Identification of *Tyrophagus putrescentiae* Allergens and Evaluation of Cross-Reactivity with *Dermatophagoides pteronyssinus*

S. Munhbayarlah[1], Jung Won Park[1], Si Hwon Ko[1] Han Il Ree[2], and Chein-Soo Hong[1]

House dust mites are the most common cause of allergic sensitization in respiratory allergic patients in the world. Tyrophagus putrescentiae (TP), which was followed by Dermatophagoides farinae (DF) and Dermatophagoides pteronyssinus (DP), has been reported as the third most common house mite in Korea. We previously reported that many respiratory allergic patients had become concomitantly sensitized to DP, DF and TP. The aims of this study were to identify the allergic components of TP and to evaluate the cross-reactivity between TP and DP. The allergenic components of TP and DP extracts were determined with SDS-PAGE and IgE immunoblotting analysis. The cross-reactivity was evaluated by ELISA inhibition and inhibitory immunoblotting experiments. According the SDS-PAGE, the protein components of the two extracts were somewhat different, although a few components displayed identical molecular weights. The 18 kD protein of TP was the most prevalent allergen in the sera of patients sensitized to TP and DP. Both of the maximum inhibition percentages of optic densities of TP-specific IgE in ELISA with TP and DP extract were 100%, respectively and the 50% inhibitory doses (ID_{50}) of TP extract and DP extract were 0.01 μg/ml and 0.02 μg/ml, respectively. Maximum inhibition of optic densities of DP-specific IgE in ELISA with TP and DP extracts were 29% and 100%, respectively and the ID_{50} of DP extract was 0.007 μg/ml. On inhibitory immunoblotting of DP specific IgE, 5 μg/ml of TP extract completely inhibited 16 kD without inhibiting the other allergic component. Whereas, on inhibitory immunoblotting of TP-specific IgE, 5 μg/ml of DP extract completely inhibited all the IgE binding components of TP. These results suggested that the major allergen of TP may be the 18 kD component and we also concluded that TP allergens have a strong cross-reactivity with DP extracts, but that DP allergens only have partial cross-reactivity with TP extracts.

Key Words: *Tyrophagus putrescentiae, Dermatophagiodes pteronyssinus,* major allergen, cross reactivity

---

Present address of S. Munhbayarlah, M.D., M.Sc : Division of Pathophysiology & Allergology, National Medical University of Mongolia, Ulaanbaatar, Mongolia
Address reprint request to Dr. C.S. Hong, Division of Allergy and Immunology, Department of Internal Medicine, Yonsei University College of Medicine, C.P.O. Box 8044, Seoul 120-752, Korea

ELISA inhibition

The atopic sera were incubated with 0.001 to 2 μg/ml of TP or DP extracts overnight at 4°C. The 96-well polystylene microtiter plate was coated with 20 μg/ml of TP and DP extract, respectively and left overnight at 4°C and then blocked for 1 hour at room temperature (RT) with 1% BSA-PBS-T. Fifty μl of the preincubated sera was added to each well and incubated for 1 hour at room temperature. After washing with PBS-T, 50 μl of streptavidin peroxidase conjugated goat antihuman IgE (1:500 v/v in PBS Tween-20) was added to each well and incubated at room temperature for 30 minutes. With the methods described in the previous section, we measured the optic densities of TP and DP-specific IgE. The percent of inhibition was calculated in comparison with the absence of inhibitors in the system. The amounts of inhibitor producing 50% inhibition were analyzed by linear regression.

SDS-PAGE and IgE immunoblotting

SDS-PAGE was carried out by the methods of Laemmlli under reducing conditions (Laemmlli, 1979). For allergen isolation, 2 mg/ml of TP/DP crude antigen was dissolved in sample buffer (60 mM Tris-HCl, 25% glycerol, 2% SDS, 14.4 mM 2-mercaptoethanol, 0.1% bromophenol blue) and boiled for 5 minutes. Two hundred micrograms of DP or TP extracts was applied to the 4% acrylamide stacking gels (80 mm preparative slots) and the proteins were separated with 15% acrylamide gels (Mighty Small electrophoresis Unit; Hoeffer, San Francisco, CA, USA) at 50 V for 30 minutes and 100 V for 2 hours. A part of the gels was stained with Coomassie Brilliant Blue (Sigma Immuno-Chemicals, St. Louis, MO, USA) and the remaining part was used for immunoblotting. The proteins of polyacrylamide gel were transferred onto a nitrocellulose membrane with a pore size of 0.45 μm (Amersham, Buckinghamshire, UK). The membrane was blocked for 1 hour at room temperature with 1% BSA-PBS-T. After blocking the membrane was cut in 4 mm wide strips and incubated with 1:10 diluted sera overnight at 4°C and followed by incubation with antihuman IgE conjugated with alkaline phosphatase (goat origin, 1: 2,000 dilution; Sigma Immuno-Chemicals, St. Louis,

