, the bacterial and antigen elimination required effector compartment and self-tolerance regulator compartment, which are associated with colitis symptoms (*3*). Granulocytes and macrophages produce proinflammatory cytokines such as IL-6, TNF-α and are reported to be directly involved in the pathogenesis of UC (*4,5*). On the contrary, B cells are mainly located within the Peyer's patches and after cell differentiation, IgA plasmablasts migrate to the lamina propria and secrete IgA into the gut lumen (*6-9*). In addition, Treg cells also play an essential role in mucosal immune tolerance (*10,11*). The other essential protective cytokines related to B cells and Tregs are transforming growth factor (TGF)-β and IL-10 (*12-16*). Although the etiology and pathogenesis of colitis are still unclear, inappropriate activation of the GALT immune system has played an important role in the pathogenesis of mucosal inflammation (*3*). However, whether the expression of those GALT immune compartments are involved in the degree and the recovery of colitis have not yet been completely identified.

In this study, we used 1% DSS and 3.5% DSS to induce mild and severe colitis respectively. By comparing the immune compartments in the GALT during mild or severe colitis, we demonstrated that regulators such as Tregs, B cells, and IgA increased in the recovery phase of mild colitis. While during severe colitis, the amount of GALT regulators did not change or decreased which resulted in irreversible colitis.

**Address correspondence to:*
Dr. Yiwei Chu, Department of Immunology, Shanghai Medical School, 138 YixueYuan Road, Shanghai 200032, China.
E-mail: yiwei_chu@126.com

2. Materials and Methods

2.1. *Mice*

Wild-type (WT) C57BL/6 mice were obtained from the Chinese Academy of Science (Shanghai, China). All mice used were 8-12 weeks of age and were housed in the animal facility of Fudan University, China. All animals received care in accordance with the Animal Care and Use Committee at Fudan University.

2.2. *DSS-induced colitis*

Mice were given 1% DSS (molecular weight 36-50 kDa) (MP Biomedicals, Inc., Santa Ana, CA,USA) or 3.5% DSS in their drinking water for 7 days followed by regular drinking water (*17*). The Disease activity index (DAI) was scored on a grade from 0 to 4 based on daily assessment of body weight, stool consistency, and detection of rectal bleeding, and then averaged for each parameter. Parameters were scored as follows: body weight loss scores (0, none; 1, 1-5%; 2, 6-10%; 3, 11-15%; 4, over 15%), stool scores (0, normal stools; 2, loose stools; 4, diarrhea) and fecal bleeding scores (0, negative hemoccult test; 1, positive hemoccult test; 2, blood visibly present in the stool; 3, blood visibly and blood clotting on the anus; 4, gross bleeding) as previously described (*17*).

2.3. *Histology*

At day 7 and day 10 after DSS administration, mice were sacrificed, the colon was removed and the segments were fixed in 10% buffered formalin. After paraffin embedding, five-micrometer thick sections were cut transversely and stained with hematoxylin and eosin (H&E). Assessment of histological colon injury was performed at 200× as previously described (*18*). A histology score was generated on a grade from 0 to 4 as follows: 0, normal tissue; 1, mild inflammation with scattered inflammatory cells; 2, moderate inflammation with multiple foci and/or mild epithelial ulcerations; 3, severe inflammation with massive infiltrating mononuclear cells and/or ulcerations in more than 30% of the tissue; and 4, inflammation with transmural inflammatory cell infiltration and/or more than 75% of the tissue section affected.

