



MicroRNA-181a, -146a and -146b in spleen CD4⁺ T lymphocytes play proinflammatory roles in a murine model of asthma

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ABSTRACT

CD4⁺ T lymphocytes can be primarily polarized to differentiate into Th2 cells, and are heavily involved in the Th2 inflammation of asthma. Little is known about the correlation between microRNAs and Th2 inflammation in asthma, therefore we explore the roles of five microRNAs (microRNA-181a, -155, -150, -146a and -146b) in Th2 inflammation of asthma by tracking their expression levels in splenic CD4⁺ T lymphocytes under different conditions. Using quantitative real-time polymerase chain reaction (qPCR), the dynamic changes of these microRNAs in murine models of acute asthma (i.e. the OVA group) were analyzed, in comparison to a control group. The effects of dexamethasone on the miRNA expression levels were also investigated. The results showed that the expression levels of microRNA-181a, -150, -146a and -146b were higher in the OVA group compared to the control group in the beginning of the disease, and after 5 days dropped to control group levels because there was no new airway challenge. Moreover, the miRNA-146a expression was down-regulated by treatment with dexamethasone. MicroRNA-181a had a positive linear correlation with the numbers of inflammatory cells (i.e. the numbers of total cells or of the eosinophils in the BALF) by Spearman correlation analysis, so did miRNA-146a and miRNA-146b. These observations suggest that microRNA-181a, -146a and -146b are proinflammatory factors in asthma, and that down-regulation of miRNA-146a may partially account for the anti-inflammatory effect of dexamethasone.

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1. Introduction

Asthma is a chronic inflammatory disease of the airway with significant morbidity and mortality worldwide [1,2]. It is characterized by the infiltration of the airway by several types of inflammatory cells, including lymphocytes and eosinophils. Th2 lymphocytes have been suggested to play a key role in the pathogenesis of asthma. They can produce Th2 cytokines such as interleukin (IL)-4, IL-5, and IL-13, which induce immunoglobulin (Ig) E production and eosinophil activation. In fact, asthma is described as a Th2 inflammation of the airway [3,4].

MicroRNAs (miRNAs) are a group of 19–22 nucleotide-long noncoding RNAs that predominantly silence target messenger RNA (mRNA) [5,6]. Recently, many miRNAs have been reported to participate in the regulation of the immune system, modulate immune responses such as proliferation, differentiation, survival and function of immune cells, and affect cytokine responses and intracellular signaling pathways

[7–10]. Evidence also suggests roles for miRNAs in lung diseases [11–13], including airway diseases such as chronic obstructive pulmonary disease [14] and asthma [15–21]. Therefore miRNAs appear to be attractive new drug targets. Using microarray analysis, it has been shown that about 114 or so miRNAs have been expressed abnormally in asthma, including miRNA-146b, -223, -29b, -29c, -483, -574-5p, -672, and -690 [15,17,18,21–25]. However, microarray analysis shows only the expression profile of miRNAs rather than the specific miRNA function in asthma. Research of the role miRNA plays in the regulation of asthma is in its infancy, and the contribution of miRNA to the pathogenesis of asthma, especially to the Th2 inflammation of asthma, remains unknown.

Recently, 5 miRNAs (miRNA-181a, -155, -150, -146a, and -146b) are reported to be related to the activation and differentiation of T lymphocytes [6,8,26]. To find more relative miRNAs involved in Th2 inflammation of asthma, the 5 miRNAs are selected for a pioneering study.

It is well known that CD4⁺ T lymphocytes are precursors of Th2 lymphocytes, and the changes of CD4⁺ T cells in a murine asthma model are often used to indicate the changes of Th2 lymphocytes in asthma [17,27,28]. The expression of miRNAs in CD4⁺ T lymphocytes in asthma would reflect the role of miRNAs in Th2 inflammation of asthma. In this study, we observed the dynamic changes of the expression of miRNA-181a, -155, -150, -146a and -146b in splenic CD4⁺ T lymphocytes in a murine asthma model, and analyzed the effect of

Abbreviations: AHR, airway hyper-responsiveness; AICD, activation-induced cell death; BALF, bronchoalveolar lavage fluid; DEX, dexamethasone; FAF1, Fas associated factor 1; IFN- γ , interferon- γ ; Ig, immunoglobulin; IL, interleukin; miRNA, microRNA; mRNA, messenger RNA; OVA, ovalbumin; qPCR, quantitative real-time polymerase chain reaction; TCR, T cell receptor.

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dexamethasone (DEX) on the expression of these miRNAs. We introduce the idea that miRNA-181a, -146a and -146b are proinflammatory factors in asthma, and that the down-regulation of miRNA-146a may be involved in the anti-inflammatory effect of DEX.

2. Materials and methods

2.1. Mice

Female BALB/c mice (10 weeks old) purchased from Guangdong Medical Laboratory Animal Center (Guangzhou, China), were maintained in the animal facility at the Shenzhen Center for Disease Control and Prevention. Animals were housed in a specific pathogen-free environment. All experiments were approved and performed according to the experiment animal guidelines of the University of Jinan.

