



Original article

Allergic asthma accelerates atherosclerosis dependent on Th2 and Th17 in apolipoprotein E deficient mice

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ABSTRACT

Aims. The chronic inflammation of atherosclerosis is regulated by Th1, while allergic asthma is controlled by Th2. The direct relationship between atherosclerosis and asthma is contradictory. The aim of this study was to investigate the role of allergic asthma in atherosclerotic plaque formation and the change of CD4⁺ T cells subsets. **Methods and results.** Six-week C57BL/6J or apoE^{-/-} mice were sensitized on day 0, 7 and 14, then exposed to aerosolized 1% Ovalbumin (OVA) or PBS 30 min/day, 3 times/week for 8 or 16 weeks from day 14 onward. The results showed that allergic asthma mice models were successfully established and the accelerated atherosclerosis induced by allergic asthma accompanied with increased Th2 and Th17 cells but not Th1 cells in spleen. Moreover, the expression and production of Th2 and Th17 biomarkers including IL-4 and IL-17A were significantly elevated in asthmatic apoE^{-/-} mice. After 8-week treated with the neutralizing antibody of IL-4 or IL-17A, the lesion area in the aortic root of asthmatic apoE^{-/-} mice was markedly decreased, and more dramatical result was observed after the combined treatment with IL-4 and IL-17A mAbs. The expression of IgE and FcεR1α in the aortic root of apoE^{-/-} mice was markedly increased but was significantly reduced after 8-week treatment with IL-4 mAb. **Conclusion.** Allergic asthma accelerates atherosclerosis by modulating the balance of Treg/Teff cells in apoE^{-/-} mice, which is associated with increased Th2 and Th17 cells but not Th1 cells.

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1. Introduction

The chronic inflammation of atherosclerosis (AS) and asthma is associated with the activation of CD4⁺ T cells, especially with the balance of CD4⁺ T cell subsets [1]. CD4⁺ T cells recognize their specific antigens and develop into effector T cells (Teffs) or regulatory T-cells (Tregs). The Teffs cells can be divided into distinct functional subsets according to the patterns of cytokine secretion: IFNγ-producing Th1 cells, IL-4-secreting Th2 cells, and IL-17A-secreting Th17 cells, etc. Generally, Teffs are pro-inflammatory and Tregs suppress the activity of autoreactive Teffs, but IL-4, the prototypical Th2 cytokine, can inhibit Th1 response and IFNγ secretion [2]. It is the classical Th1/Th2 yin-yang paradigm that involves in many different inflammatory diseases including atherosclerosis and asthma [3,4].

It is commonly accepted that Th1 is a regulator for the plaque formation [3]. The proliferation of Th1 cells affects the balance of Th1/Th2, and then the anti-inflammatory Tregs are decreased but the pro-inflammatory Th17 is activated, therefore the atherosclerosis is

accelerated. Moreover, our previous studies found Th17/Th1 and Th1 cell subsets were significantly increased in acute coronary syndrome (ACS) patients but Tregs were decreased [5], and pioglitazone (PIO) treatment markedly ameliorated atherosclerosis in apolipoproteins E deficient (apoE^{-/-}) mice by modulating the balance of Tregs/Teffs [6,7]. However, inconsistent with atherosclerosis, antigen-specific allergic asthma is classically thought to be a Th2-driven inflammatory disease [4]. Activated Th2 cells secrete cytokines such as IL-4, IL-5 and IL-13. It has been demonstrated that IL-4 is important for allergic sensitization and Immunoglobulin E (IgE) production, and IgE promotes inflammation progress by binding and activating the FcεR in the macrophage and mast cells [8–11]. Meanwhile, the reduced anti-inflammatory Treg cells and the activated pro-inflammatory Th17 cells also play important roles in asthmatic process [4].

Asthma and atherosclerosis represent different directions of inflammatory responses of CD4⁺ T cells, so we are interested in the influence of asthma on atherosclerosis plaque formation. Some clinical researches showed asthma is a significant risk factor for coronary heart disease (CHD) and stroke after the adjustment for traditional risk factors [12–14], especially in women [13]. However, another study showed that asthma seems to have a protective effect on obesity-related increase in atherosclerosis [15]. Schanen et al. suggested that asthma may be an independent risk factor for incident stroke but not CHD in

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middle aged adults [16]. Thus, the evidence indicates that the direct relationship between atherosclerosis and asthma is still uncertain.

Therefore, in the present study, we used an asthmatic AS animal model (asthmatic apoE^{-/-} mice) to investigate the relationship between atherosclerosis and allergic asthma in mice, and the effects of the balance of CD4⁺ T cell subsets on the atherosclerosis.

2. Material and methods

2.1. Animals

ApoE^{-/-} mice (C57BL/6J background) and C57BL/6J mice were maintained in a temperature- and humidity-controlled facility on a 12 h light–dark cycle, food and water were provided ad libitum. Mice were weaned at 4-week-old and then fed normal food. The experimental protocols were approved by the institutional ethics committee for animal experiments of Xi'an Jiao Tong University. All procedures conformed to the Guide for the Care and Use of Laboratory Animals published by the United States National Institutes of Health.

2.2. Allergen sensitization/challenge protocol

Six to eight-week-old mice were used throughout the study. ApoE^{-/-} and C57BL/6J mice were randomly assigned to the asthma group (n = 6) or control group (n = 6). Sensitization and challenge protocol were performed as previously described [17]. Briefly, the experimental mice were sensitized by intraperitoneal injection (i.p.) of 100 µg OVA (0.2 ml of 500 µg/ml in normal saline) with alum on day 0, 7 and 14, while the control mice received i.p. saline with alum. From day 14 onward, the mice were exposed to aerosolized 1% OVA (allergen exposure) or PBS (sham exposure) 30 min/day, 3 times a week for 8 (early group) or 16 (advanced group) weeks (n = 6 per group). Mice were sacrificed at 24 h after the last challenge.

