The dual effect of MBD2 regulating Th2 cell differentiation

Aijun Jia

Hunan Provincial People's Hospital(The first-affiliated hospital of Hunan normal university)

Yueling Wang

Xiangya Hospital Central South University

Jianghong Wei

The Affiliated Hospital of Guilin Medical University

Wenjin Sun

Sichuan University West China Hospital

Bing Xiao

The Second Xiangya Hospital of Central South University

Lulu Qiu

The Third People's Hospital of Shenzhen

Xia Pan

University of Tebingen

Shaozu Liu

Hunan Provincial People's Hospital(The first-affiliated hospital of Hunan normal university)

Jingyi Sun

The Affiliated Hospital of Guilin Medical University

Yan Jiang

The Affiliated Hospital of Guilin Medical University

Linqiao Li

The Affiliated Hospital of Guilin Medical University

Xudong Xiang

The Second Xiangya Hospital of Central South University

Libing Ma (✉ malibing1984@163.com)

The Affiliated Hospital of Guilin Medical University

Research

Keywords: MBD2, Th2 cell, IL-4, IRF4, asthma

Posted Date: February 10th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1192628/v1>

License: This work is licensed under a Creative Commons Attribution 4.0 International License.
[Read Full License](#)

The dual effect of MBD2 regulating Th2 cell differentiation

Aijun Jia ^a, Yueling Wang ^b, Jianghong Wei ^c, Wenjin Sun ^d, Bing Xiao ^e, Lulu Qiu ^f, Xia Pan ^g, Shaozu Liu ^a, Jingyi Sun ^c, Yan Jiang ^c, Linqiao Li ^c, Xudong Xiang ^e, Libing Ma ^c

^a Department of The Third Emergency of Yuelushan Hospital District, Hunan Provincial People's Hospital(The first-affiliated hospital of Hunan normal university), No.90 Pingchuan Road, Changsha, Hunan410006, China

^b Department of Anesthesiology, Xiangya Hospital, Central South University, No.87 Xiangya Road, Changsha, Hunan 410008, China

^c Department of Respiratory and Critical Care Unit, The Affiliated Hospital of Guilin Medical University, No.15 Lequn Road, Guilin, Guangxi 541001, China

^d Department of General Medicine, West China Hospital, Sichuan University, No.37 Guoxue Alley, Chengdu, Sichuan 610041, China

^e Department of Emergency, The Second Xiangya Hospital, Central South University, No.139 Middle Renmin Road, Changsha, Hunan 410011, China

^f Department of Tuberculosis, The Third People's Hospital of Shenzhen, No.29 Bulan Road, Longgang District, Shenzhen, Guangdong 518000, China

^g Department of Experimental and Clinical Pharmacology and Toxicology, University of Tübingen, Tübingen72074, Germany

Corresponding author:

Libing Ma: Tel:+86 13737729999. E-mail: malibing1984@163.com.

Institution: the Affiliated Hospital of Guilin Medical University, No.15 Lequn Road, Guilin, Guangxi 541001, China. Institutional email address: kykgyfy@163.com.

Abbreviations: **MBD2**,Methyl-CpG binding domain protein 2;**IRF4**, interferon regulatory factor 4;**HDM**, house dust mite; **OVA**, ovalbumin; **LPS**, lipopolysaccharide; **NEU**, neutrophile granulocyte; **EOS**, Eosinophilic granulocyte; **AHR**,airway hyperresponsiveness

