SJMHE1 Peptide from *Schistosoma japonicum* Inhibits Asthma in Mice by Regulating Th17/Treg Cell Balance via miR-155

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Purpose: Helminths and their products can regulate immune response and offer new strategies to control and alleviate inflammation, including asthma. We previously found that a peptide named as SJMHE1 from *Schistosoma japonicum* can suppress asthma in mice. This study mainly investigated the molecular mechanism of SJMHE1 in inhibiting asthma inflammation.

Methods: SJMHE1 was administered to mice with OVA-induced asthma via subcutaneous injection, and its effects were detected by testing the airway inflammation of mice. The Th cell distribution was analyzed by flow cytometry. Th-related transcription factor and cytokine expression in the lungs of mice were analyzed using quantitative real-time PCR (qRT-PCR). The expression of miR-155 and levels of phosphorylated STAT3 and STAT5 were also investigated after SJMHE1 treatment in mice by qRT-PCR and Western blot analysis. The in vitro mouse CD4+ T cells were transfected with lentivirus containing overexpressed or inhibited miR-155, and the proportion of Th17, Treg cells, CD4+ p-STAT3+, and CD4+ p-STAT5+ cells were analyzed by flow cytometry.

Results: SJMHE1 ameliorated the airway inflammation of asthmatic mice, upregulated the proportion of Th1 and Treg cells, and the expression of Th1 and Treg-related transcription factor and cytokines. Simultaneously, SJMHE1 treatment reduced the percentage of Th2 and Th17 cells and the expression of Th2 and Th17-related transcription factor and cytokines. SJMHE1 treatment decreased the expression of miR-155 and p-STAT3 but increased p-STAT5 expression. In vitro, the percentage of Th17 and CD4+ p-STAT3+ cells increased in CD4+ T cells transfected over-expression of miR-155, but SJMHE1 inhibited the miR-155-mediated increase of Th17 cells. Furthermore, SJMHE1 increased the proportion of Treg and CD4+ p-STAT5+ cells after transfected over-expression or inhibition of miR-155.

Conclusion: SJMHE1 regulated the balance of Th17 and Treg cells by modulating the activation of STAT3 and STAT5 via miR-155 in asthma. SJMHE1 might be a promising treatment for asthma.

Keywords: *Schistosoma japonicum* peptide, SJMHE1, Th17/Treg cell balance, miR-155, asthma

Introduction

Asthma is a heterogeneous disease characterized by chronic airway inflammation and caused by various immune cells and inflammatory factors. Although the exact molecular mechanism of asthma remains unknown, CD4+ T cells, especially the imbalance of Th1/Th2 and Th17/regulatory T cells (Tregs), participate in asthma pathogenesis. Although inhaled corticosteroids are effective for the treatment of...
asthmatic patients, many side effects and resistance are caused by corticosteroids in patients, prompting us to seek new therapeutic targets in asthma.

Helminth infection and helminth-derived products reduce allergic, autoimmune, and inflammatory reactions, including asthma. Helminths have developed several mechanisms to modulate the immune response of hosts, thereby inhibiting the occurrence of asthma. Helminth infection or helminth-derived molecules induce regulatory T cells (Tregs), IL-10-producing regulatory B cells, dendritic cells, and alternatively activated macrophages to prevent allergic airway inflammation in asthmatic mice. In comparison with helminth infection or most macromolecules derived from parasites, we have identified a small-molecule peptide from Schistosoma japonicum and it named as SJMHE1; it could induce CD4+CD25+ Treg cells and suppress the delayed-type hypersensitivity in mice. Furthermore, SJMHE1 can inhibit the inflammatory response in collagen-induced arthritis (CIA) and asthma in mice. Asthma was induced by OVA in mice, they were treated by SJMHE1 at the time of OVA administration (day 0), and SJMHE1 suppressed airway inflammation in asthma by downregulating Th2 cells and upregulating Th1 and Treg cells in asthmatic mice. However, the molecular mechanism of the downregulation of inflammation in asthma caused by SJMHE1 is not well understood.

