

Cloning and Expression of cDNA Coding for a New Allergen from the House Dust Mite, *Dermatophagoides farinae*: Homology with Human Heat Shock Cognate Proteins in the Heat Shock Protein 70 Family¹

Tsunehiro Aki,* Akihiro Fujikawa,* Takeshi Wada,** Toshihiko Jyo,*** Seiko Shigeta,* Yoshikatsu Murooka,* Satoru Oka,* and Kazuhisa Ono*²

*Department of Fermentation Technology, Faculty of Engineering, Hiroshima University, Higashi-Hiroshima, Hiroshima 724; **Development and Administration Section, Fumakilla Ltd., Hiroshima 739-04; and ***Third Department of Internal Medicine, Hiroshima Prefectural Hospital, Hiroshima 734

Received for publication, October 25, 1993

An immunoreactive clone, which shares no homology with the major allergens, *Der f* I and *Der f* II, was isolated by screening of a *Dermatophagoides farinae* cDNA library with rabbit antiserum raised against an extract of the house dust mite and sera from patients with mite allergy. The deduced amino acid sequence of the cDNA of the clone is significantly homologous, up to 65.5%, to the carboxyl-terminal region of the heat shock cognate protein (hsc) 71 in the heat shock protein (hsp) 70 family. A pool of the recombinant protein-specific IgE purified from mite-allergic sera recognized a 67 kDa protein on a blot of ATP-binding components partially purified from the mite body extract, while the antibody did not bind to the major allergens. We conclude that the recombinant protein, one of several important allergens, may be a protein in the heat shock protein 70 family from the house dust mite, *D. farinae*.

Key words: cDNA, *Dermatophagoides farinae*, heat shock protein, mite allergen.

Mite allergens, which appear to be intact or decomposed components of the mite body and feces, present in house dust are major triggers of allergic diseases such as bronchial asthma, perennial rhinitis, and atopic dermatitis in humans (1, 2) and consist of a complex mixture of antigenic components (3, 4).

These antigenic components may arise by modification of the antigenic molecular species by hydrolytic enzymes during the breeding of mites and/or additional modifications occurring during the purification process (5, 6). Thus, the total number of mite allergens identified by immunoelectrophoretic analyses has been reported as 4-26 in extracts of mite bodies and 5-19 in extracts of mite culture (7, 8). However, only the major allergens of *Der* I, II, and III groups in the small quantities of extract available have been extensively studied (9-12) because of difficulty in isolating other components by conventional methods. Thus, important allergens remain to be characterized (4, 5, 7, 8, 13).

¹This study was supported in part by Grants-in-Aid for Scientific Research (Nos. 01560097, 03660088, and 05650802) from the Ministry of Education, Science and Culture of Japan. The nucleotide sequence data reported in this paper will appear in the DDBJ, EMBL, and GenBank Nucleotide Sequence Databases with the accession number D17676.

²To whom correspondence should be addressed.

Abbreviations: Dfb, mite body extract from *Dermatophagoides farinae*; Dff, mite feces extract from *D. farinae*; GST, glutathione S-transferase; hsc, heat shock cognate protein; hsp, heat shock protein; IPTG, isopropyl- β -D-thio-galactopyranoside; mag, cDNA coding for recombinant mite antigen; PBS, phosphate-buffered saline; SSC, 15 mM sodium citrate buffer, pH 7.0, containing 150 mM NaCl; TBS, Tris-buffered saline.

A DNA cloning technique has been recently applied to the major allergens of the *Der* I and II groups to determine their primary sequences (14-17), to identify the IgE-binding regions (18, 19), and to supply recombinant allergens for clinical diagnosis. Tovey *et al.* (20) and Aki *et al.* (21) reported the sequences of two cDNA clones coding for ~14 and 39 kDa fragments of one other important allergen. Many other important allergens remain to be examined. We are, therefore, isolating cDNAs encoding new allergens by monitoring the reactivities of clones to IgE from mite-allergic patients. The amino acid sequence deduced from a newly isolated clone was found to be highly homologous with a protein in the heat shock protein (hsp) 70 family. Hsp is known to be a molecular chaperone expressed in cells stressed by heat, ionizing radiation, cytotoxic agents, or microbial infections, and may be involved in the mechanism of autoimmune diseases (22-24).

Kumar *et al.* reported the isolation of cDNA coding for an IgE-binding protein (65 kDa), which is homologous to hsp 90, from *Aspergillus fumigatus* cDNA library (25). Here, we show that one of the allergenic components from the house dust mite, *Dermatophagoides farinae*, is related to a heat shock cognate protein (hsc 71) in the hsp 70 family.

MATERIALS AND METHODS

Materials—Mites were supplied by Fumakilla (Hiroshima) and were used for preparation of mite body extracts (Dfb) as described previously (26). Restriction endonucleases, T4 DNA ligase, and Klenow fragment were purchased from Takara Shuzo (Kyoto). The major allergens,

Der f I and *Der f II*, were kindly provided by Dr. H. Ueda of National Sagami Hospital (Kanagawa).

