

# Therapeutic potential of anti-IL-1 $\beta$ IgY in guinea pigs with allergic asthma induced by ovalbumin

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## ABSTRACT

**Background:** Interleukin-1 beta (IL-1 $\beta$ ) plays pivotal roles in the progression of allergic airway inflammation. This study aims to determine whether the blockade of IL-1 $\beta$  can inhibit airway inflammation in guinea pigs with allergic asthma induced by the inhalation of aerosolized ovalbumin (OVA).

**Methods:** Healthy guinea pigs treated with saline were used as normal controls (group C). The guinea pigs with allergic asthma induced by the inhalation of aerosolized OVA were randomly divided into three groups: (1) the M group containing negative control animals treated with saline; (2) the Z<sub>1</sub> group containing animals treated by the inhalation of atomized 0.1% anti-IL-1 $\beta$  immunoglobulin yolk (IgY); and (3) the Z<sub>2</sub> group containing positive control animals that were treated with budesonide. The inflammatory cells in the peripheral blood (PB) and bronchoalveolar lavage fluid (BALF) were evaluated using methylene blue and eosin staining. Cytokine concentrations were measured using an enzyme-linked immunosorbent assay. Pulmonary sections were examined using hematoxylin-eosin staining.

**Results:** Allergic inflammation and damage to the pulmonary tissues were decreased in the Z<sub>1</sub> group compared to the M group. Eosinophils and neutrophils in the PB and BALF were significantly decreased in the Z<sub>1</sub> group compared to the M group ( $P < 0.05$ ). Treatment with anti-IL-1 $\beta$  IgY significantly reduced the levels of IL-1 $\beta$ , IL-4, IL-8, IL-13, TNF- $\alpha$ , TGF- $\beta$ <sub>1</sub> and IgE in the BALF ( $P < 0.05$ ).

**Conclusion:** The inhalation of aerosolized anti-IL-1 $\beta$  IgY inhibits pathological responses in the pulmonary tissues of guinea pigs with allergic asthma. The inhibitory activity may be due to the decrease in the numbers of eosinophils and neutrophils and the reduced levels of inflammatory cytokines and IgE in the PB and BALF.

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## 1. Introduction

Allergic asthma is an inflammatory disease of the airways. T helper type 2 (Th2) cells and proinflammatory cytokines play an important role in the development and progression of airway hyper-responsiveness (AHR), mucus production, infiltration of eosinophils, neutrophils and lymphocytes and airway remodeling.

It has been shown that the levels of eosinophils, neutrophils, lymphocytes and cytokines, such as IL-4, IL-5, IL-13, TNF- $\alpha$  and TGF- $\beta$ <sub>1</sub>, and IgE, are increased in the peripheral blood (PB), bronchoalveolar lavage fluid (BALF) and bronchi and pulmonary tissues in patients with allergic asthma (Holgate et al., 2009; Brown et al., 2012; Manuyakorn et al., 2008) as well as ovalbumin (OVA)-sensitized guinea pigs (Franova et al., 2013; Toward and Broadley, 2004; Mahajan and Mehta, 2011; Chang et al., 2011), rats and mice (Kang et al., 2010; Yadav et al., 2011).

Although Th2 cytokines have been shown to be the main drivers of allergic asthma, treatment with anti-IL-4, anti-IL-13 or combined IL-4/IL-13 antagonism (Antoniu, 2009) has been found to have limited efficacy in clinical studies and in animal models of allergy-related asthma. Recent studies have shown that the progression of airway inflammation is significantly improved in patients with allergic asthma and in OVA-sensitized/challenged mice when anti-TNF- $\alpha$  antibody (infliximab), soluble TNF- $\alpha$  receptor (sTNFR) and TNF- $\alpha$  antagonists were administered intravenously and

**Abbreviations:** AHR, airway hyperresponsiveness; BALF, bronchoalveolar lavage fluid; HE, hematoxylin-eosin; IgE, immunoglobulin E; IgY, immunoglobulin yolk; IL-1 $\beta$ , interleukin-1beta; IL-1ra, IL-1 receptor antagonist; IL-4, interleukin-4; IL-8, interleukin-8; IL-13, interleukin-13; Infliximab, anti-TNF- $\alpha$  antibody; Omalizumab, anti-IgE antibody; OVA, ovalbumin; PB, peripheral blood; sRaw, specific airway resistance; sTNFR, soluble TNF- $\alpha$  receptor; TGF- $\beta$ <sub>1</sub>, transforming growth factor-beta 1; Th2, T helper type 2; TNF- $\alpha$ , tumor necrosis factor-alpha.

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intraperitoneally (Deveci et al., 2008; Nam et al., 2009; Berry et al., 2006). However, anti-TNF- $\alpha$  therapies in asthma might be limited by the unfavorable risk/benefit ratio associated with their long-term use (Antoniou, 2009). The proinflammatory cytokine IL-1 $\beta$  is an important inducer of the pathological progression of allergic asthma. Mature IL-1 $\beta$  creates a proinflammatory milieu that produces airway inflammatory responses (Besnard et al., 2012; Lappalainen et al., 2005). In OVA-sensitized rats, the intratracheal administration of IL-1 $\beta$  has been shown to significantly increase AHR, bradykinin and neutrophil counts in the BALF (Tsukagoshi et al., 1994). However, Li et al. demonstrated that the asthmatic symptoms in guinea pigs were obviously attenuated after administration of rhIL-1ra solution via ultrasonic spraying (Li et al., 2006).

In the current study, we used the inhalation of aerosolized anti-IL-1 $\beta$  IgY to treat allergic asthma in guinea pigs induced by aerosolized OVA inhalation. We have investigated the curative effect of anti-IL-1 $\beta$  IgY and explored the potential mechanisms. Our studies provide strong experimental evidence that supports a novel therapeutic strategy against allergic asthma, which may avoid the adverse reactions associated with systemic therapy and alleviate the pathological inflammatory response.

## 2. Materials and methods

### 2.1. Animals

Hartley guinea pigs (male, 7 weeks old, 230 g  $\pm$  40 g) were purchased from Changsha City Kaifu District Dongchuang Animal Science and Technology Service (Production license SCXK (Xiang) 2009-0012, Changsha, China). The experimental studies in guinea pigs were performed in accordance with animal experiment guidelines established by The Ministry of Science and Technology of the People's Republic of China. The animal experimental procedures have been approved by the Jiangxi Province People's Hospital Ethics Committee.

### 2.2. Establishment of a guinea pig model of allergic asthma and experimental groups

After adaptation for 7 days, the guinea pigs were divided into a healthy control (group C) ( $n=15$ ) and allergic asthma groups. The guinea pig model of allergic asthma was established with 1.0% OVA in saline (Grade II, Sigma, USA), which was atomized by ultrasonic atomization every other day (days 1, 3, 5 and 7) for a total of four times. Each administration delivered 20 mL over 10 min. In group C, the guinea pigs were administered only atomized saline. After a 1-week rest period, the guinea pigs were administered atomized 1.5% OVA in saline (20 mL over 10 min) every day from day 15 to day 25 for a total of eleven times in the allergic asthma groups. In group C, the guinea pigs were administered only atomized saline. A total of 48 guinea pigs with allergic asthma were randomly divided into three groups. (1) A negative control group (the allergic asthma model group, group M) ( $n=16$ ) was treated with 0.9% saline. (2) The 0.1% anti-IL-1 $\beta$  IgY treatment group (group Z<sub>1</sub>) ( $n=16$ ) was treated with 0.1% anti-IL-1 $\beta$  IgY (prepared in the laboratory, purity 85%, valence combined recombinant human IL-1 $\beta$ : 1:3200 mg<sup>-1</sup> protein). Laying hens were inoculated with recombinant human IL-1 $\beta$ . Anti-IL-1 $\beta$  IgY was extracted from the yolk using the water dilution method and purified by salt fractionation. The purity of the IgY was determined by nonreducing SDS-polyacrylamide gel electrophoresis. The valence of anti-IL-1 $\beta$  IgY was determined by an enzyme-linked immunosorbent assay (ELISA) using 1 mg of purified IgY. (3) The positive control group (the budesonide suspension treatment group, group Z<sub>2</sub>) ( $n=16$ ) was treated with a budesonide suspension (1 mg/2 mL, AstraZeneca Pty Ltd.). Beginning on the 21st

day after challenge (on the seventh day to the eleventh day in the challenge phase), the asthmatic guinea pigs in the allergic asthma model group (group M) received atomized 0.9% saline, those in the 0.1% anti-IL-1 $\beta$  IgY treatment group (group Z<sub>1</sub>) received 0.1% anti-IL-1 $\beta$  IgY and those in the budesonide suspension treatment group (group Z<sub>2</sub>) received budesonide for 5 days. Each group received 20 mL over 10 min.