MO, USA) at room temperature for 1 hour. After washing with TBS-T (50 mM Tris with 0.1% Tween-20, pH 7.5), the IgE-binding components were developed with BCIP/NBT solution (Promega, Madison, WI, USA).

Immunoblotting inhibition

Nine sera with IgE reactivity to both TP and DP allergens were pooled for positive standard atopic sera. The pooled sera were incubated with 0.1 μg/ml and 5 μg/ml of TP and DP extracts overnight at 4°C. For positive control, the pooled sera was incubated with saline. The TP and DP proteins blotted onto nitrocellulose membranes after SDS-PAGE were incubated with pretreated pooled sera overnight at 4°C. The subsequent steps of this experiment were performed by the same methods described in the immunoblotting section.

RESULTS

TP and DP specific IgE - ELISA

For this study, 46 atopic sera with higher levels of DP-specific IgE (FAST class 4 or more) were chosen and used for determination of TP-and DP-specific IgE on ELISA. With our ELISA methods, TP-specific IgE and DP-specific IgE were detected
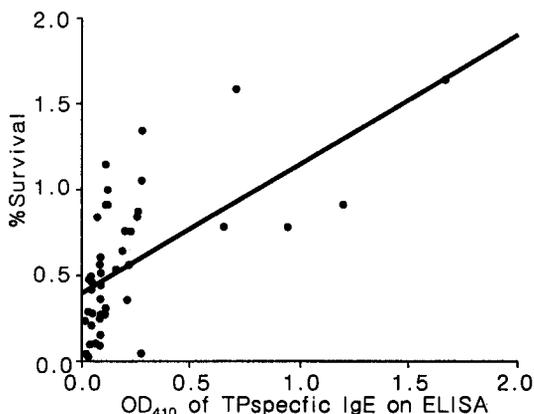

Fig. 1. The correlation of ELISA optic densities between TP- and DP-specific IgE in atopic sera (n=46)
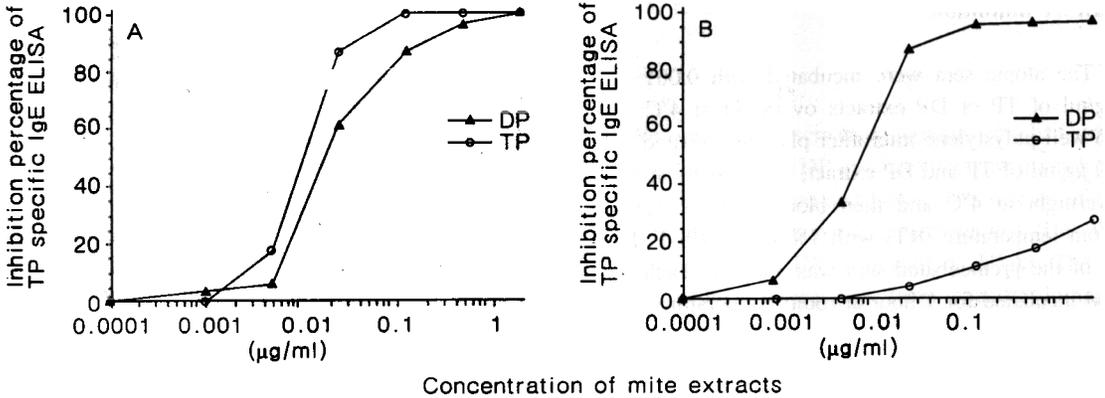
Fig. 2. ELISA inhibition of TP- and DP-specific IgE. Panel A shows the inhibition curves of TP-specific IgE with TP (-o-) and DP (-▲-) extracts. Panel B shows the inhibition curves of DP-specific IgE with (-o-) and DP (-▲-) extracts.

in 15 atopic sera (32.6%) and 43 atopic sera (93.4%), respectively. The ELISA optic densities of TP-and DP-specific IgE in 46 atopic sera were correlated with each other (r=0.6123, p<0.001, Fig. 1).

