

Serine protease inhibitors nafamostat mesilate and gabexate mesilate attenuate allergen-induced airway inflammation and eosinophilia in a murine model of asthma

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Background: Serine proteases such as mast cell tryptase and certain allergens are important in the pathogenesis of allergic inflammation of asthma.

Objective: We sought to investigate the effects of serine protease inhibitors nafamostat mesilate (FUT), gabexate mesilate (FOY), and ulinastatin (UTI) on airway inflammation in a mouse model of allergic asthma.

Methods: BALB/c mice were sensitized to *Dermatophagoides pteronyssinus* (Der p) and intratracheally challenged with Der p (0.5 mg/mL). Therapeutic doses of FUT (0.0625 mg/kg), FOY (20 mg/kg), and UTI (10,000 U/kg) were intra-peritoneally injected into 3 corresponding sensitized mice during the sensitization phase (protocol 1) or 24 hours after allergen challenge (protocol 2).

Results: Both FUT-treated and FOY-treated sensitized mice had reduced mast cells activation, airway hyperresponsiveness, attenuated eosinophils infiltrations, and decreased Der p-induced IL-4 and TNF- α , but increased IL-12 cytokine production in bronchoalveolar lavage fluid compared with nontreated mice. Furthermore, FUT treatment downregulated the expression of IL-1 β , TNF- α , IL-6, eotaxin, inducible NO synthase, CD86, and nuclear factor- κ B activation, but enhanced the expression of IL-12 and IL-10 in Der p-stimulated alveolar macrophages. UTI-treated mice have no

significant change of the aforementioned measurements compared with nontreated sensitized mice.

Conclusion: Nafamostat mesilate and FOY exerting the therapeutic effect in allergen-induced airway inflammation was a result not only of their inhibitory action in the early phase of mast cells activation but also of immunoregulatory function in the late phase of allergic inflammation. Such properties of FUT and FOY might be a potential therapeutic approach for asthma. **Clinical implications:** The clinical used of serine protease inhibitors FUT and FOY may also have implications for treating airway inflammation of asthma. (J Allergy Clin Immunol ■■■■;■■■:■■■-■■■.)

Key words: Serine protease inhibitor, allergy, airway inflammation, eosinophils

There is mounting evidence that proteases are involved in the pathogenesis of asthma.¹ The serine protease tryptase, localized in mast cell granules, constitutes as much as 25% of the total mast cell protein² and is increased in bronchoalveolar lavage fluid (BALF) of patients with asthma after allergen challenge.³ In *in vitro* studies, tryptase promotes human mast cell degranulation,⁴ induces eosinophil and neutrophil migration,⁵ amplifies the bronchoconstrictor effects of histamine on lung tissue,⁶ and stimulates the growth of airway fibroblasts, smooth muscle cells, and epithelial cells in the process of airway remodeling.⁷ Furthermore, many aeroallergens associated with asthma, such as house dust mite allergens and various fungal allergens, are proteases.⁸⁻¹⁰ Ino et al¹¹ first characterized Der p 1 as a cysteine protease, which can stimulate production of IL-6, IL-8, and prostaglandin D₂ in airway epithelial cells and activate eosinophils.¹² In an *in vitro* study, after a short phase of cytokine production, Der p 1, like tryptase, caused detachment of the epithelial cells.¹³ Group 3 (Der p 3) and 9 (Der p 9) mite allergens are serine proteases that can increase vascular permeability and detach epithelial cells.¹⁴ Although the receptors for these proteases have not yet been characterized, it is highly probable that the primary basis for this response is activation of protease-activated receptors or similar molecules.^{15,16} The stimulation was blocked by cysteine and serine protease inhibitors.¹⁷

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Supported by grant NSC89-2320-B006-029 and the National Science Council, Taiwan, Republic of China.

Disclosure of potential conflict of interest: The authors have declared that they have no conflict of interest.

Received for publication October 19, 2005; revised February 15, 2006; accepted for publication February 17, 2006.