Human Sera and Antisera—Forty-one mite-allergic sera were collected from 138 patients with bronchial asthma; the criteria of selection were that the cases had positive skin reaction for mite and house dust extracts and had received no hyposensitization therapy. On dot blot assay, the frequencies of the reactivities of IgE in the selected sera to *Der f I* or *Der f II* (1 µg) were 51% (21/41) or 56% (23/41), respectively. Sera were also used from patients allergic to cedar pollen or *Candida*, but insensitive to mite and house dust extracts.

A rabbit was immunized with 1 mg of Dfb emulsified in Freund's complete adjuvant and boosted with the same antigen every week. Antiserum was collected 10 weeks after the first immunization.

Construction and Screening of *D. farinae* cDNA Library—Seventeen micrograms of poly(A)-mRNA was isolated from an extract prepared by grinding of about 3.3 g of mites in 5.5 M guanidinium isothiocyanate solution using a Fast Track mRNA isolation kit (Invitrogen, San Diego, CA). A cDNA with *EcoRI*-*NotI* adaptors at both ends was synthesized using 5 µg of the poly(A)-mRNA and a cDNA synthesis kit from Pharmacia LKB Biotechnology, Uppsala, Sweden) and was inserted into *EcoRI*-digested pTZ19 (Stratagene, La Jolla, CA) vector in accordance with the manufacturer's directions. Recombinant phages (8×10^7 pfu) prepared by use of an *in vitro* packaging kit (Packag II PLUS, Stratagene) were spread with an overnight culture of *Escherichia coli* Y1090 to form about 100 recombinant plaques per LB medium plate. The plate was incubated at 42°C for 3 h, then overlaid with a nitrocellulose filter (Hybond-C, Amersham International plc, Little Chalfont, U.K.) impregnated with 10 mM IPTG and incubated at 37°C for an additional 3 h. The filter was blocked with 3% skim milk in 10 mM Tris-HCl, pH 7.5, containing 150 mM NaCl, 0.02% Thimerosal, and 0.05% Tween 20 (blocking solution), and then incubated with rabbit anti-Dfb serum diluted 300-fold with the blocking solution. Immunoreactive clones were detected with peroxidase-conjugated goat anti-rabbit IgG (Tago, Burlingame, CA), 3,3'-diaminobenzidine tetrahydrochloride, and NiCl_2 .

RNA Cross-Hybridization—After agarose gel electrophoresis of λ recombinant DNA digested with *NotI*, cDNA fragments coding for immunoreactive proteins were excised from the gel with a GeneClean II kit (Toyobo, Tokyo, Japan). One microgram of the respective cDNA fragments was spotted onto a nylon membrane (Hybond-N, Amersham International plc) and the binding of the fragments to the membrane was completed under UV transillumination for 3 min. DNA probes were labeled with digoxigenin and diluted to a final concentration of 50 ng/ml in hybridization buffer ($5 \times \text{SSC}$ containing 0.1% sodium *N*-lauroylsarcosine, 0.02% SDS, and 1% blocking reagent supplied with the kit). Hybridization was performed at 65°C for 12 h and the membrane was washed twice with $0.2 \times \text{SSC}$ containing 0.05% SDS at 65°C for 15 min. Detection was as directed by the manufacturer (DIG DNA labeling and detection kit, Boehringer Mannheim GmbH, Mannheim, Germany).

Nucleotide Sequencing—The *NotI*-ended cDNAs coding for mite antigens were subcloned into the same site on a pEM6 vector (pBluescript II SK(+)) DNA. Fragments excised from the subcloned plasmid by digestions with various

restriction endonucleases were ligated with suitable sites on M13-derived vectors, mp18 or mp19. Single-stranded DNA was prepared according to the method of Sambrook et al. (27) and was used as the sequencing template. Nucleotide sequencing by the dideoxynucleotide chain termination method (28) was performed using an AutoRead Sequencing kit and A.L.F. DNA sequencer (Pharmacia LKB Biotechnology).

