



Therapeutic potential of combined anti-IL-1 β IgY and anti-TNF- α IgY in guinea pigs with allergic rhinitis induced by ovalbumin[☆]



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ARTICLE INFO

Article history:

Received 29 August 2014

Received in revised form 13 November 2014

Accepted 1 December 2014

Available online 11 December 2014

Keywords:

Allergic rhinitis

Cytokines

Guinea pigs

Immunoglobulin yolk

Interleukin-1beta

OVA

Tumor necrosis factor-alpha

ABSTRACT

We have previously demonstrated that anti-IL-1 β immunoglobulin yolk (IgY) inhibits pathological responses in allergic asthma guinea pigs induced by ovalbumin (OVA). This study aims to determine whether the combined blockade of IL-1 β and TNF- α can more effectively inhibit allergic inflammation in allergic rhinitis (AR) guinea pigs induced by OVA. Healthy guinea pigs treated with saline were used as the healthy control. The AR guinea pigs induced by OVA were randomly divided into (1) the AR model group containing negative control animals treated with intranasal saline; (2) the 0.1% non-specific IgY treatment group treated with non-specific IgY; (3) the 0.1% anti-TNF- α IgY treatment group treated with 0.1% anti-TNF- α IgY; (4) the 0.1% anti-IL-1 β IgY treatment group treated with 0.1% anti-IL-1 β IgY; (5) the 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group treated with 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY; and (6) the fluticasone propionate treatment group treated with fluticasone propionate. Cytokines were measured using an enzyme-linked immunosorbent assay. The results showed that IL-1 β , IL-5, IL-9, IL-13, IL-18, IL-22, IL-33, TNF- α , TGF- β ₁ and OVA-specific IgE levels in the peripheral blood (PB) and nasal lavage fluid (NLF) significantly decreased at 2 h, 4 h or 8 h in the 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group compared to the AR model group and the 0.1% non-specific IgY treatment group ($P < 0.05$). The data suggest that blockade of IL-1 β and TNF- α by intranasal instillation of combined anti-IL-1 β IgY and anti-TNF- α IgY could be a potential alternative strategy for preventing and treating allergic rhinitis.

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1. Introduction

Allergic rhinitis (AR) is an IgE-mediated type I hypersensitivity inflammation disease of the nasal mucosa, which occurs early-phase reaction within 5–30 minutes (vasodilation, vascular permeability, swelling, sneezing, itching, nasal rubbing, rhinorrhoea or coughing) and late-phase reactions within 2–24 h (inflammatory cells, proinflammatory cytokines and Th2 cytokines) after exposure to an allergen [1–3]. In AR guinea pigs and mice induced by ovalbumin (OVA) and AR patients, the sneezing, rubbing frequencies, lacrimation, nasal congestion, and total and OVA-specific IgE, IL-1 β , IL-4, IL-5, IL-6, IL-8,

IL-9, IL-13, IL-18, IL-22, IL-33, TNF- α and eosinophilic cationic protein-1 levels in the serum and nasal lavage fluid (NLF) significantly were increased in early-phase and late-phase reactions [1,4–12]. However, the curative effect of blockage of Th2 cytokines for allergic patients using anti-IL-4, IL-5, IL-9 and IL-13 antibodies were no efficacy or of limited efficacy in clinical studies [13,14]. Proinflammatory cytokines are anterior to Th2 cytokines in pathological response procedures. In mice with AR, the symptoms, OVA-specific IgE, Th2 cytokines and inflammatory cell levels significantly decreased after anti-TNF- α (infliximab) and anti-IL-33 antibodies treatment [15,16]. Our previous studies have shown that the inhalation of aerosolized anti-IL-1 β IgY inhibited pathological responses in the pulmonary tissues, decreases eosinophils in the peripheral blood and BALF, and reduces the levels of IL-1 β , IL-4, IL-8, IL-13, TNF- α , TGF- β ₁ and IgE in the BALF in allergic asthma guinea pigs induced by OVA [17].

In the present study, we used the intranasal therapy of combined anti-IL-1 β IgY and anti-TNF- α IgY to treat AR guinea pigs induced by OVA, and provided strong experimental evidence that supports a more efficiently therapeutic strategy against AR by using combined anti-IL-1 β IgY and anti-TNF- α IgY.

Abbreviations: AR, allergic rhinitis; IgE, immunoglobulin E; IgY, immunoglobulin yolk; IL-1 β , interleukin-1 beta; IL-4, interleukin-4; IL-5, interleukin-5; IL-9, interleukin-9; IL-13, interleukin-13; IL-18, interleukin-18; IL-22, interleukin-22; IL-33, interleukin-33; Infliximab, anti-TNF- α antibody; NLF, nasal lavage fluid; OVA, ovalbumin; PB, peripheral blood; TGF- β ₁, transforming growth factor-beta 1; Th2, T helper type 2; TNF- α , tumor necrosis factor-alpha

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

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2. Materials and methods

2.1. Animals

Hartley guinea pigs (male, 7 weeks old, 230 g $\pm$ 40 g) were purchased from National Center for Experimental Animal Seed Rodent Shanghai Sub-Centres (Production license SXCK(Hu)2012-0008, Shanghai, 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. The room where experiments were carried out was free of noise and strong odors, was provided with controlled temperature of 23 $\pm$ 2 °C and 60 $\pm$ 5% relative humidity. Guinea pigs freely drank water and fed onself.

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

After adaptation for 7 days, the guinea pigs were divided into a healthy control (n = 17) and AR groups. Sensitization and challenge protocol as described by Bahekar et al. [18] was used with some modifications. In the procedure for systemic sensitization, guinea pigs were sensitized on day 1, 3, 5, 7, 9, 11 and 13 using 1.0 ml 0.9% saline by intraperitoneal injection for seven times in the healthy control, as well as using 1.0 ml OVA (300 μ g/animal) (Grade II, Sigma, USA) and aluminium hydroxide (30 mg/animal) (Thermo scientific, USA) in 0.9% saline in the AR groups. In the procedure for topical challenge after 7 days of last systemic sensitization, guinea pigs were challenged by instilled nostril with 0.2 ml 0.9% saline (bilateral nostrils/animal, 1.0 ml/each nostril) from day 21 to day 30 for ten times in the healthy control, as well as by instilled nostril with 0.2 ml OVA solution (4.0 mg/bilateral nostrils/animal, 2.0 mg/each nostril) in the AR groups. After three times OVA challenge, guinea pigs with AR according symptom scores (guinea pigs in each group including strong, mild and weak allergic reactions) were randomly divided into six groups. 1) The AR model group (n = 15) was treated with 0.9% saline for seven days (by instilled nostril with 0.2 ml OVA after instilled nostril with 0.9% saline). 2) The 0.1% non-specific IgY treatment group (n = 18) was treated with 0.1% non-specific IgY (prepared in the laboratory, purity 85%, valence combined recombinant human IL-1 β and TNF- α : 1:8/mg protein) for seven days (by instilled nostril with 0.2 ml OVA after instilled nostril with 0.1% non-specific IgY). Laying hens were inoculated with 0.9% saline. Non-specific 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 IgY valence of anti-IL-1 β and anti-TNF- α was determined by an enzyme-linked immunosorbent assay (ELISA) using 1 mg of purified IgY. 3) The 0.1% anti-TNF- α IgY treatment group (n = 17) was treated with 0.1% anti-TNF- α IgY (prepared in the laboratory, purity 85%, valence combined recombinant human TNF- α : 1:3200/mg protein) for seven days. Laying hens were inoculated with recombinant human TNF- α . Anti-TNF- α 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-TNF- α IgY was determined by an enzyme-linked immunosorbent assay (ELISA) using 1 mg of purified IgY. 4) The 0.1% anti-IL-1 β IgY treatment group (n = 17) was treated with 0.1% anti-IL-1 β IgY [17] for seven days. 5) The 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group (n = 18) was treated with 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY for seven days (half of the 0.1% anti-IL-1 β IgY and half of the anti-TNF- α IgY together mixed into 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY solution). 6) The fluticasone propionate treatment group (the positive control) (n = 17) was treated with a fluticasone propionate suspension

(0.05%, Glaxsmithk Ltd. UK) for seven days. In the experimental destination, the plasma and nose lavage fluid were obtained from guinea pigs of all experimental groups at 2 h, 4 h and 8 h after last treatment and challenge, and inflammatory cytokines were examined.

