Journal of Microbiology, Immunology and Infection (2017) xx, 1–10

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Original Article

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

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Received 3 January 2017; received in revised form 17 May 2017; accepted 16 June 2017
Available online ■ ■ ■

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 in lung 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.

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http://dx.doi.org/10.1016/j.jmii.2017.06.008

Please cite this article in press as: Lee Y-T, et al., Fungal immunomodulatory protein-*fve* could modulate airway remodel through by affect IL17 cytokine, Journal of Microbiology, Immunology and Infection (2017), http://dx.doi.org/10.1016/j.jmii.2017.06.008

Conclusion: FIP-*fve* had anti-inflammatory effects on OVA-induced airway inflammation and an effect to inhibited Th17 cells to reduced airway remodeling and collagen expression. Moreover, FIP-*fve* might be a potential alternative therapy for allergic airway diseases.

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.[1] 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.[2]

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.[3] 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.[4] 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.[5]

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.[6] 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.[7]

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).[8] 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.[9]

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.[10] 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.[11] 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[12] 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.[12] 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%).[13] Ko JL et al. reported[14] 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[15] and Hsieh KY et al.[16] 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.[15–18]

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

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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[13,14] and each mouse was treated orally with 200 μg FIP-*fve*.[15,16]

Corticosteroids

A corticosteroid is a synthetic drug that is particularly effective as an immunosuppressant drug. It is used to treat certain inflammatory diseases (such as moderate allergic reactions).[19] 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.[20] 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 animals were randomly allocated to the experimental groups, and the normal control group (NC) had 6 mice; the positive control group (PC) had 8 mice; and the pre- and post- treatment FIP-*fve* or corticosteroid group had 8 mice, respectively.

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.[21,22]

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 $NaHCO_3$ 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 Pharmingen™) 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

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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 recombinant 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).

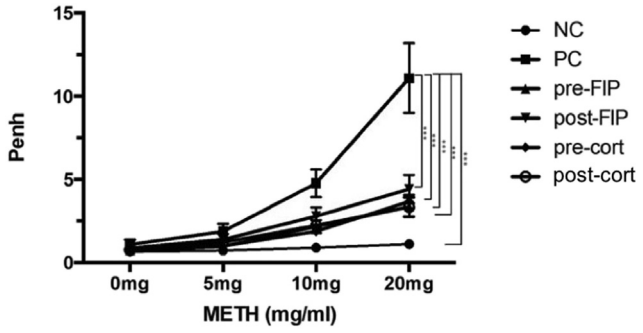

Figure 1. FIP-*fve* or corticosteroids affect AHR. The female BALB/c mice received normal saline (normal control group) or were sensitized/challenged with OVA ± treatment with FIP-*fve* or a corticosteroid during the challenge phase. The airway hyperresponsiveness (AHR) to methacholine was assessed with the Buxco system to record enhanced pauses (Penh). NC group was show in (◆), PC group was show in (■), pre- and post-FIP-*fve* group were show in (▲, ✳) and pre- and post-corticosteroid group were show in (×, ●).

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.[23] In our results, the serum IgE and IgG2a levels were significantly elevated after the OVA sensitization/challenge compared with the normal control group. In the experimental groups that received FIP-*fve* or corticosteroid treatment during the sensitization/challenge to OVA, the serum IgE level decreased significantly (Fig. 2). Moreover, the serum IgG2a level increased significantly.

FIP-*fve* or corticosteroid treatment affected infiltrating cells of the lungs

The hallmark of chronic asthma is the infiltration of inflammatory cells not only eosinophils but also neutrophils into the lung. BALF was used to evaluate cell infiltration of the lung. Differential BALF cell counts included eosinophils, lymphocytes, neutrophils and monocytes. According to our results, infiltrating cells in the lungs of OVA-sensitized mice increased significantly compared to the control group. However, the infiltrating cells in the lungs of the FIP-*fve* or corticosteroid groups decreased significantly, while the decrease in neutrophils in the FIP-*fve* groups was superior to the corticosteroid groups (Fig. 3).