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0091-6749/\$32.00

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doi:10.1016/j.jaci.2006.02.047

Abbreviations used

AHR:	Airway hyperresponsiveness
AM:	Alveolar macrophage
BAL:	Bronchoalveolar lavage
BALF:	Bronchoalveolar lavage fluid
FOY:	Gabexate mesilate
FUT:	Nafamostat mesilate
iNOS:	Inducible nitric oxide synthase
i.t.:	Intratracheally
mMCP-1:	Mouse mast cell protease 1
NF- κ B:	Nuclear factor- κ B
Penh:	Enhanced pause
UTI:	Ulinastatin

Recently, serine and trypsin inhibitors have been shown to reduce antigen-induced airway hyperreactivity in allergic guinea pigs and sheep.^{17,18} Gabexate mesilate (FOY) as well as nafamostat mesilate (6-amidino-2-naphthyl p-guanidinobenzoate dimethane sulfonate [FUT]) are nonantigenic synthetic inhibitors of trypsinlike serine proteases^{19,20} that have been therapeutically used in the treatment of pancreatitis and disseminated intravascular coagulation, and as a regional anticoagulant for hemodialysis.^{21,22} Human trypsin inhibition by FOY may account for the reported prevention of inflammation, erosion, ulceration of mucosa, and the symptom of allergic rhinitis.^{23,24} FUT inhibited purified lung trypsin and eliminated pulmonary dysfunction induced in rats by a radiographic contrast medium.²⁵ A purified human urinary trypsin inhibitor (ulcinastatin [UTI]), also a serine protease inhibitor, has been shown to have various beneficial effects in treating patients with disseminated intravascular coagulation, shock, and pancreatitis by inhibiting the activation of leukocytes.^{26,27} It also can improve peripheral microcirculation and bronchospasm associated with systemic anaphylaxis in rabbits *in vivo*.²⁸

The precise modes of anti-inflammatory mechanisms of these synthetic and purified serum trypsin inhibitors are still unclear. The purpose of this study was to determine the therapeutic potential of protease inhibitors such as FUT, FOY, and UTI on allergy airway inflammation and eosinophil infiltration in a mouse model of asthma.

METHODS

Animals and reagents

Female BALB/c mice age 6 to 8 weeks were obtained from the Laboratory Animal Center of the College of Medicine, National Cheng Kung University. All experimental animal care and treatment followed the guidelines set up by the National Institutes of Health Guide for the Care and Use of Laboratory. Der p extract (1 g lyophilized whole body extract in ether; Allergon, Engelholm, Sweden) was dissolved in pyrogenic-free isotonic saline, filtered through a 0.22- μ m filter, and stored at -70°C before use. LPS concentration of the Der p preparations was <0.96 EU/mg of Der p (limulus amoebocyte lysate test, E-Toxate; Sigma-Aldrich, St Louis, Mo). FUT

(Futhen; Torii Pharmaceuticals Co, Chiba, Japan), FOY (Ono Pharmaceutical Co, Osaka, Japan), and UTI (Mochida Pharmaceuticals Co, Tokyo, Japan), which have been used clinically, were purchased and each dissolved in PBS before use.

In vivo experimental design

Each experiment had the following groups ($n = 6$). Controls included naive mice, and the sensitized and allergen-challenged mice that were subcutaneously immunized twice at 1-week intervals with a mixture containing 40 mg Der p in incomplete Freund adjuvant (Difco, Detroit, Mich) and subsequently intratracheally (i.t.) challenged with 50 μ L Der p (0.5 mg/mL) at 3 days after the last immunization. Two experimental designs to test the effect on protease inhibitors of FUT, FOY, and UTI on allergen-induced airway inflammation were applied. In protocol 1, treated mice were given 2 intraperitoneal injections of recommended therapeutic doses of FUT (0.0625 mg/kg), FOY (20 mg/kg), and UTI (10,000 U/kg), respectively, during the sensitization periods (preventive). In protocol 2, treated mice were given these medications in the same dosages, respectively, as protocol 1 at 24 hours after i.t. allergen challenge (therapeutic). The effects of the protease inhibitors on a more robust airway remodeling model were measured in groups of mice by repeated i.t. inoculation with 5 doses of either Der p or ovalbumin (50 μ L, 0.5 mg/mL) at 1-week intervals as in our previous report.²⁹

Measurement of airway hyperresponsiveness and bronchoalveolar lavage

The airway resistance of mice was measured by a single-chamber, whole-body plethysmograph (Buxco Electronics, Inc, Troy, NY) as described in the manual provided. Airway resistance was expressed as enhanced pause (Penh). Increasing doses of methacholine were administered by nebulization for 3 minutes, and Penh was measured over the period of the subsequent 3 minutes. Mice were killed with an overdose of sodium pentobarbitone. The trachea was exposed and cannulated, and bronchoalveolar lavage (BAL) was performed with two 1-mL aliquots of saline. A total of 1.8 to 1.9 mL BALF was consistently recovered by this technique. The total cells in BALF in cytocentrifuged preparations were stained with Liu stain for differential cell counts.

