



Administration of mycobacterial Ag85A and IL-17A fusion protein attenuates airway inflammation in a murine model of asthma



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ABSTRACT

Interleukin (IL)-17A contributes to the development of asthma, especially in severe asthma which has characteristic neutrophil infiltration in airways. However, IL-17A-blocking antibody could escalate T helper (Th) 2 cytokines, such as IL-13, IL-4 in murine models. We aimed at determining the effect of mycobacterial Ag85A and IL-17A fusion protein—Ag85A-IL-17A on airway inflammation in a murine model of asthma. IL-17A recombinant protein fused mycobacterial immunodominant antigen Ag85A was constructed, expressed and purified. The fusion protein was then administrated into BALB/c mice and its anti-inflammatory effects in the infiltration of inflammatory cells, Th2/Th17 cytokines in BALF, histopathological changes of lung tissues as well as chemokines in lung tissues were evaluated in the murine model of asthma. We found that administration of mycobacterial Ag85A and IL-17A fusion protein induced IL-17A specific immunoglobulin (Ig)G in sera and significantly decreased IL-17A and IL-6 levels in bronchoalveolar lavage fluid (BALF). Ag85A-IL-17A vaccinated mice also showed marked reduction in the infiltration of inflammatory cells in peribronchiolar region and significant decrease in total cells, eosinophil cells and neutrophil cells in BALF. The increased levels of IL-13 and IL-4 in BALF of ovalbumin-sensitized mice were significantly reduced by the administration of Ag85A-IL-17A. Furthermore, CD3⁺CD4⁺IL-13⁺ splenocytes stimulated with OVA and CXCL1 mRNA, CCL2 mRNA and GATA-3 mRNA expressed in lung tissues were decreased markedly in Ag85A-IL-17A vaccinated group. Our results demonstrate remarkable antiallergic effects of Ag85A-IL-17A in a murine model of asthma and it may have protective effects on allergic asthma.

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1. Introduction

Asthma has long been considered as a chronic airway inflammation of deregulated immune responses to environmental allergens. The inflammatory response in the asthmatic lung is characterized by infiltration of airways by mast cells, lymphocytes and eosinophils and is associated with the increased expression of various inflammatory mediators, cytokines and adhesion molecules [1,2]. T helper (Th) 2 cytokines, including interleukin (IL)-4, IL-5, and IL-13, are essential for generating the pathophysiological features of asthma. IL-4 and IL-13 play important roles in immunoglobulin (Ig)E switching in B cells, mucus hypersecretion and eosinophil infiltration into lung tissue [3–7].

Emerging evidences suggest that IL-17A contributes to the development of asthma, especially in severe asthma which has a characteristic neutrophil infiltration in the airways [8–10]. Patients with severe asthma have a higher level of IL-17A in induced sputum

compared to healthy people [11–13]. Thus antibody to IL-17A might be beneficial in neutralizing IL-17A in sera of patients with severe asthma. However, it has been shown that administration of IL-17A-blocking antibody could escalate Th2 cytokines such as IL-13 in murine models [14].

Mycobacterial antigen Ag85A is one of a family of three immunodominant protein antigens secreted by Bacille Calmette-Guérin (BCG) that might have a role in stimulating Th1 responses, which in turn inhibit cytokines from Th2-like cells [15–17]. Under normal conditions, self-reactivity is prevented by a number of processes resulting in immunological tolerance of self-antigens. Therefore cytokines don't normally induce autoantibody in vivo. Marie-Christophe Boissier et al. [18] speculated that when a self protein was associated with a foreign protein-carrier, it could activate T cells and induce B cells to produce autoantibodies via Th epitopes of foreign protein. In previous study, we constructed a recombinant protein using Ag85A as a protein-carrier to provide Th epitopes to induce murine IL-17A autoantibody [19].

In the present report, we investigated the anti-inflammatory and immunomodulatory effects of mycobacterial Ag85A and IL-17A fusion protein (Ag85A-IL-17A) with regard to antigen-induced inflammatory cells infiltrating in the airways, local Th2/Th17 cytokine production,

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histopathological changes of lung tissues and chemokines in lungs in a murine model of allergic asthma.

2. Materials and method

2.1. Animals

Female BALB/c mice, 4–6 weeks old (Experimental Animal Center, Shanghai Public Health Clinical Center, Shanghai, China), bred and maintained in a specified pathogen-free (SPF) facility in this study. All experiments were performed in accordance with protocols approved by the Ethics Committee of Shanghai Public Health Clinical Center, Shanghai, China (Permit Number: 2012–0079).

