

Cross-reactivity of *Tyrophagus putrescentiae* with *Dermatophagoides farinae* and *Dermatophagoides pteronyssinus* in urban areas

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Background: *Tyrophagus putrescentiae* (TP) have cohabited with *D. pteronyssinus* (DP) and *D. farinae* (DF) in more than 25% of houses in urban areas of Korea, and many atopic subjects have also been cosensitized to TP and *Dermatophagoides* species.

Objective: We evaluated the cross-reactivity of TP with DF and DP in atopic subjects of urban inhabitants.

Methods: The cross-reactivity was evaluated with inhibition ELISA and immunoblotting. Allergenic components of TP were evaluated with IgE immunoblotting of the sera from 25 individual atopics. All enrolled subjects lived in urban areas.

Results: In ELISA inhibition with pooled sera, all TP, DP, and DF extract inhibited TP-specific IgE by more than 90%, and the 50% inhibitory concentrations of TP, DP, and DF extract were 0.4 µg/mL, 0.8 µg/mL and 0.8 µg/mL, respectively. The maximum inhibition, however, of DP-specific and DF-specific IgE by TP extracts was 32% and 29%, respectively. With six individual sera, the TP-specific IgE was also inhibited by more than 88% with DF extract in all cases. In inhibition immunoblotting, all of the TP, DP, and DF extracts completely inhibited the TP-specific IgE bands at a concentration of 2.0 µg/mL. Fifteen allergenic components in TP were found. Among them, the 16-kD allergen was most prevalent (52%) and its IgE binding was completely inhibited by 0.1 µg/mL of purified Der f2 and it also bound with 2 different monoclonal antibodies to the group 2 allergen of *Dermatophagoides* species.

Conclusions: Our results suggested considerable cross-reactivity between TP and the two *Dermatophagoides* species in urban areas where TP and *Dermatophagoides* species cohabit. The 16-kD allergen, which shared common epitopes with the group 2 allergen of *Dermatophagoides*, is one of the most prevalent allergens of TP.

Ann Allergy Asthma Immunol 1999;83:533-539.

INTRODUCTION

Tyrophagus putrescentiae (TP), is recognized as one of the important storage mites in rural regions.¹⁻³ According to epidemiologic study in Korea, *Dermatophagoides farinae* (DF), *Dermatophagoides pteronyssinus* (DP),

and TP cohabit in more than 25% of investigated houses, including urban areas, and TP have been reported as the third most common domestic mite in Korea.⁴ Recently, other investigators have also reported TP as one of the prevalent domestic mites in urban areas and cosensitization of TP and *Dermatophagoides* species have been reported in urban areas of various countries.⁵⁻⁸ These results have suggested two possibilities, including multiple sensitization or the cross-reactivity of TP and *Dermatophagoides* species. Previously, we reported the possibility of marked cross-reactivity between TP and DP.⁹ The degree of

cross-reactivity between TP and *Dermatophagoides* species, however, has been quite different according to the investigators and the areas studied.^{2,5,7,10,11} Until now, the presence of cross-reactivity between TP and *Dermatophagoides*, especially in urban areas, has been one of the most controversial issues.

In this study, we evaluated the cross-reactivity between TP and 2 *Dermatophagoides* species by inhibition ELISA and immunoblotting analysis in urban areas where TP and 2 *Dermatophagoides* cohabit.

METHODS

Subjects

From June 1997 to February 1998, 509 patients complaining of allergic symptoms visited our allergy clinic and had a skin prick test done with TP extract (1:50 wt/vol in 50% glycerol, 0.4% phenol saline). Among them, 148 subjects (29%) showed at least equal skin responsiveness compared with a histamine positive control (1 mg/mL; Bencard Co, UK). For this study, we selected 25 patients who had higher ELISA optic densities to TP extract from among those 148 subjects. All of the enrolled subjects had the symptoms of respiratory allergy and three patients also had the manifestation of atopic dermatitis. Their mean age was 29.0 ± 14.6 years and the male:female ratio was 16:9. All subjects lived in urban areas and 23 patients of them lived in Seoul, the capital city of Korea (more than 10 million inhabitants), or its satellite cities. The presence of cosensitization to DP and DF were confirmed by skin prick test (Bencard Co, UK) and serologic measurement of specific

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This work was supported by a grant from The Korea Science and Engineering Foundation (KOSEF 97-04-03-02-01-3).