Lung histology

Lungs were inflated with 0.5 mL formalin (Sigma-Aldrich) via trachea. The lungs were then removed from the chest cavity and placed in formalin overnight at 4°C . Tissues were embedded in paraffin, cut into 5- μ m sections, and stained with hematoxylin and eosin stain. Inflammatory infiltrates and lung architecture were assessed by using light microscopy.

Total IgE and Der p-specific IgG₁ and IgG_{2a/2b} Abs

Serum samples were collected from the retro-orbital venous plexus 3 days after the i.t. allergen challenge. The total IgE and Der p-specific IgG₁ and IgG_{2a/2b} serum antibody titers were determined by ELISA methods with the required reagents and mAbs purchased from BD PharMingen (San Jose, Calif).²⁸

BALF cytokine measurement

The levels of IL-4, IL-6, IL-12, and TNF- α in the BALF supernatants were evaluated by using an appropriate ELISA kit (R&D, Minneapolis, Minn). These assays have a threshold of detection of 30 and 2 pg/mL, respectively.

Mast cell activation *in vivo*: mouse mast cell protease 1 ELISA

Mouse mast cell protease 1 (mMCP-1) is a protease specific for mouse mast cells and appears in the blood of mice after mast cell activation.²⁹ Blood samples in each group of mice were collected 30, 60, 120, and 180 minutes after allergen challenge. After centrifugation, sera were stored at -70°C until assay for mMCP-1 (Moredun Scientific, Midlothian, United Kingdom) according to the manufacturer's instructions.

Alveolar macrophages cytokine mRNA detection

RT-PCR was performed to estimate mRNA expression of cytokines and inducible nitric oxide synthase (iNOS) in alveolar macrophages (AMs) isolated from BALF. Gene-specific primer pairs (sense and antisense, respectively) were used for TNF- α , iNOS, IL-1 β , IL-6, IL-10, IL-12, eotaxin, and internal control of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as previously described.³⁰ PCR products were electrophoresed on 2% agarose and stained with ethidium bromide. Bands were quantified by using densitometry analysis (Bio-1D; Vilber Lourmat, Marne La Vallee, France).

Nuclear factor- κB activation and cellular marker of nitric oxide synthase II and CD86 expression in AMs

Nuclear extracts were prepared from AMs (1×10^6) exposed to Der p for 24 hours by the method of previously described.³⁰ Supershift experiments were performed by using polyclonal Abs to the p65 of nuclear factor- κB (NF- κB ; Santa Cruz Biotechnology, Santa Cruz, Calif). Competitive assays with unlabeled oligonucleotides used a 100-fold molar excess relative to the radiolabeled oligonucleotides. To detect costimulation molecules expression, AMs were cytocentrifuged into the slides coated with poly-L-lysine (Sigma-Aldrich), fixed in ice-cold acetone, and incubated with fluorescein isothiocyanate-conjugated Abs against nitric oxide synthase II (Serotec, Atlantic) and CD86 (GL1; BD PharMingen, San Jose, Calif). The number of positive staining cells was counted in 10 fields in each of 2 duplicate slides under a fluorescent microscope ($\times 400$, Leica DM IRE2 and TRB; Leica, Wetzlar, Germany).

Statistical analysis

Mediator contents in culture supernatants and antibody production values were analyzed by 1-way ANOVA test, followed by a post hoc comparison between groups. The cellular accumulation and mediator contents in BALF and serum Ab titer were analyzed by using the Mann-Whitney *U* test. Results are expressed as means $\pm$ SEMs. A *P* value $< .05$ was considered significant.

RESULTS

Effect of protease inhibitors on cellular composition in the BALF and lung histology

In the BALF of Der p-sensitized mice, there were markedly increased inflammatory cells and eosinophils infiltration compared with naive mice (Fig 1). When treated with different protease inhibitors intraperitoneally during Der p sensitization periods (protocol 1, preventive treatment), FUT-treated mice showed significantly decreased numbers of total cells, neutrophils, and eosinophils in BALF after allergen challenge (68.7 ± 5.7 , 9.3 ± 5.5 , and $2.7 \pm 0.2 \times 10^4$ cells; $P < .05$) compared with