### 2.3. Plasma and BALF collection

Plasma and BALF were collected according to standard procedures (Nam et al., 2009). In brief, the guinea pigs were anesthetized using 10% chloral hydrate via intraperitoneal injection. Each heart was exposed and heart blood was withdrawn using a 5.0-mL syringe with EDTA. Blood was centrifuged and plasma was stored at  $-80^{\circ}\text{C}$ . The left trachea was exposed and cannulated with silicone tubing attached to a 23-gauge needle on a 5.0-mL syringe. After instillation of 2.0 mL of sterile PBS through the trachea into the lung, BALF was withdrawn and centrifuged. Supernatants were stored at  $-80^{\circ}\text{C}$ . Plasma was collected at 2 h (there were three guinea pigs in group C and four guinea pigs each in groups M, Z<sub>1</sub> and Z<sub>2</sub>), 4 h (there were four guinea pigs in each group), 8 h (there were four guinea pigs in each group) and 24 h (there were four guinea pigs in each group) in random order. BALF was collected at 2 h (there were three guinea pigs in group C and four guinea pigs each in groups M, Z<sub>1</sub> and Z<sub>2</sub>), 4 h (there were three guinea pigs in group C and four guinea pigs each in groups M, Z<sub>1</sub> and Z<sub>2</sub>), 8 h (there were four guinea pigs in each group) and 24 h (there were three guinea pigs in the Z<sub>1</sub> group and four guinea pigs in groups C, M and Z<sub>2</sub>) in random order.

### 2.4. Cell staining

Drops of the blood and precipitated cells in the BALF were placed onto a slide and smeared. After drying at room temperature, methylene blue was dripped onto the slide. The cells were fixed and stained for 1–2 min, and eosin was dripped onto the slide and the cells were stained for an additional 10 min. The slide was then rinsed with distilled water. More than 200 cells were randomly counted in a high power field, and eosinophils, neutrophils, lymphocytes, macrophages, basophils and epithelial cells were counted.

### 2.5. Pathological examination of lung tissue

Guinea pigs were anesthetized using 10% chloral hydrate via intraperitoneal injection. After the blood and BALF were drawn, air was injected into heart and the guinea pigs were sacrificed. The right lung was exposed and removed. The right lung was removed at the same time that the plasma and BALF were collected. Paraffin section preparation: the lung tissue was fixed in 10% formaldehyde for 4 h, treated with 70% ethanol for 3 h, 85% ethanol twice for 2 h, 95% ethanol twice for 1.5 h, anhydrous alcohol twice for 1 h and dimethylbenzene twice for 1 h. The treated lung tissues were paraffin embedded and cooled, and a 5- $\mu\text{m}$  slice was cut and adhered to a slide treated with polylysine. The hematoxylin-eosin (HE) staining was performed according to the standard procedure (Lillie, 1965). In brief, the slide containing the paraffin section was placed in a slider and then treated as follows: xylene 4 times  $\times$  5 min, 100% ethanol 2 times  $\times$  5 min, 95% ethanol 2 times  $\times$  5 min, 80% ethanol 2 times  $\times$  5 min, rinsed for 5 min with tap water, hematoxylin solution for 10 min, rinsed for 5 min with tap water, 1.0% hydrochloric acid alcohol solution for 5–10 s, rinsed for 5 min with tap water, eosin solution for 1–2 min, rinsed for 5 min with tap water, 75% ethanol for 2 min, 85% ethanol for 2 min, 95% ethanol for 2 min,

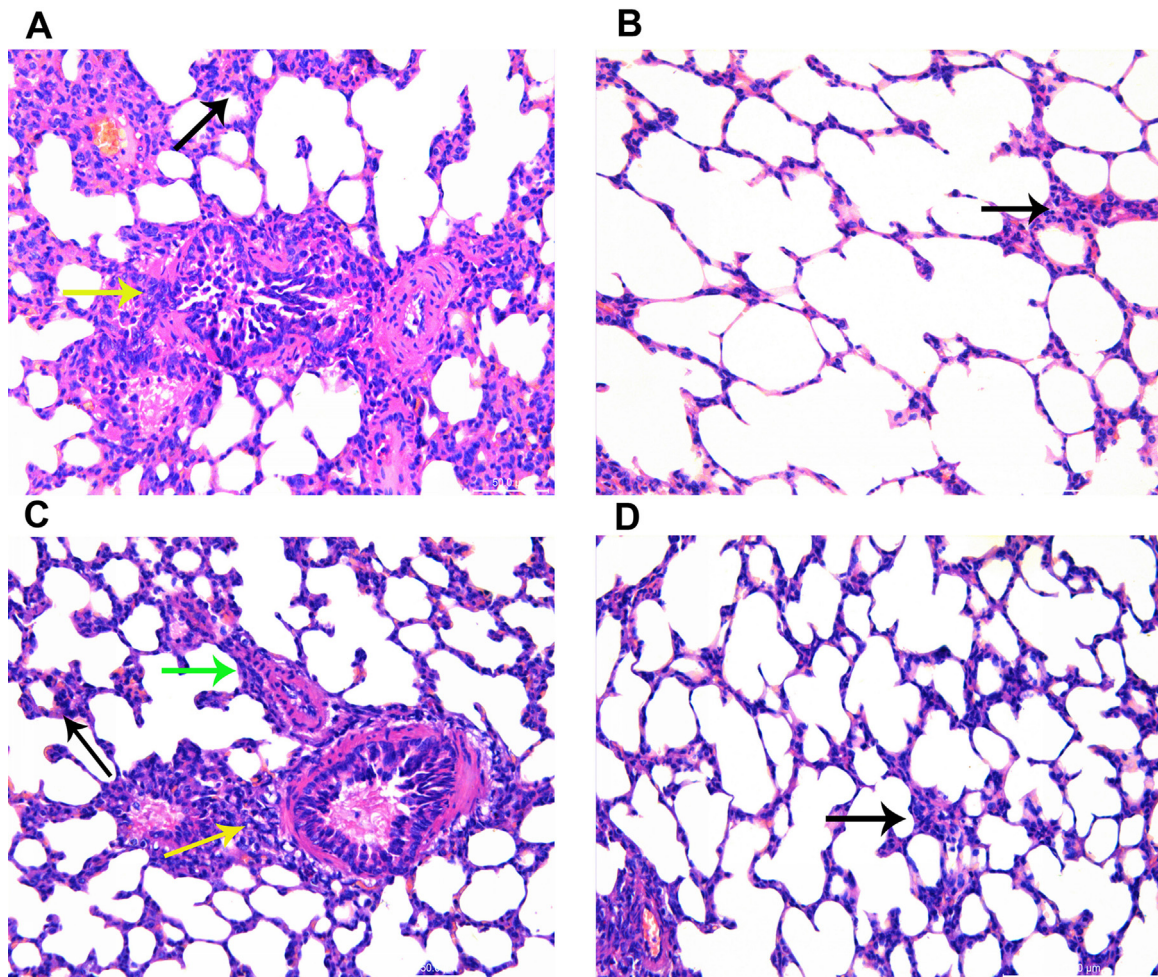


**Fig. 1.** HE staining of lung sections from the healthy guinea pigs (group C). (A) 2 h (200 $\times$ ); (B) 4 h (200 $\times$ ); (C) 8 h (200 $\times$ ) and (D) 24 h (200 $\times$ ). There were a small number of lymphocytes and neutrophils in the alveolar septa between 2 h and 24 h (A–D, the black arrows) and around the bronchioli at 2 h and 8 h (A and C, the yellow arrows), and a few enlarged alveolar spaces in the lungs at 8 h (C, the green arrows). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

100% ethanol 2 times  $\times$  1 min, xylene for 1–2 min. The slides were then sealed with neutral resin.