ABSTRACT

Background: Th2 cells and IL-4 are involved in the pathogenesis of eosinophil infiltration in almost all types of asthma. In peripheral blood of asthmatic patients, methyl-CpG binding domain protein 2 (MBD2) expression in CD4⁺ T cells is increased. So, the study's objective: 1. to investigate the expression of MBD2, IRF4, Th2 cell and IL-4 in severe asthma mice; 2. to investigate whether MBD2 affects the differentiation and function of Th2 cell in mice through IRF4. **Methods:** 1. C57BL/6J mice were divided into three groups: severe asthma group, conventional asthma group, and saline group. HE pathology, immunohistochemistry and WB were used to observe the expression of eosinophil cationic protein (ECP) and IL-4 in lung tissue. The positive rate of CD4⁺ Th2 cell from lung and spleen were detected by flow cytometry; 2. The CD4⁺ T cells derived from mouse spleen were divided into control group, empty transfection group, MBD2 silencing group (M(-)), MBD2 overexpression group (M(+)), IRF4 silencing group (I(-)), IRF4 overexpression group (I(+)). Th2 cells were cultured under directed differentiation conditions. The positive rate of Th2 cell was detected by flow cytometry. The expressions of IL-4, IRF4 and MBD2 were measured by WB; 3. Mouse spleen-derived CD4⁺ T cells were divided into control, empty transfection (M(0)/I(0)), M(-)/I(0), M(+)/I(0), M(-)/I(-), M(-)/I(+), M(+)/I(-) and M(+)/I(+) group. Th2 cells were cultured under directed differentiation conditions. The positive rate of Th2 cell was detected by flow cytometry. The expression of IL-4, IRF4 and MBD2 were measured by WB. Data were analyzed with SPSS version 25.0. The Independent-samples t-test was used for comparison of continuous variables between groups. P<0.05 was considered to indicate a statistically significant difference. **Results:** 1. There was no significant difference in ECP, IL-4 and Th2 positive rates between severe asthma group and conventional asthma group; 2. The positive rate of Th2 cells and expression of IL-4 in M (-) group increased, while the expression of IRF4 decreased. The positive rate of Th2 cells and expression of IL-4 in M (+) group decreased, while the expression of IRF4 in M (+) group was higher than empty transfection group. The expression of IL-4 decreased in I (-) group and increased in I (+) group; 3. The positive rate of Th2 cells and expression of IL-4 in M(+)/I(-) group decreased, while the positive rate of Th2 cells and expression of IL-4 in M(-)/I(+) group increased significantly. **Conclusions:** 1. MBD2 inhibits the differentiation and function of Th2 cells in spleen-derived CD4⁺ T cells of mice directly, but MBD2 promotes the expression of IL-4 through enhancing the expression of IRF4; 2. MBD2 participates in the differentiation and function of

Th2 cells in severe asthma through promotion of IRF4 indirectly and inhibition of Th2 cells directly, so IL-4 expression and Th2 positive rates were no significant difference; 3.MBD2 could play dual immune effect regulating Th2 cell differentiation through IL-4 in severe asthma.

Keywords: MBD2, Th2 cell, IL-4, IRF4, asthma

1.Introduction

Asthma is one of the most common chronic immunological diseases in humans, affecting people from childhood to old age. Reversible airflow obstruction, airway hyperresponsiveness (AHR) and airway inflammation are distinguishing feature of asthma, and a phenotypic subset of late-onset severe asthmatics has debilitating fixed airflow obstruction, increased neutrophilic inflammation and a history of pneumonia[1]. Eosinophilic airway inflammation and the imbalance between Th1/Th2 cells[2] are generally recognized as pivotal factors in the pathogenesis of asthma. In general, corticosteroid therapy is beneficial for the eosinophilic asthma (EA) patients[3]. However, one review including 21 clinical studies revealed that 50% of all asthma cases exhibit weak eosinophilic airway inflammation, which is defined as non-eosinophilic asthma[4]. These non-eosinophilic asthma cases are characterised by predominantly neutrophilic and mixed granulocytic types, and are associated with severe asthma and poor response to corticosteroids[4,5,6]. A conducted cluster analysis study agreed with the previous studies, the result showing a higher frequency of eosinophilic inflammatory phenotype in the fair asthma control cluster, while predominantly neutrophilic and mixed granulocytic patterns were most frequent in the severe asthma cluster[7]. There are also some clinical studies[4,8,9] which have indicated that sputum NEU in patients with severe persistent bronchial asthma has been attributed to the long term side effects of inappropriate use of corticosteroids. Therefore, whether the increased NEU population is an independent risk factor to severe asthma or a side effect of exposure to corticosteroids is not clear. So in our previous studies[10,11], we established a neutrophil-predominant severe asthma model successfully by using 100µg of HDM and OVA combined with 15 µg LPS. Here, we tried to further study the relationship between the changes of Th2 cells and the epigenetic alterations.