Asthmatic apoE^{-/-} mice model was similarly prepared according to the above protocol, and was administered i.p. with anti-mouse IL-4 mAb (100 µg; R&D Systems) or IL-17A mAb (100 µg; R&D Systems) or dual antibodies (n = 6 per group), once a week for 8 weeks.

2.3. Bronchoalveolar lavage and lung histopathology

Mice were anesthetized with ketamine through i.p. (60 mg/kg) and the adequacy of anesthesia was continually monitored by assessing reflexes and respiration. After exsanguinations via cardiac puncture, bronchoalveolar lavage fluid (BALF) was collected (0.4 ml saline, three times) by tracheal intubation. The death was confirmed by cervical dislocation. Allergic inflammatory responses were assessed in situ by lung histopathology and ex vivo by eosinophils counts of BALF (experimental details see the supplementary material, Methods).

2.4. Cytokine, antibody and lipid in serum

Blood samples were taken before sacrifice and then collected the serum. Circulating IgE, IFN γ , IL-4 and IL-17 were measured using mouse enzyme-linked immunosorbent assay according to the manufacturers' instructions (IgE kit from Cayman Chemical, others from RayBiotech, Inc). Serum total cholesterol (TC), high-density lipoprotein-cholesterol (HDL-C), triglyceride (TG), and low-density lipoprotein-cholesterol (LDL-C) levels were measured using assay kits according to manufacturers' instructions (LDL-C kit from Rongsheng, others from Dongou, China).

2.5. Quantitative evaluation of atherosclerotic lesions in aortic root

The aortic root was dissected under a microscope and frozen in OCT embedding medium for serial 7 µm cryosectioning that covers 500 µm of the root. Lipids were detected by Oil Red O staining (Sigma). Collagen

was stained with Sirius Red (Sigma). Macrophage content was analyzed by immunofluorescence staining with rat anti-mouse CD68 monoclonal antibody (AbD serotec) followed by a FITC-labeled rabbit anti-rat IgG antibody (Abcam). SMCs and IgE were identified by immunofluorescence staining using FITC-labeled α -actin monoclonal antibody (Sigma) and goat anti-mouse IgE polyclonal antibody (Novus), respectively. Fc ϵ RI α was evaluated by immunohistochemical staining with rabbit anti-mouse polyclonal antibody (Santa Cruz). All sections were photographed with identical exposure settings for each section and all images were quantified using Image-pro plus 6.0 software.

2.6. RNA isolation and quantitative real-time PCR

Total RNA was isolated from the spleen using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. Total RNA was reversely transcribed using a RevertAid™ First Strand cDNA Synthesis Kit (Fermentas), and analyzed by quantitative real-time PCR with a SYBR® Premix Ex Taq™ II RT-PCR Kit (TaKaRa, Japan). All real-time reactions were performed on the iQ5™ Multicolor Real-Time PCR detection system (Bio-Rad, USA). GAPDH was used as housekeeping gene (internal control). The primer sequences are shown in supplementary material, Table 2.

2.7. Flow cytometry

Splenocytes were isolated with a nylon cell strainer, and red blood cells were lysed and subsequently washed. The percentage of CD4⁺CD25⁺Foxp3⁺ Tregs in total splenocyte CD4⁺ T cells was analyzed by using a Mouse Regulatory T Cell Staining Kit (eBioscience, USA) according to the manufacturer's protocol. To analyze Th1, Th2 and Th17 cells, the activated splenocytes were stained with PE-labeled anti-CD4 and IL4, FITC-labeled anti-CD4, IFN γ and IL-17A respectively (all Abs were from eBioscience, the experimental details see supplementary material, Methods). Analysis was carried out on a FACScan flow cytometer with CellQuest software (Becton–Dickinson). Data were analyzed as the percentage of cells with positive staining, calculated the difference between the cellular fluorescence and background non-specific fluorescence.

2.8. Statistical analyses

All data were expressed as means $\pm$ SD. Data were analyzed for normality by using the Shapiro–Wilk normality test. One-way ANOVA was used to determine the statistical significance of the differences among the groups if values followed a normal distribution (otherwise a Kruskal–Wallis H test would be used). A level of $p < 0.05$ was considered significant.

3. Results

3.1. Assessment of asthmatic mice model

Allergic inflammatory responses were assessed in situ by lung histopathology and ex vivo by eosinophils counts of BALF. The results indicate that the modeling of asthmatic mice is successful (see supplementary material, Suppl. Figure).

3.2. Serum lipid among the groups

As shown in Table 1, serum TC and LDL-C were significantly elevated in the asthmatic mice whatever in C57BL/6J or apoE^{-/-} mice ($p < 0.01$). Serum HDL-C was also elevated but TG was reduced in the asthmatic apoE^{-/-} mice ($p < 0.05$) but not in C57BL/6J mice.

Table 1
Serum lipoprotein level (mmol/L, n = 6).