2.3. Identification of hypersensitivity reactions

The OVA-sensitized guinea pigs were identified using intradermal injection of OVA (Intracutaneous testing). A procedure for intradermal injection of antigen as described by Magnusson and Kligman [19] and Bahekar et al. [18]. In brief, a week after last sensitization, animals were shaved on dorsal skin area with the electric razor and challenged by intradermal injection of OVA in 0.9% saline (25 μ l of 200 μ g/ml). Successful sensitization was confirmed by edema and redness at the site of injection (diameter of swelling and redness $\geq$ 1.0 cm after injection 20 min ~ 40 min) and the success rate of sensitization was 95.65% (132 animals/138 animals).

2.4. Symptom scores of allergic rhinitis

Symptom of AR included sneezing, nose rubbing and rhinorrhea. Number of sneezing and nasal rubbing were counted continuously, and the amount of nasal secretion (rhinorrhea, watery fluid) was scored in guinea pig with AR within 30 min after intranasal challenge with 2.0% OVA in 0.9% saline. A procedure for symptom scores of AR as described by Brozmanová et al. [20] was used with some modifications. Symptom scores were graded on a three-point scale. Each grade was assigned a numerical score (1–3), and data were analyzed both as separate symptoms and as a total symptom score. Nasal rubbing: 1–2 times for one point, continuous to nasal rubbing for three points and between both of them for two points; Sneezing: 1–3 times for one point, 4–10 times for two points and above 11 times for three points; Rhinorrhea: snot to the nostrils for one point, snot out of his nostrils for two points and face covered with snot for three points. More than or equal to 5 points in symptom scores of AR are the success of the modeling.

2.5. Plasma and NLF collection

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 °C. NLF was collected according to a procedure described by Bahekar et al. [18]. The guinea pig head was fixed to lie on one's back, a cannula attached to the nasal cavity of one nostrils was connected to a 5.0-mL syringe with 2 ml 0.9% saline, 0.9% saline slowly was perfused into the nasal cavity at a rate of 0.1 ml/min, and nasal lavage liquid was collected with micropipette on the other nostrils. NLF was centrifuged and supernatant was stored at –80 °C. Plasma and NLF were collected at 2 h, 4 h and 8 h (there were three guinea pigs in each group) in random order.

2.6. Cytokine analysis

Cytokines were measured using ELISA kits (Shanghai Westang Bio-Tech Co., Ltd., China). The levels of IL-1 β , IL-5, IL-9, IL-13, IL-18, IL-22, IL-33, TNF- α , TGF- β ₁ and OVA – specific IgE in the plasma and NLF 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. Changes in symptom scores of guinea pig with allergic rhinitis

Guinea pigs in systemic sensitization and intranasal challenge with OVA appeared to allergy symptoms of nasal rubbing, sneezing and rhinorrhea, even severe dyspnea, lips purple, muscle spasms and death, particularly in OVA challenge phase. The pathological anatomy showed pulmonary atelectasis in died guinea pigs induced by OVA. The symptoms of nasal rubbing, sneezing and accelerated breathing appeared within the first ten minutes in guinea pigs with OVA intranasal challenge in all AR groups, however number of nasal rubbing and sneezing obviously decreased and the symptom of rhinorrhea appeared within the second ten minutes, and moreover, the symptoms of nasal rubbing and sneezing were no obvious and there was the symptom of rhinorrhea within the third minutes. The symptoms of nasal rubbing, sneezing and rhinorrhea in AR guinea pigs were aggravated following the increase of the number of OVA challenge. The various treatments all improved symptom scores from the fourth to tenth OVA challenge compared with the AR model group and the 0.1% non-specific IgY treatment group ($P < 0.05$) (E Fig. 1 (at the fourth, sixth and eighth OVA challenge) and Fig. 1 (at the tenth OVA challenge)). The additional detailed material can be viewed at the Online Repository), particularly, symptom scores were lighter in the 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group than that in the 0.1% TNF- α IgY treatment group in the 10th OVA challenge (Fig. 1) ($P < 0.05$). The symptom scores of AR guinea pigs were less than 5 points at the tenth OVA challenge in the 0.1% anti-IL-1 β IgY treatment group, and after the eighth challenge in the 0.1% combined anti-IL-1 β and TNF- α IgY treatment group and the fluticasone propionate treatment group.

3.2. Changes in the inflammatory cytokine content in the PB

In the PB, significantly lower levels of IL-1 β , TNF- α , IL-5, IL-9, IL-13, IL-18, IL-33, TGF- β and OVA-specific IgE at 2 h (E Fig. 2); IL-1 β , TNF- α , IL-5, IL-9, IL-13, IL-18, IL-22, IL-33, TGF- β and OVA-specific IgE at 4 h (Fig. 2); and IL-1 β , IL-5, IL-9, IL-13, IL-18, IL-22, IL-33 and TGF- β at 8 h (E Fig. 2) in the 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group were observed when compared with the AR model group. Additionally, the levels of TNF- α , IL-9 and IL-13 at 2 h (E Fig. 2); IL-1 β , TNF- α , IL-9 and IL-33 at 4 h (Fig. 2) and TGF- β and IL-33 at 8 h (E Fig. 2) in the 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group were also significantly lower than that in the 0.1% anti-TNF- α IgY treatment group ($P < 0.05$). The levels of IL-1 β and IL-9 at 2 h (E Fig. 2); OVA-specific IgE, IL-1 β , IL-9, IL-13 and IL-33 at 4 h (Fig. 2) and IL-33 at 8 h (E Fig. 2) in the 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group were also significantly lower than that in the 0.1% anti-IL-1 β IgY treatment group ($P < 0.05$).

In addition, significantly lower levels of TNF- α , IL-5, IL-9, IL-13, IL-33, TGF- β and OVA-specific IgE at 2 h; IL-5, IL-13, IL-18, IL-22, IL-33, TGF- β and OVA-specific IgE at 4 h; and IL-5, TGF- β , IL-22 and IL-33 at 8 h in the 0.1% anti-TNF- α IgY treatment group, the 0.1% anti-IL-1 β IgY treatment group and the fluticasone propionate suspension treatment group were observed when compared with the AR model group in the PB ($P < 0.05$) (Fig. 2 and E Fig. 2). Significantly lower levels of IL-18 at 2 h (E Fig. 2); and IL-1 β and IL-9 at 4 h (Fig. 2) in the 0.1% anti-IL-1 β IgY treatment group and the fluticasone propionate suspension treatment group were observed when compared with the AR model group in the PB

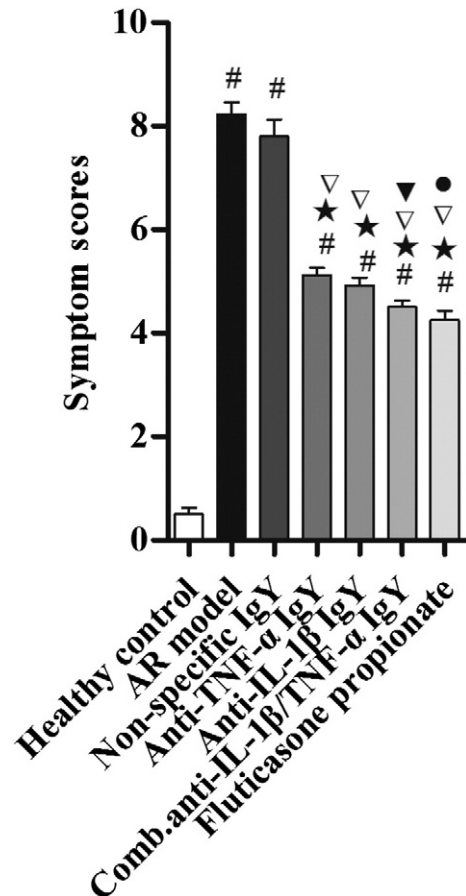


Fig. 1. Symptom scores ($X \pm S$) from the tenth OVA challenge in guinea pigs with allergic rhinitis after the different treatments. Healthy control, the healthy control group; AR model, the AR model group; Non-specific IgY, the 0.1% non-specific IgY treatment group; Anti-TNF- α IgY, the 0.1% anti-TNF- α IgY treatment group; Anti-IL-1 β IgY, the 0.1% anti-IL-1 β IgY treatment group; Comb. anti-IL-1 β /TNF- α IgY, the 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group; Fluticasone propionate, the fluticasone propionate treatment group. #: $P < 0.05$ compared the healthy control, ★: $P < 0.05$ compared with the AR model group, ∇: $P < 0.05$ compared with the 0.1% non-specific IgY treatment group, ★: $P < 0.05$ compared with the 0.1% anti-TNF- α IgY treatment group, ●: $P < 0.05$ compared with the 0.1% anti-IL-1 β IgY treatment group.