Methyl-CpG binding domain 2 (MBD2) has been demonstrated to be able to specifically bind to methylated DNA, independent of sequence context. This function involves the presence of a highly

conserved protein motif termed the methyl-CpG binding domain[12]. MBD2 is involved in many biochemical processes, affecting gene transcription, cell differentiation and other processes [13,14]. In our previous studies in lungs and spleen of mice[10,11], the expression of MBD2 enhanced noticeably after accepting stimulus. We also found that MBD2 expression increased in CD4⁺ T cells in peripheral blood of asthma patients compared with healthy people. Thus, MBD2 is an essential role in pathogenesis of severe asthma.

IRF4 is a tightly regulated transcription factor involved in growth and differentiation of normal T lymphocytes[15]. It was found in DCs by which IRF4 modulates IL-10 and IL-33 cytokine production to specifically promote Th2 differentiation and inflammation[16]. In an HDM-induced asthmatic mouse model, when IRF4 decreased, Th2-mediated inflammation was inhibited[17]. And we also found[10,11] that IRF4 expression significantly higher in neutrophil-predominant severe asthma mice. Therefore, IRF4 is an vital factor in the pathogenesis of asthma.

At previous study, we indicated that MBD2 can participate in the differentiation of Th17 cells and IL-17 secretion through IRF4 expression, and so participates in the pathogenesis of the neutrophilic predominant severe asthma model, but it is not known whether MBD2 can regulate Th2 cells through IRF4 expression. Here, we demonstrated it through the following experiments.

2.Materials and methods

2.1. Mice

Six to seven weeks old female C57BL/6 mice were used in the study. Mice were raised in a SPF facility and had free access to food and water during the experimental period.

2.2. Experimental study design

Mice were randomly divided into three groups. We used the experimental protocol previously published [18]. Briefly, the first group mice (severe asthma model) were sensitized by i.p. of HDM(GREER, USA), OVA(Sigma, USA), LPS(Sigma, USA), and aluminum hydroxide(Sigma, USA) (day 0, 1 and 2) ; then challenged by OVA solution before HDM i.n. (day 14, 15, 18, and 19). The second group mice received saline as control. The third group mice (conventional asthma model) were sensitized by i.p. of OVA and aluminum hydroxide (day 0,7), then challenged by OVA solution (day 14, 15, 18, and 19)[19]. The experiment was terminated after the exacerbation phase(day 21).

2.3. Lung histology and homogenates

Right lung lobes were perfused with- and stored in the fixative 4% formaldehyde for 24 h, level dehydration, and embedded in paraffin. Paraffin embedded lung tissue was sectioned into 4 μ m thick sections and stained with Haematoxylin & Eosin (H&E) , to assessed the lung tissue inflammation score as previously described [20].

Additionally, immunohistochemistry was performed. Lung tissue sections reacted with antibodies for detecting eosinophils (anti-ECP, Biorbyt) or goat anti-mouse IL-4 antibody (ABBIOTEC). Tissue sections were scanned with the microscope and using software of image processing system (Image-Pro Plus 6.0), then the ECP or IL-4 scores assessed.

2.4. Western blot analysis

Total proteins were prepared using RIPA lysis buffer supplemented with protease inhibitors. Proteins were separated in 10% SDS/PAGE and transferred to nitrocellulose membrane. The membrane was blocked for 1 h in blocking buffer for fluorescent Western blot. Proteins were detected by Western blot by using anti-ECP, anti-IL-4, anti-MBD2 and anti-IRF4 Ab respectively. HRP-conjugated secondary antibody was used to detect the protein bands.

2.5. Lung tissue single-cell suspensions

Lungs were collected and single-cell suspensions were prepared, as previous reported[10].Whole lungs were first washed with 5X antibiotic, and then cut into small pieces as possible, digested with digestive juice composed of collagenase1 and DNase ,passed through a 70- μ m nylon net filter (BD Falcon) , centrifugated and resuspended.

