

Immune Responses to *Tyrophagus putrescentiae*–Induced Airway Inflammation in Mice

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■ Abstract

Background: Storage mites are a source of aeroallergens that affect patients with allergic rhinitis and asthma. *Tyrophagus putrescentiae* is a causative factor of airway hypersensitivity, but the mechanisms and pathogenesis of *T putrescentiae*–induced allergy are not well understood.

Objective: This study aimed to develop a murine model of *T putrescentiae*–induced allergic asthma.

Methods: Immune responses and physiologic variations in immunoglobulins (Ig), leukocyte subpopulations, cytokines, gene expression, pulmonary function, and lung pathology were evaluated after intraperitoneal sensitization and intratracheal challenge with crude extract of *T putrescentiae*.

Results: After sensitization with aluminum hydroxide and challenge with *T putrescentiae* in mice, levels of *T putrescentiae*–specific IgE and IgG1 in sera increased significantly compared to the normal saline group ($P < .01$). Values for inflammatory leukocytes (neutrophils and eosinophils) and cytokines (interleukin [IL] 4, IL-5, and IL-13) increased significantly after sensitization. In terms of pulmonary function, pause values were significantly enhanced in *T putrescentiae*–sensitized mice after intratracheal challenge with *T putrescentiae* ($P < .05$). Expression of type 2 helper T cell (T_H2)–related genes (IL4, IL5, IL13, and RANTES), T_H2 –specific transcription factor (*GATA-3*), and proinflammatory genes (IL6), and T_H17 –related genes (*IL17F*) increased significantly after airway challenge. Sensitization with *T putrescentiae* crude extract led to inflammation of lung tissue, thickening of the tracheal wall, and tracheal rupture.

Conclusions: Intraperitoneal sensitization followed by intratracheal challenge with crude extract of *T putrescentiae* can induce airway inflammation in BALB/c mice. The symptoms observed in a mouse model of allergic asthma, in terms of immune and clinical parameters, are reminiscent of the symptoms of allergic asthma in humans. A mouse model can be used to evaluate the therapeutic effectiveness of drugs on *T putrescentiae*–induced airway inflammation in humans.

Key words: Storage mites. *Tyrophagus putrescentiae*. Mouse model. Airway inflammation. IL-17F.

■ Resumen

Antecedentes: Los ácaros de almacenamiento son una fuente de aeroalérgenos en pacientes con rinitis y asma alérgica. El *Tyrophagus putrescentiae* (Tp) es causante de reacciones de hipersensibilidad de las vías respiratorias, sin embargo los mecanismos y la patogenia de esta enfermedad están aún por dilucidar.

Objetivo: El presente estudio tuvo como objetivo el establecer un modelo murino de asma alérgica inducida por el Tp.

Métodos: Los ratones fueron sensibilizados con un extracto crudo de Tp por vía intra-peritoneal y, posteriormente, provocados con el mismo, por vía intratraqueal. Se cuantificaron diferentes parámetros de la respuesta inmunitaria como: variaciones en la concentración de inmunoglobulinas, subpoblaciones leucocitarias, citocinas y expresión de genes, así como la función pulmonar y, finalmente, se realizaron estudios histológicos.

Resultados: Los ratones sensibilizados y provocados con Tp desarrollaron un aumento significativo de los niveles de IgE e IgG1 específicas de Tp en suero, en comparación con el grupo NS ($p < 0,01$). Asimismo, se observó un incremento significativo, después de la sensibilización, en la cifra de leucocitos inflamatorios (neutrófilos y eosinófilos) y de algunas citocinas (IL-4, IL-5 e IL-13). En la función pulmonar, se obtuvieron valores significativamente mayores de Penh ($p < 0,05$) en los ratones sensibilizados a Tp. En el estudio de la expresión génica, se observó que los genes relacionados con la respuesta Th2 (IL-4, IL-5, IL-13, y RANTES), Th2 específica del factor de transcripción GATA-3, pro-inflamatoria (IL-6), y Th17 (IL-17F), aumentó significativamente tras la provocación intratraqueal. En los ratones sensibilizados con extracto crudo Tp se confirmó una histología pulmonar con inflamación del tejido pulmonar y alteraciones traqueales.

Conclusión: La sensibilización intraperitoneal con extracto crudo de *T. putrescentiae*, seguida de una provocación intratraqueal, puede inducir la inflamación de las vías aéreas en ratones. Este modelo murino pudiera servir de base para evaluar la eficacia terapéutica de fármacos en la inflamación de las vías respiratorias, inducida por *T. putrescentiae*, dadas las similitudes encontradas en aspectos inmunológicos y clínicos, con el asma alérgica de los seres humanos.

Palabras clave: Ácaros de almacenamiento. *Tyrophagus putrescentiae*. Modelo animal murino. Inflamación de las vías aéreas. IL-17F.

Introduction

Increasing evidence suggests that sensitization to indoor allergenic mites is a causative factor in the development of airway hypersensitivity among people who are genetically predisposed to immunoglobulin (Ig) E–mediated responses [1-3]. Mites are generally divided into 2 categories, namely, house dust mites and storage mites [4,5], and both types have been shown to cause allergic symptoms of bronchial asthma, allergic rhinitis, conjunctivitis, and atopic dermatitis [6,7]. Concomitant sensitization to house dust and storage mites in patients with allergic rhinitis and patients with asthma is strongly associated with airway hypersensitivity [8]. In clinical practice, the importance of house dust mites grows daily, and, to date, 21 different groups of allergens have been characterized in house dust mites and found to elicit varying degrees of IgE reactivity and T-cell responses [7]. Recently, storage mites have been reported to be key allergenic components of dust samples worldwide, thus highlighting their clinical significance [9].

