Elevated IL-23R Expression and Foxp3⁺Rorγt⁺ Cells in Intestinal Mucosa During Acute and Chronic Colitis

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Statistical Analysis C
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Background: IL-23/IL-23R signaling plays a pivotal role during the course of inflammatory bowel diseases (IBD). However, the underlying mechanisms are poorly characterized. Foxp3⁺ regulatory T cells are critical in the maintenance of gut immune homeostasis and therefore are important in preventing the development of IBD. This study was performed to clarify the association between IL-23/IL-23R signaling and Foxp3⁺ regulatory T cells in colitis.

Material/Methods: Acute and chronic mouse colitis models were established by administering mice DSS in drinking water. IL-23R, IL-23, IL-I7, and IFN-γ expression level, as well as regulatory T cell, Th17-, and Th1-related transcription factors Foxp3, RORγt, and T-bet were assayed by real-time PCR. The frequency of Foxp3⁺ RORγt⁺ cells in a Foxp3⁺ cell population in colon mucosa during acute and chronic colitis was evaluated through flow cytometry. The signaling pathway mediated by IL-23R in the colon mucosa from acute colitis mice and chronic colitis mice was monitored by Western blot analysis.

Results: We detected elevated IL-23R, IL-23, and IFN-γ expression in colon mucosa during acute and chronic colitis and found increased IL-17 in acute colitis mice. Transcription factors Foxp3 and T-bet were elevated in colon mucosa during acute and chronic colitis. Phosphorylation of Stat3 was greatly enhanced, indicating the activation of IL-23R function in colitis mice. The percentage of Foxp3⁺ T cells in acute and chronic colitis mice was comparable to control mice, but there was a 2-fold increase of Foxp3⁺ RORγt⁺ cells among the Foxp3⁺ cell population in acute and chronic colitis mice compared to control mice.

Conclusions: These findings indicate that the induction of Foxp3⁺ RORγt⁺ T cells could be enhanced during inflammation in the intestine where IL-23R expression is greatly induced. Our study highlights the importance of IL-23R expression level and the instability of Foxp3⁺ regulatory T cells in the development of inflammatory bowel diseases.

MeSH Keywords: Forkhead Transcription Factors • Inflammatory Bowel Diseases • Interleukin-17 • Interleukin-23 Subunit p19

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/896827

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Background

Inflammatory bowel diseases are severe inflammatory disorders of the gastrointestinal tract caused by imbalanced immune responses to gut commensal microbiota [1]. IL-23, a heterodimeric cytokine comprising IL-12p40 and IL-23p19, is now well documented to be critical in the pathogenesis of intestinal inflammation [2–4]. A functional receptor for IL-23 is a heterodimeric receptor complex consisting of IL-23R and IL-12Rβ1, which is highly expressed on the cell membrane of memory T cells and other immune cells, such as natural killer cells, monocytes, and dendritic cells [5,6]. Factors inducing IL23R mRNA expression include IL-23 itself, IL-6, IL-21, and T cell activation. Genome-wide association studies have identified single-nucleotide polymorphisms in the IL-23R gene as Crohn's disease susceptibility regions [7]. IL-23R variants are risk factors for both Crohn's disease and ulcerative colitis [8]. In a mouse colitis model, deletion of IL-23R on T cells led to decreased inflammatory response in the intestine [9]. Taken together, these data strongly suggest IL-23R as a key player in the pathogenesis of colitis in humans and mice. The functional activity of the IL-23/IL-23R pathway is primarily linked to the T helper 17 (Th17) cell subset [10]. However, the underlying mechanisms through which IL-23 promotes inflammatory response *in vivo* are poorly characterized.

Foxp3⁺ regulatory T cells are involved in the maintenance of gut immune homeostasis and oral tolerance through the suppressive activity of effector (proinflammatory) T cells that have differentiated into Th1, Th2, or Th17, as well as innate immune cells [11,12]. In a mouse model, transfer of Foxp3⁺ regulatory T cells successfully prevented the development of colitis and was used to treat established colitis [13]. Thus, FoxP3-positive T cells are critical in preventing the development of IBD. It has been shown that many inflammatory cytokines can inhibit the differentiation and function of Foxp3⁺ regulatory T cells [14]. However, the abundance of Foxp3⁺ regulatory T cells residing in gut mucosa of active IBD was increased in some studies, suggesting the impaired regulatory functions of these cells during inflammation [15]. A study using an IL-23R deletion mouse model revealed that the severity of colitis was reduced and the number of Foxp3⁺ cells in the colon was increased in the absence of IL-23R compared to WT mice, suggesting the possibility of a direct effect of IL-23/IL-23R signaling on Foxp3⁺ regulatory T cell differentiation [9].

