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Dermatophagoides pteronyssinus and *Tyrophagus putrescentiae* Allergy in Allergic Rhinitis Caused by Cross-reactivity Not Dual-Sensitization

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Abstract *Tyrophagus putrescentiae* and *Dermatophagoides pteronyssinus* are causative factors for the development of airway hypersensitivity. The main objective in this study was to identify the cross-reactive allergens between *T. putrescentiae* and *D. pteronyssinus* and investigate their sensitization in patients with allergic rhinitis. The prevalence of sensitization to mites was determined by skin prick tests and histamine release assays. Both immunoblot and ELISA inhibition assays were performed by using the recombinant allergens of *T. putrescentiae* and *D. pteronyssinus*. The cross-reactive allergens were identified by using IgE-binding inhibition analysis. The correlations of specific IgE between *T. putrescentiae* and *D. pteronyssinus* to group 2 and group 3 mite allergens were compared. A total of 117 allergic rhinitis patients, aged between 16 and 40 years old were recruited to be included in this study. The results showed that 70% (82/117) of allergic rhinitis subjects had skin test positive reactions to *D. pteronyssinus* or *T. putrescentiae*. Among these mite-sensitive subjects, there were 81 subjects (81/82) sensitive to *D. pteronyssinus* and 34 subjects (34/82) sensitive to *T. putrescentiae*. Among the *T. putrescentiae* hypersensitive subjects, 97% (33/34) were also sensitized to *D. pteronyssinus*. In the IgE-binding inhibition analysis, 59% (13/22) subjects had IgE-binding activity of *T. putrescentiae* that was completely absorbed by *D. pteronyssinus*, especially components with MW at 16 kDa. In ELISA inhibition testing, 69% of IgE-binding was inhibited by rTyr p 2, and 45% inhibited by rTyr p 3. The titers of IgE antibodies to rTyr p 2 and rDer p 2 were well correlated, but not rTyr p 3 and rDer p 3. In conclusion, most *T. putrescentiae* sensitized subjects were also sensitized to *D. pteronyssinus* in young adult allergic rhinitis patients. The complete absorption of IgE binding activity by *D. pteronyssinus* indicates that *T. putrescentiae* hypersensitivity might be due to the cross-reactivity, not dual-sensitization of *D. pteronyssinus* and *T. putrescentiae*. The IgE-binding titers of group 2 allergens were well correlated and the binding activity of Tyr p 2 could be absorbed by Der p 2, suggesting that group 2 allergens are the major cross-reactive allergen of *D. pteronyssinus* and *T. putrescentiae*.

Keywords Allergic rhinitis · *Dermatophagoides pteronyssinus* · *Tyrophagus putrescentiae* · cross-reactivity · Der p 2 · Tyr p 2

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Introduction

There is increasing evidence that sensitization to indoor allergens is a causative factor for the development of airway hypersensitivity among people who are genetically predisposed to making IgE antibody responses [1–3]. More than

60,000 species of mites have been described from various regions of the world; the most prevalent and well-investigated indoor perpetual allergen sources are domestic mites in tropical, subtropical, and humid areas [4, 5]. According to their abundance and allergenic importance, domestic mites are classified into two categories, Pyroglyphid and non-Pyroglyphid mites, commonly referred to as house dust mites and storage mites [6, 7], respectively. House dust mites (*Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*) and storage mites (*Blomia tropicalis* and *Tyrophagus putrescentiae*) occur with high frequency and at high levels of infestation in houses around the world [7–9], including North America (USA) [10], Europe (Spain, Holland, and UK) [11], and Asia (Korea, Singapore, and Taiwan) [12, 13]. Our epidemiologic research with regards to allergic rhinitis and asthmatics has shown that sensitization to these mites is associated strongly with airway hypersensitivity [14].

Dermatophagoides pteronyssinus is the major cause of allergy in more than 80% of allergic patients with bronchial asthma in Taiwan [15], and the major allergens of *D. pteronyssinus* have been identified as Der p 1, Der p 2, and Der p 3 [16]. Recently, storage mites have been reported to be clinically important allergenic components of dust samples throughout the world, supporting their clinical significance [17]. In our previous report, we demonstrated that 63% of asthmatic patients had concurrent sensitization to storage mite-*B. tropicalis* and house dust mite-*D. pteronyssinus* by skin tests, and the extent of cross-reactivity between Blo t 5 and Der p 5 was estimated at 33% to 43% by IgE inhibition [18]. This suggests that several IgE-binding proteins of storage mites are cross-reactive components, which can be inhibited by *D. pteronyssinus*, but the others are species-specific allergens [18]. However, which allergenic components belong to the cross-reactive or species-specific allergens between *D. pteronyssinus* and *T. putrescentiae* remains unclear and needs to be investigated in more detail.

Although it is well known that *T. putrescentiae* can cause allergic respiratory symptoms after occupational exposure in farms and grain elevator stores, currently, more attention is being paid to its allergenicity in non-occupational environments. At least 20 IgE-binding allergenic components of *T. putrescentiae* have been detected [19]. So far, only five allergens have been cloned and characterized, and the major allergens have been identified as Tyr p 2 and Tyr p 3 [13, 20].

The objectives of this study were to investigate the relationship between *T. putrescentiae* and *D. pteronyssinus* allergy in allergic rhinitis individuals using skin prick tests, basophil histamine release assays in vitro, and specific IgE measurements. Immunoblot inhibition was used to evaluate the cross-reactivity between *T. putrescentiae* and *D. pteronyssinus* and to identify components of cross-reacting allergens and species-specific allergens. The major recombinant allergens were also cloned, expressed, and purified to detect the titers of specific IgE. The correlation of IgE responses to group 2 or group 3 allergens among *T. putrescentiae* and *D. pteronyssinus* were also investigated.