Expression and Purification of Recombinant Mite Allergen—The cDNA insert digested with *NotI* was end-repaired with Klenow fragment in the presence of dCTP and dGTP and was extracted after separation by agarose gel electrophoresis. The fragment was inserted into the *SmaI* site on plasmid pGEX-2T (Pharmacia LKB Biotechnology) with T4 DNA ligase and the resultant plasmid was transformed into *E. coli* JM105. The transformant was grown at 37°C in $2 \times \text{YT}$ medium (1 liter) containing streptomycin (30 µg/ml) and ampicillin (100 µg/ml) to 1.0 A_{600} unit. Cells were harvested 3 h after addition of IPTG to a final concentration of 1 mM and washed once with PBS, pH 7.4. Pelleted cells were thoroughly sonicated in PBS, pH 7.4, containing 2 mM EDTA and 0.1% 2-mercaptoethanol, and centrifuged. The supernatant was applied to 10 ml of 50% (v/v) glutathione Sepharose 4B suspension (Pharmacia LKB Biotechnology). Recombinant protein fused to glutathione S-transferase (GST) was specifically eluted with the buffer containing 5 mM glutathione. Recombinant allergenic component (Mag29) was separated from the fusion protein by digestion with thrombin (1 µg/500 µg fusion protein) in 50 mM Tris-HCl buffer, pH 8.0, containing 150 mM NaCl and 2.5 mM CaCl_2 and further purified on a butyl-Toyopearl column (Tosoh, Tokyo).

Immunoblotting Analysis—Proteins were resolved by SDS-PAGE according to the discontinuous buffer system of Laemmli (29) on a slab gel of 12.5% acrylamide. Standard protein markers used were obtained from Pharmacia LKB Biotechnology. The separated proteins were electrotransferred from the gel to a polyvinylidene difluoride membrane (Immobilon, Millipore Corporation, Bedford, MA) at a constant voltage of 10 V/cm for 30 min in 25 mM Tris-192 mM glycine buffer, pH 8.3, containing 0.1% SDS and 20% methanol. Positions of the standard protein markers were visualized with 0.25% Coomassie Brilliant Blue R-250. The blot was washed with Tris-buffered saline (TBS), pH 7.5, followed by blocking of non-specific binding with TBS containing 3% skim milk, 0.02% Thimerosal, and 0.05% Tween 20 (blocking solution) for 1 h at room temperature. The blot was then incubated for 2 h in the blocking solution containing human serum diluted tenfold. Specific binding of IgE was detected with biotin-conjugated goat anti-human IgE ($\times 500$, The Binding Site, Birmingham Research Park, Birmingham, U.K.), followed by peroxidase-conjugated streptavidin ($\times 500$, Tago).

Identification of the Native Antigen That Shares Antigenic Determinants with the Cloned Protein—A protein blot of recombinant allergenic component (Mag29) was blocked with TBS, pH 8.0, containing 3% skim milk, 0.02% Thimerosal, and 0.05% Tween 20 (blocking buffer) and coated with pooled mite-allergic sera ($\times 10$) preabsorbed with *E. coli* lysates. One portion of the blot was immunostained. From the remaining portion of the blot, the band ($2-3 \text{ cm}^2$) corresponding to about 50 µg of Mag29 was excised and washed four times with the blocking buffer.

Antibody (equivalent to 1 ml of a mite allergic serum) specific for Mag29 was eluted three times with 1 ml of 200 mM glycine-HCl, pH 2.5, and immediately dialyzed against TBS.

ATP-binding proteins were partially purified from Dfb on an ATP-agarose column (Sigma, A9264, St. Louis, MI) by a modification of the method of Mizzen *et al.* (30). Briefly, 200 μ l of ATP-agarose (50%, v/v) suspension equilibrated with 20 mM Tris-acetate buffer, pH 7.4, containing 5 mM MgCl₂, 1% Triton X-100, and 15 mM 2-mercaptoethanol (buffer M) was poured into 10 ml of Dfb (100 mg) in the same buffer and rocked for 1 h. After extensive washing with buffer M, the gel was packed in a column and further washed with buffer M supplemented with 1 M NaCl. ATP-binding components were eluted specifically with 200 μ l of buffer M containing 10 mM ATP, Mg-salt (Sigma), and one-tenth of the eluate was loaded on SDS-PAGE.

After immersing a blot of ATP-binding proteins in the

blocking buffer, the blot was overlaid with the Mag29-specific antibody solution. The membrane was thoroughly washed and incubated successively with biotin-conjugated goat anti-human IgE ($\times 500$, The Binding Site) and peroxidase-labeled streptavidin ($\times 500$, Tago), followed by staining with 3,3'-diaminobenzidine tetrahydrochloride and NiCl₂.

RESULTS

Cloning and Nucleotide Sequencing of Mite Antigen cDNAs—A *D. farinae* cDNA library containing 1.38×10^7 recombinants yielded 273 immunoreactive clones when screened with rabbit antiserum raised against Dfb. The mite antigen cDNA fragments residing in these clones were numbered from *mag1* to *mag273* in the order of their isolation in primary screening. The fragments excised from the vector λ gt11 were cross-hybridized to classify them into at least 9 groups (data not shown). The nucleotide