In the present study, we investigated the expression level of IL-23R and downstream cytokines in the intestines in mice with acute colitis and in mice with chronic colitis. We found that IL-23R and IL-23 were greatly increased during acute and chronic intestinal inflammation. The percentage of pathogenic Foxp3⁺RORγt⁺ cells was subsequently increased. These findings indicate that the induction of Foxp3⁺ RORγt⁺ T cells can be enhanced during inflammation in the intestine where IL-23R expression is greatly induced. Thus, our study highlights the importance of IL-23R expression level and the instability of Foxp3– positive T cells in the development of inflammatory bowel diseases.

Material and Methods

Acute and chronic DSS colitis induction

Acute colitis was induced through feeding mice with 2.5% DSS in drinking water for 4 days. Mice were sacrificed on day 6. Chronic colitis was induced through administering mice 2% DSS in drinking water for 6 days followed by drinking water without DSS for 7 days as 1 cycle, with a total of 3 cycles. Daily evaluation of the progression of colitis was monitored by weighing and examination for rectal bleeding, loose stools, and diarrhoea.

Male C57BL/6 mice (Slac Laboratory Animal, Shanghai, China) aged 6–10 weeks were used in this study. All experiments were approved by the Animal Care and Use Committee of Zhongshan Hospital, Fudan University.

Histological evaluation

Colons were removed and fixed with 4% phosphate-buffered paraformaldehyde and embedded in paraffin. Tissue sections (5-µm) were prepared and stained with hematoxylin and eosin (H&E) according to standard procedures.

Quantitative real-time PCR (RT-PCR)

Colon biopsy specimens were homogenized and total RNA was extracted using TRIZOL Reagent (Life Technologies, Carlsbad, CA) following the manufacturer's instructions. Extracted RNA was reverse transcribed to cDNA using the ReverTra Ace®qPCR RT Kit (TOYOBO) according to the manufacture's protocol (TaKaRa Bio Inc., Dalian, China). The number of mRNA copies was determined by real-time PCR (ABI7500 system) using the SYBR Premix Ex Taq RT-PCR Kit according to the manufacturer's instructions (TaKaRa Bio Inc., Dalian, China). The cycle parameters were: 95°C for 30 s, 40 cycles at 95°C for 10 s, and 60°C at 30 s. Housekeeping gene GAPDH was examined under identical conditions as an internal control. All the primers used in this study are listed in Table 1. The expression of transcripts was evaluated by $2^{-\Delta\Delta Ct}$.

Flow cytometry

Single-cell suspensions were prepared from spleens and mesenteric lymph nodes by passing the tissue through nylon mesh.

Table 1. List of primers.

Primer name	Primer sequence
Mouse IL-23R sense	5'-GAGGACATCCTGCTTCAGGTAAT-3'
Mouse IL-23R anti-sense	5'-AGCCACTTTGGGATCATCAGTA-3'
Mouse IFNγ sense	5'-ACAGCAAGGCGAAAAAGGATG-3'
Mouse IFNγ anti-sense	5'-TGGTGGACCACTCGGATGA-3'
Mouse RORγt sense	5'-GACAGGGAGCCAAGTTCTCAG-3'
Mouse RORγt anti-sense	5'-TCGGTCAATGGGGCAGTTC-3'
Mouse T-bet sense	5'-AACCGCTTATATGTCCACCCA-3'
Mouse T-bet anti-sense	5'-CTTGTTGTTGGTGAGCTTTAGC-3'
Mouse GAPDH sense	5'-TGGCCTTCCGTGTTCCTAC-3'
Mouse GAPDH anti-sense	5'-GAGTTGCTGTTGAAGTCGCA-3'
Mouse IL-23 sense	5'-AATAATGTGCCCCGTATCCAGT-3'
Mouse IL-23 anti-sense	5'-GCTCCCCTTTGAAGATGTCAG-3'
Mouse IL17A sense	5'-CTGCTGAGCCTGGCGGCTAC-3'
Mouse IL17A anti-sense	5'-CATTGCGGTGGGAGAGTCCAGGG-3'
Mouse FoxP3 sense	5'-ACCATTGGTTTACTCGCATGT-3'
Mouse FoxP3 anti-sense	5'-TCCACTCGCACAAAGCACTT-3'

Separated cells were washed with phosphate-buffered saline (PBS) and immunostained with anti-mouse CD4 FITC (BD Pharmingen, NJ, USA) at 4°C for 15 min. For intracellular staining, cells were then fixed and permeabilized in fix/permeabilization solution followed by labeling of Foxp3 PE and RORγt APC (all from eBioscience) for 30 min according to the manufacturer's instructions. All fluorescence analysis was performed on a BD FACS Calibur device.