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

Eight kinds of cytokines were detected in our results including the cytokines IFN-γ, IL-4, IL-5, IL-12, IL-13, IL-17 and IL-22 which were also detected in the serum and BALF. In our allergy mouse model, the Th2 cytokines IL-4, IL-5 and IL-13 increased in the mice sensitized/challenged with OVA. However, The Th1 cytokines IFN-γ, and IL-12 did not increase in the OVA-sensitized group (Fig. 4). According to the results, only the Th1 cytokines IL-12 and IFN-γ increased significantly but the cytokine TGF-β decreased significantly in FIP-*fve* treated groups compared with the OVA-sensitized group (Fig. 4). Moreover, the FIP-*fve* or corticosteroid treatment during the challenge phase significantly decreased the cytokines IL-4, IL-5 and IL-13 in

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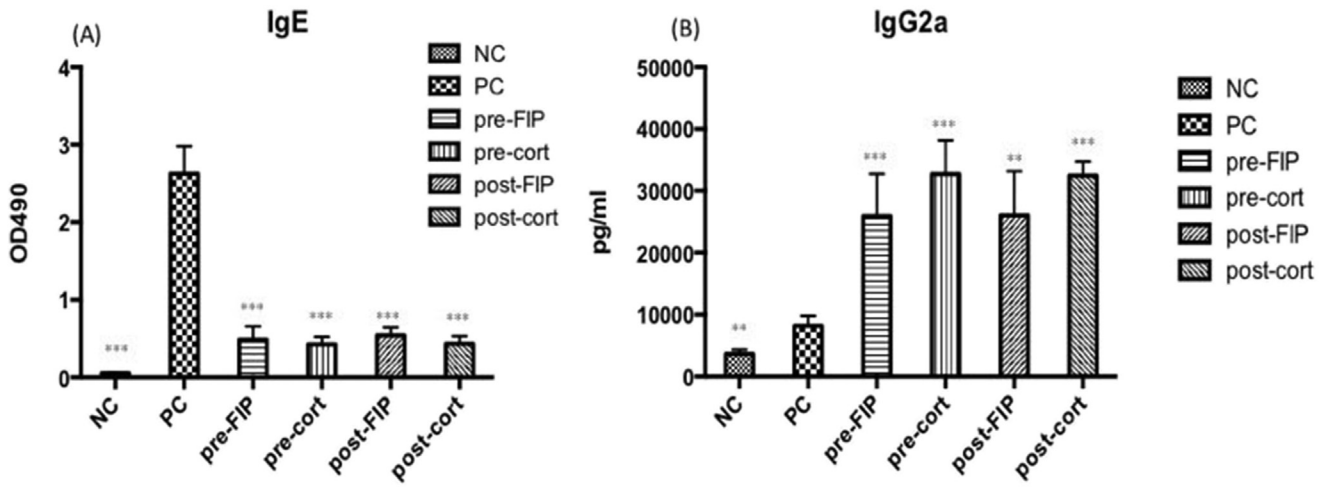

Figure 2. FIP-fve or corticosteroid treatment on the reversal of allergen-induced serum ovalbumin-specific antibodies in serum. Serums in OVA-specific-IgE and OVA-specific-IgG2a concentrations were obtained from the NC group and the OVA sensitized/challenged mice (PC) or mice treated with FIP-*fve* and a corticosteroid. Fig. 2(A) The OVA-specific-IgE in the each group. Fig. 2(B) The OVA-specific-IgG2a expression in serum in the each group. The statistical analysis compared OVA-treated mice and is represented as: $^{**}p < 0.05$; $^{***}p < 0.001$.

