

Prevalence of *Tyrophagus putrescentiae* Hypersensitivity in Subjects over 70 Years of Age in a Veterans' Nursing Home in Taiwan

En-Chih Liao^a Chau-Mei Ho^a Jaw-Ji Tsai^{a–c}

^aInstitute of Clinical Medicine and Department of Tropical Medicine, National Yang Ming University, Taipei,

^bDepartment of Medical Education and Research, Taichung Veterans General Hospital, and

^cCollege of Life Sciences, National Chung Hsing University, Taichung, Taiwan, ROC

Key Words

Tyrophagus putrescentiae • Elderly subjects • Mite hypersensitivity • Tyr p 2 • Tyr p 3

Abstract

Background: Domestic mites are present in house dust samples throughout the world. Reports have shown a high prevalence of *Tyrophagus putrescentiae* (Tp) sensitization in Europe and Asia, and its importance and clinical relevance in elderly subjects have grown rapidly. The main objective of this study was to investigate the prevalence of Tp sensitization in elderly subjects in a veterans' nursing home using mite allergen extracts and recombinant allergens. **Methods:** A total of 199 subjects were enrolled in this study: 112 elderly subjects from a nursing home and 87 healthy young adults from the hospital staff as controls. The prevalence of Tp hypersensitivity was determined by specific IgE measurements and basophil histamine release. Immunoblotting with or without inhibition with *Dermatophagoides pteronyssinus* (Dp) was performed to identify the major allergens and species-specific allergen to Tp. **Results:** It was determined that 39.3% (44/112) of the elderly population were sensitized to Tp and 17.9% (20/112) to Tp alone. There was a significantly higher prevalence of Tp hypersensitivity in elderly subjects in comparison with the young adult population. In the age association study of Tp and Dp sensitization, the elderly sub-

jects were more sensitized to Tp than to Dp ($p = 0.02$). Among the elderly subjects with chronic obstructive pulmonary disease (COPD) 45.8% (11/24) were Tp sensitive. The major allergens, Tyr p 2 and Tyr p 3, were identified with molecular weights of 16 kDa (53%) and 26 kDa (50%) as determined by ELISA and immunoblot inhibition analyses. **Conclusion:** The prevalence of Tp sensitization was higher in elderly subjects, especially in patients with COPD. The high percentage of IgE-binding components to the allergens Tyr p 2 and Tyr p 3 indicated that both allergens may play a role in the pathogenesis of IgE-mediated allergic diseases in elderly populations.

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Introduction

The most prevalent and well-investigated indoor perpetual allergen sources are domestic mites. Domestic mites can be divided into 2 categories: pyroglyphid and nonpyroglyphid mites and are commonly referred to as house dust mites (HDMs) and storage mites, respectively. Among storage mites, *Tyrophagus putrescentiae* (Tp)

C.-M.H. and J.-J.T. both contributed to the supervision of this work.

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1018–2438/10/1524–0368\$26.00/0

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Correspondence to: Prof. Jaw-Ji Tsai
Department of Medical Education and Research
Taichung Veterans General Hospital
160, Section 3, Chung-Kang Road, Taichung 407, Taiwan (ROC)
Tel. +886 42359 2525, ext. 4000, 4032, Fax +886 42359 2705, E-Mail jawji@vghtc.gov.tw

is particularly predominant and has high allergenicity [1]. In clinically relevant studies, the importance of *Dermatophagoides pteronyssinus* (Dp) and Tp has recently grown [1] insofar as both kinds of mites can cause allergic respiratory symptoms after sensitization and exposure. Sensitization to Dp and Tp is an important risk factor for the development of bronchial asthma [2–4]. A previous study demonstrated that Dp is highly prevalent in Taiwan, where the climate is subtropical [5]. More than 80% of asthmatic patients are allergic to Dp as determined by skin tests and specific IgE measurements [6]. Dp and Tp have been demonstrated to be related to allergic diseases in Taiwan [5, 7]. Although Tp can cause allergic respiratory symptoms after occupational exposure in farms and grain elevator stores, currently, a lot of attention is being paid to its allergenicity in nonoccupational environments [8].

There is a high prevalence of Tp sensitization in general adult populations in both Europe [8] and Asia [9]. In Spain, where the climate is rainy and temperate, HDM-allergic patients also show a very high prevalence (73–83%) of storage mite sensitization [8]. There, 52% of adult individuals over the age of 50 are sensitive to Tp, which is much higher than Dp (22%) in the same age group. Similar findings have been reported in Korea. Tp is the third most common mite in the indoor environment and around one third of individuals are allergic to it [9]. Despite the high prevalence of Tp sensitization in the general adult population, especially in individuals over 50, there are few reports on its clinical significance in elderly populations. It is of interest to evaluate the allergenicity, prevalence of hypersensitivity and clinical relevance of Tp sensitization in elderly populations.