2.4. *Gut cell isolation*

MLN, Peyer's patches, intraepithelial lymphocytes (iEL) and lamina propria lymphocytes (LPL) were isolated by the method of Lefrancois and Lycke (19). Briefly, MLN were isolated and then the whole intestine was flushed with $Ca^{2+}Mg^{2+}$-free 100 mM HBSS (CMF) (Sigma-Aldrich, St. Louis, MO, USA). RPMI was injected into the intestine to highlight Peyer's patches, which were then collected with a fine forceps and scissors. The intestines were opened longitudinally and cut into small sections, shook with CMF solution containing HEPES, $NaHCO_3$, DTT and EDTA (all from Sigma-Aldrich, St. Louis, MO, USA), and 2% fetal bovine serum (FBS) (Gibco®, Portland, OR, USA) at 37°C. IELs were then purified using discontinuous Percoll gradients (GE Healthcare, Buckinghamshire, UK). For the isolation of LPLs, intestinal pieces were digested with complete RPMI-1640 containing collagenase (Roche Applied Science, Upper Bavaria, Germany), dispase (Sigma-Aldrich, St. Louis, MO, USA) and Dnase I (Sigma-Aldrich, St. Louis, MO, USA) at 37°C. LPLs were then purified using Percoll gradients.

2.5. *Flow cytometry and antibodies*

Single cell suspensions from the MLN, Peyer's patches, epithelium (iEL) and LP were incubated with the 2.4G2 antibody (eBioscience, San Diego, CA, USA) to block FcR II/III receptors before staining with specific Ab. The anti-mouse antibodies B220-PE-Texas Red, CD3-PerCP-Cy7, CD4-APC-Cy7, Foxp3-APC and CD11b-Pacific Blue were purchased from eBioscience (San Diego, CA, USA). Surface and intracellular Foxp3 staining was performed using the manufacturer's recommended protocol. Cells were applied to a CyAn™ ADP Analyzer (Beckman, Coulter, Inc., Carlsbad, CA, USA) and data were analyzed using FlowJo software (Tree Star, Ashland, Or, USA).

2.6. *ELISA*

The intestines were isolated and flushed with 40 ml endotoxin free PBS. Mucus containing fluid was harvested and stored at –80°C until assayed. The concentration of IL-6, IL-10, TNF-α, TGF-β, IgM, and IgA was evaluated using the Mouse Ready-SET-Go! ELISA kits from eBioscience (San Diego, CA, USA) according to the manufacturer's instructions.

2.7. *Statistical analysis*

Multiple group comparisons were performed using one-way ANOVA, followed by Bonferroni Correction to compare two individual groups. The Spearman correlation was used to evaluate the relationship between immune factors and DAI. Statistical analysis was performed using STATA 8.0 or Prism 5.0. p values < 0.05 were considered statistically significant.

3. Results and Discussion

To induce UC assembled colitis, mice were treated with 1% or 3.5% DSS respectively, percentage change in body weight, DAI, colon length and histology of colonic sections of DSS treated and healthy animals

