Cross-reactivity between group-5 and -21 mite allergens from *Dermatophagoides farinae*, *Tyrophagus putrescentiae* and *Blomia tropicalis*

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Abstract. Group-5 and group-21 allergens, produced by house dust mites and storage mites are 36.6-55.8% identical in their sequences and are recognized by at least 50% of immunoglobulin (Ig)E from the sera of individuals allergic to dust mites. In the present study, recombinant group-5 and -21 allergens from three mite species, *Dermatophagoides farinae* (rDer f 5 and 21), *Tyrophagus putrescentiae* (rTyr p 5 and 21), and *Blomia tropicalis* (rBlo t 5 and 21), were purified from *Escherichia coli*, and the IgE reactivities and cross-reactivities of these allergen variants were assessed. The IgE binding frequencies of rDer f 5, rDer f 21, rTyr p 5, rTyr p 21, rBlo t and rBlo t 21 proteins were 64.95, 65.98, 30.41, 41.24, 30.93 and 21.65%, respectively. The IgE reactivity of rDer f 5 correlated highly with that of rDer f 21 (r=0.733). rTyr p 5 exhibited the highest level of correlation with rTyr p 21 (r=0.950), while the correlation of rBlo t 5 with rBlo t 21 was the lowest observed (r=0.104). The binding of IgE to rDer f 5 and rDer f 21 was not inhibited by any allergens but themselves. While rDer f 5 inhibited only 60.3% of IgE binding to rBlo t 5, rDer f 21 exhibited a high inhibitory effect against rTyr p 5 (93.01%), rTyr p 21 (92.12%), rBlo t 5 (86.97%) and rBlo t 21 (70.30%), implying cross-reactivity among mite species. The results of the present study demonstrated that the majority of the IgE reactivity to group-5 and -21 storage mite allergens is due to cross-reaction. It is therefore imperative to develop an accurate, component-resolved diagnosis for dust mite allergies.

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Introduction

House dust mites (HDMs) and storage mites (SMs) are the major source of indoor or occupational allergens that cause allergic diseases worldwide (1,2). To date, 24 groups of allergens have been identified in HDMs and SMs (3,4). A large number of these mite allergens are cross-reactive. For example, tropomyosin, a group-10 allergen, is a well-known pan-allergen that causes cross-reactivity among mites, a variety of invertebrates (5,6) including shrimps and crabs, and parasites, including *Anisakis* nematodes and roundworms (7-9). Group-8 mite allergens (glutathione *S*-transferase) have also been shown to be cross-reactive with other arthropod allergens (10,11). The group-2 allergens are cross-reactive among mite species (12,13) and are the primary allergens responsible for the cross-reactivity between HDM and SM in Korea (14). It has been suggested that a majority of the immunoglobulin (Ig)E reactivity to SM allergens is due to cross-reactivity between group-2, -8 and -10 allergens from HDMs. However, Son *et al* (15) reported that IgE cross-reactivity was observed between *Dermatophagoides farinae* and *Acarus siro* extracts, even though strong IgE reactive components were not detected by IgE immunoblot analysis under reducing conditions. These results indicated the importance of conformational epitopes for cross-reactivity (15). *Tyrophagus putrescentiae* is one of the predominant SM species in Korea. Five *Tyrophagus putrescentiae* allergens have been described to date: Tyr p 2, Tyr p 3, Tyr p 8, Tyr p 10 and Tyr p 24 (16). Of these, Tyr p 2, Tyr p 8 and Tyr p 10 have been shown to cause cross-reactivity (6,11,15,17).

The allergenicity of group-5 allergens is moderate, as only ~50% of HDM allergic patients are sensitized (18). In tropical regions, however, the *Blomia tropicalis* allergen Blo t 5 causes reactions in 70-92% of patients with mite allergies, making it the predominant reaction-associated group-5 allergen in that region (19,20). Group-21 allergens, which are homologous with the group-5 allergens, have only been described in *Blomia tropicalis* and *Dermatophagoides pteronyssinus* (21,22). The primary as well as secondary structures of group-5 and -21 allergens are highly conserved. Blo t 21

and Der p 21 exhibit moderate IgE reactivity and were found to co-localize in the mid-gut epithelium, lumen and feces of mites (21-23). Lin *et al* (18) reported that Der p 5 was not abundant in house dust, with a concentration of <100 ng/g in dust. Der p 5 and -21 are heat stable and can sensitize allergic individuals in domestic environments over a long period.

Arruda *et al* (19) failed to observe a correlation between Blo t 5 and Der p 5 in eliciting IgE responses in *Blomia tropicalis*-exposed and -unexposed individuals, despite 43% identity at the amino acid level. Similarly, in a study on Malaysian and Taiwanese patients, Kuo *et al* (20) reported a low correlation of IgE reactivity between Blo t 5 and Der p 5 (Taiwanese, r=0.452; Malaysian, r=0.346) as well as limited cross-reactivity. Gao *et al* (21) demonstrated that Blo t 21 was not highly cross-reactive to Blo t 5 despite a certain amount of sequence and structural identity, while Weghofer *et al* (22) showed that Der p 21 exhibited low IgE reactivity to Der p 5, Lep d 5 and Blo t 5 according to tests using sera from Der p 21-sensitized patients. In addition, Blo t 21 was reported to have low to moderate cross-reactivity with Blo t 5, Der p 5 and Der f 21 (24). While multiple studies have focused on Der p 5 and Blo t 5, group-5 and -21 allergens from *Dermatophagoides farinae* and *Tyrophagus putrescentiae,* which are the predominant mite species in Korea, have yet to be examined.