Methods

Study Subjects

A total of 117 allergic rhinitis subjects (70 males and 47 females) who attended the Clinic in the Division of Allergy, Immunology, and Rheumatology at Taichung Veterans General Hospital were recruited for this study. The subjects who had allergic rhinitis were defined as having a history of recurrent nasal stuffiness, sneezing, and rhinorrhea, which were associated with exacerbation resulting from allergens exposure. The ages of these subjects ranged from 16 to 40 years old (with a mean age of 28±10.5). The healthy volunteers (H1 and H2) with negative responses to *D. pteronyssinus* by skin prick tests and specific IgE measurements were selected as negative controls. Written informed consent was obtained from each participant before being enrolled in the study. The Institutional Review Board of Taichung Veterans General Hospital reviewed and approved the ethical issues of this study. Blood samples were drawn and sera were stored for the measurement of specific IgE to *T. putrescentiae* and *D. pteronyssinus*. Among the 117 allergic rhinitis subjects, there were 33 patients with positive prick test to both *D. pteronyssinus* and *T. putrescentiae* mites. Among these 33 mite-sensitive patients, only 30 cases donated blood samples for sera collection to examine the relationship of IgE titers by ELISA. Unfortunately, only 22 cases had enough sera for all assays.

Skin-prick Testing

All allergic rhinitis patients were selected for skin-prick testing. The skin of the forearm was pricked with *T. putrescentiae* and *D. pteronyssinus* using a disposable lancet (Greer Derma Pit system; Greer Laboratories, Lenoir, NC, USA) at the concentration of 100 μg/ml following the endpoint titration. Histamine (10 mg/ml, Sigma, St. Louis, MO, USA) and phosphate-buffered saline (PBS, pH 7.2) were used as positive and negative controls, respectively. The responses were observed after 15 min and a wheal 3 mm in diameter greater than negative control was considered a positive result.

Preparation Crude Extracts and Recombinant Allergens of T. putrescentiae and D. pteronyssinus

The preparation of *D. pteronyssinus* and *T. putrescentiae* mite crude extracts was described previously [21]. In brief,

crude extracts of *D. pteronyssinus* were prepared from lyophilized whole mite bodies purchased from Allergon (Angelholm, Sweden). *Tyrophagus putrescentiae* mites were cultured in our laboratory with a medium consisting of yeast extract and mouse chow, and 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 *T. putrescentiae* mites were homogenized and extracted with PBS. Protein concentration was determined by the Bradford assay (Bio-Rad, Hercules, CA, USA) using bovine serum albumin as a standard.

The recombinant allergens of *T. putrescentiae* (rTyr p 2 and rTyr p 3), *D. pteronyssinus* (rDer p 2 and rDer p 3), and *D. farinae* (rDer f 1 and rDer f 2) were prepared and used to analyze the relationship of IgE reactivity [13, 20, 22–25]. Total RNA of mites was isolated with Trizol reagent and cDNA was synthesized with primer dT_{17}, MMLV reverse transcriptase (Gibco-BRL, New York, NY, 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 a 6× His-tagged protein using 1 mM isopropyl-β-D-thiogalactopyranoside (Promega, Madison, WI, USA) induction. The proteins were purified by nickel-nitrilotriacetic acid agarose metal affinity column chromatography under native conditions.

Determination of IgE Antibodies in Sera Against *T. putrescentiae* and *D. pteronyssinus* Crude Extracts

A total of 22 allergic subjects positive to *T. putrescentiae* and *D. pteronyssinus* by skin prick test were selected for further analysis, including enzyme-linked immunosorbent assay (ELISA), immunoblot, and histamine release assay. The IgE antibodies against *T. putrescentiae* and *D. pteronyssinus* crude extracts in sera determined by an ELISA were described previously [21]. Mite crude extracts (5 μg/ml) were diluted with coating buffer 0.1 M $NaHCO_3$ (pH 8.4) (100 μl/well) and coated to plates, incubated overnight with diluted (1:5) sera at 4°C. The alkaline phosphatase (AP)-conjugated mouse anti-human IgE antibodies were diluted (1:2,000) (Southern Biotech Association, Birmingham, AL, USA) and added for detection the human IgE. The bound enzyme substrate was detected using r-nitrophenyl phosphate (Kirkegaard and Perry, Gaithersburg, MD, USA). The reaction was stopped after 30 min and the optical density measured at 405 nm. Ten normal healthy individuals that had negative skin test results to both *T. putrescentiae* and *D. pteronyssinus* mite crude extracts were selected from our hospital staff. The negative cut-off values were defined as values below mean ±2 standard deviations; the specimen of *T. putrescentiae* exceeding 0.21±0.04 units or specimen of *D. pteronyssinus* exceeding 0.24±0.04 units was considered as a positive reaction.