Received for publication January 23, 1999.

Accepted for publication in revised form August 10, 1999.

Table 1. Incidence of DP and DF Sensitization in Enrolled Patients (n = 25)

FAST Class D. pteronyssinus D. farinae		
Negative	1	1
1+	1	0
2+	2	4
3+	11	8
4+	10	12

IgE (3M FAST; Biowhiteker, USA) (Table 1). When the mean wheal size was larger than histamine control, we considered the skin prick test positive.

MATERIALS

TP, DP, and DF Extracts

The TP, DP, and DF allergens were extracted from the cultured mites.^{12,13} Five grams of mites were defatted by ethylether and stirred in 250 mL of phosphate buffered saline for 72 hours at 4°C and centrifuged at 12,000 rpm for 30 minutes. The supernatants were dialyzed with distilled water and centrifuged again. The supernatants were then lyophilized. Protein concentrations were measured by Bradford’s method. We measured the Der p1 and Der f1 concentrations in TP extract with a 2-site ELISA kit (Indoor Biotechnologies, Chester, UK) according to the manufacturer’s guidelines. In TP extract, Der p1 was minimally detected as 0.08 µg/mg extract, which was less than 0.5% of the group 1 allergen in the extract of DP species, and Der f1 was not detected by the 2-site system (Table 2).

SDS-PAGE and IgE Immunoblotting

SDS-PAGE was carried out by the method of Laemmli under reducing conditions.¹⁴ *Tyrophagus putrescentiae*, 1 mg/mL, crude antigen, was dissolved in sample buffer (12.5 mM Tris-HCl, pH 6.8, 5% glycerol, 0.4% SDS, 1% 2-mercaptoethanol, 0.02% bromophenol blue) and boiled at 100°C for 5 minutes. Two hundred micrograms of TP antigen was separated in 15% polyacrylamide gels with a 4% polyacrylamide stacking gel (Mighty

Table 2. Measurement of the Group 1 Major Allergens of Dermatophagoides in TP, DP, and DF Extracts

Extracts (L mg extract)	Group 1 Major Allergen (µg)	
	Der p1	Der f1
TP	0.08	0
DP	19.8	0
DF	1.7	14.5