ELISA inhibition

The results of ELISA inhibition are shown in Fig. 2. On TP-specific IgE, 50% inhibitory doses (ID_{50}) of TP and DP extracts were 0.01 $\mu g/ml$ and 0.02 $\mu g/ml$, respectively. With 2.0 $\mu g/ml$ of TP and DP extract, optic densities of TP-specific IgE were completely inhibited (Fig. 2A). DP extract completely inhibited the optic density of DP-specific IgE and ID_{50} was 0.007 $\mu g/ml$. But TP extracts partially inhibited DP-specific IgE. Even with a large amount of TP extracts (2 $\mu g/ml$), the DP-specific IgE was inhibited by 29% (Fig. 2B).

Electrophoretic profiles of TP and DP and immunoblotting patterns

Protein components of the TP and DP extracts were identified by Coomassie Blue staining of the polyacrylamide gel (Fig. 3). There were numerous protein bands between 110 kD and 10 kD. The protein profiles of the two mite extracts showed different patterns. TP-specific IgE immunoblotting with atopic pooled sera showed 9 IgE binding bands; one band with a molecular weight (MW) of about 18 kD and 8 bands with molecular weights between 30 and
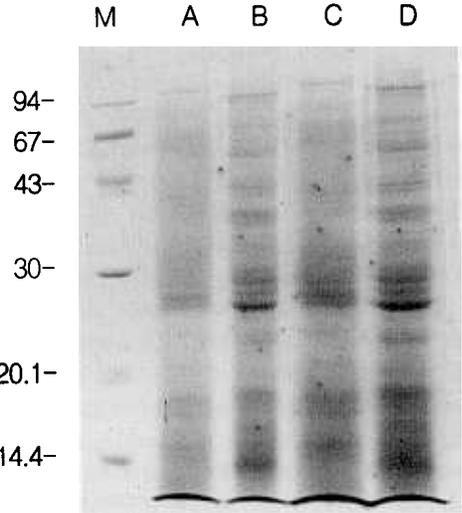
Fig. 3. SDS-polyacrylamide gel electrophoresis of TP (lanes A and C) and DP (lanes B and D) extracts. Lane M, molecular weight marker (kD); lanes A and B, loaded protein amounts were 20 μg; lanes C and D, loaded protein amounts were 30 μg.

