Original Article

Fungal immunomodulatory protein-fve could modulate airway remodel through by affect IL17 cytokine

Yu-Tzu Lee, Chia-Ta Wu, Hai-Lun Sun, Jiunn-Liang Ko, Ko-Haung Lue

Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan
Department of Emergency Medicine, Changhua Christian Hospital, Changhua, Taiwan
School of Medicine, Chung Shan Medical University, Taichung, Taiwan
Department of Pediatrics, Chung Shan Medical University Hospital, Taichung, Taiwan
College of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan

KEYWORDS
Asthma;
Airway remodeling;
Collagen deposition;
FIP-fve;
IL-17;
IL-22

Abstract
Background: Asthma is one of the most common allergic diseases. Our previous studies have reported that FIP-fve in acute allergic mouse model can reduce inflammation, improve the balance of the Th1/Th2 system. However, the effects of reducing airway remodeling on FIP-fve is still unknown.
Objective: We hypothesized that orally administrated FIP-fve should be able to reduce airway remodeling in chronic allergic models.
Methods: The chronic asthma animal model was established with 6-8 weeks female Balb/c mice. After intranasal challenges with OVA, the airway inflammation and AHR were determined by a BUXCO system. BALF was analyzed with Liu’s stain and ELISA assay. Lung histopathologic changes and Collagen deposition were assayed with H&E, Masson’s trichrome and IHC stain.
Results: FIP-fve significantly decreased the number of infiltrating inflammatory cells and Th2 cytokines and increased Th1 cytokines in BALF and serum compared with the OVA sensitized mice. FIP-fve had a better effect than corticosteroid could reduce infiltrating cells especially neutrophils and eosinophils. We also found that the oral FIP-fve group suppressed IL-17 and enhanced IL-22 in the serum and BALF. In addition, oral FIP-fve decreased MMP9 expression, collagen expression and airway remodeling in lung tissues.

Abbreviations used: FIP-fve, fungal immunomodulatory protein-Flammulina velutipes; COPD, Chronic Obstructive Pulmonary Disease; LZ-8, ling zhi-8 protein; AHR, airway hyper-responsiveness; BALF, Bronchoalveolar lavage fluid; NC, Normal control; PC, positive control; Pre-/post- costi, pre-/post- corticosteroid; Pre-/post- FIP, pre-/post- fungal immunomodulatory protein-Flammulina velutipes; MMP9, Matrix metallopeptidase-9.

* Corresponding author. Division of Allergy, Asthma and Rheumatology, Department of Pediatrics, Chung Shan Medical University Hospital, No. 110, Sec. 1, Chien-Kuo N. Road, Taichung, 402, Taiwan. Fax: +886 4 2475 9950.
E-mail addresses: tsz312@hotmail.com (Y.-T. Lee), cshy095@csh.org.tw, kohuang.lue@gmail.com (K.-H. Lue).

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Introduction

Asthma is one of the most common chronic inflammatory diseases of the airways, and the prevalence is increasing in many countries, especially in developed countries. Moreover, severe asthma is one of the main causes of school and work absence. The World Health Organization has defined severe asthma as "uncontrolled asthma" which can result in risk of frequent severe exacerbations (or death) and/or adverse reactions to medications and/or chronic morbidity.

Inhaled corticosteroids are widely used to treat asthma patients. However, there are concerns about the systemic effect of inhaled corticosteroids, particularly as they are likely to be used over long periods of time, in infants, children and older adult. It is clear that asthma is a syndrome with many distinct and overlapping phenotypes with severe glucocorticoid refractory asthma at one end of spectrum of a glucocorticoid responsiveness. Severe and corticosteroid-resistant forms of asthma actually lead to life-threatening attacks, and the mortality rate for this disease is showing an increasing trend. Thus, there is an urgent need to devise more effective treatments with fewer undesirable side effects for asthma.

There have been many clinical and experimental studies over the years which have implied that allergic asthma is much more heterogeneous and complex than just the Th2 mechanisms. These findings suggested that non-Th2 factors such as interferon-γ, IL-17 and neutrophils are frequently found in the lungs of patients with asthma, particularly those with severe asthma or asthma usually resistant to corticosteroid treatment. However, mice exposed to a secondary aeroallergen challenge develop severe AHR associated with the presence of airway neutrophils rather than eosinophils. Neutrophils might also have a causal role in human asthma and establish a new approach to the study of Th17 immune responses in the lung.