2.2. Sensitization and airway challenges

Mice were divided into three groups randomly: (1) the control group, (2) the ovalbumin (OVA) group, and (3) the DEX group. The control group and OVA group were split into 2 subgroups: mice sacrificed at Day 32 (subgroup 1), and sacrificed at Day 37 (subgroup 2). In other words, there were the subgroup 1 of the control group (con/sub 1), the subgroup 2 of the control group (con/sub 2), the subgroup 1 of the OVA group (OVA/sub 1) and the subgroup 2 of the OVA group (OVA/sub 2), respectively. Mice in the OVA or the DEX group were sensitized and challenged as described previously with slight modifications [29]. In brief, mice were sensitized at Days 0, 7 and 14 through intraperitoneal (i.p.) injection of 1 mg/kg OVA (grade V, Sigma-Aldrich) emulsified in 0.1 g/kg of aluminum hydroxide gel (Sigma-Aldrich). At Days 28, 29 and 30 after the initial sensitization, the mice were anesthetized, and challenged by aspiration with 50 μ l of 2 g/l OVA solution with a saline. Mice in the control group were sensitized and challenged with saline instead. The DEX group mice were i.p. administered with 5 mg/kg of water-soluble DEX 1 h before OVA challenge at Days 28, 29 and 30. The mice in the control group and OVA group received injections of saline instead of DEX. The mice in the con/sub 1, OVA/sub 1 and DEX group were prepared for collection of bronchoalveolar lavage fluid (BALF), lung tissues and spleen at Day 32, while the mice in the con/sub 2 and OVA/sub 2 were prepared at Day 37 (Fig. 1, Table 1).

2.3. Bronchoalveolar lavage

Mice were anesthetized with sodium pentobarbitone (50 mg/kg b.w.), the trachea was cannulated and the right bronchus was tied for histological analyses. After the instillation of saline (0.5 ml) into the left lung, BALF was collected using three aspirations (total volume

of 1.5 ml) via tracheal cannulation. The total numbers of inflammatory cells and the eosinophils were counted by a hemocytometer. BALF was centrifuged and the supernatant was stored at -80°C for cytokine analysis. The cell pellets were suspended in 0.5 ml saline, and 100 μ l of each solution was smeared onto a slide. After slides were dried, cells were fixed and stained with hematoxylin and eosin (H&E) for cytological examination.

2.4. Histopathology

The right lungs were taken from mice under terminal anesthesia, fixed in 10% neutral formalin, paraffinized, cut into 5- μ m sections, and stained with H&E to detect cellular infiltration. The degree of airway inflammatory cell infiltration was analyzed in a double-blind screen by two independent investigators.

2.5. Enzyme-linked immunosorbent assay (ELISA) for IL-4 and IFN- γ

Levels of IL-4 and interferon- γ (IFN- γ) cytokines in BALF supernatant were measured by ELISA (Dakewe, SHENZHEN, CHINA), according to the manufacturer's instructions.

2.6. Splenic CD4+ T lymphocyte isolation

Splenic monocytes of the mice were isolated by density-gradient centrifugation with EZ-sep mouse $1\times$ lymphocyte separation medium (Dakewe, SHENZHEN, CHINA), and CD4+ T cells were purified by a mouse CD4+ T cell isolation kit II (Miltenyi Biotec, Bergisch Gladbach, Germany). The purity of the splenic CD4+ cells was determined by flow cytometry, and the purity ranged from 92% to 97.5% after the isolation.

2.7. Isolation of total RNA from the splenic CD4+ T lymphocyte

Total RNA was isolated from the splenic CD4+ T lymphocyte using Trizol reagent (Invitrogen, NY, USA) according to the manufacturer's protocol. RNA purity was evaluated using the Eppendorf BioPhotometer (Eppendorf, Hamburg, Germany), and only samples with A260/A280 that lied between 1.8 and 2.0 were used. RNA integrity was judged by agarose gel electrophoresis—usable samples had sharp 28S and 18S rRNA bands.

2.8. Real-time PCR for miRNA expression

miRNA abundance was quantified using the All-in-One miRNA qRT-PCR Detection Kit (GeneCopoeia, Rockville, MD). Briefly, total RNA was poly(A) tailed and reverse transcribed using a poly dT-adaptor primer. Quantitative RT-PCR was carried out using a miRNA-specific forward primer and universal reverse primer. PCR products were subjected