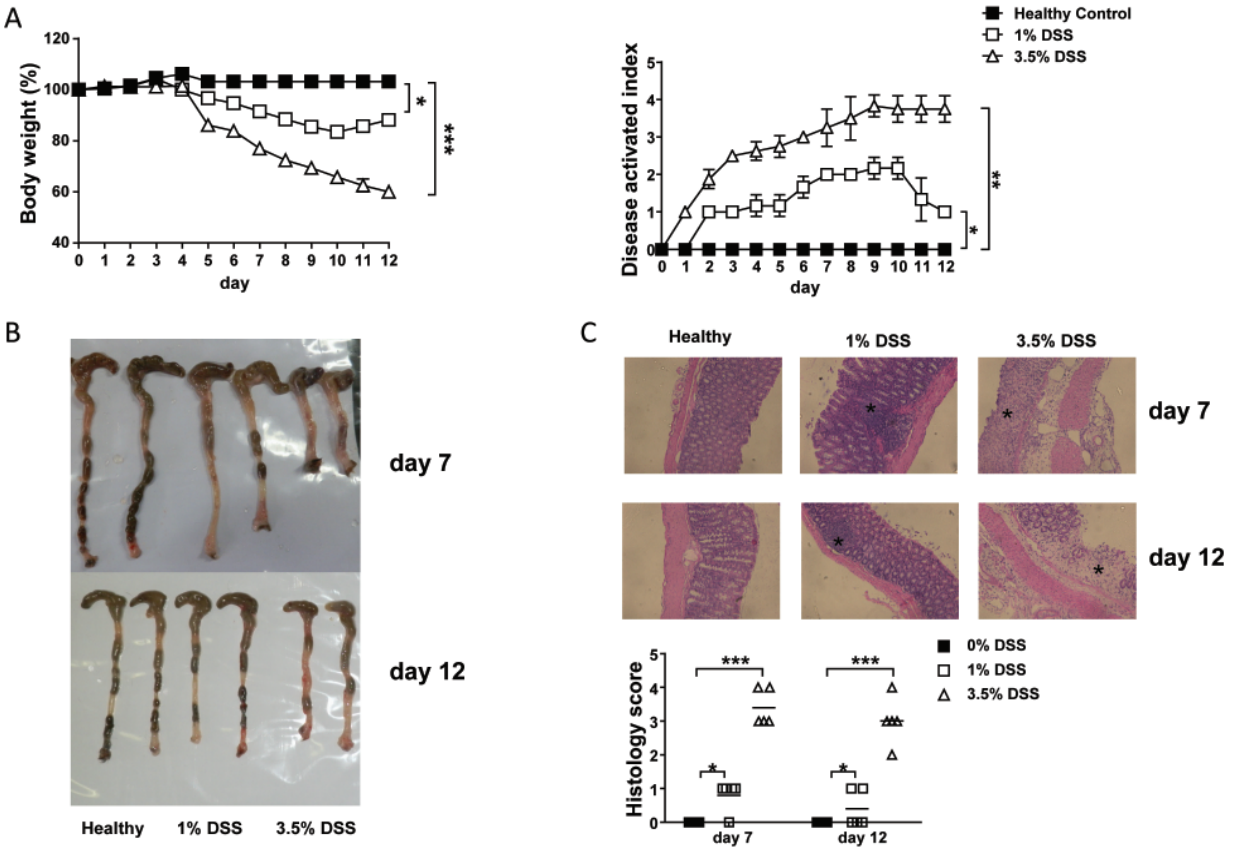

Figure 1. Figure 1. Clinical and macroscopic signs of inflammation. Mice were administered 1% or 3.5% DSS in drinking water for seven days followed by regular drinking water for an additional five days. Controls only received regular drinking water. Mice were evaluated daily for weight loss and DAI scores were calculated **(A)**. Upon sacrifice of the mice, colons were harvested for length determination and gross comparison **(B)**. Representative colon histological sections stained with H&E are shown for the WT and μMT groups **(C)**. Images are shown at 200×. An asterisk indicates the position of the leukocyte infiltration in the histological images. Histological sections were blindly scored on a scale of 0 to 4 to generate a histological score **(C)**. The data shown are the mean ± SEM of one experiment with five mice and was repeated three times with similar results. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

were observed (Figure 1). Mice showed mild symptoms of colitis at a dose of 1% DSS, but showed significantly more weight loss and more severe disease as measured by the disease activity index of 3.5% DSS. 1% DSS treated mice developed diarrhea and lost more than 10% of their body weight starting on day 8, which was accelerated in 3.5% DSS treated mice to day 5. 1% DSS treated mice began to recover starting on day 10, whereas, 3.5% DSS mice continued to progress with no diminution of disease symptoms or severity (Figure 1A). The macroscopic findings continued to be significantly different within groups, 3.5% DSS treated mice had significantly shorter colons (Figure 1B). Finally, histological examination of the colonic sections from 3.5% DSS-induced colitis mice showed severe inflammatory changes in the colon, such as ulcerations, crypt dilation, goblet cell depletion as well as inflammatory cell infiltration preferentially in the acute (day 8) and recovery phase (day 12), when compared to healthy control animals. These tissue changes were milder in mice treated with 1% DSS (Figure 1C). Thus, we demonstrated that higher concentrations of DSS induced more severe and irreversible intestinal injury, which is consistent with a previous study (*20*). Thus, we asked whether the severity of disease related to the mucosal immune responses.