In the present study, six recombinant (r) allergens, rDer f 5, rDer f 21, rTyr p 5, rTyr p 21, rBlo t 5 and rBlo t 21, were purified using an *Escherichia* (*E.*) *coli* expression system, and the IgE reactivity to each allergen was determined using sera from Korean HDM-sensitized patients. The cross-reactivities of these six recombinant allergens were then examined using a competitive ELISA approach. Furthermore, the potential of using rTyr p 5 and rTyr p 21 for mite species-specific diagnoses was examined.

Materials and methods

Subjects and serum samples. Serum samples were obtained from patients attending the Allergy-Asthma Clinic at Severance Hospital, Yonsei University College of Medicine in Seoul, Korea. Allergy diagnoses were based on any history of allergic reactions and skin prick testing. Sera from patients were tested for IgE antibodies specific for HDM (*Dermatophagoides farinae*) allergens using the Uni-CAP system (Phadia, Uppsala, Sweden) and ELISA analysis. Sera from 194 HDM-sensitized subjects (males/females, 103:91; average age, 25.4 years; age range, 4-67 years) and 20 healthy controls were used to assess the IgE reactivity of recombinant proteins. Serum samples were collected after consent of the patients, and experiments using the collected sera were approved by the Institutional Review Board (no. 4-2009-0180). The study was approved by the ethics committee of Sevrance Hospital (Yonsei University College of Medicine).

Cloning and expression of recombinant proteins in E. coli. Frozen *Dermatophagoides farinae*, *Tyrophagus putrescentiae* and *Blomia tropicalis* mites were obtained from the Arthropods of Medical Importance Resource Bank (AMIB) at the Department of Environmental Medical Biology, Yonsei University College of Medicine (Seoul, Korea). Total RNA was isolated from frozen mite bodies using TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA), according to the manufacturer's instructions. First-strand cDNA was synthesized from 5 μg total RNA using avian myeloblastosis reverse transcriptase (Promega, Madison, WI, USA) and an oligo (dT_{18}) primer. Primers were designed based on the published full-length nucleotide sequence of Der f 5 (GenBank accession no. AY283283), Der f 21 (EF027122), Tyr p 5 (AY800358), Tyr p 21 (AY800360), Blo t 5 (U59102) and Blo t 21 (AY800348), and sequences were flanked by restriction enzyme sites for either *Bam*H I/*Xho* I or *Bam*H I/*Sal* I. The primer sequences were as follows: Der f 5 forward, 5'-GGATCCATGAAATTCATCATTGCTATTGCTG-3' and reverse, 5'-CTCGAGTTAAACTTCAATCTTCTTCACACG TTGCTC-3'; Der f 21 forward, 5'-GGATCCATGAAATTC ATTATTTTCTGTGCCA-3' and reverse, 5'-CTCGAGTTA ATCATCCGATTTTACAGCTTTCACCTT-3'; Tyr p 5 forward, 5'-GGATCCATGAAGTTCGCCATTCTCGC-3' and reverse, 5'-CTCGAGTTAGCGAGTCTTGACAGCCT-3'; Blo t 5 forward, 5'-GGATCCATGAAGTTCGCCATCGTT CTTA TTG-3' and reverse, 5'-CTCGAGTTATTGGGTTTG AATATCCTTCACTTTTTG-3'; Tyr p 21 forward, 5'-GGA TCCATGAAGTTCGTCATCGCCCT-3' and reverse, 5'-GTC GACTTAGACCTTGATGGCGTTCACT-3'; Blo t 21 forward, 5'-GGATCCATGAAATTTATCATCGCATTGGCTG-3' and reverse, 5'-GTCGACTTATTCGGAATCTTGGACTCG CTTT-3'. The primers were synthesized by GeneTech Corporation (Daejeon, Korea). The conditions for PCR amplification were as follows: Pre-denaturation at 94˚C for 5 min, 30 cycles of denaturation at 94˚C for 30 sec, annealing at 55˚C for 30 sec, extension at 72˚C for 1 min and, following the final cycle, an additional extension at 72˚C for 5 min. The PCR-amplified cDNA sequences encoding Der f 5, Der f 21, Tyr p 5, 21, Blo t 5 and 21 were cloned into the pCR4-TOPO vector (Invitrogen Life Technologies), sub-cloned into the pET-28a expression vector (Novagen, San Diego, CA, USA), and transformed into *E. coli* Rosetta™ 2 (DE3) cells (Novagen). The expression of recombinant proteins was induced by addition of 1 mM isopropyl-1-thio-β-galactopyranoside (BioShop Canada Inc., Burlington, ON, Canada), and protein was purified from the insoluble fraction of cell lysates using Ni sepharose excel resin (GE Healthcare, Uppsala, Sweden) under denaturing conditions (20 mM sodium phosphate, 500 mM NaCl, 500 mM imidazole, 6 M urea, pH 7.4). The concentration of each of the recombinant allergens was determined using a Bradford Assay (Bio-Rad Laboratories, Hercules, CA, USA). The purified recombinant allergens were separated by 15% SDS-PAGE, followed by staining with Coomassie Blue R250 (Amresco, Solon, OH, USA).