The most important allergenic mite components in terms of prevalence of IgE binding are the 16-kDa group 2 allergen, which is the more ubiquitous and dominant allergen in both Dp and Tp [9], and the 26-kDa group 3 allergen, and there is a limited level of allergen cross-reactivity between Dp and Tp [7]. Blo t 5 is the major allergen of *Blomia tropicalis*; however, it was found to have limited cross-reactivity with Dp in our previous report [10]. It is important to identify the species-specific major allergens in Tp and their cross-reactivity with Dp.

In this study, we aimed to investigate the prevalence of Tp hypersensitivity and identify the major allergen affecting elderly subjects over 70 years of age. The clinical significance was evaluated by means of specific IgE measurements and basophil histamine release in individuals with chronic obstructive pulmonary disease (COPD). Immunoblot inhibition analysis was also per-

formed to avoid cross-reactivity with Dp. The major allergens were identified by their high frequency of IgE binding and confirmed by the inhibition of rTyr p 2 and rTyr p 3.

Materials and Methods

Study Subjects

A total of 112 elderly subjects with ages ranging from 70 to 90 years were recruited in this study and all of these subjects were males. Most of the subjects were veterans who had been in the army for more than 10 years, then retired and resided in the public veterans' nursing home. The criteria of the patients' symptoms followed 'Resident Assessment Instrument Minimum Data Set in the nursing home, MDS Version 2.1'. COPD was diagnosed according to the Global Initiative for Chronic Obstructive Lung Disease Criteria and further confirmed by gerontologists. All of the patients were in a stable condition and ambulatory. A total of 87 young adults with an age range from 18 to 40 years from the hospital staff and young adults who underwent health exams in Taichung Veterans General Hospital (TCVGH) were recruited as controls. The veterans' nursing home and TCVGH are located in a rural area of the middle of Taiwan.

This study was performed in cooperation with the Geriatric Research, Education and Service Group of TCVGH. The Institutional Review Board of TCVGH reviewed and approved the ethical issues of this study. Verbal consent was obtained from the study participants after detailed explanations had been given. Serum samples were obtained for the IgE measurement of sensitization to Tp and Dp. Since the skin test reaction in response to histamine is poor and comorbidity with chronic diseases is higher in elderly compared with younger subjects, basophil histamine release assays were performed to identify the allergen-specific IgE-triggering hypersensitivity in these subjects.

Crude Extracts of Mite Bodies

Two kinds of mite crude extracts were used in this study. The crude extracts of Dp were prepared from lyophilized whole mite bodies purchased from Allergon (Angelholm, Sweden). The Tp mites were cultured in our laboratory with medium consisting of yeast extract and mouse chow, then mite bodies were separated from the medium. Separation of the mites from the medium was achieved by gently stirring the medium with a glass rod following overnight culture. The mites that migrated to the cover were collected. This collection included a great proportion of mite bodies and was almost free of medium. Frozen Tp mites were homogenized and extracted with phosphate-buffered saline (PBS, pH 7.2). Protein concentration was determined by the Bradford assay (Bio-Rad, Hercules, Calif., USA) using bovine serum albumin as a standard.

Determination of IgE Antibodies in Sera against Tp and Dp Crude Extracts

The IgE antibodies against Tp and Dp crude extracts in sera were determined by an enzyme-linked immunosorbent assay (ELISA). Tp or Dp crude extracts (5 µg/ml per well) were diluted with coating buffer 0.1 mol/l NaHCO₃ (pH 8.4), then added to plates and left overnight at 4°C. The human sera were diluted 1:5

with phosphate-buffered saline, 1% Triton X-100, pH 7 (PBST) before use; a total of 100 µl of diluted sera was added and incubated overnight at 4°C. For the detection of antibodies, alkaline phosphatase-conjugated mouse antihuman IgE antibodies diluted 1:2,000 in PBST (Southern Biotech Association, Birmingham, Ala., USA) were added for the assay. Each step was continued after washing with PBST 3 times, and the bound enzyme substrate was detected using *p*-nitrophenyl phosphate (Kirkegaard and Perry Laboratories Inc., Gaithersburg, Md., USA). The reaction was stopped after 30 min and the optical density measured at 405 nm. Normal healthy individuals were selected from our hospital staffs that were skin tests negative to both Dp and Tp mite allergens. The negative cutoff values were defined as values below mean $\pm$ 2 standard deviations; samples exceeding 0.25 units were considered as a positive reaction. For the inhibition preabsorption assay, each serum sample was preincubated with 50 µl of PBS, rTyr p 2 or rTyr p 3 (50 µg/ml), then left overnight at 4°C and subsequently reacted with 5 µg/ml of Tp extract coated on the plates. The following processes for determining IgE antibodies by ELISA were the same as those described previously.