	C57BL/6J			apoE ^{-/-}		
	Control	Asthma	p	Control	Asthma	p
TC	1.72 ± 0.16	1.99 ± 0.21	0.005	8.84 ± 1.54	12.31 ± 2.94	0.005
LDL-C	0.76 ± 0.12	0.92 ± 0.16	0.002	6.67 ± 0.81	7.66 ± 0.87	0.000
TG	1.34 ± 0.31	1.31 ± 0.27	0.86	1.56 ± 0.11	1.14 ± 0.13	0.000
HDL-C	0.62 ± 0.12	0.58 ± 0.09	0.279	2.31 ± 0.29	3.26 ± 0.77	0.000

3.3. Quantification and histological evaluation of atherosclerosis lesions

C57BL/6J mice did not show the atherosclerosis lesion whatever in the early or advanced groups. In apoE^{-/-} mice, fatty streaks and advanced fibrofatty plaques in the aortic root were formed in early groups and advanced groups, respectively (Fig. 1A). In the asthmatic apoE^{-/-}

mice, the fraction area of lesion were markedly increased both in early and advanced groups (Figs. 1A and B; vs. apoE^{-/-} mice, $p < 0.05$). The plaque composition of asthmatic apoE^{-/-} mice also been changed, macrophages content was significantly increased (Figs. 1C and F; vs. apoE^{-/-} mice, $p < 0.05$), and the collagen content was markedly decreased (Figs. 1C and E; vs. apoE^{-/-} mice, $p < 0.05$), the SMCs content was reduced slightly but not significantly (Figs. 1C and D). These results suggest that asthmatic condition increases the atherosclerotic size but decreases the plaque stability in the apoE^{-/-} mice.

3.4. The percentages of Treg and Teff in splenocytes of mice

To investigate the changes of CD4⁺ T cell subsets, we utilized flow cytometry to measure the percentages of Treg, Th1, Th2 and Th17 cells in the splenocytes of mice. Both in the early and advanced groups, the percentages of Th2 and Th17 cells were markedly increased in

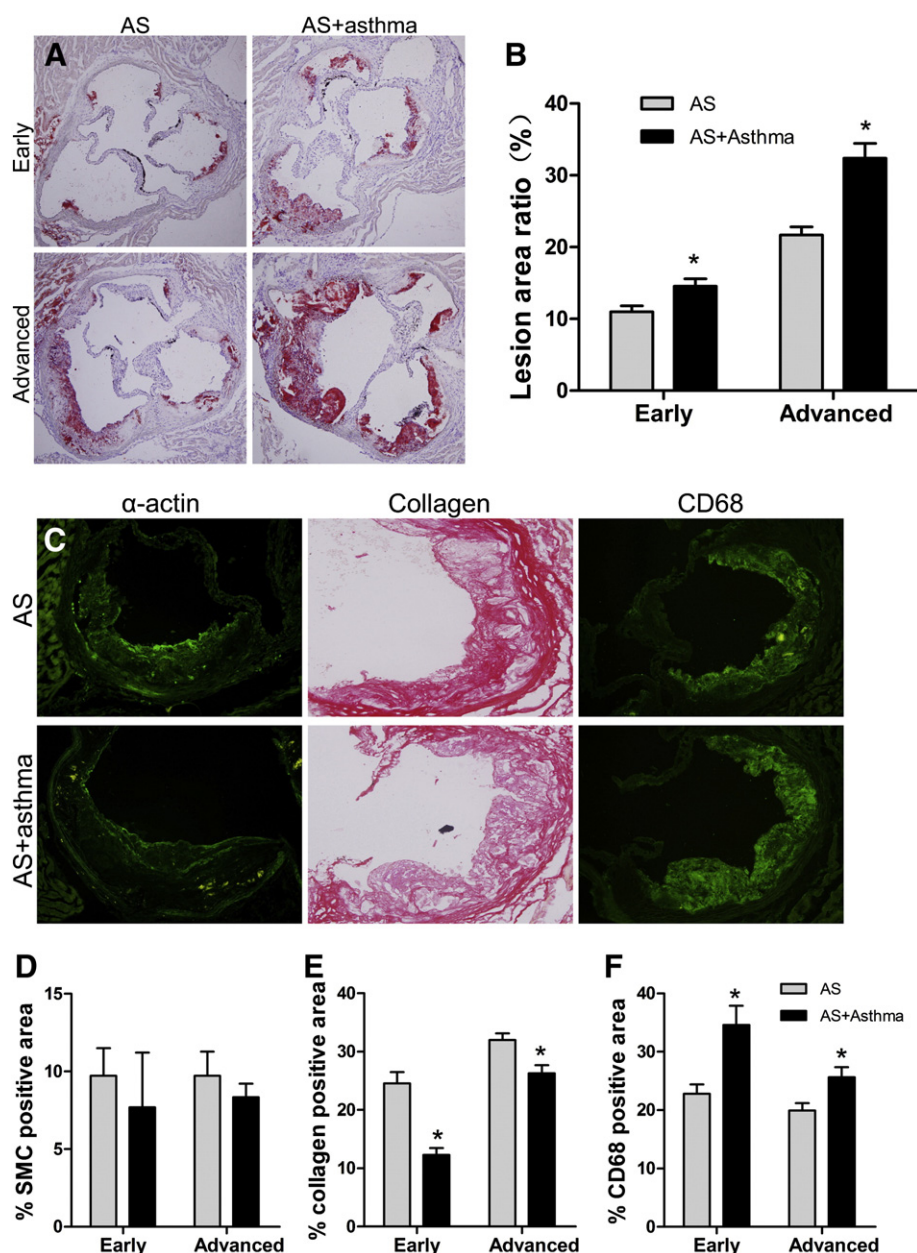


Fig. 1. Effect of allergic asthma on the lesion area and plaque stability in apoE^{-/-} mice. ApoE^{-/-} mice were randomly assigned to AS (apoE^{-/-} mice) and AS + Asthma (asthmatic apoE^{-/-} mice) groups. The fraction area of lesion was measured by Oil-red O Staining (A and B), magnification × 100; α-actin (represented SMCs, C and D) and CD68 (represented macrophages, C and F) content were evaluated by immunofluorescence staining; The collagen (C and E) content was assessed by Sirius Red Staining; magnification × 200; Data are represented as means ± SD (n = 6). * $p < 0.05$ vs AS groups.