Analysis of specific IgE binding to recombinant proteins. Recombinant Der f 2 was obtained from the AMIB and used as a control. Microtiter plates were coated with 100 μl recombinant protein (2 μg/ml in 50 mM sodium carbonate, pH 9.6), and washed with phosphate-buffered saline (PBS) containing 0.05% Tween 20 (PBST). The plates were blocked with 1% bovine serum albumin (BSA; EMD Millipore, Kankakee, IL, USA) in PBST for 1 h at room temperature and then incubated for 1 h with 100 μl serum per well diluted at 1:9 in PBST containing 1% BSA. IgE antibodies were detected

Table I. Sequence identity (%) of group-5 and group-21 allergens from *Dermatophagoides farinae, Tyrophagus putrescentiae* and *Blomia tropicalis*.

Amino acid sequence	Der f 5	Tyr p 5	Blo t 5	Der f 21	Tyr p 21	Blo t 21
Der f 5	100	38.0	44.4	36.7	40.3	36.6
Tyr p 5	-	100	52.9	41.6	45.3	43.4
Blo t 5	-	-	100	41.2	37.4	40.3
Der f 21	-	-	-	100	39.3	37.5
Tyr p 21	-	-	-	-	100	55.8
Blo t 21	-	-	-	-	-	100

The amino acid sequence identity of each allergen was calculated using ALIGN Query (http://xylian.igh.cnrs.fr). Der, *Dermatophagoides farinae*; Tyr, *Tyrophagus putrescentiae*; Blo, *Blomia tropicalis*.

Table II. Characteristics of patients used for the competitive inhibition ELISA.

No.	Age (years)	Gender (M/F)	Clinical symptoms	ELISA results (A_{450nm})[a]					
				rDer f 5	rDer f 21	rTyr p 5	rTyr p 21	rBlo t 5	rBlo t 21
1	23	M	Rhinitis	2.916	3.281	2.739	3.251	0.239	0.723
2	14	M	Asthma	3.029	3.991	1.898	2.035	1.899	0.146
3	31	M	Rhinitis	2.128	2.285	0.420	1.544	0.071	1.519
4	25	F	Rhinitis	2.560	3.252	0.402	1.114	0.078	1.602
5	55	M	Asthma	2.343	1.804	1.404	1.641	0.800	0.074
6	9	M	Asthma	2.866	3.168	1.932	2.483	0.703	2.855
7	16	M	Asthma	2.559	3.113	2.084	2.495	0.785	0.071
8	12	M	Asthma	2.336	2.994	1.588	1.909	1.377	0.104
9	24	M	Rhinitis	2.838	2.999	1.798	2.214	1.884	0.090
10	16	M	Atopic dermatitis	2.623	3.289	2.190	2.672	1.578	0.087

[a]The cut-off values were 0.0699 for rDer f 5, 0.1252 for rDer f 21, 0.1724 for rTyr p 5, 0.24 for rTyr p 21, 0.086 for rBlo t 5 and 0.0798 for rBlo t 21, respectively. M, male; F, female; r, recombinant; A, absorbance; Der, *Dermatophagoides farinae*; Tyr, *Tyrophagus putrescentiae*; Blo, *Blomia tropicalis*.

using biotinylated goat anti-human IgE (Vector Laboratories, Burlingame, CA, USA) and streptavidin-peroxidase (Sigma-Aldrich, St. Louis, MO, USA). The assay was developed with 3,3',5,5'-tetramethylbenzidine (Kirkegaard and Perry Laboratories, Gaithersburg, MD, USA), which undergoes a color change in the presence of the antibody/allergen complexes. The absorbance (A) at 450 nm was measured using a Tecan sunrise microplate reader (Tecan, Salzburg, Austria) and the Magellan CE software after addition of 0.5 M H_2SO_4 to stop color development. The cut-off value was determined as the mean absorbance plus twice the value of the standard deviation of 20 negative controls.

Inhibition assay. The inhibitory effects of each allergen were examined using a competitive ELISA approach. For these experiments, recombinant protein was suspended in coating buffer (2 μg/ml, 50 mM carbonate buffer, pH 9.6) and added to the wells of ELISA plates. After blocking with 1% BSA in PBST for 1 h at room temperature, wells were incubated with selected serum samples (1:9; a pooled serum of 10 subjects) that had been pre-incubated overnight at 4˚C with solutions containing various concentrations (0.001, 0.01, 0.1, 1.0 or 10.0 μg/ml) of recombinant proteins. IgE antibodies were detected as described above. The percentage of inhibition was calculated as $(1-A_i/A_0)x100$, where A_i stands for absorbance at 450 nm with an inhibitor and A_0 for the absorbance at 450 nm without an inhibitor. These assays were conducted in duplicate.

