

HIGH-MOLECULAR-SIZE MITE ALLERGENS WITH HIGH CARBOHYDRATE
CONTENT IN MITE EXTRACT CONTAINING MITE FECES OF
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A series of mite allergens with high carbohydrate content was fractionated from mite extract containing mite feces by ultrafiltration, and successive chromatography on Ultrogel AcA 54, Sepharose CL-6B, and DEAE Toyopearl 650S. The final fractions isolated were termed HM1-2-Eff, -0.1A, -0.1B, -0.3A, -0.3B, and -1M according to the eluted salt concentration. These six fractions were stained weakly with Coomassie Brilliant Blue but strongly with periodate-Schiff's reagent on SDS-PAGE. Their molecular weight were estimated to be 150-155 KD on Sepharose CL-6B. All of these six fractions provoked strong erythema in skin of mite-sensitive and asthmatic patients. HM1-2-Eff, -0.1A, -0.1B and -0.3B reacted with 73% of mite-sensitive patients' IgE by the nitrocellulose dot blotting. One ng/ml of 4 fractions (HM1-2-0.1A, 0.3A, 0.3B and -1M) released histamine from mite allergic patient's blood cells. HM1-2-0.3A induced proliferation of T cell from immunized mouse by mite extract containing mite feces (Dff).

Key words: *Dermatophagoides* — glycoprotein — high molecular size allergen — mite allergen — T cell activation

INTRODUCTION

Sensitization to allergens derived from the house dust mites of the genus *Dermatophagoides* is closely related with symptoms of asthma and rhinitis¹⁾⁻⁵⁾ in many parts of the world. Many studies have dealt with the characterization of the allergens they produced⁴⁾⁻¹¹⁾. In all products found up to today, *Der f* 1 and *Der f* 2 from *D.*

farinae or their equivalents from *D. pteronyssinus* have been recognized to show reactivity at least in 80% of mite-allergic patients' sera¹²⁾⁻¹⁴⁾. *Der f* 1 allergen in mite feces which is cysteine protease¹⁵⁾⁻¹⁷⁾ is heat-labile glycoprotein having molecular weight 24 K. The third group of *Dermatophagoides* allergens, *Der f* 3 and *Der p* 3, are trypsin-like protease corresponding to which has 34K on SDS-PAGE¹⁸⁾¹⁹⁾. A new set of *Dermatophagoides* allergens named *Der f* 6 and *Der p* 6 was found and purified by Yasueda et al. which have chymotrypsin like activities. They showed reactivity in 50% of mite-allergic patients²⁰⁾. *Der p* 7 produced by an IgE-binding clone was reported to be a predicted MW of 22,177D. The antigen reacted with sera from 37% of allergic children and showed reactivity in 53% in skin test of allergic patients²¹⁾. Stewart et al. have reported high molecular weight allergen Dpt 4 having Mr of

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Abbreviations: **ABTS** 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonate); **CFA** complete Freund's adjuvant; **DAB** 3,3-diaminobenzidine tetrahydrochloride; **Dfb** mite body extract; **Dff** mite extract containing mite feces; **H** house dust extract; **HM** high-molecular-size fraction of Dff; **M** mite extract; **PAS** periodate-Schiff reagent; **TBS** 10 mM Tris buffer, pH 8.0, containing 0.9% NaCl; **TBST** TBS containing 0.05% Tween 20

274 KD which was a highly dispersed lipoprotein²²⁾²³⁾. Haida et al. also found a high molecular weight mite antigen having molecular weight of 80 KD²⁴⁾.

We report a series of new mite allergens containing high carbohydrate moiety with estimated molecular weight of about 150 KD on gel filtration. These allergens are characteristics of weak and strong staining for protein and carbohydrate, respectively. They reacted strongly with IgE and IgG in asthmatic patients sensitive to mite and stimulated mouse lymph node cells.

MATERIALS AND METHODS

Materials

A growth spent medium, in which *Dermatophagoides farinae* was bred at 25°C and 75% humidity for 30 days, was supplied by Fumakilla Ltd. (Hiroshima, Japan). House dust extract (H), mite extract (M) and cedar pollen extract were obtained from Torii & Co. (Tokyo, Japan) and used for *in vivo* skin and dot blot tests. *Der f* 1, *Der f* 2, rabbit anti-*Der f* 1 and anti-*Der f* 2 were kindly given from Dr. H. Yasueda in the National Sagamihara Hospital. Sera were obtained from mite-sensitive patients with asthma and cedar pollen-allergic patients, who came to either the Third Department of Internal Medicine, Hiroshima Prefectural Hospital, or Otsuka Clinic (Hiroshima) as regular outpatients, and stored at -20°C until use.

Mice

Six to eight week old C3H/HeN (H-2k) mice were purchased from the Yoshitomi Pharmaceutical Co. (Ohita, Japan).