2.2. Preparation of recombinant protein Ag85A-IL-17A

The plasmids pET28a-Ag85a and pET28a-IL-17a were constructed by our laboratory. Murine IL-17a (NM_010552.3) was identified and amplified by PCR using the following primers with *Acc* I restrictive endonuclease sites and PCR conditions: forward, 5'-CCG GTCTAC GCA GCG ATC ATC CCT -3', reverse, 5'-CG GTAGAC GGC TGC CTG GCG GAC -3'; 94 °C 30 s, 56 °C 40 s, 72 °C 30 s for 30 cycles, followed by extension for 6 min at 72 °C. The PCR product was cloned into pET28a-Ag85a via T4 DNA ligase (Fermentas) and transformed to competent *E. coli* DH5 α . Transformants were identified by endonuclease digestion and sequencing. The confirmed plasmid with correct sequence was transformed into competent *E. coli* BL21(DE3) for protein expression and induced by isopropyl- β -D-thiogalactoside (IPTG; Sango, Shanghai, China) for 4 h at 37 °C. Then the protein was purified as described before [19]. The concentration of these purified proteins was determined with Bradford assay. Endotoxin contamination in these desalting proteins was assayed by using E-TOXATE Kits (Sigma–Aldrich) and the material under study is endotoxin free.

2.3. 3D structure of recombinant protein Ag85A-IL17A

The amino acid sequence of recombinant protein was sent to Swiss-Model Workspace to get a PDB file and then PyMol CPH software was applied to display the 3D structure of recombinant protein Ag85A-IL-17A.

2.4. Immunization, sensitization and allergen exposure protocol

Mice were divided into four groups (PBS, OVA, OVB, Ag85A-IL-17A treated group) and treated as shown in Fig. 1. Briefly, Ag85A-IL-17A treated group got subcutaneous injection of 1:1 emulsion of 50 μ l purified protein in complete Freund's adjuvant (Sigma) on the first day and then got intraperitoneal injection of 50 μ l purified protein in incomplete Freund's adjuvant (Sigma) on day 14 and day 35. Then

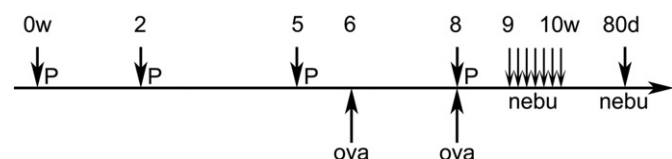


Fig. 1. Immunization, sensitization and allergen exposure protocol. Ag85A-IL-17A treated groups were injected 50 μ g purified protein in complete Freund's adjuvant on the first day, 50 μ g purified protein in incomplete Freund's adjuvant on days 14, 35 and got protein booster on day 56. Mice of OVA group, OVB group and Ag85A-IL-17A group were sensitized by 10 μ g of OVA absorbed on 1.6 mg of alum in 100 μ l of PBS on day 42 and day 56. Then they were challenged locally with 200 μ g OVA in 20 μ l PBS into their nostrils from day 63 to day 69. PBS group were injected and challenged with PBS each time. The PBS group and OVA group were sacrificed on day 70. OVB and Ag85A-IL-17A treated mice were challenged again with 4 mg OVA in 20 μ l PBS on day 79 and then sacrificed on day 80.

they got 50 μ g purified protein booster on day 56. Mice in OVA group, OVB group and Ag85A-IL-17A treated group were sensitized by subcutaneous injections with 10 μ g of ovalbumin (OVA, grade V; Sigma) absorbed on 1.6 mg of alum in 100 μ l of phosphate buffered saline (PBS) on day 42 and day 56. Then they were challenged with 200 μ g OVA nebulized in 20 μ l PBS from day 63 to day 69. PBS group were injected and nebulized with PBS each time. PBS group and OVA group were sacrificed on day 70. OVB group and Ag85A-IL-17A treated group were challenged again with 4 mg OVA nebulized in 20 μ l PBS on day 79 and then sacrificed on day 80.

2.5. IL-17A specific IgG in sera

Sera levels of IL-17A-specific IgG were measured by enzyme-linked immunosorbent assay (ELISA). Briefly, microtiter plates were coated with 100 μ l/well IL-17A (2.5 μ g/ml; eBioscience) in PBS/Tween-20 and antibodies in serum were detected using isotype-specific secondary antibodies (Goat anti-mouse IgG-HRP, KPL; 1:6000 diluted in 2% BSA). Substrate solution (eBioscience) was then added to wells and incubated for 3 min and reactions were stopped with 2 M H₂SO₄. Data are presented as mean of optical density (OD) value at 450 nm per group.

2.6. Preparation of bronchoalveolar lavage fluid (BALF)

BALF was performed as described previously [20]. In brief twenty-four hours after the last OVA or PBS challenge, we sacrificed the mice. The trachea was cannulated, the right main bronchial was ligated and then the left lung was carefully washed three times with 0.4 ml PBS. The samples were immediately centrifuged (20 °C, 300 $\times$ g, 10 min), the pellet was resuspended in PBS and the supernatant was frozen at -80 °C. The total number of leukocytes in the BALF was immediately determined in a hemocytometer using Trypan blue. Differential cell counts were obtained by using Wrights-Giemsa-stained cytopsin preparations. A differential count of at least 100 cells was made in a blind fashion in accordance with standard morphologic criteria.

2.7. Cytokine levels in BALF

ELISA was performed according to the manufacturer's instructions. The concentrations of IL-17A, IL-6, IL-4, IL-13, IL-12p70 and IL-10 in BALF were measured using specific mouse IL-17A, IL-6, IL-4, IL-13, IL-12p70 and IL-10 ELISA kits (eBioscience).