asthmatic mice (Figs. 2A, B, D and E; vs. non-asthmatic mice, $p < 0.05$), but Th1 cells were not affected significantly (Fig. 2C). The percentage of Treg cells in the splenocytes showed a different tendency between early and advanced groups. In the early group, Treg cells were significantly decreased in asthmatic mice (Figs. 3A and C; vs. non-asthmatic mice, $p < 0.05$); however, in the advanced group, Treg cells were markedly increased in the asthmatic apoE^{-/-} mice but not C57BL/6J mice (Figs. 3A and C; vs. apoE^{-/-} mice, $p < 0.05$).

3.5. The gene expression of Treg and T_H1 in the splenocytes of mice

Consistent with the percentage of CD4⁺ T cells in the spleen, mRNA expression of the prototypic CD4⁺ T cell-related cytokines in the spleen was also changed, IL-4 (Th2 related cytokine) and IL-17A (Th17 related cytokine) were significantly increased in the asthmatic mice (Figs. 4D and F; vs. non-asthmatic mice, $p < 0.05$), but IFN γ (Th1 related cytokine) was not changed significantly (Fig. 4B).

The mRNA expression of prototypic transcription factors in CD4⁺ T cells in the spleen exhibited a different tendency, in asthmatic mice, Foxp3 (represented Treg) and GATA3 (represented Th2) were markedly reduced (Figs. 3B and 4C; vs. non-asthmatic mice, $p < 0.05$), but ROR γ t (represented Th17) was slightly decreased (Fig. 4E) and Tbet (represent Th1) was not changed significantly in the early and advanced groups (Fig. 4A).

In addition, the mRNA expression of IFN- γ and Tbet was significantly elevated in the spleen of apoE^{-/-} mice (Figs. 4A and B, vs. C57BL/6J mice, $p < 0.05$).

3.6. Serum IL-4 and IL-17 levels are enhanced in asthmatic mice

The serum concentrations of IFN γ , IL-4 and IL-17 were measured by ELISA. The serum concentrations of IL-4 and IL-17 in asthmatic mice were significantly elevated in the early and advanced groups (Figs. 4G and H; vs. non-asthmatic mice, $p < 0.05$). Whereas the serum concentration of IFN γ was not significantly changed between the asthma and control groups (data not shown).

3.7. Reduced atherosclerosis in asthmatic mice injected with neutralizing antibody

We treated the asthmatic apoE^{-/-} mice using anti-mouse IL-4 mAb or IL-17A mAb or dual antibodies, respectively. After 8 week treatment, we observed the lesion area in the aortic root of mice was markedly decreased in both IL-4 mAb (Fig. 5C; $p = 0.033$) and IL-17A mAb treated groups (Fig. 5C; $p = 0.027$), but the most significant effect was found after treatment with dual antibodies (Fig. 5C; $p < 0.001$).