($P < 0.05$). Significantly lower level of IL-13 at 8 h in the 0.1% anti-TNF- α IgY treatment group and the 0.1% anti-IL-1 β IgY treatment group was observed when compared with the AR model group in the PB ($P < 0.05$) (E Fig. 2). Significantly lower levels of IL-1 β at 2 h; and IL-1 β and IL-9 at 8 h in the fluticasone propionate suspension treatment group were observed when compared with the AR model group in the PB ($P < 0.05$) (E Fig. 2).

Moreover, significantly lower levels of TNF- α , IL-13, IL-33, TGF- β and OVA-specific IgE at 2 h (E Fig. 2); IL-5, IL-18, TGF- β , IL-22 and IL-33 at 4 h (Fig. 2); and TNF- α , IL-5, IL-18, IL-33 and TGF- β at 8 h (E Fig. 2) in the 0.1% anti-TNF- α IgY treatment group, the 0.1% anti-IL-1 β IgY treatment group and the fluticasone propionate suspension treatment group were observed when compared with the 0.1% non-specific IgY treatment group in the PB ($P < 0.05$). Significantly lower levels of IL-5 at 2 h (E Fig. 2); IL-9 and IL-13 at 4 h (Fig. 2) in the 0.1% anti-IL-1 β IgY treatment group and the fluticasone propionate suspension treatment group were observed when compared with the 0.1% non-specific IgY treatment group in the PB ($P < 0.05$). Significantly lower levels of IL-18 at 2 h (E Fig. 2) in the 0.1% anti-IL-1 β IgY treatment group and OVA-specific IgE at 4 h (Fig. 2) in the 0.1% anti-TNF- α IgY treatment group were observed when compared with the 0.1% non-specific IgY treatment group in the PB ($P < 0.05$). Significantly lower levels of IL-1 β and IL-22 at 2 h (E Fig. 2); OVA-specific IgE and IL-1 β at 4 h (Fig. 2); and IL-9 at 8 h (E Fig. 2) in the fluticasone propionate

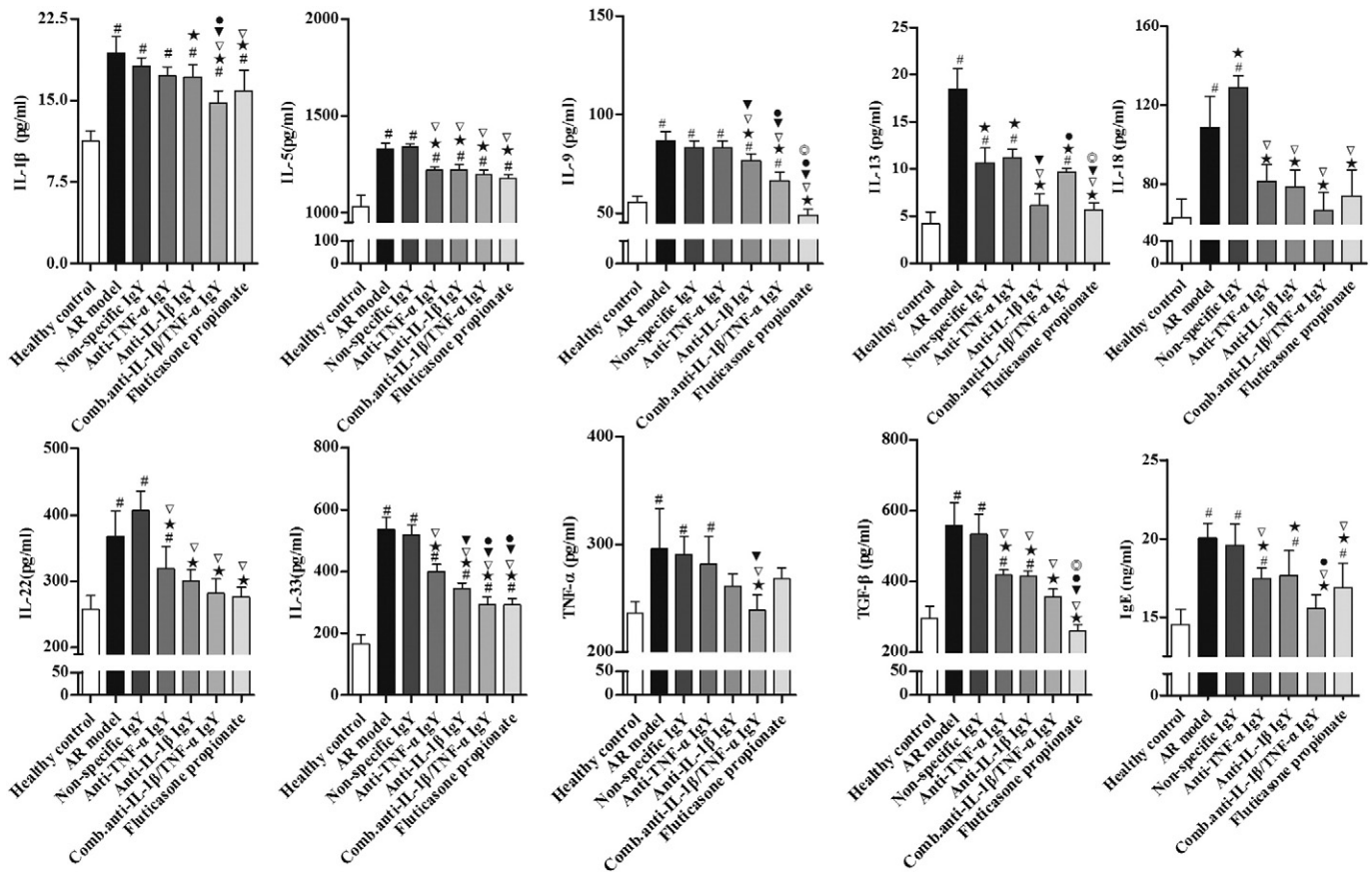


Fig. 2. Levels of inflammatory cytokines ($X \pm S$) in the PB at 4 h in guinea pigs with allergic rhinitis after the different treatments. $n = 3$ per group. Healthy control, the healthy control group; AR model, the AR model group; Non-specific IgY, the 0.1% non-specific IgY treatment group; Anti-TNF- α IgY, the 0.1% anti-TNF- α IgY treatment group; Anti-IL-1 β IgY, the 0.1% anti-IL-1 β IgY treatment group; Comb. anti-IL-1 β /TNF- α IgY, the 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group; Fluticasone propionate, the fluticasone propionate treatment group. #: $P < 0.05$ compared with the healthy control, *: $P < 0.05$ compared with the AR model group, ∇: $P < 0.05$ compared with the 0.1% non-specific IgY treatment group, ▼: $P < 0.05$ compared with the 0.1% anti-TNF- α IgY treatment group, ●: $P < 0.05$ compared with the 0.1% anti-IL-1 β IgY treatment group, ⊙: $P < 0.05$ compared with the 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group.

suspension treatment group were observed when compared with the 0.1% non-specific IgY treatment group in the PB ($P < 0.05$).

Additionally, the levels of TNF- α , IL-5, IL-13, IL-18 and TGF- β at 2 h (E Fig. 2); TNF- α , OVA-specific IgE, TGF- β , IL-18 and IL-22 at 4 h (Fig. 2); and OVA-specific IgE, TNF- α , IL-9, IL-13 and IL-18 at 8 h (E Fig. 2) in the 0.1% combined anti-IL-1 β and TNF- α IgY treatment group were no obvious differences compared to the normal control in the PB ($P > 0.05$). The levels of IL-18 at 4 h (Fig. 2) and TNF- α , IL-13 and IL-18 at 8 h (E Fig. 2) in the 0.1% anti-TNF- α IgY treatment group were no obvious differences compared to the normal control ($P > 0.05$) in the PB. The levels of IL-13 and IL-18 at 2 h (E Fig. 2); IL-13, IL-18, IL-22 and TNF- α at 4 h (Fig. 2); and IL-13, IL-18 and TNF- α at 8 h (E Fig. 2) in the 0.1% anti-IL-1 β IgY treatment group were no obvious differences compared to the normal control in the PB ($P > 0.05$). The levels of IL-1 β , IL-5, TNF- α , TGF- β and OVA-specific IgE at 2 h (E Fig. 2); IL-9, IL-13, IL-18, IL-22, TNF- α and TGF- β at 4 h (Fig. 2); and IL-1 β , IL-9, IL-13, IL-18, TNF- α , TGF- β and OVA-specific IgE at 8 h (E Fig. 2) in the fluticasone propionate suspension treatment group were no obvious differences compared to the normal control in the PB ($P > 0.05$).