Statistical analysis. Correlations between the IgE reactivities to recombinant proteins were analyzed by Pearson's correlation using GraphPad Prism 6.0 (GrahPad, Inc., La Jolla, CA, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Sequence analysis of Der f 5, Der f 21, Tyr p 5, Tyr p 21, Blo t 5 and Blo t 21 suggests cross-reactivity. The amino

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Der f 5      MKFIIAIAVC TLAVVCVSGE P------KKH DYQNEFDFLL MQRIHDQMRK  26
Tyr p 5      ...A.LALAF LG..MAQG.P K----VPA.G .FR....H.. VSTLMEG.Y.  30
Blo t 5      .....VLIA. FA.S.LAQEH K-----P..D .FR....H.. IEQANHAIE.  28
Der f 21     .....FC.IV MAVS.SGFIV D----VDTED KWR.A..HM. MEEFEEK.DQ  29
Tyr p 21     ...V..L.AL LAVACAAPTP KPARPTPA.G .FR....H.I VAAAVQRFHD  34
Blo t 21     ......L.AL IAVACALPVS N--------D NFRH...HMI VNTATQRFHE  26

Der f 5      GEEALLHL-H QINTFEENPT KEMKEQILAS EMDTIIALID GVRGVLNRLM  75
Tyr p 5      ..QH..R.SE E.AHL.STK. .VEQDR.VR- .IEVTV.F.E .GVR.VEQEL  79
Blo t 5      ..HQ..Y.Q. .LDELN..KS ..LQ.K.IR- .L.VVC.M.E .AQ.A.EREL  77
Der f 21     I.HG..M.SE .YKEL.KTKS ..L.....R- .LTIAENYLR .ALKFMQQEA  78
Tyr p 21     L..F..K.SE .VADL.KTKN R.E.AK..R- .ITIADG.VV .GRTYFEKEL  83
Blo t 21     I.KF...IT. EVDDL.KTGN .DE.ARL.R- .LTVSE.F.E .S..YFQ.EL  75
                                                          *

Der f 5      KRTDLDIFER YNVEIALKSN EILERDLKKE EQRVKKIEV- -  114
Tyr p 5      .....N.L.. F.F.EVQALS KL.VK...EA .VK..AVKTR -  119
Blo t 5      .....N.L.. F.Y.E.QTLS K..LK...ET ..K..DIQTQ -  117
Der f 21     .....NM... ..F.T.VSTI ...VK..AEL AKK..AVKSD D  119
Tyr p 21     ......LV.K F.F.AV.ATI G..D....AL AT..NA.K.- -  132
Blo t 21     ......LL.K F.F.A..ATG DL.LK...AL QK..QDS.-- -  123
               *    *     *
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Figure 1. Sequence comparison of recombinant Der f 5 and other group-5 and group-21 allergens. Sequence alignment of Der f 5 with Der f 21, Tyr p 5, Tyr p 21, Blo t 5 and Blo t 21. Four of the charged residues were previously identified as the crucial amino acids for immunoglobulin E reactivity in Blo t 5 (marked by asterisks). The signal peptide regions, predicted using the SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP/), are underlined. Der, *Dermatophagoides farinae*; Tyr, *Tyrophagus putrescentiae*; Blo, *Blomia tropicalis*.