IgE ELISA Reactivity and Inhibition Assays with Recombinant Allergens

The IgE antibodies against recombinant allergens in serum samples were detected by ELISA. The preliminary test showed that the optimal level of absorption could be obtained by coating a plate with 5 μg/ml recombinant allergen in 0.1 M $NaHCO_3$, pH 8.4. One hundred microliters of diluted (1:5) human sera was added and incubated overnight at 4°C. Plates were washed and incubated with AP-conjugated mouse anti-human IgE antibodies diluted 1:2,000 in PBST (phosphate-buffered saline, 1% Triton X-100, pH 7.2) for 1 h at room temperature. The colorimetric reaction was presented by r-nitrophenyl phosphate and stopped after 30 min and the optical density measured at 405 nm. The cut-off value was mean ±2× standard deviation as 0.23±0.02 unit of the sera of 10 healthy volunteers who did not have a history of allergic symptoms, and samples exceeding 0.25 unit were considered as positive. The titers of antigen-specific IgE were further classified as follows: $OD_{405nm} \leq 0.25$ of titer "−"; OD_{405nm}: 0.25~0.34 of titer "1±"; OD_{405nm}: 0.35~0.44 of titer "2±"; OD_{405nm}: 0.45~0.54 of titer "3±"; OD_{405nm}: 0.55~0.64 of titer "4±"; OD_{405nm}: 0.65~0.74 of titer "5±"; $OD_{405nm} \geq 0.75$ of titer "6±". For the IgE ELISA inhibition assay, each serum sample was pre-incubated with 50 μl of 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 are 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 *T. putrescentiae* allergenic components as previously described [21]. In brief, *T. putrescentiae* crude extract was transferred onto a polyvinylidene difluoride (PVDF) membrane. After blocking, the blots were incubated with diluted serum (1:5) at 4°C for

16 h. The membranes were incubated with AP-conjugated monoclonal antihuman IgE antibodies at room temperature for 2 h. The blots were developed in a substrate solution for 30 min and the antigen-antibody binding was recorded photographically.

Each serum sample (100 μl) was pre-incubated with 50 μl of *D. pteronyssinus* crude extracts or recombinant allergen rDer p 2, rDer p 3 (50 μg/ml) and left overnight at 4°C. After the pre-absorption, the subsequent procedures for determining IgE by immunoblot were the same as those described previously. Furthermore, five subjects (B6, B9, B12, B14, and B17) who had IgE antibodies against Tp extracts with MWs both of 16 and 26 kDa were selected for inhibition immunoblots with 50 μl of rDer p 2 or rDer p 3 (50 μg/ml).

Basophil Histamine Release Assay

The sera from the allergic rhinitis patients (B1~B22) and healthy volunteers (H1 and H2) were selected for basophil histamine release assay, and the selection of these subjects was described above. Washed polymorphonuclear cells were obtained from the venous blood of non-atopic donors using Polymorphpret^{TM} solution (Axis-Shield, Oslo, Norway). The cells were resuspended in medium RPMI-1640 by adjusting to 2×10^6 cells/ml using trypan blue assessment. Passive sensitization of basophils with specific IgE from the subject sera (B1~B22) was performed for 4 h at 37°C. The sensitized cells were triggered with 50 μg/ml of allergen for 30 min at 37°C, and then the supernatant was collected and reacted with *O*-phthalaldehyde (OPA, 5 mM) for 7 min [26]. The reaction was stopped by adding H_2SO_4 (0.04 M). The histamine released into the supernatant was measured by a fluorescence spectrophotometer. The percentage of histamine release was calculated by the following formula: (stimulated released histamine − spontaneous released histamine) / total released histamine × 100%.

Statistical Analysis

The SPSS Sample Power 2.0 was used for power calculation analysis. Statistical comparisons of the mite-triggering histamine release between *D. pteronyssinus* and *T. putrescentiae* were performed by paired *t* test. Statistical comparisons of the histamine release between allergic rhinitis patients or healthy volunteers were performed by student's *t* test. Two-tailed *p* values lower than 0.05 were considered to be statistically significant.

Results

The Prevalence of Sensitization to *D. pteronyssinus* and *T. putrescentiae*

The sensitization to *D. pteronyssinus* and *T. putrescentiae* was determined by skin prick tests. The results showed that 70% (82/117) of allergic subjects were sensitized to either *D. pteronyssinus* or *T. putrescentiae*. There were more subjects sensitized to *D. pteronyssinus*, 69% (81/117) than to *T. putrescentiae* 29% (34/117), and 28% (33/117) of the subjects were sensitive to both *D. pteronyssinus* and *T. putrescentiae.* Among the *T. putrescentiae*-sensitive allergic subjects, 97% (33/34) of the subjects were also sensitive to *D. pteronyssinus*, with only one subject being sensitized to *T. putrescentiae* alone.