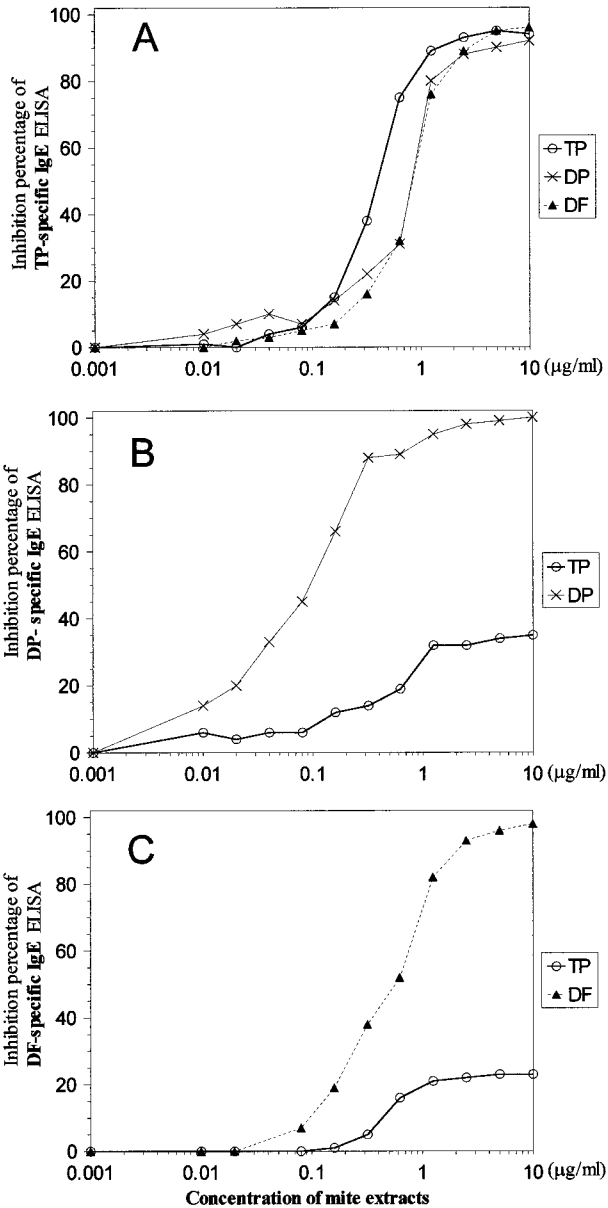


Figure 1. ELISA inhibition of TP-specific, DP-specific, and DF-specific IgE. (A) The inhibition curves of TP-specific IgE with TP (—○—), DP (—×—) and DF (—▲—) extracts. (B) The inhibition curves of DP-specific IgE with TP (—○—) and DP (—×—) extracts. (C) The inhibition curves of DF-specific IgE with TP (—○—) and DF (—▲—) extracts.

tae, 1 mg/mL, crude antigen, was dissolved in sample buffer (12.5 mM Tris-HCl, pH 6.8, 5% glycerol, 0.4% SDS, 1% 2-mercaptoethanol, 0.02% bromophenol blue) and boiled at 100°C for 5 minutes. Two hundred micrograms of TP antigen was separated in 15% polyacrylamide gels with a 4% polyacrylamide stacking gel (Mighty

Small electrophoresis unit; Hoeffer, San Francisco, USA) at 50 V for 30 minutes and at 180 V for 2 hours. Transfer of the proteins onto nitrocellulose membranes (pore size is 0.45 µm; Amersham, Buckinghamshire, UK) was performed on a Transpor TE 42 Unit (Hoeffer, San Francisco, CA) in transfer buffer (25 mM Tris, 192

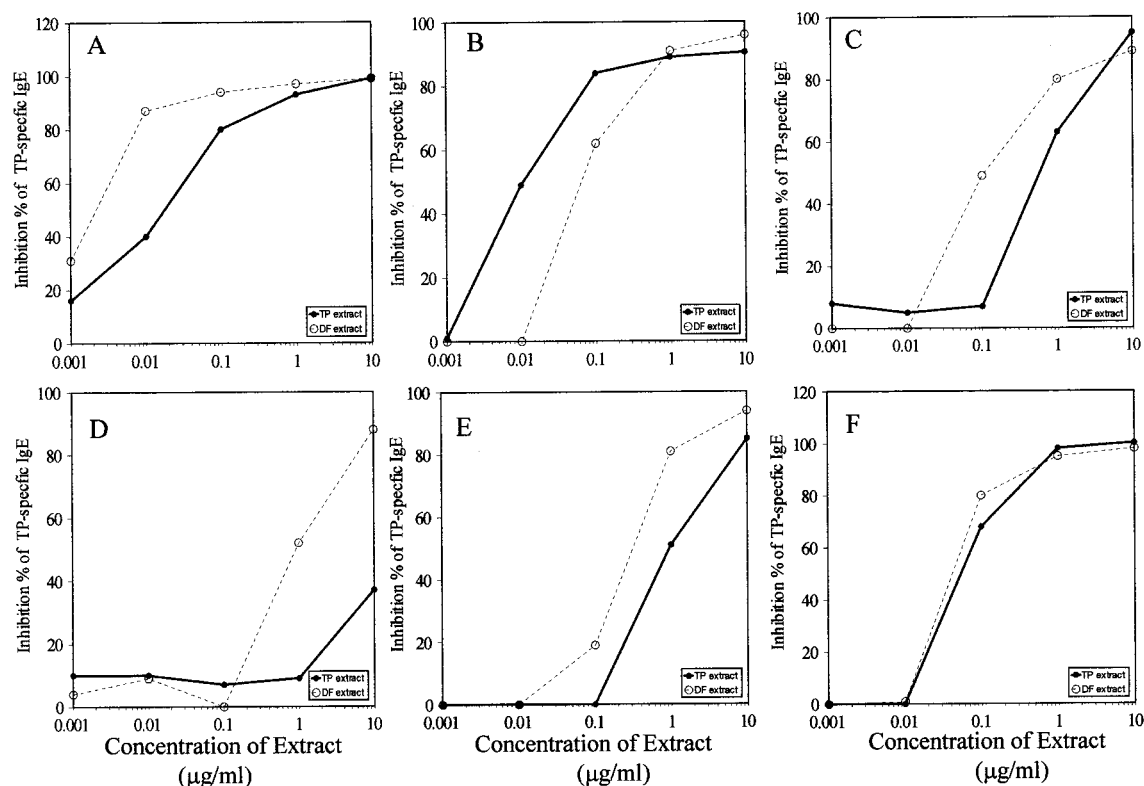


Figure 2. ELISA inhibition of TP-specific IgE by TP (●) and DF (○) extract in six individual TP-sensitized atopic subjects.

