

ORIGINAL ARTICLE

Serum Specific IgE Reactivity to Recombinant Der f 11 in Asthmatic Children

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ABSTRACT

This study was designed to examine the prevalence of positive serum IgE reactivity to the recombinant group 11 Dermatophagoides farinae allergen (rDer f 11) in asthmatic children in Taiwan. Using immunoblot analysis in a preliminary study of 18 asthmatic children, 13 (72.2%) reacted positively to rDer f 11 and 16 (88.9%) showed positive reactivity to D. farinae extracts. The allergenicity of rDer f 11 was further evaluated with in vivo skin tests and in vitro IgE immunodot assays in 24 mite skin-test-positive asthmatic children. Whereas 17 (70.8%) had positive skin tests to rDer f 11, 18 (75.0%) had positive serum IgE reactivity to rDer f 11. A good coincidence (87.5%) between the immunodot assay and the skin test was confirmed in these asthmatic children. Moreover, the prevalence of serum IgE reactivity to rDer f 11 was further investigated in a large panel of 49 mite skin-test-positive asthmatic children. Again, 38 (77.6%) had positive serum IgE reactivity to rDer f 11 in immunodot assays. Taken together, the positive IgE reactivity to rDer f 11 in immunodot analysis ranged from 75 to 77.6% in two groups of 73 mite skin-test-positive asthmatic children. High incidence of serum IgE antibodies specific for rDer f 11 in the present study suggests that Der f 11 is a novel major allergen of house dust mites.

KEY WORDS: Asthmatic children; IgE antibodies; Recombinant Der f 11.

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INTRODUCTION

Allergens derived from house dust mites have been regarded as an important source of indoor aeroallergens and are the major risk factors in extrinsic asthma, allergic rhinitis, and atopic dermatitis (1–3). House dust mite species of clinical significance, *Dermatophagoides pteronyssinus* and *D. farinae*, are ubiquitous worldwide (4). Immunoblotting analysis using patients' IgE antibodies has detected a large number of allergenic components with a wide range of molecular masses in house dust mites (4). In the past few years several important mite allergens have been serially characterized by molecular cloning of cDNA (5–7). More than 10 protein components from these mite allergens have been identified, and most of them are low-molecular-weight proteins or glycoproteins with diverse structures and biological functions (6,7). Several studies, however, reported that some high-molecular-weight components (approximately 100–177 kDa) in the crude mite extract had significant serum IgE-binding activities (8–10). We have subsequently characterized a novel high-molecular-weight (98 kDa) house dust mite allergen from *D. farinae* extracts by affinity chromatography with a specific monoclonal antibody mAb642 (11). The allergenicity of this affinity-purified native protein, Df642, examined in asthmatic children with positive skin reactions showed high IgE reactivity with more than 80% of 67 serum samples (11). Although high allergenic reactivity of Df642 was demonstrated, it is difficult to prepare a large amount of Df642 for clinical use, since it is a minor component in the crude mite extract.

Conversely, a large amount of recombinant protein could be uncomplicated to prepare by genetic engineering technique. Thus, it may facilitate clinical application. Indeed, the cDNA of Df642 has been recently isolated and characterized (12) and nominated as Der f 11 (13,14). This cDNA insert was then subcloned in the PGEX-2T *Escherichia coli* expression system, and the recombinant Der f 11 (rDer f 11) was expressed as a fusion protein containing glutathione-S-transferase (GST) (12,13). The allergenicity of rDer f 11 was further evaluated by using in vitro IgE-binding assays and in vivo skin tests in this study. The results of this study demonstrated that rDer f 11, a novel recombinant protein of mite allergens, has potential for clinical application in the diagnosis of allergic diseases.

METHODS

Mite Extracts

Dry *D. farinae* obtained from Allergon, Inc. (Engelholm, Sweden) was extracted with 0.01 M cold phosphate

buffered saline (PBS, pH 7.2) at a ratio of 1:100 (g/mL) as previously described (15). The extract was dialyzed and freeze-dried in aliquots for storage at -20°C . The protein levels in mite extracts were analyzed by using a protein assay kit (Bio-Rad, Richmond, CA).