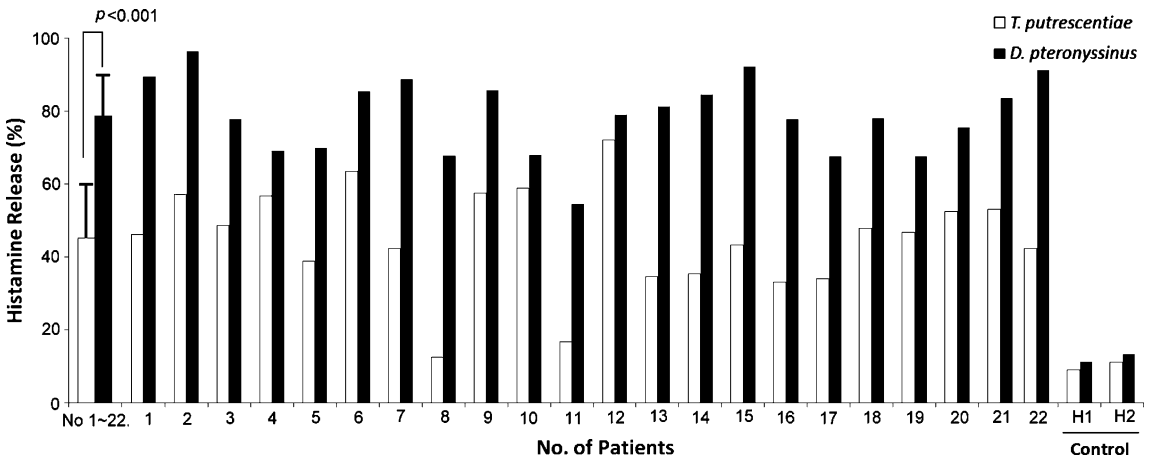


Fig. 1 The percentage of histamine release from basophils. The basophils were pre-sensitized by sera from subjects with allergic rhinitis and healthy volunteers were then triggered by *T. putrescentiae* or *D. pteronyssinus* at a concentration of 50 μg/ml. Tp (*open bars*): crude extract of *T. putrescentiae*; Dp (*closed bars*): crude extract of *D. pteronyssinus*, B1–B22: allergic rhinitis subjects, H1–H2: normal subjects

Table I The Titers of Mite-Specific IgE in the Sera

No. of cases allergic rhinitis subjects	Tp	Dp	No. of cases healthy volunteers	Tp	Dp
B1	0.67[a]	0.83	C1	0.18	0.31
B2	0.45	0.62	C2	0.17	0.22
B3	0.56	0.62	C3	0.27	0.26
B4	0.66	0.89	C4	0.16	0.23
B5	0.37	0.52	C5	0.18	0.18
B6	1.21	1.67	C6	0.22	0.19
B7	0.75	0.84	C7	0.21	0.25
B8	0.34	0.62	C8	0.23	0.27
B9	1.31	1.62	C9	0.22	0.26
B10	1.26	1.34	C10	0.26	0.24
B11	0.36	0.52			
B12	1.73	1.84			
B13	0.52	0.84			
B14	1.01	1.24			
B15	0.67	0.84			
B16	0.85	0.96			
B17	1.41	1.65			
B18	1.35	1.63			
B19	0.76	1.22			
B20	0.87	1.42			
B21	0.94	1.21			
B22	0.74	0.89			
Mean	0.85	1.08	Mean	0.21	0.24
SD	0.38	0.41	SD	0.04	0.04

[a] Absorbance was measured at an optical density of 405 nm

Dp D. pteronyssinus, *Tp T. putrescentiae*

Different Percentages of *D. pteronyssinus*- and *T. putrescentiae*-Triggered Histamine Release by Basophils After Sensitization with Sera

A total of 24 samples, 22 from allergic rhinitis patients (B1–B22) and two from non-allergic healthy volunteers (H1 and H2) were used for the basophil histamine release assay. When the basophils were pre-incubated with the sera (H1 and H2) and then triggered by *T. putrescentiae* or *D. pteronyssinus*, the mean percentage of baseline histamine release was 9.0±3.5 % (7.8–13.6%). While the basophils pre-incubated with sera (B1~B22), the percentage of histamine release was compared between *T. putrescentiae* and *D. pteronyssinus* challenge. The results show that the mean percentage of histamine release triggered by *D. pteronyssinus* was apparently higher than *T. putrescentiae* (78.6±10.4% vs. 45.2±14.2%; $p<0.001$) (Fig. 1).

The Prevalence of Sensitization to the Crude Extracts and Major Allergens of *D. pteronyssinus* and *T. putrescentiae*

The results of ELISA with *D. pteronyssinus* and *T. putrescentiae* crude extracts reactive to sera from the 22 subjects (B1~B22) are shown in the Table I. Further, these 22 sera were performed for analysis of the major allergen sensitization. The major allergens were determined by specific IgE measurement against rDer f 1, rDer f 2, rDer p 2, rTyr p 2, rDer p 3, and rTyr p 3. The results showed that 82% (18/22) of subjects were sensitive to rDer p 2 and more subjects were sensitized to group 2 allergens than group 1 (rDer f 1; 59%) or group 3 allergens (rDer p 3; 55%). There were also more subjects that were sensitized to group 2 allergens of *D. pteronyssinus* (82% for rDer p 2) than to *D. farinae* (77% for rDer f 2) and *T. putrescentiae* (73% for rTyr p 2) (Table II).

Identification of *T. putrescentiae* Specific Allergenic Components by IgE Immunoblot Inhibition with *D. pteronyssinus*

The same 22 sera were selected for IgE immunoblot inhibition, and a PVDF membrane of the *T. putrescentiae* crude extract was used to identify *T. putrescentiae* specific allergens reacting with the sera after the absorption of *D. pteronyssinus*. The results of immunobot for *T. putrescentiae* and immunoblot inhibition with *D. pteronyssinus* are shown in Fig. 2a; 59% (13/22) of the subjects possessed the

Table II The Titers of Recombinant Allergen-Specific IgE in the Sera

	rDer f 1	rDer f 2	rDer p 2	rTyr p 2	rDer p 3	rTyr p 3
B1	–	2+	3+	1+	3+	2+
B2	1+	5+	6+	1+	1+	–
B3	3+	1+	1+	–	–	2+
B4	2+	5+	5+	2+	1+	–
B5	–	–	–	–	–	1+
B6	2+	3+	2+	2+	–	2+
B7	2+	–	–	–	1+	3+
B8	–	–	–	–	1+	–
B9	3+	2+	2+	6+	–	1+
B10	–	1+	1+	1+	–	1+
B11	–	–	–	–	–	–
B12	3+	6+	6+	6+	2+	1+
B13	1+	–	1+	–	1+	–
B14	1+	2+	3+	3+	2+	4+
B15	–	2+	3+	1+	–	–
B16	–	1+	2+	2+	–	–
B17	2+	1+	1+	1+	–	1+
B18	–	1+	1+	1+	1+	–
B19	1+	1+	1+	1+	–	–
B20	–	1+	2+	1+	1+	–
B21	2+	1+	3+	1+	3+	–
B22	1+	1+	1+	1+	2+	–
Positive cases	13	17	18	16	12	10
%	59%	77%	82%	73%	55%	46%