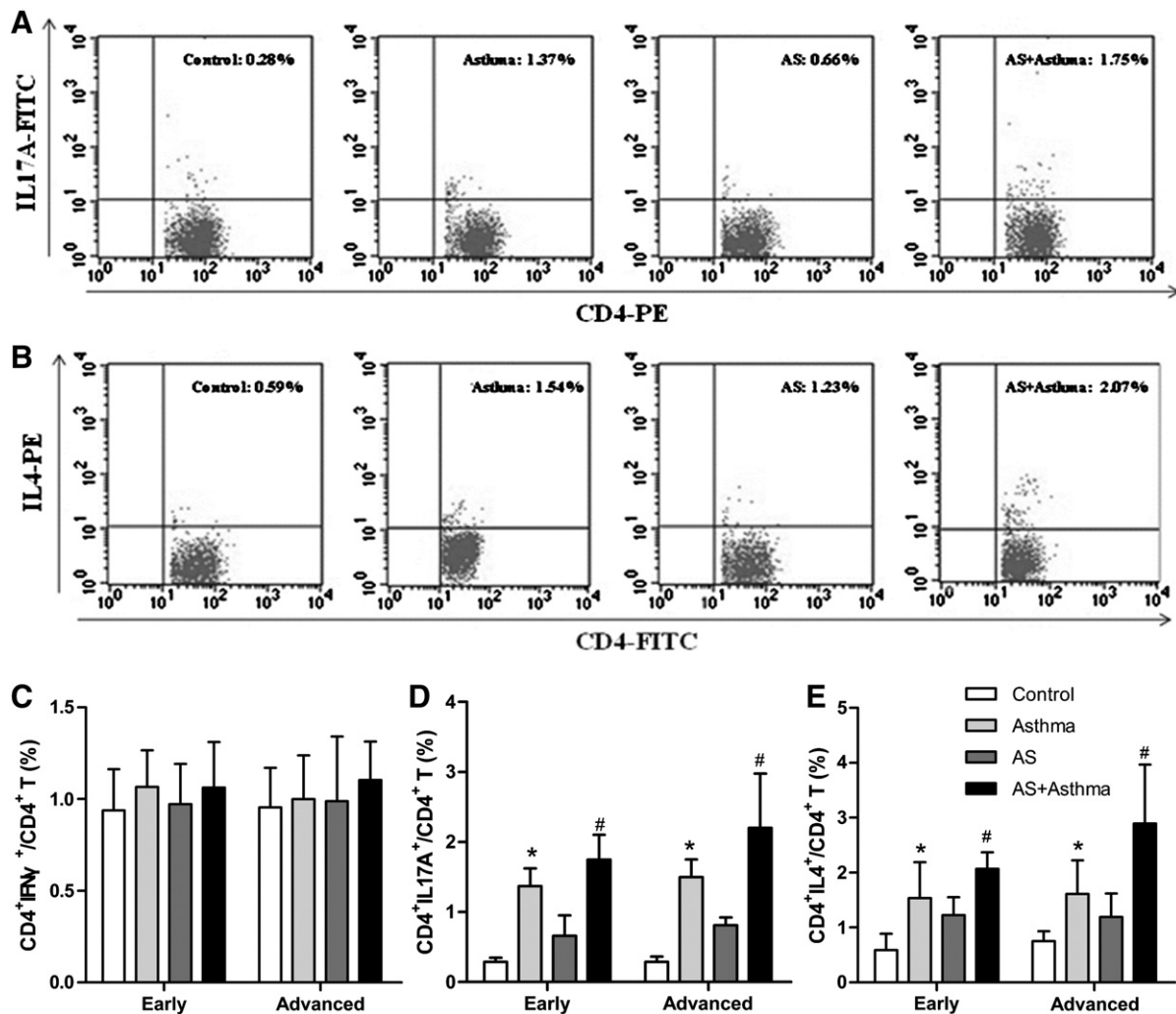


Fig. 2. Effect of allergic asthma on the contents of Tregs in the spleen. Mice were randomly assigned to four groups: Control (C57BL/6J mice), Asthma (asthmatic C57BL/6J mice), AS (apoE^{-/-} mice) and AS + Asthma (asthmatic apoE^{-/-} mice). (A) Representative FACS results of Th17 from single mouse in four groups. The percentage of positive cells is shown in each panel. (B) Representative FACS results of Th2 from single mouse in four groups. The percentage of positive cells is shown in each panel. Comparisons of Th1 (C), Th17 (D) and Th2 (E) contents among four groups. Data are represented as means $\pm$ SD ($n = 6$). * $p < 0.05$ vs control group, # $p < 0.05$ vs AS groups.

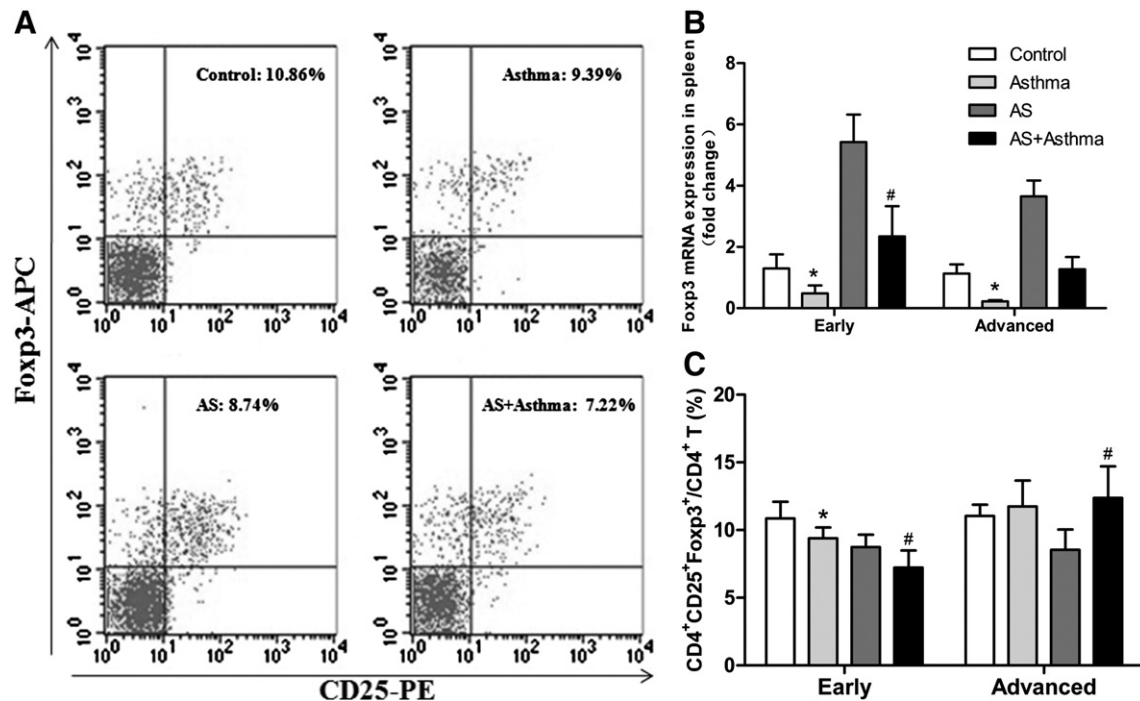


Fig. 3. Effect of allergic asthma on the contents of Tregs in the spleen. (A) Representative FACS results from single mouse in four groups (gated by CD4⁺ T cells). The percentage of positive cells is shown in each panel. (B) The mRNA expression of Foxp3 (prototypical transcription factors of Treg) in four groups. (C) Comparison of the Treg content among four groups. Data are represented as means $\pm$ SD (n = 6). * $p < 0.05$ vs control group, # $p < 0.05$ vs AS groups.