2.8. Immunohistochemistry

After BALF was acquired, the right lung was isolated and incubated in formalin for 24 h. Then lung tissues were embedded in paraffin and processed for analysis. Tissue sections of 4 to 5 μ m were stained with hematoxylin and eosin staining to assess the infiltration of inflammation cells. The severity of peribronchial inflammation was scored according to a published guideline [21]. A value of 0 was assigned when no inflammation was detectable. A value of 1 was assigned for occasional cuffing with inflammatory cells. A value of 2 was assigned for most bronchi or vessels surrounded by a thin layer (one to five cells thick) of inflammatory cells. A value of 3 was given when most bronchi or vessels were surrounded by a thick layer (more than five cells thick) of inflammatory cells. Total lung inflammation was defined as the average of the peribronchial and perivascular inflammation scores. Samples were scored in a blinded fashion.

2.9. Intracellular staining and flow cytometry analysis.

The spleen was minced using 200 μ m nylon nets. Splenic cell suspension was collected and centrifuged for 3 min at 2000 r/min and the supernatant was discarded. The red blood cells were lysed with red blood cell lysing solution (MultiScience). Cells (1×10^7 cells/well)

were washed thrice and stimulated with endotoxin-free OVA (grade V, Sigma; 10 µg/ml) for 24 h. Permeabilised by 100 µl Cytotfix/Cytopern (BD Biosciences) for 10 min at RT, cells were pelleted and incubated with 10 µg/ml of CD3-APC-Cy7 (eBiosciences), 10 µg/ml CD4-FITC (eBiosciences) and 10 µg/ml IL-13-PE-Cy7 (eBiosciences) for 30 min at RT. After washing with PBS, cells were analyzed on a FACStar Plus cytometer (BD Biosciences). For comparison between experiments, fluorescein quantitative standard beads (BD Biosciences) were used to quantify the fluorescence signal.

2.10. RT-PCR analysis

Lung samples (about 20–30 mg of tissue for each sample) isolated and flash-frozen in liquid nitrogen were mechanically homogenized using a variable speed homogenizer. Total RNA was isolated from homogenized lungs using TRIzol reagent (Invitrogen). RNA samples were used to synthesize cDNA using the first strand cDNA synthesis kit (Fermentas). Real-time polymerase chain reaction (RT-PCR) was carried out in a 23 µl final volume in duplicates using SYBR Green as a fluorescent detection dye. PCR amplification was performed with the following sense and antisense primers: CXCL1 sense, 5'-CACCCAAA CCGAAGTCAT-3'; CXCL1 antisense, 5'-GGGACACCTTTAGCATCT-3'; CCL2 sense, 5'-CAAGAAGGAATGGGTCCAGACA-3'; CCL2 antisense, 5'-GCTTCAGATTTACGGGTCAACT-3'; GATA-3 sense, 5'-GAAGGCATCCAG ACCCGAAAC-3'; GATA-3 antisense, 5'-ACCATGGCGGTGACCATGC-3'; ROR-γt sense, 5'-AGGATGAGATTGCCCTCTACAC-3'; ROR-γt antisense, 5'-AGATGATGATGAAAGCCAGTT-3' and GAPDH sense, 5'-TGCAGTG GCAAAGTGAGATTGTG-3'; GAPDH antisense, 5'-GGTCTCGCTCCTGG AAGATGGTGAT-3'. PCR efficiency of both the target and reference genes were calculated from the derived slopes of the standard curves.

2.11. Statistical analysis

Statistical analyses were performed using SPSS software, version 17.0. The Student–Newman–Keuls was applied to compare the difference between groups. Data were expressed as mean values ± SD. $P < 0.05$ was considered statistically significant.

3. Results

3.1. IL-17A specific IgG induced by Ag85A-IL-17A in sera

As Fig. 2A showed, recombinant protein Ag85A had a molecular mass of 35 kDa while fusion protein Ag85A-IL-17A had a molecular mass of 50 kDa. Fig. 2B shows 3D structure of recombinant protein Ag85A-IL17A. Fig. 2C shows mice of PBS group, OVA group and OVB group had no IL-17A specific IgG in sera while Ag85A-IL-17A treated mice had high titers of IgG specific to IL-17A in sera. We also

immunized mice ($n = 3$) with recombinant protein Ag85A in the same protocol and found there was no IL-17A specific IgG in sera [19].

3.2. Effect of Ag85A-IL-17A on airway inflammation and cytokine levels in BALF

We examined whether Ag85A-IL-17A had effect on inflammatory cells in BALF (Fig. 3). Total cells, macrophages in OVA group and OVB group after the last challenge were significantly increased in BALF compared with PBS group. Eosinophils in BALF of OVA group were significantly increased compared with PBS group. Neutrophils in BALF of OVB group were significantly increased compared with PBS group. These increases in the numbers of total cells, neutrophils and eosinophils in BALF were significantly declined in Ag85A-IL-17A treated group.