Recent studies reveal that non-coding RNAs (ncRNA), especially microRNAs (miRNAs), participate in the regulation of inflammatory response in asthma. Asthmatic patients up-regulates miR-498, miR-187, and miR-143 and down-regulates miR-18a, miR-126, miR-155, and miR-224 in nasal biopsies. The upregulation of miR-155, miR-21, and miR-18a is correlated with Th2 cytokines in bronchoalveolar lavage fluid of asthmatic mice. Among these miRNAs involved in the pathogenesis of asthma, miR-155 is an important immune modulator and is upregulated in various activated immune cells. Furthermore, miR-155 regulates Th2, Th17, and Treg differentiation and participates in the pathogenesis of various diseases, including asthma. Malmhäll and colleague reported that miR-155 knockout (KO) mice decrease eosinophilic inflammation and mucus hypersecretion and reduce the levels of Th2 cells and Th2-related cytokines, suggesting that miR-155 is essential for Th2 response in asthma. miR-155 also regulates Th1/Th2 balance. The overexpression of miR-155 in CD4+ T cells promotes Th1 differentiation, whereas the inhibition of miR-155 induces Th2 response. Furthermore, miR-155 is involved in the regulation of Th17/Treg balance in various inflammations. Whether miR-155 is involved in the regulation of Th cells in asthmatic mice by SJMHE1 needs further analysis.

In the present study, we further investigated the role of SJMHE1 in the development of asthma and treated mice with SJMHE1 at days 14 and 21 beginning at the third OVA sensitization. We demonstrate that the SJMHE1 treatment still suppresses the inflammation of the airway and regulates Th cell distribution, the expression of key transcription factors, and cytokines for Th cell differentiation in the lungs of asthmatic mice. Furthermore, SJMHE1 treatment downregulated miR-155, and this condition may suppress airway inflammation by regulating Th17/Treg balance in the asthmatic mice.

Materials and Methods

Mice

Male BALB/C mice aged 6–8 weeks were obtained from Comparative Medicine Centre of Yangzhou University (Yangzhou, China) and bred with specific pathogen-free condition at the Animal Experimental Centre of Jiangsu University. Mouse handling and experimentation were conducted in strict compliance with the Regulations for Administration of Affairs Concerning Experimental Animals of Jiangsu University. All protocols were approved by the Jiangsu University Institutional Animal Care and Use Committee (Permit Number: JSU 18–012).

Peptides

The synthesis and purification of SJMHE1 peptide from Schistosoma japonicum (VPGGGTALLRCIPVLDTLSTKNED) are entrusted to Chinapeptides (Shanghai, China). Polymyxin B-agarose was used to remove possible LPS contamination as described previously.

Induction of Experimental Asthma and SJMHE1 Treatment

Asthma was induced in mice as described previously. In brief, mice were randomly divided into four groups, namely, PBS, OVA, OVA/PBS, and OVA/SJMHE1 group. On days 0, 7, and 14, each mouse in PBS group was immunized by intraperitoneal injection of 200 µL of PBS, while the mice in the other groups were immunized by 50 µg OVA (Sigma-Aldrich, Steinheim, Germany) and 2 mg of 10% aluminum hydroxide gel in PBS. On days 14 and 21, mice in OVA/PBS and OVA/SJMHE1 groups were separately treated with PBS and SJMHE1 (10 µg) emulsified with incomplete Freund’s adjuvant.
On days 21–28, the mice were challenged in the form of atomization with OVA (2%) or PBS as previously described. All the mice were sacrificed on the day 29 to evaluate airway inflammation and immune response.

Histopathologic Analysis
The left lung of mice was soaked in 10% formalin for paraffin embedding. The lung tissue was then stained with hematoxylin and eosin (H&E) and periodic acid-Schiff (PAS) to determine the histopathologic analysis.

Cell Analysis in Bronchoalveolar Lavage Fluid (BALF)
The left main bronchus was ligated, and the right lung of each mouse was washed twice with 0.5 mL of sterile phosphate buffer solution (PBS) to collect BALF. BALF was centrifuged, and the supernatant was removed. Cell precipitation was resuspended with 1 mL of PBS, and the total number of inflammatory cells was counted using a hemocytometer. Wright and Giemsa staining was used to count the eosinophils in BALF as described previously.

Anti-OVA-specific IgE Detection in Serum
Anti-OVA-specific IgE was detected using an enzyme-linked immunosorbent assay (ELISA) as described previously. In brief, the plate was coated with 100 µL of OVA (100 µg/mL) per well and blocked with 5% skim milk. After washing, goat anti-mouse IgE (Abcam, 1:250 diluted) and HRP-conjugated rabbit anti-goat secondary IgG (Multisciences, 1:5000 diluted) were used for detection.