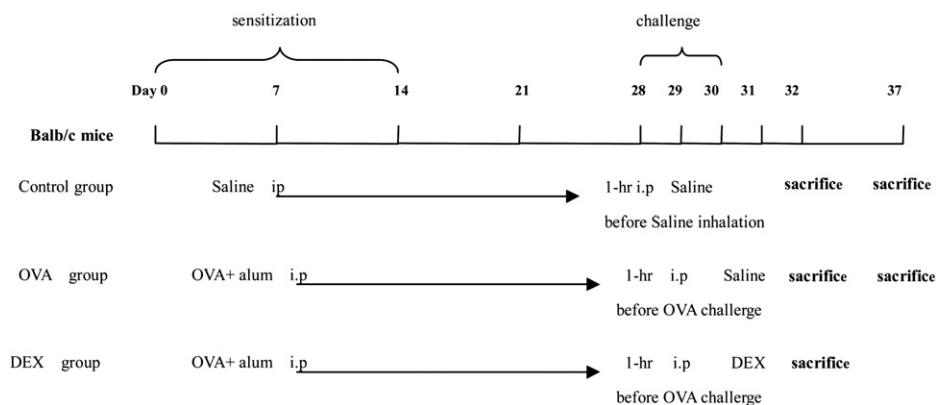


Fig. 1. Protocol for inducing experimental asthma and DEX treatment.

Table 1
Comparison of each group in the protocol.

Group	Sensitization	Challenge	Treatment	Sacrifice time
Con/sub 1	Saline	Saline	Saline	Day 32
Con/sub 2	Saline	Saline	Saline	Day 37
OVA/sub 1	OVA/aluminum hydroxide gel	OVA	Saline	Day 32
OVA/sub 2	OVA/aluminum hydroxide gel	OVA	Saline	Day 37
DEX	OVA/aluminum hydroxide gel	OVA	Dexamethasone	Day 32

to dissociation curve and gel electrophoresis analyses to ensure that single, mature miRNA products were amplified. Data were normalized to small RNA U6 levels. The forward primers for the miRNAs were as follows: miRNA-181a 5'-AACAUUCAACGUCGUGAGU-3', miRNA-155 5'-UUAUUGCUAAUUGUGAUAGGGU-3', miRNA-150 5'-UCUCCC-AACCUUGUACCAGUG-3', miRNA-146a 5'-UGAGAACUGAAUCCAUG-GGUU-3', and miRNA-146b 5'-UGAGAACUGAAUCCAUGGCU-3'. The reverse primers and the primers for small RNA U6 were proprietary (GeneCopoeia). Amplification was performed according to the manufacturer's cycling protocol and done in triplicate. Each real-time PCR assay was run with a negative control (PCR-grade water).

2.9. Data analysis and statistical analysis

For the real-time PCR data, efficiency was calculated as $E = 10^{-1/\text{slope of standard curve}}$. Using the miRNAs of interest (miRNA-181a, -155, -150, -146a and -146b) as targets and small RNA U6 as the reference, the relative gene expression ratio was determined as such:

$$R = \frac{(E_{\text{target}})^{\Delta C_P^{\text{target}}(\text{calibrator-sample})}}{(E_{\text{ref}})^{\Delta C_P^{\text{ref}}(\text{calibrator-sample})}}$$

where E_{target} and E_{ref} were the efficiencies of the target miRNAs and small RNA U6, respectively, and ΔC_P is the difference of the crossing points between calibrator and samples [30]. The relative expression ratios (R) were used to determine differences in miRNA expression among the

different groups, excluding the outliers. The expression levels of normalized target genes were analyzed using the relative expression software tool (REST-MCS) [31].

With regard to the levels of cytokines (i.e. IL-4 and IFN- γ) and the numbers of cells (i.e. total cells and eosinophils) in the BALF, data were presented as mean $\pm$ standard error of the mean (SEM). The statistical significance of the differences among groups was determined by analysis of variance. Spearman correlation analyses were taken to evaluate the relationship between miRNAs (miRNA-181a, -150, -146a and -146b) and inflammatory cells in the BALF. All tests were performed using the SPSS 13.0 system. A p value of less than 0.05 was accepted as statistically significant.

3. Results

3.1. Histological examination

Evidence of inflammatory cell infiltration and the effects of DEX treatment were investigated by H&E staining quantification of lung tissue sections. Sections in the control group (i.e. con/sub 1 and con/sub 2) showed normal bronchial and alveolar architecture (Fig. 2A and G). Sensitized mice after OVA challenge showed increased numbers of eosinophils and lymphocytes in the lung tissue. Histological evaluation of the lungs from OVA/sub 1 (Fig. 2B, I and L) revealed marked eosinophil and lymphocyte influx into the lungs. The BALF smear among these groups also reflected the phenomena. Macrophage-predominant BALF was seen from con/sub 1 (Fig. 2D) and con/sub 2 (Fig. 2J), and eosinophil-predominant BALF was presented from OVA/sub 1 (Fig. 2E), OVA/sub 2 (Fig. 2K) and the DEX group (Fig. 2F).