2.6. T cell purification and isolation

Splenocytes were collected by pressing the spleen through a 70- μ m nylon net filter, incubated with CD4 microbeads , selected by MASC columns (Miltenyi Biotec, Germany) for the positive selection of CD4⁺ T cells, and then restimulated by phorbol-12-myristate-13-acetate, ionomycin and monensin as previous method[10]. Lung cells were stained for antiCD4 antibody, fixed and permeated (Multi Sciences Company, China) for 15 min. Finally, lung and spleen cells were stained with anti-IL-4

antibody (BioLend, USA) for 20 min. Finally, lung and spleen cells were isolated and purified with fluorescence-activated cell sorting (FACS) respectively. The data were analyzed by FlowJo version X software.

2.7. Th2 cell differentiation

CD4⁺ T cells from C57BL/6 mice were cultured on anti-CD3-coated flat-bottomed 96-well plates with anti-CD28(BioLend,USA) for 3 days in the presence of T cell differentiation mixtures for Th2. 10µg/ml anti-INF-gamma and 10µg/ml anti-IL-12 were added for neutralization of IFN-gamma and IL-12[21,22]. The above cytokines were obtained from BioLend Co.(BioLend,USA).

2.8. Th2 cell transduction

Splenic CD4⁺ Th2 lymphocytes were transfected with chemosynthesis MBD2 siRNA sequence (S) 5'-GTTTGGCTTAACACATCTCAA-3' and IRF4 siRNA sequence(S) 5'-GCCAGACAAGTGTATTACTTT-3'. Transduction was performed for 72 h at a MOI of 20 concomitantly with activation in 12-well flat-bottom plates. Finally, the cells were stimulated with PMA, ionomycin and monensin in the last 5h as above.

2.9 Statistical analysis

Data were analyzed with SPSS version 25.0 (SPSS, Inc., Chicago, IL, USA). Values are expressed as the mean $\pm$ standard deviation. The Independent-samples t-test was used for comparison of continuous variables between groups. P<0.05 was considered to indicate a statistically significant difference.

3. Results

3.1 The expression of ECP in the severe asthma mice

Histological analyses showed that lungs from the severe group and the conventional group markedly increased peribronchial inflammation with infiltrated eosinophils, but not obviously between them(Fig.1a). Results of western blot analyses were similar to histological analyses(Fig.1b).

a saline conventional severe

Fig.1 (a) Lung tissues were stained with H&E, immunohistochemistry eosinophil antibody (anti-ECP) of the three groups. Histological analyses showed that lungs from the severe group and the conventional group markedly increased peribronchial inflammation with infiltrated eosinophils, but not obviously different between them. (b)Western blot analyses detected ECP protein expression in the three groups. The ECP protein expression from the lungs of the severe group and the conventional group were higher, but not obviously different between them.

3.2 Th2 cells were involved in the process of severe asthma mice

IL-4 is the main representative cell factor of Th2 cells. Histological analyses of lungs from the severe group exhibited markedly enhanced cells with stained IL-4, but not obviously different compared with the conventional group (Fig.2a). Results of western blot analyses were similar to histological analyses (Fig.2b). Th2 cells in bronchial lung tissue suspension cells and splenocytes from the severe and the conventional group were more than the saline group(Fig.2c).

a saline conventional severe

Fig.2 Th2 cells were involved in the process of severe asthma mice. (a) Lung tissues were stained for immunohistochemistry (anti-IL-4) of the three groups. Histological analyses of lungs from the severe group exhibited enhanced cells with stained IL-4 positively, but not obviously different compared with the conventional group. (b) Western blot analyses detected IL-4 protein expression in the three groups. The IL-4 protein expression from the lungs of the severe and the conventional group were higher than that from the saline group. (c) Th2 cells were tested in bronchial lung tissue suspension cells and splenocytes, and the cells were then subjected to intracellular staining of PE-anti-IL-4 by flow cytometry analyses. Th2 cells in bronchial lung tissue suspension cells and splenocytes from the severe

and the conventional group compared with the saline group, but not obviously different between the severe and the conventional group.