Immunization

C3H/HeN (H-2k) was immunized with 50 µg of antigen (Dff) in 200 µl of CFA in food pad on day 0 and boosted with same amount of antigen on day 14.

Proliferative assay of lymph node cells

Lymph node cells were suspended in Hanks balanced salt solution for 10 min and centrifuged at 600 g for 10 min. The lymph node cells were cultured at 2×10^5 cells in 200 µl of Eagle-Hanks amino acid medium in 96 well flat bottomed tissue culture plates (Falcon Dickinson, CA, USA). Antigen was added at varying concentrations and the cells were cultured for 6 days in 5% CO₂ at 37°C²⁵⁾. Cells were labeled with 0.5 µCi of [³H]-thymidine (specific activity of 6.0 µCi/mmol; Amersham, England) during last 18 hours and were harvested and washed with water twice. Thymidine incorporation was assessed using liquid scintillation spectrometer²⁶⁾²⁷⁾.

Preparation of mite body and mite feces extracts

Mites and feces were separated according to essentially the same method of Sasa et al.²⁸⁾, mentioned precedingly²⁹⁾. A whole mite culture (4.4 kg) was suspended into saturated NaCl solution and centrifuged at 1400 ×g for 30 min. Floating mites were collected by filtration and filtrate were dialyzed against water, freeze-dried, and used as the crude *D. farinae* extract containing feces (Dff). The filtered mites were ground with quartz in 10 mM phosphate buffered saline, followed by centrifugation. The supernatant was dialyzed, lyophilized and used as the mite body extract (Dfb). From 4.4 kg of a whole mite culture, 0.56 g of Dfb and 26.8 g of Dff were obtained.

Ultrafiltration

Dff was applied to UF-20CS ultrafiltration apparatus (Tosoh, Shin-Nanyo, Japan) equipped with 20 sheets (11 × 14 cm) of UF-20CS-10PS (10⁴Mr-cut-off) filters (Tosoh) to eliminate filtrable fraction. After the solution retained in the apparatus was dialyzed and lyophilized as high-molecular-size fraction (HM) of Dff, 24.1 g of HM was obtained.

Purification of HM

HM (1 g) was applied to a 4.4 × 100 cm

column of Ultrogel AcA 54 (IBF, France) and eluted with 0.9% NaCl at a flow rate of 120 ml/h. The fraction was monitored with an absorbance at 280 nm, amount of saccharide and protein by using the modified phenol sulfuric acid method³⁰⁾ and Lowry method³¹⁾, respectively, and for immunoreactivity using rabbit anti-Dff, human IgG and IgE in pooled sera from mite-asthmatic patients. The allergenically active fractions were pooled, lyophilized and applied to Sepharose CL-6B (4.4 × 70 cm, Pharmacia Biotechnology, Uppsala, Sweden) under the same monitoring condition.

The allergen fractionated on gel filtration was applied to DEAE-Toyopearl 650S column (2.6 × 10 cm, Toso). After equilibration with 10 mM phosphate buffer at pH 6.0, 200 mg of sample was applied to the column. The column was washed with 3-fold of the column volume of same buffer and the allergen was eluted stepwise with 10 mM phosphate buffer containing 0.1 M, 0.3 M, and 1 M NaCl.

Estimation of the molecular weight

The molecular weight was estimated by the gel filtration of Sepharose CL-6B comparing the standard proteins (ferritin, 440 KD; catalase, 232 KD; aldolase, 158 KD; BSA, 67KD; ovalbumin, 43 KD) obtained by Pharmacia (Sweden).

Immunoreactivity of purified allergen

Reactivities of chromatographically separated fractions to rabbit anti-Dff, human IgG and IgE in mite allergic patients' sera were measured by ELISA³²⁾. A polystyrene microplate was used as antigen carrier. Rabbit anti-Dff, mite-allergic patients' sera as for IgG and IgE were diluted 2,000-fold, 300-fold and 10-fold as first antibody, respectively. For second antibody, horseradish peroxidase-conjugated goat anti-rabbit IgG (4,000-fold, Zymed CA, USA) or IgE (5,000-fold, Zymed) was used. ABTS (0.1%, Boehringer Mannheim, GFR) solution containing 0.03% hydrogen peroxide was used as a substrate. Ten mM NaN₃ was added as a stopping reagent. The reacted wells were measured

at 415 nm with a microplate reader (MPR A4i, Tosoh).