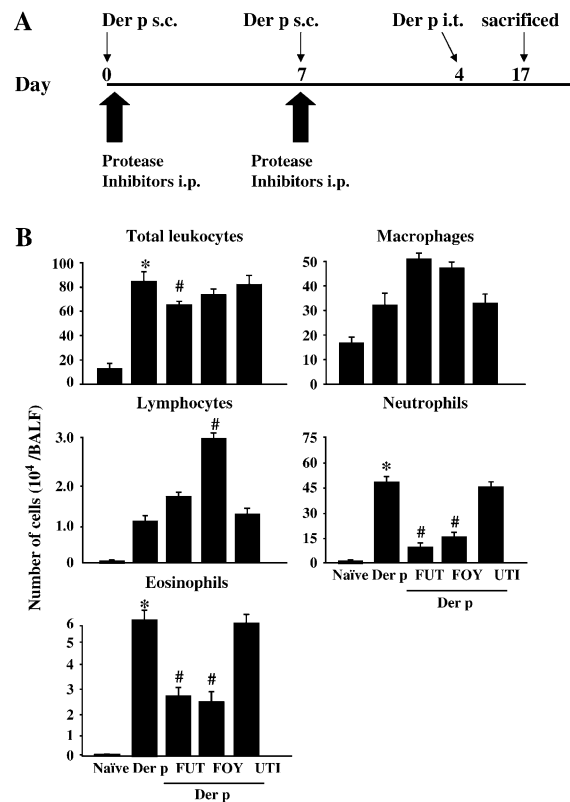


FIG 1. The protocol of preventive treatment (A), and the total and differential counts of BAL cells in various groups of mice (B). Values represent the means $\pm$ SEMs of 6 mice. Naive, Nonsensitized and saline-exposed; Der p, sensitized and Der p-exposed; s.c., subcutaneously; i.p., intraperitoneously. * $P < .05$ (vs naive group); # $P < .05$ (between Der p-exposed groups).

those in untreated sensitized mice. FOY-treated mice also have the same inhibitory effects on the recruitment of neutrophils and eosinophils (23.1 ± 1.2 , $2.5 \pm 1.0 \times 10^4$ cells; $P < .05$, compared with untreated mice) into BALF as FUT-treated mice. Moreover, the lymphocytes in BALF in FOY-treated mice were significantly increased compared with untreated sensitized mice. UTI-treated mice did not have a significant change of total cell numbers and differential cell counts in BALF after allergen challenge compared with those of untreated sensitized mice. In the therapeutic experiment of protocol 2 (Fig 2), the results showed a similar pattern to protocol 1 in decreasing total cell counts, macrophage, neutrophil, and eosinophil cells counts in the BALF of FUT-treated and FOY-treated mice compared with nontreated sensitized mice. UTI-treated mice did not show any significant change of cell numbers and distributions in BALF compared with nontreated mice as seen in protocol 1.

From examination of lung histology (see this article's Fig E1 in the Online Repository at www.jacionline.org), the infiltration of eosinophils was markedly reduced in the FUT-treated and FOY-treated mice in both protocols

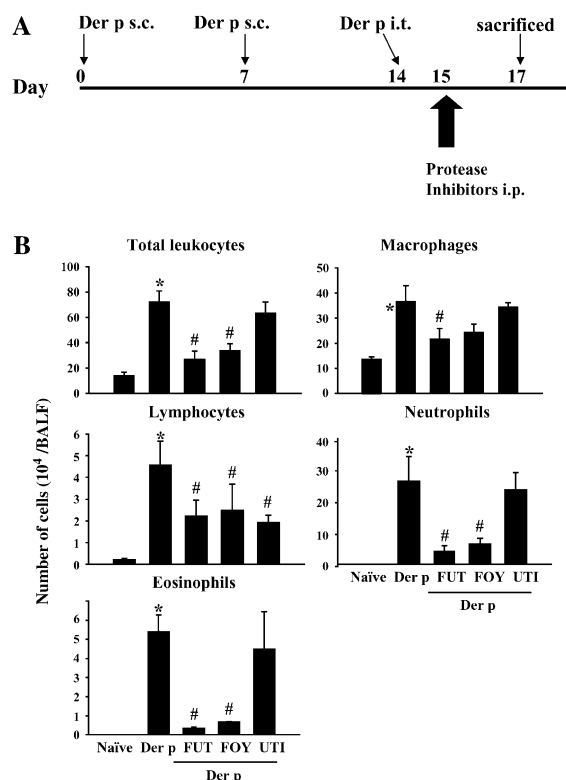


FIG 2. The protocol of therapeutic treatment (A), and the total and differential counts of BAL cells in various groups of mice (B). The number of mice in each group and abbreviation notes are the same as in Fig 1.

compared with nontreated sensitized mice. Both FUT and FOY also showed significantly alleviating of eosinophil infiltrations and decreasing T_H2 cytokines and inflammatory mediators production in BALF of treated mice (see this article's Table E1 in the Online Repository at www.jacionline.org), in either an airway remodeling model by chronic exposure of Der p or using a nonproteolytic allergen such as ovalbumin as a sensitizing agent (see this article's Fig E2 in the Online Repository at www.jacionline.org).