mM glycine, and 20% methanol, pH 8.3) at 350 mA for 1 hour. The membrane was sliced into 4-mm widths. The membranes were blocked by 5% skim milk in TBS-T (50 mM Tris with 0.1% Tween, pH 7.5) and then incubated with primary antibodies. For IgE immunoblotting, the transferred NC membranes were incubated with patient sera (1:5 diluted in TBS-T, 0.02% Na₂S₂O₃) at 4°C for 18 hours and washed with TBS-T. For inhibition immunoblotting, the pooled sera (n = 10) was preincubated with each concentration of mite extracts for 3 hours at room temperature and then the transferred NC membranes were incubated with the preincubated serum overnight at 4°C. Consequently, the goat anti-human IgE conjugated with alkaline phosphatase (Sigma, St. Louis, MO) was diluted 1:1000 in TBS-T before incubation with the blots. After washing with TBS-T, the bound goat antibodies were detected with a BCIP/NBT kit (Promega, Madison, WI).

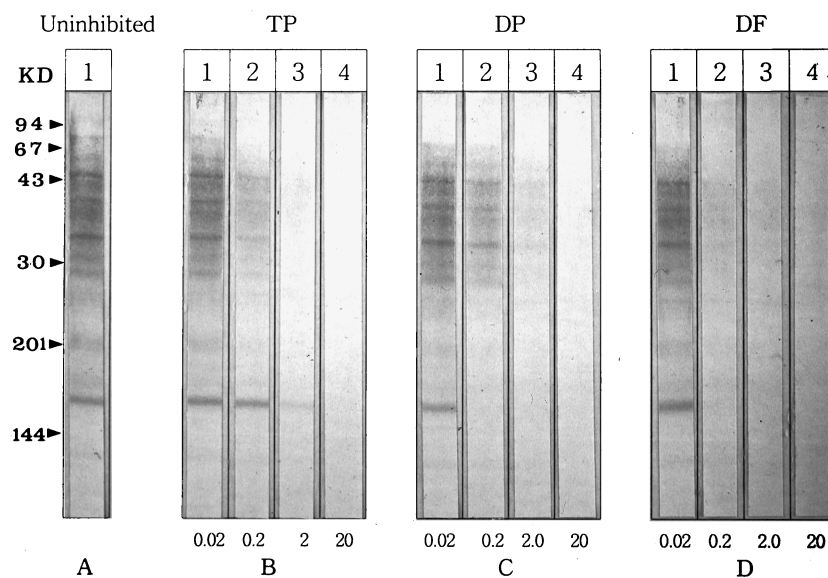


Figure 3. Inhibition IgE immunoblotting of TP-specific IgE with TP (B), DP (C), and DF (D). A represents uninhibited immunoblotting features of TP-specific IgE. Lane 1 (except A) represents inhibition with 0.02 μg/mL of extract; lane 2, inhibition with 0.2 μg/mL of extract; lane 3, inhibition with 2.0 μg/mL of extract; and lane 4, inhibition with 20 μg/mL of extract.

