



MBD2-mediated Th17 differentiation in severe asthma is associated with impaired SOCS3 expression

Wenjin Sun^{a,b,c}, Bing Xiao^{d,e}, Aijun Jia^f, Lulu Qiu^{a,b,c}, Qingping Zeng^{a,b,c}, Da Liu^{a,b,c}, Yu Yuan^{a,b,c}, Jingsi Jia^{a,b,c}, Xiufeng Zhang^{g,*}, Xudong Xiang^{d,e,**}

^a Department of Respiratory Medicine, the Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China

^b Research Unit of Respiratory Disease, Central South University, Changsha, Hunan 410011, China

^c Hunan Diagnosis and Treatment Center of Respiratory Disease, Changsha, Hunan 410011, China

^d Department of Emergency, The Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China

^e Institute of Emergency and Difficult Diseases, Central South University, Changsha, Hunan 410011, China

^f Department of Respiratory Medicine, The Affiliated Hospital of Guilin Medical University, Guilin 541001, China

^g Department of Respiratory Medicine, The Second Hospital, University of South China, 30 Jiefang Road, Hengyang, Hunan 421001, China

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ABSTRACT

T helper 17 (Th17) cells has proven to be crucial in the pathogenesis of severe asthma. Although it is known that Suppressor of cytokine signaling 3 (SOCS3) is involved in differentiation of Th17 cells but, how it affects severe asthma is uncertain. Since previous studies indicated that Methyl-CpG binding domain protein 2 (MBD2) null mice was deficient in Th17 cell differentiation, the aim of the present study was to understand how MBD2 interacts with SOCS3 to regulate Th17 cell differentiation in severe asthma. Here, we show that SOCS3 expression was significantly decreased in Th17-mediated severe asthmatic mice, accompanied by elevated STAT3 phosphorylation and ROR γ t expression. Knock-down of SOCS3 promoted the differentiation of naïve T cells into Th17 cells through STAT3/ROR γ t pathway. Meanwhile, MBD2 was overexpressed in Th17-mediated severe asthmatic mice. Intervention of MBD2 expression lead to a negative change of SOCS3 expression, whereas the differentiation of Th17 cells showed positive change. In addition, MBD2 knockout (MBD2-KO) mice displayed increased SOCS3 expression and decreased Th17 differentiation after severe asthma modeling. Taken together, our results suggest that MBD2 might facilitate Th17 cell differentiation via down-regulating SOCS3 expression in severe asthma. These findings uncover new roles for SOCS3 and MBD2, and provide a potential target for treatment of severe asthma.

1. Introduction

Asthma is a heterogeneous disease, with different “phenotypes” of “endotypes”, which may reflect different underlying disease processes [1–3]. Unlike allergic asthma, non-allergic asthma which has a neutrophilic airway inflammation often respond less well to inhaled corticosteroid, hence it's often referred to as “severe asthma” [1,4]. It is estimated that severe asthma represents 5–10% of all asthma cases, but they have a much higher economic burden than milder asthma, so there is an urgent need to understand the pathogenesis of severe asthma [5]. The recent study confirmed that T helper 17 (Th17) is in the kernel of severe asthma [6–12]. Th17 cell differentiation starts at T cell receptor

stimulation plus accessory factors activate the expression of STAT3, which in term facilitates the expression of ROR γ t, the master regulator for Th17 cells. Although previous results revealed that pro-inflammatory cytokines, oxidative stress and epigenetic regulation involved in Th17 cell-mediated severe asthma, the underlying mechanisms are still largely unknown.

Suppressor of cytokine signaling is a protein family of eight members (SOCS1–7 and CIS) that responds to a wide range of cytokines, growth factors and hormones [13]. In resting cells, SOCS is expressed at a minimum level, and its expression could be induced by a wide range of signals. SOCS3 is one of the key factors involved in negative regulation of STAT3 [14–16]. Furthermore, Previous studies have found

Abbreviations: SOCS3, Suppressor of cytokine signaling 3; MBD2, Methyl-CpG binding domain protein 2; STAT3, Signal transducers and activators of transcription phosphorylation 3; Th17, T helper 17 cells; ROR γ t, Retinoic acid receptor-related orphan receptor gamma; IL-17, Interleukin-17

* Corresponding author.

** Corresponding author at: Department of Emergency, The Second Xiangya Hospital, Central South University, Changsha, Hunan 410011, China.

E-mail addresses: zxf96178@126.com (X. Zhang), xudongxiang@csu.edu.cn (X. Xiang).