In a previous study, phenotypic changes in immune compartments associated with DSS-induced colitis were determined within splenic cells and MLN. They showed that adaptive immune responses are induced during colitis (*21*). Thus, we asked whether the local GALT immune cells are recruited and activated during mild (1% DSS treated) or severe colitis (3.5% DSS treated). GALT cells were isolated and analyzed by flow cytometry. On day 12, the recovery phase, there was a significant increase in the percentage of Tregs in the MLN, Peyers' patch, iEL and LPL of 1% DSS treated mice while the frequencies didn't alter in the GALT of 3.5% DSS treated mice. During the 1% DSS treatment, the frequencies of B cells increased in the initial compartment MLN and effected the iEL compartment of GALT while the frequencies decreased in the MLN, Peyers' patch and LPL of 3.5% DSS treated mice (Figure 2).On the other hand, the effector compartment of GALT-$CD11b^+$ cells were significantly increased in the MLN, Peyers' patch, and iEL during 3.5% DSS induced colitis while the percentage changed mildly in 1% DSS treated mice. At sites of intestinal inflammation, $CD11b^+$ cells such as granulocytes and macrophages produced high levels of

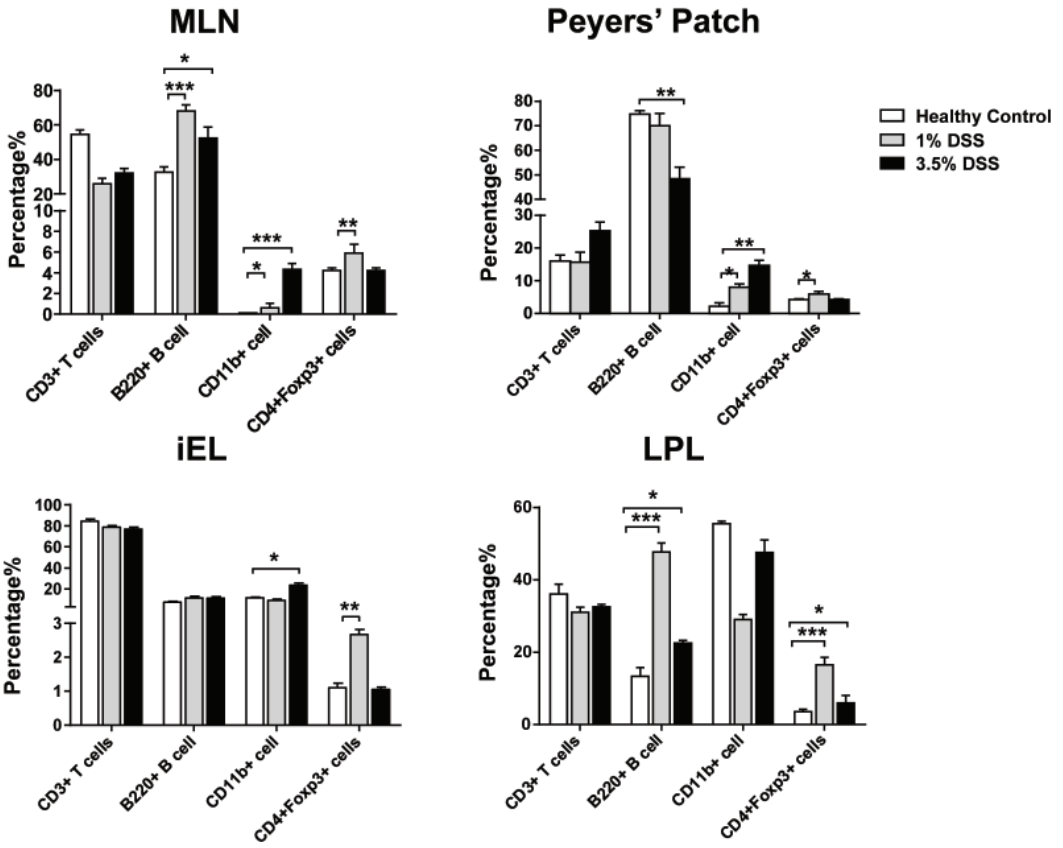

Figure 2. Percentage of immune cell populations in GALT on day 12. Colitis was induced as in Figure 1. Flow cytometry was used to determine the percentage of $CD3^+$ T cells, $B220^+$ B cells, $CD11b^+$ cells and Tregs ($CD4^+Foxp3^+$) in Peyers' patch, iEL, MLN and LPL of colitis mice and healthy mice 12 days after colitis induction. The data shown are the mean ± SEM from one experiment with five mice and was repeated three times with similar results. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