3.2. The dynamic change of lung BALF inflammation in sensitized and challenged mice

The numbers of total inflammatory cells (Fig. 3A) and eosinophils (Fig. 3B) recovered in BALF from the control and OVA groups at 2 different time-points (i.e. Days 32 and 37), showed that the total numbers of inflammatory cells and eosinophils in BALF of the OVA group

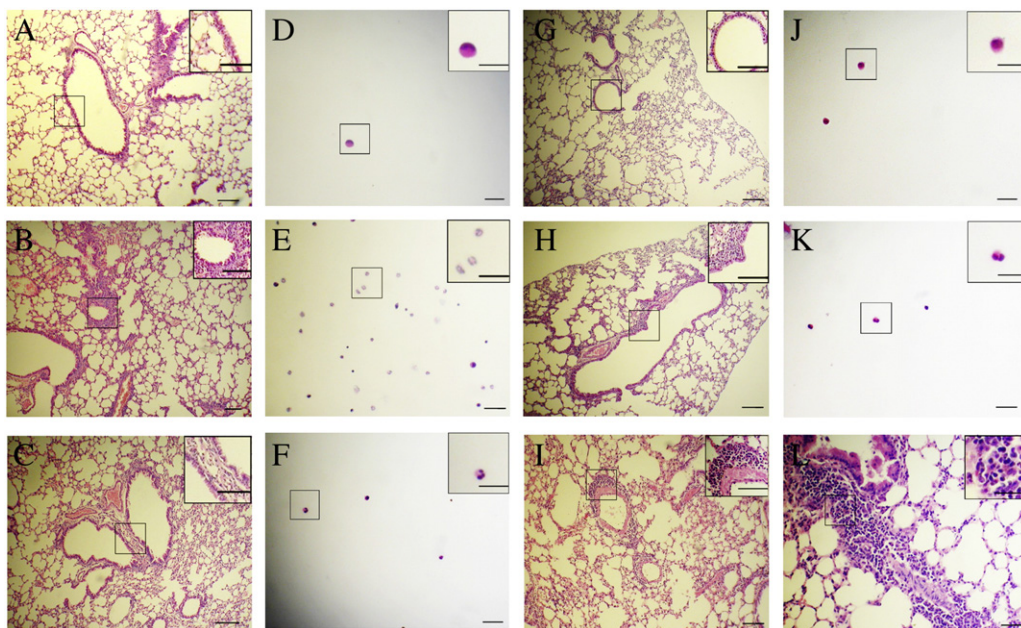


Fig. 2. Formalin-fixed lung tissue sections and BALF smears from each group. Representative photomicrographs of the lung sections from con/sub 1 (A), OVA/sub 1 (B, I and L), the DEX group (C), con/sub 2 (G), and OVA/sub 2 (H). The sections were subjected to H&E staining. Representative examples are shown at 200 $\times$ magnification (lower panel). Detailed images of the histological lung sections (400 $\times$ magnification, upper panel). Macrophage-predominant BALF from con/sub 1 (D) and con/sub 2 (J), and eosinophil-predominant BALF from OVA/sub 1 (E), OVA/sub 2 (K) and DEX group (F). These sections were also subjected to H&E staining. Representative examples are shown at 400 $\times$ magnification (lower panel).

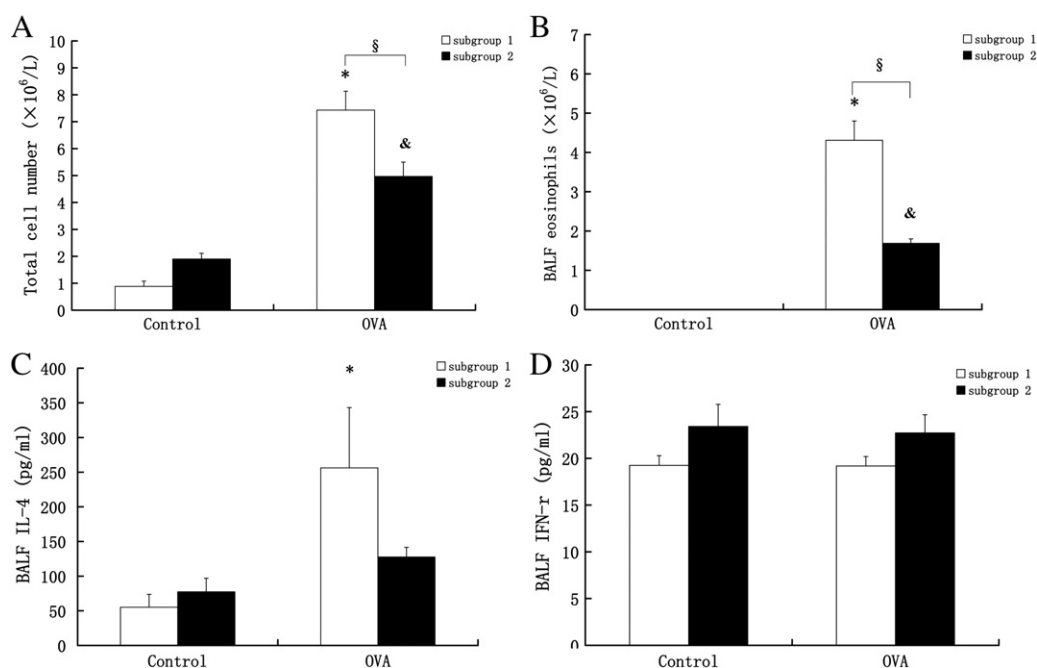


Fig. 3. Dynamic changes in the inflammation in lung BALF in sensitized and challenged mice. The numbers of the total inflammatory cells (3A) and eosinophils (B) recovered in BALF of the control and OVA groups at 2 different time-points (i.e. Days 32 and 37), respectively. Collected BAL fluid assayed for the indicated Th2 cytokine IL-4 (C) and Th1 cytokine IFN- γ (D) production at the same time. Values presented are means $\pm$ SEM ($n=6$ mice per group). * $p<0.05$, compared to con/sub 1. & $p<0.05$, compared to con/sub 2. § $p<0.05$, compared to OVA/sub 1.