Histological analyses revealed numerous inflammatory cells had infiltrated around the bronchiole and airway epithelium was thickened in OVA group and OVB group compared to PBS group (Fig. 3). However, mice treated with Ag85A-IL-17A showed marked reductions in thickening of the airway epithelium and infiltration of inflammatory cells in peribronchiolar region.

We further examined Th17 and Th2 cytokine levels in BALF (Fig. 4). ELISA showed that levels of IL-17A, IL-6 in BALF were significantly increased in OVB group after the last OVA challenge compared with PBS group and OVA group. The levels of IL-13, IL-4 in BALF were significantly increased in OVA group and OVB group compared with PBS group. The increased levels of IL-17A, IL-6, IL-13, IL-4 in BALF were significantly reduced by administration of Ag85A-IL-17A. It also showed that levels of IL-12p70, IL-10 in BALF were significantly decreased in OVA group and OVB group after the last OVA challenge compared with PBS group. However, administration of Ag85A-IL-17A didn't increase levels of IL-10 and IL-12p70 in BALF.

3.3. Effect of Ag85A-IL-17A on IL-13-producing T cells in vivo and chemokine levels in lung tissues

To investigate whether Ag85A-IL-17A specifically skewed Th2 response in vivo, IL-13 producing T cells of spleen was analyzed. After the last challenge, spleen cells were harvested and proportions of IL-13 producing T cells in them were flow-cytometrically analyzed after being light-scatter gated for lymphocytes (Fig. 5). The population of IL-13 producing T cells increased from 0.010% in PBS group to 0.241% in OVA group and 0.341% in OVB group. Administration of recombinant protein Ag85A-IL-17A significantly reduced IL-13 producing CD3⁺CD4⁺ T cells in spleen.

We further assessed chemokine mRNA levels of CXCL1, CCL2 as well as Th2 transcription factor- GATA-3 and Th17 transcription factor- ROR-γt in lung tissues. Lung tissues of four different groups were harvested

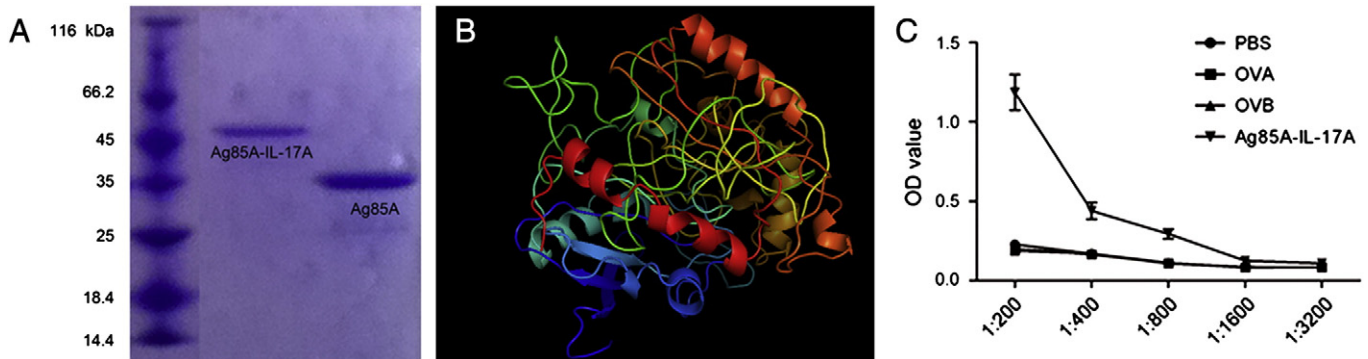


Fig. 2. IL-17A specific IgG induced by Ag85A-IL-17A in sera. (A) After expression in *E. coli* BL21(DE3) cells, recombinant proteins Ag85A, Ag85A-IL-17A were purified and analyzed by SDS-PAGE. (B) The 3D structure of recombinant protein Ag85A-IL-17A. The protein amino acid sequence was sent to Swiss-Model Workspace to get a PDB file and then PyMol CPH software was applied to display the 3D structure of recombinant protein Ag85A-IL-17A. (C) Sera of mice were collected after the last challenge and ELISA was applied to analyze IL-17A specific IgG in sera. Results are presented as mean values ± SD; $n = 8$.