Immunoblot Analysis

For the analysis of the components recognized by IgE antibodies, protein components of mite extracts were separated by SDS-PAGE and immunoblot analysis of Tp allergenic components as previously described [11]. Tp crude extract (300 µg) was separated by SDS-PAGE (12.5%) and transferred onto a polyvinylidene difluoride membrane. After being blocked with 3% skimmed milk in PBST, the blots were incubated with diluted serum samples (5× dilution in PBST) and 1% skimmed milk for 16 h at 4°C. After washing with PBST, the blots were incubated with alkaline phosphatase-conjugated monoclonal antihuman IgE antibodies (Pharmingen, Calif., USA) for 2 h at room temperature. The blots were developed in a substrate solution of carbonate buffer (0.1 mol/l NaHCO₃, 1 mol/l MgCl₂, pH 9.8) containing 0.35 mol/l 5-bromo-4-chloro-3-indolyl phosphate *p*-toluidine salt (BCIP) and 0.37 mol/l *p*-nitroblue tetrazolium chloride (NBT) for about 30 min. After washing with H₂O, antigen-antibody binding was recorded photographically. For the inhibition immunoblot assay, serum samples were selected from 22 elderly allergic subjects (A1–A22) who had IgE antibodies against Tp crude extracts using ELISA. Each serum sample was preincubated with 50 µl of Dp crude extract (50 µg/ml) and left overnight at 4°C. After preabsorption, the subsequent procedures for determining IgE by immunoblot were the same as those described previously. Furthermore, 6 subjects (A4, A10, A12, A16, A20 and A21) who had IgE antibodies against Tp extracts with molecular weights of 16 and 26 kDa were selected for inhibition immunoblots with 50 µl of rTyr p 2 or rTyr p 3 (50 µg/ml).

Recombinant Allergens of Tp

The major allergens of Tp (Tyr p 2 and Tyr p 3) were used to analyze the differences of IgE reactivity between the different age groups. Recombinant Tyr p 2 and Tyr p 3 were expressed and purified as previously described [7, 12]. Total RNA of mites was isolated with Trizol reagent and cDNA was synthesized with primer dT₁₇, MMLV reverse transcriptase (Gibco-BRL, New York, N.Y., USA). The specific primers were designed based on the accession number in the National Center for Biotechnology Information, Tyr p 2 (O02380) and Tyr p 3 (EU302595). The PCR

products were ligated into a pQE30 vector and transformed into *Escherichia coli*-M15. Transformants were selected by kanamycin (25 µg/ml) and ampicillin (100 µg/ml). Expressions of recombinant allergens were performed according to the methods described in the QIA Expressionist™ Kit manufacturer's directions (Qiagen, Hilden, Germany). The recombinant proteins were expressed as 6× His-tagged proteins using 1 mmol/l isopropyl-β-D-thiogalactopyranoside (Promega, Madison, Wisc., USA) induction. The proteins were purified by nickel-nitrilotriacetic acid agarose metal affinity column chromatography under native conditions.

Basophil Histamine Release Assay

Washed polymorphonuclear cells were obtained from venous blood of nonatopic donors using Polymorphpret™ solution (Axis-Shield, Oslo, Norway). The cells were resuspended in RPMI-1640 medium by adjusting to 2×10^6 cells/ml using trypan blue assessment. Passive sensitization of basophils with specific IgE from subject sera was performed. The sensitized cells were triggered with 50 µg/ml of allergen for 30 min at 37°C, the supernatant was collected and reacted with *o*-phthalaldehyde (OPA, 5 mmol/l) for 7 min. The reaction was stopped by adding H₂SO₄ (0.04 mol/l). The histamine released into the supernatant was measured with a fluorescence spectrophotometer [13]. The percent histamine release was calculated by the following formula: (stimulated released histamine – spontaneously released histamine)/total released histamine $\times$ 100%.

Statistical Analysis

The SPSS Sample Power 2.0 was used for power calculation analysis. We used the χ^2 test with Yates' correction for continuity to test the significance of associations between mite sensitization and different age groups. Statistical comparisons between individuals allergic or nonallergic to mites relative to the occurrence of COPD were performed by the χ^2 test. Two-tailed *p* values lower than 0.05 were considered statistically significant.

Results

Prevalence of Mite Sensitization in Elderly Subjects over 70 Years of Age

Sensitization of the 112 elderly subjects to Tp and Dp was determined by measuring serum IgE antibodies against Tp and Dp crude extracts. The results showed that 45 subjects (40.1%) were sensitized to mites and more subjects were sensitized to Tp in comparison with Dp. Forty-four subjects (39.3%) sensitized to Tp, 25 subjects (22.3%) were sensitized to Dp, 24 subjects (21.4%) to both Tp and Dp, and 67 subjects (59.8%) nonsensitized to either Dp or Tp (table 1). When different age subgroups were compared, the results showed that the prevalence of sensitization to Tp was apparently higher than to Dp in all subgroups.