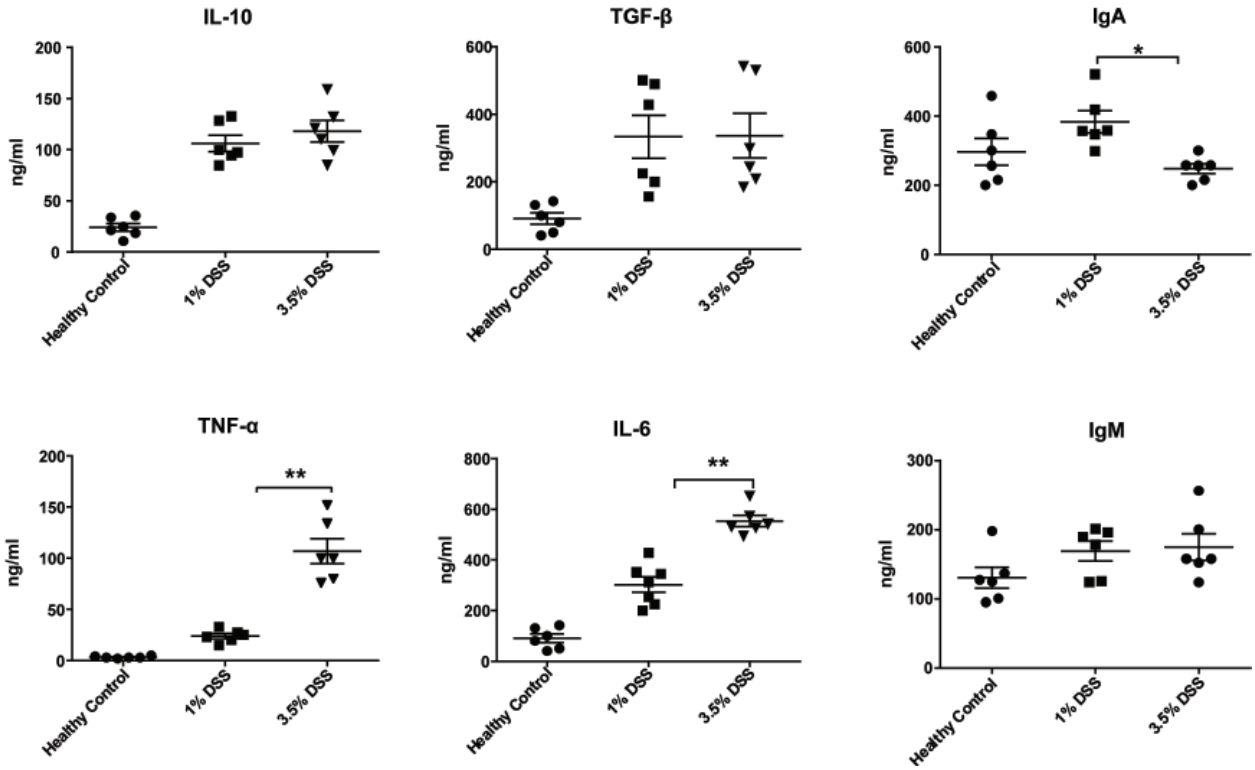

Figure 3. DSS-induced colitis induces significant changes in cytokines and antibodies within the gut. Colitis was induced as in Figure 1. On day 12, the intestines were flushed with 20 mL endotoxin and FCS free PBS. Mucus containing fluid was harvested and the level of IL-6, TNF-α, IL-10, TGF-β, IgM and IgA in the supernatant was determined by ELISA. Each data point represents a single mouse with the mean shown. Data shown are the mean ± SEM of six mice and was repeated three times with similar results. * $p < 0.05$; ** $p < 0.01$.