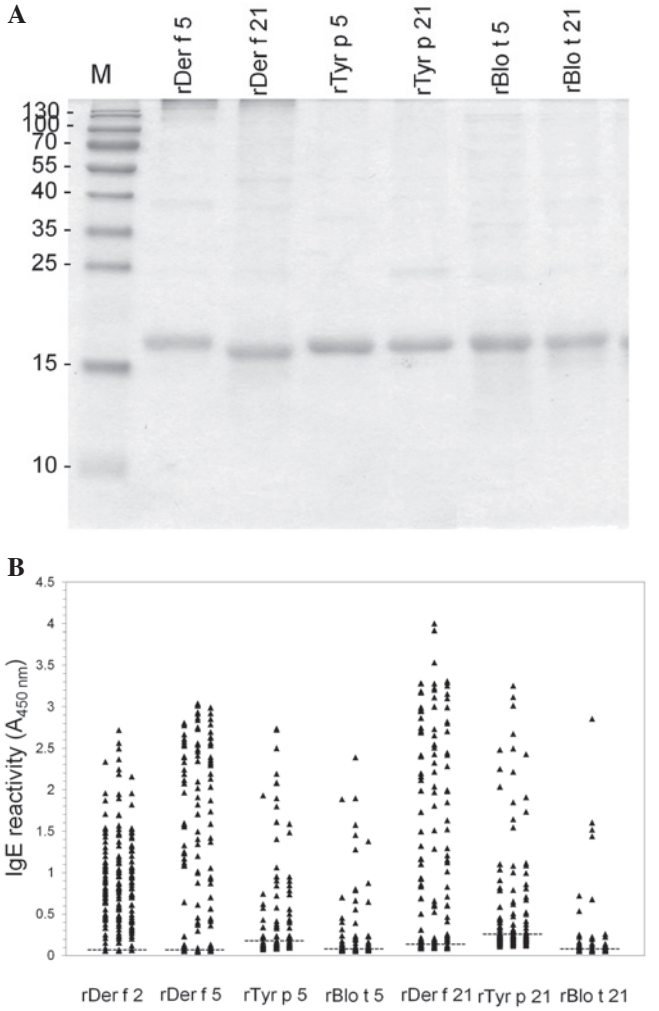

Figure 2. Human IgE reactivity to purified recombinant allergens. (A) Coomassie-stained 15% SDS-PAGE gel containing a molecular weight marker (lane M) and 3 μg of purified recombinant allergens. (B) The IgE reactivities to recombinant group-5 and -21 allergens were evaluated by ELISA using sera from 194 house dust mite-sensitized patients and 20 normal control sera. Each dot indicates an individual serum. The cut-off values (dashed lines) were set as the mean plus two-fold the standard deviation of the control sera. IgE, immunoglobulin E; r, recombinant; A, absorbance; M, marker, Der, *Dermatophagoides farinae*; Tyr, *Tyrophagus putrescentiae*; Blo, *Blomia tropicalis*.

acid sequences of the six group-5 and group-21 allergens, Der f 5, Der f 21, Tyr p 5, Tyr p 21, Blo t 5 and Blo t 21, were compared. As shown in Table I, the sequence of each allergen was 36.6-55.8% identical to that of the other group-5 and -21 allergens. While Der f 5 exhibited the highest level of identity to Blo t 5 (44.4%), Der f 21 was most similar to Tyr p 5 (41.6%), Tyr p 5 was most similar to Blo t 5 (52.9%) and Tyr p 21 was most similar to Blo t 21 (55.8%). The lowest level of similarity was observed between Der f 5 and Blo t 21 (36.6%), while the highest sequence identity was observed between Tyr p 21 and Blo t 21 (55.8%) (Table I). This high level of sequence identity between allergens suggested cross-reactivity between group-5 and group-21 allergens. As depicted in Fig. 1, four amino acids that were previously found to be crucial for IgE binding, were shown to be conserved in five of the allergens: Der f 21 (E-77, D-82, E-87 and E-92), Tyr p 5 (E-78, D-83, E-88 and E-93), Tyr p 21 (E-82, D-87, E-92 and E-97) Blo t 5 (E-76, D-81, E-86 and E-91) and Blo t 21 (E-74, D-79, E-84 and E-89). Der f 5 (L-74, D-79, E-84 and E-89) was the only allergen in which the binding site was not conserved.

Production of recombinant group-5 and group-21 allergens. Recombinant Der f 5, Der f 21, Tyr p 5, Tyr p 21, Blo t 5 and Blo t 21 were inducibly expressed in *E. coli*. The recombinant proteins were purified from *E. coli* inclusion bodies by affinity chromatography using a Ni-sepharose resin. Purified proteins were then separated by 15% SDS-PAGE and visualized by Coomassie Brilliant Blue staining. As expected, the purified proteins migrated slightly slower than the 15-kDa marker, which was consistent with the predicted 17-kDa size of the allergens (Fig. 2A).

IgE reactivity is correlated between HDM-associated recombinant proteins. The IgE reactivity of each purified recombinant allergen was examined by ELISA using sera from 194 Korean patients who were allergic to HDM. Der f 2 was used as a positive control. While 187 of the 194 sera (96.39%) tested showed reactivity to rDer f 2, 126 (64.95%) to rDer f 5, 128 (65.98%) to rDer f 21, 59 (30.41%) to rTyr p 5, 80 (41.24%) to rTyr p 21, 60 (30.93%) to rBlo t 5 and 42 (21.65%) to rBlo t 21 (Fig. 2B). The IgE reactivity of rDer f 5 was also strongly correlated with that of rDer f 21 (r=0.733). The IgE reactivity to rTyr p 5 exhibited the highest correlation with that to rTyr p 21 (r=0.950). It also moderately correlated with those of rDer f 5 (r=0.563), rDer f 21 (r=0.613) and Blo t 5 (r=0.742). The IgE reactivity to rTyr p 21 correlated the highest with the reactivities to rDer f 5 (r=0.571), rDer f 21 (r=0.651) and Blo t 5 (r=0.704), while the reactivity to rBlo t 5 was highly correlated with those to rTyr p 5 (r=0.742) and rTyr p 21 (r=0.704). However, the IgE reactivity to rBlo t 21 exhibited a poor correlation with those to rDer f 5, rDerf 21, rTyr p 5 and rTyr p 21 (r=0.270, 0.305, 0.254, 0.349, respectively) (Fig. 3). These results demonstrated that the IgE reactivity of all six recombinant allergens exhibited at least a certain level of correlation to one another.