3.8. Decreased local IgE and FcεRIα expression and serum IgE levels in asthmatic apoE^{-/-} mice treated with IL-4 mAb

In the aortic root of asthmatic apoE^{-/-} mice, IgE and FcεRIα expression were markedly increased, but were significantly reduced after 8 week treatment with IL-4 mAb (Figs. 5A and B; $p < 0.05$). Similarly, serum IgE level was enhanced in asthmatic mice, but was reduced after 8 week treatment with IL-4 mAb (Fig. 5D; $p < 0.001$). We also measured several macrophage related inflammatory factors MCP-1, MIP1α and IL-6 mRNA expression in asthmatic apoE^{-/-} mice spleen. Compared with apoE^{-/-} mice, all of them were significantly elevated, but were significantly reduced after 8 week treatment with IL-4 mAb (Fig. 5E; $p < 0.05$).

4. Discussion

Although previous studies demonstrated that Th1/Th2 yin-yang modulated the progress of atherosclerosis, and the chronic inflammation of atherosclerosis is Th1 dominant [3,5,6]. Our present study clearly showed that allergic asthma accelerates atherosclerosis by modulating the balance of Teff/Treg cells in apoE^{-/-} mice, which is associated with increased Th2 and Th17 cells but not Th1 cells.

The chronic inflammation of atherosclerosis is regulated by Th1 [3], but asthma is controlled by Th2 [4]. Asthma and atherosclerosis thus represent different directions of inflammatory responses of CD4⁺ T cells, and the direct relationship between atherosclerosis and asthma is contradictory. In the present study, we used an asthmatic mice model to investigate the effect of asthma on atherosclerosis plaque formation. Our finding showed that allergic asthma increased the plaque size and decreased the stability of plaques in the apoE^{-/-} mice.

Studies on the roles of different CD4⁺ T-cell subsets showed that deficiency of Tregs accelerates the progression of atherosclerotic plaques [18]. Our group also found that the suppression of atherosclerosis lesion in apoE^{-/-} mice by PIO treatment was associated with the activated

Tregs [6,7], and Treg cells were significantly decreased in ACS patients [5]. Additionally, in the airway of children with asthma, the percentage of Treg cells is lower than that in healthy children [19]. Transfusion of Treg cells ameliorates the development of airway inflammation and prevents the allergen-induced activation of DCs in the airway [20,21]. In the present study, accompanied by accelerated atherogenesis, the percentage of Tregs was markedly decreased in asthmatic mice from early group, but increased in asthmatic apoE^{-/-} mice from advanced group. It could be a homeostasis between anti-inflammatory and pro-inflammatory processes. The reduced Tregs in asthmatic mice from early groups may be due to the deterioration of disease, while the increased Tregs in asthmatic apoE^{-/-} mice from advanced groups may be caused by the anti-inflammatory epiphenomenon against the aggravated disease. These results suggest that the aggravated atherosclerosis in asthmatic mice may be associated with the change of Tregs. In addition, the Foxp3 mRNA expression was markedly decreased in the spleen of asthmatic mice, it may be due to the feedback regulation and different time points for detection and the mRNA may start to degrade when the level of protein reaches the peak.

Th17 cells, a new subset of IL-17-producing T cells with effector functions, are distinct from the classic Th1 and Th2 cells [22]. Many recent studies reported that Th17 cells can be isolated from the atherosclerotic plaques and blockade of IL-17A may alleviate atherosclerosis [23,24]. Our previous studies also found Th17 cells are significantly increased in ACS patients [5]. Furthermore, IL-17A is upregulated in the lung of asthma patients and correlates with disease severity [25,26], and abrogation of IL-17A function during allergic sensitization is protective in mouse asthma models [27]. Similarly, Th2 cells are present in the lungs of almost all asthma patients, particularly patients with allergic asthma [28]. Th2 cells produce cytokines such as IL-4 that regulates the allergen-specific synthesis of IgE [29], IgE up-regulates and binds to the FcεRI on effector cells [30–32], and thus develop progressive allergic inflammation. During the development of atherosclerosis, the roles of Th2 cells and IL-4 appear to be more multifaceted. Some studies have shown that IL-4 has a protective effect, whereas others found that absence of IL-4 reduces disease [33–35]. In the present study, we