IL-17-secreting Th17 cells which were neither Th1 nor Th2 cells, led to a major revision of the Th1/Th2 hypothesis. Th17 cells secrete IL-1β, IL-6, IL-17, IL-21, IL-22, IL-23 and transforming growth factor β (TGFβ). IL-17 acts on a variety of cells such as neutrophils, endothelial cells, epithelial cells and fibroblasts. It is a major cytokine for the recruitment and activation of neutrophils and has been shown to attract neutrophil migration in the lung. IL-17 can enhance airway smooth muscle contraction and proliferation and epithelial permeability of the airways to allergens.

IL-17A produced by Th17 cells contributes to allergen-induced airway hyper-responsiveness through direct effects on airway smooth muscle.

Airway hyper-responsiveness has been diminished and less airway remodeling has been displayed after the chronic allergen challenge in response to house dust mites and ovalbumin sensitization/challenge in Th17-deficient mice. Early childhood asthma attacks and the respiratory syncytial virus might play an important role. In our previous study, we found that FIP-fve could inhibit inflammation and replication of the human respiratory syncytial virus. Moreover, FIP-fve reduced the neutrophils in the above model. Thus, the role of neutrophils in asthma is important in identifying additional options for the treatment of neutrophilic airway inflammation for chronic asthma following FIP-fve treatment.

An earlier study showed that LZ-8, a protein derived from Ganoderma lucidum which is an oriental medicinal mushroom widely used in Asia to promote health and longevity, has immunomodulatory capacities. LZ-8 can effectively promote the activation and maturation of immature DCs, preferring a Th1 response, suggesting that LZ-8 may possess a potential effect in regulating immune responses. Moreover, the amino acid sequence of the immunomodulatory protein FIP-fve from the edible golden needle mushroom is similar to LZ-8 (invariant amino acid residues more than 61.4%). Ko JL et al. reported that FIP-fve stimulates blast-forming activity of human peripheral blood lymphocytes and gene expression of IL-2, IFN-γ, TNF-α, and administration of FIP-fve inhibits systemic anaphylaxis reactions in mice. We have previously demonstrated and Hsieh KY et al. reported that in an animal model of acute allergic response the administration of FIP-fve modifies the immune system by maintaining a Th1/Th2 balance, reducing Th2 cytokine production and reducing inflammatory cells such as eosinophil infiltration in the lung, indicating the effects and features of those compounds on allergic pulmonary inflammation. Moreover, some studies have also reported that the oral immunomodulatory protein can reduce allergic diseases by adjusting the regulatory T cell system.

In this study, using a model of chronic allergic airway inflammation with subepithelial fibrosis, we investigated the effects of alleviating airway inflammation and airway remodeling using FIP-fve and corticosteroids in the pathogenesis.

Methods

Ethics statement

All animal experiments, care and housing requirements and all procedures were performed in accordance with the...
Institutional Animal Care and Use Committee at Chung Shan Medical University (Reference No. 1136).

Mice

Female BALB/c mice at 6–8 weeks of age with body weights of 20–25 g were purchased from the National Laboratory Animal Center (Taipei, Taiwan).

Reagents and challenge

FIP-fve-fungal immunomodulatory protein-fve (FIP-fve) was isolated from Flammulina velutipes. We followed the isolation protocol based on previous studies and each mouse was treated orally with 200 μg FIP-fve. The corticosteroid given as was an oral dose of 75 μg/day.

