

LOW-MOLECULAR-SIZE ALLERGENS, LM-1s, IN FECES EXTRACT OF
DERMATOPHAGOIDES FARINAE WHICH ELICIT HISTAMINE
RELEASE FROM WASHED BLOOD CELLS OF PATIENTS
WITH BRONCHIAL ASTHMA

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A significant activity to elicit histamine release was found in an ultrafilterable (Mr-cut-off <10 kD) fraction of a mite feces extract from a spent mite medium. The activity was divided into two fractions (LM1 and LM2) on an Ultrogel AcA 54 column when monitored by histamine release assay using washed peripheral blood cells from mite-allergic patients. The larger-molecular-size antigen, LM1 was further separated into three allergenic fractions by consecutive chromatography on Sephadex G-50, GM-DEAE, and TSKgel ODS-120T. All the purified allergens, LM1s (LM1a, LM1b, and LM1c), which produced positive reactions in skin tests on allergic patients, were glycoproteins (molecular weight: 8 kD on SDS-PAGE; 12.5 kD on Gel filtration) with different carbohydrate contents and pI values ranging from 4 to 5 on an IEF plate. LM1s were cross-reactive with anti-*Der f* II but not with anti-*Der f* I. The reaction of LM1c to anti-*Der f* II serum was completely inhibited with the other antigens, while the reactions of LM1a and LM1b to the serum were partially inhibited with LM1c or the other antigens, respectively.

Key words: bronchial asthma — *Dermatophagoides farinae* — histamine release assay — house dust mite — low-molecular-size mite allergen

INTRODUCTION

Intact or decomposed components of mite body and feces from two closely related mite species, *Dermatophagoides farinae* and *D. pteronyssinus*, are well known as potent allergens causing allergic diseases such as bronchial asthma, perennial rhinitis, and atopic dermatitis^{1)–3)}.

Commercially available allergic extracts for clinical diagnosis, therapy, and research of associated diseases, and mite body or feces extracts from whole mite cultures are intricate mixtures of

allergenic and non-allergenic components. Attention has been mainly focused on group (Der f I and Der p I), group II (Der f II and Der p II), and group III (Der f III) as the major allergens (molecular weights: 24, 14, and 29 kD, respectively^{4)–12)}), because of difficulty in isolating other components by conventional methods. However, of the 30 or more IgE-binding components detected by immunoelectrophoretic analyses using allergic sera, at least six have been reported to be possibly important allergens on the basis of their frequency and intensity of IgE-binding on blots^{13)–15)}. Furthermore, low-molecular-size antigens are reported to have haptenic properties^{16)–18)}. Much information on these allergens still remains to be accumulated.

Low-molecular-size allergens of ragweed pollen, timothy pollen, and green pea are characterized as components having haptenic properties^{19)–21)}. Especially in sea squirt allergy, low-molecular-size

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Abbreviations: Dfb mite body extract; Dff mite feces extract; ELISA enzyme-linked immunosorbent assay

antigen (~10 k) has been found to be a component capable of inducing asthmatic attack in allergic patients²¹⁾. Using a system for histamine release assay²²⁾, we have found new low-molecular-size allergenic components which pass through 10⁴ Mr-cutoff filters in *Dermatophagoides farinae* feces (Dff) extracts. The antigens can be distinguished from the previously-reported low molecular components^{6)(16)–18)} by their molecular weights, isoelectric points, and the reactions they cause in skin tests.

This report describes the isolation, physico-chemical properties, and allergenicity of purified low-molecular-size mite allergens (LM1s) in relation to the major allergens (*Der f* I and *Der f* II).

MATERIALS AND METHODS

Materials

A growth spent medium of *Dermatophagoides farinae* was supplied by Fumakilla Ltd. (Hiroshima, Japan). The culture medium used in this study was 30% of wheat bran in a powdered animal food for laboratory mice and rats MF (Oriental Yeast Co., Ltd., Tokyo, Japan). House dust extract and mite extract solutions (Torii & Co., Tokyo, Japan) were used for *in vivo* skin tests. *Der f* I, *Der f* II, rabbit anti-*Der f* I serum, and rabbit anti-*Der f* II were kindly supplied by Dr. Hiroshi Yasueda, National Sagamihara Hospital, Kanagawa, Japan. Ultrafiltration membranes (UF-20CS-10PS, 10⁴ Mr-cutoff) and TSKgel ODS-120T were purchased from Tosoh Co. (Tokyo, Japan). GM-DEAE was kindly provided by Kurita Kogyo (Kanagawa, Japan). Ultrogel AcA 54 (IBF, France) and Sephadex G-50 and G-25 (Pharmacia LKB Biotechnology, Uppsala, Sweden) were used for gel filtration chromatography. Ampholine PAG plates were purchased from Pharmacia LKB Biotechnology.

Subjects

Blood samples from 48 patients with bronchial asthma who came to the Hospital, Katsutani Clinic, Otsuka Clinic, or Tsuboi Clinic as regular out-patients, were used in this study. In 48 patients, 19 are allergic to both house dust and mite, 6 are allergic to only house dust, 4 are allergic to

only mite and 19 are allergic to the other, respectively. In the 19 patients who are allergic to the other allergy 8 have high titer of IgE (<500 U).

Purification of low-molecular-size allergen, LM-1s

Mite feces extract (Dff, lot 1) was prepared from a portion of a pooled supernatant after successive extraction of a spent mite medium with saturated sodium chloride solution and phosphate buffered saline according to the method described in the preceding paper²²⁾. Mite body extract (Dfb, lot 1) was prepared by grinding mites in 10 mM phosphate buffered saline. The rest of the pooled supernatant was ultrafiltrated on an UF-20CS-10PS (10⁴ Mr-cutoff) membrane filter to separate low-molecular-size substances. The retentate and the filtrate were lyophilized and referred to as high-molecular-size antigen (HM) and low-molecular-size antigen (LM) fractions, respectively.