3.3. Changes in the inflammatory cytokine content in the NLF

In the NLF, significantly lower levels of IL-1 β , TNF- α , IL-5, IL-9, IL-13, IL-18, IL-22, IL-33, TGF- β and OVA-specific IgE at 2 h (Fig. 3); IL-1 β , TNF- α , IL-5, IL-9, IL-13, IL-22, IL-33, TGF- β and OVA-specific IgE at 4 h (E Fig. 3); and IL-1 β , TNF- α , IL-5, IL-9, IL-13, IL-22, IL-33, TGF- β and OVA-specific IgE at 8 h (E Fig. 3) in the 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group were observed when compared

with the AR model group ($P < 0.05$). Additionally, the levels of OVA-specific IgE and IL-13 at 2 h (Fig. 3); IL-13 and IL-33 at 4 h (E Fig. 3); and OVA-specific IgE, IL-33 and TGF- β at 8 h (E Fig. 3) in the 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group were also significantly lower than that in the 0.1% anti-TNF- α IgY treatment group in the NLF ($P < 0.05$). The levels of OVA-specific IgE at 2 h, IL-13 at 4 h (E Fig. 3); and OVA-specific IgE and TGF- β at 8 h (E Fig. 3) in the 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group were also significantly lower than that in the 0.1% anti-IL-1 β IgY treatment group in the NLF ($P < 0.05$).

In addition, significantly lower levels of IL-1 β , TNF- α , IL-5, IL-9, IL-18, IL-22, IL-33, TGF- β and OVA-specific IgE at 2 h (Fig. 3); IL-1 β , TNF- α , IL-5, IL-9, IL-33 and OVA-specific IgE at 4 h (E Fig. 3); and IL-1 β , TNF- α , IL-5, IL-9 and IL-33 at 8 h (E Fig. 3) in the 0.1% anti-TNF- α IgY treatment group, the 0.1% anti-IL-1 β IgY treatment group and the fluticasone propionate suspension treatment group were observed when compared with the AR model group in the NLF ($P < 0.05$). Significantly lower levels of IL-13 at 2 h (Fig. 3); IL-13 and TGF- β at 4 h (E Fig. 3); and IL-13 and IL-22 at 8 h (E Fig. 3) in the 0.1% anti-IL-1 β IgY treatment group and the fluticasone propionate suspension treatment group were observed when compared with the AR model group in the NLF ($P < 0.05$). Significantly lower levels of IL-22 in the fluticasone propionate suspension treatment group at 4 h (E Fig. 3); and TGF- β in the 0.1% anti-TNF- α IgY treatment group and the fluticasone propionate suspension treatment group at 8 h (E Fig. 3) were observed when compared with the AR model group in the NLF ($P < 0.05$).

Moreover, significantly lower levels of IL-1 β , TNF- α , IL-5, IL-9, IL-33 and OVA-specific IgE at 2 h (Fig. 3); IL-1 β , IL-5, IL-9, IL-22, IL-33, TGF- β

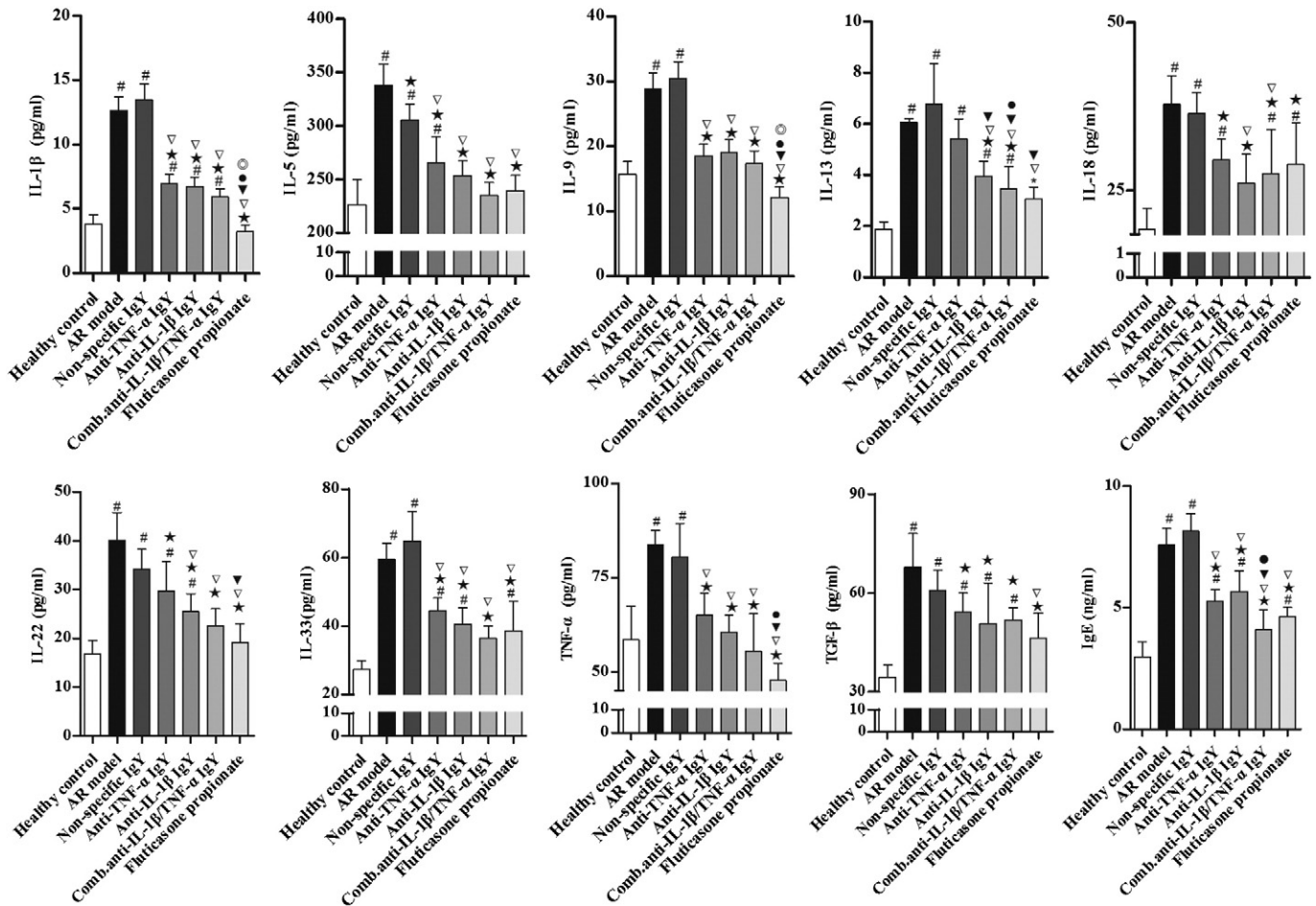


Fig. 3. Levels of inflammatory cytokines ($\bar{X} \pm S$) in the NLF at 2 h in guinea pigs with allergic rhinitis after the different treatments. $n = 3$ per group. Healthy control, the healthy control group; AR model, the AR model group; Non-specific IgY, the 0.1% non-specific IgY treatment group; Anti-TNF- α IgY, the 0.1% anti-TNF- α IgY treatment group; Anti-IL-1 β IgY, the 0.1% anti-IL-1 β IgY treatment group; Comb-anti-IL-1 β /TNF- α IgY, the 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group; Fluticasone propionate, the fluticasone propionate treatment group. #: $P < 0.05$ compared with the healthy control; ★: $P < 0.05$ compared with the AR model group; ▽: $P < 0.05$ compared with the 0.1% non-specific IgY treatment group; ●: $P < 0.05$ compared with the 0.1% anti-IL-1 β IgY treatment group; ⊙: $P < 0.05$ compared with the 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group.