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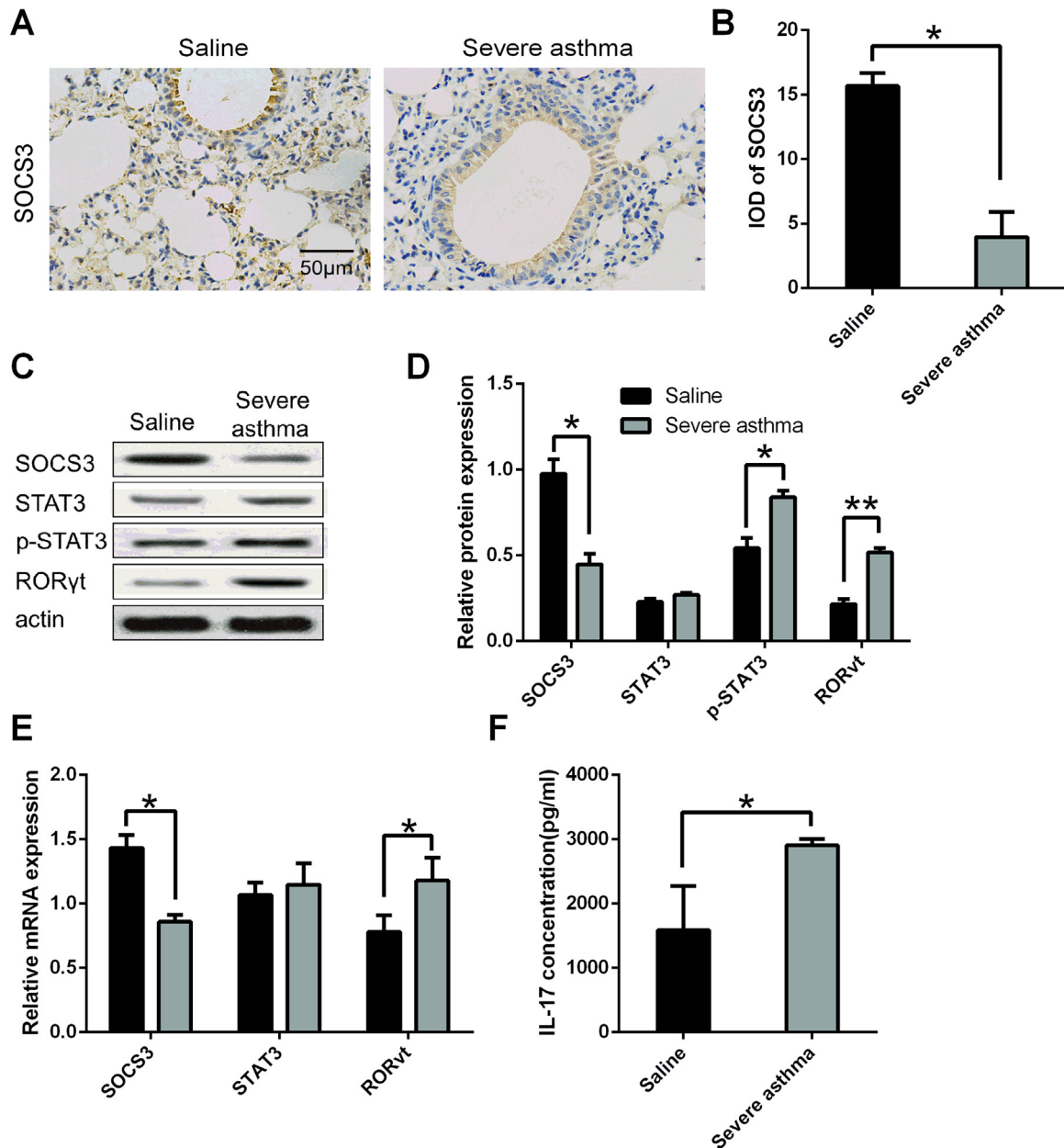


Fig. 1. Expression of SOCS3 and its downstream targets in Th17 cell-mediated severe asthmatic mice and saline control mice. Mice received allergens (severe asthma) ($n = 6$) or saline separately, 48 h after last challenge, Lungs and spleens were harvested for various studies. (A-B) IHC results of SOCS3 staining and quantification of SOCS3 staining integrated optical density (IOD) in lung tissues. Scale bar, 50 μ m. (C-D) Protein expression of SOCS3, STAT3, p-STAT3 and ROR γ t in spleen CD4 + T cells by western blotting, actin as an internal control. (E) mRNA expression of SOCS3, STAT3 and ROR γ t in spleen CD4 + T cells by quantitative real-time PCR, actin as an internal control. (F) Secreted IL-17 in blood serum using ELISA. * $p < 0.05$. ** $p < 0.01$. Data are the representative of at least three separate experiments.

that SOCS3 played key roles in Th17 cell biology, and forced expression of SOCS3 could impair production of IL-17, the hallmark cytokine of Th17 [17–20]. These evidences have made SOCS3 a promising therapeutic target to intervene Th17-related diseases. However, the expression pattern and functional roles of SOCS3 in Th17 cell-mediated severe asthma pathogenesis remain yet unidentified.

The methyl-CpG-binding domain (MBD) proteins interpret the DNA methylation marks and thus are critical mediators of many epigenetic processes [21,22]. MBD2 is a member of the MBD family which is ubiquitously expressed, research has found emerging roles for MBD2 in immunity [21]. Loss of MBD2 tilts cells differentiation towards Th2 polarization [23], and further study revealed that in MBD2 null mice, there is a deficiency in Th17 cell differentiation due to unsuppressed T-bet/HLx axis, resulting in protection from experimental autoimmune encephalomyelitis (EAE) [24]. Given the forementioned role of Th17

cells in severe asthma, MBD2 might as well be a potential target of severe asthma.

We sought to identify the role of MBD2 and SOCS3 in the pathogenesis of Th17 cell-mediated severe asthma. Since our previous study successfully established a Th17 cell-mediated severe asthma mouse model [25], we initiated to assess the expression of SOCS3 in severe asthma. Also, the impact of SOCS3 intervention on the differentiation of naïve T cells into Th17 cells was analyzed. Next, we found the reverse expression between SOCS3 and MBD2. Moreover, the impact of MBD2 intervention on the expression of SOCS3 and the differentiation of naïve T cells into Th17 cells was detected. We further show that MBD2 may induced SOCS3 suppression and the inhibition of Th17 cell differentiation in MBD2-knockout mice. Taken together, these data indicated that MBD2 might promote the differentiation of Th17 cell by down-regulating SOCS3, hence promote the pathogenesis of severe asthma.