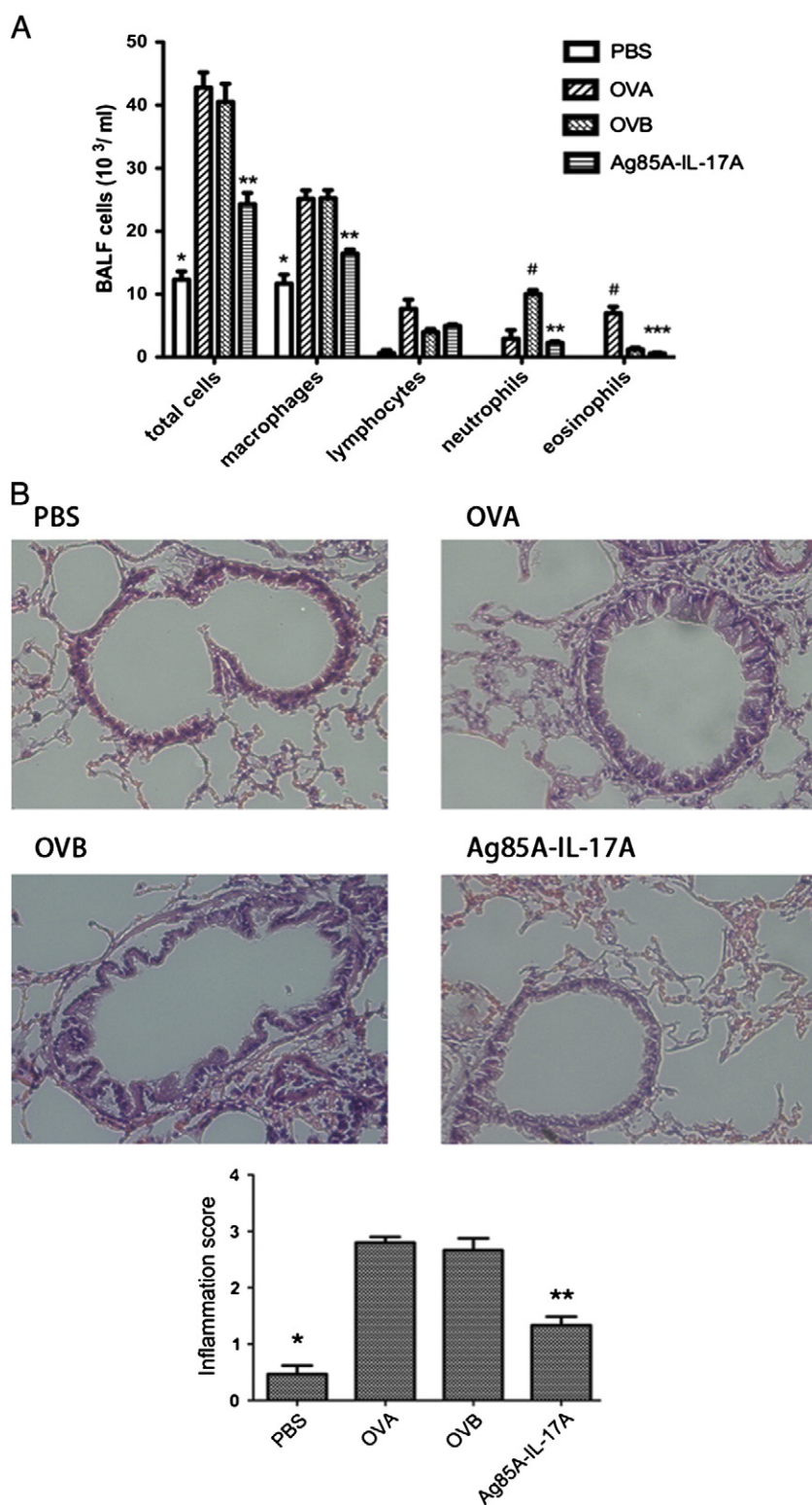


Fig. 3. Effect of Ag85A-IL-17A on airway inflammation. (A) Total cells and differential cellular components were counted after stained with Wright-Giemsa. * $p < 0.05$ vs. OVA, OVB; ** $p < 0.05$ vs. OVB; *** $p < 0.05$ vs. OVA; # $p < 0.05$ vs. PBS. Results are presented as mean values $\pm$ SD; $n = 8$. (B) Lung tissues were fixed, sectioned at 4–5 μ m thicknesses and stained with H&E (magnification $\times 400$). Lung inflammation was measured and defined as average of inflammation scores. * $p < 0.05$ vs. OVA, OVB; ** $p < 0.05$ vs. OVB. Results are presented as mean $\pm$ SD; $n = 8$.

for RT-PCR analysis (Fig. 6). Mice of OVB group and OVA group expressed significantly increased levels of CXCL1 mRNA, CCL2 mRNA and GATA-3 mRNA in lungs compared with PBS group. The administration of Ag85A-IL-17A significantly decreased levels of CXCL1 mRNA, CCL2 mRNA and GATA-3 mRNA in lungs. In contrast, expression of ROR- γ t didn't show any difference in four groups.

4. Discussion

In this study, we evaluated protective effects of mycobacterial Ag85A and IL-17A fusion protein—Ag85A-IL-17A on airway inflammation using an OVA-induced allergic asthmatic murine model. Administration of fusion protein Ag85A-IL-17A induced IL-17A specific autoantibody in