Flow Cytometry
Single-cell suspensions of the lungs were prepared and analyzed for Th1, Th2, TH17, and Treg as previously described. For Th1, Th2, and Th17 cell subset analysis, lung cell suspensions were stimulated with PMA/Ionomycin mixture (Multisciences, Hangzhou, China) and BFA/Monensin Mixture (MultiSciences) for 5 h, and then stained with PerCP anti-CD3 mAbs (eBioscience, San Diego, CA, USA) and FITC-anti-CD4 (eBioscience). After fixation and permeabilization with Cytotox/Cytoperm (BD Biosciences), the cells were stained with PE-anti-p-STAT3 (BD Biosciences) and PE-anti-p-STAT5 (BD Biosciences), respectively.

Quantitative Real-Time PCR (qRT-PCR)
Total RNA extracted from the lungs of mice and CD4+ T cells was reverse-transcribed into cDNA by using the Prime Script 1st Strand cDNA synthesis kit (Takara) and All-in-one™ miRNA First-Strand cDNA synthesis kit 2.0. All-in-One™ qPCR primer for GAPDH (MQP027158), IFN-γ (MQP027401), IL-4 (MQP032451), IL-5 (MQP029462), IL-13 (MQP092962), IL-17A (MQP029457), IL-10 (MQP029453), IL-35 (MQP027412), TGF-β (MQP030343), T-bet (MQP034192), GATA3 (MQP027165), FoxP3 (MQP067272), ROR-γt (MQP054636), miR-155-5p (MmiRQP0890), and U6 (MmiRQP9002) were obtained from GeneCopoeia (GeneCopoeia, Germantown, MD, USA). The PCR amplification of miR-155-5p was conducted using the All-in-one™ miRNA qPCR kit (GeneCopoeia, Germantown, MD, USA), and the other primers were analyzed via RT-PCR with ChamQ Universal SYBR qPCR Master Mix (Vazyme, Nanjing, China) according to the manufacturer’s protocol.

Western Blot Analysis
Protein samples were extracted from mouse lungs for Western blot analysis as previously described. The rabbit monoclonal antibodies of p-Stat3 (Tyr705; Cell Signaling staining kit (eBioscience) was used to determine Treg cells as previously described.

For the detection of CD4+p-STAT3+ and CD4+p-STAT5+ cells, the CD4+T cells were separately stimulated with 100 ng/mL IL-6 (Pepro Tech, Cranbury, NJ, USA) and 100 ng/mL IL-2 (Pepro Tech, Cranbury, NJ, USA) at 37°C for 30 mins following the manufacturer’s protocol. Then, the cells were collected and stained with BV421-anti-CD4 (eBioscience). After fixation and permeabilization with Cytotox/Cytoperm (BD Biosciences), the cells were stained with PE-anti-p-STAT3 (BD Biosciences) and PE-anti-p-STAT5 (BD Biosciences), respectively.
Technology; 1:1000 dilution), p-Stat5 (Tyr694; Cell Signaling Technology; 1:1000 dilution), SOCS1 (Cell Signaling Technology; 1:1000 dilution), and GAPDH (Cell Signaling Technology; 1:1000 dilution) were used as the primary antibodies. HRP-conjugated anti-rabbit IgG (AB Clonal, Wuhan, China) was used as the second antibody. The chemiluminescent detection and image analysis were carried out as previously described.21

CD4+ T Cell Isolation and Activation
CD4+ T cells were separated from the splenocyte suspension of naïve mice by using the EasySep™ Mouse CD4+ T cell isolation kit (Stem Cell Technologies, Vancouver, Canada) following the manufacturer’s protocol. The purity of CD4+ T cells was >95% as determined by flow cytometry. CD4+ T cells (2×10^6/mL) were cultured in the presence or absence of MACSiBead Particles (T cell activation/expansion kit, Miltenyi Biotec, Bergisch Gladbach, Germany) for 24 h. MACSiBead particles are loaded with mouse CD2, CD3, and CD28 antibodies.

CD4+ T Cell Transfection by Lentivirus
Upregulated (overexpression) and knocked down miR-155 (inhibition) in the GV369 and GV280 lentivirus vector (Genechem, Shanghai, China) were transfected with CD4+ T according to the manufacturer’s instructions. In brief, according to the MOI and the titer of lentivirus, 150 µL of GV369, 56 µL of GV280 lentivirus vector, and 20 µL of 250× Histrans G P (Genechem) were added into CD4+ T cells (5 × 10^5 cells/well) containing 500 µL of PRMI 1640 in a 24-well plate. After 24 h, the medium was replaced by 1 mL of RPMI 1640 containing 10% FBS. The cells were then stimulated by 5 µL of loaded anti-biotin MACSiBead particles (Miltenyi Biotec) for 96 h. At the same time, certain wells were added with 1 µg/mL SJMHE1. Then, the percentages of Th17 and Treg cells, and p-STAT3, p-STAT5, and SOCS1 expression levels in CD4+ T cells were analyzed by flow cytometry.