Allergic Sera

Serum samples were obtained from 91 asthmatic children (aged 5–14 years, mean = 9.7 years) attending the Pediatric Allergy Clinics of the Taipei-Veterans General Hospital, Taiwan. Among them, 73 had a history of recurrent wheezing episodes, positive skin tests to the commercial mite extract, and serum IgE reactivity to mite allergens extracted from *D. farinae* or *D. pteronyssinus*. The other 18 patients were similarly diagnosed but without skin tests. Serum samples collected from 13 mite skin-test-negative asthmatic children and from four healthy children were used as controls.

Preparation of Recombinant Der f 11

A BamHI fragment containing the entire Df642 cDNA sequence was produced by polymerase chain reaction (PCR) amplification. The PCR product was subcloned into the expression vector pGEX-2T and expressed as a GST-containing fusion protein in *E. coli* (16). Production and purification of rDer f 11 were performed as previously described (12,13). In brief, bulk culture of rDer f 11-generating *E. coli* was maintained on Luria broth containing 0.1 mg/mL ampicillin to log phase and then induced with 1 mM isopropyl β -D-thiogalactopyranoside. After harvesting, cell lysate was prepared by sonification, and rDer f 11 was purified by glutathione affinity chromatography (Amersham Pharmacia Biotech AB, Uppsala, Sweden). The purified rDer f 11 was dialyzed, freeze-dried, and stored at -20°C until required.

Immunoblotting

The protein components of rDer f 11 and *D. farinae* extracts were separated by sodium dodecyl sulfate-polyacrylamide gel (12.5%) electrophoresis (SDS-PAGE) and transferred electrophoretically onto polyvinylidene fluoride (PVDF) membrane (Millipore Corp., Bedford, MA) with a semidry blotting apparatus as previously described (8,13). The subsequent procedures for immunostaining of the blot were performed as described in the immunodot assay.



Immunodot Assay for Human IgE

To determine the serum IgE reactivity to rDer f 11, the immunodot assay was performed as previously described (12,13). Briefly, rDer f 11, rDer p 2 (0.5 μ g/dot) a kind gift from Dr. C. K. Chua, university of singapore, GST (0.5 μ g/dot), or *D. farinae* extracts (1.5 μ g/dot) were spotted on a PVDF membrane. GST was used as a control. After blocking with 5% skim milk, the blot was washed with TBS containing 0.05% Tween-20 and sequentially incubated with double-diluted samples for 12–16 h and 1/3000 diluted horseradish peroxidase-conjugated rabbit antihuman IgE antibodies (DAKO, Denmark) for 4–6 h at room temperature. The signal of the reaction was induced by using an enhanced chemiluminescent detection reagent (Amersham, Buckinghamshire, England) for 1–2 min and exposed to X-ray films within 2 min. The intensity of the IgE-reacting dot was determined by transmission densitometer (ImageQuaNT Analyzer; Molecular Dynamics Inc., Sunnyvale, CA) with ImageQuaNT software according to the user's guide. The intensity of defined reactive dots from patients was compared with that from healthy controls. The mean of the controls plus 2 SD was used as a cutoff value for the positive reaction.

Skin Test

The skin test was performed as previously described (17). Briefly, 20 μ L of rDer f 11 (100 μ g/mL) was injected intradermally into the forearm skin of asthmatic children, and the reaction was scored 15 min later. The commercial crude mite extract (GREERPick™) was purchased from Greer Lab (Lenoir, NC). The size of a wheal greater than 5 mm was scored as a positive reaction. The test was performed simultaneously with a positive control (aqueous histamine) and a negative control (pyrogen-free saline).