AAA GAC GAT ATT GAA CGT ATG GTC AAA GAG GCT GAA TCA TAT AAA GAA GAA GAT GAC AAA	60
Lys Asp Asp Ile Glu Arg Met Val Lys Glu Ala Glu Ser Tyr Lys Glu Glu Asp Asp Lys	
CAA CGT GAC CGT ATT GCA GCC AAA AAT TCA TTG GAA GGT TAT GCT TTC CAG ATG AAA GCC	120
Gln Arg Asp Arg Ile Ala Ala Lys Asn Ser Leu Glu Gly Tyr Ala Phe Gln Met Lys Ala	
ACT TTG GAT GAA GAA GCA ATT AAA TCT AAA GTT TCT GAA GAA GAT CGT AAA AAA ATC CTG	180
Thr Leu Asp Glu Glu Ala Ile Lys Ser Lys Val Ser Glu Glu Asp Arg Lys Lys Ile Leu	
GAC AAA GTT GAT GAA GTG CTC AAA TGG TTG GAC GCT AAT GCT TTA GCA GAA AAA GAT GAA	240
Asp Lys Val Asp Glu Val Leu Lys Trp Leu Asp Ala Asn Ala Leu Ala Glu Lys Asp Glu	
TTT GAA CAT CAA CGT AAA GAA TTG GAA AGT GTA TGC AAT CCG ATC ATT ACT AAA TTA TAT	300
Phe Glu His Gln Arg Lys Glu Leu Glu Ser Val Cys Asn Pro Ile Ile Thr Lys Leu Tyr	
CAA CAA GCT GGT GGT GCA GGC GCT GGG GGT ATG CCA GGT GGA TTT CCA GGT GGT TTT CCC	360
Gln Gln Ala Gly Gly Ala Gly Ala Gly Gly Met Pro Gly Gly Phe Pro Gly Gly Phe Pro	
GGT ACT GAT GGT TCT GGT GGT GGT GCA GCC GGT GGT GAT GGT GGT AAA TCT GGG CCC ACC	420
Gly Thr Asp Gly Ser Gly Gly Gly Ala Ala Gly Gly Asp Gly Gly Lys Ser Gly Pro Thr	
ATT GAA GAA GTA GAT TAA TTTCATATCGTAGTCATCTTTTGTCTTCGGCGCCGACCTTCCTTGAAGCATT	480
Ile Glu Glu Val Asp ***	
TTTTTTATCCGAAACAAAATGGTCTGTGTGTGTGTTTGAAAAAAATTCAAATTAATTCCTCCTCATAT	540
ATATGATAATTGCTTTTATTTCTCTCTTTGTAATATCACTATGAGATGATAATTAATTAATTCGAATGTCGTCG	600
TCGTCCGTTATAGTCAAGCATTATTTAAACAAAAAAATTTGTCATTTTATTAATTAATTCATTGTATAATTTAT	660
AATTTCAATTAACTTTGAATTC	720

Mag29	1	25	50
KDDIERMVKAEASYKEEDDKQRDRIAAKNSLEGYAFQMKATLDEEAIKSK			
Hsc71	512	535	560
KEDIERMVQEAKEYKADEKQDKVSSKNSLESYAFNMKATVEDEKLQKG			
Mag29	51	75	100
VSEEDRKKILDKVDEV LKWL DANALAEKDEFEHQRKELESVCNPIITKLY			
Hsc71	562	585	610
INDEKQKILDKCNEIINWLDKNQTAEEFEHQKELEKVCNPIITKLY			
Mag29	101	125	145
QQAGGAGAGGMPGGFPGGFGPTDGSGGGAAGGDGKSGPTIEEVD			
Hsc71	612	635	646
QSAGGM-PGGMPGGFPGG--GAPPSGGAS-----SGPTIEEVD			

Fig. 1. Nucleotide sequence of *mag29* and amino acid homologies between Mag29 and human Hsc 71. (A) The nucleotide sequence of the *mag29* gene in the 5'→3' orientation is indicated. The nucleotide numbers are represented on the right. The deduced amino acid sequence is shown by three letters. The terminal codon (TAA) is marked with asterisks and the sequence confirmed with a protein sequencer (Model 471A, Applied Biosystem Japan, Tokyo) is indicated by the underline. (B) Alignment of the deduced amino acid sequence of *mag29* (upper) and the partial amino acid sequence of human Hsc 71 (lower) is shown by one letter codes. Colons indicate the matched residues and gaps indicated by dashes were introduced to obtain the best alignment.

sequences of representative fragments in each group were determined and the corresponding amino acid sequences were deduced.

Unexpectedly, *mag29* (Fig. 1A) was significantly homologous to the primary amino acid sequence at the carboxyl-terminal region of hsc 71 in the hsp 70 family. This sequence is highly conserved in bacteria, insects, plants, and mammals. *Mag29*, consisting of 752 base pairs, codes for 145 amino acids, with the first amino acid residue corresponding to the 512th residue on the human hsc 71 sequence (Fig. 1B). The highest homology score (65.5%) was obtained in the composite alignment of amino acid sequences between *Mag29* and human heat shock cognate protein of 71 kDa (31). A strikingly high homology score was also observed to hsp 70 of *Drosophila melanogaster* (32) (64.8%), but homology scores to *Mycobacterium prae* (33) (20.7%) and *E. coli* (34) (15.4%) were relatively low (data not shown).