Effect of the protease inhibitor on the airway hyperresponsiveness of mice

Seventy-two hours after allergen challenge, all groups of mice were subjected to the methacholine stimulation test. The mice, which were treated with FUT in the protocol 1, had a significant decrease in airway hyperreactivity, as assessed by their response to increase the dose of inhaled methacholine (Fig 3, A). In contrast, mice treated with FOY or UTI had a similar airway hyperresponsiveness (AHR) as those of the nontreated sensitized group. In the therapeutic experiment, both FUT-treated and FOY-treated mice showed a significant decrease of AHR compared with nontreated sensitized mice, whereas UTI-treated mice did not have any change of AHR (Fig 3, B).

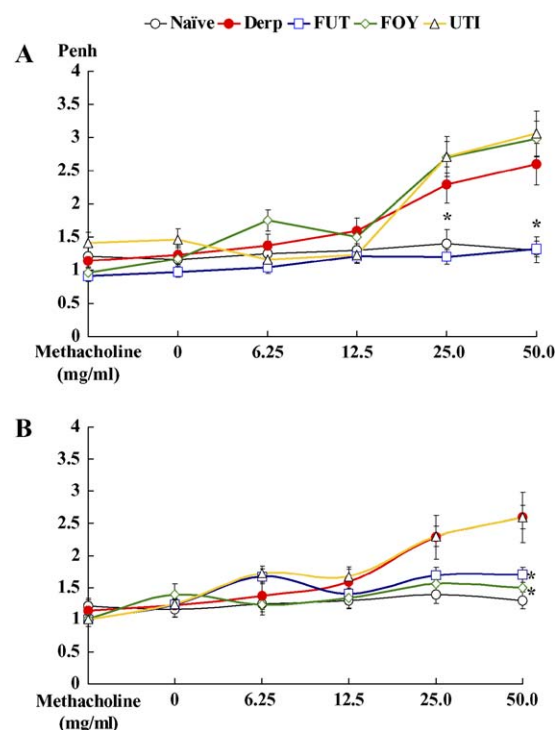


FIG 3. The AHR in mice treated with different serine protease inhibitors during the allergen sensitization (A) or after allergen challenge (B). Values were expressed as means $\pm$ SEMs of 6 mice. *The difference is statistically significant compared with the nontreated sensitized mice ($P < .05$).

FUT, FOY significantly inhibited serum IgE but did not affect Der p-specific IgG₁ and IgG_{2a/2b} Abs

A significant increase in the levels of serum IgE was observed in the untreated sensitized mice compared with the nonsensitized control mice (261 ± 15.3 vs 66.4 ± 4.3 pg/mL; Fig 4). After treatments with FUT, FOY, or UTI respectively in the sensitization period (protocol 1), the serum concentrations of IgE were decreased significantly on FUT-treated and FOY-treated mice compared with untreated sensitized mice. On the contrary, there was no significant difference in the levels Der p-specific IgG₁ and IgG_{2a/2b} Abs between sensitized mice with or without protease inhibitors treatment (Fig 4, A). In protocol 2, the serum levels of IgE were not changed significantly between treated and nontreated mice, whereas the concentrations of Der p-specific IgG₁ showed markedly decreased and Der p-specific IgG_{2a/2b} increased in the FUT-treated and FOY-treated mice compared with nontreated sensitized mice (Fig 4, B).