Storage mites have been reported to cause anaphylaxis through ingestion of mite-contaminated foods [10]. Based on its allergenic potential and abundance, the storage mite *Tyrophagus putrescentiae* is a major cause of allergic asthma and allergic rhinitis. *T. putrescentiae* is found worldwide, mainly in contaminated food such as wheat flour, wheat germ, cheese, and cereal [11]. *T. putrescentiae*–induced allergy is mostly reported as occupational exposure among farmers, grain workers, bankers, and food industry workers and is considered closely associated with respiratory diseases in these individuals [12]. Sensitization to *T. putrescentiae* has been reported in both urban and rural areas [13]. However, while it is well known that *T. putrescentiae* can cause allergic respiratory symptoms after occupational exposure, more attention is given to its allergenicity in

uninhabited environments and to its role in the pathogenesis of allergic diseases. At least 20 IgE-binding allergenic components of *T. putrescentiae* have been detected in human sera [11], but their effects on sensitization and pathogenesis have not yet been clarified. The mechanisms involved in intraperitoneal sensitization and intratracheal challenge with *T. putrescentiae* have not been determined.

Given that no suitable animal model of *T. putrescentiae*–induced allergic inflammation has been developed, it would be interesting to apply such a model to evaluate the effectiveness of therapy. This study aimed to observe physiologic changes during immunization using *T. putrescentiae* in conjunction with aluminum hydroxide in a mouse model. Specifically, this study aimed to evaluate physical variations in Ig, leukocyte subpopulations, cytokines, gene expression, and pulmonary function after intraperitoneal sensitization and intratracheal challenge with crude *T. putrescentiae* extract.

Materials and Methods

Animals and Experimental Schedule

Female BALB/c mice (6-8 weeks old) were obtained from the National Laboratory Breeding Research Center in Taiwan. The Animal Committee of the Taichung Veterans General Hospital approved the experimental procedures. Groups of 6 mice were caged separately according to their treatment and raised in a specific pathogen-free environment. The mice were sensitized 3 times by intraperitoneal injection with crude *T. putrescentiae* extracts emulsified in aluminum hydroxide (Whitehall Lab Ltd.) (Figure 1). After sensitization, the mice underwent intratracheal challenge twice with crude

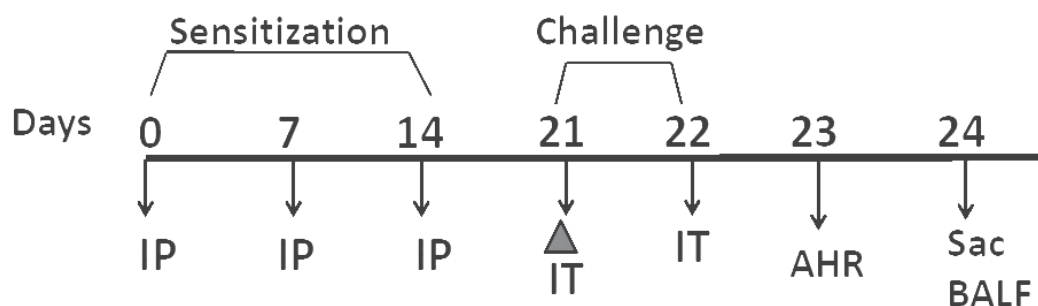


Figure 1. Schedule for intraperitoneal sensitization and intratracheal challenge. Intraperitoneal sensitization was divided into 3 groups: normal saline, Tp-40 µg with 4 mg aluminum hydroxide, and Tp-10 µg with 4 mg aluminum hydroxide. Intratracheal challenge involved inoculation with 5 µg/20 µL of crude *T. putrescentiae* extract. Blood samples were taken at day 21. IP indicates intraperitoneal sensitization; IT, intratracheal challenge; AHR, airway hyperresponsiveness; Sac, sacrifice after the AHR measurement; BALF, bronchoalveolar lavage fluid.

T putrescentiae extract (5 µg/20 µL), and pulmonary function was assessed by measuring airway hyperresponsiveness. The control group received an intraperitoneal injection of normal saline with no intratracheal challenge. Bronchoalveolar lavage fluid (BALF) and blood were collected after the mice were sacrificed.

Determination of Serum Allergen-Specific Ig

Serum was isolated using Ficoll-paque Premium (GE Healthcare), and *T putrescentiae*-specific IgE, IgG1, and IgG2a titers were determined using enzyme-linked immunosorbent assay (ELISA), as described previously [14]. Briefly, the crude *T putrescentiae* extract was prepared and added (5 µg/mL) to buffer-coated plates (0.05 M carbonate/bicarbonate; pH, 9.6) at 4°C overnight [15]. Diluted mouse sera (1:5 dilution for IgE, 1:100 dilution for IgG1, and 1:25 dilution for IgG2a) were incubated and washed with phosphate-buffered saline/0.05% Tween-20 (PBST). Goat antimouse horseradish peroxidase-conjugated antibody (1:800 dilution for IgE and 1:2000 dilution for IgG1 and IgG2a) (Southern Biotech Associates, Inc) was added and developed with 2,2'-azino-bis-3-(ethylbenzothiazoline-6-sulfonic acid) (Zymed). The reaction was stopped with 50 µL of 4N H₂SO₄, and the absorbance of optical density was determined at 405 nm using a PowerWare-X340 ELISA reader (Bio TEK). The results were expressed as ELISA units.