## 2.6. Cytokine analysis

Cytokines were measured using ELISA kits (Shanghai Westang Bio-Tech Co., Ltd., China). The levels of IL-1 $\beta$ , IL-4, IL-8, IL-13, TNF- $\alpha$ , TGF- $\beta$ <sub>1</sub> and IgE in the plasma and BALF supernatant were determined using the sandwich-ELISA method. The experiments were performed following the manufacturer's instructions exactly. The concentrations of cytokines were calculated according to the standard curves.

## 2.7. Statistical analysis

The experimental data were expressed as the mean  $\pm$  standard deviation ( $X \pm S$ ). The tests for normal distribution and homogeneity of variances were performed among different groups at each time point. If the experimental data conformed to a normal distribution and homogeneity of variance, the comparison among the groups was performed using a one-way ANOVA, and post hoc tests were used for comparison between 2 groups. If the experimental data did not fit a normal distribution or homogeneity of variance, a non-parametric test was used to compare the differences among groups.  $P < 0.05$  was considered statistically significant.

## 3. Results

### 3.1. Pathology of lung tissue inflammation

We found a small number of lymphocytes and neutrophils in the lung tissue between 2 h and 24 h in the healthy guinea pigs (group C) (Fig. 1A–D), and a few alveolar spaces in the lungs were enlarged at 8 h (Fig. 1C). However, in the allergic asthma model guinea pigs (group M), a large number of lymphocytes and neutrophils were observed around the bronchioli and blood vessels, and we observed evidence of telangiectasis at 2 h (Fig. 2A). Bullae in the lung, exudate and necrotic alveolar epithelial cells in the alveolar cavity, increased lymphocyte, neutrophil and eosinophil infiltration, thickening of the bronchial mucosa and increased goblet cell numbers in the epithelial lamina were observed in the lungs at 4 h (Fig. 2B). By 8 h to 24 h, we observed damage to the alveolar septa (Fig. 2C and D), an increased thickness of the basement membrane and hyaline degeneration in the lungs (Fig. 2D). In addition, smooth muscle cell hypertrophy, bronchial wall hyperplasia and pulmonary interstitial edema were observed in the lungs (Fig. 2D). We also discovered lymphocyte, neutrophil and eosinophil infiltration in the submucosal and muscle layers as well as inflammatory cell infiltration in the bronchial lumen and lamellar eosinophils adjacent to the lumen with fiber hyperplasia in the lungs (Fig. 2C and D).

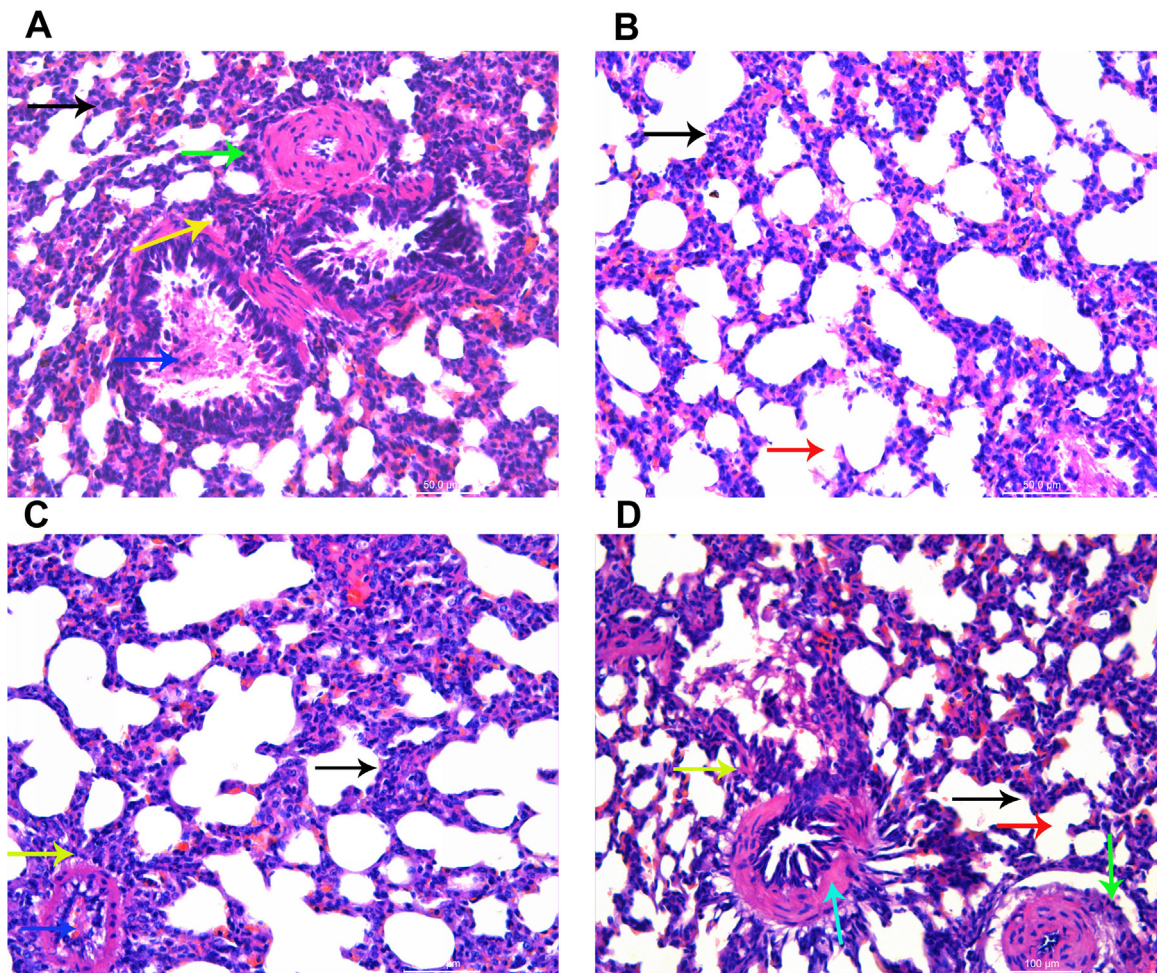


**Fig. 2.** HE staining of lung sections from the allergic asthma model guinea pigs (group M). (A) 2 h (200 $\times$ ); (B) 4 h (200 $\times$ ); (C) 8 h (200 $\times$ ) and (D) 24 h (200 $\times$ ). There were a large number of lymphocytes, neutrophils and eosinophil in the alveolar septa between 2 h and 24 h (A–D, the black arrows), around the bronchioli at 2 h, 8 h and 24 h (A, C and D, the yellow arrows) and blood vessels at 2 h and 24 h (A and D, the green arrows) and in the bronchial lumen at 2 h and 8 h (A and C, the blue arrows). Damaged alveolar septa were observed at 8 h and 24 h (C and D, the red arrows). An increased thickness of the basement membrane, hyaline degeneration, smooth muscle cell hypertrophy and bronchial wall hyperplasia were observed in the bronchial at 24 h (D, the skyey arrows). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

In the guinea pigs with allergic asthma treated with 0.1% anti-IL-1 $\beta$  IgY (group Z<sub>1</sub>), damage to the alveolar septa and the infiltration of a large number of lymphocytes, neutrophils and eosinophils were observed in the lungs at 2 h (Fig. 3A). Alveolar retraction and lymphocyte and neutrophil infiltration were observed in the lungs at 4 h (Fig. 3B). By 8–24 h, we observed a reduced inflammatory cell infiltration and severe broadening of the alveolar septa in the lungs (Fig. 3C and D). However, compared to the allergic asthma model guinea pigs (group M) (Fig. 2C and D), the animals treated with 0.1% anti-IL-1 $\beta$  IgY showed less severe tissue damage (Fig. 3C and D).

In the budesonide treated guinea pigs (group Z<sub>2</sub>), we observed the infiltration of a large number of lymphocytes, neutrophils and eosinophils in the bronchioli and in lung tissue blood vessels. Substantial lung tissue damage was also observed at 2 h (Fig. 4A). At 4 h, the infiltration of lymphocytes, neutrophils and eosinophils in the bronchioli and blood vessels was slightly reduced, and the broadening of the alveolar septa and hyperemia were severe in the lungs (Fig. 4B). By 8–24 h, few lymphocytes, neutrophils and eosinophils were present around the bronchia and blood vessels, and the number of inflammatory cells was slightly reduced compared to 2 h (Fig. 4C and D). The broadening of the alveolar septa and hyperemia were less severe (Fig. 4D); however, the observed pathological damage did not significantly differ between the animals treated with the budesonide and those treated with anti-IL-1 $\beta$  IgY.