and OVA-specific IgE at 4 h (E Fig. 3); and IL-1 β , IL-9, IL-22, TGF- β and OVA-specific IgE at 8 h (E Fig. 3) in the 0.1% anti-TNF- α IgY treatment group, the 0.1% anti-IL-1 β IgY treatment group and the fluticasone propionate suspension treatment group were observed when compared with the 0.1% non-specific IgY treatment group in the NLF ($P < 0.05$). Significantly lower levels of IL-13 and IL-22 at 2 h (Fig. 3); IL-5, IL-33 and TNF- α at 8 h (E Fig. 3) in the 0.1% anti-IL-1 β IgY treatment group and the fluticasone propionate suspension treatment group were observed when compared with the 0.1% non-specific IgY treatment group in the NLF ($P < 0.05$). Significantly lower levels of IL-18 at 2 h (Fig. 3) in the 0.1% anti-IL-1 β IgY treatment group and IL-13 at 4 h (E Fig. 3) in the 0.1% anti-TNF- α IgY treatment group and the fluticasone propionate suspension treatment group were observed when compared with the 0.1% non-specific IgY treatment group in the NLF ($P < 0.05$). Significantly higher level of IL-18 at 8 h (E Fig. 3) was observed in the 0.1% anti-IL-1 β and TNF- α IgY treatment group compared to the normal control, the allergic rhinitis model group and the 0.1% non-specific IgY treatment group in the NLF ($P < 0.05$).

Additionally, the levels of OVA-specific IgE, TNF- α , IL-5, IL-9, IL-22 and IL-33 at 2 h (Fig. 3); IL-1 β , IL-5, IL-9, IL-13, IL-33, TNF- α and OVA-specific IgE at 4 h (E Fig. 3); and OVA-specific IgE, TNF- α , IL-1 β , IL-5, IL-22 and TGF- β at 8 h (E Fig. 3) in the 0.1% combined anti-IL-1 β and TNF- α IgY treatment group were no obvious differences compared to the normal control in the NLF ($P > 0.05$). The levels of IL-9 and TNF- α at 2 h (Fig. 3); IL-1 β , IL-5, IL-18, TNF- α and OVA-specific IgE (E Fig. 3)

at 4 h; and IL-1 β , IL-18 and TNF- α at 8 h (E Fig. 3) in the 0.1% anti-TNF- α IgY treatment group were no obvious differences compared to the normal control ($P > 0.05$) in the NLF. The levels of IL-5, IL-9, IL-18 and TNF- α at 2 h (Fig. 3); IL-1 β , IL-5, IL-18, TNF- α and OVA-specific IgE at 4 h (E Fig. 3); and IL-1 β , IL-5, IL-18 and TNF- α at 8 h (E Fig. 3) in the 0.1% anti-IL-1 β IgY treatment group were no obvious differences compared to the normal control in the NLF ($P > 0.05$). The levels of IL-1 β , IL-5, IL-9, IL-13, IL-22, TNF- α and TGF- β at 2 h (Fig. 3); IL-1 β , IL-5, IL-9, IL-18, IL-22, IL-33, TNF- α , TGF- β and OVA-specific IgE at 4 h (E Fig. 3); and IL-1 β , IL-13, IL-18, IL-22, TNF- α and TGF- β at 8 h (E Fig. 3) in the fluticasone propionate suspension treatment group were no obvious differences compared to the normal control in the PB ($P > 0.05$).

4. Discussion

When sensitized animals again exposing to an allergen, IgE bound to Fc ϵ RI on eosinophils and mast cells is crosslinked by allergen, resulting in the release of the cells' diverse preformed and newly synthesized mediators, which cause early-phase reaction. IgE-mediated type I early-phase reaction occurs within 5–30 minutes in allergic rhinitis, which shows swelling of nasal mucosa, sneezing, itching, nasal rubbing, rhinorrhoea and airway hyperresponsiveness [3]. In guinea pig AR model, guinea pigs appeared typical allergic symptoms (itching, sneezing, nasal rubbing, rhinorrhoea and airway hyperresponsiveness). The guinea pigs death was due to airway hyperresponsiveness, such as

tachypnea, lip cyanoderma and muscle spasms in OVA-sensitized/challenged stages, and showed pulmonary atelectasis in pathological anatomy. However, the AR symptom scores (nasal rubbing, sneezing and rhinorrhea) of guinea pigs challenged with OVA were significantly reduced after the 0.1% anti-TNF- α IgY, 0.1% anti-IL-1 β IgY or 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY intranasal instillation therapy, particularly 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY, compared to the allergic rhinitis model group ($P < 0.05$) (Fig. 1 and E Fig. 1). Zhang et al. [21] demonstrated that in murine tracheal segments culture, presence of IL-1 β increased 5-hydroxytryptamine induced airway contraction in a time- and concentration-dependent way. Makwana et al. [22] demonstrated that in guinea pig tracheal segments culture, the trachea exposed to TNF- α concentration-dependently potentiated contractions to 5-hydroxytryptamine. Haenueki et al. [23] demonstrated that IL-33-stimulated mast cells and basophils contributed to the early-phase AR manifestation through increasing histamine release. Human nasal fibroblasts and airway smooth muscle cells exposed to IL-1 β or TNF- α increased IL-33 production [24,25]. The above mentioned results suggest that proinflammatory cytokine IL-1 β and TNF- α are prior to proinflammatory cytokine IL-33 in allergic inflammation pathological reaction process, as well as blockade of IL-1 β and TNF- α can largely reduce allergic inflammation pathological reaction. Our data showed that the intranasal instillation therapy of 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY effectively alleviated the allergic early-phase reaction symptoms in OVA-challenged guinea pigs. Although it is thought that glucocorticosteroid still is the best drug for allergic disease therapy, the therapeutic effect of 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY was same as glucocorticosteroid, fluticasone, which indicates that the combined anti-IL-1 β IgY and anti-TNF- α IgY may be to substitute glucocorticosteroid for treating allergic rhinitis via nasal drip, meanwhile can avoid various side effects of glucocorticosteroid.

When sensitized mammals again exposing to an allergen, late-phase reactions occurs within 2–24 h in allergic rhinitis. Proinflammatory cytokines, Th2 cytokines and inflammatory cells lead to the occurrence and development of pathological responses in allergic rhinitis [1–3]. In fact, the serum total and OVA-specific IgE [1,26–28], TNF- α [6], IL-9 [5], IL-18 [7,29], IL-22 [12,30] and IL-33 [8,31] levels significantly were increased in guinea pigs, mice and patients with AR. Moreover, the levels of OVA-specific IgE [1], IL-4, IL-5, IL-13, IL-33 [11,32], IL-18 [10,33] and TGF- β [16] in the NLF significantly increased in AR guinea pigs, mice and patients with AR. Our studies also showed that IL-1 β , TNF- α , IL-18, IL-22, IL-33, TGF- β (proinflammatory and inflammatory cytokines); IL-5, IL-9, IL-13 (Th2 cytokines); and OVA-specific IgE levels in the PB and NLF elevated and contributed to the development of late-phase reaction of allergic inflammation in AR guinea pigs induced by OVA (Fig. 2, Fig. 3, E Fig. 2 and E Fig. 3). However, the intranasal administration of the combined anti-IL-1 β IgY and anti-TNF- α IgY to AR guinea pigs showed a significant reduction in the levels of OVA-specific IgE, TNF- α , IL-1 β , IL-5, IL-9, IL-13, IL-18, IL-22, IL-33 and TGF- β during 2–8 h in the PB ($P < 0.05$), as well as the decrease in the levels of IL-1 β , TNF- α , IL-9, IL-13, IL-33 and TGF- β and the decrease in the levels of OVA-specific IgE, IL-1 β , IL-9, IL-13 and IL-33 were more remarkable than that after the 0.1% anti-TNF- α IgY and the 0.1% anti-IL-1 β IgY alone intranasal administration ($P < 0.05$) (Fig. 2 and E Fig. 2). Iwasaki et al. [34] also demonstrated that the serum OVA-specific IgE level significantly was reduced in TNF- α (–/–)AR mice induced by OVA. Anti-IL-33 antibody treatment significantly reduced the serum total and OVA-specific IgE levels in AR mice induced by OVA [26,28]. The intranasal administration of the combined anti-IL-1 β IgY and anti-TNF- α IgY to AR guinea pigs showed a significant reduction in the levels of OVA-specific IgE, TNF- α , IL-1 β , IL-5, IL-9, IL-13, IL-22, IL-33 and TGF- β during 2–8 h in the NLF ($P < 0.05$), in addition, the decrease in the levels of OVA-specific IgE, IL-13, IL-33 and TGF- β and the decrease in the levels of OVA-specific IgE, IL-13 and TGF- β were more remarkable than that after the 0.1% anti-TNF- α IgY and the 0.1% anti-IL-1 β IgY alone intranasal administration ($P < 0.05$) (Fig. 3 and E