Bronchoalveolar Lavage Fluid Collection and Cytokine Measurement

In order to obtain BALF, 1 mL of sterile endotoxin-free saline was injected into the lungs of each mouse via the trachea. Approximately 0.85 mL of the washing solution was recovered. In the total leukocyte count, 100 µL of BALF was used for the cytopsin preparations before application of Liu stain (Tonyar Diagnostic Inc). The cytokines interleukin (IL) 4, IL-5, and IL-13 were measured using commercially available ELISA kits (R&D Minneapolis) containing mouse monoclonal antibody recognizing different epitopes of the cytokine molecules. The kits were used according to the manufacturer's instructions, and results were expressed as pg/mL in BALF. The lowest detectable concentration was 10 pg/mL.

Determination of Pulmonary Function

Airway hyperresponsiveness was measured by methacholine-induced airway obstruction as described previously [16] using 4 individual whole-body plethysmography chambers (Buxco Electronics). The results were analyzed using the BioSystem XA program (Buxco Electronics). The enhanced pause (Penh) was calculated following the manufacturer's instructions for monitoring airway hyperresponsiveness [17]. The aerosol was generated by placing 5 mL of PBS or methacholine solution (Sigma, 6.25-25 mg/mL) in the cup of an ultrasonic nebulizer (De Vilbiss). A dose-response curve was calculated for methacholine starting at a low dose of 6.25 mg/mL and increasing to 25 mg/mL. The Penh value obtained for each methacholine dose was presented as mean (SD). Differences in respiratory parameters between the groups were investigated using analysis of variance.

Cell Culture, Immunofluorescence Staining, and Flow Cytometry

Cytokines in activated murine helper T (T_H) cells were assayed according to the method of Assenmacher et al [18]. Three-color staining methods were used to analyze expression of IL-4 and interferon (IFN) γ in CD4⁺ cells. Leukocytes from the peripheral blood of 6 mice were pooled and stimulated with phorbol myristate acetate (PMA; 50 ng/mL), ionomycin (2 µM), and GolgiStop (Cytofix/Cytoperm Plus, Pharmingen) for 5 hours before being washed twice with PBS. The cells were stained with peridinin chlorophyll-a protein (PerCP)-conjugated rat antimouse CD4 monoclonal antibody (BD Biosciences) at room temperature for 30 minutes and washed with PBS. Cells were fixed with cytofix/cytoperm at room temperature for 30 minutes, washed with PBS, and stained with fluorescein isothiocyanate-conjugated rat antimouse IFN-γ monoclonal antibody (BD Biosciences) and R-phycoerythrin-conjugated rat antimouse IL-4 monoclonal antibody (BD Biosciences) at room temperature for 30 minutes and washed with PBS. The cells were resuspended in 0.5 mL PBS with 0.1% (wt/vol) sodium azide. Mean fluorescence was measured using a flow cytometer (Becton Dickinson). A total of 5000 cells were analyzed in each sample.

Statistical Analysis

SPSS Sample Power 2.0 was used to analyze the statistical power. The differences between the groups were analyzed using the Mann-Whitney test. Statistical significance was set at *P*<.05. Results were expressed as arithmetic mean (2SD) for each group.

Results

Effects of Intraperitoneal Sensitization on Serum Immunoglobulin Production

To determine the effect of crude *T putrescentiae* extract on immunoglobulin production, different doses of extract (10 or 40 µg) were used for intraperitoneal sensitization. The results showed that baseline levels of *T putrescentiae*-specific IgE and IgG1 were undetectable before sensitization on day 0. However, *T putrescentiae*-specific IgE and IgG1 in sera from the 40-µg group increased significantly (*P*<.01) when compared with those of the normal saline group on day 21 after sensitization (Figure 2). Although IgE and IgG1 were elevated in the 10-µg group, only IgG1 was significantly elevated. Sensitization with *T putrescentiae* had no effect on IgG2a production.

Effects of Intraperitoneal Sensitization and Intratracheal Challenge on Inflammatory Leukocytes and Cytokines in the Airways

Airway inflammation was compared by investigating inflammatory leukocyte subpopulations and cytokines in BALF. After intraperitoneal sensitization, the body weights of both experimental groups were similar to those of the normal saline group. Neutrophilic and eosinophilic

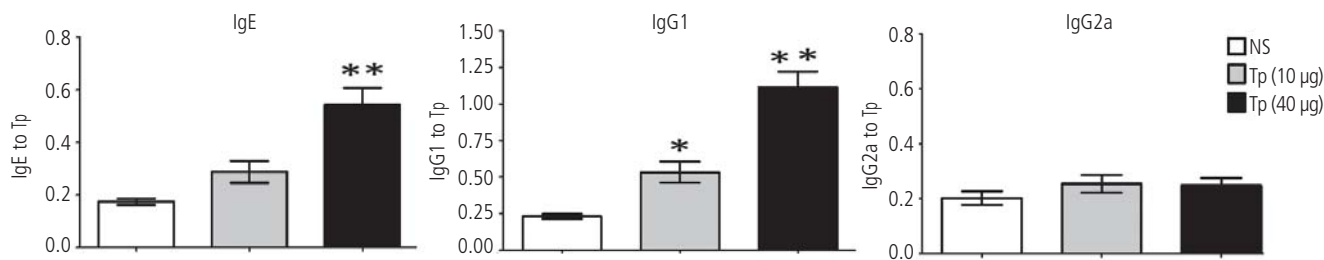


Figure 2. Serum concentrations of Tp-specific IgE, IgG1, and IgG2a from the 3 groups. Serum concentrations of Tp-specific IgE, IgG1, and IgG2a were measured by enzyme-linked immunosorbent assay on day 21. Values are expressed as mean (SD) optical density 405 nm for each group (n=6). **P*<.05 when compared to the NS group; ***P*<.01 when compared to the NS group. Ig indicates immunoglobulin; Tp, *Tyrophagus putrescentiae*; NS, normal saline.