Western blot analysis

Protein samples from colon tissues were incubated at 94°C for 5 min, separated on 10% SDS-PAGE gels, and transferred onto PVDF membranes (Millipore, Bedford, MA). The nonspecific sites were blocked with a solution containing 5% non-fat milk powder in TBS/Tween20 (TBS/T) for 2 h at room temperature. The membrane was incubated with antibodies against phospho-Stat3 (Tyr705) (Cell Signaling Technology, Danvers, CT) in TBS/T containing 5% bovine serum albumin (BSA) overnight at 4°C, then with HRP-conjugated anti-rabbit IgG (Sigma) at a dilution of 1/5000. Protein bands were visualized using chemiluminescence reagent (Thermo Scientific, Waltham, MA). The membrane was stripped using Restore Western Blot Stripping Buffer (Thermo Scientific) then re-probed with anti-Stat3 antibody (Cell Signaling Technology, Danvers, CT) followed by HRP-conjugated anti-rabbit IgG.

Statistical analysis

The statistical significance of differences between DSS and control groups was assessed using Student's t test. A p value <0.05 was considered statistically significant in all experiments.

Results

Acute and chronic inflammation in colon mucosa was induced through DSS feeding

Acute inflammation in colon mucosa was induced through administering mice 2.5% DSS in drinking water for 4 days. Mice were then sacrificed on day 6. The mice were assessed daily for weight, rectal bleeding, and changes in stool consistency. From day 4 of DSS exposure, weight loss, occurrence of rectal bleeding, and change of stools were observed. Histological analysis clearly displayed epithelial erosions, massive inflammatory response, and destruction of the normal crypt structure of the colon in DSS mice (Figure 1A).

Chronic colitis was induced through administering 2% DSS in drinking water for 6 days followed by drinking water without DSS for 7 days as 1 cycle, with total of 3 cycles. We observed weight loss, loose stool, and rectal bleeding during the DSS administration period and recovery after switching to plain drinking water. Histological analysis displayed epithelial erosions, massive inflammatory response, and destruction of the normal crypt structure of the colon in DSS mice (Figure 1B).

IL-23R, IL-23, and downstream inflammatory cytokines were greatly elevated *in situ* in colon mucosa during acute and chronic colitis