Expression of *mag29*—Expression plasmid carrying *mag29* was constructed on a plasmid pGEX-2T and trans-

formed into *E. coli* JM105. IPTG-Induced cells in 2 liters of culture yielded about 25 mg of purified GST-fused *mag29* product (GST-*Mag29*) after affinity purification on a glutathione Sepharose column (Fig. 2, lane 3). After digestion of the fusion protein with thrombin, *Mag29* was further purified by successive column chromatography on glutathione Sepharose and on butyl-Toyopearl. The sequence of the first seventeen amino acids at the amino terminus of the purified *Mag29* was identical to that deduced from the cDNA clone indicated by the underline in Fig. 1A.

The mobility of the GST-*Mag29* product on SDS-PAGE (Fig. 2, lane 3) corresponds to a molecular size of 43 kDa. On this basis, the molecular size of *Mag29* was calculated to be 17 kDa, but the mobility of the purified *Mag29*, which migrated as a single band on the same gel (Fig. 2, lane 4), corresponds with a size of 20 kDa. On the other hand, the

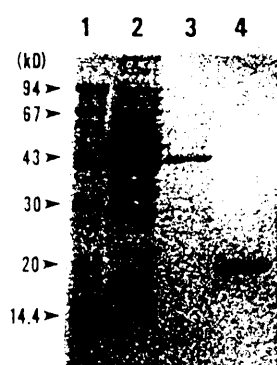


Fig. 2. Purification of the recombinant mite allergen. Proteins denatured in the presence of 2-mercaptoethanol were separated on a 12.5% polyacrylamide gel and visualized by staining with Coomassie Brilliant Blue R-250. Molecular sizes in kilodaltons are represented on the left. Lane 1, molecular weight markers; lane 2, total cell lysate (10 µl) of *E. coli* JM105 transformant carrying *mag29*-inserted pGEX-2T; lane 3, GST-*Mag29* (3 µg) purified on a glutathione Sepharose column; lane 4, purified *Mag29* (3 µg).

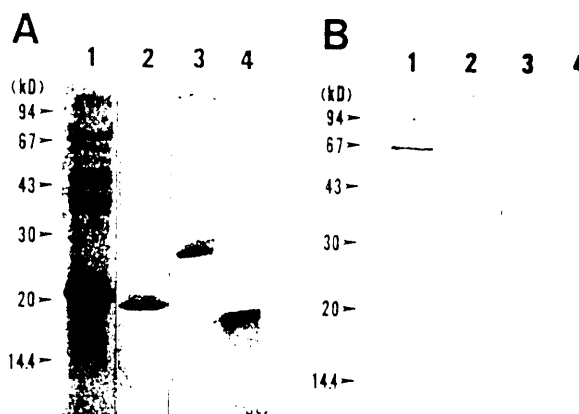


Fig. 4. Identification of the native antigen bound to *Mag29*-specific IgE. Molecular size in kilodaltons is represented on the left. (A) Proteins denatured in the absence of 2-mercaptoethanol were separated on a 12.5% polyacrylamide gel and visualized by staining with Coomassie Brilliant Blue R-250. (B) Protein blot was coated with *Mag29*-specific antibody prepared from mite-allergic sera as described in the text, and specific IgE-binding was detected as in Fig. 3. In each panel, proteins were loaded as follows: lane 1, ATP-binding fraction from 10 mg of Dfb; lane 2, the recombinant *Mag29* (2 µg); lane 3, native *Der f I* (2 µg); lane 4, native *Der f II* (2 µg).