Table 1. Prevalence of sensitization to Tp and Dp in different age groups of elderly subjects

Sensitization	Age groups			
	70–79 years (n = 33)	80–89 years (n = 71)	90–99 years (n = 8)	Total (n = 112)
Tp+	12 (36.4)	29 (40.8)	3 (37.5)	44 (39.3)
Dp+	4 (12.1)	19 (26.8)	2 (25.0)	25 (22.3)
Tp+ and Dp+	4 (12.1)	18 (25.3)	2 (25.0)	24 (21.4)
Tp+ or Dp+	12 (36.4)	30 (42.3)	3 (37.5)	45 (40.1)
Negative	21 (63.6)	41 (57.7)	5 (62.5)	67 (59.8)

Figures in parentheses are percentages of affected subjects among the total subjects of the same age group.

Table 2. Difference in sensitization between different age groups among aged subjects and association between age of and sensitization to Tp and Dp**A** Difference of sensitization between different age groups

Age	Age group		p value
	≥70 years	<40 years	
Cases, n	112 (100)	87 (100)	
Tp+	44 (39.3)	16 (18.4)	0.003
Dp+	25 (22.3)	25 (28.7)	0.365
Tp+ only	20 (17.9)	4 (4.6)	0.009
Dp+ only	1 (0.9)	13 (14.9)	<0.001
Tp+ and Dp+	24 (21.4)	12 (13.8)	0.241
Tp+ or Dp+	45 (40.1)	29 (33.3)	0.427

B Association between age and sensitization to Tp and Dp

Age ¹	Sensitization to Tp	Sensitization Dp	p value
70 years or above	44	25	0.020
Under 40 years	16	25	

Figures in parentheses are percentages. Yates' correction of contingency was used.

¹ Age was associated with Tp and Dp sensitization.

Differences of Mite Sensitization between Elderly and Young Adult Subjects

Differences in sensitization to Tp and Dp were compared between elderly and young adult subjects. The results showed there were 39.3% (44/112) Tp-sensitive elderly subjects while there were only 18.4% (16/87) Tp-sensitive young adult subjects; there was thus a significantly

higher prevalence of Tp sensitivity in the elderly subjects ($p = 0.003$) (table 2A). There were 14.9% (13/87) Dp-sensitive young adults and 0.9% (1/112) Dp-sensitive elderly subjects; there was thus a significantly higher prevalence of Dp sensitivity in the young adults than in the elderly subjects ($p < 0.001$). The data showed that the prevalence of subjects who were only sensitized to Tp was also higher in the elderly than in the young adult subjects (17.9 vs. 4.6%, $p = 0.009$). The age association of Dp and Tp sensitization between the elderly and young subjects was further analyzed. The prevalence of sensitization to Tp was significantly higher in the elderly subjects, while the prevalence of sensitization to Dp was significantly higher in the young adults. Tp and Dp sensitizations were associated with age ($p = 0.02$) (table 2B).

Major Allergenic Components of Tp

A total of 44 sera with ELISA-positive reactions to Tp from the 112 elderly subjects were selected for identification of the major allergenic component by IgE immunoblotting. The results showed there were at least 16 IgE-binding components in the Tp crude extract (fig. 1) with molecular weights ranging from 10 to 100 kDa. The three major IgE-binding components were proteins with molecular weights of 16 kDa (53%), 26 kDa (50%) and 24 kDa (38%).

Identification of Tp-Specific Allergenic Components by IgE Immunoblot Inhibition with Dp

Among the 16 IgE-binding proteins in the Tp crude extract as determined by the Tp-positive sera, only two major allergens with molecular weights around 16 and 26 kDa reacted with more than 50% of Tp-positive sera (fig. 2). To identify the Tp-specific allergenic components, a total of 22 sera from the elderly subjects were se-

Fig. 1. Frequency of IgE-binding components in Tp crude extract. The sera from elderly subjects with ELISA-positive reactions to Tp were selected for immunoblot analysis against Tp.