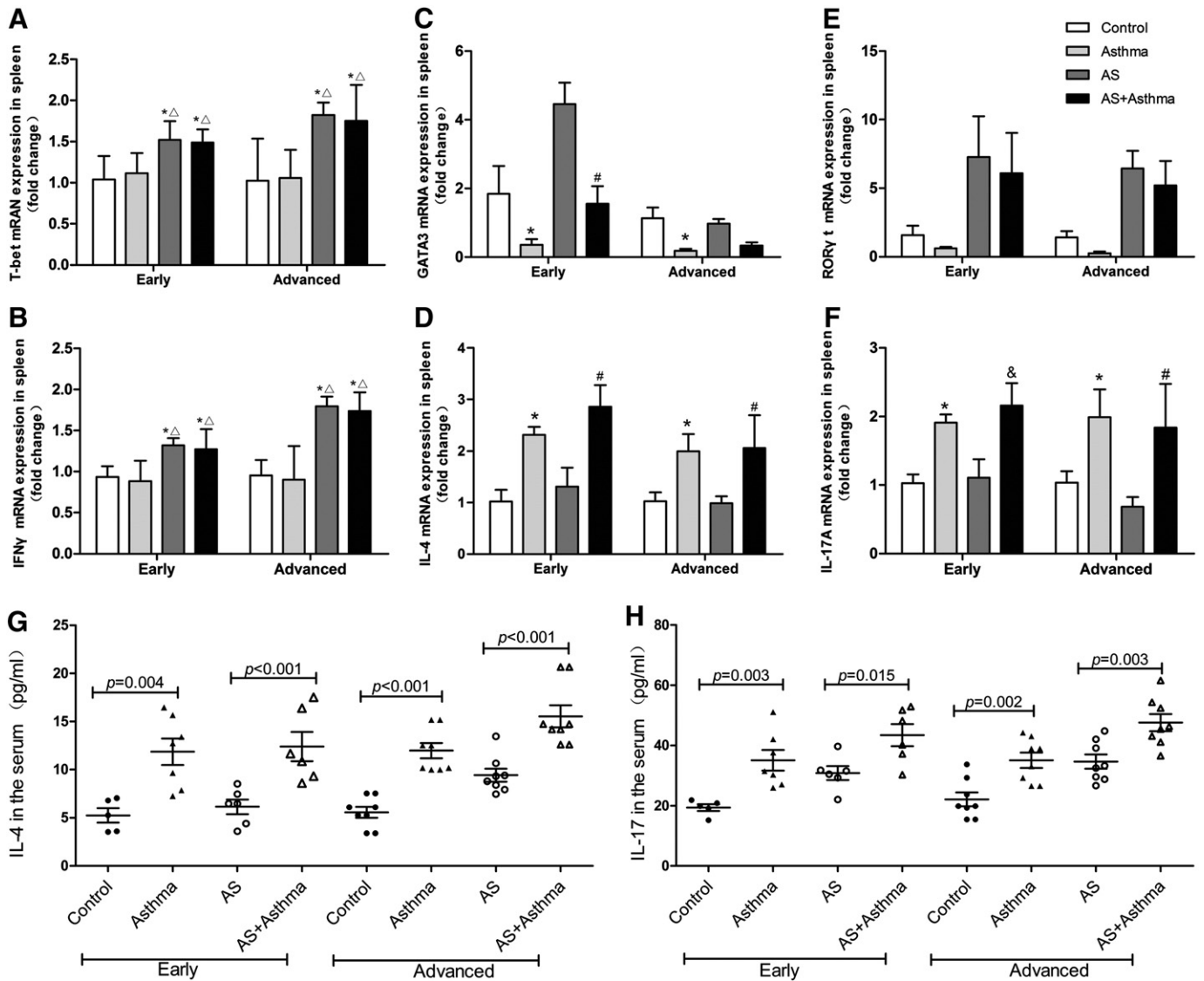


Fig. 4. Effect of allergic asthma on cytokine production and transcription factors expression of Tregs. Relative mRNA expression of Tregs in the spleen was measured by RT-PCR. IFN γ and Tbet represent Th1 (A and B), IL-4 and GATA3 represent Th2 (C and D), IL-17A and ROR γ t represent Th17 (E and F). The serum concentrations of IL-4 and IL-17 were measured by ELISA (G and H). Data are represented as means $\pm$ SD ($n = 6$). * $p < 0.05$ vs control group, # $p < 0.05$ vs AS groups, $\Delta p < 0.05$ vs asthma groups, & $p = 0.074$ vs AS groups.

observed that the contents of Th17 and Th2 cells and their relative cytokine mRNA expression were markedly increased in the spleen of asthmatic mice accompanied by aggravated atherosclerosis, and abrogation of IL-17A or IL-4 function in asthmatic apoE $^{-/-}$ mice obviously decreased the lesion area in the aortic root, suggesting that the aggravated atherosclerosis in asthmatic mice is associated with the increased Th17 and Th2 subsets. Interestingly, we noted that the subsets of Th2 and Th17 cells were increased in the spleen of asthmatic mice, but their prototypical transcription factors, GATA 3 was significantly reduced and ROR γ t was slightly decreased. We considered it may be due to the feedback regulation and different time points for detection.

IL-4 could induce IgE production, and IgE promotes inflammation progress by binding and activating the Fc ϵ R in the macrophage and mast cells [8–10]. Furthermore, activated Fc ϵ R1 affects IFN- γ -mediated proinflammatory and antiinflammatory cytokine production in dendritic cells [36]. Wang et al. reported that IgE and the Fc ϵ R α were present in human atherosclerotic lesions and localized particularly to macrophage-enriched areas. They also demonstrated that the absence of Fc ϵ R α reduced inflammation and apoptosis in atherosclerotic

plaques and reduced the burden of disease in mice [11]. Hence, it is a logical speculation that increased Th2 cells and IL-4 expression stimulate the synthesis of IgE, then IgE promotes inflammation progress through up-regulating and binding to the Fc ϵ R1 in the macrophages and mast cells. To investigate the hypothesis, we detected the expression of IgE and Fc ϵ R α in the aortic root of apoE $^{-/-}$ mice, and their expression levels were markedly increased in the aortic root of asthmatic apoE $^{-/-}$ mice, but were significantly reduced after 8 weeks treatment with IL-4 mAb. We also measured the macrophage related inflammatory factors expression, and the level of MCP-1, MIP1 α and IL-6 mRNA expression in asthmatic mice spleen were significantly elevated, but were significantly reduced after 8 weeks treatment with IL-4 mAb. It is the possible mechanism for accelerated atherosclerosis in allergic asthmatic mice.