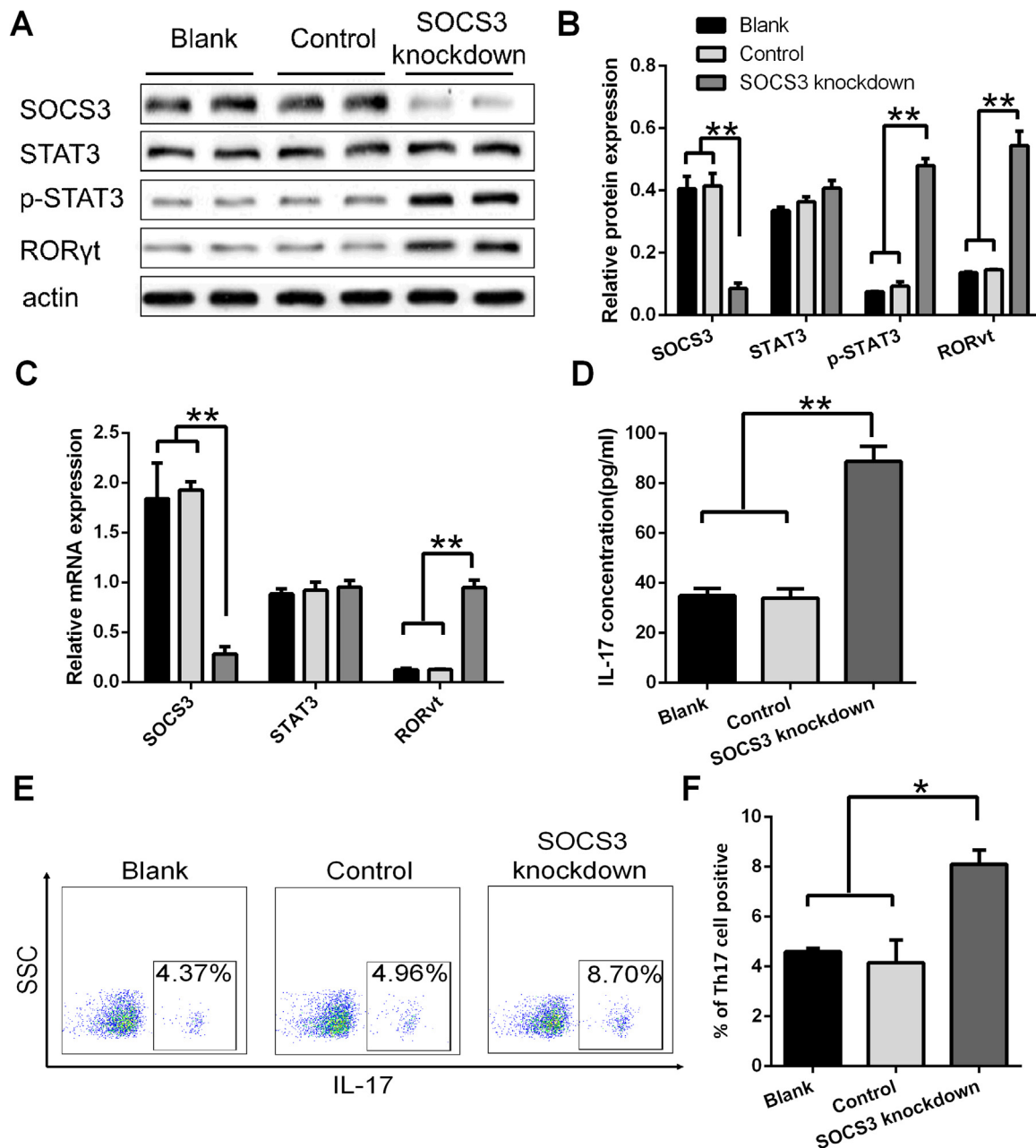


Fig. 2. Effects of SOCS3 knockdown on Th17 polarized differentiated CD4⁺ T cells. CD4⁺ T cells were transfected with SOCS3 siRNA (SOCS3 knockdown) or the sham siRNA (control) or nothing (blank). 72 h after Th17 polarized differentiation, cells were harvested for various studies. (A-B) Protein expression of SOCS3, STAT3, p-STAT3 and RORγt by western blotting, actin as an internal control. (C) mRNA expression of SOCS3, STAT3 and RORγt by quantitative real-time PCR, actin as an internal control. (D) Secreted IL-17 in culture supernatants using ELISA. (E-F) Ratio of positive Th17 cells assessed by flow cytometry. * $p < 0.05$. ** $p < 0.01$. Data are the representative of at least three separate experiments.

2. Materials and methods

2.1. Animal model

Wild-type (WT) C57BL/6 mice were provided by animal center of the Second Xiangya hospital of Central South University (Changsha, China), MBD2 global knockout (MBD2-KO) C57BL/6 mice were purchased from Cyagen Biosciences Co., Ltd (Guangzhou, China). Female mice, aged 6–7 weeks, weighing 18–20 g, were used in the experiments. The severe asthma model was established as previously reported [25]. The severe asthma group ($n = 6/\text{group}$) were given an intraperitoneal sensitization injection with 100 μg house dust mite (HDM, Greer Laboratories, USA), 100 μg Ovalbumin (OVA, Grade V, Sigma Aldrich,

USA) and 15 μg lipopolysaccharide (LPS, Sigma) with 2 mg aluminum hydroxide (Sigma) on days 0, 1 and 2; then challenged with OVA solution atomized for 30 min before HDM intranasal excitation on days 14, 15, 18, and 19. For the saline group, mice received saline only. All studies were performed in compliance with the Second Xiangya Hospital and Central South University Animal Care and Use Committee guidelines. Mice were sacrificed on day 21.