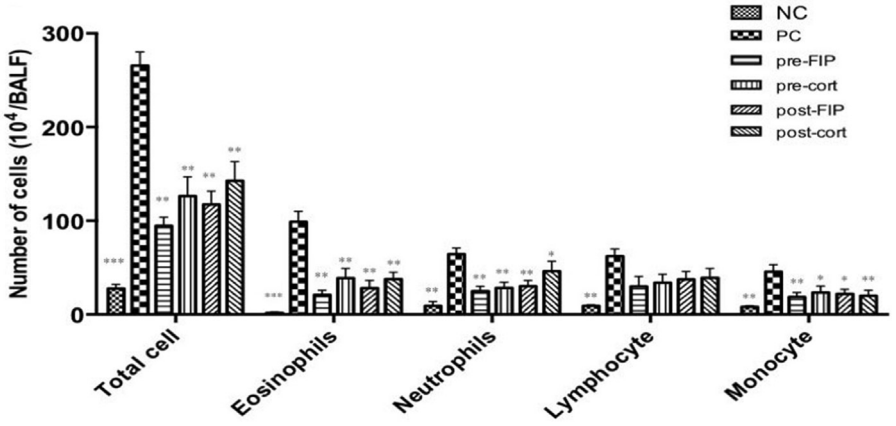

Figure 3. FIP-*fve* or corticosteroid treatment affects the infiltrating cells of the lungs. All cell counts were obtained from the NC group and the OVA sensitized/challenged mice (PC) or mice treated with FIP-*fve* and a corticosteroid. The total cells and inflammatory cells were counted ($\times 10^4$) from the BALF in millimeters by morphometric evaluations of cytospin preparations. The statistical analysis compared OVA-treated mice and is represented as: $^{**}p < 0.05$; $^{***}p < 0.001$.

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

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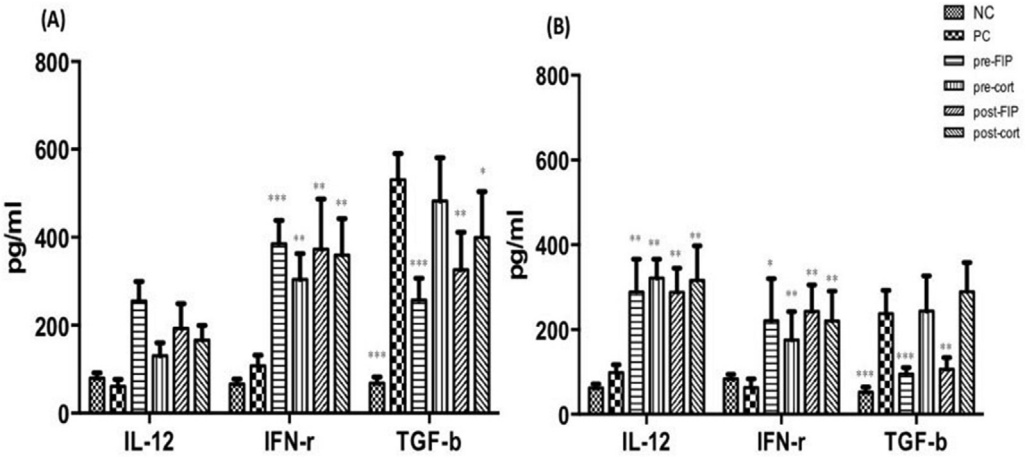

Figure 4. FIP-*fve* and corticosteroid treatment on the Th1 (IL-12 and IFN-γ) and Treg (TGF-β) cytokine levels in OVA-treated mice in the serum and BALF. Th1 and Treg cytokine concentrations in the serum and BALF were obtained from the NC group and OVA sensitized/challenged mice (PC) or the mice treated 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: $^{**}p < 0.05$; $^{***}p < 0.001$.