Absorbance was measured at an optical density of 405 nm. –: <0.25; 1+: 0.25~0.34; 2+: 0.35~0.44; 3+: 0.45~0.54; 4+: 0.55~0.64; 5+: 0.65~0.74; 6+: 0.75~0.84

IgE-binding components of *T. putrescentiae*, which could be completely absorbed by *D. pteronyssinus*. Eighteen out of 22 sera possessed an IgE-binding component with a MW of 16 kDa, except for patients B5, B7, B8, and B11. The IgE-binding component with a MW of 16 kDa could be totally absorbed by *D. pteronyssinus* from the sera of these 16 patients and partially absorbed from sera B12 and B20. The results indicate that this allergenic component showed a high level of cross-reactivity between *D. pteronyssinus* and *T. putrescentiae.* Six out of 22 subjects possessed an IgE-binding component with a MW of 26 kDa, which was not absorbed by *D. pteronyssinus* in four sera (B1, B3, B6, and B12) or partially absorbed in two sera (B14 and B17), indicating that this component might be a *T. putrescentiae* specific allergen (Fig. 2a). Five sera from subjects who had positive reactions to the IgE-binding component of *T. putrescentiae* with MWs of 16 and 26 kDa were selected for further analysis. These five sera were absorbed by either rDer p 2 or rDer p 3, followed by IgE immunoblot assay. The results show that all the *T. putrescentiae* IgE-binding components of 16 kDa could be absorbed by rDer p 2 and components of 26 kDa could not be absorbed by rDer p 3 (Fig. 2b). The complete absorption of the 16 kDa allergen suggested that the IgE-binding component of *T. putrescentiae* displayed high level cross-reactivity of Der p 2.

To evaluate the IgE binding activity of Tyr p 2 and Tyr p 3 in *T. putrescentiae*-induced IgE-mediated reaction, the previous 22 sera were used for ELISA IgE inhibition assay. Immune inhibition was performed after pre-incubation with either rTyr p 2 or rTyr p 3 (100 μg/ml). The results showed that there was more inhibition by rTyr p 2 (68.5±19.0%) than by rTyr p 3 (45.3±20.1%)(Fig. 3).

The Relationship of IgE Antibody Titers, Sequence Homology, and 3D Structure Prediction Between rTyr p 2 and rDer p 2

A total of 30 sera from *T. putrescentiae* sensitive allergic rhinitis patients were further examined in this experminent. The results show that the titers of IgE to rTyr p 2 and rDer p 2 were well correlated (R^2=0.89; p<0.01) (Fig. 4a), but the titers of IgE to rTyr p 3 and rDer p 3 were not well correlated (R^2=0.35) (Fig. 4b). The protein sequence homology between rDer p 2 and rTyr p 2 was 38%, as performed using the ExPASy server (www.expasy.ch/tools/). Howerver, the 3D structure predictions of rTyr p 2 and rDer p 2 were

compared and showed that there was a high degree of similarity between rDer p 2 and rTyr p 2 (Fig. 5a, b).

Discussion

Sensitization to house dust mites is an important risk factor for the development of allergic diseases such as asthma and rhinitis. In our previous study, the prevalence of sensitization to *T. putrescentiae* was significantly higher in elderly subjects (age over 70), while the prevalence of sensitization to *D. pteronyssinus* was significantly higher in young adults (age under 40). *Tyrophagus putrescentiae* and *D. pteronyssinus* sensitization are associated with age (p=0.02) [21]. Similar results were found in this study; there were more allergic rhinitis patients sensitized to *D. pteronyssinus* than to *T. putrescentiae* in the young adult less than 40 years of age. Therefore, a selection bias may exist. Since all patients in the study were 40 years old or younger, their possibility to be sensitized to *D. pteronyssinus*