Fig. 3). These data suggest that the combined anti-IL-1 β IgY and anti-TNF- α IgY intranasal treatment has better curative effect for AR therapy than that of a single agent, as well as the therapeutic effect is similar to glucocorticosteroid, which can avoid side effects of glucocorticosteroid. It is worth noting that the level of IL-18 in the NLF only was lower at 2 h in the 0.1% combined anti-IL-1 β and TNF- α IgY treatment group than that in the AR model group, but was higher during 4–8 h in the 0.1% combined anti-IL-1 β and TNF- α IgY treatment group than that in the AR model group. Salagianni et al. [35] showed that IL-18 also plays an essential role in IgE suppression. In vitro, IL-18 synergized with IL-12 to promote Th1/T cytotoxic 1 and inhibit Th2/T cytotoxic 2 differentiation. In vivo, IL-18(–/–) mice exhibited higher IgE and IgG1 levels compared with wild-type mice after immunization with OVA/alum. Our data showed that level of IL-18 in the NLF increased during 4 h to 8 h in the 0.1% combined anti-IL-1 β and TNF- α IgY therapy, which suggest that elevation of IL-18 may be advantageous because it perhaps can inhibit Th2 cytokines production.

Present studies have demonstrated that the TNF- α , IL-1 β , IL-5, IL-9, IL-13, IL-22, IL-33 and TGF- β etc cytokines are involved in allergic inflammation pathological occurrence and development. However these cytokines can interact to promote them further production and aggravate allergic inflammation pathological responses. Such as, IL-9 stimulated human airway smooth muscle cells released Th2-associated chemokines (eotaxin1/CCL11) [36]. IL-13 significantly stimulated airway fibroblasts to produce collagen type-1 and increased total and active forms of TGF- β 1 in asthma, which potentiates airway remodeling [37]. Treatment of primary human bronchial epithelial cells with TGF- β 1 significantly induced the epithelial – mesenchymal transition, and IL-1 β enhanced the transition [38]. IL-33 increased the levels of IL-4, IL-5 and IL-13 secreted by nasal mucosa in AR mice [32]. IL-33 more potently induced expansion of IL-13-producing type 2 innate lymphoid cells, correlating with airway contraction [39]. IL-33 or IL-33 and IL-13 synergistical stimulation of OVA-sensitized mice' lung fibroblasts resulted in release of eotaxin1/CCL11 [40]. IL-33 promoted collagen synthesis both from asthmatic fibroblasts from pediatric patients and after intranasal administration in mice [41]. When OVA-sensitized mice were exposed to the OVA with IL-1 β or IL-33, they developed IgE antibodies, in particular, IL-33 induced robust and long-lasting Th2 cells that produced a large quantity of IL-5 and IL-13 and asthma-like airway pathology [42]. Airway exposure to allergen in sensitized individuals causes the release of ATP and uric acid, activating the NLRP3 inflammasome complex and cleaving pro-IL-1 β to mature IL-1 β through caspase-1. IL-1 β creates a pro-inflammatory milieu with the production of IL-6 and chemokines which mobilize neutrophils and enhance Th17 cell differentiation in the lung [43]. Human nasal fibroblasts exposed to IL-1 β or TNF- α increased IL-33 production [24], as well as TNF- α upregulated IL-33 expression by cultured airway smooth muscle cells derived from patients with severe asthma [25]. However, antibodies to IL-1 β or IL-1 α alone blocked airway inflammation and the expression of IL-4 and adhesion molecules in the lung in mice exposed to TDI [44]. Mo et al. [15] also demonstrated that Infliximab reduced allergic symptoms, total and OVA-specific IgE levels and IL-4 production in AR mice induced by OVA. Our previous study also demonstrated that IL-1 β may be the initiating and aggravating proinflammatory cytokine response in the development and progression of allergic asthma inflammation, as well as local treatment using an aerosolized anti-IL-1 β IgY antibody could effectively treat allergic asthma [17]. In the present study, our results showed remarkable decrease in the levels of Th2 cytokines, proinflammatory cytokines, inflammatory cytokines and OVA-specific IgE in the PB and NLF after intranasal treatment of combined anti-IL-1 β IgY and anti-TNF- α IgY in AR guinea pigs induced by OVA, as well as therapy of combined anti-IL-1 β IgY and anti-TNF- α IgY was better than that in anti-TNF- α IgY or anti-IL-1 β IgY alone therapy. In addition, our results also indicates that in cytokines network, IL-1 β and TNF- α are anterior to the other inflammatory cytokines in pathological response procedures. Therefore,

the combined blockade of IL-1 β and TNF- α using combined anti-IL-1 β IgY and anti-TNF- α IgY can more effectively suppress allergic inflammation pathological responses, treat allergic rhinitis and avoid side effects of glucocorticosteroid. Additionally, oral IgY antibodies against bacteria and viruses have been applied to treat human diseases (rotavirus diarrhea [45], pulmonary pseudomonas aeruginosa infections [46]), and no negative side effects of IgY treatment have been noted during these 10 years [46].

In summary, we demonstrated that intranasal treatment using combined anti-IL-1 β IgY and anti-TNF- α IgY may be an more effective therapy for allergic rhinitis than that using anti-IL-1 β IgY or anti-TNF- α IgY alone therapy, as well as can avoid side effects of glucocorticosteroid. The blockade of IL-1 β and TNF- α by the intranasal instillation of combined anti-IL-1 β IgY and anti-TNF- α IgY could be a potential alternative strategy for preventing and treating allergic rhinitis.

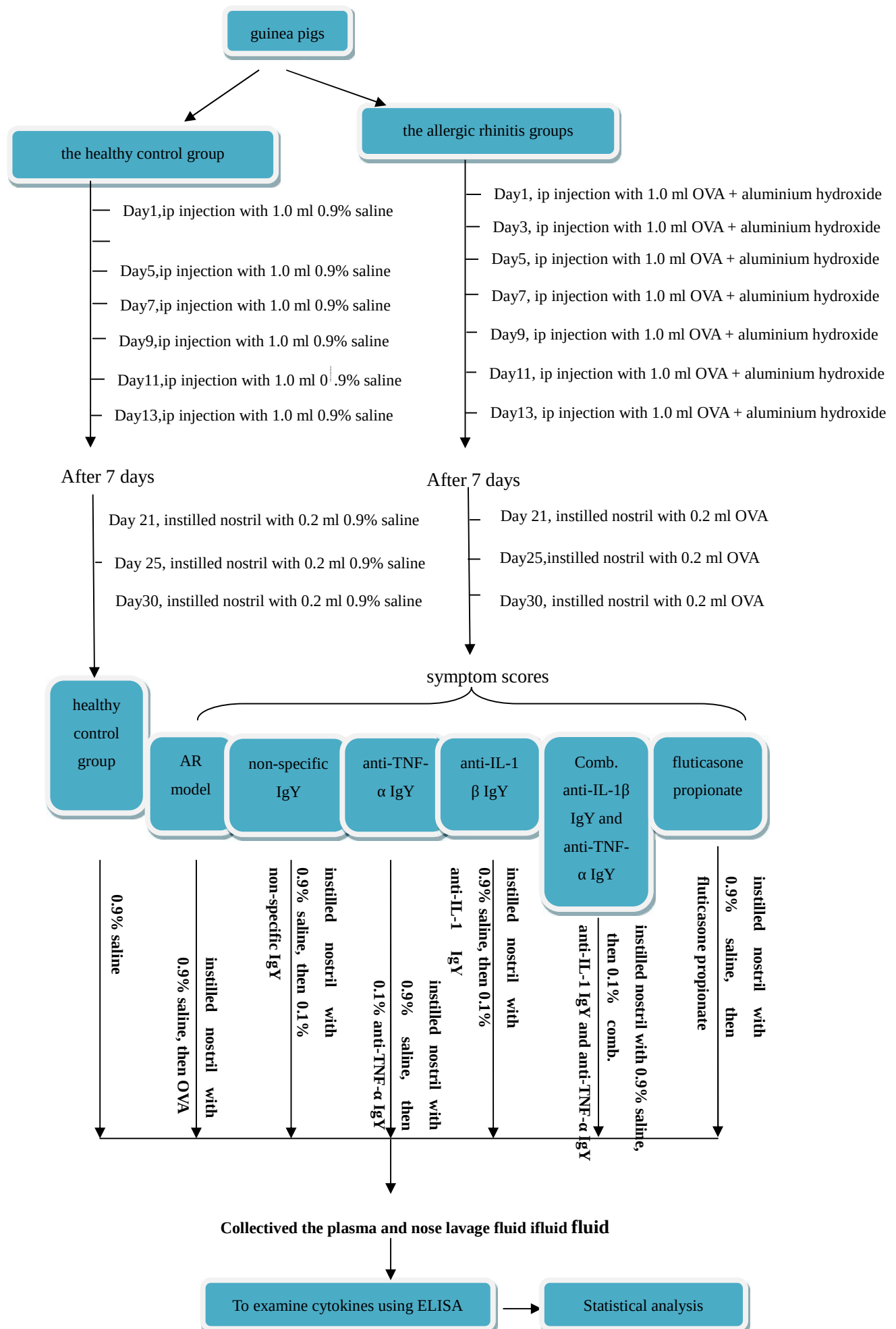
Refer to Web version on PubMed Central for supplementary material. Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.intimp.2014.12.002>.