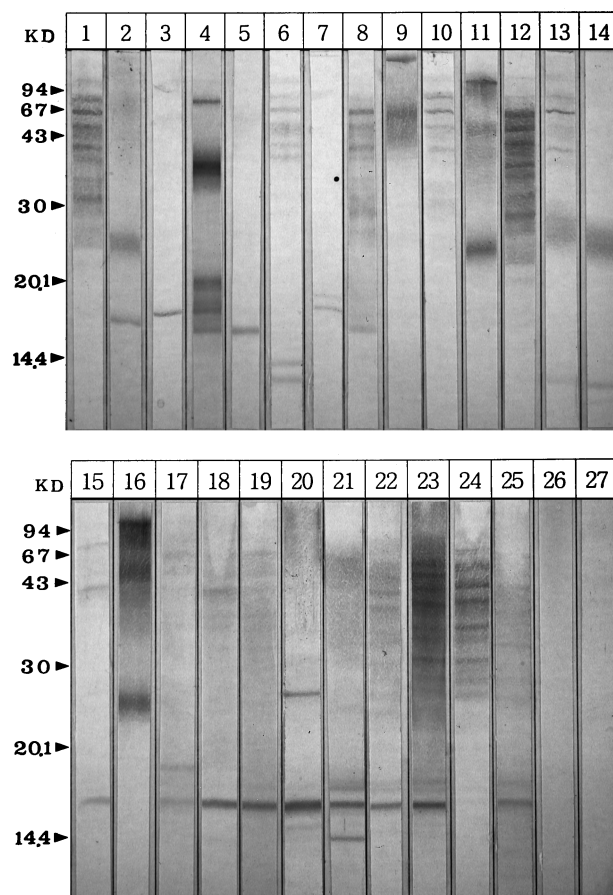


Figure 4. Immunoblotting of TP-specific IgE with 25 individual atopic sera. Lanes 1 through 25, atopic sera; lane 26, nonatopic serum; and lane 27, buffer control.

For evaluation of the presence of common epitopes between *Dermatophagoides* species and TP, skim milk-blocked NC membranes were incubated with monoclonal antibodies to group 1 [5H8 (anti-Der p1), 4C1 (anti-Der f1), and 6A8]¹⁵ and group 2 allergen (1D8, 7A1) of *Dermatophagoides* (1:1000 diluted in TBS-T; Indoor Biotechnologies, Chester, UK).¹⁶ After washing with TBS-T, the NC membranes were incubated with alkaline phosphatase conjugated goat anti-mouse IgG antibodies (1:1000 diluted in TBS-T; Vector, Burlingame, CA) for 1 hour at room temperature. After washing with TBS-T, the bound goat antibodies were also detected with a BCIP/NBT kit (Promega, Madison, WI).

ELISA Inhibition Tests

Polystyrene microplates (Costar, Cambridge, MA) were coated with 50 μ L of 20 μ g/mL of mite extract in 0.05 M carbonate buffer (pH 9.6) overnight at 4°C. The nondiluted pooled sera from 10 atopic patients or individual patient's serum was preincubated with each concentration of mite extracts, respectively, overnight at 4°C. After washing with PBS-T (137 mM NaCl, 1.8 mM KH_2PO_4 , 10 mM Na_2HPO_4 , 27 mM KCl, 0.1% Tween-20, pH 7.4), each microplate well was blocked by incubation with 200 μ L of 1% bovine serum albumin in PBS-T for 1 hour at room temperature. Specific IgE detection was carried out by incubating 50 μ L of preincubated pooled sera for 1 hour at room temperature. After wash-

ing 3 times with PBS-T, 50 μ L of 1:1000 biotin-labeled goat antihuman IgE (Vector, Burlingame, CA) was added to the well and incubated for 1 hour. The wells were then washed 3 times with PBS-T and incubated with 1:1000 vol/vol streptavidin-peroxidase (Sigma, St. Louis, MO) for 30 minutes before another washing step. Colorimetric reaction was developed with 50 μ L of ABTS solution [25 mg of 2,2-azino-bis-3-ethylbenzthiazoline-sulfonic acid in 50 mM citrate phosphate buffer, 50 μ L of 30% H_2O_2] for 5 minutes. The reaction was stopped by the addition of 50 μ L of 2 mM NaN_3 , and the optic densities were determined at 410 nm of UV by an automated microplate spectrophotometer (Dynatec, Alexandria, CA). All assays were performed in duplicate.

RESULTS

Co-sensitization of the TP Reactive Subjects to DP and DF

Most of the enrolled subjects were co-sensitized to DP or DF. In skin prick tests, 88% and 92% showed positive responsiveness to DP and DF, respectively. In addition, all patients except one (96%) had positive responses to DP or DF with FAST-specific IgE measurement.