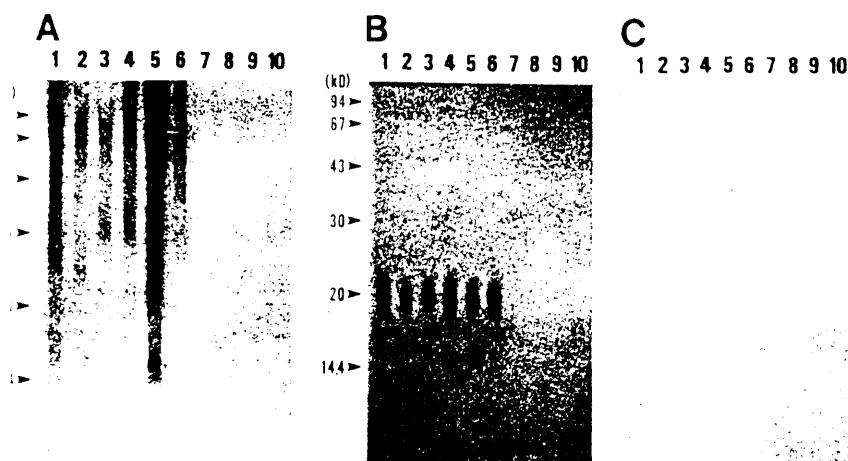


Fig. 3. IgE immunoblotting. Molecular sizes in kilodaltons are represented on the left. Dfb (20 µg, lane, panel A) denatured in the absence of 2-mercaptoethanol and the purified *Mag29* (1 µg, lane, panels B and C) denatured in the presence of 2-mercaptoethanol were separated on 12.5% polyacrylamide gels and transferred to the membranes. Specific IgE-bindings on blots were detected with biotin-conjugated goat anti-human IgE and subsequently peroxidase-conjugated streptavidin. Human sera were used as follows: lanes 1-4, sera from individual patients allergic to mites; lanes 5 and 6, pooled sera from ten mite-allergic patients who are either sensitive (lane 5) or insensitive to *Der f I* or *Der f II* (lane 6); lanes 7 and 8, sera from patients specifically allergic to cedar pollen and *Candida* with high IgE concentration (1,500 and 1,600 IU/ml); lane 9, sera from non-atopic donor; lane 10, no serum. In panel C, human sera were pretreated with Dfb (10 µg/100 µl of diluted serum) before the incubation of a blot.

molecular weight deduced from the cDNA sequence was estimated to be 15,594. The reason for this discrepancy is not known.

Immunoblotting of the Purified Mag29—The specific IgE-binding capacity of the purified Mag29 was compared on immunoblotting analyses with that of Dfb (Fig. 3).

Four sera were selected, on the basis of their IgE-activity to the purified GST-Mag29, from 41 patients allergic to mite and house dust (9.8% frequency, data not shown). Lanes 1-4 in Fig. 3B show positive reactions of purified Mag29 to IgE in the four individual allergic sera, but a wide variation of IgE-binding to Dfb was observed with the four sera (lanes 1-4, Fig. 3A). Sera from 10 mite-allergic patients were combined to give one pool that strongly reacted with the major allergens, *Der f* I and *Der f* II (lane 5, Fig. 3A) and a second pool that was unreactive with them (lane 6, Fig. 3A). The reactivity of the second pool with purified Mag29, however, was slightly greater (lane 6, Fig. 3B) than that of the first pool (lane 5, Fig. 3B). This indicates that IgE epitopes in the purified Mag29 molecule are not related to those in the major allergens. On the other hand, common immunoreactive bands over the range of 60-80 kDa were detected in each lane and the binding activity of specific IgE to the purified Mag29 was completely inhibited in the presence of Dfb (Fig. 3C). Specificity of the IgE immunoreactive bands was confirmed by the negative results in 2 sera from asthmatic patients responding specifically to cedar pollen and *Candida* (lanes 7 and 8, Fig. 3, A and B) and further in 21 sera from atopic patients insensitive to mite and house dust in addition to 2 sera from healthy donors (data not shown).

Identification of the Native Mite Component Corresponding to the Recombinant Protein—Recombinant Mag29-specific IgE in pooled sera of mite-allergic patients, who are sensitive to the major allergens, was eluted from a blot of the GST-Mag29. The IgE antibody reacted specifically with a 67 kDa protein on a blot of partially purified ATP-binding components in Dfb (lane 1, Fig. 4B), but not with *Der f* I or *Der f* II (lane 3 or 4). These results suggest that the 67 kDa protein having ATP-binding activity is the hsp 70 molecule of the house dust mite and shares IgE-binding determinants with the cloned protein.

DISCUSSION

Essential biological roles in the life of the host have been ascribed to some natural protein allergens on the basis of their biochemical properties or primary sequences (16, 35-37). That these proteins always behave as allergens, however, is not yet established. Many immunodominant antigens from a wide variety of bacteria, fungi, and parasites have been identified on the basis of sequence homology as hsps (38). The pleiotropic role of hsps in important processes within the cell has drawn a great deal of attention, because of the universality of the heat shock response, the high degree of conservation of the hsps, and their potential immunogenicity.

The amino acid sequence deduced from the cDNA encoding Mag29 shows significant homologies with amino acid sequences of several *hsc* 71 in the *hsp* 70 family. Hsp 70 proteins have been shown to associate with newly synthesized, unfolded, aberrantly folded, or aberrantly glycosylated proteins, to play a role in antigen presentation,

and to bind to ATP with high affinity (22-24). Recombinant Mag29-specific IgE antibody from mite-allergic patients bound to a 67 kDa protein possessing a high affinity for ATP. The binding frequency of IgE (9.8%) in mite-allergic sera (41 cases) to a blot of Mag29 was significantly higher than that (0%) in other allergic (non-mite-allergic) sera (26 cases), including sera from 3 healthy individuals. Thus, we propose that Mag29 may be a protein in the hsp 70 family from the house dust mite, *D. farinae*, and that the protein may be an important agent in mite allergies.