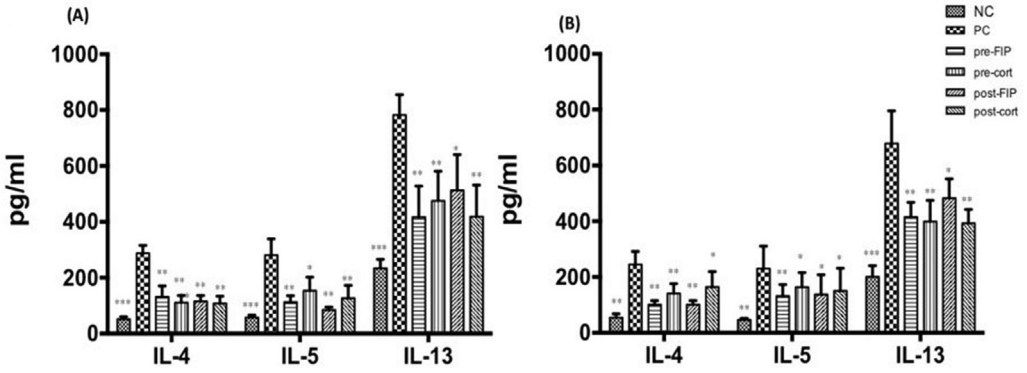

Figure 5. FIP-*fve* and corticosteroid treatment on the Th2 (IL-4, IL-5, IL-13) cytokine levels in OVA-treated mice in the serum and BALF. Th2 cytokine concentrations in the serum and BALF were obtained from the NC group and OVA sensitized/challenged mice (PC) or the mice treated with FIP-*fve* and a 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.

Samples	Group					
	NC (n = 6)	PC (n = 8)	pre-FIP (n = 8)	pre-cort (n = 8)	post-FIP (n = 8)	post-cort (n = 8)
(a) Serum						
IL-17 (pg/ml)	38.0 ± 5.598***	230.17 ± 63.73	63.71 ± 12.02***	202.0 ± 72.52	113.0 ± 39.22**	203.29 ± 32.94
IL-22 (pg/ml)	19.75 ± 7.6***	216.83 ± 63.24	452.43 ± 111.33**	238.14 ± 68.76	363.43 ± 146.32**	254.06 ± 25.82
MMP9 (ng/ml)	0.08 ± 0.02***	2.59 ± 0.37	0.99 ± 0.13***	1.41 ± 0.23	1.04 ± 0.07**	1.36 ± 0.35*
(b) BALF						
IL-17 (pg/ml)	41.0 ± 15.41***	250.5 ± 78.71	102.14 ± 18.17**	273.0 ± 99.4	127.0 ± 48.44**	266.29 ± 95.22
IL-22 (pg/ml)	13.5 ± 5.3***	276.33 ± 92.43	500.14 ± 99.46***	168.29 ± 43.5*	380.57 ± 118.21*	149.57 ± 57.0*
MMP9 (ng/ml)	0.06 ± 0.01***	2.18 ± 0.74	0.67 ± 0.12***	1.22 ± 0.28*	1.07 ± 0.32**	1.05 ± 0.07**

(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: $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