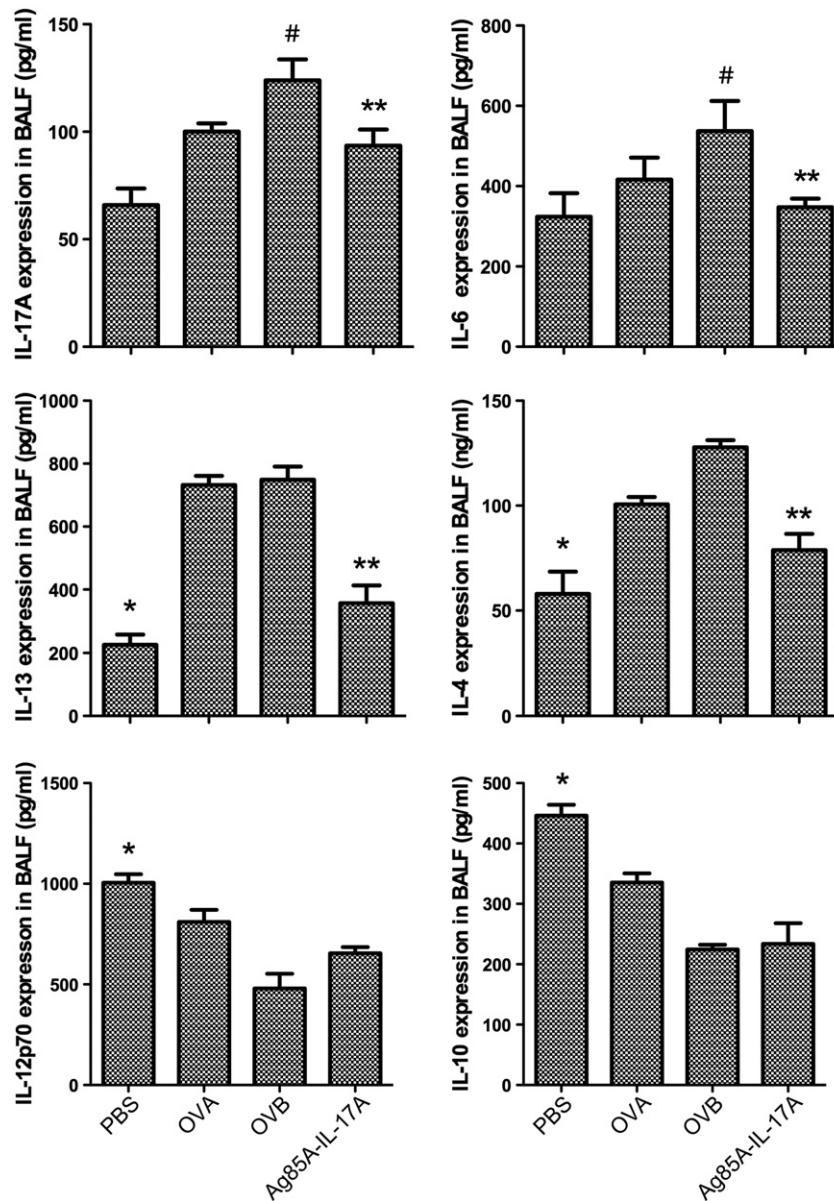


Fig. 4. Effect of Ag85A-IL-17A on cytokine levels in BALF. Levels of IL-17A, IL-6, IL-13, IL-4, IL-12p70 and IL-10 in BALF were detected by ELISA. * $p < 0.05$ vs. OVA,OVB; ** $p < 0.05$ vs. OVB; # $p < 0.05$ vs. PBS. Results are presented as mean values $\pm$ SD; $n = 8$.

sera attenuated OVA-induced inflammatory cell infiltration into lung tissues and reduced Th17 and Th2 cytokine levels in BALF as well as chemokine levels of lung tissues in murine model of allergic asthma.

OVA-induced allergic asthma is recognized as a disease that results from chronic airway inflammation characteristically associated with infiltration of lymphocytes, eosinophils, macrophages and neutrophils into the bronchial lumen [1,22–25]. Similar alterations, including histopathological changes in lung tissues and inflammatory cells in BALF were observed in present study. However, administration of mycobacterial Ag85A and IL-17A fusion protein—Ag85A-IL-17A showed marked anti-inflammatory effect on airway inflammation in the murine model of allergic asthma.

To study the effect of Ag85A-IL-17A on Th17 cytokines in BALF of murine model of allergic asthma, we constructed the murine model characterized with high concentration of IL-17A as well as IL-6 in BALF. We followed the protocol of Peter W. et al, which had one higher dose of OVA nebulization after general OVA sensitization and challenge

of airway [26]. Then we found that increased levels of IL-17A, IL-6 in BALF as well as numbers of neutrophils infiltrated into airways were reduced by administration of mycobacterial Ag85A and IL-17A fusion protein—Ag85A-IL-17A. The suppression of Th17 cytokines, such as IL-17A and IL-6, and neutrophils infiltrated into airways could be explained by the induction of IL-17A specific IgG in sera of mice. This phenomenon verified Marie-Christophe Boissier's speculation that self protein which is associated with foreign protein-carrier could activate T cells and B cells to secrete autoantibodies via Th epitopes of the foreign protein [18]. Under normal conditions, self-reactivity is prevented by a number of processes resulting in immunological tolerance of self-antigens because self cytokine doesn't have T cell epitopes to activate T cell in vivo. Therefore cytokines don't normally induce autoantibody in vivo. When self cytokine carried by foreign protein, the carrier provide T cell epitopes which play prominent role in immunologic reaction of T cell and B cell. If the fusion protein has B cell epitopes, it would then induce B cell secret specific IgG. In our murine model, mycobacterial Ag85A and IL-17A fusion protein—Ag85A-IL-17A may