3.3 IL-4 expression and Th2 cell differentiation under MBD2 gene silencing or over expression

We found that IL-4 expression was significantly increased than that of the empty transfection group (M(0)) under MBD2 gene silencing, and IL-4 expression was markedly lower compared to that of the empty transfection group when the MBD2 gene over expressed (Fig.3a). Th2 cells were obviously increased or decreased under MBD2 gene silencing or over expression (Fig.3b).

Fig.3 IL-4 expression and Th2 cell differentiation under MBD2 gene silencing or over expression. (a) Under MBD2 gene silencing(M(-)), MBD2 protein expression was significantly lower than that of the empty transfection group(M(0)) by western blot analyses, but IL-4 protein expression showed oppositely; with MBD2 gene over expression(M(+)), MBD2 protein expression was markedly increased than that of M(0) , but IL-4 protein expression showed oppositely. (b) Under MBD2 gene silencing(M(-)), Th2 cell differentiation was significantly increased than that of M(0) according to flow cytometry analyses; with MBD2 gene over expression(M(+)), Th2 cell differentiation was markedly lower compared to that of M(0).

3.4 IL-4 expression under IRF4 gene silencing or over expression

We confirmed that IL-4 expression was significantly lower than that of the empty transfection group

(I(0)) under IRF4 gene silencing, and IL-4 expression was markedly increased compared to that of the empty transfection group when IRF4 gene over expression (I(+)) (Fig.4).

Fig.4 IL-4 expression under IRF4 gene silencing(I(-)) or over expression(I(+)). Under I(-), IL-4 and IRF4 protein expression was significantly lower than that of the empty transfection group(I(0)) according to western blot analyses; under I(+), IL-4 and IRF4 protein expression was markedly increased compared to that of I(0).

3.5 IL-4 expression and Th2 cell differentiation under joint MBD2 and IRF4 gene silencing or over expression

We found that IL-4 expression was significantly the lowest when joint both MBD2 gene over expression and IRF4 gene silencing, and IL-4 expression was the highest when joint both MBD2 gene silencing and IRF4 gene over expression (Fig.5a). Th2 cells showed the same trend changes as IL-4 expression while both MBD2 and IRF4 underwent joint gene silencing or over expression(Fig.5b).

b control M(0)/I(0) M(-)/I(0) M(+)/I(0)

Fig.5 IL-4 expression and Th2 cell differentiation under joint MBD2 and IRF4 gene silencing or over expression. (a) Under joint M(+)/I(-), IL-4 protein expression was significantly the lowest according to western blot analyses ; under joint M(+)/I(0), IL-4 protein expression was higher than M(+)/I(-). While under joint M(-)/I(+), IL-4 protein expression was significantly the highest; under joint M(-)/I(0), IL-4 protein expression was lower than M(-)/I(+). IL-4 protein expression showed no significant difference between the control group, M(0)/I(0), M(-)/I(-) and M(+)/I(+). (b) Under joint M(+)/I(-), Th2 cell differentiation was significantly the lowest according to flow cytometry analyses; under joint M(+)/I(0), Th2 cell differentiation was higher than M(+)/I(-). Under joint M(-)/I(+), Th2 cell differentiation was significantly the highest; under joint M(-)/I(0), Th2 cell differentiation was lower than M(-)/I(+). No significant difference in Th2 cell differentiation was observed between the control group, M(0)/I(0), M(-)/I(-) and M(+)/I(+).

Discussion

In order to the follow study the mechanisms of severe asthma, we repeated the neutrophil-predominant severe asthma mice model utilizing 100 μ g HDM +100 μ g OVA +15 μ g LPS again[10,11]. Then analysis of lung tissues revealed that there was no difference in the expression of ECP between the severe group and the conventional group. Since lung tissue as one of the effector organs of immunity, our further research found IL-4 expression in lung tissue was no difference in the HDM/OVA/LPS sensitized group when compared with the OVA group. While the spleen as the

classical immune target, Th2 cells in the splenocytes from severe group were no difference with conventional group according to flow cytometry. The research also showed that compared to moderate asthma, IL-4 expression were decreased in severe asthma[6]. Now came the question, why did ECP, IL-4 and Th2 cells expression have no significant difference in the two groups?. Hence we continued further experimentation to find out it.