A question arises concerning the origin of the immunogen for rabbit or sensitized antigen for human, because of the high homology of hsp 70 among species. Rabbit Mag29-specific IgG was confirmed to be an antibody raised against mite body extract, because no positive band was detected on a blot of mite-free medium extract when stained with Mag29-specific antibody. The possibility that hsp 70 in microorganisms attached to the mite body might be an immunogen can be excluded on the basis of the high homology of DNA and the deduced amino acid sequences of *mag*29 with those of mammalian hsp 70 rather than microbial hsp 70. These possibilities may apply to the human case. The possibility that rabbit Mag29-specific antibody might be an autoantibody to hsp 70 induced by the stimulus of antigen injection can also be ruled out since we observed no reactivity of several anti-sea squirt antigens and anti-mite other allergens sera to Mag29.

The allergenic nature of hsp has been the subject of many reports. Giudice *et al.* (39) reported that IgG specific to mycobacterial hsp molecule (65 kDa) could be induced in human or mouse by vaccination with the trivalent vaccine against tetanus, diphtheria, and pertussis. Burnie and Matthews (40) demonstrated IgG, IgM, and IgE antibodies against *Aspergillus* hsp 90 protein in patients with allergic bronchopulmonary aspergillosis. In a review, Yoshikai (41) concluded that antibodies to hsp 60 and 70 families were induced in patients with autoimmune diseases and that the carboxyl-terminal regions of hsp 60 and 70 were required for recognition of the antibodies. Ours is the first report to show that IgE specific to hsp 70 is elicited in patients allergic to mites.

The epitope binding to mite-specific IgE antibody in human serum resides in the translated product of *mag*29 which encodes the carboxyl-terminal region of hsp 70 or *hsc* 71 corresponding to about one-fifth of the entire sequence of the polypeptide chain. The IgE in the clinically assessed mite-allergic sera bound at a rather low frequency (9.8%) to the purified Mag29. However, the true frequency needs to be determined after purification of the complete native or recombinant hsp 70 molecule from *D. farinae*, because other epitopes binding to IgE may be contained in the extrapolated region of the *mag*29 gene. This is currently under investigation.

Also, only weak or no binding of the specific IgE was detected on a blot of *mag*29 product fused to β -galactosidase produced in *E. coli* (data not shown). This phenomenon and the low frequency of IgE-binding to Mag29 indicate that primary screening of the cDNA library utilizing human serum may not be suitable for detection of all clones coding for a relatively minor allergen.

The characterization of other recombinant allergen cDNAs represented in the library and the identification of their native proteins should provide insight into the anti-

genic make-up of *Dermatophagoides farinae*. Information on nucleotide sequences of the genes involved will be useful in the mapping of the allergenic epitopes responsible for mite allergy.

We are very grateful to Prof. Eric E. Smith of the Department of Molecular Biology and Biochemistry, University of Miami School of Medicine for his revising English.