Effect of serine protease inhibitors on the early phase mast cell activation

A 6-fold increase in serum mMCP-1 was found in nontreated sensitized mice as early as 30 minutes after Der p challenge in comparison with naïve mice (6.20 ± 0.24

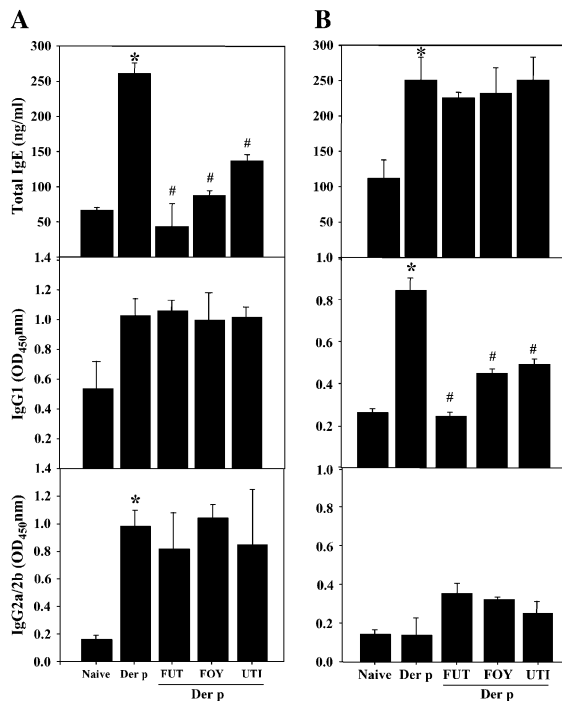


FIG 4. Serum Der p-specific antibody levels in mice treated with different serine protease inhibitors during the allergen sensitization (A), or after allergen challenge (B). Values were expressed as means $\pm$ SEMs of 6 mice. * $P < .05$ (vs naive group); # $P < .05$ (between Der p-exposed groups).

ng/mL serum vs 0.92 ± 0.63 ng/mL serum; $P < .05$; $n = 6$ mice per group). Elevated mMCP-1 levels lasted for at least 180 minutes after challenge. FUT-treated mice in the preventive or therapeutic experiments decreased mite allergen-induced enhancement in serum mMCP-1 by 50% to 70% (see this article's Fig E3, A and B, in the Online Repository at www.jacionline.org). FOY-treated mice showed significantly decreased mast cell activation of mMCP-1 release only in the therapeutic experiment (protocol 2). UTI-treated mice showed no effect in mast cell activation.

FUT and FOY attenuate proinflammatory and T_H2 cytokines production

In protocol 1 (Fig 5, A), FUT and FOY significantly attenuated Der p-induced IL-4 (from 138.7 ± 14 to 75.7 ± 8.7 , 90.2 ± 10.2 pg/mL, $P < .05$), IL-6 (from 1168 ± 39 to 812 ± 45.9 , 944 ± 44.3 pg/mL, $P < .05$) cytokines, but enhanced the IL-12 cytokines production (from 311.5 ± 32 to 596 ± 20 , 511 ± 19.1 pg/mL) compared with those in untreated sensitized mice. UTI did not have any effect on Der p-induced IL-4, IL-6, and IL-12 production. In protocol 2, FUT-treated and FOY-treated mice also showed significantly decreased proinflammatory cytokine TNF- α , but not IL-6, and increased IL-12 in the BALF compared with nontreated sensitized mice. UTI-treated mice had significantly decreased TNF- α , but no change of IL-6 and IL-12.

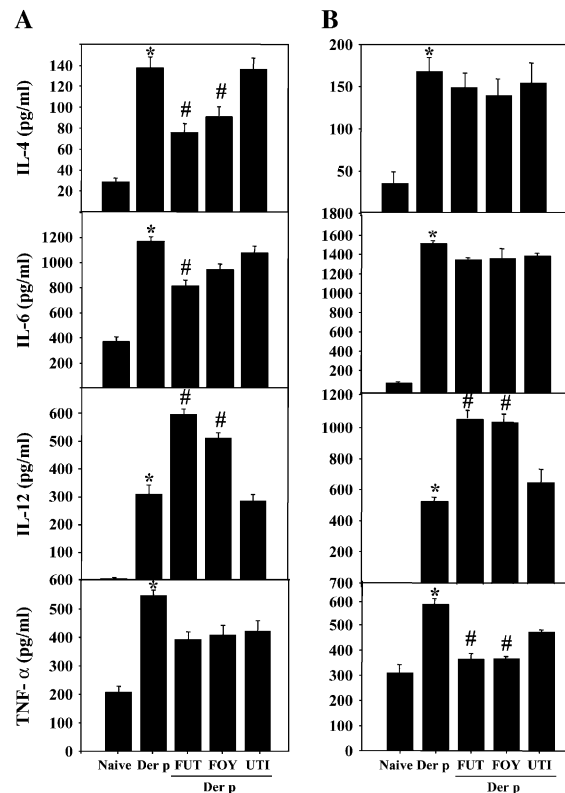


FIG 5. Cytokine levels in BALF of groups of sensitized mice, with different serine protease inhibitors administrated during sensitization (A), or after allergen challenge (B). Values were expressed as means $\pm$ SEMs of 6 mice. * $P < .05$ (vs naive group); # $P < .05$ (between Der p-exposed groups).