Statistical Analyses
GraphPad Prism 5.01 (GraphPad Software, 2007, La Jolla, CA, USA) was used for statistical analyses. Data were expressed as mean ± standard error of the mean. One-way ANOVA with Tukey Kramer was used to compare the differences among the groups. Spearman correlation was used for the correlation analysis, and P-value < 0.05 was considered statistically significant.

Results
SJMHE1 Treatment Ameliorated Ongoing Asthma Inflammation in Allergic Mice
We further investigated whether SJMHE1 could treat ongoing asthma in mice.21 SJMHE1 treatment began with the third OVA sensitization, and the treatment regimen is illustrated in Figure 1A. Consistent with previous results,21 the infiltration of inflammatory cell in the peribronchial and perivascular regions, mucus hypersecretion, structural changes in lungs, such as goblet cell hyperplasia, and increased airway smooth muscle mass, were displayed in OVA- or OVA/PBS-treated mice (Figures 1B and C). By contrast, inflammatory cell infiltration, mucus secretion, and structural changes in the airway were ameliorated in OVA/SJMHE1 groups. Similarly, SJMHE1 treatment reduced the total cell, eosinophilia, and neutrophil numbers in the BALF (Figure 1D–F) but did not affect the IgE levels relative to the OVA or OVA/PBS group (Figure 1G). These results suggest that SJMHE1 treatment can ameliorate ongoing asthma in mice.

SJMHE1 Treatment Modulates Th Cell Distribution and Transcription Factor and Cytokine Expression in Lungs of Asthmatic Mice
The lungs serve as the inflammatory site in asthmatic mice, and CD4+ T cells are at the forefront of airway inflammation in asthma.2 We investigated the effect of SJMHE1 treatment on the Th cell distribution in the lungs of asthmatic mice. Consistent with previous results in the splenocytes of allergic mice,21 the proportion of Th1 and Treg cells increased in SJMHE1-treated mice compared with OVA or OVA/PBS-treated mice (Figure 2). Similarly, the percentage of Th2 and Th17 cells decreased in SJMHE1-treated mice compared with OVA or OVA/PBS-treated mice (Figure 2). T-bet, GATA3, RORγt, and Foxp3 are the dominant transcription factors of Th1, Th2, Th17, and Treg cells, respectively. Consistent with Th cell distribution, SJMHE1 treatment displayed upregulation of T-bet and Foxp3 mRNA expression and downregulation of GATA3 and RORγt mRNA expression (Figure 3). Consistent with the regulation of Th cells by SJMHE1, SJMHE1 treatment upregulated the expression of IFN-γ, IL-10, TGF-β, and IL-35 mRNA and
downregulated the expression of IL-4, IL-5, IL-13, and IL-17 mRNA in the lungs of asthmatic mice (Figure 4). Therefore, SJMHE1 ameliorated ongoing asthma, which was associated with the regulation of Th differentiation and cytokine expression in the lungs of asthmatic mice.