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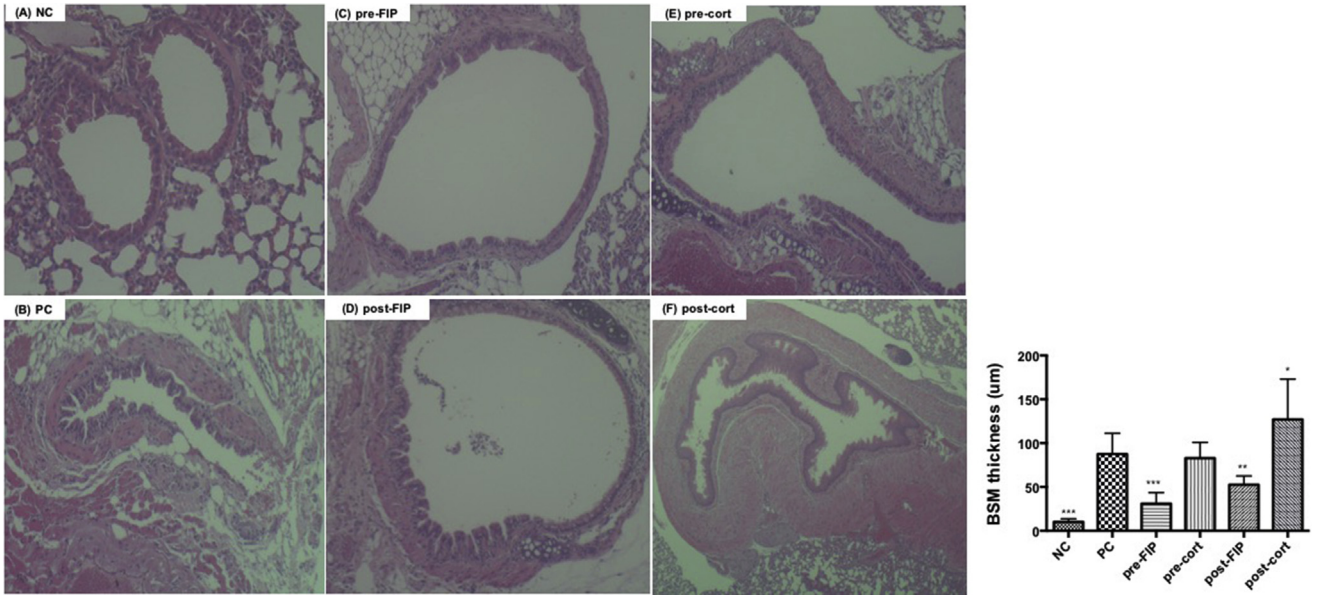

Figure 6. FIP-*fve* and corticosteroid treatment on allergen-induced airway inflammation and remodeling. Lung tissue samples were obtained on day 76 to determine airway inflammation. Fig. 6(A) the NC group 6(B) the OVA sensitized/challenged mice (PC), 6(C) and 6(E) the groups pre-treated with FIP-*fve* or a corticosteroid respectively 6(D) and 6(F) the groups post-treated with a corticosteroid or FIP-*fve* which were stained with **hematoxylin and eosin**. The Image analysis were use Image-plus pro software and the statistical analysis compared OVA-treated mice 4(G) and is 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