2.2. Histopathology

Lungs were first fixed with 10% formalin via the trachea, and then removed and stored in 10% formalin. Fixed lung tissues were paraffinized and sectioned (5 μm) for immunohistochemistry (MBD2 antibody

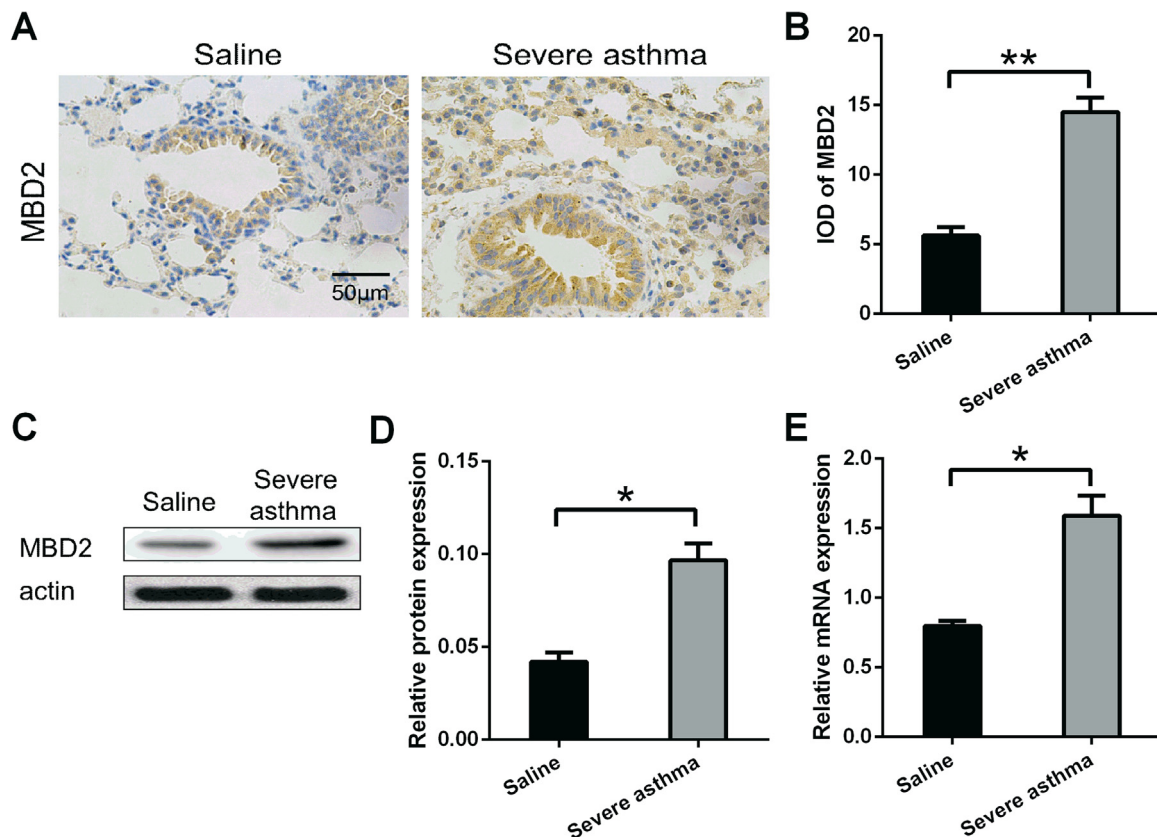


Fig. 3. Expression of MBD2 in Th17 cell-mediated severe asthmatic mice and saline control mice. Mice received allergens (severe asthma) ($n = 6$) or saline separately, 48 h after last challenge, Lungs and spleens were harvested for various studies. (A-B) IHC results of MBD2 staining and quantification of MBD2 staining integrated optical density (IOD) in lung tissues. Scale bar, 50 μ m. (C-D) Protein expression of MBD2 in spleen CD4 + T cells by western blotting, actin as an internal control. (E) mRNA expression of MBD2 in spleen CD4 + T cells by quantitative real-time PCR, actin as an internal control. * $p < 0.05$. ** $p < 0.01$. Data are the representative of at least three separate experiments.

(Abcam, Cambridge, USA) and SOCS3 antibody (Proteintech, Chicago, USA). Select stained sections per group were collected and assessed for MBD2 and SOCS3 protein expression using a microscope and computer image processing system (Image-Pro Plus 6.0) by two pathologists blinded to treatments.

2.3. CD4 + T cell isolation, activation, and staining

Spleen CD4 + T cells of mice were isolated by microbead sorting (130-049-201, Miltenyi Biotec, Germany), and were then seeded in 12-well flat bottom plates for 24 h. Five hours later, cells were restimulated with 50 ng/ml of phorbol-12-myristate-13-acetate (PMA) (Multi Sciences Company, China), 1 μ g/ml of ionomycin (Multi Sciences Company), and 3 μ g/ml of Monensin (Multi Sciences Company) for 5 h. The cells were then fixation and permeabilization with fixation and permeabilization buffer (Multi Sciences Company) for 15 min. After washing with permeabilization buffer, the cells were stained with intracellular markers APC-anti-IL-17 and PE-anti-IL-4 cytokine antibodies (BioLend, USA) in the permeabilization buffer for 20 min. Flow cytometry was conducted and the data were analyzed using the FACSCalibur and FlowJo V10 software.

2.4. Lentiviral transduction

Isolated CD4 + T cells from female wild-type C57BL/6 mice were transfected with chemosynthesis MBD2 siRNA sequence (S) 5'-GTTTG GCTTAACACATCTCAA-3'; SOCS3 siRNA sequence (S) 5'-GGATTCTAC TGGAGCGCGGTGAC-3'. The CD4 + T cells were cultured in 1640 medium without serum. Cells were resuspended according to

1.5×10^6 /tube and MOI = 20, and the appropriate amount of virus was used for transfection. In blank group cells received medium only. In control group cells received medium and sham siRNA.