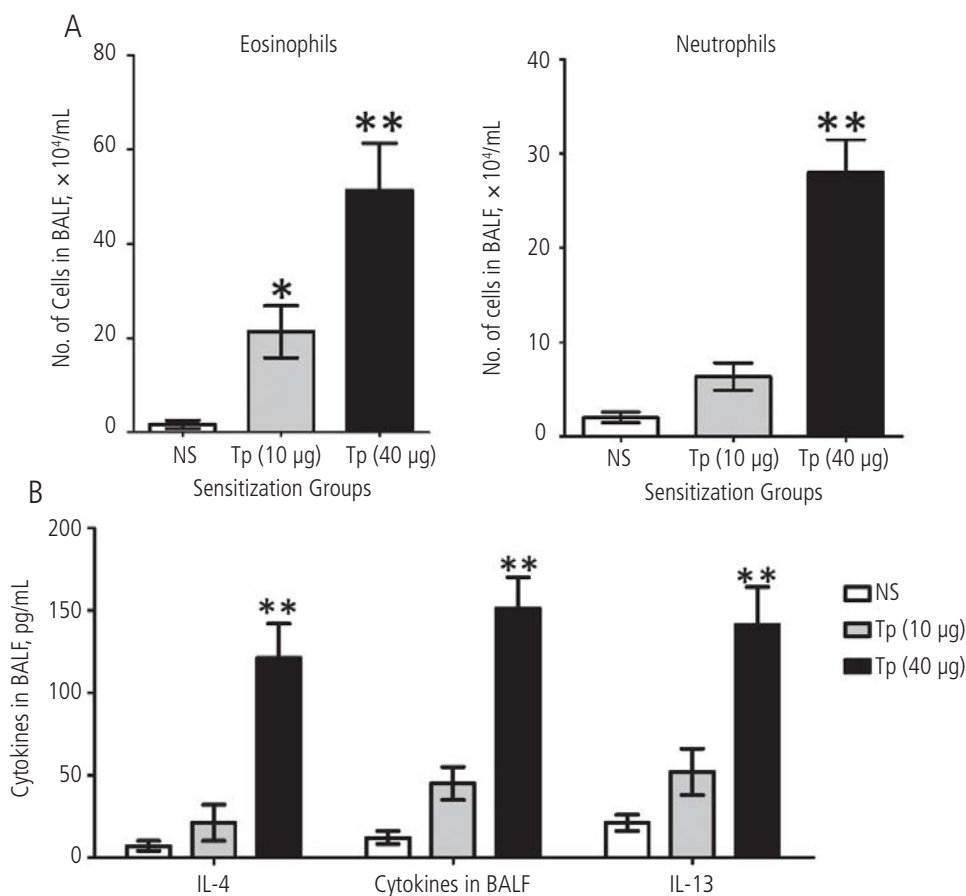


Figure 3. Differences in inflammatory leukocyte subpopulations and cytokine levels in BALF after sacrifice in the 3 groups of mice. A, Inflammatory leukocyte subpopulations. B, Cytokines in BALF. Data for neutrophils and eosinophils are expressed as mean (SD) × 10⁴ cells/mL for each group (n=6). Values of cytokines were presented as mean (SD) of OD 405 nm of each group (n=6). **P*<.05 when compared to the normal saline group; ***P*<.01 when compared to the normal saline group. BALF indicates bronchoalveolar lavage fluid; NS, normal saline; Tp, *Tyrophagus putrescentiae*; IL, interleukin.

infiltrations in BALF were significantly higher after sensitization with 40 µg of *T putrescentiae* extract than with normal saline (*P*<.01; Figure 3A). In *T putrescentiae*-sensitized mice, levels of IL-4, IL-5, and IL-13 in BALF were higher than in the normal saline group, but the

difference was only significant for the 40-µg group (*P*<.01; Figure 3B). Levels of inflammatory leukocytes (neutrophils and eosinophils) and cytokines (IL-4, IL-5, and IL-13) increased significantly after sensitization with crude *T putrescentiae* extract (40 µg).

Effects of Intraperitoneal Sensitization and Intratracheal Challenge on Airway Hyperresponsiveness

Because airway hyperresponsiveness was a functional trademark of asthma, hypersensitivity to methacholine was increased after intratracheal allergen challenge. Airway hypersensitivity to methacholine was evaluated 48 hours after 2 intratracheal challenges with *T putrescentiae*. Penh was increased after inhalation of methacholine in both the 10- μ g and the 40- μ g groups, but not in the normal saline group. In the *T putrescentiae*-sensitized mice, the Penh values were significantly enhanced ($P < .05$) after the intratracheal challenge (40 μ g) and methacholine challenge (at 12.5 and 25 mg/mL) (Figure 4).