Total mRNA was freshly extracted from colons of sacrificed mice with acute DSS colitis. IL-23R and IL-23 mRNA levels were assessed through RT-PCR. As shown in Figure 2, IL-23R and IL43 were greatly elevated in the DSS group compared to control mice. The downstream cytokines of IL-23/IL-23R axis IL-17 were subsequently elevated. IFN-γ is a marker of intestinal inflammation and was greatly increased in the DSS group compared to control mice, indicating that strong inflammation was present in DSS mice in this study. The role of IL-23/IL-23R axis and IL-17 in chronic colitis was less clear. We established a chronic DSS colitis model and assessed the IL-23/IL-23R axis and IL-17, as well as IFN-γ, and found that IL-23R, IL-23, and IFN-γ were increased in chronic DSS colitis, but we did not observe a significant increase of IL-17 in chronic colitis mice.
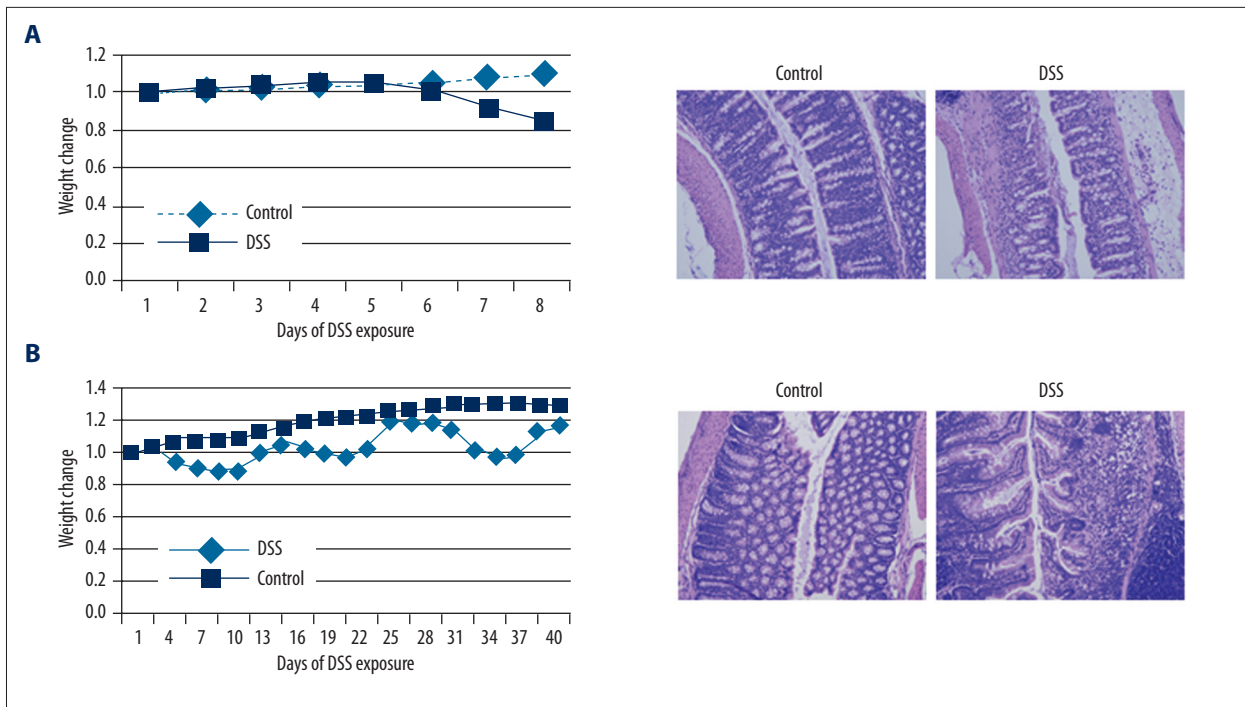
Figure 1. Acute and chronic inflammation of colon mucosa was induced through administration of DSS. (**A**) Acute colitis was evaluated through weight loss of mice and histological analysis of colon tissue. (**B**) Chronic colitis was evaluated through weight loss of mice and histological analysis of colon tissue. Each group contained 6 mice. Experiments were repeated 3 times.
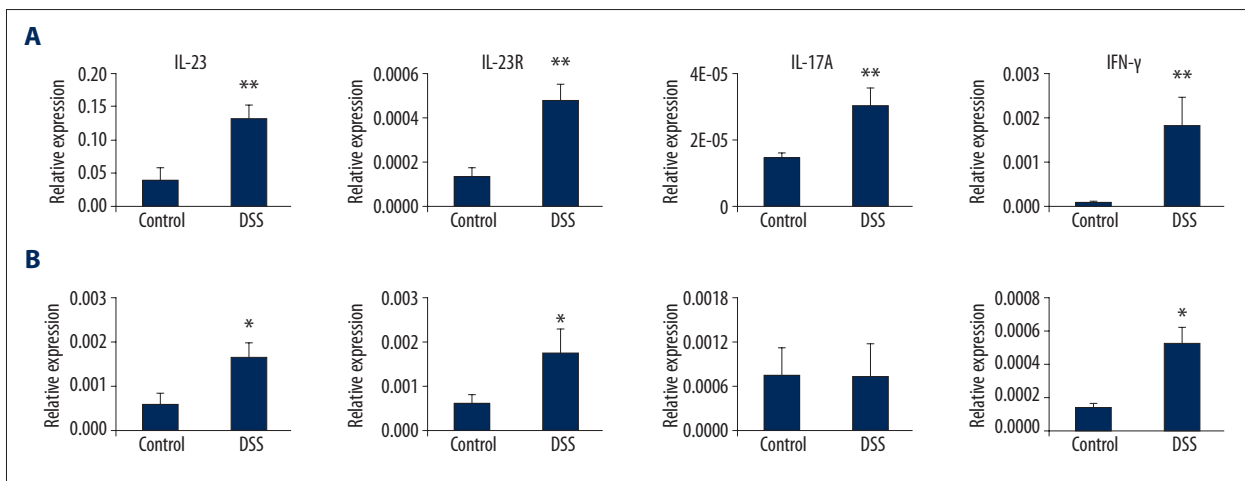