### 3.2. Content variation of inflammatory cells in the PB and BALF

In the current study, we found that inflammation in the allergic asthma model guinea pigs (group M) was due primarily to the increase of eosinophils in the PB from 2 h to 24 h ( $P < 0.05$ ) (Fig. 5A–D). The most significant increase was observed at 2 h (Fig. 5A). In the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group Z<sub>1</sub>) and in the budesonide treated guinea pigs (group Z<sub>2</sub>), the number of eosinophils was significantly reduced compared to the allergic asthma model guinea pigs (group M) at 2 h, 8 h and 24 h (Fig. 5A, C and D) and 2 h and 24 h (Fig. 5A and D), respectively ( $P < 0.05$ ), and no difference was observed when compared with the healthy guinea pigs (group C) (Fig. 5A–D) ( $P > 0.05$ ). However, we did not observe a significant difference between the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group Z<sub>1</sub>) and the budesonide treated guinea pigs (group Z<sub>2</sub>). We found a significant reduction of neutrophils in the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group Z<sub>1</sub>) at 2 h (Fig. 5A) ( $P < 0.05$ ), whereas lymphocytes were significantly increased in the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group Z<sub>1</sub>) when compared with the allergic asthma model guinea pigs (group M) at 2 h ( $P < 0.05$ ) (Fig. 5A–D).

Inflammation in the allergic asthma model guinea pigs (group M) was correlated with increases in eosinophils, neutrophils and lymphocytes in the BALF from 2 h to 24 h (Fig. 6A–D) ( $P < 0.05$ ); however, these increases were the most significant between 2 h

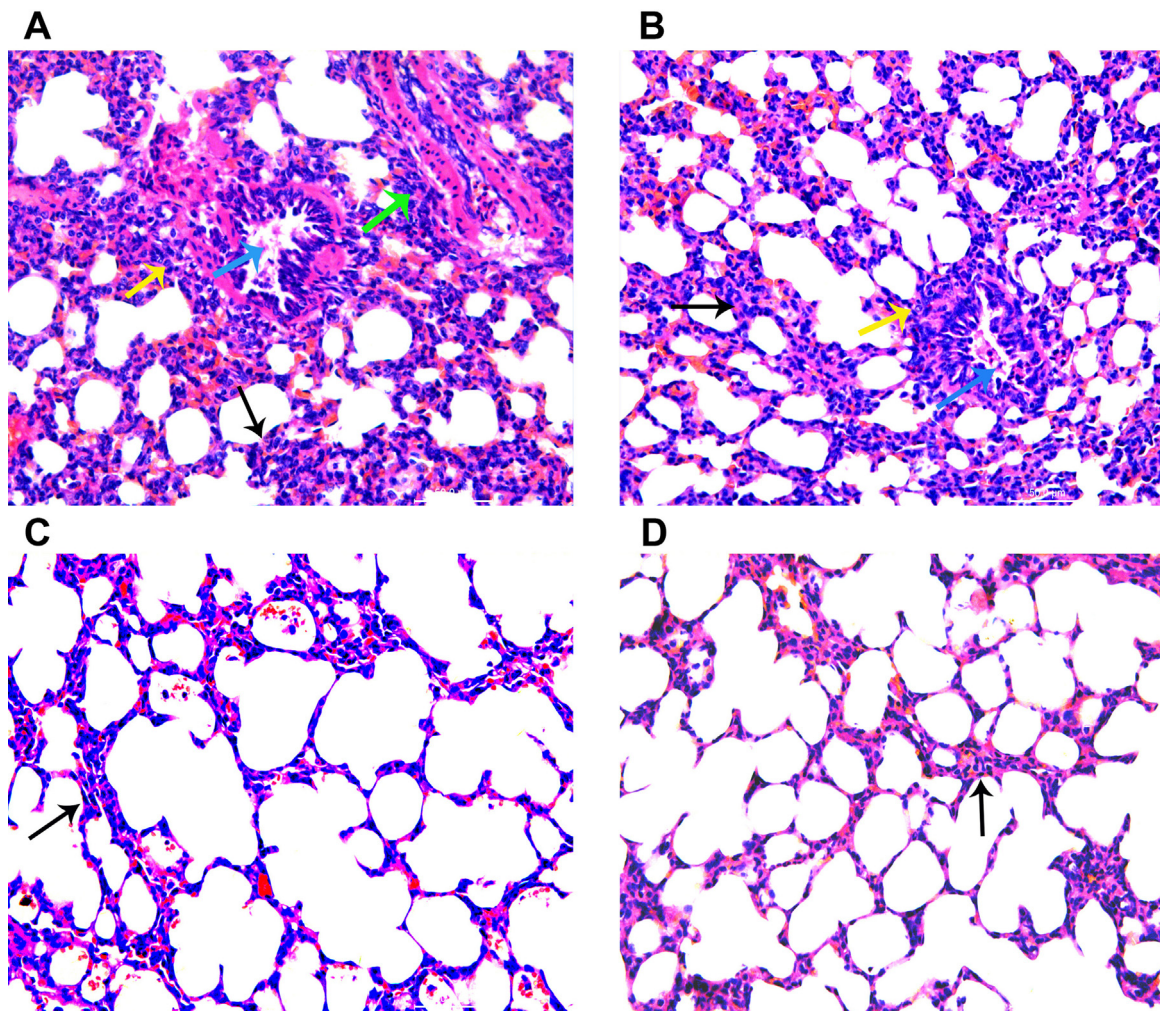


**Fig. 3.** HE staining of lung sections from the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group Z<sub>1</sub>). (A) 2 h (200 $\times$ ); (B) 4 h (200 $\times$ ); (C) 8 h (200 $\times$ ) and (D) 24 h (200 $\times$ ). There were a large number of lymphocytes, neutrophils and eosinophil in the alveolar septa at 2 h and 4 h (A and B, the black arrows), around the bronchioli at 2 h and 4 h (A and B, the yellow arrows) and blood vessels at 2 h (A, the green arrows) and in the bronchial lumen at 2 h and 4 h (A and B, the blue arrows). Reduced inflammatory cell infiltration was observed in the alveolar septa at 8 h and 24 h (C and D, the black arrows). Compared to the allergic asthma model guinea pigs (group M), the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs showed less severe tissue damage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

and 8 h (Fig. 6A–C). In the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group Z<sub>1</sub>), the numbers of eosinophils, neutrophils and lymphocytes were significantly reduced in the BALF compared with the allergic asthma model guinea pigs (group M) from 2 h to 8 h (Fig. 6A–C) ( $P < 0.05$ ). In addition, lymphocytes were significantly reduced in the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group Z<sub>1</sub>) compared to the allergic asthma model guinea pigs (group M) at 24 h (Fig. 6D) ( $P < 0.05$ ). Moreover, the number of macrophages was significantly reduced in the allergic asthma model guinea pigs (group M) compared to the healthy guinea pigs (group C) from 2 h to 24 h (Fig. 6A–D); however, the number was significantly increased in the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group Z<sub>1</sub>) compared to the allergic asthma model guinea pigs (group M) at 2 h and 8 h ( $P < 0.05$ ) (Fig. 6A and C).

### 3.3. Changes in the inflammatory cytokine content in the PB and BALF

In the current study, we observed a significant increase in the level of TGF- $\beta_1$  from 4 h to 24 h in the PB in the allergic asthma model guinea pigs (group M) when compared with the healthy guinea pigs (group C) (Fig. 7, TGF- $\beta_1$ ). Significantly lower levels of IL-1 $\beta_1$  were observed in the budesonide treated guinea pigs

(group Z<sub>2</sub>) at 8 h and in the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group Z<sub>1</sub>) at 24 h compared with the allergic asthma model guinea pigs (group M) (Fig. 7 IL-1). Additionally, TNF- $\alpha$  in the budesonide treated guinea pigs (group Z<sub>2</sub>) at 8 h (Fig. 7, TNF- $\alpha$ ), TGF- $\beta_1$  in the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group Z<sub>1</sub>) at 4 h and 24 h and in the budesonide treated guinea pigs (group Z<sub>2</sub>) at 8 h and 24 h (Fig. 7, TGF- $\beta_1$ ) and IgE in the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group Z<sub>1</sub>) at 24 h and in the budesonide treated guinea pigs (group Z<sub>2</sub>) from 8 h to 24 h (Fig. 7, IgE) were also significantly lower compared to the allergic asthma model guinea pigs (group M) ( $P < 0.05$ ).