were higher than those of the control group (Fig. 3A and B). The numbers of total inflammatory cells and eosinophils in OVA/sub 2 were lower than those in OVA/sub 1 (Fig. 3A and B).

Th2 inflammation of the airway manifests not only inflammatory cell infiltration, but also accompanying increase of Th2 cytokines (such as IL-4, IL-5 and IL-13) in the BALF. Collected BALF was assayed for the indicated Th2 cytokine IL-4 and Th1 cytokine IFN- γ production at 2 time-

points (i.e. Days 32 and 37). A higher expression of Th2 cytokine IL-4 was seen in OVA/sub 1 than in con/sub 1 ($p<0.05$). There was no significant difference in IL-4 between OVA/sub 2 and con/sub 2. IL-4 between the con/sub 1 and con/sub 2 subgroups showed similar expression levels, as did IL-4 between OVA/sub 1 and OVA/sub 2 subgroups (Fig. 3C). There was no difference in Th1 cytokine IFN- γ among any of the four subgroups (Fig. 3D).

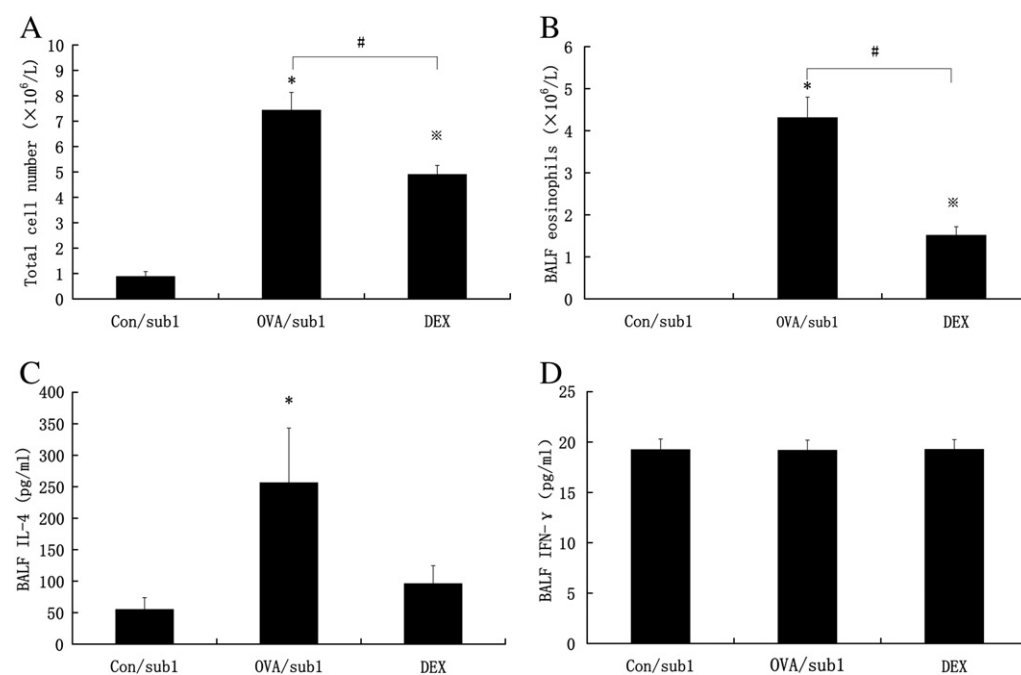


Fig. 4. Effects of DEX on lung BALF inflammation in sensitized and challenged mice. The numbers of the total inflammatory cells (A) and eosinophils (B) in the BALF of three groups (i.e. con/sub 1, OVA/sub 1 and DEX group) were determined at Day 32, as described in Materials and methods. Th2 cytokine IL-4 (C) and Th1 cytokine IFN- γ (D) production in BALF among the three groups was measured at the same time. Values presented are means $\pm$ SEM ($n=6$ mice per group). * $p<0.05$, compared to con/sub 1. ※ $p<0.05$, compared to con/sub 1. # $p<0.05$, OVA/sub 1 versus the DEX group.