RESULTS

SDS-PAGE and Immunoblot Analysis

A DNA fragment containing BamHI restriction enzyme sites was PCR amplified from Df642 cDNA and subcloned into the pGEX-2T expression vector (12,13). The resulting rDer f 11 expressed as a fusion protein with GST (GST-Der f 11) was estimated on a SDS-PAGE gel (Fig. 1, lane 1). The GST-Der f 11 could be fragmented by reduction with 2-mercapthal ethanol into GST (26 kDa) and rDer f 11 (83 kDa) (Fig. 1, lane 2). The rDer f 11 is very susceptible to degradation and will give rise to a number of smaller fragments ranging from 40 to 83 kDa in size.

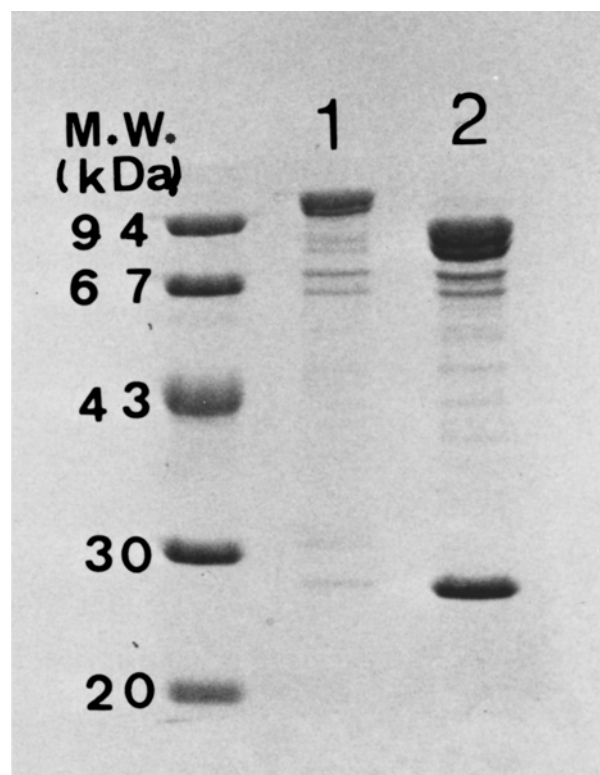


Figure 1. SDS-PAGE analysis of rDer f 11 containing GST (0.5 μ g, lane 1) and 2-ME-treated rDer f 11 (1.5 μ g, lane 2). Proteins were separated under reducing conditions on 12.5% polyacrylamide gel. Separated proteins were visualized by staining with Coomassie brilliant blue. The visible protein bands are about 110 kDa for rDer f 11 containing GST (lane 1), and 83 and 26 kDa for rDer f 11 and GST respectively (lane 2).

Using immunoblot analysis, the reactive components of *D. farinae* extracts show a wide range of different sizes (14.4–130 kDa) (Fig. 2a), but the reactive components of rDer f 11 have a major band (83 kDa) and several degraded fragments (Fig. 2b). The positive IgE reactivity of patient sera was found in 16 of 18 (88.9%) to *D. farinae* extracts and in 13 of 18 (72.2%) to rDer f 11.

Comparison of In Vitro Immunodot Assays and In Vivo Skin Tests

Using an immunodot assay in 24 mite skin-test-positive asthmatic children, their serum IgE reactivity to the rDer f 11 was first compared with that of rDer p 2 and *D. farinae* extracts (Fig. 3). The intensity of the antigen-specific IgE reactivity was evaluated by imaging analysis and is shown in Table 1. The serum IgE reactivity to the