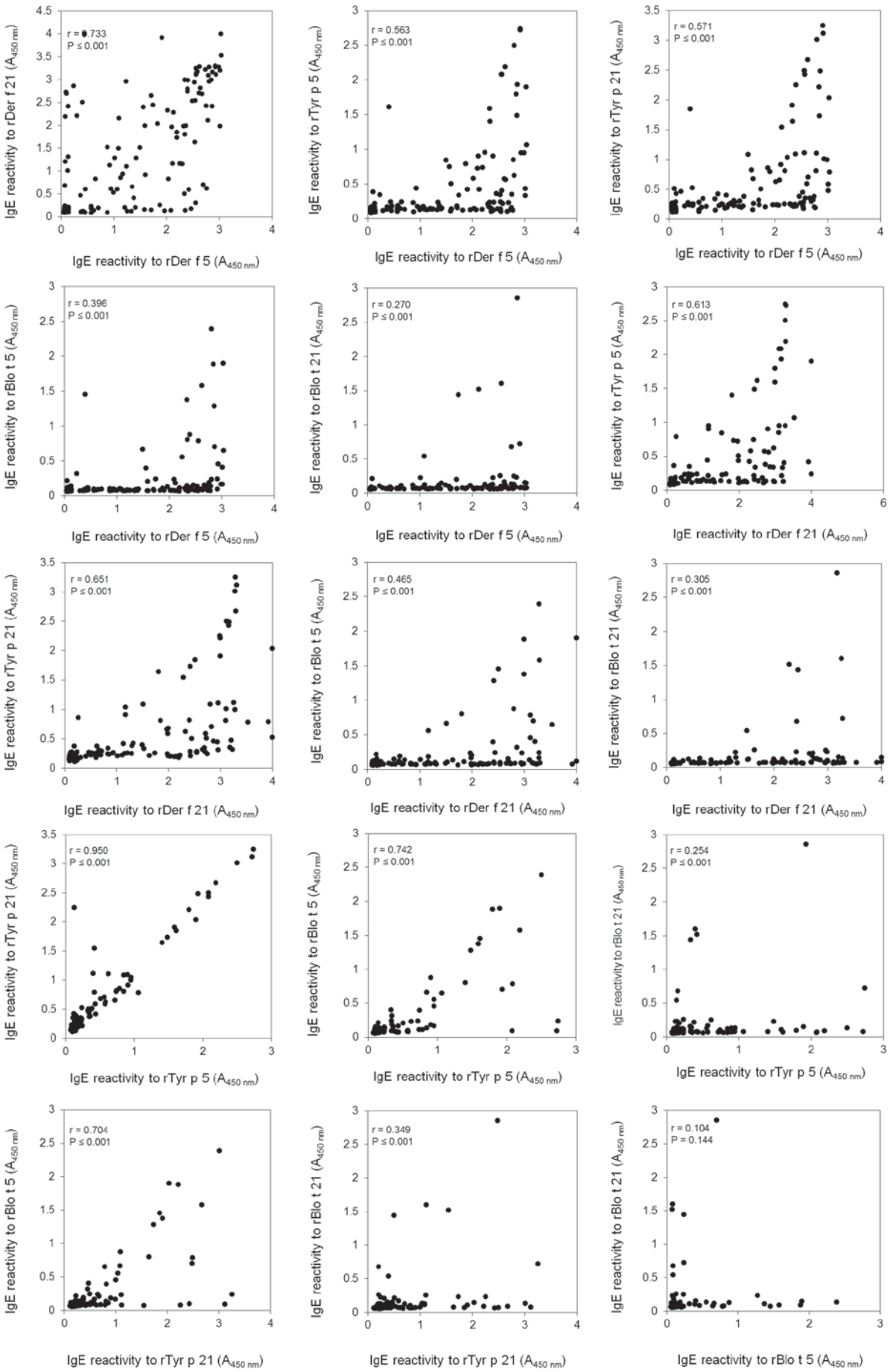

Figure 3. Correlations between the IgE reactivities of recombinant allergens. A total of 194 serum samples were used in the direct human IgE ELISA. IgE, immunoglobulin E; r, recombinant; Der, *Dermatophagoides farinae*; Tyr, *Tyrophagus putrescentiae*; Blo, *Blomia tropicalis*.