Skin test

Saline (0.02 ml) containing 0.0005% of the fraction on the purification steps was intracutaneously injected. House dust (0.005%) and mite extracts (0.1%) were simultaneously used as standard antigens. Scores of 0, 0.5, 1, 2 and 3 were assigned for erythema of 10, 10 to 20, 20 to 30, 30 to 40 and larger than 40 mm in diameter at 15 min, respectively. The allergenicity of a sample was expressed by the ratio of the total scores obtained in 7-14 patients with the sample to those obtained with commercial house dust and mite allergens in the same patients³³⁾. For example, when scores of 1, 3, 0.5, 2, 0 and 0.5 were obtained by a sample, score of 0.5, 3, 0, 0, 0 and 0 by house dust allergen (H) and 0.5, 2, 1, 1, 1, 1 and 1 by mite allergen (M) in patients a, b, c, d, e, f and g, respectively, the ratio of allergenicity was shown the ratio of a sample to H and M and was calculated as $(1+3+0.5+2+0+0+0.5)/[(0.5+3+0+3+0+0+0), (0.5+2+1+1+1+1+1)]=7/(6.5, 7.5)$ in 7 patients (n=7).

Dot Immunoblotting

One μ l of three different concentrations of 0.001%, 0.01% and 0.1% of samples was blotted on nitrocellulose film (Hybond-C Nitrocellulose, 0.4 μ , Amersham International plc, UK) and soaked with 3% skim milk solution at room temperature for 1 hour. Then, for IgG staining, a mite-allergic patient's serum diluted 100-fold with 3% skim milk was poured on the sheet which was incubated for 12 hours at room temperature. For IgE staining, 10-fold diluted serum was used. After thorough washing with TBST (10 mM Tris-HCl saline, pH 7.5 containing 0.05% tween 20), the sheet was soaked with goat anti-human IgG conjugated with horseradish peroxidase (500-fold, Zymed) or goat anti-human IgE conjugated with biotin (300-fold, KPL, Gaithersburg, MD, USA) at room temperature for 1 hour. This sheet was immersed in

0.2% peroxidase-conjugated streptavidin (300-fold, Tago Inc., Buringame, CA, USA) at room temperature for 1 hour. After thorough washing with TBST, immunoreactive spots were visualized by incubation with 0.04% DAB (Dojindo, Kumamoto, Japan) in TB solution containing 0.01% hydrogen peroxide. After the sheet was dried, the color spots were compared with control BSA, symbols +, ++, and +++ indicate the visualized concentration of antigen at 0.1%, 0.01% and 0.001%, respectively.

SDS-polyacrylamide gel electrophoresis and immunoblotting

SDS-PAGE was carried out in 7.5% polyacrylamide gel at pH 7.5³⁴⁾. The gel was stained with both Coomassie Brilliant Blue³⁵⁾ and periodate-Schiff's reagents (PAS)³⁶⁾. After electrophoresis, a gel was blotted to an Immobilon membrane (Millipore, MA, USA) at 1 mA/cm² for 90 min³⁷⁾ using the apparatus of Atto Electrophoresis Model AE-6678 (Atto, Tokyo, Japan). After blotting, the membrane was soaked overnight in 10 fold diluted patient's serum in 3% skim milk. As the second antibody, peroxidase-conjugated goat anti-rabbit IgG (5000-fold, Capel, PA, USA) or biotin conjugated goat anti-human IgE (300-fold) were used. Peroxidase conjugated streptavidin (300-fold) was covered over goat anti-human IgE. The staining reagent was same as the case of blot test.

Histamine release

Histamine release assay of mite-allergic patients' blood cells was carried out by the method of Yamaguchi et al.³⁸⁾. Washed peripheral blood cells (200 μ l) from mite-allergic patients were challenged with a series of an appropriately diluted antigen solution for 30 min at 37°C. After 10 min centrifugation, the supernatant was deproteinized with one tenth volumes of 60% perchloric acid and the released histamine was analyzed an HPLC system LC-6A liquid chromatograph (Simadzu) equipped with a Simadzu RF-535 fluorescence monitor.

Table 1 Allergenicity of the fractions on purification steps

Fraction	Allergenicity in skin* HM/(HD, M)	Number of patients (Number)
HM-1	15/(5.5, 6)	10
HM-2	9/(5.5, 6)	10
HM-3	9/(5.5, 6)	10
HM-4	8/(5.5, 6)	10
HM1-1	5/(11, 14.5)	12
HM1-2	12/(11, 14.5)	12
HM1-3	11/(11, 14.5)	12
HM1-2-EFF	14/(20, 21)	14
HM1-2-0.1A	18.5/(20, 21)	14
HM1-2-0.1B	16.5/(20, 21)	14
HM1-2-0.3A	7/(6.5, 7.5)	7
HM1-2-0.3B	6/(6.5, 7.5)	7
HM1-2-1M	4.5/(6.5, 7.5)	7

*The allergenicity of a sample was expressed by the ratio of total skin test score obtained in 7 to 14 patients by the sample to that obtained by control allergen of house dust and mite in the same patient as total skin test score for the sample/(Total score for house dust, Total score mite). See Materials and Methods.

Total histamine was determined by perchloric acid lysis of cell aliquote.

Composition analysis

Amino acids were analyzed with an amino acid analyzer (Hitach 835, Hitachi, Japan) after hydrolysis of a sample in 6 M HCl at 110°C for 24 h.