Table 3. Frequency of TP Allergen-Specific IgE in Immunoblotting Analysis (n = 25)

Molecular Weight (kD)	Frequency	Percentage (%)
13	3	12
14	2	8
15	2	8
16	13	52
17	6	24
18	3	12
20	1	4
25	7	28
27	6	24
30	6	24
33	6	24
38	10	40
40	10	40
50	13	52
67	12	48

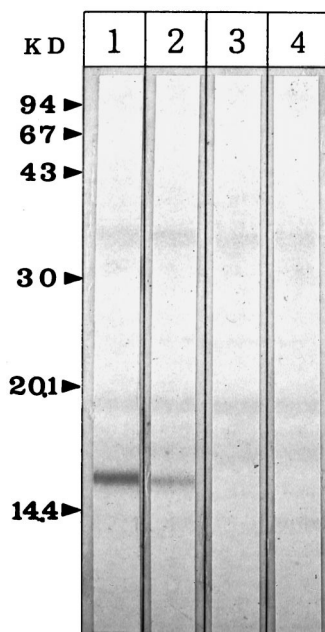


Figure 5. Inhibition immunoblotting of TP-specific IgE with purified natural Der f2. Lane 1 represents uninhibited; lane 2, inhibited with 0.01 $\mu\text{g/mL}$ of Der f2; lane 3, inhibited with 0.1 $\mu\text{g/mL}$ of Der f2; and lane 4, inhibited with 1.0 $\mu\text{g/mL}$ of Der f2.

ELISA Inhibition of TP-Specific IgE with TP, DP, and DF Crude Extract

With ELISA inhibition, TP-specific IgE was inhibited by 95% with 2 $\mu\text{g/mL}$ of TP extract. Both DP and DF extract inhibited TP-specific IgE by 92% and 95%, respectively. A 50% inhibitory concentration (IC_{50}) of TP extract was 0.4 $\mu\text{g/mL}$, which is about half of IC_{50} of DP (0.8 $\mu\text{g/mL}$) and DF extracts (0.8 $\mu\text{g/mL}$) (Fig 1A); however, 10 $\mu\text{g/mL}$ of TP extract maximally inhibited DP- and DF-specific IgE by 32% and 29%, respectively (Fig 1B, C). In ELISA inhibition test with six individual atopic sera, the TP-specific IgE was inhibited by more than 88% with DF extract in all subjects (Fig 2).

Inhibition Immunoblotting of TP-Specific IgE with TP, DP, and DF Extracts

The TP-specific IgE was almost completely inhibited with the incubation of the TP reactive subjects pooled sera ($n = 10$) with 2.0 $\mu\text{g/mL}$ of TP, DP,

and DF extracts, respectively. We could not find any differences in degree of inhibition among these three extracts at the concentrations of 0.02 $\mu\text{g/mL}$, 0.2 $\mu\text{g/mL}$, 2.0 $\mu\text{g/mL}$, and 20 $\mu\text{g/mL}$ (Fig 3).

IgE Immunoblotting Features of TP with Atopic Patients' Sera

With 25 atopic patients' sera, we performed SDS-PAGE and IgE immunoblotting. We identified 15 allergenic components in TP extract. Among them, the 16 kD and 50 kD allergens were the most prevalent allergens of TP (Fig 4 and Table 3).

Cross-Reactivity of 16-kD Allergen with Group 2 Allergen of Dermatophagoides

We performed inhibition IgE immunoblotting with TP-reactive subjects' pooled sera ($n = 10$). The IgE binding to the 16-kD allergen was completely inhibited with 0.1 $\mu\text{g/mL}$ of purified natural Der f2, which was kindly provided by Dr. Okudaira (Fig 5). We could not, however, find any inhibition of TP-specific IgE bands with the incubation of 1.0 $\mu\text{g/mL}$ of purified Der f1, which was also kindly provided by Dr. Okudaira (data not shown). We also found that the two monoclonal antibodies to the group 2 allergen of Dermatophagoides species, ID8 and 7A1, bound to the 16-kD allergen (Fig 6).

DISCUSSION

Tyrophagus putrescentiae has been recognized as one of the important storage mites and many investigators have reported the prevalence of TP sensitization in rural areas.¹⁻³ Recent studies have reported that TP is also one of the important inhalant allergens in urban areas.⁶⁻⁸ Previously, we reported that TP cohabited with Dermatophagoides species in more than 25% of domestic houses in Korea and that the prevalence of TP is about one-tenth of Dermatophagoides.⁴ One of the difficult aspects in studying the cross-reactivity between TP and Dermatophagoides species is obtaining uncontaminated TP extract. Using 2-site ELISA to measure the group 1 major allergen of DP and DF, as well as by

microscopic examination, we confirmed that the TP extract used in this study was not contaminated by DP or DF.