Figure 2. IL-23R and IL-23, as well as downstream inflammatory cytokine, were greatly elevated *in situ* in colon mucosa during acute and chronic colitis. (**A**) Acute colitis was induced through administering 2.5% DSS in drinking water for 4 days. Mice were sacrificed on day 6. Colon tissues were collected and total mRNA was extracted. IL-23, IL-23R, IL-17, and IFN-γ expression were assessed through RT-PCR. (**B**) Chronic colitis was induced through administering 2% DSS in drinking water for 4 days followed by drinking water without DSS for 7 days as 1 cycle, with a total of 3 cycles. Colon tissues were collected and total RNA was extracted. IL-23, IL-23R, IL-17, and IFN-γ expression were assessed through RT-PCR. Each group contained 5 mice. Data are shown as mean ± SD from 3 independent experiments. * $p<0.05$, ** $p<0.01$.
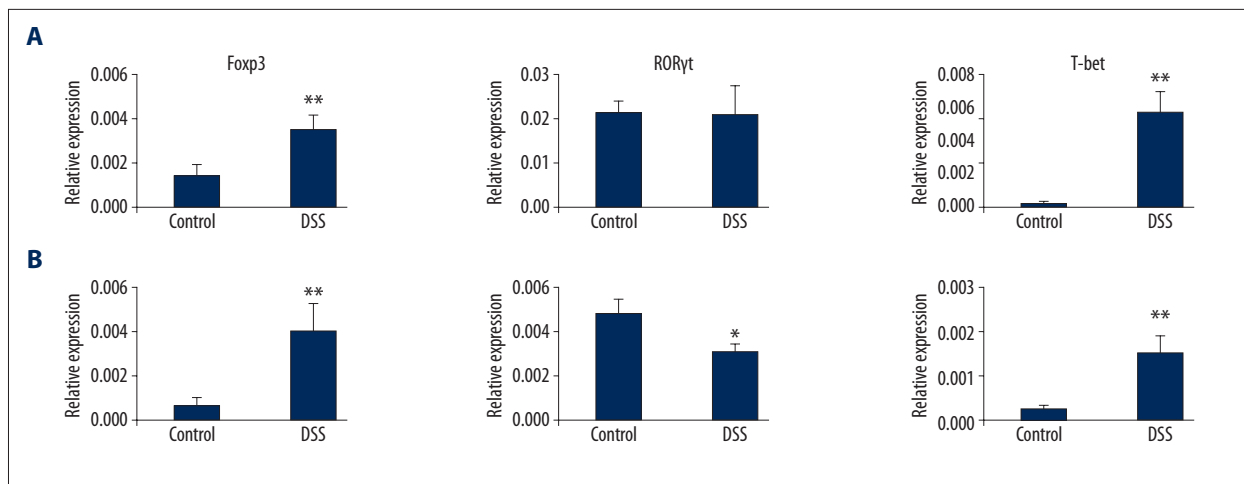
Figure 3. Transcriptional factors driving regulatory T cells and Th1 cells were increased in colon mucosa during acute and chronic colitis. Foxp3, RORγt, and T-bet expression levels in colon tissues from acute DSS colitis (**A**) and chronic DSS colitis (**B**) were assessed through RT-PCR. Each group contained 5 mice. Data are shown as mean ±SD from 3 independent experiments. * $p < 0.05$, ** $p < 0.01$.

Transcriptional factors driving regulatory T cells and Th1 cells were increased in colon mucosa during acute and chronic colitis

To determine the dynamic change in Foxp3⁺ regulatory T cells in colon mucosa during colitis, we assessed the Foxp3 mRNA expression level in colons from DSS colitis mice. We found a 2-fold increase in acute colitis mice ($p < 0.01$) and a 5-fold increase in chronic DSS colitis mice ($p < 0.01$) compared to control mice (Figure 3). The expression of RORγt, a transcriptional factor driving Th17 cell differentiation, was not increased in DSS colitis mice. Again, the expression of T-bet, a transcriptional factor that drives Th1 cell differentiation, was remarkably increased in acute colitis mice and in chronic colitis mice.

Increased Foxp3⁺RORγt⁺ cells during acute and chronic colitis

The above results showed that Foxp3 expression was increased during acute and chronic colitis, but the immune homeostasis in gut mucosa was impaired and the pathological inflammation was not inhibited. It is unlikely that this result was due to the decreased number of Foxp3-positive T cells in gut mucosa, because we and others have reported that the number of Foxp3-positive T cells was increased during inflammation [16]. To further explore the phenotype of Foxp3-positive T cells during DSS colitis, we established an acute DSS colitis model, then examined the RORγt expression among Foxp3-positive cells in mesenteric lymph nodes and spleens. We found the percentage of Foxp3-positive T cells among total CD4⁺ T cells in MLN and spleens from DSS colitis mice was comparable to that in control mice, but RORγt⁺ cells among Foxp3⁺ cells increased 2-fold in spleens and MLN from DSS mice compared to

control mice (Figure 4). These results suggest that the number of Foxp3⁺ cells did not decrease, but the phenotype of Foxp3⁺ cells changed during DSS colitis.

Enhanced cell signaling pathway downstream of IL-23/IL-23R in gut mucosa during acute and chronic colitis

Activation of IL-23R through IL-23 induces Stat3 (signal transducer and activator of transcription) phosphorylation. Next, we investigate the signaling pathway downstream of IL-23R/IL-23 through examining the phosphorylation of Stat3 in colon samples from acute and chronic DSS colitis mice. We observed remarkable phosphorylation of Stat3 in the colon from DSS-treated mice but control mice did not show phosphorylation of Stat3, indicating that the increased expression of IL-23R and IL-23 induced pronounced activation of the cell signaling pathway on target cells in the DSS-treated mice (Figure 5).