RESULTS

Purification of high-molecular-size allergen (HM) derived from mite extraxt containing mite feces

HM antigen (24.1 g) was chromatographed on a column of Ultrogel AcA 54 and the eluate was divided into four fractions (HM1 to 4, Fig. 1A). The yields of HM1, HM2, HM3, and HM4 were 5.2 g, 5.9 g, 6.0 g and 0.2 g, respectively. HM1, which reacted most strongly with rabbit anti-Dff serum, human IgG, and IgE among the 4 fractions (Fig. 1a), and skin test (Table 1), was purified again on a column of Sepharose CL-6B.

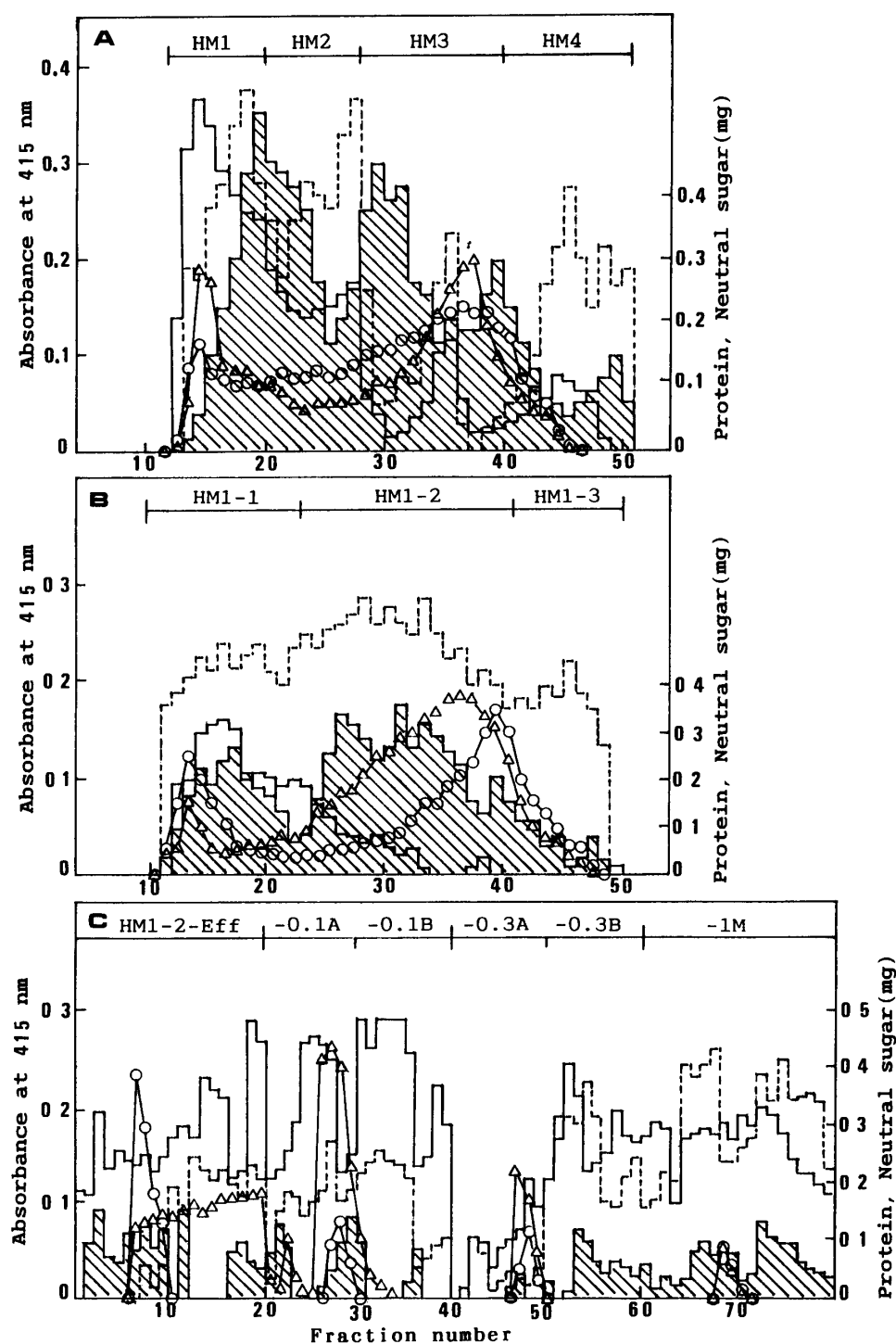


Fig. 1A. Gel chromatography of HM antigen on Ultrogel AcA 54. HM (1 g/30 ml) was applied to a column (4.4×70 cm) and eluted with 0.9% NaCl at a flow rate of 2 ml/min and collected 32-ml each. The samples were monitored by UV 280 nm (solid line), protein ($-\bigcirc-$) and sugar ($-\triangle-$). The antigen activities were monitored using rabbit anti-Dff (solid histogram), pooled mite-allergic patients' IgG (oblique line histogram) and IgE (dotted histogram) by ELISA.