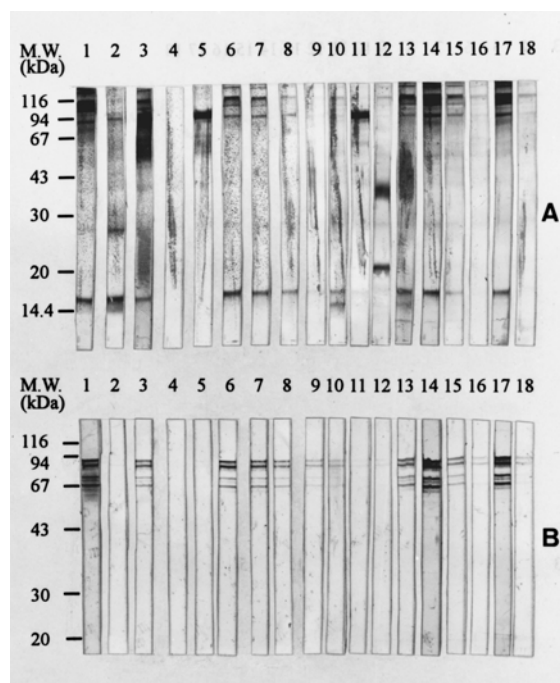


Figure 2. Immunoblot analysis of serum IgE-binding activities to crude *D. farinae* extracts (a) and rDer f 11 (b) in 24 asthmatic children. The positive IgE reactivity to *D. farinae* extracts (a) and to rDer f 11 (b) was approximately 89% (16 of 18) and 72% (13 of 18), respectively.

fusion protein rDer f 11 containing GST, rDer p 2 containing GST, and *D. farinae* extracts was 75% (18/24), 87.5% (21/24), and 79.2% (19/24), respectively. There was no serum IgE reactivity to GST. The intensity of serum IgE reactivity to rDer p 2 was obviously higher than that to rDer f 11 and *D. farinae* extracts (Fig. 3). The skin test with rDer f 11 was further performed in these mite skin-test-positive asthmatic children (Table 2). Approximately 70% (17 of 24) of asthmatic children showed positive skin tests to rDer f 11.

Prevalence of Serum IgE Reactivity to rDer f 11 in Immunodot Analysis

The incidence of serum IgE reactivity to rDer f 11 in the immunodot assay was further examined in a large panel of 49 mite skin-test-positive asthmatic children (Fig. 4). Again, the positive serum IgE reactivity to rDer f 11 was up to 77.6% (38 out of 49). There was no serum IgE reactivity to rDer f 11 in 13 mite skin-test-negative asthmatic children (No. 50–62) and four healthy children

Table 1

Specific Serum IgE Reactivity to rDer f 11, rDer p 2, and D. farinae Extracts in Immunodot Analysis

Patients	Serum IgE Reactivity ^a			
	GST	rDer f 11	rDer p 2	<i>D. farinae</i> Extracts
1	–	++	+++	+
2	–	++	+++	++
3	–	+	+	–
4	–	++	+++	+
5	–	++	+++	+
6	–	++	+++	+
7	–	++	+++	++
8	–	–	++	–
9	–	–	+++	+
10	–	+	++	+
11	–	–	–	+
12	–	–	+	+
13	–	++	+++	+
14	–	++	+++	+++
15	–	–	++	–
16	–	–	–	–
17	–	+++	+++	+++
18	–	++	–	–
19	–	++	+++	++
20	–	+	+++	+
21	–	++	++	+++
22	–	+	++	+
23	–	+	+++	++
24	–	++	+++	++
Positive rate	0%	75%	87.5%	79.2%

^aThe serum IgE reactivity to each mite allergen was quantitatively analyzed by immunodot analysis. The mean of serum IgE-binding activities to GST plus 2SD (115) was used as the negative control. The serum IgE-binding activities to rDer f 11, rDer p 2, and *D. farinae* extracts were scored as follows: – (less than 115), + (116–500), ++ (501–1000), or +++ (higher than 1000).

(No. 63–66). Taken together, the prevalence of positive serum IgE reactivity to rDer f 11 in immunodot analysis was ranged from 75% (18/24) to 77.6% (38/49) in two groups of 73 mite skin-test-positive asthmatic children.