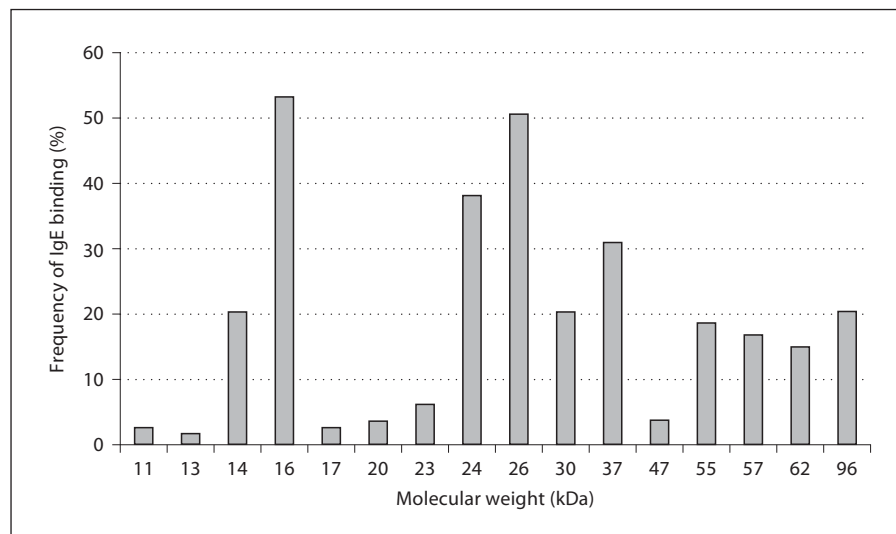


Fig. 2. Tp-specific allergenic components were identified by immunoblot inhibition analysis of IgE reactivity by sera from the elderly subjects. Sera pretreated with or without Dp crude extract were used to identify the specific IgE-binding components to Tp crude extract. M: protein marker; Tp crude: the crude extracts of Tp were separated by SDS-PAGE, transferred onto a PVDF membrane and stained with Coomassie blue. ▲: Sera pre-absorbed with 50 µg/ml Dp crude extract. Arrows point to the IgE-binding components with molecular weights of 16 and 26 kDa.

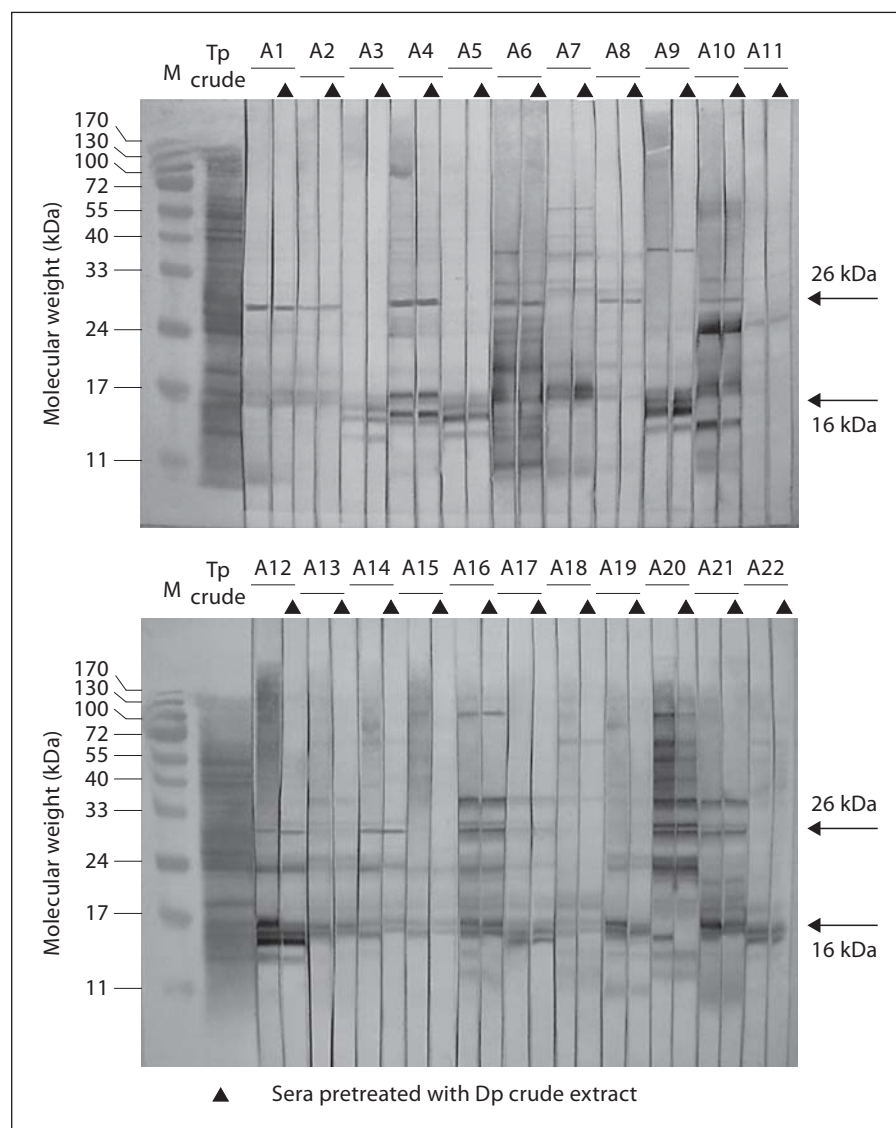
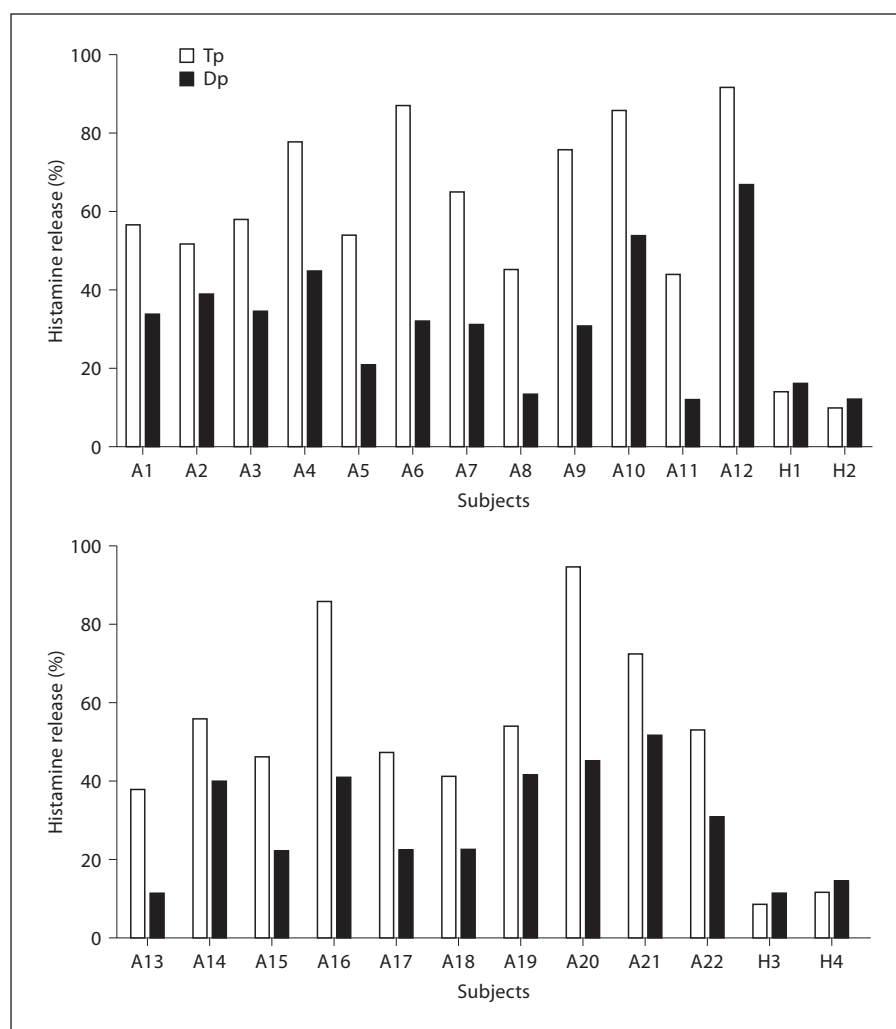