93 kD (Fig. 4A, Lane 1, 2). On DP-specific IgE immunoblotting with the same atopic pooled sera, strong IgE binding bands were shown at 16, 28, and 30 kD (Fig. 4B, Lane 1, 2). The patterns of IgE binding bands in TP-and DP-specific IgE immunoblotting were quite different. With 10 atopic individual sera, we determined the most prevalent allergen of DP and TP extract with IgE immunoblotting.
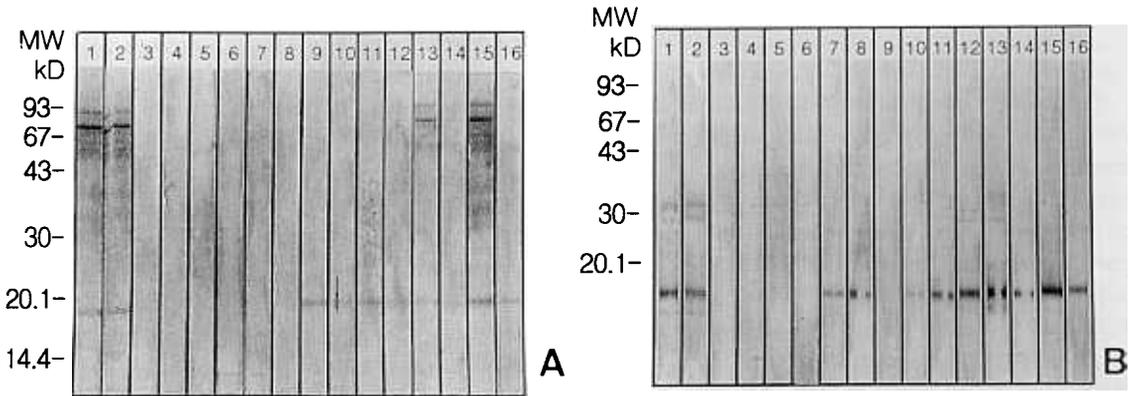
Fig. 4. IgE Immunoblotting patterns of TP (panel A) and DP extracts (panel B) with atopic pooled sera. Lanes 1 and 2, atopic pooled sera with high ELISA optic densities of TP- and DP-specific IgE; lanes 3~6, non-atopic sera; lanes 7~8 and 10~16, atopic individual sera with high optic densities of TP- and DP-specific IgE; lane 9, atopic serum from the patient with high TP-specific IgE but not with DP-specific IgE.
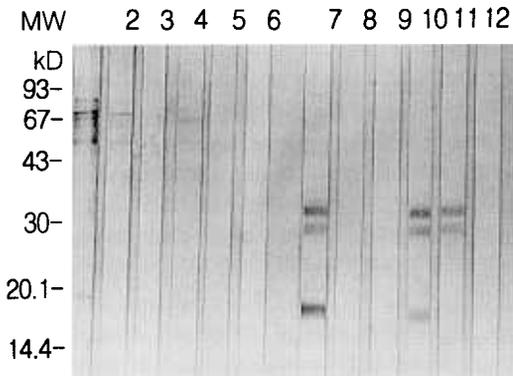
Fig. 5. Inhibition IgE immunoblotting to the components of TP (lanes 1~6) and DP (lanes 7~12). Lanes 2, 3, 8 and 9, atopic pooled sera inhibited with DP extract (lanes 2, 3 and 8, 9); lanes 4~5 and 10~11, atopic sera inhibited with TP extract; lanes 1 and 7, uninhibited atopic pooled sera; lanes 6 and 12, non-atopic sera.

Eight of 10 sera showed IgE binding bands with the 18 kD component of TP and two sera had several IgE binding bands at higher MW components (Fig. 4A, Lane 13, 15). Nine of 10 sera had IgE binding bands with the 16 kD component of DP and two sera showed additional IgE binding bands around the 30 kD component of DP (Fig. 4B, Lane 12, 13). One serum which was positive on TP-ELISA but negative on DP-ELISA showed an IgE binding band

at the 18 kD fraction of TP immunoblotting, but did not show IgE-bound fractions on DP-immunoblotting (Fig. 4A and B, Lane 9).

Inhibition immunoblotting

The presence of a cross-reactive IgE binding component in the TP and DP major allergens was investigated by IgE inhibition immunoblotting experiments. On the inhibition studies of TP-specific IgE immunoblotting, 0.1 μg/ml of DP showed complete inhibition of the 18 kD band (Fig. 5, Lane 2) and 5 μg/ml of DP showed complete inhibition of all IgE binding bands (Fig. 5, Lane 3). With 0.1 μg/ml of TP extract, the IgE binding band of the 18 kD component was completely inhibited and 5 μg/ml of TP extracts completely inhibited all IgE binding bands (Fig. 5, Lane 4, 5). On the inhibition studies of DP-specific IgE immunoblotting, 0.1 and 5 μg/ml of DP extracts completely inhibited all the IgE binding bands (Fig. 5, Lane 8, 9). With 0.1 μg/ml of TP extracts, IgE binding band of the 16 kD component was partially inhibited (Fig. 5, Lane 10). Five μg/ml of TP extracts completely inhibited the IgE binding band of the 16 kD components of DP, while the IgE binding bands of the 28 and 30 kD components of DP were not inhibited (Fig. 5, Lane 11).

DISCUSSION

The storage mite has been recognized as one of the mites present in the house dust of rural or highly humidified areas and sensitization to storage mites has been reported as an important cause of respiratory allergy in these areas. Several studies have reported on sensitization to storage mites among urban populations (Harfast *et al.* 1992; Ebner *et al.* 1994; Johansson *et al.* 1994). Bernd *et al.* Reported on the importance of sensitization to storage mites in patients previously considered non-allergic, based on negative reactions to skin tests with common inhalant allergens (Bernd *et al.* 1996). Recently, storage mites have been recommended as an item of skin test or in vitro specific IgE measurements in areas which favor the growth of storage mites.