DISCUSSION

Both *D. pteronyssinus* and *D. farinae* have been proved clinically to be the most important aeroallergens in mite species. Several of the major mite allergens have been cloned by genetic engineering techniques, and their



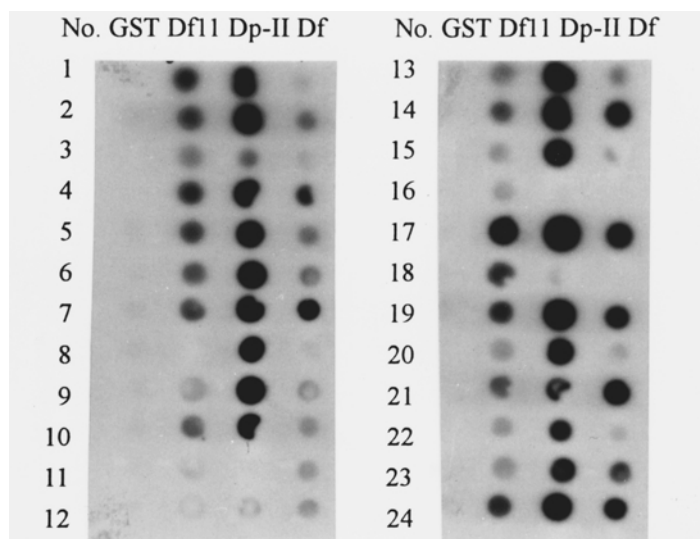


Figure 3. The serum IgE-binding activities of 24 mite skin-test-positive children with rDer f 11 (0.5 μ g/dot) in immunodot analysis. Both rDer p 2 (0.5 μ g/dot) and *D. farinae* extracts (1.5 μ g/dot) were used as positive controls. The mean plus 2 SD (115) of serum IgE-binding activities to GST (0.5 μ g/dot) was used as the negative control. The serum IgE-binding activities to rDer f 11, rDer p 2, and *D. farinae* extracts were scored as follows: – (less than 115), + (116–500), ++ (501–1000), or +++ (higher than 1000).

molecular structures, amino acid sequences, and biochemical properties have been characterized (6,7). Most protein components in crude (*D. farinae*) extracts have been identified as important allergens, and their molecular size ranges from 14 to 60 kDa (6,7). In contrast, we found that one of its protein components with higher molecular weight (approximately 100 kDa) had significant serum IgE-binding activity (8). Indeed, the clinical significance

of high-molecular-weight mite allergens in allergic asthma has been also disclosed by other studies (9,10). Subsequently, a novel 98-kDa house dust mite allergen Der f 11 was isolated from *D. farinae* extracts in our laboratory (11), and its cDNA was then characterized (12). The present report further revealed that the prevalence of IgE-binding activities of mite-allergic sera to the high-molecular-weight components of the *D. farinae* extract and rDer f 11 were

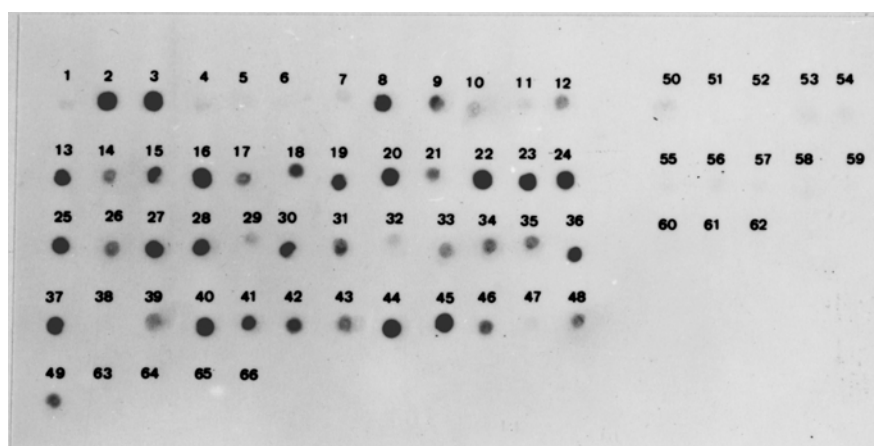


Figure 4. Comparison of serum IgE reactivity to rDer f 11 in immunodot analysis among 49 mite skin-test-positive children (No. 1–49), 13 mite skin-test-negative children (No. 50–62), and 4 healthy children (No. 63–66).