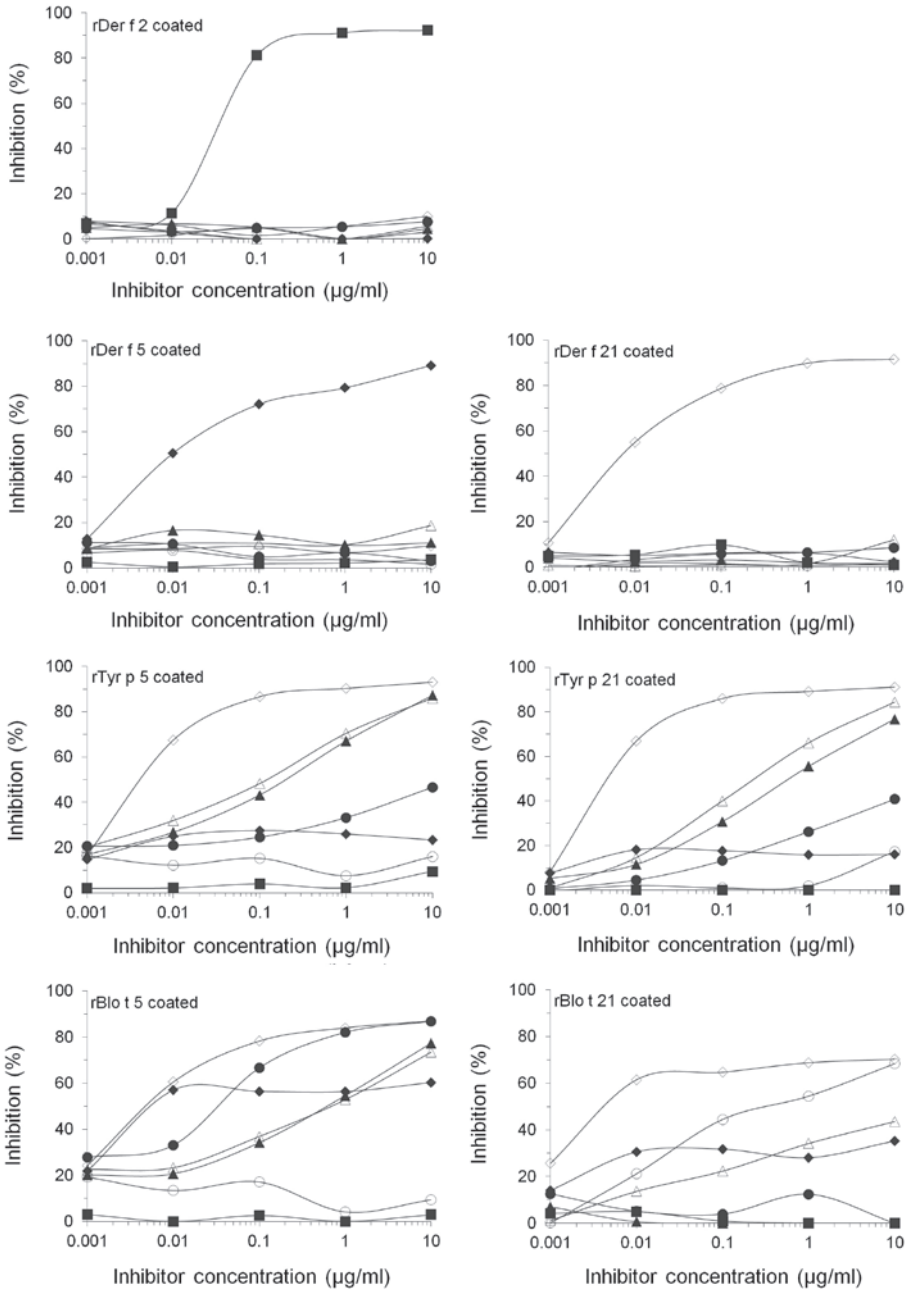

Figure 4. Inhibition analyses of six recombinant allergens. Competitive IgE inhibition ELISA was conducted using pooled sera from 10 patients, pre-absorbed with 0.001, 0.01, 0.1, 1 or 100 μg/ml of each allergen (■, rDer f 2; ♦, rDer f 5; ◊, rDer f 21; ▲, rTyr p 5; Δ, rTyr p 21; ●, rBlo t 5; ○, rBlo t 21). IgE, immunoglobulin E; r, recombinant; Der, *Dermatophagoides farinae*; Tyr, *Tyrophagus putrescentiae*; Blo, *Blomia tropicalis*.

IgE cross-reactivity is present among recombinant group-5 and group-21 allergens. In order to investigate the cross-reactivity between the allergens, the present study performed an IgE inhibition on each allergen, using a competitive ELISA approach with serum pooled from 10 allergy patients (Table II). The IgE reactivities to rDer f 2, rDer f 5 and rDer f 21 were only inhibited by the selfsame allergens. rDer f 21 was found to be the strongest inhibitor of IgE reactivity to the other allergens, inhibiting 93.01% of IgE reactivity to rTyr p 5, 92.12% of that to rTyr p 21, 86.99% of that to rBlo t 5 and 70.30% of IgE reactivity to rBlo t 21, at an inhibitor concentration of 10 μg/ml (Fig. 4). The IgE reactivity to rBlo t 21 was inhibited by up to 35.27% by rDer f 5, and by up to 43.57% by rTyr p 21. rTyr p 5 inhibited 76.77% of IgE reactivity to rTyr p 21, while rTyr p 21 inhibited 86.0% of the IgE reactivity to rTyr p 5. The IgE reactivity of Blo t 5 was inhibited by up to 77.35% by rTyr p 5 and by 73.44% by rTyr p 21. In addition, rBlo t 21 inhibited the IgE reactivity to rTyr p 21 by up to 43.57%. These results suggested that each of the allergens tested exhibited a certain degree of cross-reactivity to one another; however, there was an extremely low degree of cross-reactivity between Der f 5 and the other allergens tested, which may be due to the lack of a conserved IgE binding site of Der f 5.