TP is the third most common mite (6.5% of total mites) in the indoor environment in Korea (Ree *et al.* 1997a). Previously we reported high rates of skin reactivity in DP- or DF-sensitized respiratory allergic patients (Park *et al.* 1988). These results suggested the possibilities of cross-allergenicity between TP and *Dermatophagoides* species. Miyamoto *et al.* showed evidence of cross-reactivity between DF and several storage mites species (Miyamoto *et al.* 1969). Green and Woolcock demonstrated with skin-prick tests that atopics sensitized to TP were as common as DF reactive atopics (Green and Woolcock, 1978). Using immunodiffusion, they found that TP and DF had at least one cross-reactive allergen. They suggested that TP should be included as a possible inhalant allergen whenever HDM was considered to be the causative allergen. Blainey *et al.* with the skin prick and RAST found a significant correlation between DP-specific IgE and *Acarus siro*-specific IgE, and between DP and TP in atopic subjects (Blainey *et al.* 1988, 1989). These results suggested not only the cross-reactivity between storage mites and DP but also the multi-sensitization to storage mites and DP. But Wraiht *et al.* failed to show any correlation between positive skin prick test reactions to DP and the four storage mites, whereas highly-significant correlations between *Glycyphagus destructor*, *G. domesticus* and TP were found (Wraiht *et al.* 1979).

One great difficulty in exploring cross-reactivity

is primarily the result of a lack of well characterized and purified mite allergens. There have been a few reports on characterization of storage mites and cross allergenicity between Pyrogliphids and storage mites. CIE and CRIE studies have revealed 20 antigens and 5 allergens in TP extract (Arlian *et al.* 1984a). They also demonstrated cross-antigenicity and cross-allergenicity between DF and TP (Arlian *et al.* 1984b). They determined that two allergenic determinants were shared by the two species. But van Hage-Hamsten *et al.* could not find any significant allergenic cross-reactivity between DP and four storage mites, *G. destructor*, TP, *G. domesticus*, and *A. siro* by RAST inhibition experiments (van Hage-Hamsten *et al.* 1987). However, Johansson *et al.* reported that one allergenic determinant located on the 25 kD component shared TP and DP on the blotting inhibition method (Johansson *et al.* 1994). They reported the 16 kD component of TP as a major allergenic component.

In this study, the crude extracts of TP and DP were made from the bodies of mites which were cultured in different systems developed by Ree *et al.* (1997b). We ascertained the different protein profiles between TP and DP with SDS-PAGE. On ELISA inhibition, small amounts of DP and TP extract completely inhibited TP-specific IgE, respectively, and the ID_{50} of DP extract was only two-times higher than that of TP extract. However, TP extract could only minimally inhibit DP-specific IgE. These results suggested strong cross-reactivity between the major allergens of TP and DP. But the major allergens of DP extracts have less cross-reactivity with TP allergens.

With atopic pooled sera, we found 9 IgE binding components in TP extract on immunoblotting. The most prevalent IgE binding band was the 18 kD component. A few strong IgE binding bands between 43~93 kD showed only in 2 of 10 sera. In immunoblotting of DP extract with atopic pooled sera, only 4 IgE binding bands were noted. All nine sera (9/9) with higher ELISA optic densities of DP-specific IgE showed an IgE binding band onto the 16 kD component of DP extract. The results of inhibitory immunoblotting experiments were consistent with the results of ELISA inhibition. The IgE binding band of the 18 kD component of TP was inhibited with small amounts of DP and all IgE binding bands

were completely inhibited with large amounts of DP extract. But large amounts of TP extract only inhibited the 16 kD IgE binding component of DP while other allergenic components were not inhibited. We thought that the 16 kD component of DP would be Der p II major allergen. Recently, Erickson *et al.* reported the cloned sequence of the group II allergen of TP, which had 50% homology with the group II allergen of the *Dermatophagoides* species (Erickson *et al.* 1997).

From these results, we thought that a major allergen of TP might be the 18 kD component. The TP allergens had strong cross-reactivity with DP allergens but the major allergen of DP had only partial or minimal cross-reactivity with TP extract. The 16 kD allergenic component of DP is partially cross-reactive with TP allergen. Further study for characterization of the 18 kD allergenic component of TP remains to be evaluated.