The mouse model of allergic asthma and challenge

The allergic asthma models were modified according to the method of Chi EY et al. The sensitization protocol was as follows: mice received an intraperitoneal injection of 50 μg of ovalbumin (OVA) complexed with alum on days 1, 2, 3 and an intranasal dose of 5% OVA 50 μl on days 14, 17, 21, 24, 27, 60, 69, 71, 73, 74, and 75. The control group received normal saline with Alum intraperitoneally. Other groups of OVA-treated mice were given FIP-fve or a corticosteroid. The pre-groups were treated with FIP-fve or a corticosteroid on days 1–14 and days 45–60. The post-groups were treated with FIP-fve or a corticosteroid on days 14–28 and days 60–75. On day 76 following the methacholine challenge, airway hyperresponsiveness (AHR) was determined in all the experimental groups, and the mice were sacrificed as well. Animals known to be high IgE responders were used, and the mice were maintained on an ovalbumin (OVA)-free diet and were individually housed in rack-mounted stainless steel cages with free access to food and water. Ovalbumin (OVA) was prepared in 1 mg/ml solution with normal saline. Six groups of mice were treated as follows: (1) the normal control group received normal saline plus Alum intraperitoneally and normal saline intranasally; (2) the positive group received 50 μg OVA plus Alum intraperitoneally and 5% OVA (50 μl) intranasally; (3) the pre- and (4) post-FIP-fve groups received 50 μg OVA plus Alum intraperitoneally and 5% OVA (50 μl) intranasally, and were fed 200 μg of FIP-fve. (5) The pre- and (6) post-corticosteroid groups received 50 μg OVA plus Alum intraperitoneally and 5% OVA (50 μl) intranasally, and 75 μg of corticosteroid orally.

The adverse events in the pre- and post oral FIP-fve groups was only weight loss during the FIP-fve feed day, but there were no other adverse reactions in the laboratory data and after stopping FIP-fve feed weights would increase.

Airway hyperresponsiveness and isolation of bronchoalveolar lavage fluid

Airway hyperresponsiveness (AHR) was assessed in unrestrained mice through whole-body barometric plethysmography (Model PLY 3211; Buxco Electronic Inc., Sharon, CT) in order to record enhanced pauses (Penh). Penh, a dimensionless parameter, was used to measure the pulmonary resistance, which was calculated by changing the chamber pressures through methacholine (sigma-Aldrich, St. Louis, MO) challenges during inspiration and expiration. After a brief acclimation to the chamber, the mice received an initial baseline challenge of saline, followed by increasing doses of nebulized methacholine. During exposure to methacholine, each mouse was given either 0 (saline), 5, 10 or 20 mg/ml. Mice remained in the chamber for three minutes. The respiratory rate was counted during the three minute methacholine challenge. After that, the Penh values were averaged and reported as baseline saline values in percentages.

Bronchoalveolar lavage fluid (BALF) was isolated in 1 ml of normal saline. The BALF cellularity was determined using a hemocytometer. The cells were centrifuged, transferred to slides, and were fixed and stained using Liu’s stain. To classify the individual leukocyte populations, 500 cells per slide were counted.

OVA-specific antibodies in serum (IgE, IgG2a)

First, the serum levels of OVA-specific IgE and IgG2a were determined. In short, the 96-well microtiter plates were coated with 100 μl of 100 mg/ml OVA in 0.1 M NaHCO₃ at 4 °C and left unattended overnight. The plates were washed with Tween 20 solution (0.05%) and then blocked with bovine serum albumin (BSA) (3%) for 1 h at 37 °C. After washing, 50 μl of serially diluted sera in 3% BSA was added and incubated at 4 °C and was left unattended overnight. After washing, HRP conjugated with the anti-mouse isotype-specific antibody (BD Pharningen™) and optimal dilutions were added. The plates were incubated at 37 °C for 2 h and were then washed. To determine the IgE/IgG2a, p-nitrophenyl phosphate substrate (Sigma Chemical Co., St Louis, MO, USA) was added and the absorbance at 490 nm was measured.

The measurement of cytokines and MMP9 with ELISA

Serum and BALF samples were collected from each group of mice after the mice were sacrificed. The samples were assayed for the presence of cytokines using the R&D system per the manufacturer’s protocol. After that, IFN-γ, IL-4, IL-5, IL-10, IL-12, IL-13, IL-17, IL-22, TGF-β and MMP9 were assayed according to the manufacturer’s protocol (Quantikine ELISA Kit, R&D system, USA), and the ELISA plate was
read at 450 nm using a Bio-Rad ELISA Reader. The data were then analyzed using SoftMax Pro software. The unknowns were compared using a standard curve containing at least five to seven dilution points, which were the relevant recombiant cytokines on each assay plate.