Figure 1 SJMHE1 treatment alleviates ongoing airway inflammation in allergic mice. (A) Experimental scheme. BALB/c mice were sensitized with OVA on days 0, 7, and 14 and challenged with OVA daily from day 21 to day 28. Mice were injected with SJMHE1 emulsified in IFA on days 14 and 21. The mice were killed on day 29. (B) Lung sections from mice were stained by hematoxylin and eosin (H&E), and (C) periodic acid-Schiff reagent (PAS) at 20× magnification. Images represent three independent experiments (n = 6 mice per group). (D) Total cells, (E) eosinophil number, and (F) neutrophil number in BALF. Eosinophil and neutrophil in BALF cells were determined by Wright and Giemsa staining. (G) OVA-specific IgE antibody levels in sera of mice. Results are presented as mean±SEM (n=18) from three-independent experiments. *P < 0.05, ***P < 0.001.
miR-155 regulates the balance of Th1/Th2 and is a novel target for asthma, while SJMHE1 effectively modulates Th cell distribution to suppress inflammatory response in asthmatic mice based on the above results.
miR-155 could regulate Th17 and/or Treg response in various diseases, including asthma. Thus, we further explored whether SJMHE1 could modulate the balance of Th17 and Treg of asthma via miR-155 in vitro. Mouse CD4⁺ T cells were transfected by a lentivirus containing over-expressed and knocked down miR-155, and then stimulated with MACsiBead particles in the presence or absence of SJMHE1. miR-155 expression significantly increased in CD4⁺ T cells transfected with lentivirus encoding miR-155 (overexpression), whereas no change was observed in cells transfected with miR-155 sponge (inhibition, Figure S1). As expected, the over-expression...
of miR-155 increased the proportion of Th17 cells in CD4⁺ T cells, but the proportion of Th17 cells decreased in the presence of SJMHE1 (Figure 7). In comparison with the vector control, the proportion of Treg had no significant change in the over-expression or inhibition of miR-155 group. However, the addition of SJMHE1 increased the proportion of Treg regardless of the over-expression or inhibition of miR-155 (Figure 7). In general, STAT3 and STAT5 signaling is critical for Th17 and Treg differentiation, respectively. Consistent with the findings on Th17 and Treg cells, the proportion of CD4⁺p-STAT3⁺ increased after transfecting lentivirus with over-expressed miR-155, but treatment with SJMHE1 reduced the expression of p-STAT3 in CD4⁺ T cells overexpressed miR-155, while that of p-STAT5 was elevated in CD4⁺ T cells after SJMHE1 treatment regardless over-expressed or inhibited miR-155 (Figure 8). In comparison with vector control or over-expressed miR-155, SJMHE1 treatment increased the percentage of CD4⁺SOCS1⁺ cells (Figure 8). Therefore, miR-155 promotes Th17 response, but SJMHE1 inhibits the miR-155-mediated Th17 cell response and promotes the induction of Treg bias in CD4⁺ T cells in vitro by regulating the activation of STAT3 and STAT5.

**Discussion**

Asthma is an uncontrolled airway inflammatory response characterized by immune imbalance, especially when the imbalance of CD4⁺ T cells plays a critical role in the pathogenesis of asthma. Drug intervention can correct the imbalance of CD4⁺ T cell and improve respiratory symptoms in asthma.39,40 Interestingly, helminth parasite can balance between immune cell activation through co-evolving with the host, thus introducing a new pathway to control untoward immune responses, including asthma.4 Our previous research found that the schistosoma peptide SJMHE1 can inhibit airway inflammation in asthmatic mice.21 SJMHE1-treated macrophages and dendritic cells (DCs) show a tolerogenic phenotype and induce CD4⁺CD25⁺Foxp3⁺ Treg cell differentiation.18 Although innate lymphoid cells, macrophages, eosinophils, mast cells, and T and B cells play an important role in the pathogenesis of asthma, the type 2
response is usually the hallmark of asthma.\textsuperscript{41} The Th17 cell response has been related to the severity of asthma,\textsuperscript{42} and high levels of IL-17 indicate resistance to corticosteroid treatment in asthmatic mice and patients.\textsuperscript{43,44} Thus, in the current study, we evaluated the effect of SJMHE1 on Th17 and Treg cells in asthmatic mice and demonstrated that SJMHE1 inhibits the development of asthma by regulating the activation of STAT3 and STAT5 via miR-155, thereby modulating the balance of Th17/Treg.

Similar to the intervention with SJMHE1 at the beginning of OVA-induced asthma,\textsuperscript{21} SJMHE1 still relieved the development of asthma, reduced the infiltration of inflammatory cells in the lungs, and decreased the total cell, eosinophilia, and neutrophil number in the BALF of mice, which were treated by SJMHE1 from the third OVA sensitization. SJMHE1 treatment upregulated the proportion of Th1 and Treg cells and downregulated the proportion of Th2 and Th17 cells in the lungs of asthmatic mice. Consistent with the regulation of Th cells by SJMHE1, SJMHE1 treatment upregulated the expression of T-bet and Foxp3 mRNA and downregulated the expression of GATA3 and ROR-γt mRNA of lungs in asthmatic mice. Consistent with our previous study,\textsuperscript{21} SJMHE1 treatment reduced the expression of IL-4, IL-5, and IL-13 mRNA and increased the expression of IFN-γ, IL-10, TGF-β, and IL-35 mRNA in the lungs of asthmatic mice. Treg cells usually suppress inflammatory responses via the production of IL-10, IL-35, and TGF-β.\textsuperscript{13} The increase in IFN-γ, IL-10, TGF-β, and IL-35 mRNA expression was consistent with the increase in Th1 and Treg cell