Fig. 3. Percentage of histamine release from basophils. Histamine release of basophils presensitized with serum samples from elderly subjects was then triggered by Tp or Dp at a concentration of 10 µg/ml. Tp (□): crude extract of Tp; Dp (■): crude extract of Dp; A1–A22: elderly subjects; H1–H4: normal subjects.



lected for IgE immunoblot inhibition. A PVDF membrane of the Tp crude extract was used to identify Tp-specific allergens reacting with the sera after the absorption of Dp. Almost none of the Tp IgE-binding components were absorbed by Dp, indicating that the level of cross-reactivity between Dp in elderly subjects was limited, except for the IgE-binding component with a molecular weight of 16 kDa, which was partially absorbed by Dp.

Histamine Release by Basophils after Sensitization with Sera of Elderly Subjects

A total of 22 sera from elderly subjects were used for the basophil histamine release assay. Basophils were pre-treated with sera followed by either Tp or Dp activation. The results showed that the Tp-triggered percent histamine released by basophils presensitized with sera of all

elderly subjects apparently higher than that triggered by Dp (fig. 3).

Percentage of Mite-Sensitive Elderly Subjects with COPD

The clinical relevance of mite sensitivity in elderly subjects with COPD was analyzed. Among the 112 elderly subjects, 24 (21.2%) had COPD. Among the 24 subjects with COPD, there were 45.8% (11/24) Tp-sensitive subjects and 25% (6/24) Dp-sensitive subjects (table 3). All of the Dp-sensitive subjects were also Tp-sensitive. The results showed that the elderly subjects with COPD had a higher prevalence of sensitivity Tp than to Dp. However, the differences between the COPD and non-COPD groups were not statistically significant.

Table 3. Cases of mite allergy in elderly subjects with COPD

Cases		Allergic			Nonallergic
		Tp+	Dp+	Tp+ and Dp+	
COPD	24 (100)	11 (45.8)	6 (25.0)	6 (25.0)	13 (54.2)
Non-COPD	88 (100)	33 (37.5)	19 (21.6)	18 (20.5)	54 (61.4)

Nonallergic = Sensitization to neither Tp nor Dp.

Role of Group 2 or Group 3 Allergens in Tp Sensitization in Elderly Subjects

To evaluate the role of Tyr p 2 and Tyr p 3 in the Tp-induced IgE-mediated hypersensitivity, IgE ELISA inhibition by rTyr p 2 or rTyr p 3 from the serum samples of elderly subjects was performed. The results showed that in 13 out of 22 sera (59%) the IgE-binding activity could be inhibited by over 50% by rTyr p 2. In 11 of 22 sera (50%) IgE-binding activity could be inhibited by over 50% by rTyr p 3 (fig. 4A). To identify the major allergens of Tp, 7 sera which reacted to both Tyr p 2 and Tyr p 3 were selected for Western blot inhibition. The results showed that all positive sera could be absorbed by either rTyr p 2 or rTyr p 3 (fig. 4B).