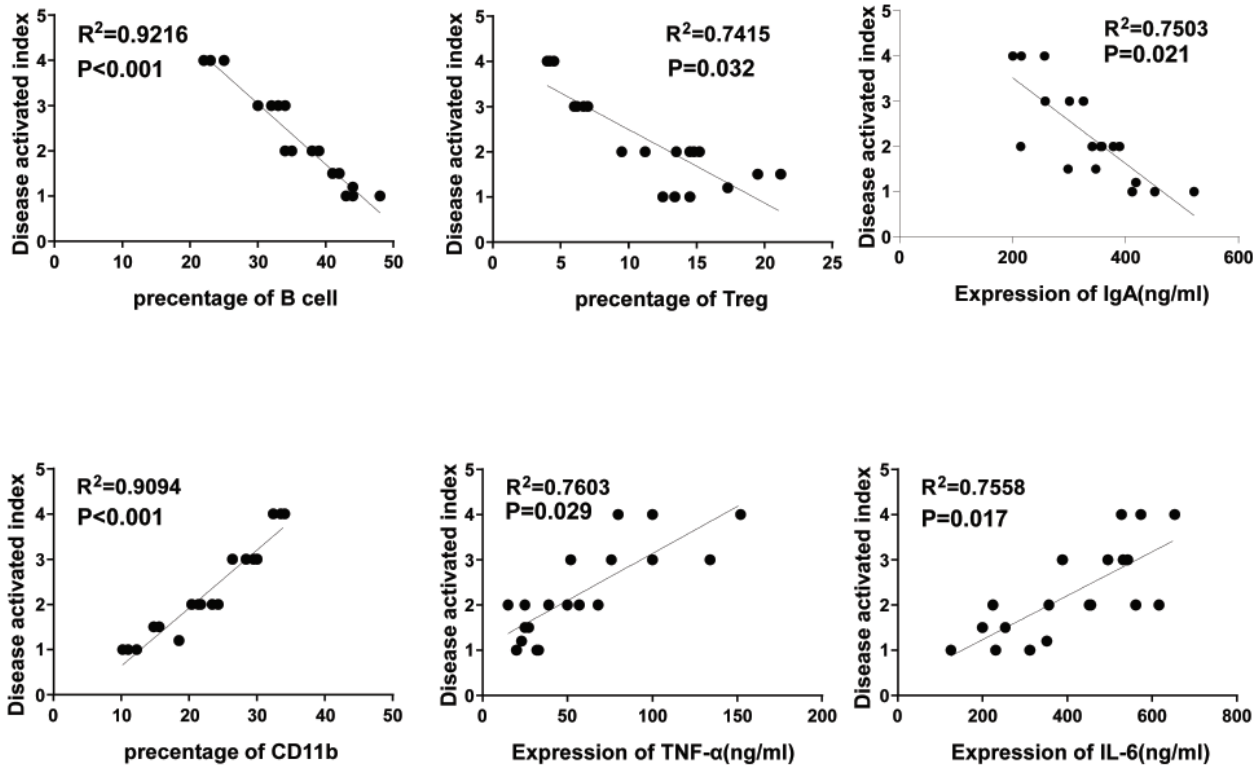

Figure 4. The expression of immune cells and cytokines is related to disease symptoms. Relative frequencies of $B220^+$ B cells, $CD11b^+$ cells, Tregs, IL-6, TNF-α, IgA and DAI in healthy and colitis mice. Each data point represents a single mouse with the mean shown. Data were repeated three times with similar results.

pro-inflammatory cytokines, including IL-6, and TNF-α, which are directly involved in the pathogenesis of UC (*4,5*). On the contrary, a protective role for B cells and serum Ig was described in the TCR-$\alpha^{-/-}$ spontaneous model of colitis (*22*) and DSS induced colitis (*23*). And also, Tregs are considered one of the most important immune cell regulators of intestinal homeostasis (*15,24-27*). Thus, the deficiency of GALT B cells and Tregs during 3.5% DSS may cause the infiltration of $CD11b^+$ cells and lead to an irreversible colitis.