Table 2
Prevalence of Positive Skin Tests with rDer f11 in 24 Asthmatic Children

Patients	Age (Years)	Sex	rDer f11	Crude Mite Extract
1	9	M	+	+
2	11	M	+	+
3	12	M	-	+
4	12	F	++	+
5	13	M	+	+++
6	12	M	+	+
7	8	F	-	+++
8	10	F	-	+
9	13	F	+	+++
10	9	F	+	++
11	10	M	-	+
12	9	M	-	+
13	10	F	+	+++
14	9	F	+	++
15	11	M	-	+
16	9	M	-	+
17	12	F	+++	+++
18	10	F	++	+
19	12	M	+	+
20	13	M	+	++
21	10	M	+	+
22	8	M	+	++
23	13	M	+	+
24	11	M	++	+++
Positive rate ^b			70.8%	100%

^aThe skin test was examined by an intradermal injection of rDer f 11 (2 µg) in 24 mite skin-test-positive asthmatic children. The results of skin tests were scored according to the size of the wheal: < 5 mm (-), 5–6 mm (+), 7–9 mm (++), and ≥10 mm (+++) in diameter.

^bPositive rate = Number of positive subjects/Number of total subjects.

approximately 89% and 72%, respectively, in immunoblot analysis (Figs. 2a and b). In fact, it is too difficult to prepare a large amount of affinity-purified Der f 11 for clinical tests. Our data imply that this problem can be solved through clinical application of rDer f 11. Thus, the present result suggests that a recombinant allergen can be used, instead of the native allergen, in the skin test.

It is well known that group 1 and 2 house dust mite allergens are the major allergens that have approximately 80–90% positive rate of serum IgE reactivity in mite-allergic patients (5,18). We have recently shown that more than 82% of the mite skin-test-positive asthmatic patients have

positive serum IgE reactivity to the affinity-purified native Der f 11 (11). In the present study of two panels of mite skin-test-positive asthmatic children, the serum IgE reactivity to rDer f 11 was also higher than 70% in the immunodot assay (Table 2 and Fig. 4). However, the positive rate of serum IgE reactivity to rDer f 11 was lower than that to native Der f 11. It is probably because the deduced polypeptide of rDer f 11 is truncated at both N- and C-terminuses (12). On the other hand, the result of immunodot assays for rDer f 11 reveals a very high agreement (87.5%; 21 of 24) with that of skin tests (Tables 1 and 2). Usually, a major allergen should have a positive rate up to 50% (19). Since the positive rate of serum IgE reactivity to rDer f 11 in both in vivo and in vitro tests are higher than 70%, the present study confirms that Der f 11 is one of the major mite allergens.

The study of amino acid sequence alignment of Der f 11 has shown that the deduced polypeptide is a paramyosin (12). Paramyosin is a prominent immunogen in parasites and a most promising vaccine candidate for parasitic diseases such as filariasis (20) and schistosoma infection (21). For instance, *Taenia solium* (Cestoda) paramyosin is a strong prominent antigen (antigen B) in human cysticercosis and may have a role as a modulator of the host immune response (22,23). Previous studies have reported that most mite allergens are enzyme (6) except tropomyosin (24). Like the mite tropomyosin, the group 10 mite allergen, paramyosin is a protein associated with muscle. Our finding is not an exception because tropomyosin from *D. farinae* (24) and shrimp (25) has also been identified as an allergen. It has been postulated that the exposure to mite paramyosin may be accompanied by the exposure to other cross-reactive mite proteins with enzymatic activity (14). However, our recent report has shown that rDer f 11 or its fragments can effectively induce specific IgE antibodies in mice (13). In conclusion, the high prevalence of serum IgE reactivity to rDer f 11 in both in vivo and in vitro tests suggests that rDer f 11 is a unique high-molecular-weight mite major allergen.