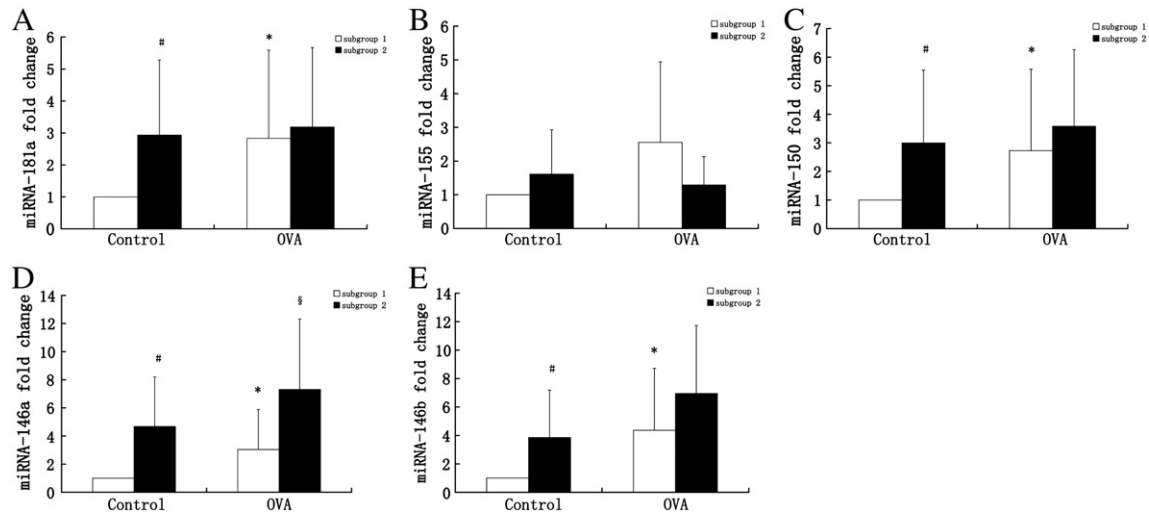


Fig. 5. Dynamic change of the expression levels of five miRNAs in splenic CD4+ T lymphocytes. Q-PCR analyzed expression levels of five miRNAs in the control group and the OVA group on Day 32 and Day 37, respectively. These miRNAs include: miRNA-181a (A), miRNA-155 (B), miRNA-150 (C), miRNA-146a (D) and miRNA-146b (E). Expression levels were calculated relative to U6 SnRNA and the data are expressed as fold-increase under input subgroup 1 of the control group, which was assigned a value of "1". n ranged from 3 to 5 mice per group. Data represent three independent experiments. # $p < 0.05$, compared to con/sub 1; * $p < 0.05$, compared to con/sub 1; § $p < 0.05$, compared to OVA/sub 1.

3.3. Effects of DEX on lung inflammation in sensitized and challenged mice

Mice in OVA/sub 1 had higher total inflammatory cells and eosinophils in BALF than mice in con/sub 1. Eosinophil inflammation in the lungs was observed in the histological section from OVA/sub 1. The numbers of total inflammatory cells and eosinophils in the DEX group were lower than those in OVA/sub 1 (Fig. 4A and B).

The IL-4 level in OVA/sub 1 was higher than in con/sub 1 ($p < 0.05$). However there was no significant difference in IL-4 between the DEX group and con/sub 1 or between the DEX group and OVA/sub 1, the mean values of IL-4 among these 3 groups (i.e. con/sub 1, OVA/sub 1 and the DEX group) were apparently different, with 55.13 ± 18.65 pg/ml, 256.27 ± 86.89 pg/ml and 96.08 ± 28.54 pg/ml respectively. These results indicated that the high level of IL-4 in OVA/sub 1 had been

decreased by DEX treatment (Fig. 4C). The production of Th1 cytokine IFN- γ in BALF was not significantly changed by DEX treatment (Fig. 4D).

3.4. The dynamic change of miRNA expression levels in splenic CD4+ T lymphocytes

In order to elucidate the role the miRNAs (-181a, -155, -150, -146a, and -146b) played in Th2 inflammation of asthma, the expression levels of these miRNAs in CD4+ T lymphocyte between the control group and the OVA group at Day 32 and at Day 37 were investigated. The expression levels of miRNA-181a, -150, -146a and -146b, but not miRNA-155, were higher in OVA/sub 1 than in con/sub 1 (Fig. 5A, C, D and E). There was no difference in the five miRNAs between OVA/sub 2 and con/sub 2 (Fig. 5A–E). Higher expression levels of miRNA-181a,