MBD2, as an epigenetic regulation factor[23], has been linked to disease such as immune system function and tumorigenesis. In the absence of MBD2, DCs display reduced phenotypic activation and a markedly impaired capacity to initiate Th2 immunity against helminths or allergens, and it indicated MBD2 played a dominant role in controlling Th2 induction[13]. To ascertain the relationship between MBD2 and IL-4 in the pathogenesis of severe asthma, we conducted *in vitro* splenocyte experiments, and found that IL-4 protein expression increased significantly along with the silencing of the MBD2 gene, and decreased with over expression of the MBD2 gene. At the same time, the number of Th2 cells showed the same trend with IL-4 when the silencing or over expression of the MBD2 gene. Through these tests, MBD2 presents the reverse regulation of the differentiation of Th2 cells and IL-4 secretion in severe asthma. At this point, the theoretical conclusion should be IL-4 expression and the differentiation of Th2 cells were decreased in severe asthma, which consistent with the above research [6]. But why do we have different results?. The following experiments demonstrated it further.

Next, we tested the important role of IRF4 in asthma. IRF4, as a transcription factor, plays pivotal roles in the immune response, and when inhibiting IRF4 expression, thereby Th2 cell activation was suppressed [24]. IRF4 can promote the expression of a subset of Th2 cytokines and Th2 cell-inducing function [25]. We found that both IRF4 and IL-4 had an increased expression in the lung and the spleen cells of the severe group and the conventional group, but no difference between them. From the *in vitro* cell experiment, IL-4 protein expression increased significantly with over expression of the IRF4 gene, and decreased with the silencing of the IRF4 gene. These findings show that IRF4 participates in severe asthma by affecting the differentiation of IL-4 secretion.

Finally, the differentiation of Th2 cells and IL-4 secretion were the lowest when joint over expression of MBD2 and silencing of IRF4 genes, and the differentiation of Th2 cells and IL-4 secretion were the highest while joint silencing of MBD2 and over expression of IRF4 genes. Under joint over expression of MBD2 and silencing of IRF4 genes, the differentiation of Th2 cells and IL-4 secretion were the lowest, while joint silencing of both MBD2 and of IRF4 genes, the differentiation of

Th2 cells and IL-4 secretion were increased. The result shows that demethylation of MBD2 can promote the differentiation of Th2 cells and IL-4 secretion. Under conditions of joint silencing of MBD2 and over expression of IRF4 genes, the differentiation of Th2 cells and IL-4 secretion were the highest, while joint over expression of both MBD2 and of IRF4 genes, the differentiation of Th2 cells and IL-4 secretion were declined. It indicates that methylation of MBD2 can restrain the differentiation of Th2 cells and IL-4 secretion. In line with previous studies[26], MBD2 can directly bind to the IL-4 promoter. So the problem can be readily solved. MBD2 can promote the IRF4 expression, and then IRF4 can promote the IL-4 secretion and Th2 cell differentiation. But MBD2 can be directly combined with the IL-4 promoter, inhibited the effects of IL-4, and decreased the differentiation of Th2 cells further, and IRF4 expression of Th2 cells decreased finally. This forms a negative feedback. So in the severe asthma group, while MBD2 and IRF4 increased, due to the offset effect, Th2 cells and IL-4 did not increase or decrease significantly, resulting in the secretion of eosinophil relatively reduced and no difference compared with the conventional asthma group. This way maybe the best answer for MBD2 to participate in the Th2 cell differentiation of the neutrophilic-predominant severe asthma model.

Conclusion

Altogether, MBD2 promoting IL-4 secretion leads to Th2 cell differentiation through stimulating IRF4, but MBD2 decreased Th2 cell differentiation by combined with the IL-4 promoter directly in the mice model of severe asthma. So MBD2 plays dual immune effect regulating Th2 cell differentiation through IL-4 in severe asthma.

Ethics approval and consent to participate

Experiments were approved by the Animal Care and Use Committee of the Second Xiangya Hospital of Central South University.

Consent for publication

The authors agree to publish.