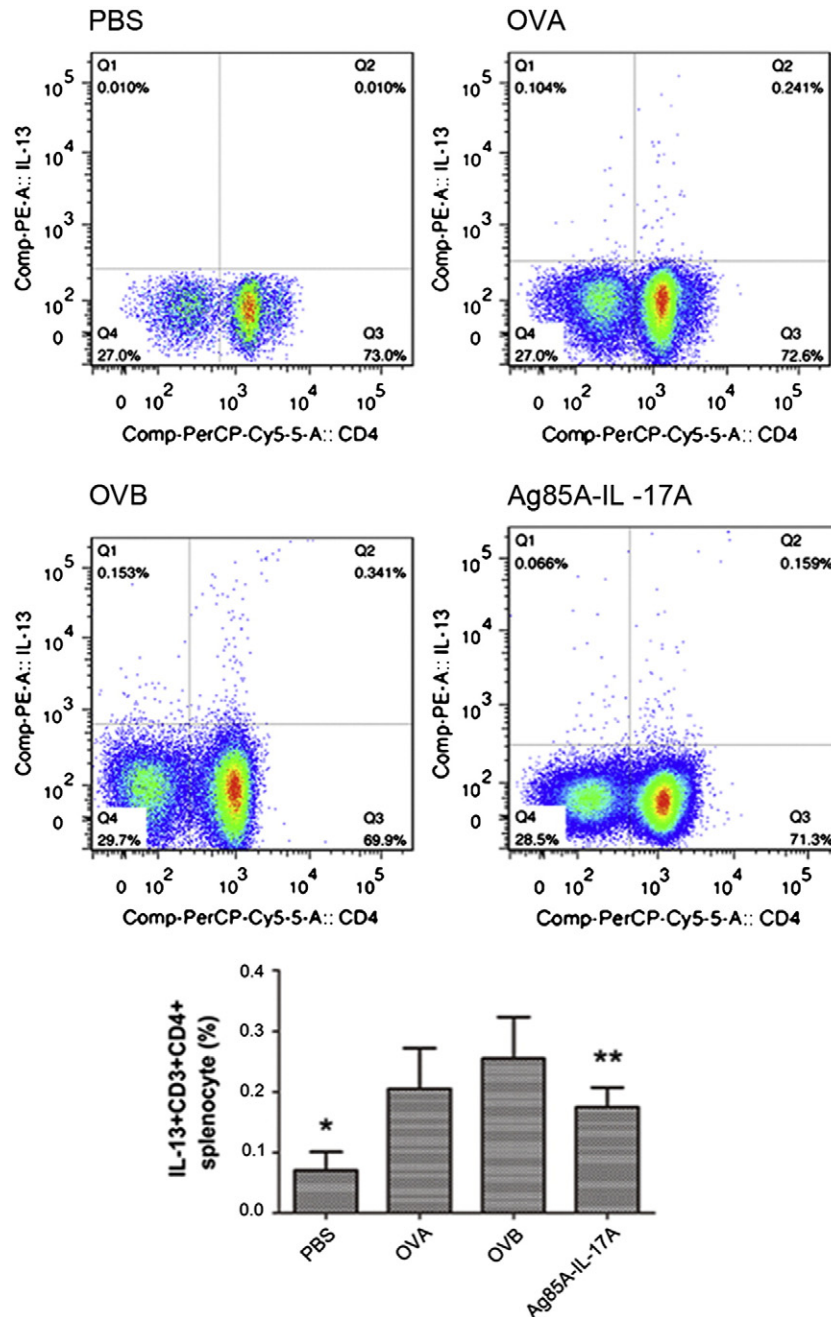


Fig. 5. Effect of Ag85A-IL-17A on IL-13-producing T cells in spleen. After stimulated with OVA for 24 h, spleen cells were permeabilised and incubated with 10 μ g/ml of CD3-APC-Cy7, 10 μ g/ml CD4-FITC and 10 μ g/ml IL-13-PE-Cy7 for 30 min at RT. After washed with PBS, spleen cells were analyzed by FACS. Results are presented as mean $\pm$ SD of two independent experiments. * $p < 0.05$ vs. OVA,OVB. ** $p < 0.05$ vs OVB.

be recognized by antigen presenting cells (APC) because of Th epitopes of Ag85A. Then T cells reacting to Th epitopes deliver helper signals to B cells which stimulates production of autoantibody to IL-17A. IL-17A specific IgG in sera could neutralize IL-17A in BALF which plays an important role for neutrophil infiltration in airways in severe asthma. Therefore, downstream cytokines such as IL-6 in inflammation response is decreased in airways [27,28].