In the BALF, compared with the healthy guinea pigs (group C), there were significant increases in the levels of the inflammatory cytokines IL-1 $\beta$ , IL-4, IL-8 and IL-13 and IgE from 2 h to 24 h as well as the levels of TNF- $\alpha$  at 2 h, 8 h and 24 h and TGF- $\beta_1$  at 4 h and 8 h in the allergic asthma model guinea pigs (group M) (Fig. 8, IL-1, IL-4, IL-8, IL-13, IgE, TNF- $\alpha$  and TGF- $\beta_1$ ) ( $P < 0.05$ ). However, the levels of IL-1 $\beta$  in the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group Z<sub>1</sub>) from 2 h to 8 h and in the budesonide treated guinea pigs (group Z<sub>2</sub>) from 2 h to 24 h were significantly reduced compared to the allergic asthma model guinea pigs (group M) ( $P < 0.05$ ) (Fig. 8 IL-1). The following cytokines were significantly decreased compared to the allergic asthma model guinea pigs (group M) ( $P < 0.05$ ) but not

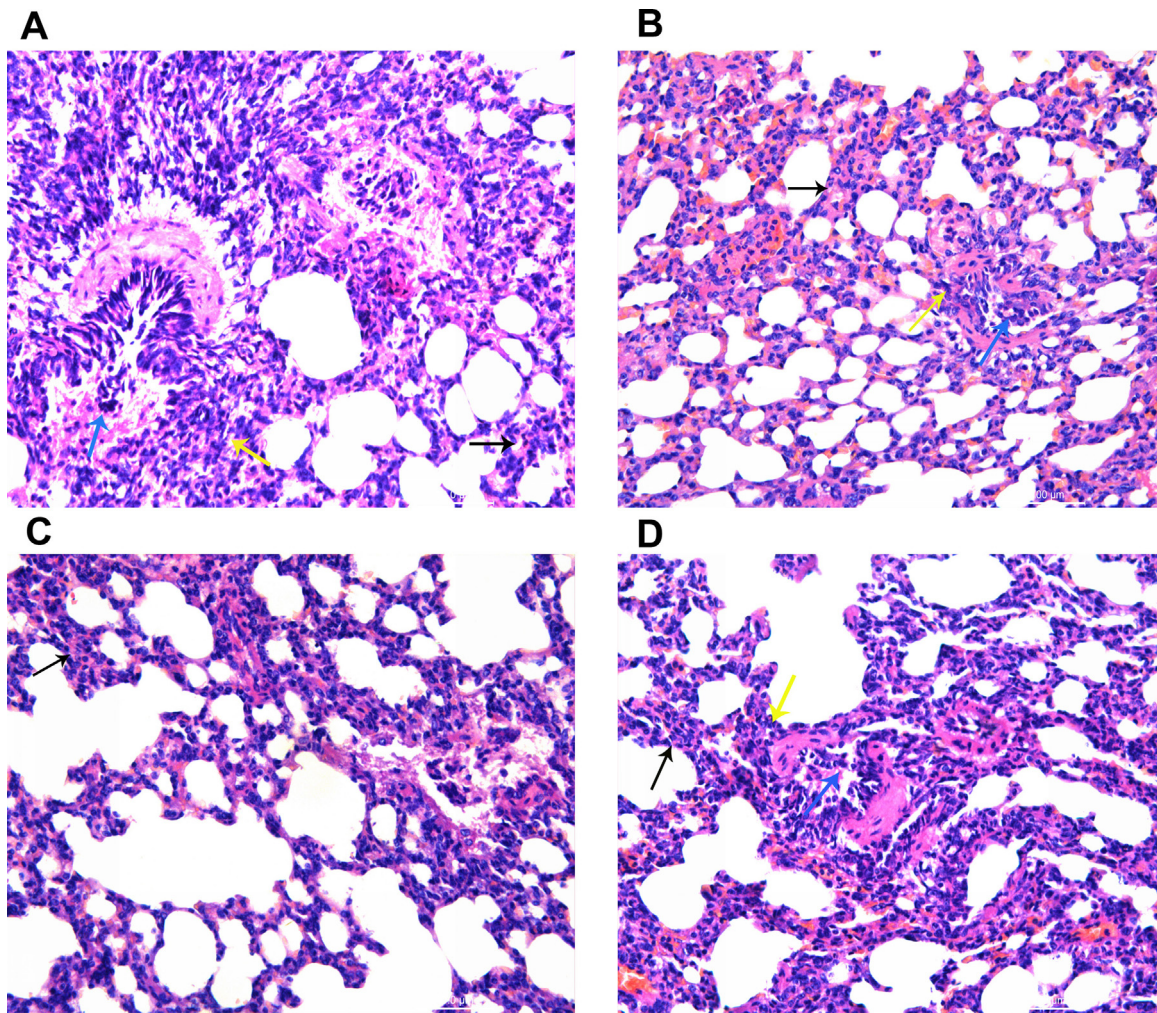


**Fig. 4.** HE staining of lung sections from the budesonide treated guinea pigs (group  $Z_2$ ). (A) 2 h (200 $\times$ ); (B) 4 h (200 $\times$ ); (C) 8 h (200 $\times$ ) and (D) 24 h (200 $\times$ ). There were a large number of lymphocytes, neutrophils and eosinophil in the alveolar septa between 2 h and 24 h (A–D, the black arrows), around the bronchioli at 2 h, 4 h and 24 h (A, B and D, the yellow arrows) and in the bronchial lumen at 2 h, 4 h and 24 h (A, B and D, the blue arrows). However, the infiltration of lymphocytes, neutrophils and eosinophils in the bronchioli gradually reduced from 4 h to 24 h. Compared to the allergic asthma model guinea pigs (group M), the budesonide treated guinea pigs showed less severe tissue damage. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

the healthy guinea pigs (group C) ( $P > 0.05$ ); TNF- $\alpha$  in the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group  $Z_1$ ) at 8 h and in the budesonide treated guinea pigs (group  $Z_2$ ) from 4 h to 8 h; IL-4 in the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group  $Z_1$ ) from 4 h to 24 h and in the budesonide treated guinea pigs (group  $Z_2$ ) from 2 h to 24 h; IL-8 and IL-13 in the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group  $Z_1$ ) at 8 h and in the budesonide treated guinea pigs (group  $Z_2$ ) from 4 h to 24 h; TGF- $\beta_1$  in the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group  $Z_1$ ) and in the budesonide treated guinea pigs (group  $Z_2$ ) from 8 h to 24 h (Fig. 8, TNF- $\alpha$ , IL-8, IL-13 and TGF- $\beta_1$ ). The levels of IgE in the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group  $Z_1$ ) from 4 h to 8 h and in the budesonide treated guinea pigs (group  $Z_2$ ) from 4 h to 24 h were significantly lower than those in the allergic asthma model guinea pigs (group M) ( $P < 0.05$ ) (Fig. 8, IgE). In the BALF, we first observed a significant reduction in the levels of IL-1 $\beta$ , and soon afterwards the levels of other inflammatory cytokines were significantly decreased.

These data demonstrate that pulmonary inflammatory pathological responsiveness as well as inflammatory cell numbers (eosinophils, neutrophils and lymphocytes), Th2 cytokines (IL-4 and IL-13), proinflammatory cytokines (IL-1, TNF- $\alpha$ , IL-8 and TGF- $\beta_1$ ) and IgE in the PB and BALF (particularly in the BALF) are alleviated and decreased in the 0.1% anti-IL-1 $\beta$  IgY treated guinea

pigs when compared with the allergic asthma model guinea pigs. These results indicate that the inhalation of aerosolized anti-IL-1 $\beta$  IgY can effectively inhibit the inflammatory pathological responses of pulmonary tissues in guinea pigs with allergic asthma induced by aerosolized OVA inhalation. The inhibitory activity of anti-IL-1 $\beta$  IgY may be a result of its ability to significantly decrease the numbers of eosinophils, neutrophils and lymphocytes and reduce the levels of IL-1 $\beta$ , IL-4, IL-8, IL-13, TNF- $\alpha$ , TGF- $\beta_1$  and IgE in the PB and BALF.