2.5. Th17 cell differentiation

12 h after lentiviral transduction, CD4 + T cells were centrifuged and resuspended in 10% 1640 medium, activated with 5 μ g/ml of anti-CD3, 1 μ g/ml of anti-CD28 for 3 days in the presence of 5 ng/ml of TGF- β , 20 ng/ml of IL-1 β , 20 ng/ml of IL-6 and 10 ng/ml of IL-21. For neutralization of IL-4 and IFN- γ , 10 μ g/ml of anti-IL-4 and 10 μ g/ml of anti-IFN- γ were added into the cultures [26,27]. All cytokines were purchased from BioLend Co. (BioLend, USA). The cells were seeded in 12-well flat-bottom plates for 72 h. In the last 5 h, the cells were restimulated with PMA, ionomycin and Monensin as above.

2.6. Western blotting

Total proteins were prepared using RIPA lysis buffer supplemented with protease inhibitors. Western blotting was carried out by probing the membranes with indicated primary antibodies followed by incubating with an HRP-conjugated secondary antibody. The MBD2 and SOCS3 antibody were the same as above.

2.7. Quantitative Real-time PCR

Total RNA from CD4 + T cells was isolated using Trizol reagent according to the manufacturer's instructions (Invitrogen). First strand cDNA was synthesized with the RevertAid First Strand cDNA Synthesis

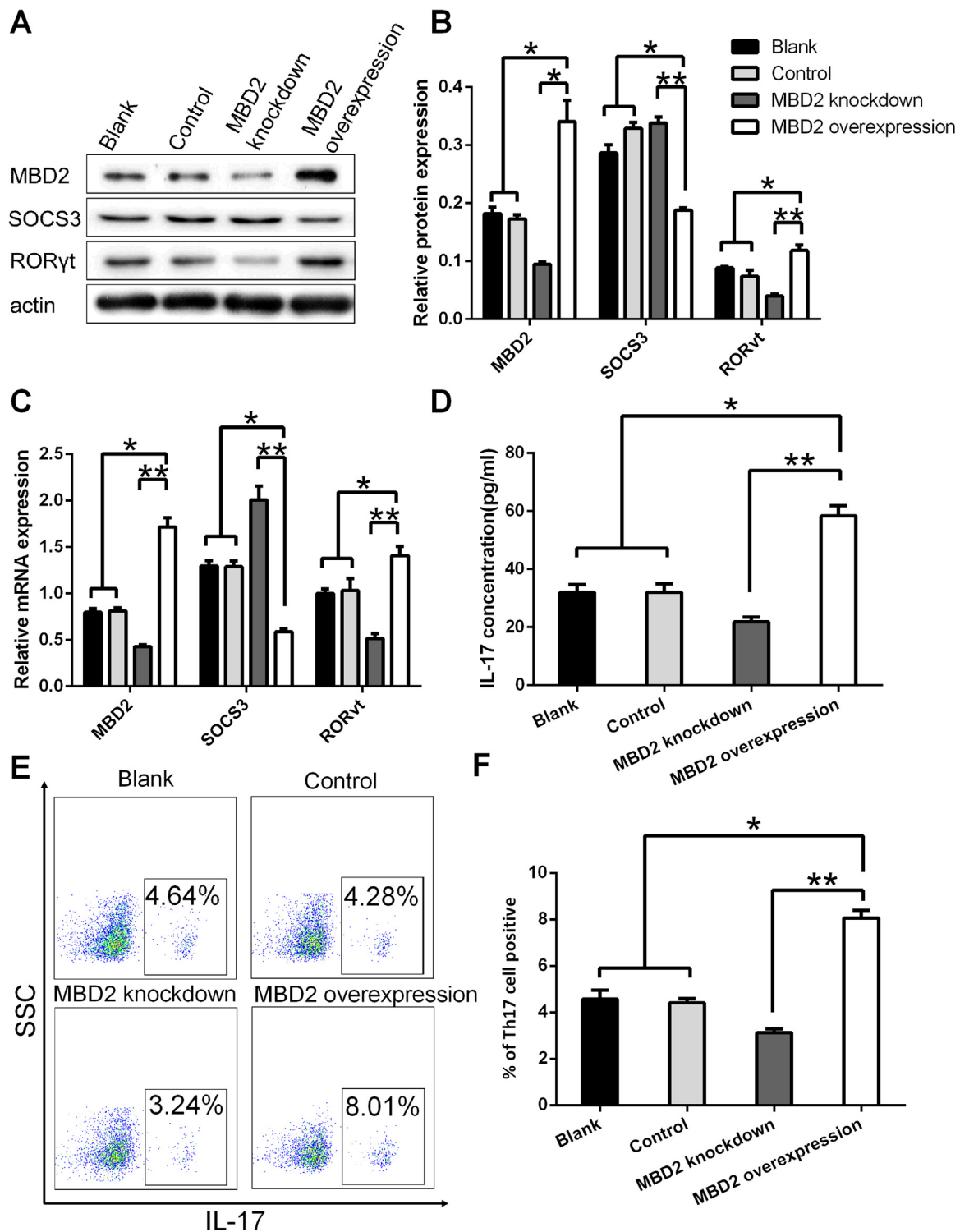


Fig. 4. Effects of MBD2 knockdown or overexpression on SOCS3 expression in Th17 polarized differentiated CD4⁺ T cells. CD4⁺ T cells were transfected with MBD2 shRNAs (MBD2 knockdown or MBD2 overexpression) or the sham siRNA (control) or nothing (blank). 72 h after Th17 polarized differentiation, cells were harvested for various studies. (A-B) Protein expression of SOCS3 and RORγt by western blotting, actin as an internal control. (C) mRNA expression of SOCS3 and RORγt by quantitative real-time PCR, actin as an internal control. (D) Secreted IL-17 in culture supernatants using ELISA. (E-F) Ratio of positive Th17 cells assessed by flow cytometry. * $p < 0.05$. ** $p < 0.01$. Data are the representative of at least three separate experiments.