**Histological analysis**

**Masson’s trichrome stain**
To assess the pathological changes, samples of the lungs were taken after the mice were sacrificed. The samples were fixed in neutrally buffered 10% formaldehyde and were embedded in paraffin. Three micrometer sections were stained with Masson’s trichrome to detect collagen deposition in the lung tissue.

**Hematoxylin and eosin stain (H&E stain or HE stain)**
To assess the pathological changes, samples of the lungs were taken after the mice were sacrificed. The samples were fixed in neutrally buffered 10% formaldehyde and were embedded in paraffin. Five micrometer sections were stained with H&E to detect inflammatory cell infiltration in the lung tissue.

**Statistical analyses**
All data points represent the median ± IQR of the individual mouse groups. Analyses were performed using GraphPad Instat software (San Diego, CA), and the Mann–Whitney nonparametric test was conducted to determine the statistical significance, where appropriate. A p value < 0.05 was considered statistically significant.

The number of animals in each group included in each analysis were NC = 6 mice; PC = 8 mice; and the pre and post treatment FIP-fve or corticosteroid group had 8 mice, respectively.

**Results**

**FIP-fve or corticosteroid affected AHR**

The allergen induced chronic asthmatic animal models were successful. The mice sensitized and challenged with OVA were significantly more sensitive to methacholine exposure than the NC (Fig. 1). In this study, we detected whether FIP-fve or corticosteroids had a beneficial effect during the allergy challenge. According to the results, not only the pre-groups but also the post-groups that received FIP-fve (p = 0.002) or a corticosteroid (p = 0.0054) during the challenge phase had significantly lower AHR after the methacholine challenge compared with the positive mice (OVA-sensitization) (analysis at 20 mg/ml level) (Fig. 1).

**FIP-fve or corticosteroid treatment on the reversal of allergen-induced ovalbumin-specific antibodies in serum**

Increased IgE serum levels are a characteristic of allergic diseases. In addition, IgG2a levels have confirmed that the Th1 response could be a trigger. In our results, the serum IgE and IgG2a levels were significantly elevated after the...
the serum and BALF (Fig. 5). IL-17 cytokine levels were significantly increased in the OVA-sensitized group (Table 1). It is noteworthy that only the groups treated with FIP-fve had decreased IL-17 expression in the serum and BALF. However, we also detect IL-17A in serum and BALF, the results were similar with IL-17 (data not show). Moreover, groups treated with FIP-fve had not only increased expression of IFN-γ, but also IL-22 in the serum and BALF (Fig. 4, Table 1).

**FIP-fve or corticosteroid treatment affected MMP9 in serum or BALF**

The results for MMP9 levels showed that the MMP9 level increased in the serum and BALF of the OVA-sensitized group. However, the MMP9 levels in the oral FIP-fve or corticosteroid groups sensitized with OVA were decreased significantly and respectively with the former having a better effect (Table 1).

**FIP-fve or corticosteroid treatment affected lung inflammation and airway remodeling**

The effect of the FIP-fve or corticosteroid treatment in the OVA-sensitized/challenged mice on overall lung inflammation was evaluated through histological staining with H&E (Fig. 6). The mice in the OVA-sensitized/challenged (Fig. 6B) group had severe inflammation compared with the NC group (Fig. 6A). After the FIP-fve or corticosteroid treatment, there was significantly less inflammation in not only the pre-groups but also the post-groups (Fig. 6C–G). However, only oral FIP-fve could significant improve airway
Figure 4. FIP-fve and corticosteroid treatment on the Th1 (IL-12 and IFN-γ) and Treg (TGF-β) cytokine levels in OVA-treated mice in the serum and BALF. Th1 and Treg cytokine concentrations in the serum and BALF were obtained from the NC group and OVA sensitized/challenged mice (PC) or the mice treated with FIP-fve and a corticosteroid. Fig. 4(A) the Th1 (IL-12, IFN-γ) and Treg (TGF-β) cytokines detected in the serum, and Fig. 4(B) the Th1 and Treg cytokines detected in the BALF. The statistical analysis compared OVA-treated mice and is represented as: **p < 0.05; ***p < 0.001.