Discussion

Inflammatory bowel disease (IBD), a chronic inflammatory condition of the intestines that is marked by remission and relapses, is derived from a dysregulated mucosal immune response to the commensal microbiota that reside within the intestine in a genetically susceptible host [17]. It remains unclear how the genetically imposed risk factors result in the development of IBD. However, it can be reasonably predicted that these genetic factors modify the intestinal epithelial cell barrier and have major effects on the function of innate and adaptive immune systems [18]. A wide variety of animal studies of mucosal inflammation that mimic IBD, as well as human IBD itself, strongly indicate the importance of Th1, Th2, and Th17 cells
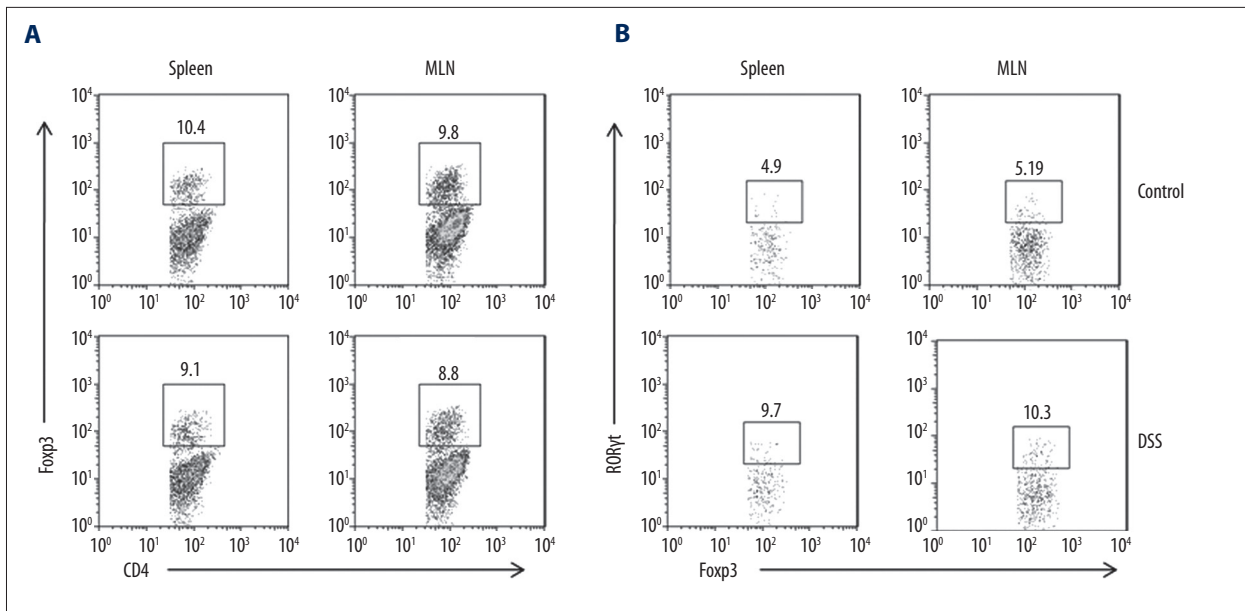
Figure 4. Increased Foxp3⁺RORγt⁺ cells during acute and chronic colitis. (**A**) Frequencies of Foxp3⁺ cells among CD4⁺ cells in spleen and mesenteric lymph nodes from DSS colitis and control mice. (**B**) Frequencies of RORγt⁺ cells among Foxp3⁺ cells in spleen and mesenteric lymph nodes from DSS colitis and control mice.
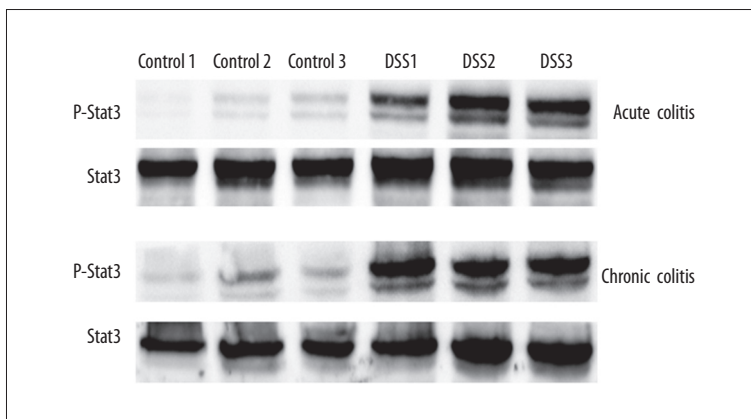

Figure 5. Enhanced cell signaling pathway downstream of IL-23/IL-23R in gut mucosa during acute and chronic colitis. Protein samples of colon tissue from acute DSS colitis mice and chronic DSS colitis mice were separated on 10% SDS-PAGE gels and transferred onto PVDF membranes. The membranes were probed with antibodies against phospho-Stat3 (Tyr705) and Stat3. Each group contained 3 mice.

and their respective cytokines, as well as environmental toxins, in the development of this disease [19–21].

Mutations in the *IL-23R* gene are linked to inflammatory bowel disease susceptibility. Individuals who carry *IL-23R* (Q381) may be less susceptible to IBD due to reductions in receptor function, downstream Stats phosphorylation, and IL-17 production [22,23]. However, only around 7% of Caucasians and even fewer individuals of other ethnic backgrounds have the R381Q allele of *IL-23R* [7,23]. The SNP of *IL-23R* does not explain the pathogenic features of the IL-23/IL-23R signaling pathway in most IBD patients, who carry the same SNP as healthy individuals, suggesting that the expression level, not the genotype, of *IL-23R* control the strength of the IL-23/IL-23R signaling pathway. Although much attention has been paid to the SNP of *IL23R,* the significance of the expression level of IL-23R

in the pathogenesis of intestinal inflammations, such as inflammatory bowel diseases, has been ignored.