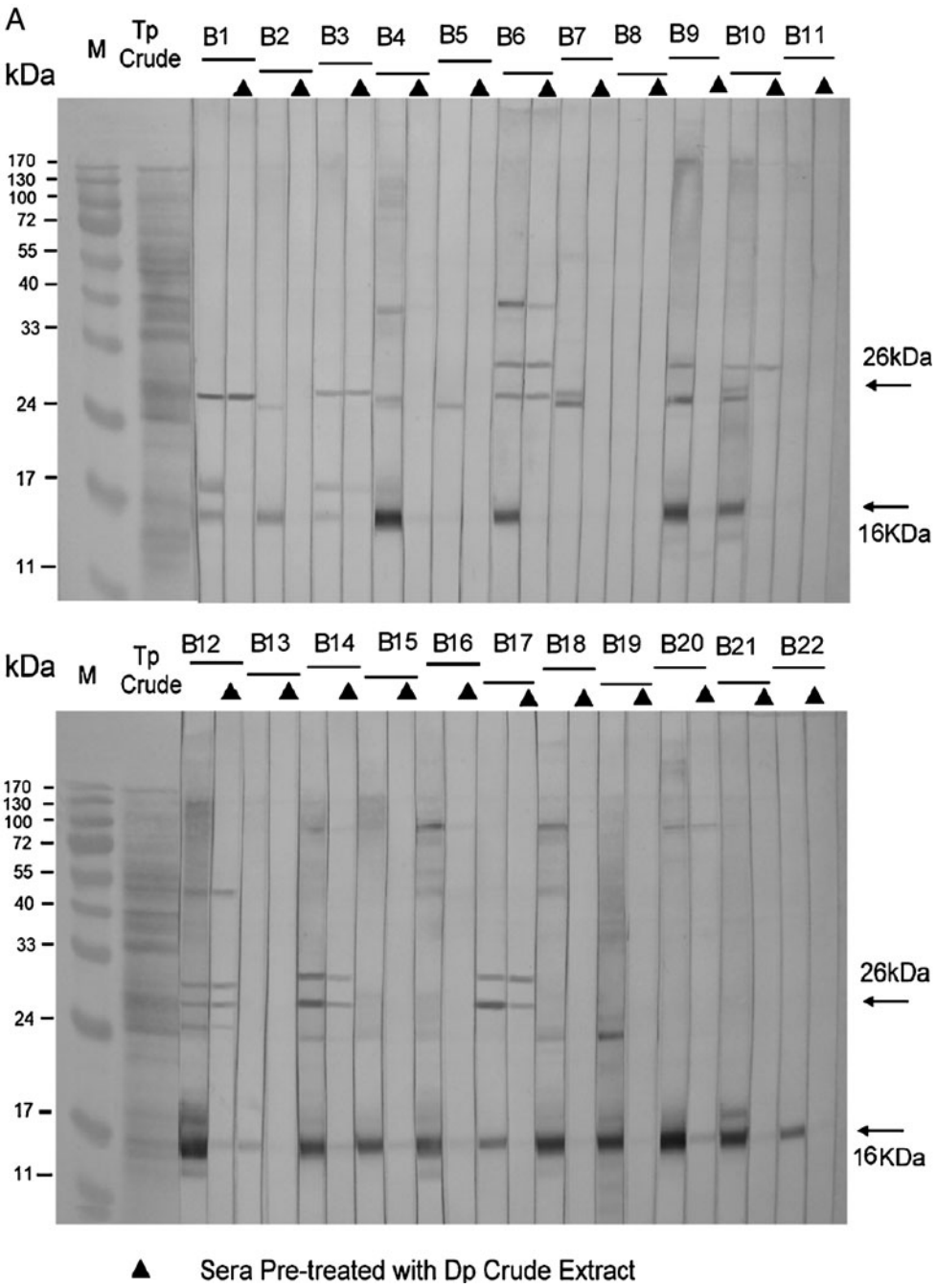
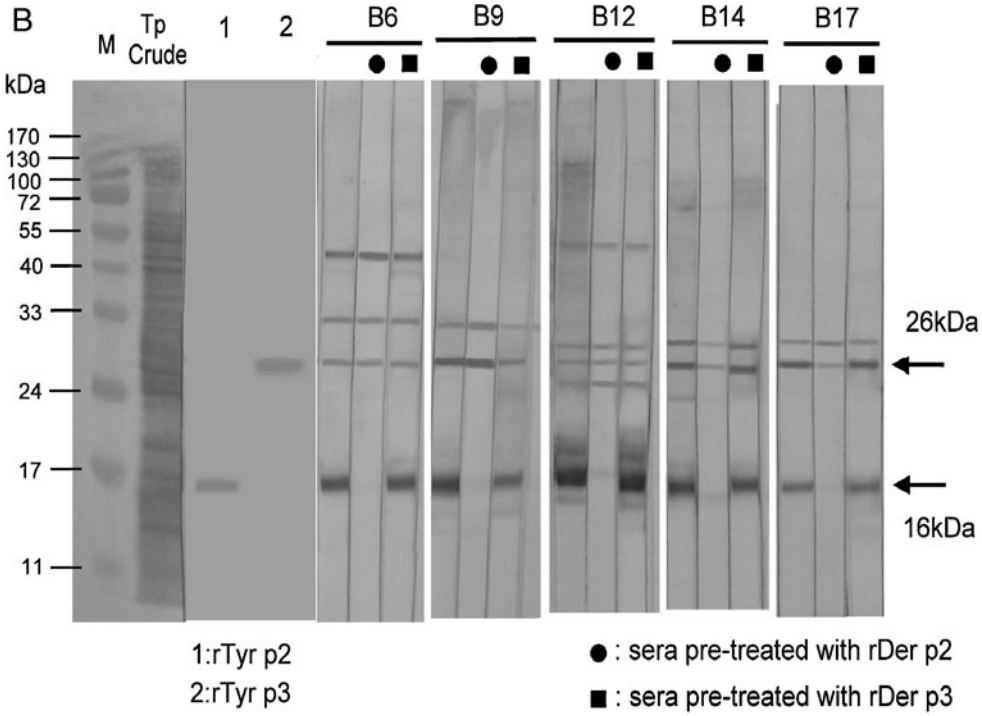


Fig. 2 Identification of *T. putrescentiae*-specific allergenic components was performed by immunoblot inhibition analysis of IgE reactivity by sera from allergic rhinitis subjects. **a** Sera pre-treated with or without *D. pteronyssinus* crude extract were used to identify the specific IgE-binding components to *T. putrescentiae* crude extract. *M*: protein marker; *Tp crude*: crude extract of *T. putrescentiae*, *B1–B22*: sera from allergic rhinitis subjects. *Triangles*: sera pre-absorbed with 50 μg/ml *D. pteronyssinus* crude extract. *Arrows* point to the IgE-binding components of molecular weights 16 and 26 kDa. **b** Five sera from patients who had positive reactions to the IgE-binding component of *T. putrescentiae* with MWs of 16 and 26 kDa were selected for further analysis. *Lane 1*: recombinant Tyr p 2 allergen, *lane 2*: recombinant Tyr p 3 allergen, *circles*: sera pre-treated with rDer p 2, *squares*: sera pre-treated with rDer p 3

Fig. 2 (continued)



only and react to *T. putrescentiae* due to cross-reactivity is higher compared to what would be expected if older patients had also been included.