Fig. 1B. Gel chromatography of HM1 antigen on Sepharose CL-6B. The column size, elution condition and all symbols are the same as above.

Fig. 1C. Ion exchange chromatography of HM1-2 by DEAE-Toyopearl 650S. HM1-2 (200 mg/200 ml) were applied onto a column of DEAE-Toyopearl 650S equilibrated with 10 mM phosphate buffer, pH 6.0, previously. The column was eluted with 10 mM phosphate buffer containing 0.1 M NaCl, 0.3 M NaCl and 1 M NaCl stepwisely. Symbols are the same as above.

Table 2 Physical and chemical properties of HM1-2 fractions

Component	HM1-2-Eff	-0.1A	-0.1B	-0.3A	-0.3B	-1M
Sugar ($\mu\text{g}/\text{mg}$)	770	687	143	590	75	95
Protein ($\mu\text{g}/\text{mg}$)	153	166	180	193	140	156
Amino acid (nmol/mg)						
Asp	75	56	89	90	66	72
Thr	303	593	150	103	60	65
Ser	102	99	97	70	101	105
Glu	142	183	122	94	103	95
Pro	129	119	50	40	29	42
Gly	93	51	104	107	139	159
Ala	63	44	68	67	54	66
Val	47	27	44	48	35	8
Cys	4	4	3	3	9	—
Met	5	4	1	6	1	—
Ile	31	16	41	42	29	34
Leu	52	23	44	54	41	45
Tyr	17	8	23	23	16	17
Phe	20	10	25	36	21	22
His	41	38	39	39	24	28
Lys	12	10	20	19	16	22
Arg	18	7	17	18	16	18
Amino sugar (nmol/mg)						
Glucosamine	50	24	17	416	10	4
Galactosamine	378	776	69	160	85	311

The eluate divided into 4 fractions (HM1-1, -2, -3, and -4, Fig. 1B). HM1-2 which had the highest reactivities to the patients' IgG and IgE, and provoked the biggest erythema, was dialysed and lyophilized. HM1-2 (2.7 g) was further fractionated on a DEAE-Toyopearl 650S column and divided into HM1-2-Eff, -0.1A, -0.1B, -0.3A, -0.3B, and -1M as shown in Fig. 1C. After dialysis and lyophilization, 507 mg, 267 mg, 294 mg, 534 mg, 374 mg, and 160 mg of HM1-2-Eff, -0.1A, -0.1B, -0.3A, -0.3B, and -1M were finally obtained from 4.4 kg of a starting growth spent medium, respectively.

Chemical and physical properties

Table 2 shows the neutral sugar and protein contents, amino acid and amino sugar compositions of the six fractions. HM1-2-Eff, and -0.1A had high amount of neutral sugar, galactosamine and threonine. On the other hand, HM1-3A had high glucosamine and neutral sugar content and considerable amount of

threonine, glycine and galactosamine. Recovery of amino acids and carbohydrates of HM1-2-0.1B, -0.3B and -1M were low. The six fractions had the similar molecular weight from 150 KD to 155 KD estimated on Sepharose CL-6B (Table 2, bottom).

SDS-PAGE of these fractions is shown in Fig. 2A. These fractions were stained with Coomassie Brilliant Blue weakly but with PAS strongly. Almost all of the samples were retained near the top of the separating gel.

After six fractions were blotted to Immobilon film (Fig. 2B), HM1-2-Eff, -0.1A, -0.1B and -0.3B fractions reacted with mite allergic patient's IgE. HM1-2-1M were not stained as same as the culture medium as control.

Allergenicity

The allergenicities expressed as scores in skin test are listed in Table 1. Each fraction elicited strongly specific allergenicity to mite-sensitive patients.