#### 4. Discussion

The pathological progression of OVA-induced allergic asthma differs in different species of animals. Zosky et al. demonstrated that there was no evidence of a late-phase response or AHR in mice after three aerosolized OVA exposures (Zosky et al., 2008). Other studies have demonstrated increases in airway resistance, in the numbers of eosinophils, neutrophils and macrophages and in the concentrations of TNF- $\alpha$ , IL-4 and IL-5 in the BALF and in the amount of extracellular matrix and fibrosis in the subepithelial regions of bronchi and bronchioles following the sensitization and challenge of guinea pigs with OVA (Franova et al., 2013; Toward and Broadley, 2004; Bazán-Perkins et al., 2009). Additionally,

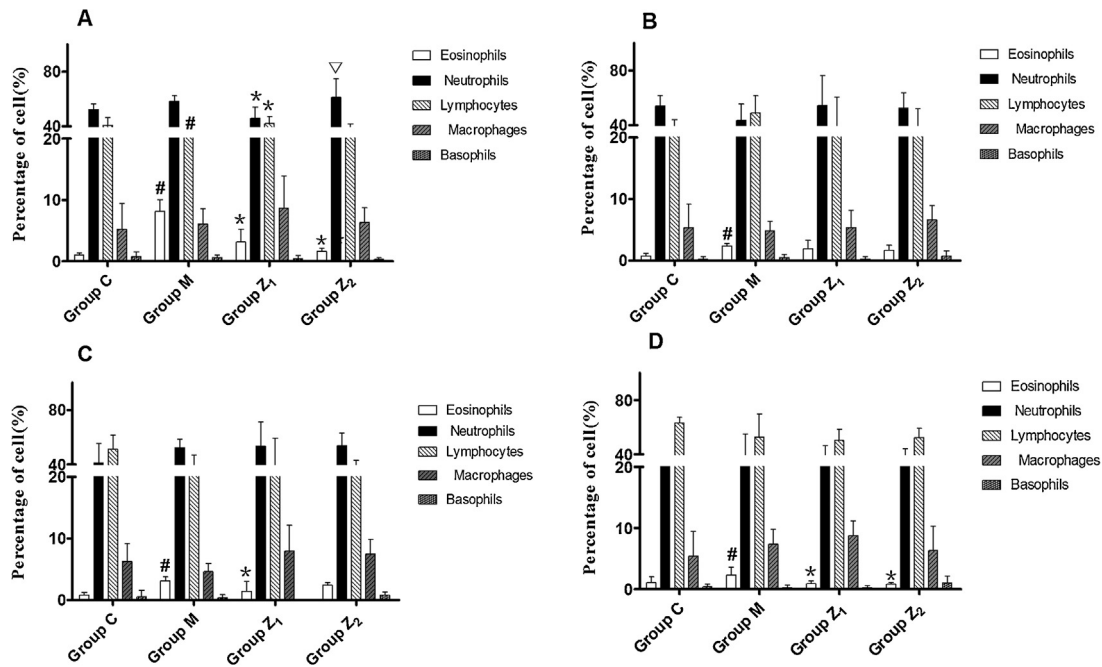


**Fig. 5.** Percentages of different inflammatory cells ( $X \pm S$ ) in the peripheral blood from guinea pigs after the different treatments. (A) 2 h; (B) 4 h; (C) 8 h and (D) 24 h.  $n = 3-4$  per group. #  $P < 0.05$  compared with group C; \*  $P < 0.05$  compared with group M, ∇  $P < 0.05$  compared with group Z<sub>1</sub>.

OVA-sensitized and OVA-challenged mice develop sustained eosinophilic airway inflammation and show increased levels of collagen, hydroxyproline, alpha-smooth muscle actin and features of airway remodeling, including increased peribronchial fibrosis, increased thickness of the peribronchial smooth muscle layer and the deposition of extracellular matrix proteins (Kang et al., 2010; Cho et al., 2011). However, in our study, we used OVA to sensitize and challenge guinea pigs by aerosol inhalation, and the guinea pigs showed typical pathological responses of allergic asthma. Our guinea pig model and results were similar to those caused by the Mizutani N' method (Mizutani et al., 2010). In our guinea pig model,

the phenomenon of high airway resistance has been observed and guinea pigs death was due to tachypnea, abdominal breathing, lip cyanoderma and muscle spasms after inhaling OVA for 5–10 min in OVA-sensitized/challenged stages, as well as pathological anatomy showing pulmonary atelectasis. Our data showed that the inhalation of aerosolized anti-IL-1 $\beta$  IgY effectively decreased the inflammatory pathological responses in pulmonary tissue and suppressed fibrosis in OVA-sensitized and challenged guinea pigs. Additionally, the infiltration of eosinophils, neutrophils and lymphocytes was reduced, and the broadening of and damage to the alveolar septa and hyperemia were also markedly alleviated

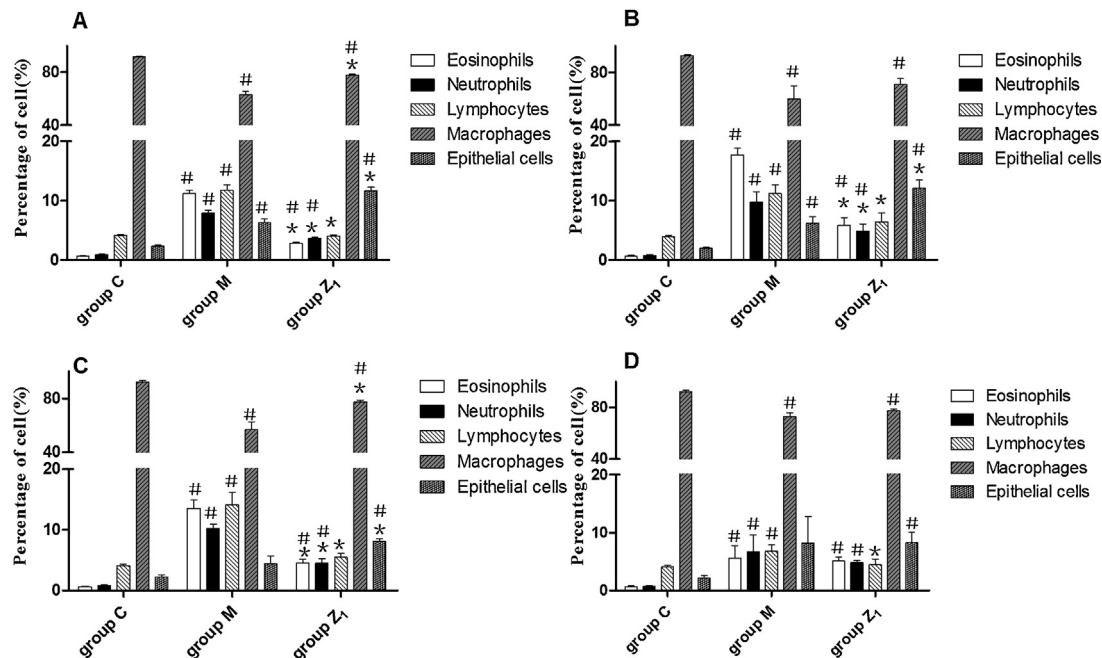


**Fig. 6.** Percentages of different inflammatory cells ( $X \pm S$ ) in the BALF from guinea pigs after the different treatments. (A) 2 h; (B) 4 h; (C) 8 h and (D) 24 h.  $n = 3-4$  per group. #  $P < 0.05$  compared with group C; \*  $P < 0.05$  compared with group M.