In initiation and progression of asthma, an aberrant Th2-type response to allergens is characterized by overproduction of IL-4, IL-5, and IL-13, which are critical for infiltration of inflammatory cells such as mast cell, eosinophil cells and basophil cells [29]. IL-4 is the Th2 cytokine that is most important for the induction of isotype switching to IgE in B lymphocytes [30]. IL-13 can also regulate recruitment of eosinophils to airways via its various effects on epithelial and smooth

muscle cells [31]. In our experiments, recombinant protein Ag85A-IL-17A induced a reduction in levels of IL-4 and IL-13. However, levels of IL-10, IL-12p70 weren't affected by administration of Ag85A-IL-17A. It showed administration of an IL-17A-blocking antibody could escalate production of Th2 cytokines such as IL-13 in murine asthmatic model [14]. In our study, although there was a high titer of IL-17A autoantibody in sera, we found administration of Ag85A-IL-17A reduced the level of IL-13 in BALF and IL-13⁺ T cell in spleen. This indicated that the anti-inflammatory effect of Ag85A-IL-17A is not only based on the induction of IL-17A specific IgG in sera but also on the suppression of Th2 cytokines. Consistent with the reduction of these inflammatory cytokines, the recruitment of inflammatory cells in BALF, especially of eosinophil cells and neutrophils, was suppressed markedly after the administration of Ag85A-IL-17A.

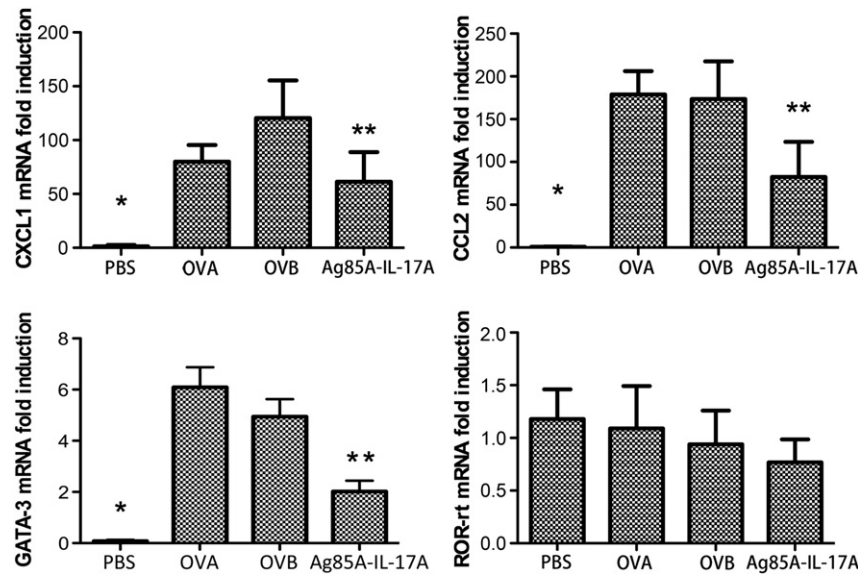


Fig. 6. Effect of Ag85A-IL-17A on chemokine and transcription mRNA levels in lung tissues. RNA was prepared from lung tissues and examined for gene expression by real-time PCR. Results are presented as mean $\pm$ SD of two independent experiments. Hypoxanthine guanine phosphoribosyl transferase (GADPH) was used as an internal control. * $p < 0.05$ vs. OVA, OVB. ** $p < 0.05$ vs OVB.

CXCL1 is a potent chemoattractant for neutrophils and basophils whereas the expression and secretion of CCL2 are well correlated to the infiltration of monocytes, basophils, eosinophils, memory T lymphocytes and natural killer cells [32–34]. Administration of mycobacterial Ag85A and IL-17A fusion protein—Ag85A-IL-17A significantly decreased CXCL-1, CCL2 and GATA-3 mRNA levels in lungs while the level of ROR- γ t mRNA was not affected. ROR- γ t induces transcription of genes encoding IL-17A and related cytokine IL-17 F in naive CD4⁺ T helper cells and is required for their expression in response to IL-6 and TGF- β [35,36]. This finding indicated that administration of Ag85A-IL-17A suppresses Th17 cytokines not through the way of the reduction of transcription factor ROR- γ t but through the neutralization of IL-17A via IL-17A specific IgG. GATA-3 belongs to GATA transcription family and has been shown to promote the secretion of IL-4, IL-5, and IL-13 from Th2 cells [37]. The reduction of GATA-3 mRNA in lung tissues explained the fact that levels of IL-13 and IL-4 in BALF were decreased in Ag85A-IL-17A treated mice. It also showed that suppression of chemokines such as CXCL1 and CCL2 is another possible mechanism that recombinant protein Ag85A-IL-17A attenuated airway inflammation in the murine model of allergic asthma.

In conclusion, administration of mycobacterial Ag85A and IL-17A fusion protein—Ag85A-IL-17A significantly suppressed airways inflammation and it may be associated with the neutralization of IL-17A, reduction of Th17 and Th2 cytokines in BALF and decrease of CXCL-1, CCL2 and GATA-3 mRNA levels in lung tissues. Hence, mycobacterial Ag85A and IL-17A fusion protein—Ag85A-IL-17A may have some protective effects on airway inflammation of allergic asthma.

Acknowledgments

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