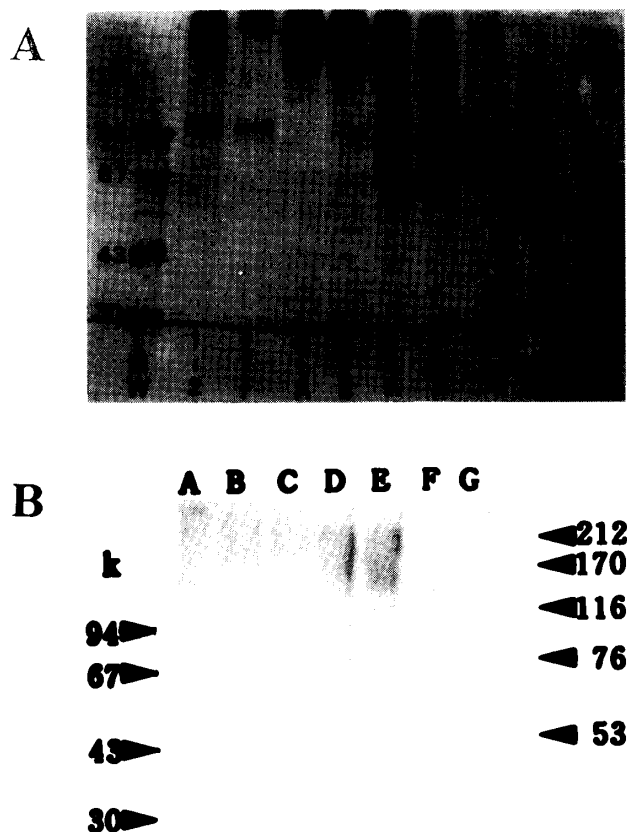


Fig. 2. SDS-PAGE of HM1-2 fractions on 7.5% acrylamide gel. The sample (100 μ g-each) was applied on the gel. After Coomassie Brilliant Blue R250 staining, the gel was stained with PAS (Fig. 2A). Fig. 2B shows IgE-immunostaining of HM1-2 fractions that were blotted on the Imobilon membrane after SDS-PAGE; lane A, HM1-2-Eff; lane B, HM1-2-0.1A; lane C, HM1-2-0.1B; lane D, HM1-2-0.3B; lane F, HM1-2-1M; and lane G, culture medium without mites.

These fractions were challenged with washed blood cells from mite-allergic patients to release histamine (Fig. 3). The highest activity to release histamine was obtained at the challenged antigen concentration of 1 ng/ml in HM1-2-0.1A, -0.3A, -0.3B and -1M. In the 4 fractions, HM1-2-0.3A and -0.3B made 90 and 94% histamine released from the mite allergic patient's blood cells, respectively. HM1-2-Eff and -0.1B released 62 and 90% histamine at the antigen concentration of 10 ng/ml, respectively.

The allergenicities of these fractions were investigated on the basis of the reactivities of

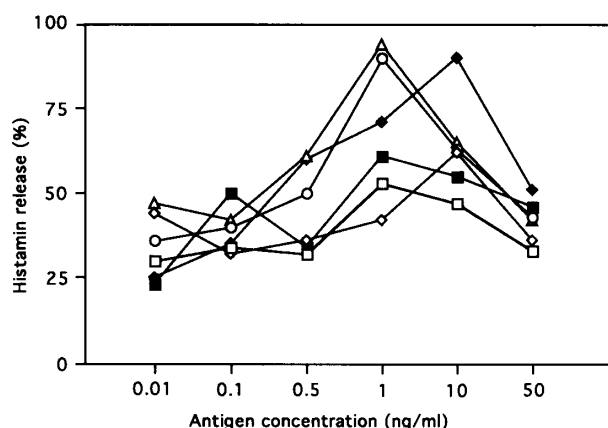


Fig. 3. Histamine release from washed blood cells of a mite-allergic patient challenged of 6 fractions in HM1-2 at varying antigen concentrations.

The value is expressed as % of total histamine. Symbols $\blacklozenge$, $\square$, $\diamond$, $\circ$, $\triangle$, $\blacksquare$ are shown for HM1-2-EFF, -0.1A, -0.1B, -0.3A, -0.3B, and -0.1M, respectively.

Total histamine content was 42 ng/ml.

IgE against 15 of mite allergic patients' sera comparing with *Der f* 1 and *Der f* 2 (Table 3, patient A to O). HM1-2-Eff, -0.1A, -0.1B, and -0.3B reacted with 11 of 15 patients' IgE (73%). These frequencies of reactivity were between that of *Der f* 1 (14 of 15 patients, 93%) and *Der f* 2 (9 of 15 patients, 60%). HM1-2-0.3A bound to 47% but -1M was reacted weakly. Culture medium with no breeding of mites were fractionated by Ultrogel AcA 54 and void volume fraction were used to examine reactivity to IgE of 10 mite-allergic patients as control (right column). Three of 10 patients were reacted weakly but the others did not. Cedar-pollen extract did not react with mite allergic patients' sera used. On the other hand, a cedar pollen allergic patient's serum, P, reacted strongly with HM1-2-0.1A, -0.3B, and *Der f* 2. The other serum Q did not react with mite allergens except for slightly reactive HM1-2-0.1A. For mite-allergic patients' IgG, these six fractions reacted strongly with all sera from 18 mite-allergic patients (data not shown).