Effects of FUT, FOY on Der p-induced proinflammatory cytokine and chemokine expression (mRNA) in AMs

After Der p allergen-challenge, the mRNA synthesis of IL-1 β , TNF- α , IL-6, iNOS, and eotaxin in AMs from untreated sensitized mice was upregulated from 2-fold to 11-fold, respectively, compared with unsensitized mice (Fig 6, A). However, IL-10 and IL-12 mRNAs were only slightly increased. These AMs from untreated sensitized mice were cocultured with various protease inhibitors and assayed for their effects on proinflammatory cytokines and chemokines production. The most significant findings of FUT treatment were the decreased synthesis of TNF- α cytokine and the markedly increased synthesis of IL-10, and IL-12 (3.5-fold, and 5.5-fold to basal levels, respectively) compared with untreated AMs. FOY also had the same effects, significantly suppressing IL-1 β , TNF- α , IL-6, and eotaxin in AMs (1.2-fold, 2.4-fold, 2.2-fold, 2.4-fold to basal levels, respectively; Fig 6, A) and upregulating IL-10 and IL-12 (3.5-fold, and 5.5-fold to basal levels, respectively). However, there were not significant changes in the expression of proinflammatory cytokines and chemokines assayed in UTI-treated AMs.

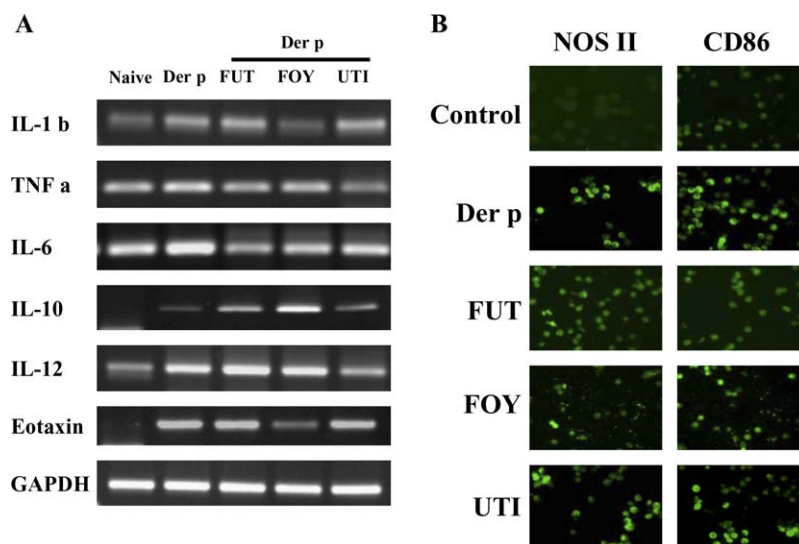


FIG 6. The profiles of expression mRNA of various cytokines in AMs obtained from the BALF of treated and nontreated mice (**A**). Nitric oxide synthase II (NOSII) and CD86 positive cells in the BALF obtained from groups of mice detected under a fluorescent microscope (400 $\times$) (**B**).

FUT suppresses B7.2 (CD86), iNOS overexpression, and NF- κ B activation in Der p-treated alveolar macrophages

Using immunohistochemistry for direct detection of iNOS and CD86 expression in AMs (Fig 6, B), FUT significantly suppress the expression of iNOS and CD86 in Der p (10 μ g/mL)-stimulated AMs, whereas FOY and UTI treatments did not show any change of iNOS and CD86 expression in stimulated AMs compared with untreated Der p-stimulated AMs from sensitized mice. The results of NF- κ B activation in alveolar macrophages of protease inhibitors in treated and untreated Der p-sensitized mice are shown in Fig E4 (see the Online Repository at www.jacionline.org). NF- κ B activation was also suppressed in both FUT-treated and FOY-treated mice, but not in UTI-treated mice.