CONCLUSION

The allergenicity of recombinant high-molecular-weight group 11 *D. farinae* allergen (rDer f 11) was evaluated by using in vivo and in vitro tests in asthmatic children in Taiwan. At first, immunoblot analysis was used in 18 asthmatic children; 13 (72.2%) reacted positively to rDer f 11. Subsequently, the positive IgE reactivity to rDer f 11 ranged from 75 to 77.6% in two groups of 73 mite



skin-test-positive asthmatic children. Among 24 asthmatic children with positive skin tests to the crude mite extract, 17 (70.8%) had positive skin tests to rDer f 11 and 18 (75.0%) had positive serum IgE reactivity to rDer f 11 in immunodot assays. A high coincidence (87.5%) between in vivo and in vitro tests to rDer f 11 was thus confirmed. The present study discloses that Der f 11 is a novel major mite allergen with very high prevalence in asthmatic children.

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REFERENCES

1. Sears, M.R.; Herson, G.P.; Holdaway, M.D.; Hewitt, C.J.; Flannery, E.M.; Silva, P.A. The Relative Risks of Sensitivity to Grass Pollen, House Dust Mite, Cat Dander in the Development of Childhood Asthma. *Clin. Exp. Allergy* **1989**, *19*, 419.
2. Tang, R.B.; Tsai, L.C.; Hwang, H.M.; Hwang, B.; Wu, K.G.; Hung, M.W. The Prevalence of Allergic disease and IgE Antibodies to House Dust Mite Allergen in School Children in Taiwan. *Clin. Exp. Allergy* **1990**, *20*, 33.
3. Marks, G.B. House Dust Mite Exposure as a Risk Factor for Asthma: Benefits of Avoidance. *Allergy* **1998**, *80*, 108.
4. Smith, J.M. Epidemiology and Nature History of Asthma, Allergic Rhinitis and Atopic Dermatitis (Eczema). In *Allergy: Principles and Practice*; Middleton, E., Jr., Reed, C.E., Ellis E.F., Alkinson, N.F., Jr., Yunginger, J.W., Eds.; Mosby: St. Louis, 1983; 771–795.
5. Chua, K.Y.; Stewart, G.A.; Thomas, W.R.; Dilworth, R.J.; Plozza, F.M. Sequence Analysis of cDNA Coding for a Major House Dust Mite Allergen, Der p 1, Homology with Cysteine Protease. *J. Exp. Med.* **1988**, *167*, 175.
6. Thomas, W. Mite Allergens Group I–VII: A Catalogue of Enzymes. *Clin. Exp. Allergy* **1993**, *23*, 350.
7. Stewart, G.A.; Thompson, P.J. The Biochemistry of Common Aeroallergens. *Clin. Exp. Allergy* **1996**, *26*, 1020.
8. Tang, R.B.; Tsai, L.C.; Hung, M.W.; Hwang, B.; Wu, K.G. Detection of House Dust Mite Allergens and Immunoblot Analysis in Asthmatic Children. *J. Asthma* **1988**, *25*, 83.
9. Tovey, E.R.; Baldo, B.A. Comparison by Electrophoresis of IgE-Binding Components in Extracts of House Dust Mite Bodies and Spent Mite Culture. *J. Allergy Clin. Immunol.* **1987**, *79*, 93.
10. Fujikawa, A.; Uchida, K.; Yanagidani, A.; et al. Altered Antigenicity of M-177, A 177 kDa Allergen from House Dust Mite *Dermatophagoides Farinae*, in Stored Extracts. *Clin. Exp. Allergy* **1998**, *28*, 1549.
11. Tsai, L.C.; Chao, P.L.; Shen, H.D.; Tang, R.B.; Chang, T.C.; Chang, Z.N.; Hung, M.W.; Lee, B.L.; Chua, K.Y. Isolation and Characterization of a Novel 98-kD *Dermatophagoides farinae* mite Allergen. *J. Allergy Clin. Immunol.* **1998**, *102*, 295.