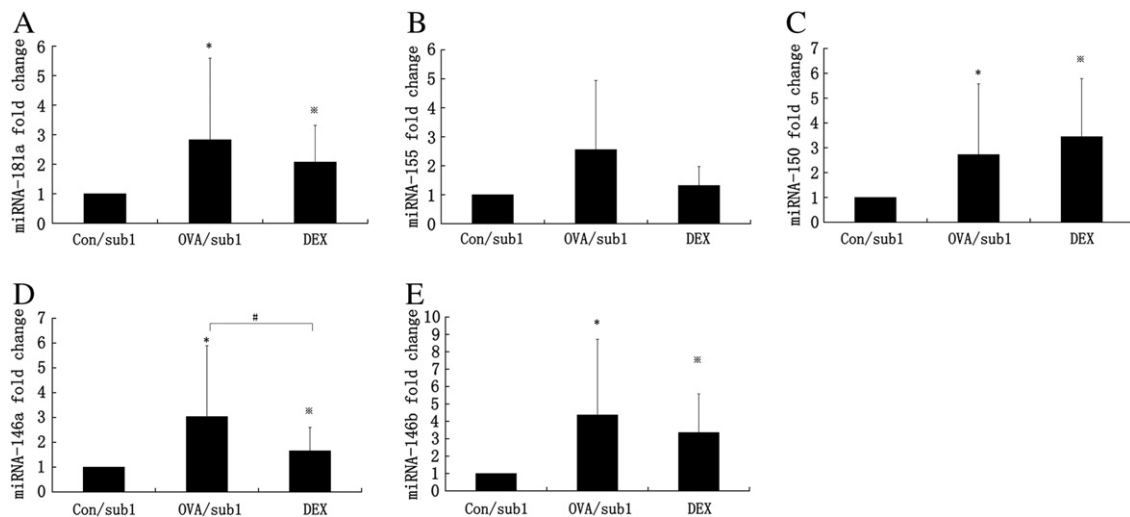


Fig. 6. Effects of DEX on the expression levels of five miRNAs in splenic CD4+ T lymphocytes. Q-PCR analyzed expression levels of five miRNAs in splenic CD4+ T lymphocytes of three groups (i.e. con/sub 1, OVA/sub 1 and DEX group). These miRNAs include: miRNA-181a (A), miRNA-155 (B), miRNA-150 (C), miRNA-146a (D) and miRNA-146b (E). Expression levels were calculated relative to U6 SnRNA and the data are expressed as fold-increase under input subgroup 1 of the control group, which was assigned a value of "1". n ranged from 3 to 5 mice per group. Data represent three independent experiments. * $p < 0.05$, compared to con/sub 1. § $p < 0.05$, compared to con/sub 1. # $p < 0.05$, OVA/sub 1 versus the DEX group.

Table 2

Correlations of miRNAs (miRNA-181a, -150, -146a and -146b) with the number of inflammatory cells in the BALF.

	miRNA-181a		miRNA-150		miRNA-146a		miRNA-146b	
	Spearman correlation	p value	Spearman correlation	p value	Spearman correlation	p value	Spearman correlation	p value
Total cell number	0.770	0.009	0.612	0.060	0.733	0.016	0.806	0.005
BALF eosinophil	0.666	0.036	0.511	0.131	0.666	0.036	0.718	0.019

-150, -146a, and -146b, were observed in con/sub 2 than in con/sub 1. The expression of miRNA-146a in OVA/sub 2 was higher than that in OVA/sub 1 (Fig. 5D).

3.5. Effects of DEX on the expression levels of five miRNAs in splenic CD4⁺ T lymphocytes

The expression levels of these miRNAs in mouse cells were compared to examine whether miRNA-181a, -155, -150, -146a and -146b can be affected by DEX. The result showed that the expression level of miRNA-146a was lower in the DEX group than in OVA/sub 1 (Fig. 6D). There were no significant difference in the expression levels of miRNA-181a, -155, -150, and -146b between the DEX group and OVA/sub 1 (Fig. 6A, B, C and E).

3.6. Correlations of miRNAs (miRNA-181a, -150, -146a and -146b) with the number of inflammatory cells in the BALF

Spearman correlation analysis proved that there was a positive linear correlation between microRNA-181a and the number of inflammatory cells (i.e. the number of total cells or of the eosinophils in the BALF), so did miRNA-146a and miRNA-146b ($p < 0.05$, Table 2).

4. Discussion

The relationship between miRNA and the Th2 lymphocytes of asthma is of great interest because of the rapid and efficient intervention miRNAs have on the target gene. Until now, few studies have contributed to this topic [15,17], with the ones that have been conducted regarding mainly miRNAs from airway biopsies or lung tissue. Th2 lymphocyte is differentiated from CD4⁺ T lymphocyte, therefore the study of miRNA expression in CD4⁺ T lymphocytes can offer a new insight into the pathology of asthma. We report here that 3 miRNAs (miRNA-181a, -146a and -146b) play proinflammatory roles in asthma, and the down-regulation of miRNA-146a might explain partially the anti-inflammatory effect of dexamethasone.