Acknowledgments

This work was supported by grants from the Jiangxi Provincial Department of Science and Technology, Major Projects (The fourth batch, 2006). We thank Prof. Ming-zhi Lu (Department of Pathology, People's Hospital of Jiangxi Province) for performing the pathological diagnoses.

References

- [1] Thakare VN, Osama MM, Naik SR. Therapeutic potential of curcumin in experimentally induced allergic rhinitis in guinea pigs. *Int Immunopharmacol* 2013;17:18–25 [PMID: 23665314].
- [2] Nam SY, Kim MH, Seo Y. The (2'S,7'S)-O-(2-methylbutanoyl)-columbianetin as a novel allergic rhinitis-control agent. *Life Sci* 2014;98:103–12 [PMID:24447626].
- [3] Galli SJ, Tsai M, Piliponsky AM. The development of allergic inflammation. *Nature* 2008;454:445–54 [PMID:18650915].
- [4] Daoud A, Xie Z, Ma Y, Wang T, Tan G. Changes of T-helper type 1/2 cell balance by anticholinergic treatment in allergic mice. *Ann Allergy Asthma Immunol* 2014;112:249–55 [PMID:24428969].
- [5] Ciprandi G, De Amici M, Marseglia GL. Serum IL-9 levels and sublingual immunotherapy: preliminary report. *J Biol Regul Homeost Agents* 2011;25:295–7 [PMID:21880201].
- [6] Boghdadi G, Marei A, Ali A, Lotfy G, Abdulfattah M, Sorour S. Immunological markers in allergic rhinitis patients treated with date palm immunotherapy. *Inflamm Res* 2012;61:719–24 [PMID:22456954].
- [7] Ando M, Shima M. Serum interleukins 12 and 18 and immunoglobulin E concentrations and allergic symptoms in Japanese schoolchildren. *J Investig Allergol Clin Immunol* 2007;17:14–9 [PMID:17323858].
- [8] Kamekura R, Kojima T, Takano K, Go M, Sawada N, Himi T. The role of IL-33 and its receptor ST2 in human nasal epithelium with allergic rhinitis. *Clin Exp Allergy* 2012;42:218–28 [PMID:22233535].
- [9] Sim TC, Grant JA, Hilsmeier KA, Fukuda Y, Alam R. Proinflammatory cytokines in nasal secretions of allergic subjects after antigen challenge. *Am J Respir Crit Care Med* 1994;149(2 Pt 1):339–44 [PMID:8306027].
- [10] Verhaeghe B, Gevaert P, Holtappels G, Lukat KF, Lange B, Van Cauwenberge P, et al. Up-regulation of IL-18 in allergic rhinitis. *Allergy* 2002;57:825–30 [PMID:12169180].
- [11] Asaka D, Yoshikawa M, Nakayama T, Yoshimura T, Moriyama H, Otori N. Elevated levels of interleukin-33 in the nasal secretions of patients with allergic rhinitis. *Int Arch Allergy Immunol* 2012;158(Suppl. 1):47–50 [PMID:22627366].
- [12] Farfariello V, Amantini C, Nabissi M, Morelli MB, Aperio C, Caprodossi S, et al. IL-22 mRNA in peripheral blood mononuclear cells from allergic rhinitic and asthmatic pediatric patients. *Pediatr Allergy Immunol* 2011;22:419–23 [PMID:21535180].
- [13] Antoniu SA. Cytokine antagonists for the treatment of asthma: progress to date. *BioDrugs* 2009;23:241–51 [PMID:19697966].
- [14] Oh CK, Leigh R, McLaurin KK, Kim K, Hultquist M, Molfino NA. A randomized, controlled trial to evaluate the effect of an anti-interleukin-9 monoclonal antibody in adults with uncontrolled asthma. *Respir Res* 2013;14:93 [PMID:24050312].
- [15] Mo JH, Kang EK, Quan SH, Rhee CS, Lee CH, Kim DY. Anti-tumor necrosis factor- α treatment reduces allergic responses in an allergic rhinitis mouse model. *Allergy* 2011;66:279–86 [PMID:21208219].
- [16] Kim YH, Park CS, Jang TY. Immunologic properties and clinical features of local allergic rhinitis. *J Otolaryngol Head Neck Surg* 2012;41:51–7 [PMID:22498269].
- [17] Wei-Xu H, Qin X, Zhu W, Yuan-yi C, Li-feng Z, Zhi-yong L, et al. Therapeutic potential of anti-IL-1 β IgY in guinea pigs with allergic asthma induced by ovalbumin. *Mol Immunol* 2014;58:139–49 [PMID:24355520].
- [18] Bahekar PC, Shah JH, Ayer UB, Mandhane SN, Thennati R. Validation of guinea pig model of allergic rhinitis by oral and topical drugs. *Int Immunopharmacol* 2008;8:1540–51 [PMID:18627797].
- [19] Magnusson B, Kligman AM. The identification of contact allergens by animal assay. The guinea pig maximization test. *J Invest Dermatol* 1969;52:268–76 [PMID:5774356].
- [20] Brozmanová M, Calkovský V, Plevková J, Bartos V, Plank L, Tatár M. Early and late allergic phase related cough response in sensitized guinea pigs with experimental allergic rhinitis. *Physiol Res* 2006;55:577–84 [PMID:16343041].
- [21] Zhang Y, Cardell LO, Adner M. IL-1 β induces murine airway 5-HT $_2A$ receptor hyperresponsiveness via a non-transcriptional MAPK-dependent mechanism. *Respir Res* 2007;8:29 [PMID:17407556].
- [22] Makwana R, Gozzard N, Spina D, Page C. TNF- α -induces airway hyperresponsiveness to cholinergic stimulation in guinea pig airways. *Br J Pharmacol* 2012;165:1978–91 [PMID:21951209].
- [23] Haenuli Y, Matsushita K, Fuutatsugi-yumikura S, Ishii KJ, Kawagoe T, Imoto Y, et al. A critical role of IL-33 in experimental allergic rhinitis. *J Allergy Clin Immunol* 2012;130:184–94 [PMID:22460070].
- [24] Nomura K, Kojima T, Fuchimoto J, Obata K, Keira T, Himi T, et al. Regulation of interleukin-33 and thymic stromal lymphopoietin in human nasal fibroblasts by proinflammatory cytokines. *Laryngoscope* 2012;122:1185–92 [PMID:22460292].
- [25] Préfontaine D, Lajoie-Kadoch S, Foley S, Audusseau S, Olivenstein R, Halayko AJ, et al. Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. *J Immunol* 2009;183:5094–103 [PMID:19801525].
- [26] Kim YH, Yang TY, Park CS, Ahn SH, Son BK, Kim JH, et al. Anti-IL-33 antibody has a therapeutic effect in a murine model of allergic rhinitis. *Allergy* 2012;67:183–90 [PMID:22050307].
- [27] Kim YH, Park CS, Lim DH, Ahn SH, Son BK, Kim JH, et al. Beneficial effect of anti-interleukin-33 on the murine model of allergic inflammation of the lower airway. *J Asthma* 2012;49:738–43 [PMID:22799279].
- [28] Liu X, Li M, Wu Y, Zhou Y, Zeng L, Huang T. Anti-IL-33 antibody treatment inhibits airway inflammation in a murine model of allergic asthma. *Biochem Biophys Res Commun* 2009;386:181–5 [PMID: 19508862].
- [29] Kurt E, Aksu K, Dokumacioglu A, Alatas O. Bronchial hyperresponsiveness in seasonal allergic rhinitis patients is associated with increased IL-18 during natural pollen exposure. *Cytokine* 2012;60:100–3 [PMID:22819246].
- [30] Besnard AG, Sabat R, Dumoutier L, Renaud JC, Willart M, Lambrecht B, et al. Dual role of IL-22 in allergic airway inflammation and its cross-talk with IL-17A. *Am J Respir Crit Care Med* 2011;183:1153–63 [PMID:21297073].
- [31] Glöck J, Rymarczyk B, Rogala B. Serum IL-33 but not ST2 level is elevated in intermittent allergic rhinitis and is a marker of the disease severity. *Inflamm Res* 2012;61:547–50 [PMID:22349136].
- [32] Lin L, Zhao X, Yan W, Guo Y, Liang S. Amelioration of Muc5b mucin hypersecretion is enhanced by IL-33 after 2-APB administration in a murine model of allergic rhinitis. *Biotech Histochem* 2014;89:273–86 [PMID:24111497].
- [33] Erin EM, Leaker BR, Zachariasiewicz AS, Higgins LA, Williams TJ, Boyce MJ, et al. Single dose topical corticosteroid inhibits IL-5 and IL-13 in nasal lavage following grass pollen challenge. *Allergy* 2005;60:1524–9 [PMID:16266385].
- [34] Iwasaki M, Saito K, Takemura M, Sekikawa K, Fujii H, Yamada Y, et al. TNF- α contributes to the development of allergic rhinitis in mice. *J Allergy Clin Immunol* 2003;112:134–40 [PMID:12847490].
- [35] Salagianni M, Wong KL, Thomas MJ, Noble A, Kemeny DM. An essential role for IL-18 in CD8 T cell-mediated suppression of IgE responses. *J Immunol* 2007;178:4771–8 [PMID:17404257].
- [36] Gouni AS, Hamid Q, Rahman SM, Hoek J, Yang J, Shan L. IL-9-mediated induction of eotaxin1/CCL11 in human airway smooth muscle cells. *J Immunol* 2004;173:2771–9 [PMID:15294996].
- [37] Firszt R, Francisco D, Church TD, Thomas JM, Ingram JL, Kraft M. Interleukin-13 induces collagen type-1 expression through matrix metalloproteinase-2 and transforming growth factor- β 1 in airway fibroblasts in asthma. *Eur Respir J* 2014;43:464–73 [PMID:23682108].
- [38] Doerner AM, Zuraw BL. TGF- β 1 induced epithelial to mesenchymal transition (EMT) in human bronchial epithelial cells is enhanced by IL-1 β but not abrogated by corticosteroids. *Respir Res* 2009;10:100 [PMID:19857272].
- [39] Barlow JL, Peel S, Fox J, Panova V, Hardman CS, Camelo A, et al. IL-33 is more potent than IL-25 in provoking IL-13-producing nuocytes (type 2 innate lymphoid cells) and airway contraction. *J Allergy Clin Immunol* 2013;132:933–41 [PMID:23810766].
- [40] Kurokawa M, Matsukura S, Kawaguchi M, Ieki K, Suzuki S, Odaka M, et al. Expression and effects of IL-33 and ST2 in allergic bronchial asthma: IL-33 induces eotaxin production in lung fibroblasts. *Int Arch Allergy Immunol* 2011;155(Suppl. 1):12–20 [PMID:21646790].
- [41] Saglani S, Lui S, Ullmann N, Campbell GA, Sherburn RT, Mathie SA, et al. IL-33 promotes airway remodeling in pediatric patients with severe steroid-resistant asthma. *J Allergy Clin Immunol* 2013;132:676–85 [e13. [PMID: 23759184]].
- [42] Kobayashi T, Iijima K, Checkel JL, Kita H. IL-1 family cytokines drive Th2 and Th17 cells to innocuous airborne antigens. *Am J Respir Cell Mol Biol* 2013;49:989–98 [PMID:23837489].
- [43] Besnard AG, Togbe D, Couillin I, Tan Z, Zheng SG, Erard F, et al. Inflammasome-IL-1-Th17 response in allergic lung inflammation. *J Mol Cell Biol* 2012;4:3–10 [PMID:22147847].
- [44] Johnson VJ, Yuceosoy B, Luster MI. Prevention of IL-1 signaling attenuates airway hyperresponsiveness and inflammation in a murine model of toluene diisocyanate-induced asthma. *J Allergy Clin Immunol* 2005;116:851–8 [PMID:16210060].
- [45] Rahman S, Higo-Moriguchi K, Htun KW, Taniguchi K, Icatto Jr FC, Tsuji T, et al. Randomized placebo-controlled clinical trial of immunoglobulin Y as adjunct to standard supportive therapy for rotavirus-associated diarrhea among pediatric patients. *Vaccine* 2012;30:4661–9 [PMID:22575165].
- [46] Nilsson E, Kollberg H, Johannesson M, Wejaker PE, Carlander D, Larsson A. More than 10 years' continuous oral treatment with specific immunoglobulin Y for the prevention of *Pseudomonas aeruginosa* infections: a case report. *J Med Food* 2007;10:375–8 [PMID:17651078].