Dermatophagoides pteronyssinus was the most common house dust mite to which allergic individuals were sensitized. Among the mite-sensitive subjects, 28% (33/117) of the subjects were sensitive to both *D. pteronyssinus* and *T. putrescentiae*, indicating that *T. putrescentiae*, as well as *D. pteronyssinus*, could contribute to the allergic reactions in allergic subjects. Since 97.1% (33/34) of the *T. putrescentiae*-sensitive subjects were co-sensitized to *D. pteronyssinus*, this could either be due to multiple mite allergen sensitizations or due to the cross-reaction between *T. putrescentiae* and *D. pteronyssinus*. It has been reported that there are cross-reactive components among *T. putrescentiae* and *D. pteronyssinus*, and that patients can be co-sensitized by both mite species [12]. Our previous report also showed that both house dust mites and storage mites can be found simultaneously in house dust collected from the houses of allergic asthmatic patients [18]. However, it has also been demonstrated that there is limited cross-inhibition between 15–16 kDa components in *T. putrescentiae* and *D. pteronyssinus* extracts [27]. This discrepancy is probably due to different patient population selection and a lack of a purified recombinant allergen preparation.

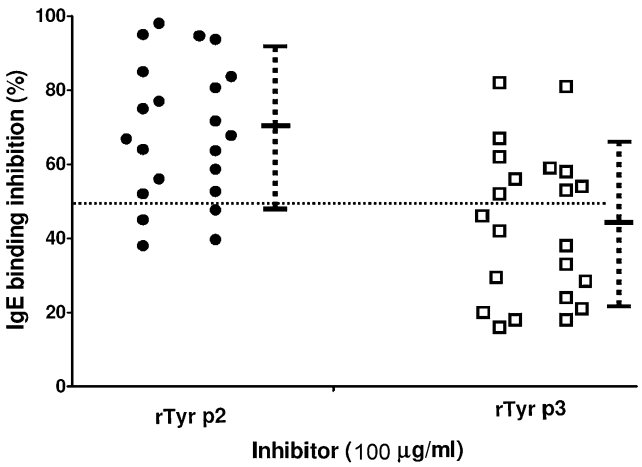


Fig. 3 The ELISA inhibition study with inhibitor-rTyr p 2 and rTyr p 3 (100 μg/ml) using sera from 22 patients (B1–22). The value of 50% inhibition is indicated as (*broken line*). *Circles*: sera absorbed with rTyr p 2, *squares*: sera absorbed with rTyr p 3

To differentiate the co-sensitization and cross-reactivity between *T. putrescentiae* and *D. pteronyssinus*, the sera from skin test positive reactions to *T. putrescentiae* and *D. pteronyssinus* were selected for immunoblot inhibition after *D. pteronyssinus* absorption. Group 2 and group 3 allergens were used to identify the cross-reactivity. Our results showed that the IgE-binding component with a MW of 16 kDa was absorbed by *D. pteronyssinus* and rDer p 2. The titers of specific IgE to rTyr p 2 and rDer p 2 were also well correlated. These results indicate that the cross-reactivity between *T. putrescentiae* and *D. pteronyssinus* is mainly due to the group 2 allergens. Although there was only 38% sequence homology between Tyr p 2 and Der p 2