Discussion

The widespread existence of Tp in urban and rural household settings is an important occupational hazard in agricultural environments or workplaces [14]. A high prevalence of Tp sensitization has been found in a general adult population in Spain, and the sensitization to Tp is apparently higher than that to Dp in subjects over 60 years of age [8]. Similar sensitization patterns have been observed in Barbados, where the sensitization to the major allergen, Bt, is higher than that to Dp in asthmatic patients over 60 years of age [15]. In this survey, a higher percentage of Tp sensitization compared with Dp was also found in an elderly population (over 70 years of age) living in a veterans' nursing home.

It has been reported that the mean duration for Tp sensitization is about 3 times longer than that of Dp [16]. In this study, the elderly subjects had a significantly higher incidence of Tp sensitivity and a high prevalence of IgE binding to the allergens in their sera. These results could be due to either a high amount of Tp present in the veterans' nursing home or due to a long duration of Tp sensitization.

Although there were at least 16 IgE-binding components in the Tp crude extract as determined by the Tp-positive sera, there were only two major allergens with molecular weights around 16 and 26 kDa that could be reacted with more than 50% of the Tp-positive sera in our results. Similar findings have been reported [7, 9], i.e., there are 21 groups of mite allergens according to the Allergen Nomenclature Sub-Committee of the International Union of Immunological Societies, but only a few major allergens have been identified in Tp.

Basophil histamine release has been reported to be correlated with the symptoms of hypersensitivity reactions [17], and the basophil histamine release assay demonstrated higher responses to Tp than to Dp in the elderly group, indicating that elderly subjects were sensitized predominantly by Tp. Tp sensitization might play an important role in the clinical symptoms of elderly subjects. It has been reported that conjunctivitis and perioral angioedema more frequently occur in patients allergic to Tp [16]. Although there were more elderly subjects with COPD sensitized to Tp in comparison with Dp in this survey, 54.2% of the elderly subjects with COPD were not sensitized to mites (Tp or Dp). This could be due to the pathogenesis of COPD in elderly subjects, which may be caused by recurrent respiratory tract infections or other causes, such as smoking or working in polluted environments, since a higher proportion of Tp-sensitized individuals with either nasal or bronchial symptoms has been reported than among nonatopic individuals [8]. Skin tests related to HDMs are good predictors of clinical allergy in most patients with asthma and COPD. Although the contribution of HDM-allergic components in the pathogenesis of COPD was less than in allergic asthma, it has been reported that there is a higher percentage of skin test reaction to HDMs in COPD than normal individuals, and 37% (19 of 51) of patients with a history-based diagnosis of COPD, skin tests reacted to HDMs [18]. A similar result was found in our study, in that there were more Tp-sensitive subjects with COPD than without