In this study we first assessed the IL-23R expression level of colon mucosa in both acute and chronic mouse colitis induced by DSS. We detected increased IL-23R level in colon mucosa from DCC colitis mice. It has been reported that IL-23 was elevated in IBD patients [2]. In our study we also observed increased IL-23 and IL-17 in colon mucosa. The ability of cells to respond to IL-23 correlates with the expression of IL-23R [6]. The elevated IL-23R expression observed during colitis in our study could explain the induction of IL-17, producing cell differentiation. The signaling pathway downstream of IL-23R is mainly mediated by STAT3 and NF-κB. We indeed detected enhanced Stat3 phosphorylation in colon mucosa during colitis, suggesting the activation of the IL-23 receptor function.

It has been demonstrated that IL-23 can also drive intestinal inflammation through the pivotal role of Th1 cells responses in T cell-mediated colitis using T-bet deficiency mice [24] as well as in IBD patients [25]. Genetic ablation of IL23a revealed that IL-23, not IL-12, drives the IFN-γ- producing Th1 subset-mediated inflammation in the intestine [26]. A recent study using IL-23R deletion mice showed that IL-23/IL-23R signaling promotes both Th17 and Th1 cell proliferations in the gut mucosa [9]. In our study we detected increased IFN-γ in colon mucosa from acute and chronic DSS colitis mice.

Foxp3-positive T cells suppress the activity of effector T cells mainly through cell membrane TGF-β. It has been reported that the expression of Foxp3 gene was positively regulated through multiple transcriptional factors, such as Smad3, NFAT, AP-1, and Stat5, and was negatively regulated through Stat3 and GATA-3 [27,28]. It has been demonstrated that some pro-inflammatory cytokines, such as IL-4, IL-6, and IL-27, can inhibit the induction of Foxp3 [14,29,30]. However, in our study the number of Foxp3⁺ cells during colitis was not reduced. A recent study reported that IL-17⁺Foxp3⁺ T cells expressed IL-23R in the synovium of subjects with active rheumatoid arthritis (RA), suggesting the pathological importance of Foxp3 instability in the generation of pathogenic TH17 cells in autoimmunity [31]. After further analysis of the phenotype of Foxp3⁺ T cells, we found that an increased percentage of these cells co-expressed RORγt, a transcriptional factor driving IL-17 production. A recent study by Yang et al. [32] demonstrated that Foxp3⁺ T cells expressing RORγt represent a stable regulatory T cell lineage, but a study by Kluger et al. [33] suggested that these cells were distinct from Th17 cells and conventional Tregs. Whether these Foxp3⁺RORγt⁺ cells are stable or can be converted back to Foxp3⁺ regulatory T cells needs to be further clarified.

Conclusions

The current study demonstrates that IL-23R was elevated in colon mucosa and the receptor function was activated during acute and chronic colitis, but the induction of pathogenic Foxp3⁺RORγt⁺ cells was increased, resulting in the enhanced immune response. Thus, our study highlights the importance of IL-23R expression level and the instability of Foxp3-positive T cells in the development of colitis.

Declaration of interest

The authors have declared there are no conflicts of interest in this work. The authors have agreed that they are responsible for the content and writing of the paper.

References:

1. Xavier RJ, Podolsky DK: Unravelling the pathogenesis of inflammatory bowel disease. Nature, 2007; 448: 427–34

2. Abraham C, Cho J: Interleukin-23/Th17 pathways and inflammatory bowel disease. Inflamm Bowel Dis, 2009; 15: 1090–100

3. Abraham C, Cho JH: IL-23 and autoimmunity: new insights into the pathogenesis of inflammatory bowel disease. Annu Rev Med, 2009; 60: 97–110

4. Croxford AL, Mair F, Becher B: IL-23: one cytokine in control of autoimmunity. Eur J Immunol, 2012; 42, 2263–73

5. Parham C, Chirica M, Timans J et al: A receptor for the heterodimeric cytokine IL-23 is composed of IL-12Rbeta1 and a novel cytokine receptor subunit, IL-23R. J Immunol, 2002; 168: 5699–708

6. Eken A, Singh AK, Oukka M: Interleukin 23 in Crohn's disease. Inflamm Bowel Dis, 20014; 20: 587–95

7. Duerr RH, Taylor KD, Brant SR et al: A genome-wide association study identifies IL23R as an inflammatory bowel disease gene. Science, 2006; 314: 1461–63

8. Zwiers A, Kraal L, van de Pouw Kraan TC et al: Cutting edge: a variant of the IL-23R gene associated with inflammatory bowel disease induces loss of microRNA regulation and enhanced protein production. J Immunol, 2012; 188: 1573–77