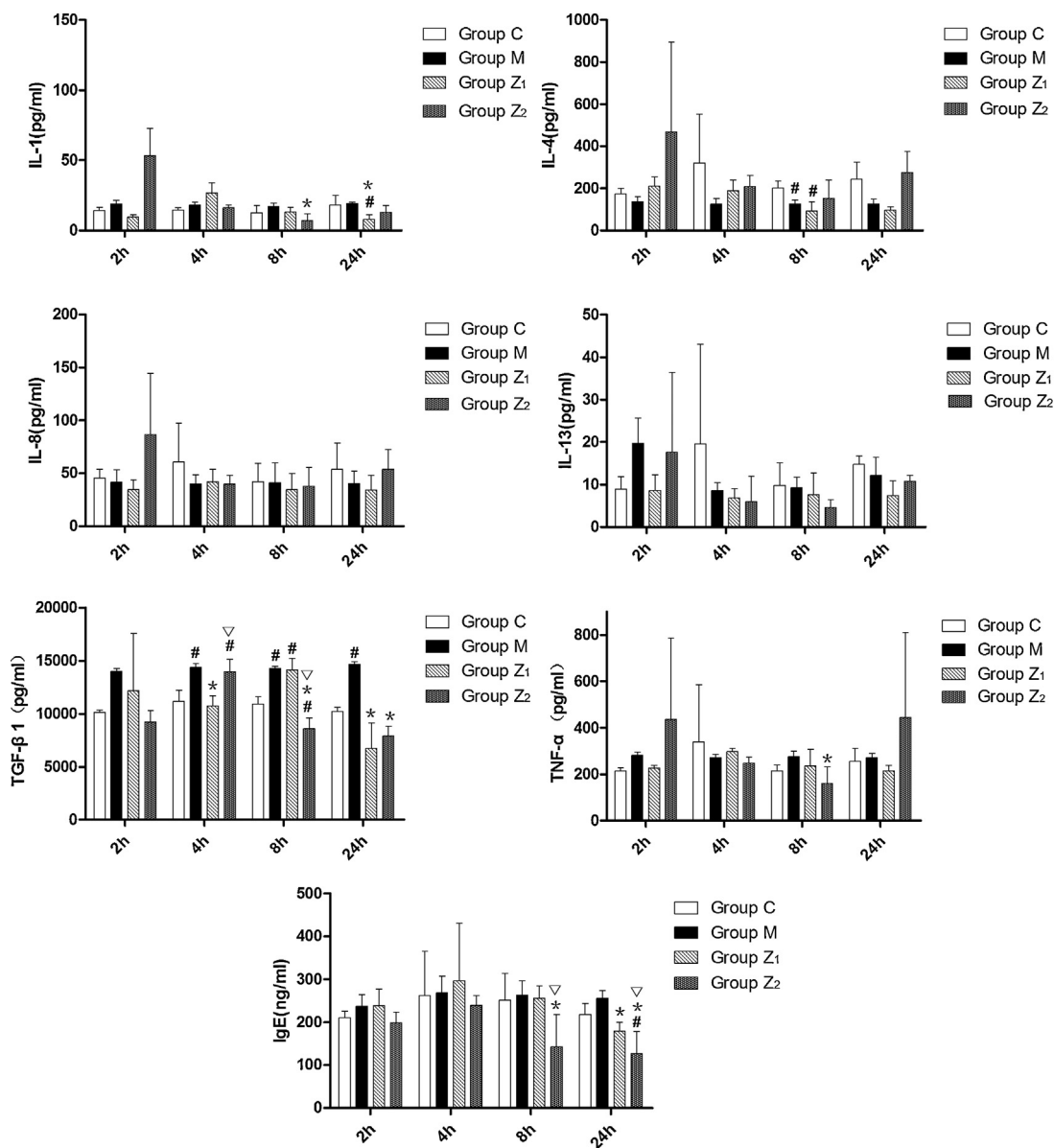


**Fig. 7.** Levels of inflammatory cytokines ( $X \pm S$ ) in the plasma from guinea pigs after the different treatments.  $n = 3-4$  per group. #  $P < 0.05$  compared with group C; \*  $P < 0.05$  compared with group M; ∇  $P < 0.05$  compared with group Z1.

after the inhalation of aerosolized anti-IL-1 $\beta$  IgY between 8 h and 24 h (Fig. 3C and D). These findings were similarly observed in the animals treated with budesonide (Fig. 4C and D).

In aerosolized OVA-challenged guinea pigs and rats, the levels of IgE, TNF- $\alpha$ , IL-4 and IL-5 as well as the number of eosinophils have been shown to increase in the PB. However, the administration of  $\beta$ -sitosterol, dexamethasone and IL-1ra to guinea pigs and rats has been shown to significantly reduce these levels (Mahajan and Mehta, 2011; Li et al., 2006). Holgate et al. demonstrated that the levels of IgE, IL-4, IL-5 and IL-13 and the number of eosinophils in the PB were reduced by omalizumab (anti-IgE antibody) infusion in patients with allergic asthma (Holgate et al., 2009). We found a significant reduction in the numbers of eosinophils and neutrophils and the levels of IL-1, TGF- $\beta$ 1 and IgE in the PB at different time points after the inhalation of aerosolized 0.1% anti-IL-1 $\beta$  IgY (in the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group Z1)) or budesonide (in the budesonide treated guinea pigs (group Z2)) compared with the allergic asthma model guinea pigs (group M) ( $P < 0.05$ ). It is worth noting that the numbers of inflammatory cells and the levels

of inflammatory cytokines in the PB were lower in OVA aerosol-sensitized and OVA aerosol-challenged guinea pigs compared to the guinea pig models of allergic asthma reported by others. The differences may be due to OVA aerosol sensitization at the lung surface, which may cause local allergic responsiveness in lung tissue (airway and lung alveoli). Other reports have used a subcutaneous or intraperitoneal injection of OVA for sensitization, which results in systemic immunization. In the clinic, sensitization and challenge by allergen inhalation may be more widespread for allergic asthma.

In OVA-sensitized/challenged guinea pigs and mice, the numbers of eosinophils and neutrophils and the levels of TNF- $\alpha$ , IL-4, IL-5, IL-13 and TGF- $\beta$ 1 were increased in the BALF (Franova et al., 2013; Toward and Broadley, 2004; Kang et al., 2010; Szelenyi et al., 2000). Previous studies have reported that oral dexamethasone and  $\beta$ -sitosterol, atomized budesonide, intravenously injected infliximab, intraperitoneally injected sTNFR and intravenously injected IL-1ra can inhibit increases in eosinophils, neutrophils, TNF- $\alpha$ , IL-4, IL-5 and IL-13 in the BALF in OVA-sensitized/challenged guinea pigs, rats and mice (Toward and Broadley, 2004; Mahajan and