REFERENCES

Arlian LG, Geis DP, Vyszenski-Moher DL, Bernstein IL, Gallagher JS: Antigenic and allergenic properties of the storage mite *Tyrophagus putrescentiae*. *J Allergy Clin Immunol 74: 166-171, 1984a*

Arlian LG, Geis DP, Vyszenski-Moher DL, Bernstein IL, Gallagher JS: Cross antigenicity and allergenic properties of the house dust mite *Dermatophagoides farinae* and the storage mite *Tyropagoides putrescentiae. J Allergy Clin Immunol 74: 172-179, 1984b*

Bernd LA, Ambrozio LC, Baggio D: Storage mite allergy in perennial rhinitis patients not to house dust mites. *J Invest Allergy Clin Immunol 6: 94-97, 1996*

Blainey Ad, Topping MD, Ollier S, Davies RJ: Respiratory symptoms in arable farm workers: role of storage mites. *Thorax 43: 697-702, 1988*

Blainey AD, Topping MD, Ollier S, Davies RJ: Allergic respiratory disease in grain workers: the role of storage mites. *J Allergy Clin Immunol 84: 296-303, 1989*

Ebner C, Feldner H, Ebner H, Kraft D: Sensitization to storage mites in house dust mite (*Dermatophagoides pteronyssinus*) allergenic patients. Comparison of a rural and an urban population. *Clin Exp Allergy 24: 347-352, 1994*

Erickson TLJ, Johansson E, Whitley P, Schmidt M, Ell-Sayed S, van Hage-Hamsten M: Cloning and characterization of group II allergen from the dust mite Tyrophagus putresentiae. *1997 (submitted)*

Fernandez-Caldas E: Mite species of allergologic importance in Europe. *Allergy 52: 383-387, 1997*

Green FW, Woolcock JA: *Tyrophagus putrescentiae*: an allergenically important mite. *Clin Allergy 8: 135-144, 1978*

Harfast B, Johansson E, Johansson SG, Van Hage Hamesten M: ELISA method for detection of mite allergens in barn dust: comparison with mite counts. *Allergy 51: 257-261, 1996*

Harfast B, van Hage Hamesten M, Ansotegui IJ, Johansson E, Johansson SG: Monoclonal antibodies to *Lepidoglyphus destructor*: delineation of cross-reactivity between storage mites and house dust mites. *Clin Exp Allergy 22: 1032-1037, 1992*

Johansson E, Johansson SG, Van Hage Hamesten M: Allergenic characterization of *Acarus siro* and *Tyrophagus putrescentiae* and their cross reactivity with *Lepidoglyphus destructor* and *Dermatophagoides pteronyssinus. Clin Exp Allergy 24: 743-751, 1994*

Laemmlli UK: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature 227: 680-685, 1970*

Marx JJ Jr, Twiggs JT, Ault BJ, Merchant JA, Fernandez-Caldas E: Inhaled aeroallergen and storage mite reactivity in a Wisconsin Farmer nested case control study. *Am Rev Respir Dis 147: 354-358, 1993*

Miyamoto T, Oshima S, Mizuno K, Sasa M, Ishizaki T: Cross-antigenicity among six species of dust mites and house dust antigens. *J Allergy 44: 228-238, 1969*

Park HS, Kang SW, Hong CS: The results of skin test and RAST to *Tyrophagus putrescentiae. Korean J Allergy 8: 118-124, 1988*

Ree HI, Jeon SH, Lee IY, Hong CS, Lee DK: Fauna and geographical distribution of house dust mites in Korea. *Korean J Parasitol 35: 9-17, 1997a*

Ree HI, Lee IY, Kim TE, Jeon SH, Hong CS: Mass culture of house dust mites, Dermatophagoides farinae and D. pteronyssinus (Acari: Pyroglyphidae). *Med Entomol Zool 48: 109-116, 1997b*

Wraiht DG, Cunnington Am, Seymour WM: The role and allergenic importance of storage mites in house dust and other environments. *Clin Allergy 9: 545-561, 1979*

van Hage-Hamsten M, Johansson SGO, Johansson E, Wiren A: Lack of allergenic cross-reactivity between storage mites and *Dermatophagoides pteronyssinus. Clin Allergy 17: 23-31, 1987*