Figure 5. FIP-fve and corticosteroid treatment on the Th2 (IL-4, IL-5, IL-13) cytokine levels in OVA-treated mice in the serum and BALF. Th2 cytokine concentrations in the serum and BALF were obtained from the NC group and OVA sensitized/challenged mice (PC) or the mice treated with FIP-fve and a corticosteroid. Fig. 5(A) the Th2 (IL-4, IL-5, IL-13) cytokines detected in the serum, and Fig. 5(B) detected in the BALF. The statistical analysis compared OVA-treated mice and is represented as: **p < 0.05; ***p < 0.001.

Table 1 The effects of the FIP-fve or corticosteroid treatment on IL-17, IL-22, and MMP9 expression in the serum and BALF. Data points represent the median ± IQR of the individual mouse groups.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Group</th>
<th>Serum (pg/ml)</th>
<th>BALF (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NC (n = 6)</td>
<td>PC (n = 8)</td>
<td>pre-FIP (n = 8)</td>
</tr>
<tr>
<td>IL-17</td>
<td>38.0 ± 5.598***</td>
<td>230.17 ± 63.73</td>
<td>63.71 ± 12.02***</td>
</tr>
<tr>
<td>IL-22</td>
<td>19.75 ± 7.6***</td>
<td>216.83 ± 63.24</td>
<td>452.43 ± 111.33**</td>
</tr>
<tr>
<td>MMP9</td>
<td>0.08 ± 0.02***</td>
<td>2.59 ± 0.37</td>
<td>0.99 ± 0.13***</td>
</tr>
<tr>
<td>IL-17</td>
<td>41.0 ± 15.41***</td>
<td>250.5 ± 78.71</td>
<td>102.14 ± 18.17**</td>
</tr>
<tr>
<td>IL-22</td>
<td>13.5 ± 5.3***</td>
<td>276.33 ± 92.43</td>
<td>500.14 ± 99.46***</td>
</tr>
<tr>
<td>MMP9</td>
<td>0.06 ± 0.01***</td>
<td>2.18 ± 0.74</td>
<td>0.67 ± 0.12***</td>
</tr>
</tbody>
</table>

(a) Serum was obtained from the NC, PC, pre-/post-FIP or pre-/post-cort groups. The statistical analyses compared PC group Represented as: *p < 0.05, **p < 0.01, ***p < 0.001.  
(b) BALF was obtained from the NC, PC, pre-/post-FIP or pre-/post-cort groups. The statistical analyses compared PC group represented as: *p < 0.05, **p < 0.01, ***p < 0.001.
remodeling (Fig. 6C and D) compared with PC group (Fig. 6B) and corticosteroid groups (Fig. 6E and F).

**FIP-fve treatment affected collagen deposition in lung tissue and IL-17 expression in lung**

The effects of the FIP-fve or corticosteroid treatment on the OVA-sensitized/challenged mice with collagen deposition in the lung were evaluated through histological staining with Masson’s trichrome stain (Fig. 7A–F). The mice sensitized/challenged with OVA in the allergic asthma models (Fig. 7B) had more collagen deposition compared with the normal control group (Fig. 7A). In the FIP-fve groups (Fig. 7C, D and G) collagen deposition was significantly decreased, but in the corticosteroid group collagen deposition was significantly expressed (Fig. 7E–G).

The effects of the FIP-fve or corticosteroid treatment on the OVA-sensitized/challenged mice with IL-17 expression in the lung were evaluated through histological staining with IHC stain (Fig. 7H–M). The mice sensitized/challenged with OVA in the allergic asthma models (Fig. 7I) had high IL-17 expression compared with the normal control group (Fig. 7H). In the FIP-fve groups (Fig. 7J and K) IL-17 expression were significantly decreased, but in the corticosteroid group IL-17 expression were significantly expressed (Fig. 7L and M).