Kit (Fermentas). Quantitative real-time PCR was performed using SYBRGreen Master Mix (ABI, Shanghai, China). Oligonucleotide primers for target genes were designed by Primer Premier 5.0 and synthesized by Sangon Biotech (Shanghai) Co., Ltd. China. The following primers were used: SOCS3-forward, 5'-CATCCTGCGTCTGGACCTGG-3' and SOCS3-reverse, 5'-CACTGGATGCGTAGGTTCTTGGTC-3'; STAT3-

forward, 5'-CATCCT

GGTGTCTCCACTTGTCT-3' and STAT3-reverse, 5'-CACTACCTGGGTCGGCTTC

G-3'; RORγt-forward, 5'-CCTGGGCTCCTCGCTGACC-3' and RORγt-reverse, 5'-TCTCTCTGCCCTCAGCCTTGCC-3'; MBD2-forward, 5'-CCCAGCGGATGA

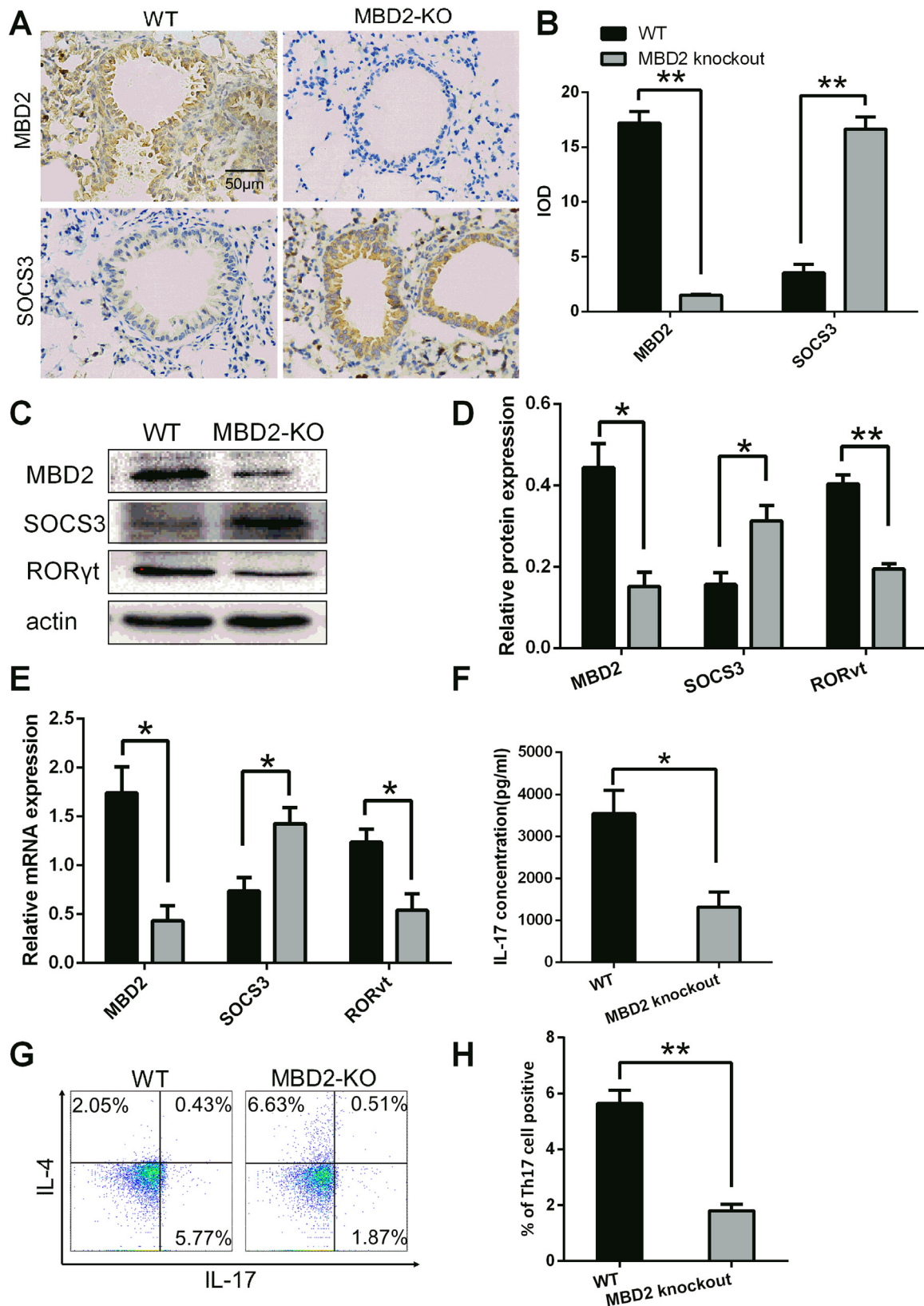


Fig. 5. Expression of SOCS3 and Th17 differentiation in MBD2-KO mice. WT and MBD2-KO mice (n = 6) were both used to build the Th17 cell-mediated severe asthma mouse model. 48 h after the last challenge, Lungs and spleens were harvested for various studies. (A-B) IHC results of MBD2 and SOCS3 staining, quantification of MBD2 and SOCS3 staining integrated optical density (IOD) in lung tissues. Scale bar, 50 μ m. (C-D) Protein expression of MBD2, SOCS3 and ROR γ t in spleen CD4 + T cells by western blotting, actin as an internal control. (E) mRNA expression of MBD2, SOCS3 and ROR γ t in spleen CD4 + T cells by quantitative real-time PCR, actin as an internal control. (F) Secreted IL-17 in blood serum using ELISA. (G-H) Ratio of positive Th17 cells from spleen CD4 + T cells assessed by flow cytometry. * p < 0.05. ** p < 0.01. Data are the representative of at least three separate experiments.