\textbf{Figure 7} SJMHE1 regulates Th17 and Treg differentiation via miR-155 in vitro. CD4\textsuperscript{+} T cells were transfected with lentivirus containing over-expressed and knocked down miR-155, and then stimulated with MACS\textsuperset{i}Bead particles in the presence or absence of 1 µg/mL SJMHE1 for 96 h. The cells were tested for Th17 and Treg subsets by flow cytometry. (A) CD4\textsuperset{i}IL-17Th17 cells and (B) CD4\textsuperset{i}CD25Foxp3 Tregs in each group are shown. Data are representative of three experiments. (C) Proportion of CD4\textsuperset{i}IL-17 Th17 cells and (D) CD4\textsuperset{i}CD25Foxp3 Tregs in each group. Results are presented as mean±SEM of three experiments performed in triplicate. *P < 0.05, **P < 0.01.
SJMHE1 regulated the balance of Th cells and cytokines in the lungs of asthmatic mice and may offer protection against the development of asthma. However, how SJMHE1 regulates the balance of Th cells remains unknown.

miRNA-mediated controlled gene expression can fine-tune the appropriate immune response, which is essential in helminth infection and immunity. The excretory/secretory (ES) antigens of *Taenia crassiceps* can inhibit LPS-induced Let-7i in human dendritic cells (DCs) related to the decrease in inflammatory response. Furthermore,
miRNAs participate in the regulation of T cell polarization and cytokine production. miR-155 was initially thought as a “Th2 enriched” miRNA, which was essential in mediating allergy and antihelminth immunity.\(^47\) Recent studies show that miR-155 is involved in controlling Treg function and Th17-mediated inflammation.\(^35,48\) Furthermore, miR-155 is a critical target in asthma, and the upregulation of miR-155 participates in the development of asthma.\(^26,30\) Whether miR-155 is involved in the SJMHE1 regulating Th cell balance in asthmatic mice has not been determined. Consistent with the upregulation of miR-155 expression in asthmatic mice,\(^30\) this study also demonstrated higher miR-155 expression in OVA and OVA/PBS groups than PBS control. However, SJMHE1 treatment significantly inhibited the expression of miR-155 in lungs of asthmatic mice (Figure 5). These results are consistent with a previous publication in which miR-155 deficiency reduced the Th2 cell and Th2 cytokine levels and contributed to the alleviation of airway inflammation in asthmatic mice.\(^30\) Th17/Treg imbalance is far beyond Th1/Th2 imbalance,\(^35\) and whether miR-155 regulates Th17/Treg balance in asthma remains unclear. The present study focused on SJMHE1 regulating Th17/Treg balance in asthmatic mice through miR-155. Indeed, miR-155 expression was positively correlated with Th17/Treg ratio and RORγt mRNA levels but negatively correlated with Foxp3 mRNA levels in asthmatic mice (Figure 5). SJMHE1 might regulate Th17/Treg balance through miR-155 in asthmatic mice.

In general, STAT signal is critical for Th17/Treg cell differentiation. IL-6/STAT3 is essential for Th17 cell differentiation, while IL-2/STAT5 is indispensable for Treg cells.\(^49,50\) To explore the regulation of Th17/Treg cells by SJMHE1, we tested the expression of p-STAT3 and p-STAT5 in lungs of mice. Consistent with the proportion of Th17 and Treg cells in asthmatic mice, OVA and OVA/PBS groups showed the upregulation of p-STAT3 and downregulation of p-STAT5. However, OVA/SJMHE1 group showed the upregulation of p-STAT5 and downregulation of p-STAT3. Moreover, consistent with miR-155 expression, as a target of miR-155 and an important negative regulator of JAK/STAT signal, SOCS1 expression was downregulated in OVA and OVA/PBS groups but upregulated in OVA/SJMHE1 (Figure 6). SOCS1 negatively regulated the Th17 cell differentiation by blocking the IL-6-induced activation of STAT3.\(^51,52\) This finding supports that miR-155 regulates Th17 and Treg cell differentiation by targeting SOCS1.\(^29,48\) SJMHE1 might regulate Th17/Treg balance by regulating the activation of STAT3 and STAT5, thereby ameliorating the airway inflammation of asthmatic mice.