REFERENCES

- Voohorst, M., Spieksma, F.T.M., Varekamp, M.J., Leupen, M.J., & Lyklema, A.W. (1967) *J. Allergy* **39**, 325-339
- Tovey, E.R., Chapman, M.D., & Platts-Mills, T.A.E. (1981) *Nature* **289**, 592-593
- Lind, P., Weeke, B., & Loewenstein, H. (1984) *Allergy* **39**, 259-274
- Baldo, B.A., Ford, S.A., & Tovey, E.R. (1989) *Adv. Biosci.* **74**, 13-31
- Sasa, M., Miyamoto, J., Shinohara, S., Suzuki, H., & Katsuhata, A. (1970) *Jpn. J. Exp. Med.* **40**, 367-382
- Tovey, E.R. & Baldo, B.A. (1987) *J. Allergy Clin. Immunol.* **79**, 93-102
- Tovey, E.R. & Baldo, B.A. (1987) *Electrophoresis* **8**, 452-463
- Arlian, L.G., Bernstein, I.L., Geis, D.P., Vyszynski-Moher, D.L., Gallagher, J.S., & Martin, B. (1987) *J. Allergy Clin. Immunol.* **79**, 457-466
- Stewart, G.A. (1982) *Int. Archs. Allergy Appl. Immunol.* **69**, 224-230
- Lind, P. (1985) *J. Allergy Clin. Immunol.* **76**, 753-761
- Heymann, P.W., Chapman, M.D., Aalberse, R.C., Fox, J.W., & Platts-Mills, T.A.E. (1989) *J. Allergy Clin. Immunol.* **83**, 1055-1067
- Yasueda, H., Mita, H., Yui, Y., & Shida, T. (1989) *Int. Arch. Allergy Appl. Immunol.* **88**, 402-407
- Van der Zee, J.S., Van Swieten, P., Jansen, H.M., & Aalberse, R.C. (1988) *J. Allergy Clin. Immunol.* **81**, 884-896
- Dilworth, R.J., Chua, K.Y., & Thomas, W.R. (1991) *Clin. Exp. Allergy* **21**, 25-32
- Trudinger, M., Chua, K.Y., & Thomas, W.R. (1991) *Clin. Exp. Allergy* **21**, 33-37
- Chua, K.Y., Stewart, G.A., Thomas, W.R., Simpson, R.J., Dilworth, R.J., Plozza, T.M., & Turner, K.J. (1988) *J. Exp. Med.* **167**, 175-182
- Chua, K.Y., Doyle, C.R., Simpson, R.J., Turner, K.J., Stewart, G.A., & Thomas, W.R. (1990) *Int. Arch. Allergy Appl. Immunol.* **91**, 118-123
- Greene, W.K., Cyster, J.G., Chua, K.Y., O'Brien, R.M., & Thomas, W.R. (1991) *J. Immunol.* **147**, 3768-3773
- Chua, K.Y., Greene, W.K., Kehal, P., & Thomas, W.R. (1991) *Clin. Exp. Immunol.* **21**, 161-166
- Tovey, E.R., Cobon, G.S., Johnson, M.C., & Baldo, B.A. (1989) *Adv. Biosci.* **74**, 33-42
- Aki, T., Ono, K., Paik, S.Y., Wada, T., Jyo, T., Shigeta, S., Murooka, Y., & Oka, S. (1994) *Int. Arch. Allergy Immunol.*, in press
- Schlesinger, M.J. (1990) *J. Biol. Chem.* **265**, 12111-12114
- Ang, D., Liberek, K., Skowrya, D., Zylics, M., & Georgopoulos, C. (1991) *J. Biol. Chem.* **266**, 24233-24236
- Winfield, J.B. & Jarjour, W.N. (1991) *Curr. Top. Microbiol. Immunol.* **167**, 161-189
- Kumar, A., Reddy, L.V., Sochanik, A.S., & Kurup, V.P. (1993) *J. Allergy Clin. Immunol.* **91**, 1024-1030
- Yamaguchi, T., Ono, K., Shigeta, S., Wada, T., Otsuka, T., Jyo, T., & Oka, S. (1993) *Jpn. J. Allergol.* **42**, 1754-1763
- Sambrook, J., Fritsch, E.F., & Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, 2nd ed., p. 13.1, Cold Spring Harbor Laboratory Press, New York
- Sanger, F., Nicklen, S., & Coulson, A.R. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 5463-5467
- Laemmli, U.K. (1970) *Nature* **227**, 680-685
- Mizzen, L.A., Chang, C., Garrels, J.I., & Welch, W.J. (1989) *J. Biol. Chem.* **264**, 20664-20675
- Dworniczak, B. & Mirault, M.E. (1987) *Nucleic Acids Res.* **15**, 5181-5197
- Ingoren, T.D., Craig, E.A., & McCarthy, B.J. (1980) *Cell* **21**, 669-679
- Garsia, R.J., Hellqvist, L., Booth, R.J., Radford, A.J., Britton, W.J., Astbury, L., Trent, R.J., & Basten, A. (1989) *Infect. Immun.* **57**, 204-212
- Bardwell, J.C.A. & Craig, E.A. (1984) *Proc. Natl. Acad. Sci. USA* **81**, 848-852
- Breiteneder, H., Pettenburger, K., Bito, A., Valenta, R., Kraft, D., Rumpold, H., Scheiner, O., & Breitenbach, M. (1989) *EMBO J.* **8**, 1935-1938
- Izumi, H., Adachi, T., Fujii, N., Matsuda, T., Nakamura, R., Tanaka, K., Urisu, A., & Kurosawa, Y. (1992) *FEBS Lett.* **302**, 213-216
- Valenta, R., Duchene, M., Perrenburger, K., Sillaber, C., Valent, P., Bettelheim, P., Breitenbach, M., Rumpold, H., Kraft, D., & Scheiner, O. (1991) *Science* **253**, 557-560
- Young, D.B., Mehlert, A., & Smith, D.F. (1990) *Stress Proteins in Biology and Medicine* p. 131, Cold Spring Harbor Laboratory, New York
- Giudice, G.D., Gervais, A., Costantino, P., Wyler, C.A., Tougne, C., De Graeff-Meeder, E.R., Van Embden, J., Van den Zee, R., Nencioni, L., Rappuoli, R., Suter, S., & Lambert, P.H. (1993) *J. Immunol.* **150**, 2025-2032
- Burnie, J.P. & Matthews, R.C. (1991) *J. Clin. Microbiol.* **29**, 2099-2106
- Yoshikai, Y. (1993) *Kagaku to Seibutsu* (in Japanese) **31**, 163-169