**Discussion**

Acute animal models have shown that oral administration of FIP-fve during allergen sensitization induces a Th1-predominant allergen-specific immune response in mice with food-allergies and allergic asthma.15,16 In this study, we developed a mouse model with the following characteristic features of airway inflammation and remodeling as a representation of patients with chronic asthma: eosinophil and neutrophil infiltration into the lung interstitium, BAL fluid, release of inflammatory cytokines and goblet cell hyperplasia with airway occlusion by mucus, and increased collagen deposition around airways. This study demonstrated that oral administration of FIP-fve in a chronic animal model alleviated airway remodeling and inhibited airway inflammation. In addition to the result that oral administration of FIP-fve in a chronic animal model could suppress the level of IgE in the serum, it also reduced the cytokines such as IL-4, IL-5, IL-13, IL-17 and TGF-β in the serum and BALF. Moreover, oral FIP-fve also could reduce inflammatory cell infiltration, especially eosinophils and neutrophils in the lung. Previous studies have reported that IL-4 is known to directly promote the features of asthma, such as eosinophil infiltration, goblet cell metaplasia, airway hyperresponsiveness, IgE production in serum, mastocytosis, airway remodeling, and Th2 induction/maintenance.24,25 Earlier studies have shown that IL-5 plays a crucial role in eosinophil growth, maturation, and activation.26 Also, anti-IL5 therapeutic strategies may potentially be effective in the treatment of asthma and airway inflammation disease.27–29 However, our results which were similar to previous studies of animal allergy models showed that mice which received FIP-fve had decreased expression of IL-5 in the serum and BALF.15,20

In this study, we found FIP-fve could not only decrease eosinophils but also neutrophils and this phenomenon was better than using oral corticosteroids in mouse models of chronic allergic pulmonary inflammation. Furthermore, an earlier study reported that certain part of asthma patients develops severe allergen-induced AHR in association with
the presence of airway neutrophils rather than eosinophils. Therefore, oral corticosteroids may cause more severe asthma in the chronic stage, but the mechanism is not clear. Moreover, FIP-fve treatment in OVA sensitized mice enhanced production of cytokines such as IFN-γ, IL-12 and IL-22 and significantly decreased IL-17 and TGF-β in the serum and BALF.

Earlier studies have reported that Th1, Th17, and cytotoxic T cells (Tc) contribute to lung inflammation through the release of a large number of cytokines, such as IFN-γ, IL-12 and IL-22 and induction of apoptosis in lung epithelial cells. IL-22 has been shown to induce the recruitment of granulocytes synergistically with IL-17 and thus increases inflammation in mouse models of lung fibrosis and allergic asthma. However, mice lacking IL-17 production in these disease models show less inflammation, decreased numbers of infiltrating cells, and reduced airway tissue damage after injection of IL-22. Moreover, IL-22 can act as an anti-inflammatory cytokine in the absence of IL-17, whereas in the presence of IL-17, IL-22 contributes to the recruitment of inflammatory cells in animal models of lung inflammation.

In previous study had reported that IL-22 inhibits inflammatory responses in a murine model of asthma by modulating the function of dendritic cells. Furthermore, IL-22 is required for the sensitization phase of allergic inflammation but exerts inhibitory functions in the effector phase. Moreover, a study also report that IL-22 may inhibit antigen-induced airway inflammation by suppressing cytokine and chemokine production from lung epithelial cells. And those studies were the same with our results that increase IL-22 could improve airway inflammation. However, Besnard et al. have shown that lung inflammation is reduced in mice deficient in IL-22 or after IL-22 antibody neutralization. Moreover, it has been reported that IL-22 levels are increased in asthma patients and are positively