We next determined cytokine and antibody production by those immune cells during mild and severe colitis. We found that IL-10, TGF-β and IgM production in the gut mucus were increased during colitis, but there was no significant difference between mild and severe coiltis, however, pro-inflammatory cytokines TNF-α and IL-6 were found to be significantly higher in the severe colitis (Figure 3). Noteworthy, IgA secreted in the gut mucus decreased in the severe colitis mice compared to the mild colitis mice (Figure 3). In order to identify the effector compartment and the regulator compartment during colitis, we performed statistical analysis between immune factors and DAI. Tregs, B cells and IgA had a negative relationship with DAI, while $CD11b^+$ cells, IL-6 and TNF-α had a positive relationship with DAI (Figure 4).

Our results indicated that Tregs, B cells, and IgA produced had a regulatory function during colitis, which can rescue colitis by confrontation of pro-inflammation cells and cytokines (Figure 3). However, during severe colitis, Tregs, B cells and IgA were exhausted in the acute phase (data not shown). They were not increased on recovery phase, thus $CD11b^+$ cells and its secreted IL-6 and TNF-α were accumulated on lamina propria and epithelia , resulting in severe and irreversible colitis. These results give us a new prospect that patients have different degrees of colitis (*28*), both immune cells such as $CD11b^+$ cells, B cells, Tregs and the immune factors produced by them are directly related to the symptoms of different degrees of colitis. Thus, the frequencies of immune cells and the level of immune factors may provide a new diagnosis of colitis. More importantly, the fact that sufficient Tregs, B cells and IgA are able to rescue colitis from pro-inflammatory factors activation while the deficiency of regulators leads to an irreversible colitis may provide innovative immune cell-based therapies for the treatment of colitis.

Acknowledgements

This work was supported by National Science Foundation of China (81273215, 91229110), the National Basic Research Program of China (973 Programs, 2011CB910404), the China Postdoctoral Science Foundation (2014M5502141), Specialized Research Fund for the Doctoral Program of Higher Education (20120071110046).