To confirm the regulation of Th17/Treg cells by SJMHE1 via miR-155, we stimulated mouse CD4\(^+\) T cells after forcing the expression or inhibition of miR-155 by lentivirus. Indeed, the over-expression of miR-155 increased the frequency of Th17 cells. However, in the presence of SJMHE1, the proportion of Th17 cells decreased (Figure 7). Although the over-expression or inhibition of miR-155 did not increase the proportion of Treg cells, with the addition of SJMHE1, the frequency of Treg cells increased (Figure 7). Consistent with the proportion of Th17 and Treg cells, the over-expression of miR-155 increased the frequency of CD4\(^+\)p-STAT3\(^+\) cells. The addition of SJMHE1 reduced the proportion of CD4\(^+\)p-STAT3\(^+\) cells increased the frequency of CD4\(^+\)p-STAT5\(^+\) cells regardless of the over-expression or inhibition of miR-155 (Figure 8). Consistent with the increase in SOCS1 expression in OVA/SJMHE1-treated mice, SJMHE1 treatment increased the proportion of CD4\(^+\)SOCS1\(^+\) cells in vitro (Figure 8). The increase of SOCS1\(^+\) cells is consistent with the decrease of p-STAT3\(^+\) cells in CD4\(^+\) T cells after SJMHE1 treatment. Nevertheless, another publication showed that the over-expression of miR-155 by pre-miR
-155 increased the frequencies of Th17 and Treg cells, and the expression of p-STAT3 and p-STAT5, but the inhibition of miR-155 by anti-miR-155 reduced the proportion of Th17 and Treg cells and the expression of p-STAT3 and p-STAT5 in CD4+ T cells. This discrepancy is associated with the difference in miR-155 expression system or CD4+ T cell treatment method, thus requiring further investigation. Our results are consistent with another publication in which JAK2 inhibitor AG490 regulates the balance of Th17 and Treg cells by modulating the activation of STAT3 and STAT5 in autoimmune arthritis mice. A490 treatment reduced the proportion of Th17 cells and the expression of p-STAT3 but increased the proportions of Treg cells and the expression of p-STAT5. A reciprocal regulation of STAT3 and STAT5 was observed under an inflammatory condition. Transfection with STAT3 siRNA in CD4+ T cells inhibited Th17 cell differentiation but increased the frequency of Treg cells from rheumatoid arthritis (RA) peripheral blood and synovial fluid. Similarly, the suppression of STAT5 decreased Treg cells and increased Th17 cells. Furthermore, the results of our previous and the present study support that reciprocal activation was observed between STAT3 and STAT5. However, the mechanism of reciprocal regulation of STAT3 and STAT5 needs to be explored. Therefore, SJMHE1 regulates Th17 and Treg balance by regulating the activation of STAT3 and STAT5 via miR-155 in vitro.

**Conclusion**

In conclusion, we confirmed that SJMHE1 ameliorated ongoing asthma by regulating the balance of Th cells. SJMHE1 suppressed the activation of STAT3 and upregulated the activation of STAT5 via miR-155, thereby regulating the balance of Th17 and Treg cells in asthma. The schematic representation of SJMHE1 regulation of Th17/Treg balance in asthma is shown in Figure 9. Our results further confirmed that *S. japonicum* peptide SJMHE1 might be a promising treatment for asthma.

**Ethics Approval**

Mouse handling and experimentation were conducted in strict compliance with the Regulations for Administration of Affairs Concerning Experimental Animals of Jiangsu University. All protocols were approved by the Jiangsu University Institutional Animal Care and Use Committee (Permit Number: JSU 18–012).

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**Disclosure**

The authors declare that they have no competing interests.