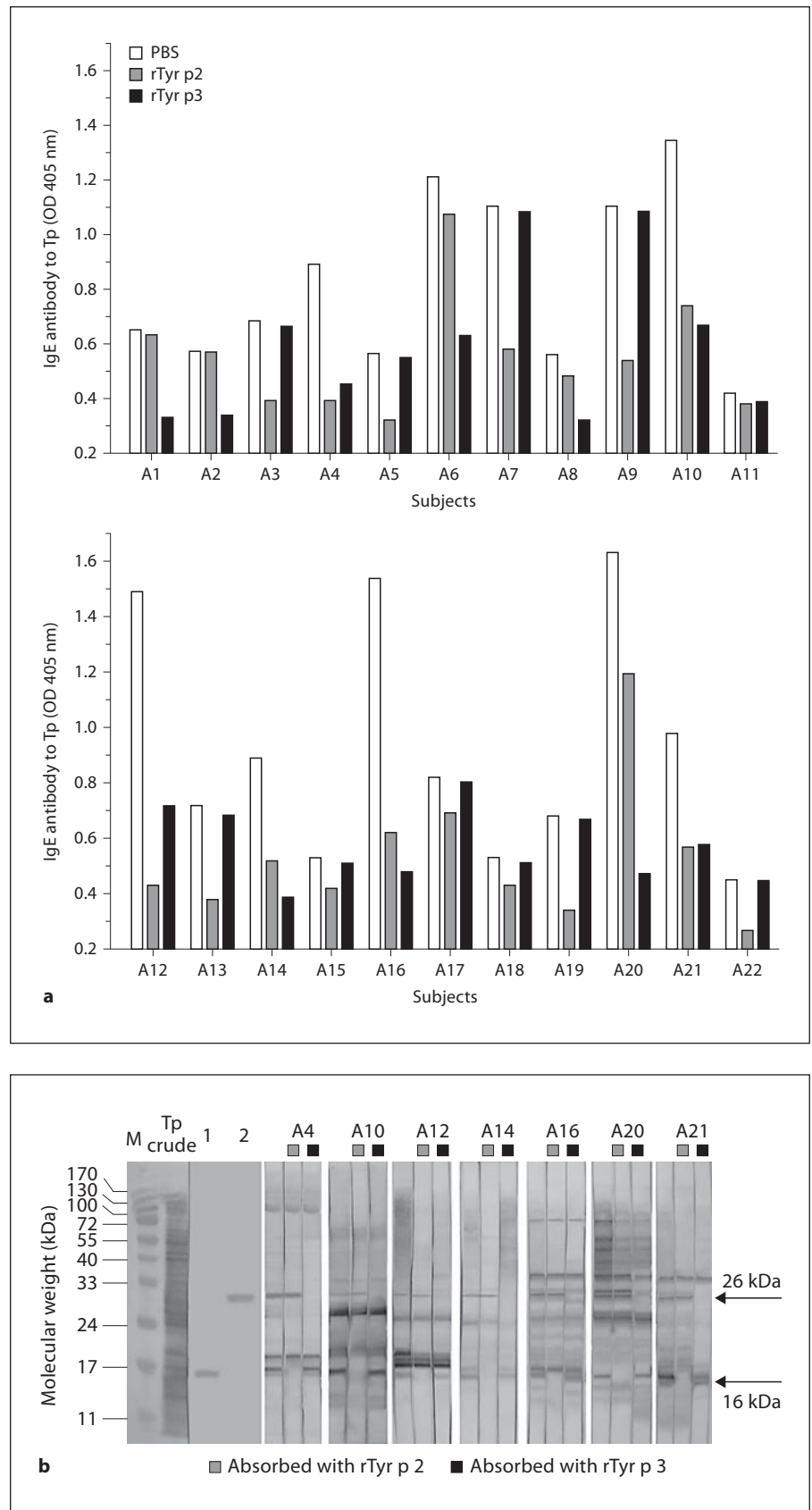


Fig. 4. Inhibitory effects of rTyr p 2 or rTyr p 3 on the IgE-binding activity to Tp. IgE-binding activity to Tp was measured after pretreatment of patient sera with PBS (white columns), rTyr p 2 (grey columns) (50 μ g/ml) or rTyr p 3 (black columns) (50 μ g/ml). The IgE-binding activity was determined by ELISA at an optical density of 405 nm (**a**) and by Western blot (**b**). Tp crude: Tp crude extracts; lane 1: rTyr p 2; lane 2: rTyr p 3. These proteins were separated by SDS-PAGE, transferred onto a PVDF membrane and stained with Coomassie blue.

COPD. However, there were no statistically significant differences between COPD and non-COPD. This may be due to the fact that the sample size was small, so more cases of COPD should be recruited to clarify the clinical significance. The clinical significance of Tp sensitization in the pathogenesis of COPD should be further investigated.

Allergen-specific IgE against Tp extracts has also been detected in sera from horses affected with chronic bronchitis [19]. Dp has also been reported to play an important role as a trigger factor in patients with atopic dermatitis [20]. Whether Tp can trigger allergic responses in elderly subjects needs further investigation. Since asthmatic patients over 65 years of age have higher hospitalization and mortality rates than other age groups [21], the risks of allergic symptoms in Tp-sensitized elderly subjects should not be underestimated.

It has been suggested that Tp and Dp possess their own unique allergens [22]. It is conceivable that the allergens in Tp might be different from those in Dp. In the inhibition study, when the sera from elderly subjects absorbed Dp, the IgE immunoblotting to Tp was only slightly affected. These results indicate that Tp plays a role as the primary sensitizing agent independently of Dp in elderly patients naturally exposed to them.

In this study, sera from elderly subjects were selected to identify the major allergens in Tp, and more than half of IgE antibody was absorbed by rTyr p 2 and rTyr p 3, indicating that group 2 and group 3 appear to play major roles in determining the allergenicity of Tp, although a

high percentage of IgE reactivity of group 1 and group 2 has been reported in young adults allergic to Dp [15]. This discrepancy might be due to differences in allergenicity between Dp and Tp, and to the fact that sera from different populations were used for allergen identification. A previous report showed that all the major allergen identification was conducted using sera from a general adult population [8], and our study is the first to use sera from elderly subjects to identify the allergenicity of Tp. Although all of the subjects were residents in a veterans' nursing home, little absorption by Dp was found in the sera, thus avoiding the confounding factor of Dp sensitivity.

In conclusion, Tp sensitization is highly prevalent among elderly subjects and should be identified, especially in patients with difficult-to-treat COPD. The major allergens, Tyr p 2 and Tyr p 3, have been demonstrated to possess a high frequency of IgE binding. However, the role of these allergens in the pathogenesis of IgE-mediated allergic diseases in elderly subjects needs to be further clarified.

Acknowledgements

The authors sincerely appreciate all the work done by members of the AGEing research group and the Biostatistics Task Force of TCVGH. This study was supported by grants (TCVGH-935701D, TCVGH-945703D and TCVGH-955701A) from TCVGH and partially by a grant (NSC-95-2320-B075A-005) from the National Science Council of Taiwan.

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