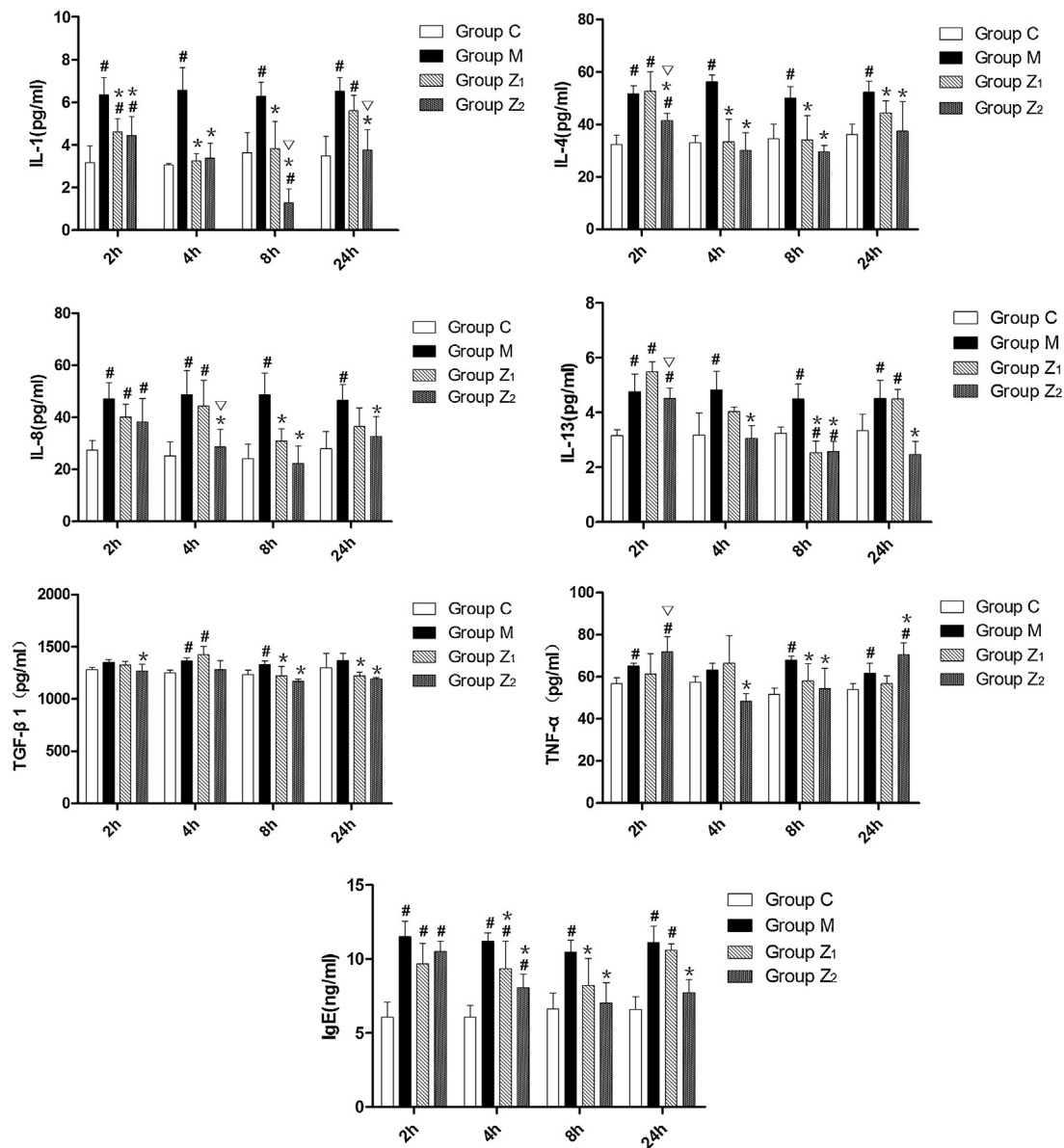


**Fig. 8.** Levels of inflammatory cytokines ( $X \pm S$ ) in the BALF from guinea pigs after the different treatments.  $n = 3-4$  per group. #  $P < 0.05$  compared with group C; \*  $P < 0.05$  compared with group M; ∇  $P < 0.05$  compared with group Z<sub>1</sub>.

Mehta, 2011; Deveci et al., 2008; Nam et al., 2009; Li et al., 2006; Szelenyi et al., 2000). In our study, the numbers of eosinophils, neutrophils and lymphocytes were significantly reduced in the BALF after the inhalation of aerosolized 0.1% anti-IL-1β IgY, particularly from 2 h to 8 h, which was a significantly greater reduction in the 0.1% anti-IL-1β IgY treated guinea pigs (group Z<sub>1</sub>) compared to the allergic asthma model guinea pigs (group M) ( $P < 0.05$ ). The levels of IL-1β, IL-4, IL-8, IL-13, TGF-β<sub>1</sub> and IgE in the 0.1% anti-IL-1β IgY treated guinea pigs (group Z<sub>1</sub>) and the budesonide treated guinea pigs (group Z<sub>2</sub>) were significantly reduced in the BALF after OVA challenge compared with the allergic asthma model guinea pigs (group M) ( $P < 0.05$ ).

Interestingly, the proinflammatory cytokine IL-1β was significantly reduced at 2 h in the BALF in the 0.1% anti-IL-1β IgY treated guinea pigs (group Z<sub>1</sub>). Other proinflammatory cytokines and Th2 cytokines, such as TNF-α, TGF-β<sub>1</sub>, IL-8, IL-4 and IL-13, and IgE antibodies were significantly decreased after 4 h in the BALF in the 0.1% anti-IL-1β IgY treated guinea pigs (group Z<sub>1</sub>) (Fig. 8).

The level of IL-1β was again increased at 24 h compared to 2 h, 4 h and 8 h in the 0.1% anti-IL-1β IgY treated guinea pigs (group Z<sub>1</sub>); however, the levels of IL-4 and TGF-β<sub>1</sub> were still significantly decreased in the BALF at 24 h in the 0.1% anti-IL-1β IgY treated guinea pigs (group Z<sub>1</sub>) ( $P < 0.05$ ). These results suggest that IL-1β may be the initiating and aggravating proinflammatory cytokine response in the immune effector stage of the development and progression of allergic asthma inflammation. Additionally, IL-1β may directly or indirectly regulate the production of IL-4, IL-13, IL-8, TNF-α, TGF-β and IgE. Cardell et al. demonstrated the following: the expression of the interleukin-1 receptor I and the TNF receptor I was increased in epithelial cells; the expression of TNF receptor I was increased in smooth muscle cells; and TNF receptor II is expressed in the epithelial layer and smooth muscle cells when a tracheal culture was performed in the presence of IL-1β (Cardell et al., 2008). Zhang et al. demonstrated that IL-1β enhanced TNF-α mRNA expression in the trachea, while the administration of anti-TNF-α antibody markedly suppresses the

IL-1 $\beta$ -induced up-regulation of bradykinin B1 and B2 receptors in the tracheal smooth muscle (Zhang et al., 2007), which indicates that IL-1 $\beta$  may be an initiating factor during the development of inflammation. IL-1 $\beta$  induced bronchial epithelial cells and smooth muscle cells to create a proinflammatory milieu with the production of IL-6 and chemokines. These inflammatory cytokines and chemokines mobilize neutrophils in the lung and induce goblet cell hyperplasia to produce mucin MUC5AC (Besnard et al., 2012; Fujisawa et al., 2009; Lai and Rogers, 2010; Zhu et al., 2011). TNF- $\alpha$  has been shown to induce goblet cell hyperplasia in the epithelial layer of the airways (Lai and Rogers, 2010), induce human primary cultured small airway epithelial cells and airway smooth muscle cells to produce IL-6, IL-8 and PGE2 (Yadav et al., 2011; Manetsch et al., 2012) and strongly up-regulate chemokine (C-C motif) ligand 15 (CCL15), a chemo-attractant for neutrophils, eosinophils, monocytes and lymphocytes in human airway smooth muscle cells isolated from the main bronchial airway segments of patients with asthma (Joubert et al., 2012). TGF- $\beta$ 1 has been shown to induce normal human lung fibroblasts to produce the extracellular matrix proteins tenascin C, fibronectin and collagen types I and IV and to induce them to differentiate into myofibroblasts, causing pulmonary fibrosis (Pegorier et al., 2010). In our study, the levels of IL-13 and TGF- $\beta$ 1 in the BALF not only significantly were reduced but also further decreased smooth muscle cell hypertrophy, bronchial wall hyperplasia, thickness of the basement membrane and hyaline degeneration in the lungs in the 0.1% anti-IL-1 $\beta$  IgY treated guinea pigs (group Z<sub>1</sub>).

In summary, we demonstrated that local treatment using an aerosolized anti-IL-1 $\beta$  IgY antibody may be an effective therapy to treat allergic asthma. This novel approach reduces the levels of proinflammatory cytokines, Th2 cytokines and IgE, and alleviates pulmonary inflammation and airway remodeling. Our data suggest that once every 8 h is an appropriate frequency for the local application of aerosolized anti-IL-1 $\beta$  IgY antibodies. In fact, oral IgY antibodies against bacteria and viruses have been applied to treat human diseases. Nilsson et al. demonstrated that when orally administered IgY against *Pseudomonas aeruginosa* prevented pulmonary *P. aeruginosa* infections in patients with cystic fibrosis, none of the IgY-treated patients became chronically colonized with *P. aeruginosa* (24% of patients became chronically colonized with *P. aeruginosa* in the control group); in addition, no negative side effects of IgY treatment have been noted during these 10 years (Nilsson et al., 2007). Orally administered IgY against human rotavirus produced a statistically significant reduction in the mean rehydration fluid intake, duration of intravenous fluid administration, mean duration of diarrhea from day of admission and duration of rotavirus clearance from stool from the day of admission in rotavirus-positive children with diarrhea (Rahman et al., 2012). In addition, Worledge et al. demonstrated that orally administered IgY against recombinant human TNF- $\alpha$  could effectively treat experimental colitis in rats. They observed that oral anti-TNF- $\alpha$  IgY, in both the acute and chronic phases of the model, significantly decreased all inflammatory end points and proved to be more effective than sulfasalazine and dexamethasone (Worledge et al., 2000). Therefore, IgY treatment produces added benefit compared to steroids and avoids steroid side effects. The blockade of IL-1 $\beta$  by the inhalation of aerosolized anti-IL-1 $\beta$  antibody could be a potential alternative strategy for preventing and treating allergic asthma and bronchial remodeling.

## Conflicts of interest

The authors declare that they have no relevant conflicts of interest.

## Acknowledgments

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