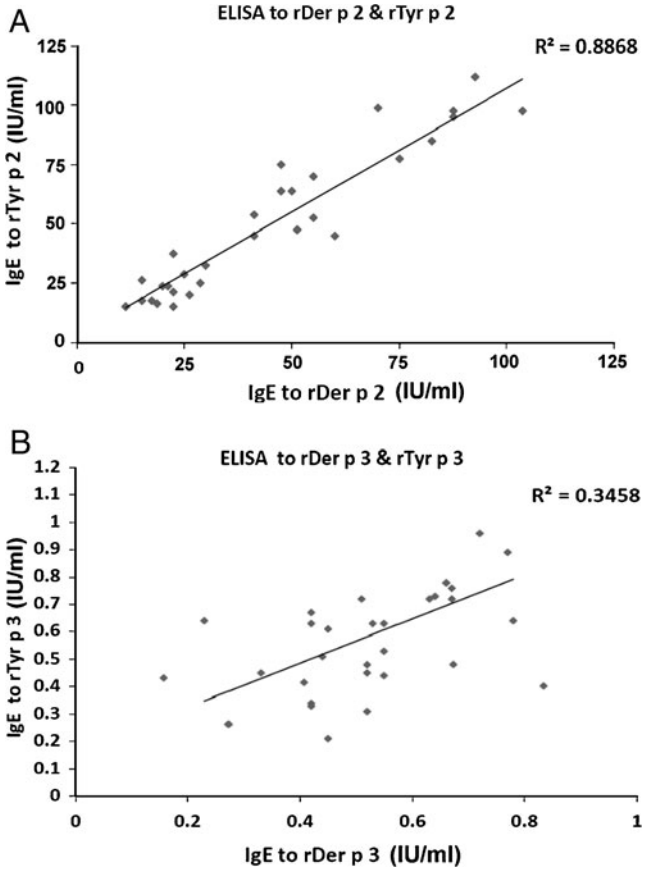


Fig. 4 The relationship between the titer of IgE antibodies to group 2 or group 3 allergens of *T. putrescentiae* and *D. pteronyssinus* (**a**). Relationship between antibodies to rTyr p 2 and rDer p 2 by ELISA. Absorbance was measured at an optical density of 405 nm. Correlation coefficient (R^2=0.89; $p \leq 0.01$) (**b**). Relationship between antibodies to rTyr p 3 and Der p 3 by ELISA. Correlation coefficient (R^2=0.35)

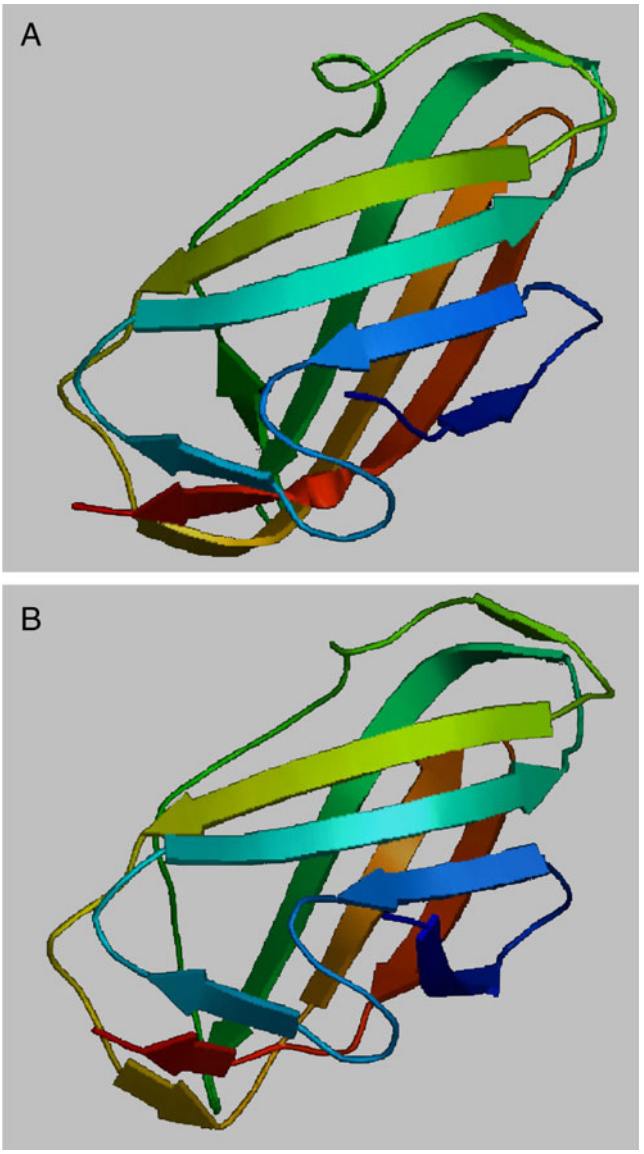


Fig. 5 The 3D tertiary structure prediction of rDer p 2 (**a**) and rTyr p 2 (**b**)

(www.expasy.ch/tools/) (data not shown), there was high structural homology between these two allergens. Whether Tyr p 2 and Der p 2 share common IgE binding epitopes requires further investigation.

Since the titer of specific IgE and its binding activity to major allergens is related to the severity of allergic reaction, ELISA was performed to measure the specific IgE binding activity using recombinant proteins. The results show that 82% and 73% of the subjects' sera were positive to rDer p 2 and rTyr p 2, respectively, and that this IgE binding activity could be inhibited by rTyr p 2. These results indicate that rTyr p 2 is the major allergen of *T. putrescentiae* and may play a major role in the IgE-mediated hypersensitive reaction to *T. putrescentiae*. The IgE-mediated hypersensitivity caused by group-2 mite allergen might be relevant to the clinical presentation of allergic rhinitis.

Each allergenic component in the crude extract can vary from each batch of mite allergen preparation; it is important to identify the specific allergens from each species. In this study, although the titers of specific IgE to rTyr p 3 were low and only 46% subjects were positive to rTyr p 3, there was non-absorbance by rDer p 3 and poor correlation of the titers of specific IgE to rDer p 3. These results suggest that Tyr p 3 is a *T. putrescentiae* specific allergen, and it could serve as a good marker for the identification of *T. putrescentiae* allergy.

In conclusion, most of our young adult allergic rhinitis patients were sensitized to either *D. pteronyssinus* or *T. putrescentiae.* Although there was cross-reactivity and co-sensitization, *T. putrescentiae*-sensitive patients were not uncommon, and most of the *T. putrescentiae*-sensitive patients were also co-sensitive to *D. pteronyssinus*. Tyr p 2 was the major allergenic component and might have cross-reactivity with Der p 2. Tyr p 3 was the major non-cross

reactive component and might serve as a *T. putrescentiae*-specific allergen. These observations could help us to better understand the reasons for co-sensitization to *D. pteronyssinus* and *T. putrescentiae* in Taiwan. The diagnosis could be accurate (dual sensitization), but the reason may be cross-reactive, and therefore, treatment for both species may not be needed.

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