ATGAACAAC-3' and MBD2- reverse, 5'-CTGGACCGACTCCTTGAA GACC-3'; actin-forward, 5'-CATCCTGCGTCTGGACCTGG-3' and actin-reverse, 5'-TAATGTCACGCACGATTCC-3'. The mRNA expression values were normalized to actin. Each experiment was repeated for 3 times in duplicates. The comparative threshold cycle (Ct) value method was used to analyze relative gene expression.

2.8. ELISA analysis

Blood serum of mice and culture supernatants of CD4 + T cells after 72 h of cytokine treatment were collected for ELISA analysis of IL-17 using an IL-17 ELISA kit following the instructions provided by the manufacturer (R&D, USA).

2.9. Statistics analysis

All quantitative data were recorded as $M \pm SD$. And all the data were analyzed using SPSS software version 22.0. Differences between groups were considered statistically significant with a P value < 0.05.

3. Results

3.1. Impaired SOCS3 signaling in Th17 cell-mediated severe asthma

SOCS3 is an important negative feedback regulator of JAK-STAT pathway, and previous evidences have shown its role in modulation of Th17 cell differentiation. We herein explored its role in Th17 cell-mediated severe asthma. The expression of SOCS3 and its downstream targets were determined in Th17 cell-mediated severe asthmatic mice and saline control mice. We applied immunohistochemistry (IHC) on lung specimens and found dramatically decreased SOCS3 immunopositivity in severe asthma group vs. saline group (Fig. 1A-B). Similarly, there was a substantial decrease in the expression of SOCS3 in spleen CD4 + T cells from severe asthma mice, either on transcriptional level or on translational level (Fig. 1C-E). The transcription and protein expression of STAT3 remained statistically unaltered in severe asthma group (Fig. 1C-E), while there was a dramatically increased protein expression in phosphorylated STAT3 (p-STAT3) (Fig. 1C-D). Furthermore, ROR γ t, the master transcription factor for Th17 differentiation, increased significantly in concomitant to the change of STAT3 phosphorylation (Fig. 1C-E). Meanwhile, IL-17, the hallmark cytokine of Th17, showing the same trend as ROR γ t (Fig. 1F). Taken together, these data indicated that impaired SOCS3 expression might contribute to enhanced Th17 response seen in severe asthma.

3.2. Intervention of SOCS3 leads to increased Th17 differentiation

It is now clear that SOCS3 could possibly be responsible for the Th17 polarized differentiation in severe asthma, we next sought to further explore these results with in vitro SOCS3 intervention. We used siRNA to knockdown expression of SOCS3 in T cells, then incubated these cells in Th17-inducing media as previously described. Quantitative real-time PCR and western blot confirmed successful knockdown of SOCS3 in T cells, while in blank and control groups the expression of SOCS3 remained largely unaffected (Fig. 2A-C). We then determined the expression of STAT3 and phosphorylated STAT3 in each group. As expected, the transcription of STAT3 remained unchanged after SOCS3 siRNA intervention, but there was a significant increased STAT3 phosphorylation (Fig. 2A-C). Subsequent to SOCS3 intervention, there were profound reduced Th17 cell differentiation, supported by results of IL-17 in the supernatant and Th17 cell count determined by flow cytometry (Fig. 2D-F). These data suggested that SOCS3 can suppress Th17 cell differentiation via modulation of STAT3 phosphorylation.

3.3. Elevated MBD2 expression in Th17 cell-mediated severe asthma

MBD2 have been found to play a critical role in immunity, and MBD2 null mice are deficient in Th17 cell differentiation. Since severe asthma is acknowledged as a Th17 cell-mediated disorder, we next tend to determine whether MBD2 plays a role in severe asthma. In severe asthmatic mice, immunohistochemistry staining showed that there were elevated expression of MBD2 in the lung (Fig. 3A-B). Quantitative real-time PCR and western blot confirmed that MBD2 over-expressed in spleen CD4 + T cells (Fig. 3C-E). These data indicated that MBD2 expression correlated with asthma severity, suggesting a role of MBD2 in severe asthma pathogenesis.

3.4. MBD2 is necessary to maintain the silencing of SOCS3

We next sought to determine whether there was any relationship between the levels of MBD2 and expression of SOCS3 in Th17 polarized CD4 + T cells. The protein levels of MBD2 and SOCS3 were determined and our data suggested a correlation between lack of SOCS3 protein expression and elevated MBD2 protein levels. To determine whether MBD2 played a direct role in SOCS3 gene silencing, the endogenous levels of MBD2 mRNA were knocked down or overexpressed by Lentiviral transfection of specific shRNA expression vectors in CD4 + T cells. After Th17 polarized differentiation, cells were collected for detection. We tested the effect of two MBD2 shRNAs first, quantitative real-time PCR and western blot confirmed successful knockdown or overexpression of MBD2 in T cells, while in blank and control groups the expression of MBD2 remained largely unaffected (Fig. 4A-C). We then determined the expression of SOCS3 in each group. As expected, transfection with MBD2- silent shRNA exhibited a significant reactivation in SOCS3 expression on transcriptional level as compared with blank and control groups. While, there was no significant difference in the upregulation of SOCS3 expression on translational level (Fig. 4A-C). Transfection with MBD2-overexpress shRNA exhibited a significant reduction in SOCS3 expression as compared with blank and control groups (Fig. 4A-C). Furthermore, the expression of ROR γ t from T cells and secreted IL-17 from culture supernatants were decreased or increased in concomitant to the changes of MBD2 expression (Fig. 4D). Meanwhile, Th17 cell count determined by flow cytometry showing the same trend as MBD2 expression (Fig. 4E-F). Together, these results show that MBD2 contributes to SOCS3 silencing and, as a result, promotes Th17 cell differentiation.