Effects of Intraperitoneal Sensitization and Intratracheal Challenge on Production of Cytokines by Peripheral Blood Leukocytes

Peripheral blood leukocytes were collected and cultured with PMA and ionomycin for 5 hours before CD4⁺ cells

producing the T_H1-type cytokine IFN- γ and T_H2-type cytokine IL-4 were analyzed. The results showed that the percentage of CD4⁺ cells producing IL-4 was higher in *T putrescentiae*-sensitized mice than in the normal saline group (Table). The percentage of CD4⁺ cells producing IL-4 increased concomitantly with increasing concentrations of crude *T putrescentiae* extract. However, the percentage of CD4⁺ cells producing IFN- γ was lower in mice sensitized with *T putrescentiae* than in the normal saline group. When the ratios of CD4⁺ cells producing IFN- γ and IL-4 were compared between the groups, the lowest ratio was found in the mice sensitized with 40 μ g of crude *T putrescentiae* extract.

Effects of Intraperitoneal Sensitization and Intratracheal Challenge on Expression of Genes in Lung Tissue

Lung tissue was acquired for real-time polymerase chain reaction assay to investigate gene expression, including that of T_H2-related genes (*IL4*, *IL5*, *IL13*, *RANTES*, and *GATA-3*), the proinflammatory gene *IL6*, and the T_H17-related gene *IL17F*.

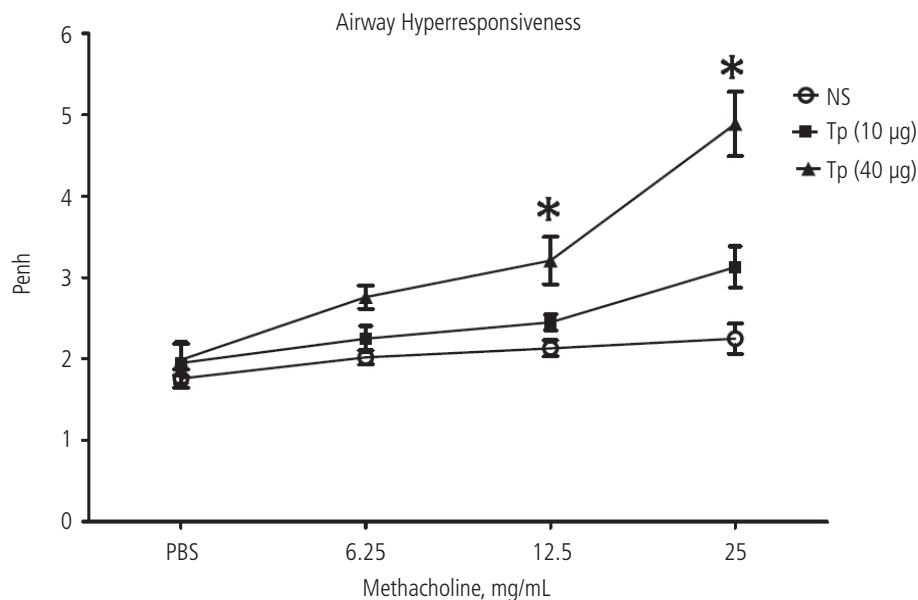


Figure 4. Differences in airway hyperresponsiveness to methacholine. Pulmonary function measurement after intratracheal challenge with crude Tp extract in NS or Tp-sensitized mice. Airway hyperresponsiveness to methacholine was measured 30 minutes after intratracheal challenge. Results are expressed as Penh. * $P < .05$ when compared to the NS group. Penh indicates enhanced pause; Tp, *Tyrophagus putrescentiae*; NS, normal saline.

Table. Percentages of Positive Cytokine-Staining Cells (IL-4 or IFN- γ) in CD4⁺ Lymphocytes From Peripheral Blood Leukocytes

Group	A (IFN- γ + CD4 ⁺ /CD4 ⁺)	B (IL4 + CD4 ⁺ /CD4 ⁺)	A/B
Normal saline	3.12	3.09	1.01
10 μ g	2.91	3.45	0.84
40 μ g	2.89	4.12	0.70