Availability of data and materials

Authors have nothing to disclose. The data are available in the manuscript.

Competing interests

The authors report no conflicts of interest.

Funding

This study was supported by National Natural Science Foundation of China (No.81560007, No.81760009, No.81370128 and No.81960007), Science and Technology Base and Talent Special Project of Guangxi Province (No.AD19110160), and General Project of Department of Education of Hunan Province (No.20C1117).

Authors' contributions

Not applicable.

Acknowledgements

Not applicable.

Authors' information

Aijun Jia ^a, Yueling Wang ^b, Jianghong Wei ^c, Wenjin Sun ^d, Bing Xiao ^e, Lulu Qiu ^f, Xia Pan ^g, Shaozu Liu ^a, Jingyi Sun ^c, Yan Jiang ^c, Linqiao Li ^c, Xudong Xiang ^e, Libing Ma ^c

^a Department of The Third Emergency of Yuelushan Hospital District, Hunan Provincial People's Hospital(The first-affiliated hospital of Hunan normal university), No.90 Pingchuan Road, Changsha, Hunan410006, China

^b Department of Anesthesiology, Xiangya Hospital, Central South University, No.87 Xiangya Road, Changsha, Hunan 410008, China

^c Department of Respiratory and Critical Care Unit, The Affiliated Hospital of Guilin Medical University, No.15 Lequn Road, Guilin, Guangxi 541001, China

^d Department of General Medicine, West China Hospital, Sichuan University, No.37 Guoxue Alley, Chengdu, Sichuan 610041, China

^e Department of Emergency, The Second Xiangya Hospital, Central South University, No.139 Middle Renmin Road, Changsha, Hunan 410011, China

^f Department of Tuberculosis, The Third People's Hospital of Shenzhen, No.29 Bulan Road, Longgang District, Shenzhen, Guangdong 518000, China

^g Department of Experimental and Clinical Pharmacology and Toxicology, University of Tübingen, Tübingen 72074, Germany

References

1. Wang H, Aloe C, McQualter J, et al. G-CSFR antagonism reduces mucosal injury and airways fibrosis in a virus-dependent model of severe asthma. *Br J Pharmacol*. 2021 Apr;178(8):1869-1885.
2. Park KH, Oh EY, Han H, et al. Efficacy of transdermal immunotherapy with biodegradable microneedle patches in a murine asthma model. *Clin Exp Allergy*. 2020 Sep;50(9):1084-1092.
3. Katsaounou P, Buhl R, Brusselle G, et al. Omalizumab as alternative to chronic use of oral corticosteroids in severe asthma. *Respir Med*. 2019 Apr;150:51-62.
4. Esteban-Gorgojo I, Antolín-Amérigo D, Domínguez-Ortega J, et al. Non-eosinophilic asthma: current perspectives. *J Asthma Allergy*. 2018 Oct 29;11:267-281.
5. Jeong JS, Kim SR, Cho SH, et al. A Novel Insight on Endotyping Heterogeneous Severe Asthma Based on Endoplasmic Reticulum Stress: Beyond the "Type 2/Non-Type 2 Dichotomy". *Int J Mol Sci*. 2019 Feb 7;20(3):713.
6. Gai XY, Zhang LJ, Chang C, et al. Metabolomic Analysis of Serum Glycerophospholipid Levels in Eosinophilic and Neutrophilic Asthma. *Biomed Environ Sci*. 2019 Feb;32(2):96-106.
7. Moore WC, Hastie AT, Li X, et al. Sputum neutrophil counts are associated with more severe asthma phenotypes using clusteranalysis. *J Allergy Clin Immunol*. 2014 Jun;133(6):1557-63.e5.
8. Cowan DC, Cowan JO, Palmay R, et al. Effects of steroid therapy on inflammatory cell subtypes in asthma. *Thorax*. 2010 May;65(5):384-90.
9. Wu W, Bang S, Bleecker ER, et al. Multiview Cluster Analysis Identifies Variable Corticosteroid Response Phenotypes in Severe Asthma. *Am J Respir Crit Care Med*. 2019 Jun 1;199(11):1358-1367.
10. Jia A, Wang Y, Sun W, et al. MBD2 Regulates Th17 Cell Differentiation and Experimental Severe Asthma by Affecting IRF4 Expression. *Mediators Inflamm*. 2017;2017:6249685.
11. Jia A, Wang Y, Sun W, et al. Comparison of the roles of house dust mite allergens, ovalbumin and lipopolysaccharides in the sensitization of mice to establish a model of severe neutrophilic asthma. *Exp Ther Med*. 2017 Sep;14(3):2126-2134.
12. Pei YF, Xu XN, Wang ZF, et al. Methyl-CpG Binding Domain Protein 2 Inhibits the Malignant Characteristic of Lung Adenocarcinoma through the Epigenetic Modulation of 10 to 11 Translocation