Response of lymph node cells to HM antigen

The response of mice lymph node cells from C3H/HeN (H-2^k) to HM antigen fractions were

Table 3 Reaction of six fractions to IgE in mite and cedar pollen allergic patients' sera on nitrocellulose film

Patient	HM1-2-EFF	-0.1A	-0.1B	-0.3A	-0.3B	-1M	Der f 1	Der f 2	Cedar*	Culture**
A	+	+	+	-	+	-	##	##	-	-
B	##	+	##	+	+	+	##	##	-	+
C	+	##	+	-	+	-	##	+	-	+
D	##	##	##	##	##	+	##	+	-	+
E	##	+	##	+	##	+	##	##	-	-
F	+	##	##	##	+	-	##	+	-	-
G	+	##	##	+	+	-	##	+	-	-
H	+	+	+	+	+	-	+	-	-	-
I	##	##	+	+	##	-	+	-	-	-
J	+	##	+	-	+	-	+	+	-	-
K	+	+	+	-	+	-	+	-	-	-
L	-	-	-	-	-	-	+	-	-	-
M	-	-	-	-	-	-	+	-	-	-
N	-	-	-	-	-	-	+	-	-	-
O	-	-	-	-	-	-	-	+	-	-
P	+	##	+	+	##	-	+	##	##	-
Q	-	+	-	-	-	-	-	-	##	-

*Cedar pollen extract, **High molecular weight fraction (MW>60K) of culture medium

The cases reacted with allergen concentration of 0.1, 0.01 and 0.001% indicate +, ++ and ##, respectively. Case A to O were mite allergic patients of asthma, and case P and Q were cedar pollen allergic patients.

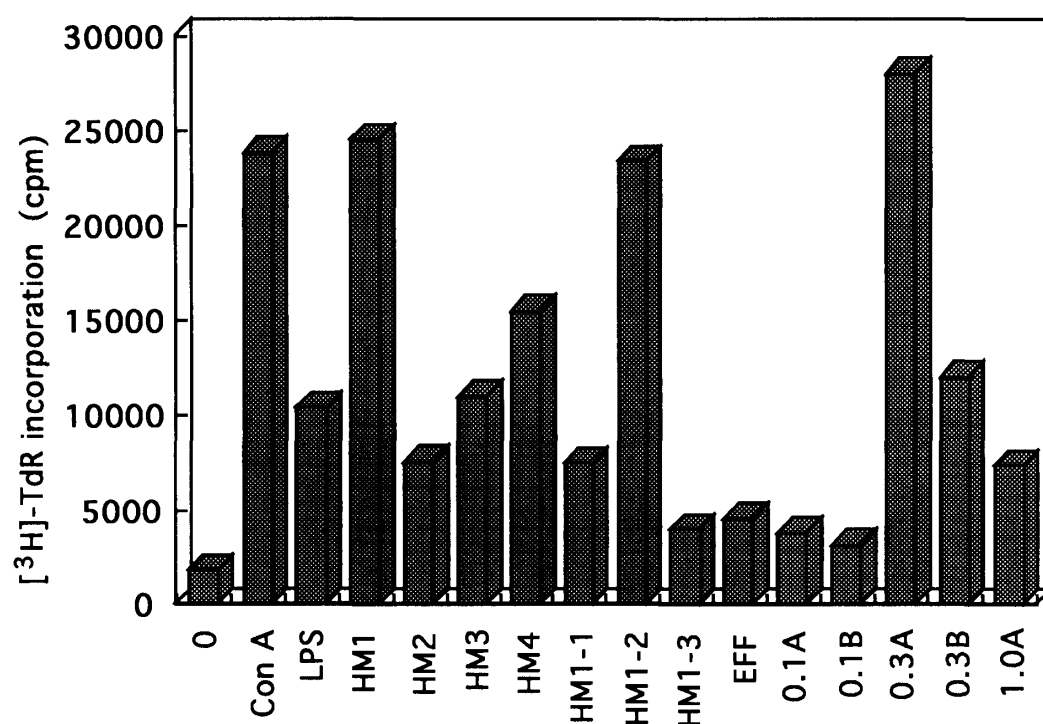


Fig. 4. Proliferative response of Dff-immunized murine lymph node cells to HM fractions.

Mice were immunized by Dff and the lymph node cells were cultured for 6 days with HM fractions of 10 μ g/ml. The proliferative response was measured by the incorporation of [³H]-TdR. (see text)

examined. The lymph node from the immunized mouse with Dff was removed at 14 days after boost and examined the proliferation of lymph node cells in response to the antigens. As shown in Fig. 4, HM1-2-0.3A made proliferation of Dff immunized mouse lymph node cells stimulated.