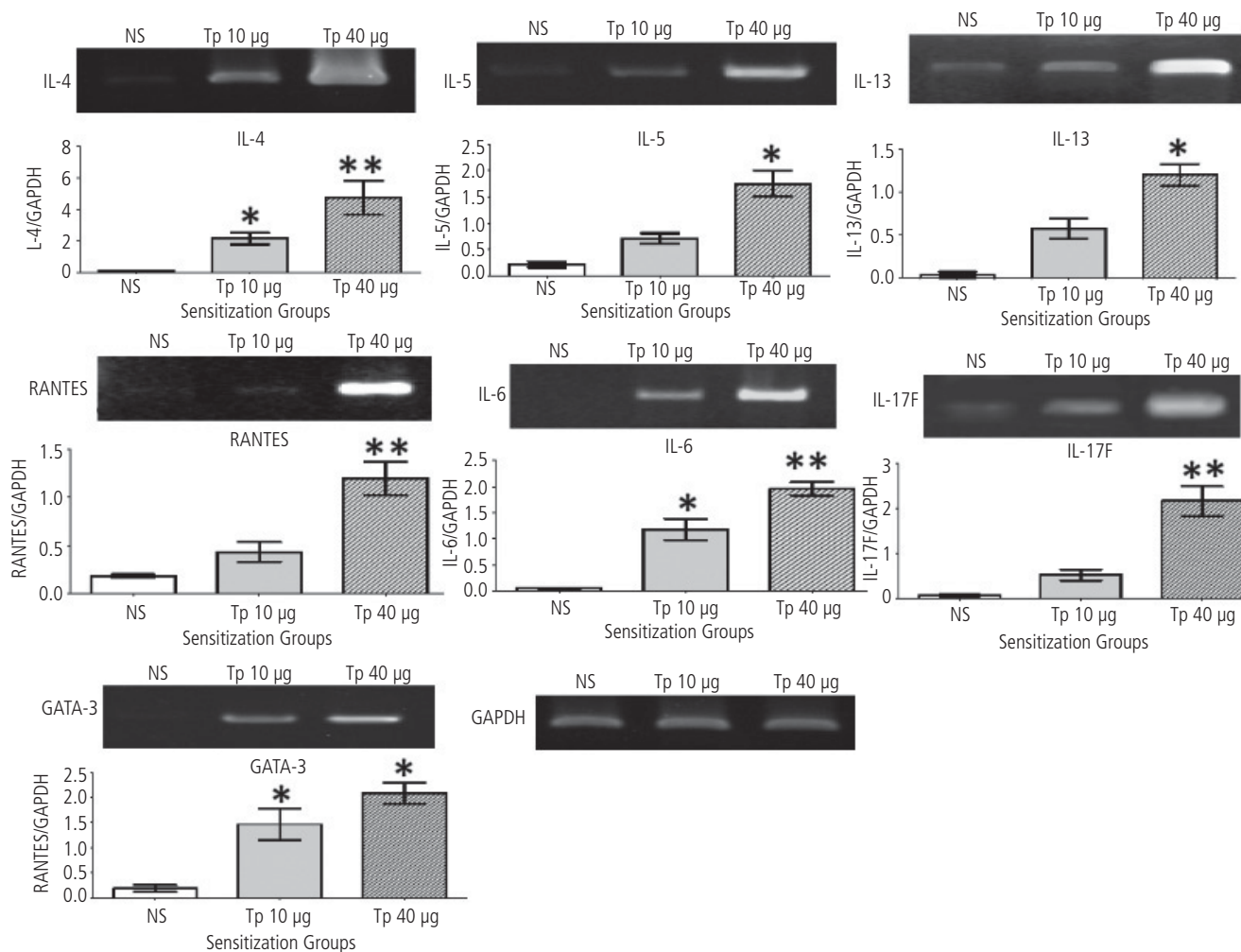


Figure 5. mRNA expression of inflammatory cytokines and transcription factors in lung tissue. Total mRNA was extracted from lung tissue after sacrifice, and mRNA expression was evaluated using reverse-transcriptase polymerase chain reaction. Data were the expressed as mean (SD) of each group (n=6). * $P < .05$ and ** $P < .01$ when compared to the NS group. NS indicates normal saline; Tp, *Tyrophagus putrescentiae*; IL, interleukin.

The results of real-time polymerase chain reaction revealed significant upregulation of the T_H2 -related cytokines IL-4, IL-5, and IL-13 (5-fold, 1.6-fold, and 1.2-fold baseline levels, respectively) in the 40- μ g group ($P < .05$; Figure 5). This group also had a 1.2-fold increase in the expression of the *RANTES* gene ($P < .01$), and a 2-fold increase over baseline in expression of *IL6* and *IL17F* ($P < .01$). Expression of the T_H2 -specific transcription factor *GATA-3* was 0.4-fold and 1.2-fold higher than that of the internal control in the 10- μ g and 40- μ g groups, respectively (Figure 5).

Effects of Intraperitoneal Sensitization and Intratracheal Challenge on Lung Pathology

A histopathology study (hematoxylin-eosin staining) was performed to determine whether intraperitoneal sensitization with crude *T putrescentiae* extract affected

lung pathology. The histologic scores for each field were based on tracheal thickness, inflammatory cell count, and tracheal rupture. The results showed that airway challenge led to marked infiltration of eosinophils in *T putrescentiae*-sensitized mice, especially in the 40- μ g group (Figure 6A). Both tracheal thickness and tracheal rupture were significantly increased in the 40- μ g group. Moreover, goblet cell metaplasia and mucus hypersecretion were more easily detected in mice sensitized to and challenged with *T putrescentiae*. The histologic scores were significantly higher in the 40- μ g group than in the normal saline group ($P < .01$) (Figure 6B). Representative photomicrographs and histological scores are shown in Figures 6A and 6C. The findings indicated that sensitization with crude *T putrescentiae* extract led to significant expression of lung tissue inflammation, tracheal thickening, and tracheal rupture.