- 1 and miR-200s. *Am J Pathol.* 2019 May;189(5):1065-1076.
13. Cook PC, Owen H, Deaton AM, et al. A dominant role for the methyl-CpG-binding protein Mbd2 in controlling Th2 induction by dendritic cells. *Nat Commun.* 2015 Apr 24;6:6920.
 14. Gigeck CO, Chen ES, Smith MA. Methyl-CpG-Binding Protein (MBD) Family: Epigenomic Read-Outs Functions and Roles in Tumorigenesis and Psychiatric Diseases. *J Cell Biochem.* 2016 Jan;117(1):29-38.
 15. Li P, Leonard WJ. Chromatin Accessibility and Interactions in the Transcriptional Regulation of T Cells. *Front Immunol.* 2018 Nov 22;9:2738.
 16. Williams JW, Tjota MY, Clay BS, et al. Transcription factor IRF4 drives dendritic cells to promote Th2 differentiation. *Nat Commun.* 2013;4:2990.
 17. Park SJ, Lee K, Kang MA, et al. Tilianin attenuates HDM-induced allergic asthma by suppressing Th2-immune responses via downregulation of IRF4 in dendritic cells. *Phytomedicine.* 2021 Jan;80:153392.
 18. Daan de Boer J, Roelofs JJ, de Vos AF, et al. Lipopolysaccharide inhibits Th2 lung inflammation induced by house dust mite allergens in mice. *Am J Respir Cell Mol Biol.* 2013 Mar;48(3):382-9.
 19. Hoffman SM, Tully JE, Nolin JD, et al. Endoplasmic reticulum stress mediates house dust mite-induced airway epithelial apoptosis and fibrosis. *Respir Res.* 2013 Dec 24;14(1):141.
 20. Bandukwala HS, Clay BS, Tong J, et al. Signaling through Fc gamma RIII is required for optimal T helper type (Th)2 responses and Th2-mediated airway inflammation. *J Exp Med.* 2007 Aug 6;204(8):1875-89.
 21. Hu G, Tang Q, Sharma S, et al. Expression and regulation of intergenic long noncoding RNAs during T cell development and differentiation. *Nat Immunol.* 2013 Nov;14(11):1190-8.
 22. Gomez-Rodriguez J, Meylan F, Handon R, et al. Itk is required for Th9 differentiation via TCR-mediated induction of IL-2 and IRF4. *Nat Commun.* 2016 Mar 3;7:10857.
 23. Cheng L, Zhou K, Chen X, et al. Loss of MBD2 affects early T cell development by inhibiting the WNT signaling pathway. *Exp Cell Res.* 2021 Jan 1;398(1):112400.
 24. Kim SB, Lee AY, Chun JM, et al. Anthriscus sylvestris root extract reduces allergic lung inflammation by regulating interferon regulatory factor 4-mediated Th2 cell activation. *J Ethnopharmacol.* 2019 Mar 25;232:165-175.
 25. Gao Y, Nish SA, Jiang R, et al. Control of T helper 2 responses by transcription factor IRF4-dependent dendritic cells. *Immunity.* 2013 Oct 17;39(4):722-32.
 26. Hutchins AS, Artis D, Hendrich BD, et al. Cutting edge: a critical role for gene silencing in preventing excessive type 1 immunity. *J Immunol.* 2005 Nov 1;175(9):5606-10.