DISCUSSION

In this study, we evaluated the effect of serine protease inhibitors on the allergen-induced airway inflammation in a murine model of asthma. In the preventive treatment of protocol 1, we found that only FUT and FOY could inhibit eosinophils infiltration in the lung, thus suppressing allergen-specific IgE production. These findings were in agreement with the decreased inflammatory cytokines and T_H2 to T_H1 cytokine profiles changes in the FUT-treated and FOY-treated mice. However, only FUT-treated mice showed a significant change of AHR compared with other protease inhibitors between treated and nontreated sensitized mice, suggesting that instead of anti-inflammatory action by serine protease inhibition, FUT may have other protective pharmacological effects on AHR. In protocol 2, the results of therapeutic effects of FUT and FOY on allergen-induced airway inflammation, such as attenuating

pulmonary eosinophilia, inflammatory cytokine production in the BAL, and reducing AHR, were similar to protocol 1, except that there was no change of allergen-specific IgE. However, the ratio of anti-Der p IgG₁/IgG_{2a} antibody was markedly increased and the mast cell activation was significantly inhibited in the sensitized mice treated with FUT and FOY after allergen challenge (protocol 2) compared with the sensitized mice when FUT and FOY were administered during the sensitization period (protocol 1). We believed that FUT and FOY administered in the sensitization period could reverse the allergic status of mice, thus decreasing allergen-induced airway inflammation. After allergen challenge, although allergic status persisted, FUT and FOY still had an inhibitory effect on non-IgE mediated inflammatory processes, such as mast cell activation and inflammatory cytokines production in the allergic inflammation of the lung. The detail mechanisms exerted by serine protease inhibitors, FOY and FUT, in allergen-induced airway inflammation still need further investigation.

It is interesting to note that UTI, a urine trypsin inhibitor purified and concentrated from a human source, had no effect on our allergen-sensitized murine model of asthma. Previous reports^{27,28} showed that by inhibiting the activation of leukocytes via its elastase inhibitor effect, UTI might play certain roles in the protection of neutrophil-activating, endotoxins-induced sepsis. However, this protective effect seems not prominent in airway inflammation in patients with asthma, who had significantly higher serum UTI levels during an asthma attack.³¹ Therefore, the administration of UTI in subjects with asthma as well as in our murine model of asthma may not have a beneficial effect for symptom controls.

Serine protease inhibitors have been shown to have various beneficial anti-inflammatory effects by inhibiting the activation of leukocytes, and fibrogenolytic activity of

human tryptase generated during the coagulation cascade and the inflammatory process,¹⁹⁻²⁴ but the mechanism is still not fully elucidated. Previously, it has been reported that FUT and FOY inhibited tryptase released from mast cells,^{19,20,25} which may partially account for their anti-asthma effect. Although we do not have direct evidence to show the change of tryptase activity, the mast cell activation, measured by mMCP-1 serum level, was significantly decreased in the FUT-treated and FOY-treated mice when administrated during sensitization or after allergen challenge (see this article's Fig E3 in the Online Repository at www.jacionline.org). Moreover, the anti-inflammatory effects of FUT and FOY were not restricted to their inhibition of tryptase activity. Yuksel et al²³ found FOY was able to inhibit LPS-induced TNF- α production in human monocytes via inhibiting activation of both NF- κ B and activator protein-1 to target sites as well as through the activation of mitogen-activated protein kinase pathways. FUT was able to suppress LPS-induced nitric oxide synthesis and apoptosis in cultured human trophoblasts,³² inducing IL-12, IL-18, and IFN- γ production, and the expression of intercellular adhesion molecule 1, B7.1, B7.2, CD40, and CD40 ligand in human PBMCs.³³ In this study, we also found that alveolar macrophages in FUT-treated and FOY-treated mice had decreased nitric oxide production and CD86 expression compared with nontreated allergen-sensitized mice. Also, the synthesis of inflammatory cytokines such as IL-1 β , IL-6, and TNF- α as well as eotaxin was decreased, whereas production of anti-inflammatory cytokines such as IL-10 was increased in AMs from FUT-treated and FOY-treated mice. These protective effects on allergen-induced airway inflammation may through FUT-initiated intracellular signal transduction for promoting anti-inflammatory cytokines production and inhibit NF- κ B activation as shown in Fig E4 (see the Online Repository at www.jacionline.org). Further study on the immunopharmacological action of FUT and/or FOY should be performed before the application of this new regimen of treating allergic asthma.

In conclusion, we found that serine protease inhibitors FUT and FOY could exert their therapeutic effect in allergen-induced airway inflammation as a result not only of the inhibitory action in the early phase of mast cells activation but also of the immunoregulatory function in the late inflammatory phase of decreasing pulmonary eosinophilia, and attenuating T_H2 as well as other inflammatory cytokines production in asthma. Such properties of FUT and FOY might be useful in combination, or as an alternative treatment with current antiasthma medications for patients with asthma.

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