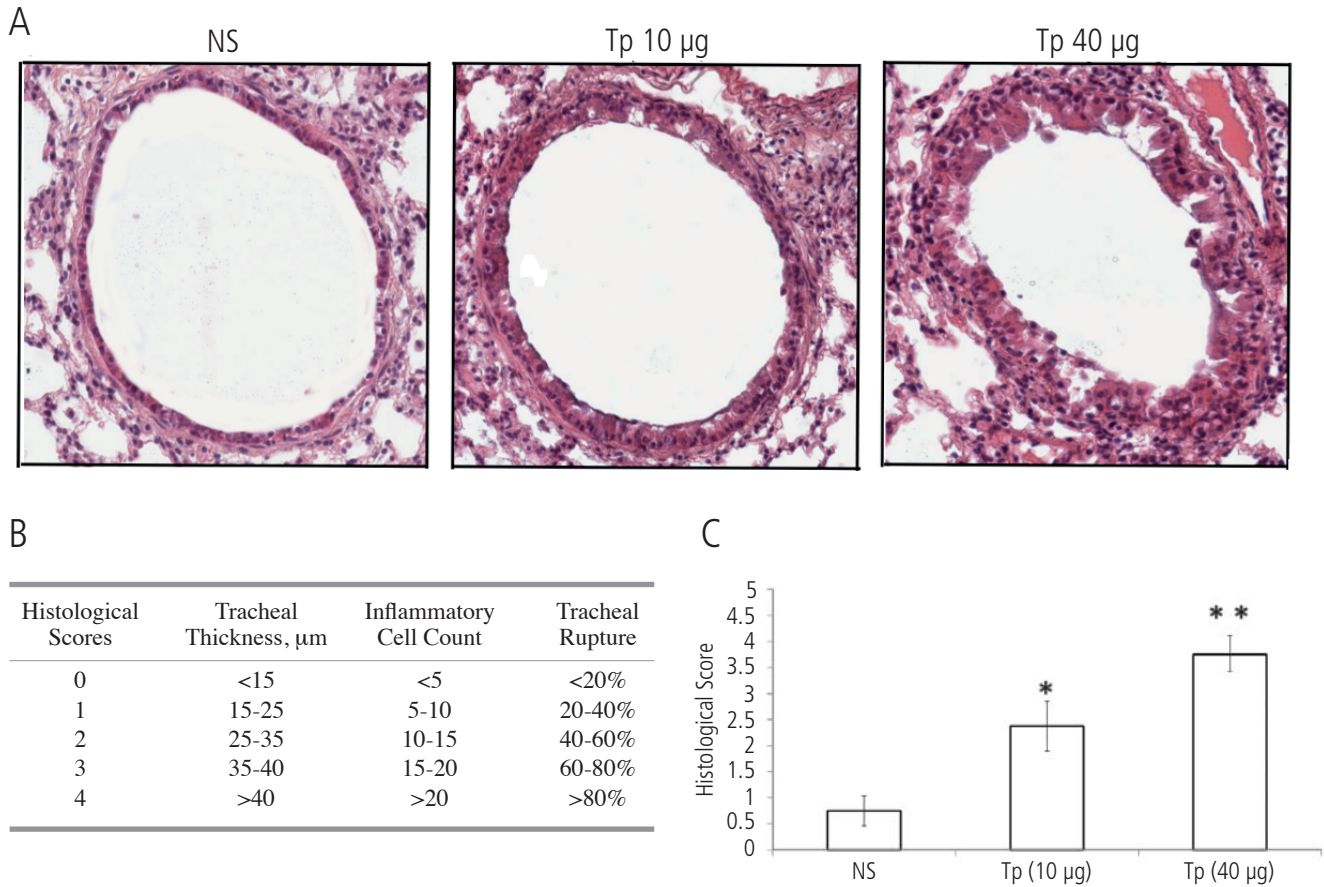


Figure 6. Histopathology of mice from different groups after sensitization and challenge with Tp. A, Representative photomicrographs from mice treated with NS, Tp 10 μ g, and Tp 40 μ g (hematoxylin-eosin, \times 100). B, The histologic score for each field is shown in terms of tracheal thickness, inflammatory cell count, and tracheal rupture. C, Semiquantitative analysis of lung inflammation. * P <.05 and ** P <.01 when compared to the NS group. NS indicates normal saline; Tp, *Tyrophagus putrescentiae*.

Discussion

Allergic disorders affect 10%-20% of the population in developed countries. Allergic symptoms are triggered mainly by allergen-mediated cross-linking of specific IgE on the surface of mast cells and basophils, leading to the release of histamine and other mediators. Storage mites, which are similar to house dust mites, have been shown to cause allergic diseases such as bronchial asthma, allergic rhinitis, and conjunctivitis. They cohabit with house dust mites in the environment, and have even caused anaphylaxis after ingestion of contaminated food [10,13]. The importance of storage mites is growing in clinical medicine, and the prevalence of sensitization to *T putrescentiae* in particular is significant, ranging from 22% to 43% in Korean [13], Taiwanese [15], and European allergy patients [19-21].

Several studies have used animal models of asthma and different allergen species to investigate airway inflammation; however, none have used *T putrescentiae* as the sensitizing allergen. The present study revealed that 40 μ g of *T putrescentiae* extract combined with 4 mg of aluminum hydroxide, not normal

saline, can upregulate production of *T putrescentiae*-specific IgE and IgG1 and significantly enhance the production of the inflammatory cytokines IL-4, IL-5, and IL-13 in BALF. We found similar results in a previous study, where serum concentrations of house mite allergen-specific IgG and IgE and the inflammatory cytokine IL-4 increased significantly after intraperitoneal sensitization and intratracheal challenge [22].

Brimnes et al [23] showed that allergen-specific IgE in serum and BALF doubled or increased 5-fold after mice were sensitized with 3 intraperitoneal injections of alum-adsorbed *Phleum pratense* extract followed by intranasal challenge. Immunoglobulin IgE has a critical role in triggering the allergic cascade, and IL-4 is the most important cytokine in IgE synthesis because of its ability to induce immunoglobulin isotypes to switch from IgM to IgE. It is now widely accepted that airway inflammation is the basis of airway hypersensitivity and that at least 3 inflammatory cytokines are involved in the pathogenesis of allergy. The first is dependent on IL-4 and mast cells, the second is dependent on IL-5 and eosinophils, and the third is mediated by IL-13 [24].