DISCUSSION

Six carbohydrate-rich fractions were isolated from the mite extract containing mite feces of *Dermatophagoides farinae*. These were termed HM1-2-Eff, -0.1A, -0.1B, -0.3A, -0.3B and -1M. Sums of carbohydrate and protein contents of HM1-2-Eff, -0.1A and 0.3A are almost 100% but those of -0.1B, 0.3B and -1M were poor about 49, 23, and 31%, respectively. It is considered that other materials, for example lipid, phosphate, or sulphate, might be contained. Stewart et al. found high molecular weight allergen with an apparent molecular weight of 274 KD and pI 4.5 which name is Dpt 4 was a lipo-protein. The six fractions were stained strongly with carbohydrate staining reagent, PAS, but weakly with Coomassie Brilliant Blue. We considered these fractions are glycoproteins. Chemical analyses of HM1-2-Eff and -0.1A which had high amount of N-acetylgalactosamine and threonine suggest that these contains O-glycosidic sugar linkages. On the other hand, HM1-2-0.3A had not only N-acetylgalactosamine but also N-acetylglucosamine. It suggests that the fraction contain N-glycosidic chain linkages and/or bigger O-glycosidic linkages. Strong erythema produced in mite-sensitive patients' skin and reactions with IgE in the patients' sera which did not react with cedar pollen allergen and did little with high molecular fraction (>60 KD) of culture medium without mites suggested that these six fractions are allergens existed in mite extract containing mite feces.

Haida et al²⁴⁾ found a high-molecular-size mite antigen named Me4 having 80 KD by

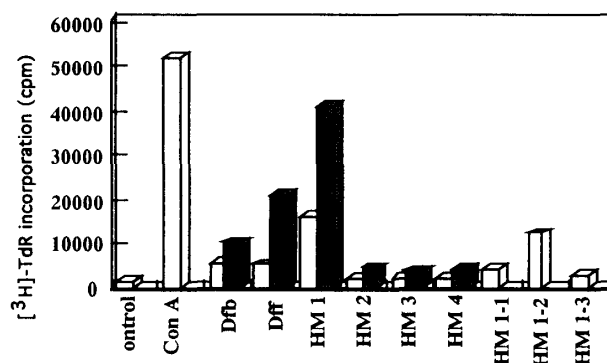


Fig. 5. HM fractions stimulated the proliferative response of non-immunized murine lymph node cells.

After non-immunized murine lymph node cells were cultured for 6 days with 10 μ g/ml ($\square$), 100 μ g/ml ($\blacksquare$) of HM fractions. The proliferation response was measured by the incorporation of [^{3} H]-TdR. (see text)

radioimmunoblotting after SDS-PAGE. As our six fractions are quite different from Dpt4 and Me4, we consider that these are new types of allergens.

These new six allergens reacted with all mite-allergic patients' IgG measured (18 patients) and four (HM1-2-Eff, -0.1A, -0.1B and -0.3B) of the six allergens reacted with 73% of mite-sensitive asthmatic patients' IgE. This is comparable to that of major allergens.

The six fractions, especially HM1-2-0.3A, gave rise to the proliferation of lymph node cells. Lymph node cells contain 55 to 65% of T cells. T cells must be responded to those allergens. On the purification steps, HM1 and HM1-2 were proliferated T cells strongly but HM2 and HM3, weakly, though HM3 included *Der f* 1 and *Der f* 2 (data not shown), and HM2 contained *Der f* 6. Moreover we found HM1 and HM1-2 stimulated the proliferation of the non-immunized mouse lymph node cells (Fig. 5). These antigens must be contained mitogenic activities. Relationship between mitogenic activity and antigenicity remains to be solved.

Among six fractions, HM1-2-0.3A and -0.3B had the strongest activity to release histamine. In the skin and blot tests, HM1-2-Eff, -0.1A, -0.1B and 0.3B widely reacted with sera from mite-sensitive asthma patients. On the other

hand, HM1-2-0.3A has the strongest ability to proliferate T cell. It is considered that the specificities for mite allergens in mite allergic patients were quite diversified one another.

These allergens which are glycoproteins containing high percentage of carbohydrate with MW of 150 to 155 KD would become the good material for hyposensitization therapy like the sea squirt antigens, Ei-M and Gi-rep which are good therapeutic agents containing high carbohydrate moieties with MW of 22.8 and 106 KD, respectively³⁹⁾⁴⁰⁾. We will investigate antigenicity, induction of anaphylaxis, and chronic toxicity of six fractions of HM1-2.

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ダニ排泄物培地中に存在する糖含量の高い新規ダニ高分子アレルゲン

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ダニ喘息患者血清に対する反応を用いて、ダニ培養中に含まれる抗原成分を検索した結果、従来言われている主要なダニアレルゲンよりもさらに高分子量の画分に、患者血清に特異的に強い反応性を示す成分の存在を確認し、それを2回のゲル濾過及びイオン交換クロマトグラフィによって6画分に分画した。この6画分の分子量は約150～155KDであった。さらに泳動後のイムノステイニングではHM1-2-1Mを除く5画分がダニアレルギー患者IgEと強く反応した。これらの画分は1ng～10ng/mlの濃度で患者の洗浄血球から最大ヒスタミン遊離率を示した。さらにHM1-2-0.3A画分はTセル活性化能を有していた。これら6画分はさらに皮内反応テスト、ニトロセルロース膜上でのダニ喘息患者血清IgE反応性等から、ダニ喘息患者の多数例が特異IgE抗体を持つ新しいアレルゲンであると考えられた。