Therapeutic Potential of Combined Anti-IL-1 β IgY and TNF- α IgY in Guinea Pigs with Allergic Rhinitis Induced by Ovalbumin

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

Running head: Therapeutic mechanism of combined anti-IL-1 β IgY and anti-TNF- α
IgY in allergic rhinitis

Results

Changes in symptom scores of guinea pig with allergic rhinitis

The symptom scores of AR guinea pigs were more than 5 points from the first to tenth OVA challenges in the AR model guinea pigs, the 0.1% non-specific IgY treated guinea pigs and the 0.1% anti-TNF- α IgY treated guinea pigs, from the first to eighth OVA challenges in the 0.1% anti-IL-1 β IgY treated guinea pigs and from the first to sixth OVA challenges in the 0.1% combined anti-IL-1 β IgY and TNF- α IgY treated guinea pigs and fluticasone propionate treated guinea pigs (Fig. 1 and E Fig. 1). Guinea pigs in systemic sensitization and intranasal challenge with OVA appeared to allergy symptoms of nasal rubbing, sneezing and rhinorrhea, even severe dyspnea, lips purple, muscle spasms and death, particularly in OVA challenge phase. The allergic symptoms significantly were alleviated in the 0.1% anti-TNF- α IgY treatment group, 0.1% anti-IL-1 β IgY treatment group, 0.1% combined anti-IL-1 β IgY and anti-TNF- α IgY treatment group and fluticasone propionate treatment group compared with the AR model group and 0.1% non-specific IgY treatment group ($P < 0.05$) (Fig. 1 and E Fig. 1) The symptoms of nasal rubbing, sneezing and accelerated breathing appeared within the first ten minutes in guinea pigs with OVA intranasal challenge in all AR groups, however number of nasal rubbing and sneezing obviously decreased and the symptom of rhinorrhea appeared within the second ten minutes, and moreover, the symptoms of nasal rubbing and sneezing were no obvious and

there was the symptom of rhinorrhea within the third minutes. All symptoms of AR disappeared after 2 hours in guinea pigs with OVA challenge. The symptoms of nasal rubbing, sneezing and rhinorrhea in AR guinea pigs were aggravated following the increase of the number of OVA challenge (Fig. 1 and E Fig. 1). In guinea pigs of a week following sensitization with OVA and after the third OVA challenge the mortality rate was 58.78% (154/262 guinea pigs). During the fourth to tenth OVA challenges the mortality rate of guinea pigs was 40.0% (6/15 guinea pigs) in the AR model group, 33.33% (6/18 guinea pigs) in the 0.1% non-specific IgY treatment group, 11.76% (2/17 guinea pigs) in the 0.1% anti-TNF- α IgY treatment group, 11.76% (2/17 guinea pigs) in the 0.1% anti-IL-1 β IgY treatment group, 11.11% (2/18 guinea pigs) in the 0.1% combined anti-IL-1 β IgY and TNF- α IgY treatment group and 29.41% (5/17 guinea pigs) in the fluticasone propionate treatment group.