**References**

1. Xue F, Yu M, Li L, et al. Elevated granulocytic myeloid-derived suppressor cells are closely related with elevation of Th17 cells in mice with experimental asthma. *Int J Biol Sci*. 2020;16(12):2072–2083. doi:10.7150/ijbs.43596
2. Muehling LM, Lawrence MG, Woodfolk JA. Pathogenic CD4+(+T cells in patients with asthma. *J Allergy Clin Immunol*. 2017;140(6):1523–1540. doi:10.1016/j.jaci.2017.02.025
3. Shi YH, Shi GC, Wan HY, et al. Coexistence of Th1/Th2 and Th17/Treg imbalances in patients with allergic asthma. *Chin Med J (Engl)*. 2011;124(13):1951–1956.
4. Corren J, Lemanske RF, Hanania NA, et al. Lebrikizumab treatment in adults with asthma. *N Engl J Med*. 2011;365(12):1088–1098. doi:10.1056/NEJMoa1106469
5. Just J, Deschildre A, Lejeune S, Amat F. New perspectives of childhood asthma treatment with biologics. *Pediatr Allergy Immunol*. 2019;30(2):159–171.
6. Maizels RM, Mc Sorley HJ. Regulation of the host immune system by helminth parasites. *J Allergy Clin Immunol*. 2016;138(3):666–675. doi:10.1016/j.jaci.2016.07.007
7. Gazzinelli-Guimaraes PH, Nutman TB. Helminth parasites and immune regulation. *F1000Res*. 2018;7:F1000 Faculty Rev–1685. doi:10.12688/f1000research.15596.1
8. Maizels RM. Regulation of immunity and allergy by helminth parasites. *Allergy*. 2020;75(3):524–534. doi:10.1111/all.13944
9. Fernandes JS, Cardoso LS, Pitrem PM, Cruz AA. Helminths and asthma: risk and protection. *Immunol Allergy Clin North Am*. 2019;39(3):417–427. doi:10.1016/j.iac.2019.03.009
10. Bohmacker S, Troisi F, de Los Reyes Jimenez M, Esser-von Bieren J. What can parasites tell us about the pathogenesis and treatment of asthma and allergic diseases. *Front Immunol*. 2020;11:2106. doi:10.3389/fimmu.2020.02106
11. Pacíflico LG, Marinho FA, Fonseca CT, et al. Schistosoma mansoni antigens modulate experimental allergic asthma in a murine model: a major role for CD4+ CD25+ Foxp3+ T cells independent of interleukin-10. *Infect Immun*. 2009;77(1):98–107. doi:10.1128/IAI.00783-07
12. Navarro S, Pickering DA, Ferreira IB, et al. Hookworm recombinant protein promotes regulatory T cell responses that suppress experimental asthma. *Sci Transl Med*. 2016;8(362):362ra143. doi:10.1126/scitranslmed.aaf8807
13. Logan J, Navarro S, Loukas A, Giacomini P. Helminth-induced regulatory T cells and suppression of allergic responses. *Curr Opin Immunol*. 2018;54:1–6. doi:10.1016/j.coi.2018.05.007
50. Zorn E, Nelson EA, Mohseni M, et al. IL-2 regulates FOXP3 expression in human CD4+CD25+ regulatory T cells through a STAT-dependent mechanism and induces the expansion of these cells in vivo. Blood. 2006;108(5):1571–1579. doi:10.1182/blood-2006-02-004747

51. Yu CR, Mahdi RR, Oh HM, et al. Suppressor of cytokine signaling-1 (SOCS1) inhibits lymphocyte recruitment into the retina and protects SOCS1 transgenic rats and mice from ocular inflammation. Invest Ophthalmol Vis Sci. 2011;52(9):6978–6986.

52. Jager LD, Dabelic R, Waiboci LW, et al. The kinase inhibitory region of SOCS-1 is sufficient to inhibit T-helper 17 and other immune functions in experimental allergic encephalomyelitis. J Neuroimmunol. 2011;232(1–2):108–118. doi:10.1016/j.jneuroim.2010.10.018

53. Park JS, Lee J, Lim MA, et al. JAK2-STAT3 blockade by AG490 suppresses autoimmune arthritis in mice via reciprocal regulation of regulatory T cells and Th17 cells. J Immunol. 2014;192(9):4417–4424. doi:10.4049/jimmunol.1300514

54. Ju JH, Heo YJ, Cho ML, et al. Modulation of STAT-3 in rheumatoid synovial T cells suppresses Th17 differentiation and increases the proportion of Treg cells. Arthritis Rheum. 2012;64(11):3543–3552. doi:10.1002/art.34601

55. Dong L, Wang X, Tan J, et al. Decreased expression of microRNA-21 correlates with the imbalance of Th17 and Treg cells in patients with rheumatoid arthritis. J Cell Mol Med. 2014;18(11):2213–2224. doi:10.1111/jcmm.12353

56. Park JS, Kwok SK, Lim MA, et al. STA-21, a promising STAT-3 inhibitor that reciprocally regulates Th17 and Treg cells, inhibits osteoclastogenesis in mice and humans and alleviates autoimmune inflammation in an experimental model of rheumatoid arthritis. Arthritis Rheumatol. 2014;66(4):918–929. doi:10.1002/art.38305

57. Jhun JY, Moon SI, Yoon BY, et al. Grape seed proanthocyanidin extract-mediated regulation of STAT3 proteins contributes to Treg differentiation and attenuates inflammation in a murine model of obesity-associated arthritis. PLoS One. 2013;8(11):e78843. doi:10.1371/journal.pone.0078843