The expression levels of four miRNAs (miRNA-181a, -150, -146a and -146b) in the OVA/sub 1 were higher than those in con/sub 1, which was accompanied by both marked eosinophil and lymphocyte influx into the lungs and the up-regulation of Th2 cytokine (IL-4) in the BALF. Without new airway challenge at Day 37, the expression levels of these 4 miRNAs in CD4⁺ T lymphocytes in OVA/sub 2 were similar as in con/sub 2, associated with the down-regulation of IL-4 in BALF and the resolution of the airway inflammation. On the other hand, microRNA-181a had a positive linear correlation with the number of inflammatory cells (i.e. the number of total cells or of the eosinophils in the BALF) by Spearman correlation analysis, so did miRNA-146a and miRNA-146b. The close relationship between the 3 miRNAs (miRNA-181a, -146a and -146b) and immunology has been recently reported. Li et al. found that a higher expression of miR-181a in mature T cells augmented T cell receptor (TCR) sensitivity, and enabled mature T cells to recognize antagonists as agonists [32]. These effects were partially due to the down-regulation of multiple phosphates, which were negative regulators in the TCR signaling pathway. These phosphates included SHP 2 (Src homology 2-domain-containing protein-tyrosine phosphatase 2), PTPN22 (non-receptor type tyrosine phosphatase 22), DUSP5 (dual-specificity phosphatase 5) and DUSP6. With the up-regulation of miRNA-181a, T cells would exhibit increased TCR signaling and reduced

T-cell activation threshold. It was well-known that modulating activation-induced cell death (AICD) and modulating Fas associated factor 1 (FAF1) were both critically involved in modulating T cell apoptosis. Curtale et al. found that miRNA-146a was induced in human primary T lymphocytes upon TCR stimulation, and acted as an anti-apoptotic factor for AICD [33]. On the other hand, Li et al. also proved that miR-146a over-expression could suppress Jurkat T cell's apoptosis by down-regulation of its target gene—FAF1 [34]. These results suggested that miR-146a was important in T lymphocyte activation. Garbacki et al. proved that miRNA-146b up-regulated in murine lung tissue in an asthma model [25], and increased the production of IgE, an important Th2 inflammatory cytokine by reducing the synthesis of TNFRSF9. These findings implied that miRNA-181a, -146a and -146b play proinflammatory roles in asthma.

Although Spearman correlation analysis showed no correlation between microRNA-150 and the number of the inflammatory cells in BALF, another study had also found that the expression level of miRNA-150 was up-regulated in the lung tissue of asthma models [25]. The role of miRNA-150 was needed to be explored further.

Interestingly, the expression levels of the 3 miRNAs (miRNA-181a, -146a and -146b) in con/sub 2 were higher than those in con/sub 1. However they did not lead to the inflammation of the lung or to the up-regulation of the Th2 cytokine IL-4 in the BALF. The up-regulation of these miRNAs was probably caused by some procedures when constructing asthma models, (e.g. intraperitoneal injection, anesthesia, etc.). This possibility was proven by analyzing the differences between the expression levels of the 3 miRNAs (miRNA-181a, -146a and -146b) in OVA/sub 1 and OVA/sub 2. Although miRNA-146a was found to be up-regulated, the airway inflammation was not aggravated, nor did the IL-4 in the BALF increase. The mechanism of the up-regulation may be the same as that in the control group, and highlighted the need for proper controls, and we took this bias into consideration when we analyzed the experimental data.

From the dynamic change in the expression of miRNAs from the control group to the OVA group, we found that up-regulation of these 3 miRNAs alone was not enough to induce the Th2 inflammation of the airway in asthma, therefore other factors may take part in the pathology, such as airway provocation. In other words, the 3 miRNAs (miRNA-181a, -146a and -146b) were not independent risk factors of asthma.

Although the 3 miRNAs (miRNA-181a, -146a and -146b) were all up-regulated in Th2 inflammation of asthma, the mechanisms for the upregulation may be different. Corticosteroids were the most potent anti-inflammatory drug used to treat asthma. They can not only significantly decrease the accumulation and activity of eosinophils, lymphocytes, macrophages, and other cells, but can also potentially inhibit the synthesis of inflammatory mediators. Our study revealed that miRNA-146a was down-regulated in the DEX group, while the expression of miRNA-181a and miRNA-146b did not change after DEX treatment. These implied that the expression level of miRNA-146a could be affected by DEX, and that the down-regulation of miRNA-146a might be partly involved in the anti-inflammatory effect of DEX.

Rodriguez et al. suggested that miRNA-155 may be related to asthma [22]. It was shown that miRNA-155-deficient mice were immunodeficient and displayed increased airway remodeling. MiRNA-155 targeted the transcription factor c-Maf, which promoted IL-4, IL-5, and IL-10 production by Th2 lymphocytes. In addition, the human BIC/miR-155 gene

was also mapped in an asthma, pollen sensitive, and atopic dermatitis susceptible region on chromosome 21q21. Therefore it was proposed that miR-155 may be an important modulator in asthma. In contrast, our results showed that there was no significant difference in miR-155 among each group. Garbacki found that there were no changes to miR-155 in acute asthma, which we confirmed [25]. This discrepancy between Rodriguez's and ours may be due to the different methods of animal model establishment. A large-scale randomized clinical trial would be ideal to determine the mechanism.

In conclusion, our results suggested for the first time that these miRNAs (miRNA-181a, -146a and -146b) were proinflammatory factors in asthma, though they were not independent risk factors. Down-regulation of miRNA-146a might be partly involved in the anti-inflammatory effect of dexamethasone. Although these results were based on murine models of asthma, the present study built a foundation for future efforts to design novel therapeutic interventions for asthma.

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