In this study, we did not observe obvious association between Th1 cells and atherosclerosis size in asthmatic mice, suggesting Th1 cells may not be involved in the process of aggravated atherosclerosis because of asthma. However, the mRNA expression of IFN- γ (Th1 related cytokines) and Tbet (Th1 prototypical transcription factors) was

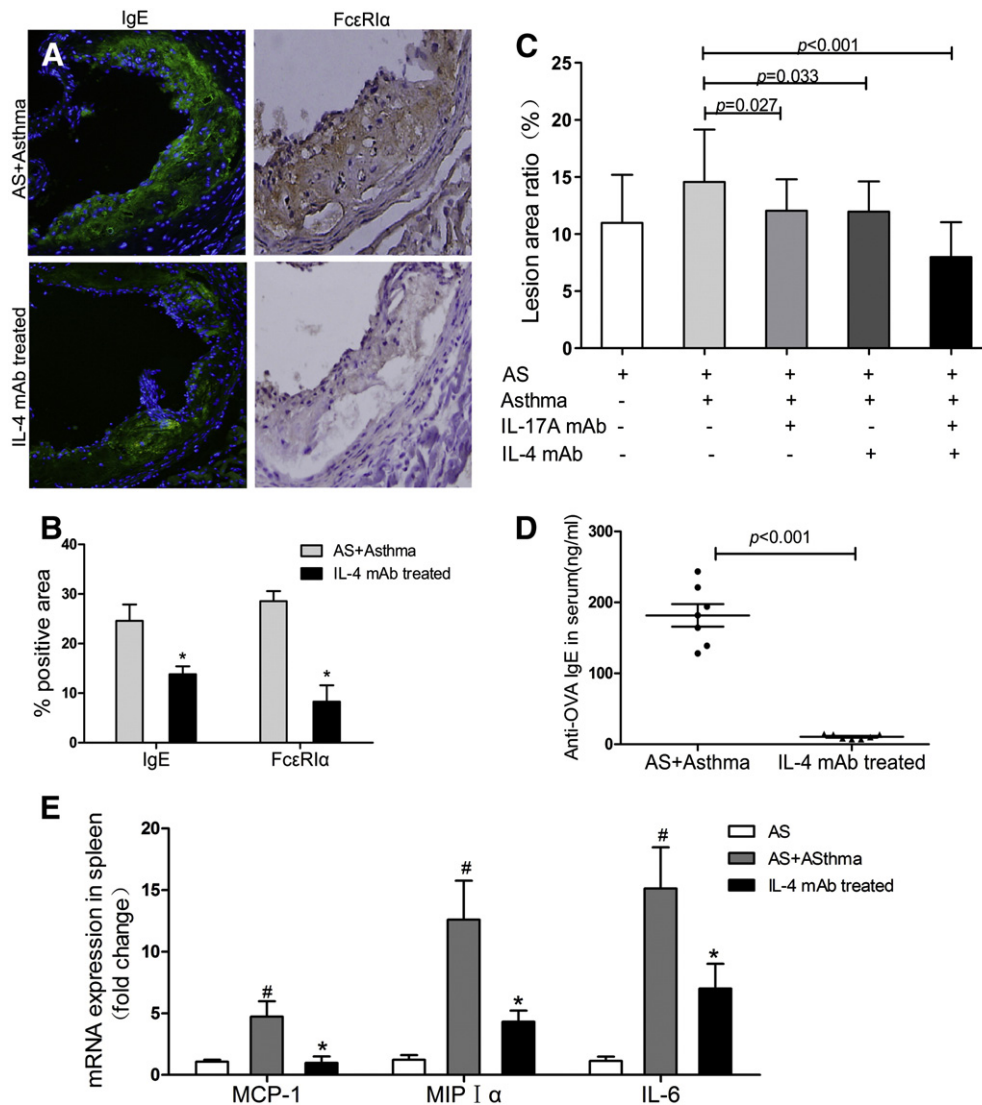


Fig. 5. Effect of neutralizing antibodies on the treatment of lesion in asthmatic apoE^{-/-} mice. Asthmatic apoE^{-/-} mice were administered i.p. with IL-4 mAb or IL-17A mAb or dual antibodies for 8 weeks. (A and B) IgE and FcεRIα expression in the aortic root were detected by Immunohistochemistry and Immunofluorescence Staining respectively, magnification ×200. (C) The lesion area in the aortic root of asthmatic apoE^{-/-} mice treated with different neutralizing antibodies. (D) The serum concentration of IgE in asthmatic apoE^{-/-} mice treated by IL-4 mAb; (E) Macrophage related inflammatory factors MCP-1, MIP1α and IL-6 mRNA expression in the spleen was measured by RT-PCR. Data are represented as means ± SD (n = 6). * p < 0.05 vs AS + Asthma groups, # p < 0.05 vs AS groups.

significantly increased in the spleen of apoE^{-/-} mice than that in C57BL/6J mice, and the percentage of Th1 in splenocytes also showed an increased tendency in apoE^{-/-} mice compared with C57BL/6J mice, suggesting the chronic inflammation of atherosclerosis is Th1 dominant. We observed that the level of serum lipid was significantly elevated in the asthmatic apoE^{-/-} mice. But after IL4 mAb or IL17A mAb treatment for 8 weeks, accompanied by ameliorated atherogenesis, the elevated serum lipid was not affected, suggesting that allergic asthma accelerates atherosclerosis is independent on the lipid profile.

A few limitations of this study should be considered. First, this model (sensitization with ovalbumin) only explored one pathway in asthma (the allergen-driven pathway). Allergic asthma is a heterogeneous inflammatory disease, the model can not reflect all forms of asthma or replace clinical studies, but can represent the features of allergic asthma in humans. Second, to abrogate the functions of Th2 and Th17, neutralizing antibody treatment was used in the present study, but it is more convincing to use knockout animal models.

In summary, allergic asthma increased the atherosclerotic plaque size and decreased the stability of plaques in apoE^{-/-} mice, which is associated with the increased Th2 and Th17 cells. The present study

provides evidence that allergic asthma accelerates atherosclerosis dependent on Th2 and Th17 cells but not Th1 cells in apoE^{-/-} mice.

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Conflict of interests

The authors declare no conflicts of interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.jmcc.2014.02.005>.

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