12. Tsai, L.C.; Sun, Y.C.; Chao, P.L.; Ng, H.P.; Hung, M.W.; Hsieh, K.H.; Liaw, S.H.; Chua, K.Y. Sequence Analysis and Expression of a cDNA Clone Encoding a 98-kD Allergen in *Dermatophagoides Farinae*. *Clin. Exp. Allergy* **1999**, *29*, 1606.
13. Tsai, L.C.; Sun, Y.C.; Chao, P.L.; Hung, M.W.; Kuo, I.C.; Liaw, S.H.; Chua, K.Y. Protein Sequence Analysis and Mapping of IgE and IgG Epitopes of an Allergenic 98 kDa *Dermatophagoides Farinae* Paramyosin, Der f 11. *Allergy* **2000**, *55*, 141.
14. Thomas, W.R.; Smith, W. Towards Defining the Full Spectrum of Important House Dust Mite Allergens. *Clin. Exp. Allergy* **1999**, *29*, 1583.
15. Tsai, L.C.; Tang, R.B.; Hung, M.W.; Chen, H.M.; Hwang, L.A. Correlation Between Serum IgE and Specific IgE Antibody Titer to House Dust Mite in Children with Asthma. *J. Asthma* **1988**, *25*, 7.
16. Smith, D.B.; Johnson, K.S. Single-Step Purification of Polypeptides Expressed in *E. coli* as Fusion with Glutathione-S-Transferase. *Gene* **1988**, *67*, 31.
17. Tsai, L.C.; Hung, M.W.; Tang, R.B. Changes of Serum-Specific IgE Antibody Titer during Hyposensitization in Mite-Sensitive Asthmatic Children. *J. Asthma* **1990**, *27*, 95.
18. Chua, K.Y.; Doyle, C.R.; Simpson, R.J.; Turner, K.J.; Stewart, G.A.; Thomas, W.R. Isolation of cDNA Coding for the Major Mite Allergen Der p II by IgE Plaque Immunoassay. *Int. Arch. Allergy Appl. Immunol.* **1990**, *91*, 118.
19. Lowenstein, H. Quantitative Immunoelectrophoretic Methods as a Tool for the Analysis and Isolation of Allergens. *Prog. Allergy* **1978**, *25*, 1.
20. Li, B.W.; Chandrasekar, R.; Weil, G.J. Vaccination with Recombinant Filarial Paramyosin Induces Partial Immunity to *Brugia Malai* Infection in Birds. *J. Immunol.* **1993**, *150*, 1881.
21. Pearce, E.J.; James, S.L.; Dalton, J.; Barrall, A.; Rammos, C.; Strand, M.; et al. Immunochemical Characterization and Purification of SM-97, a *Schistosoma Mansoni* Antigen Monospecifically Recognized by Antibodies from Mice Protectively Immunized with a Nonliving Vaccine. *J. Immunol.* **1986**, *11*, 3595.
22. Laclette, J.P.; Landa, A.; Arcos, L.; Willms, K.; Davis, A.E.; Shoemaker, C.B. Paramyosin is the *Schistosoma Mansoni* (Trematoda) Homologue of Antigen B from *Taenia Solium* (Cestoda). *Mol. Biochem. Parasitol.* **1991**, *44*, 287.



23. Landa, A.; Laclette, J.P.; Shoemaker, C.B. cDNA Cloning and Recombinant Expression of Collagen-Binding and Complement Inhibitor Activity of *Taenia Solium* Paramyosin (AgB). *Mol. Biochem. Parasitol.* **1993**, *60*, 343.
24. Aki, T.; Kodama, T.; Fujikawa, A.; Miura, K.; Shigeta, S.; Wada, T.; et al. Immunochemical Characterization of Recombinant and Native Tropomyosin as a new Allergen from the Dust mite, *Dermatophagoides Farinae*. *J. Allergy Clin. Immunol.* **1995**, *96*, 74.
25. Shanti, K.N.; Martin, B.M.; Nagpal, S.; Metcalfe, D.D.; Subba Rao, P.V. Identification of Tropomyosin as the Major Shrimp Allergen and Characterization of its IgE-Binding Epitopes. *J. Immunol.* **1993**, *151*, 5354.



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