Combining the allergen with adjuvant alum facilitates

induction of asthma in mouse models [25] and promotes a strong antibody response or other effects that could influence allergic reactions. However, mice can still be sensitized without adjuvant alum before intranasal challenge with allergen to assess the characteristics of airway responses such as reactivity to aerosolized methacholine, infiltration of airway tissues with eosinophils, and bromodeoxyuridine-positive cell counts in the airway epithelium [26]. In the present study, we did not perform intraperitoneal sensitization without adjuvant aluminum hydroxide. However, intranasal or intraperitoneal exposure in the absence of aluminium hydroxide warrants further study.

The clinical symptoms of allergic asthma are characterized by airway hyperresponsiveness to specific allergenic stimulation, chronic eosinophil inflammation, elevated serum IgE levels, and excessive mucus production. In the current study, levels of inflammatory leukocytes (neutrophils and eosinophils) and cytokines (IL-4, IL-5, and IL-13) were significantly increased after sensitization and challenge with *T putrescentiae*. Further observations of variations in lung pathology and gene expression reveal significant upregulation of T_H2-related cytokines (IL-4, IL-5, and IL-13) and eosinophilic infiltration in lung tissue. The inflammatory mediators of allergic diseases are characterized by increased levels of activated T_H lymphocytes, mast cells, and eosinophils within the tracheal and bronchial mucosa [27,28].

Liu et al [29] demonstrated that intraperitoneal sensitization using *Dermatophagoides pteronyssinus* enhanced T_H2-based allergenicity and that immunotherapy with fungal immunomodulatory peptide enhanced T_H1-based immunogenicity and reduced T_H2-based allergenicity. While T_H2 cytokines trigger an isotype switch towards the production of IgE and IgG1, the T_H1 cytokine IFN- γ induces production of IgG2a by B lymphocytes. T_H1 cells secrete IFN- γ and promote cellular immunity and production of IgG2a. In contrast, T_H2 cells secrete IL-4 and induce IgE antibody production, thereby reciprocally regulating T_H1 and T_H2 responses. Daily intranasal exposure to crude house dust mite extracts elicits T_H2-type sensitization, eosinophilic airway inflammation, and airway hyperresponsiveness via a granulocyte-macrophage colony-stimulating factor–mediated mechanism in mice [30]. However, the effects of intranasal exposure to storage mite remain unclear and should be further investigated to clarify the impact of respiratory mucosal exposure to *T putrescentiae* on the generation of allergic inflammatory responses.

In the present study, IL-6 and IL-17F are expressed in lung tissues, as are inflammatory leukocytes and neutrophils in BALF after sensitization and challenge with *T putrescentiae*. Similar results for overexpression of IL-17F and increased neutrophil levels show an additive effect on Ig-induced allergic inflammatory responses in a mouse model of asthma [31]. IL-17F is involved in the stimulation of bronchial epithelial cells to induce IL-6, IL-8, growth-related oncogene α , and intracellular adhesion molecule 1 [32]. Sequentially, bronchial epithelial cells secrete a variety of mediators and proinflammatory cytokines and chemokines that play a key role in the recruitment of inflammatory cells (eg, lymphocytes, neutrophils, and eosinophils) into the interstitial and alveolar spaces. These findings suggest that IL-17F

can cause and perpetuate airway inflammation. Therefore, blocking overexpression of IL-17F could be a suitable therapeutic strategy for modulating airway inflammatory diseases.

The World Health Organization and International Union of Immunological Societies Allergen Nomenclature Sub-committee (www.allergen.org) lists 23 groups of mite allergens. At least 20 IgE-binding allergenic components of *T putrescentiae* have been distinguished. To date, few major allergens have been identified in *T putrescentiae* [33]. As for the prevalence of IgE binding, the most important components are the 16-kDa group 2 allergens, which are also the most ubiquitous and dominant [33,34]. Tyr p 3 plays a key role in allergic reactions. Although it has been shown to possess enzymatic activity [15], the effects of Tyr p 3 on sensitization and pathogenesis have not yet been clarified. No previous animal model of *T putrescentiae*-induced allergic inflammation has evaluated the effectiveness of therapy in further investigations.

Mouse asthma models are the most popular approach to modeling allergic responses in the airway. Mice exposed to 10–70 μ g of the indoor mold *Penicillium chrysogenum* by involuntary aspiration were observed to undergo dose-dependent increases in allergic asthma parameters such as eosinophilic infiltration, IL-5 levels, total IgE levels, and severity of histopathologic lesions [35]. It has been suggested that molds differ in terms of the threshold at which they induce asthma [35]. Our results showed that the induction threshold of *T putrescentiae* extracts was >40 μ g and that this level increased dose-dependent allergic asthma-like responses in mice. Depending on the dose of antigen used, the inflammatory response to allergen challenge in sensitized mice usually results in an extensive influx of inflammatory cells (eg, eosinophils or neutrophils) into the airways. The development of human allergic diseases depends on 2 critical factors, exposure levels and sensitization thresholds, which are unknown for storage mite allergens, including *T putrescentiae*. Our findings suggest a threshold dose for the induction of allergic responsiveness to *T putrescentiae*. Both *T putrescentiae* and other dust mites can play an important role in airway inflammation and the development of systemic allergic diseases.

In conclusion, we designed a mouse model of allergic asthma to elucidate the mechanisms involved in sensitization and challenge with storage mites. Physiologic variations and immune responses after intraperitoneal sensitization and intratracheal challenge with *T putrescentiae* induced airway inflammation in BALB/c mice. In terms of immune and clinical parameters, our model closely reflects the symptoms associated with allergic asthma in humans and can be used to evaluate the effectiveness of therapy in the future.

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