9. Ahern PP, Schiering C, Buonocore S et al: Interleukin-23 drives intestinal inflammation through direct activity on T cells. Immunity, 2010; 33: 279–88

10. Sarra M, Pallone F, Macdonald TT et al: IL-23/IL-17 axis in IBD. Inflamm Bowel Dis, 2010; 16: 1808–13

11. Barnes MJ, Powrie F: Regulatory T cells reinforce intestinal homeostasis. Immunity, 2009; 31: 401–11

12. Bollrath J, Powrie FM: Controlling the frontier: regulatory T-cells and intestinal homeostasis. Semin Immunol, 2013; 25: 352–7

13. Uhlig HH, Coombes J, Mottet C et al: Characterization of Foxp3+CD4+CD25+ and IL-10-secreting CD4+CD25+ T cells during cure of colitis. J Immunol, 2006; 177: 5852–60

14. Estelle B, Carrier Y, Gao W et al: Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. Nature, 2006; 441: 235–38

15. Jochen M, Loddenkemper C, Mundt P et al: Peripheral and intestinal regulatory CD4+CD25high T cells in inflammatory bowel disease. Gastroenterology, 2005; 128: 1868–78

16. Xu L, Kitani A, Stuelten C et al: Positive and negative transcriptional regulation of the Foxp3 gene is mediated by access and binding of the Smad3 protein to enhancer I. Immunity, 2010; 33: 313–25

17. Abraham C, Medzhitov R: Interactions between the host innate immune system and microbes in inflammatory bowel disease. Gastroenterology, 2011; 140: 1729–37

18. Henderson P, van Limbergen JE, Schwarze J, Wilson DC: Function of the intestinal epithelium and its dysregulation in inflammatory bowel disease. Inflamm Bowel Dis, 2011; 17: 382–95

19. Cader MZ, Kaser A: Recent advances in inflammatory bowel disease: mucosal immune cells in intestinal inflammation. Gut, 2013; 62: 1653–64

20. Neurath MF: Cytokines in inflammatory bowel disease. Nat Rev Immunol, 2014; 14: 329–342

21. Kim A, Jung BH, Cadet P: A novel pathway by which the environmental toxin 4-Nonylphenol may promote an inflammatory response in inflammatory bowel disease. Med Sci Monit Basic Res, 2014; 20: 47–54

22. Pidasheva S, Trifari S, Phillips A et al: Functional studies on the IBD susceptibility gene IL23R implicate reduced receptor function in the protective genetic variant R381Q. PLoS One, 2011; 6: e25038

23. Kim SW, Kim ES, Moon CM et al: Genetic polymorphisms of IL-23R and IL-17A and novel insights into their associations with inflammatory bowel disease. Gut, 2011; 60: 1527–36

24. Neurath MF, Weigmann B, Finotto S et al: The transcription factor T-bet regulates mucosal T cell activation in experimental colitis and Crohn's disease. J Exp Med, 2002; 195: 1129–43

25. Kobayashi T, Okamoto S, Hisamatsu T et al: IL23 differentially regulates the Th1/Th17 balance in ulcerative colitis and Crohn's disease. Gut, 2008; 57: 1682–89

26. Hue S, Ahern P, Buonocore S et al: Interleukin-23 drives innate and T cell-mediated intestinal inflammation. J Exp Med, 2006; 203: 2473–83

27. Tone Y, Furuuchi K, Kojima Y et al: Smad3 and NFAT cooperate to induce Foxp3 expression through its enhancer. Nat Immunol, 2008; 9: 194–202

28. Xu L, Kitani A, Strober W: Molecular mechanisms regulating TGF-beta-induced Foxp3 expression. Mucosal Immunol, 2010; 3: 230–38

29. Neufert C, Becker C, Wirtz S et al: IL-27 controls the development of inducible regulatory T cells and Th17 cells via differential effects on STAT1. Eur J Immunol, 2007; 37: 1809–16

30. Huber M, Steinwald V, Guralnik A et al: IL-27 inhibits the development of regulatory T cells via STAT3. Int. Immunol, 2008; 20: 223–34

31. Komatsu N, Okamoto K, Sawa S et al: Pathogenic conversion of Foxp3+ T cells into TH17 cells in autoimmune arthritis. Nat Med, 2104; 20: 62–68

32. Yang BH, Hagemann S, Mamareli P et al: Foxp3+ T cells expressing RORγt represent a stable regulatory T-cell effector lineage with enhanced suppressive capacity during intestinal inflammation. Mucosal Immunol, 2015 [Epub ahead of print]

33. Kluger MA, Meyer MC, Nosko A et al: RORγt+Foxp3+ cells are an independent bifunctional regulatory T cell lineage and mediate crescentic GN. J Am Soc Nephrol, 2015 [Epub ahead of print]