References

1. Garcia de Tena J, Espinosa LM, Alvarez-Mon M. Inflammatory bowel disease. N Engl J Med. 2002; 347:1982-1984; author reply 1982-1984.
2. Ni J, Chen SF, Hollander D. Effects of dextran sulphate sodium on intestinal epithelial cells and intestinal lymphocytes. Gut. 1996; 39:234-241.
3. Cheroutre H, Madakamutil L. Acquired and natural memory T cells join forces at the mucosal front line. Nat Rev Immunol. 2004; 4:290-300.
4. 4 Sands BE. Inflammatory bowel disease: Past, present, and future. Journal of gastroenterology. 2007; 42:16-25.
5. Hibi T, Ogata H. Novel pathophysiological concepts of inflammatory bowel disease. Journal of gastroenterology. 2006; 41:10-16.
6. Sattler S, Ling GS, Xu D, Hussaarts L, Romaine A, Zhao H, Fossati-Jimack L, Malik T, Cook HT, Botto M, Lau YL, Smits HH, Liew FY, Huang FP. IL-10-producing regulatory B cells induced by IL-33 (Breg(IL-33)) effectively attenuate mucosal inflammatory responses in the gut. J Autoimmun. 2014; 50:107-122.
7. 7 Suzuki K, Kawamoto S, Maruya M, Fagarasan S. GALT: Organization and dynamics leading to IgA synthesis. Adv Immunol. 2010; 107:153-185.
8. Chorny A, Puga I, Cerutti A. Innate signaling networks in mucosal IgA class switching. Adv Immunol. 2010; 107:31-69.
9. Kirkland D, Benson A, Mirpuri J, Pifer R, Hou B, DeFranco AL, Yarovinsky F. B cell-intrinsic MyD88 signaling prevents the lethal dissemination of commensal bacteria during colonic damage. Immunity. 2012; 36:228-238.
10. Rudensky AY. Regulatory T cells and Foxp3. Immunol Rev. 2011; 241:260-268.
11. Kawamoto S, Maruya M, Kato LM, Suda W, Atarashi K, Doi Y, Tsutsui Y, Qin H, Honda K, Okada T, Hattori M, Fagarasan S. Foxp3$^+$ T cells regulate immunoglobulin a selection and facilitate diversification of bacterial species responsible for immune homeostasis. Immunity. 2014; 41:152-165.
12. Sonoda E, Matsumoto R, Hitoshi Y, Ishii T, Sugimoto M, Araki S, Tominaga A, Yamaguchi N, Takatsu K. Transforming growth factor beta induces IgA production and acts additively with interleukin 5 for IgA production. J Exp Med. 1989; 170:1415-1420.
13. Cazac BB, Roes J. TGF-beta receptor controls B cell responsiveness and induction of IgA *in vivo*. Immunity. 2000; 13:443-451.
14. Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell. 1993; 75:263-274.
15. Rubtsov YP, Rasmussen JP, Chi EY, Fontenot J, Castelli L, Ye X, Treuting P, Siewe L, Roers A, Henderson WR, Jr., Muller W, Rudensky AY. Regulatory T cell-derived interleukin-10 limits inflammation at environmental interfaces. Immunity. 2008; 28:546-558.
16. Zigmond E, Bernshtein B, Friedlander G, Walker CR, Yona S, Kim KW, Brenner O, Krauthgamer R, Varol C, Muller W, Jung S. Macrophage-Restricted Interleukin-10 Receptor Deficiency, but Not IL-10 Deficiency, Causes Severe Spontaneous Colitis. Immunity. 2014.
17. Murthy SN, Cooper HS, Shim H, Shah RS, Ibrahim SA, Sedergran DJ. Treatment of dextran sulfate sodium-induced murine colitis by intracolonic cyclosporin. Digestive diseases and sciences. 1993; 38:1722-1734.
18. Wirtz S, Neufert C, Weigmann B, Neurath MF. Chemically induced mouse models of intestinal inflammation. Nat Protoc. 2007; 2:541-546.
19. Lefrancois L, Lycke N. Isolation of mouse small intestinal intraepithelial lymphocytes, Peyer's patch, and lamina propria cells. Current protocols in immunology (Coligan JE, ed.). 2001; Chapter 3: Unit 3.19.
20. Rose WA, 2nd, Sakamoto K, Leifer CA. Multifunctional role of dextran sulfate sodium for *in vivo* modeling of intestinal diseases. BMC immunology. 2012; 13:41.
21. Hall LJ, Faivre E, Quinlan A, Shanahan F, Nally K, Melgar S. Induction and activation of adaptive immune populations during acute and chronic phases of a murine model of experimental colitis. Digestive diseases and sciences. 2011; 56:79-89.
22. Mizoguchi A, Mizoguchi E, Smith RN, Preffer FI, Bhan AK. Suppressive role of B cells in chronic colitis of T cell receptor alpha mutant mice. J Exp Med. 1997; 186:1749-1756.
23. Yanaba K, Yoshizaki A, Asano Y, Kadono T, Tedder TF, Sato S. IL-10-producing regulatory B10 cells inhibit intestinal injury in a mouse model. Am J Pathol. 2011; 178:735-743.
24. Schmitt EG, Haribhai D, Williams JB, *et a*l. IL-10 produced by induced regulatory T cells (iTregs) controls colitis and pathogenic ex-iTregs during immunotherapy. J Immunol. 2012; 189:5638-5648.
25. Eastaff-Leung N, Mabarrack N, Barbour A, Cummins A, Barry S. Foxp3$^+$ regulatory T cells, Th17 effector cells, and cytokine environment in inflammatory bowel disease. J Clin Immunol. 2010; 30:80-89.
26. Griseri T, Asquith M, Thompson C, Powrie F. OX40 is required for regulatory T cell-mediated control of colitis. J Exp Med. 2010; 207:699-709.
27. Uhlig HH, Coombes J, Mottet C, Izcue A, Thompson C, Fanger A, Tannapfel A, Fontenot JD, Ramsdell F, Powrie F. Characterization of Foxp3$^+$CD4$^+$CD25$^+$ and IL-10-secreting CD4$^+$CD25$^+$ T cells during cure of colitis. J Immunol. 2006; 177:5852-5860.
28. Hanauer SB. Inflammatory bowel disease. N Engl J Med. 1996; 334:841-848.

(Received August 12, 2014; Revised October 5, 2014; Re-revised October 9, 2014; Accepted October 10, 2014)