Discussion

In the present study, six recombinant group-5 and group-21 mite allergens were produced using an *E. coli* expression

system, and their IgE reactivities and cross-reactivities were examined. The recombinant proteins exhibited moderate IgE reactivities (21.65-65.98%) and low-to-moderate cross-reactivities in Korean patients with HDM allergies. Previous studies reported that group-5 and group-21 mite allergens exhibited little to no cross-reactivity (20,21,24), and Tan *et al* (24) reported low to moderate cross-reactivity between Blo t 21 and Blo t 5, Der p 5 and Der f 21. Blo t 21 and Blo t 5 encode a conserved IgE epitope, and there is little difference in their secondary structure. A linear IgE epitope of Blo t 5 was mapped in the loop region (76-ELKRTDLNILERFNYE-91). However, the IgE epitope of Blo t 21 is not thought to be linear, as one (E-89) of the four critical amino acids of the IgE binding site (E-74, D-79, E-84 and E-89) was predicted to be part of another amino acid cluster (24). It is likely that the cross-reactivity of group-5 and -21 allergens may be affected by their tertiary structure.

rDer f 5 and rDer f 21 were inhibited by the selfsame proteins, but not by any other allergens. This reflects the fact that the sera utilized in the present study are from subjects who are sensitized to *Dermatophagoides farinae*, but not to SMs. The IgE reactivity to Der f 21 was effectively inhibited by Tyr p 5, Tyr p 21, Blo t 5 and Blo t 21. This may suggest that the IgE reactivity to the group-5 and -21 allergens of SMs are cross-reactive to Der f 21. It is likely that the IgE epitopes of Der f 21 are similar to or partially overlap with those of the group-5 and -21 allergens from *Tyrophagus putrescentiae* and *Blomia tropicalis*. By contrast, rDer f 5 exhibited poor cross-reactivity to the other group-5 and -21 allergens tested. Therefore, Der f 5 may potentially be used for species-specific diagnoses. By contrast, due to its high level of cross-reactivity, Der f 21 would likely cause an allergic response in patients that are not sensitized to SMs.

Interestingly, although *Blomia tropicalis* is not native to Korea, rBlo t 5 and rBlo t 21 displayed IgE reactivity to the sera of Korean patients with HDM allergies. This may presumably be due to the cross-reactivity of *Blomia tropicalis* allergens to those of *Dermatophagoides farinae*, *Tyrophagus putrescentiae* or *Blomia kulagini*. Of note, *Blomia kulagini*, though not being common, has been found in Korea and was shown to be cross-reactive with *Blomia tropicalis* (25). In the present study, the binding of IgE to rBlo t 5 and rBlo t 21 was inhibited by rDer f 5, rDer f 21, rTyr p 5 and rTyr p 21. However, rBlo t 5 and rBlo t 21 were unable to inhibit IgE binding to the other group-5 and -21 allergens from *Dermatophagoides farinae* or *Tyrophagus putrescentiae*. In 2004, Chew and co-workers deposited the mRNA sequences of a Tyr p 5 (accession name, Tyr p 5.01 allergen; accession number, AY800358) and Tyr p 21 (Tyr p 5.03 allergen; AY800360; in the present study, this allergen was named Tyr p 21 due to its high similarity to Blo t 21) in GenBank. However, their IgE reactivity had not yet been reported. The present study provided the first analysis of the IgE reactivities to Tyr p 5 and Tyr p 21 as well as their cross-reactivities with Der f 5, Der f 21, Blo t 5 and Blo t 21. Tyr p 5 exhibited 52.9% identity with Blo t 5 and 55.8% with rTyr p 21 and Blo t 21 at the amino acid level. rTyr p 5 inhibited 77.35% of the IgE reactivity to rBlo t 5, indicating a high degree of cross-reactivity. Furthermore, rTyr p 21 inhibited 43.57% of the IgE reactivity to rBlo t 21. According to Casset *et al* (26), Der p 5 and Der p 21 were not thought to be stable in allergen extracts as they were not detected in commercially available extracts. Indeed, the batch variation of crude extracts can result in difficulties with allergy diagnosis and immunotherapy. These issues, however, may be solved by the use of recombinant allergens, which allow for easy standardization and preparation of allergen mixtures (27). Recombinant allergens may also enable component-resolved diagnoses and personalized allergen-specific immunotherapies.

In conclusion, the present study examined the IgE reactivity and cross-reactivity of recombinant group-5 and group-21 allergens from three mite species *Dermatophagoides farinae, Tyrophagus putrescentiae* and *Blomia tropicalis*. Cross-reactivity to the storage allergens (Tyr p 5, Tyr p 21, Blo t 5 and Blo t 21) was observed when high titers of IgE antibodies, specific for HDM allergens (Der f 5 and Der f 21), were detected. This was particularly the case for Der f 21. It is therefore concluded that mite group-5 and -21 allergens are at least in part responsible for these cross-reactions. However, further studies are required to identify the IgE epitope that is the cause of cross-reactivity. Understanding the cross-reactivity that occurs between allergens from different mite species may be useful for the development of improved component-resolved diagnoses and for enhancing treatment of mite-associated allergies.

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