Figure 7. FIP-fve and corticosteroid treatment on allergen-induced airway remodeling and collagen deposition and IL-17 expression in lung tissue. Lung tissues for detection of airway remodeling and collagen deposition were obtained on day 76. Fig. 7(A) the NC group 7(B) the OVA sensitized/challenged mice (PC), 7(C) and 7(E), the groups pre-treated with FIP-fve or a corticosteroid respectively. 7(D) and 7(F) the groups post-treated with a corticosteroid or FIP-fve which were stained with Masson’s trichrome. Fig. 7(H) the NC group 7(I) the OVA sensitized/challenged mice (PC), 7(J) and 7(K), the groups pre-treated with FIP-fve or a corticosteroid respectively. 7(L) and 7(M) the groups post-treated with a corticosteroid or FIP-fve which were stained with IHC stain. The Image analysis were use Image-plus pro software and the statistical analysis (collagen deposition) compared OVA-treated mice 7(G) and is represented as: *p < 0.05, **p < 0.01, ***p < 0.001.
correlated with disease severity.\textsuperscript{38} It also has been shown that IL-22R1 is expressed on airway smooth muscle cells and that IL-22 enhances their proliferation and migration, suggesting that IL-22 may involve in smooth muscle cell hyperplasia.\textsuperscript{39} So IL-22 seems to have a dual role in allergic airway inflammation and airway remodeling. In our results, oral FIP-fve could increase IL-22 and suppress IL17 and improve airway inflammation and remodeling significantly in chronic stage, but the mechanism is not clear. Therefore, the mechanisms by which IL-22 regulates allergic airway inflammation remain largely unknown need to be clarified in the future.

Previous studies have reported that another relevant factor is TGF-\(\beta\) in airway remodeling.\textsuperscript{40} TGF-\(\beta\) has been a focal point of considerable investigation as both a mediator and effector molecule in the Th2 driven immune cascade, and TGF-\(\beta\) is believed to play an important role in most of the cellular biological processes leading to airway remodeling. It has been shown to be involved in epithelial changes, subepithelial fibrosis, airway smooth muscle remodeling, and microvascular changes.\textsuperscript{40,41}

Previous studies have shown that airway remodeling puts patients with chronic airway inflammation, asthma and COPD at risk.\textsuperscript{42} Emerging research on anti-airway remodeling drugs is important if we want to improve symptoms, slow this disease and reduce mortality rates. Previous studies have demonstrated that corticosteroids can reduce airway inflammation. However, corticosteroids cannot effectively reduce airway remodeling. In this study, oral administration of corticosteroids decreased airway inflammation successfully and the result was similar to previous studies. Moreover, administration of FIP-fve not only decreased airway inflammation but also inhibited airway remodeling in OVA-sensitized mice (Fig. 6).

However, it is worth mentioning that the oral administration of FIP-fve in an acute model of sensitized mice significantly improved airway inflammation.\textsuperscript{15} Based on previous research, FIP-fve is an immunomodulatory protein extracted from fresh mushrooms.\textsuperscript{13,15} In this study, the immunomodulatory protein inhibited airway inflammation and effectively improved airway remodeling due to chronic inflammation. In fact, to the best of our knowledge this is the first study to report this finding. Therefore, according to our previous report\textsuperscript{15} and this study, it can be confirmed that oral administration of FIP-fve could be used as an emerging clinical auxiliary substance for an anti-allergic, anti-inflammatory and anti-airway remodeling effect.

Matrix metalloproteinase (MMP)-9 is an MMP that is present in low quantities in the healthy adult lung but is much more abundant in several lung diseases, including asthma, idiopathic pulmonary fibrosis (IPF), and COPD.\textsuperscript{43} Another FIP or GMI could down-regulate TNF-\(\alpha\)-induced MMP-9 via the NF-\(\kappa\)B pathway.\textsuperscript{44} However, in our study although both the oral FIP-fve and the corticosteroid groups decreased MMP9 significantly in the serum and BALF, the oral FIP-fve had a better effect. Moreover, one important result of this study was collagen deposition. The hallmark of chronic airway inflammation, asthma and airway remodeling is collagen deposition in the bronchi and lung tissue. According to our results, oral FIP-fve decreased collagen deposition significantly, but with oral administration of corticosteroids there was more collagen deposition in the bronchi and lung tissue (Fig. 7). The results indicate that FIP-fve affected collagen deposition and reduced airway remodeling.

In conclusion, asthma is a complicated disease of study interest. This study demonstrated that oral FIP-fve may not only exert an anti-inflammatory effect but may also reduce airway modeling in OVA-induced chronic airway inflammation and FIP-fve might be an additional or supplementary therapy for allergic airway diseases.

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FIP-fve could affect airway inflammation in chronic