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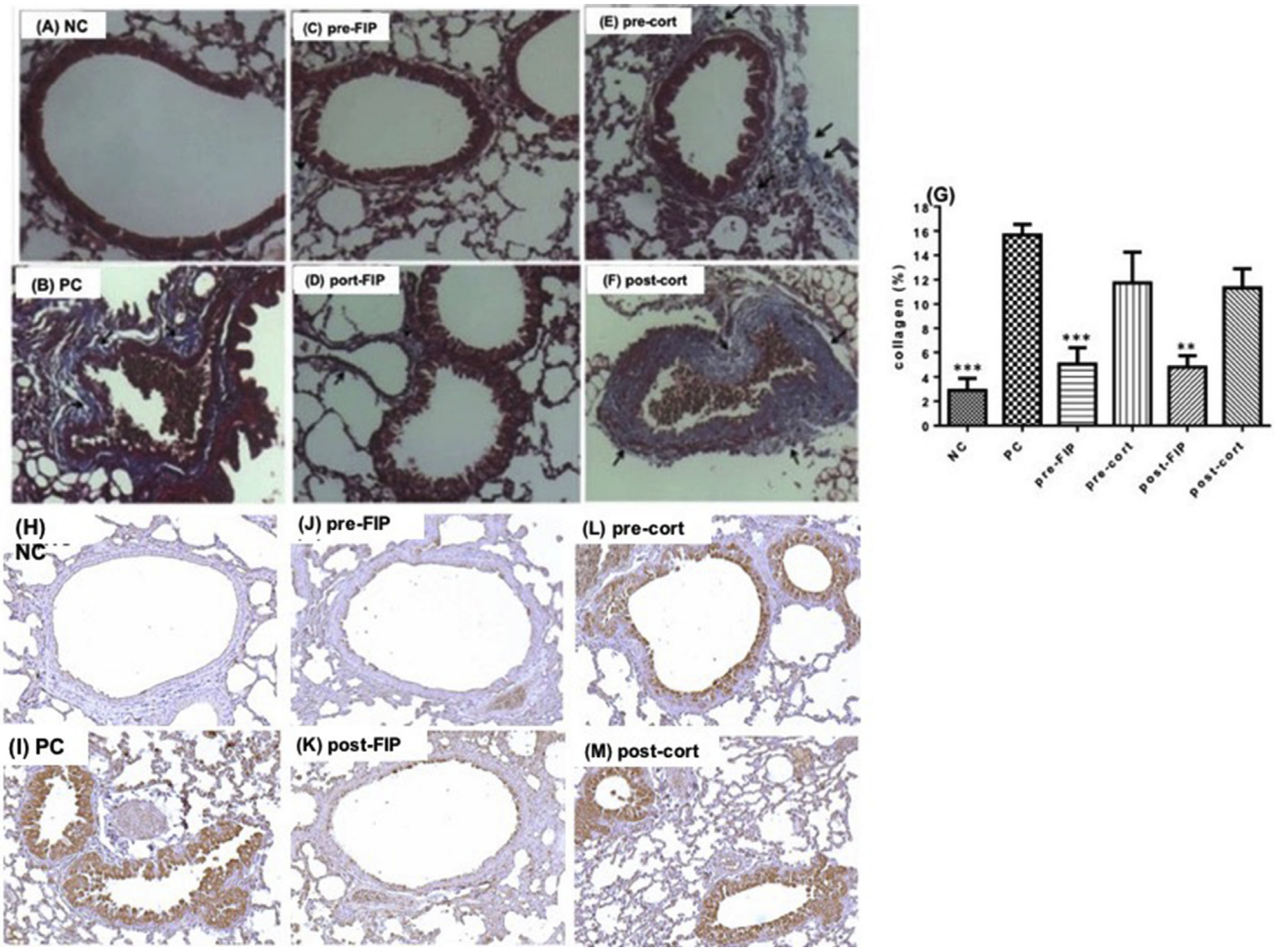

Figure 7. FIP-*fve* and corticosteroid treatment on allergen-induced airway remodeling and collagen deposition and IL-17 expression in lung tissue. Lung tissues for detection of airway remodeling and collagen deposition were obtained on day 76. Fig. 7(A) the NC group 7(B) the OVA sensitized/challenged 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: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

the presence of airway neutrophils rather than eosinophils.[30] 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-17, and IL-22 and induction of apoptosis in lung epithelial cells.[31–33] 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.[33–35] 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.[34,35]

In previous study[36] 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[37] 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.[34] Moreover, it has been reported that IL-22 levels are increased in asthma patients and are positively

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correlated with disease severity.[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.[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 were need to be clarified in the future.

Previous studies have reported that another relevant factor is TGF-β in airway remodeling.[40] TGF-β has been a focal point of considerable investigation as both a mediator and effector molecule in the Th2 driven immune cascade, and TGF-β 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.[40,41]

Previous studies have shown that airway remodeling puts patients with chronic airway inflammation, asthma and COPD at risk.[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,[15] Based on previous research, FIP-*fve* is an immunomodulatory protein extracted from fresh mushrooms.[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[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.[43] Another FIP or GMI could down-regulate TNF-a induced MMP-9 via the NF-kβ pathway.[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 or 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.

Acknowledgements

This study was supported by a research grant from Chung Shan Medical University Hospital (CSH-2014-C-003), Taichung, Taiwan, and the National Science Council (103-2314-B-040-009 and 104-2314-B-040-015-MY3), Taipei, Taiwan.

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