Our results showed that all patients except one, were also sensitized to Dermatophagoides species and the TP extract was considerably cross-reactive with the extract of Dermatophagoides species. As well, we could not find significant individual variation in pattern of marked inhibition of TP-specific IgE by DF extract at least in six individual atopic urban inhabitants. These results suggested that the extent of reactions to TP in urban areas might be attributed to cross-reactivity with Dermatophagoides species. There have been some controversies in cross-reactivity between TP and Dermatophagoides species. Some investigators have suggested minimal cross-reactivity in atopic subjects in rural areas^{2,10,17} and others have reported moderate to higher cross-reactivity between TP and Dermatophagoides species.^{5,7,11} Our results were in concordance with two other studies, which were done in urban areas^{5,7} and that reported a marked inhibition of TP-specific IgE with DP

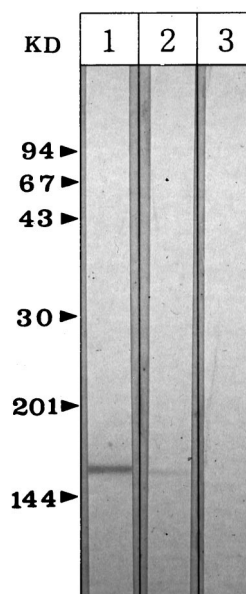


Figure 6. Immunoblotting of TP extract with mouse monoclonal antibodies to group 2 allergen of Dermatophagoides. Lane 1 is with 1D8 monoclonal antibodies; lane 2, with 7A1 monoclonal antibodies; and lane 3, buffer control.

extract. Many investigators have reported a significant relationship between the levels of TP specific IgE and *Dermatophagoides* specific IgE^{8,9,11,18,19} while others were not.² Previously, we also reported that many urban respiratory allergic subjects were cosensitized with TP and DP, and by retrospective analysis we also suggested the possibility of marked cross-reactivity between DP and TP in atopic patients with high levels of DP-specific IgE (FAST class ≥ 4).⁹ For this study, we prospectively selected the subjects who showed skin reactivity to TP and collected the sera for measurement of TP specific IgE.

Our ELISA inhibition results were in concordance with two other studies, which were done in urban areas^{5,7} and that reported a marked inhibition of TP-specific IgE with DP extract. In this study, the DP or DF specific IgE was not inhibited with TP and these results might suggest the possibility that TP, per se, does not have enough cross-reactive antigens to *Dermatophagoides*, although *Dermatophagoides* species had sufficient cross-reactive antigens to TP.

Others have reported some cases of TP-sensitized perennial allergic rhinitis without co-sensitization of *Dermatophagoides* species^{2,20} and Griffin et al. showed that some allergenic determinants of storage mites are not cross-reactive with the allergens of *Dermatophagoides*.²¹ We do not think that our study can exclude the possibility of species-specific allergenic components in TP, but we suggest that many allergenic determinants of TP may be cross-reactive to *Dermatophagoides* species. The discrepancies between our study and others may be due to the difference in the prevalence of TP in studied regions. As the prevalence of TP is slightly less than one-tenth of *Dermatophagoides* species in the Seoul area,⁴ we considered the possibility that sensitization of DP or DF allergens which may be cross-reactive to TP could be more emphasized than sensitization to species-specific TP allergens in this study.

Our results of IgE immunoblotting with atopic sera also suggested that the 16-kD allergen of TP was one of the major allergens. We showed that the 16-kD allergen of TP, considered to be the major allergen of TP,^{10,22} shared some common epitopes of the group 2 allergen of *Dermatophagoides* species by immunoblotting with 2 different monoclonal antibodies to the group 2 allergen of *Dermatophagoides*. In addition, we also demonstrated that the 16-kD allergen of TP is highly cross-reactive to the group 2 allergen of *Dermatophagoides* species by inhibition immunoblotting. Our results may be consistent with Eriksson et al who showed the similarity of cDNA sequences between the 16-kD allergen of TP and the group 2 allergen of *Dermatophagoides* species.²²

More or less, our results suggested that in urban areas where the TP and *Dermatophagoides* species cohabit, considerable cross-reactivity of TP and *Dermatophagoides* species could be present and it should be taken into consideration for the diagnosis and management of mite allergic patients in these areas.

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