3.5. Deletion of MBD2 promote the expression of SOCS3 leads to reduced Th17 differentiation in severe asthma modeling mice

To further assess the role of MBD2 in the SOCS3 regulation in vivo, the wild-type and MBD2-KO mice were both used to build the Th17 cell-mediated severe asthma mouse model. 48 h after the last challenge, lung resistance and BALF cell count were measured as previously published [25], then mice were sacrificed for detection. After severe asthma modeling intervention, MBD2-KO mice were found to be able to construct a less severe asthma model compared to WT mice (Supplementary Fig. 1). MBD2 was completely abolished in MBD2-KO mice in the lung as well as spleen CD4 + T cells (Fig. 5A-E). In the MBD2-KO mice group, SOCS3 was significantly overexpressed compared with WT mice group after severe asthma modeling treatment (Fig. 5A-E), suggesting that MBD2 can modulate SOCS3 expression. Next, we investigated the influence of overexpressed SOCS3 on the differentiation of Th17 cell in MBD2-KO severe asthma modeling mice. Real-time PCR and western blot showed a significant decrease of ROR γ t compared with WT mice group (Fig. 5C-E). Similarly, decreased IL-17 production and ameliorated Th17 response were obtained compared with WT mice group (Fig. 5F-H). Taken together, these data indicated that impaired MBD2 promote SOCS3 expression might contribute the reduced Th17 response, resulting a alleviated symptoms of severe

asthma.

4. Discussion

Severe asthma comprises only 5–10% of the total asthma population, but accounts for a large proportion of asthma morbidity and health care expenditures [28]. Th17 cell, the important subset of T cells that mediates severe asthma, is mainly mediated by ROR γ t and characterized by increased IL-17 production. Despite recent progress in understanding the pathogenesis of severe asthma, the mechanisms remain poorly understood. In present report, SOCS3 was discovered a reduction in lungs and spleen CD4 + T cells from Th17 cell-mediated severe asthmatic mice. Knockdown the expression of SOCS3 promoted Th17 differentiation in activated T cells. Meanwhile, MBD2 was found to contribute to SOCS3 silencing. Our study for the first time reported that global MBD2-KO significantly ameliorated severe asthma. Moreover, we have identified that MBD2 may induce SOCS3 suppression to facilitate ROR γ t expression, together resulting in Th17 cell differentiation in severe asthma.

Our result for the first time showed that SOCS3 was suppressed in Th17 cell-mediated severe asthma. SOCS3, as a negative regulator of cytokine signal transduction, is correlated with infection and autoimmunity [14,29–31]. SOCS3 was usually reported inhibits signaling via preventing JAK mediated activation of STAT3 [32,33]. Previous results have shown that inhibition of SOCS3 promotes Th17 cell development, and upregulation of SOCS3 inhibits IL-17 production contribute to reduced Th17 cell development, indicating that SOCS3 is a potential target to intervene Th17-related diseases [17–19]. However, the role and mechanism of SOCS3 in Th17 cell-mediated severe asthma remains unclear. Our current study shows that SOCS3 is reduced in Th17 cell-mediated severe asthma, accompanied by elevated STAT3 phosphorylation and ROR γ t expression. We further investigated that inhibition of SOCS3 expression by siRNA can promote the expression of ROR γ t by promoting the phosphorylation of STAT3, resulting in increased differentiation of Th17 cells. Therefore, the role of SOCS3 and downstream signaling pathways in the differentiation of severe asthma TH17 cells was clarified.

Although Epigenetic modifications may have a role in asthma pathogenesis [34,35], the specific regulatory molecular mechanisms remain unclear. MBD2, as a regulation factor of DNA methylation, has been reported to be involved in the differentiation of th17 cells [24], thus becoming a possible upstream regulatory target in Th17 cell-mediated severe asthma. In our study, we confirmed that MBD2 overexpressed in severe asthma. Interestingly, an inverse relationship between MBD2 and SOCS3 has been found. As MBD2 involved in methylation regulation, we guess that MBD2 may repress SOCS3 transcription by banding to methylated gene promoters. Bioinformatics analysis (<http://www.urogene.org/methprimer/>) implied that there were CpG islands in the SOCS3 promoter sequence. Currently, there is no study on whether MBD2 can regulate the expression of SOCS3. Our study for the first time suggests that inhibition of MBD2 significantly upregulates SOCS3, hence ameliorates Th17 response in vitro and in vivo. First, inhibition of MBD2 markedly increased transcriptional levels of SOCS3, and also downregulated ROR γ t to inhibit Th17 differentiation. Second, upregulation of MBD2 markedly decreased expression levels of SOCS3, and also upregulated ROR γ t to promote Th17 differentiation. Surprisingly, using MBD2 KO mice, suppressed SOCS3 expression was reversed. Moreover, Th17 differentiation were significantly reduced via downregulation of ROR γ t expression, accompanied with alleviated symptoms of severe asthma. Future studies are warranted to determine the methylation status in the SOCS3 gene promoter regions and the roles of MBD2 in SOCS3 gene transcription. In conclusion, our data demonstrated that MBD2-mediated Th17 differentiation in severe asthma is associated with impaired SOCS3 expression. This study could lead to new therapeutic prospects for the treatment of patients with severe asthma.

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Author contribution

Xudong Xiang and Xiufeng Zhang designed this project. Wenjin Sun, Bing Xiao, Aijun Jia, Lulu Qiu, Qingping Zeng, Da Liu, Yu Yuan and Jingsi Jia performed the experiments.

Conflict of interest

There is no conflict of interest.

Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.yexcr.2018.08.010.

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