Composition analysis

Amino acids were analyzed with an amino acid analyzer (Hitachi 835, Hitachi, Japan) after hydrolysis of a sample in 6 M HCl containing 0.3% phenol and 0.2% 2-mercaptoethanol at 110°C for 24 hours. The total amount of neutral sugars was determined by a modification of the phenol-sulfuric acid method²³⁾.

Histamine release assay

Histamine released by the stimulus of the washed peripheral blood cells of allergic or healthy subjects with a series of an appropriately diluted antigen (128 ng–0.2 mg/ml) solution. Total histamine was determined by perchloric acid lysis of cell aliquots (complete). Spontaneous release (blank) was determined for cell incubated in the buffer. Histamine spontaneously released from the cells were determined by the method described previously²²⁾. Results are expressed as the mean percentage of histamine released for duplicated assays where percentage histamine release = (sample-blank)/(complete-blank) × 100. The sensitivity (μg of allergen/ml of blood) of a patient to an allergen was defined as the allergen concentration necessary to release 50% of the maximum amount of histamine released.

Intracutaneous test

The allergenic activity was evaluated with *in vivo* skin tests on allergic patients by injecting intracutaneously 20 μ l of solution containing LM (5 μ g/ml), LM1s (5 μ g/ml), house dust extract (1:1,000) or mite extract (1:10,000) dissolved in 0.9% NaCl and 0.5% phenol. When erythema, appearing after 15 min, was 0 to 9, 10 to 19, 20 to 29, 30 to 39 or >40 mm in mean diameter, score of -, $\pm$, ++ or +++ was assigned, respectively.

ELISA inhibition test

ELISA plates (Falcon 3912, Becton Dickinson and Co., Oxnard, CA) coated with 50 μ l containing 10 μ g/ml (dry weight) of LM1a, LM1b, or LM1c diluted 0.1 M sodium bicarbonate buffer, pH 9.5, were incubated for 2 h at 37°C. The washed wells were blocked with 200 μ l of 50 mM Tris-HCl buffer, pH 8.0, containing 1% bovine serum albumin, 0.02% Thimerosal, and 0.05% Tween 20 (blocking solution). Rabbit anti-*Der f* II sera diluted 5,000-fold with the blocking solution were pre-incubated for 3 hours in the presence or absence of a series of properly diluted LM1a, LM1b, LM1c, or cytochrome c solutions (34 or 102 ng-25 μ g/well) and added to the antigen-coated wells. The washed wells were overlaid with horseradish peroxidase-conjugated goat anti-rabbit IgG (Cappel, Organon Teknika Corp. West Chester, PA) ($\times 10,000$) at 37°C for 1 hour. Absorbance at 415 nm was measured after 10 min of the enzyme reaction in 10 mM citrate buffer, pH 4.2, containing 1 mg/ml 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) and 0.03% H₂O₂.

RESULTS

Statistical discussion of whether allergens in LM fraction are derived from mite body and feces

LM (Lot 1, 18.8 g) and HM (Lot 1, 34.3 g) fractions were obtained from an extract of whole mite culture (1 kg). The reactivities of patients with bronchial asthma to LM fraction and house dust extract or mite extract are listed in tables 1A and 1B, respectively. House dust-sensitive patients with score of +, ++ and +++ responded to LM at frequencies of 2/8, 1/5, and 9/12 (Table 1A) while mite-sensitive case with score of +, ++, and +++ reacted to LM at frequencies of 2/17, 3/6, and 3/4, respectively (Table 1B). The culture medium used in this study produced no positive reaction in skin tests on allergic patients (13 cases) with house dust and mite except two cases (data not shown). No statistical independence of the records of skin tests to LM fraction with those to house dust or mite suggested that the LM fraction included allergens derived from mite body and feces ($p < 0.001$).

The LM fraction seemed to contain a significant amount of allergens when compared with HM and Dfb (Lot 1, 1.6 g/kg of a whole mite culture) in view of the sensitivities of patients and yields from a whole mite culture (Table 2), although the sensitivities of patients to the allergens varied among individuals.

Purification of LM

LM fraction (500 mg) was applied to a column of Ultrogel AcA 54 to remove high molecular impurities. The procedure was repeated until 18.8 g of

Table 1 A correlation between LM and house dust or mite judged by skin tests

A		LM(skin test)					B		LM(skin test)				
		-	$\pm$	+	++	+++			-	$\pm$	+	++	+++
HD (skin test)	-	13	1	1	0	0	Mite (skin test)	-	2	1	0	0	0
	$\pm$	1	2	1	0	0		$\pm$	10	1	2	1	0
	+	6	0	2	0	0		+	10	2	2	2	1
	++	4	0	0	1	0		++	3	0	1	2	0
	+++	2	1	1	5	3		+++	1	0	0	1	2

A & B, skin tests of patients with bronchial asthma allergic to mite or house dust (cases).

Table 2 Diverse sensitivities of patients to various allergen fractions

Case	Skin test		Dfb (1.60g) ^a		HM (34.3g) ^a		LM (18.8g) ^a		LM1 (0.84g) ^a		LM2 (5.07g) ^a		Total histamine ^b
	HD	M	1	2	1	2	1	2	1	2	1	2	
1	++	—	0.07	100	0.16	73	0.09	100	2.4	100	2.5	100	26.1
2	++	++	6.6	18	4.8	16	4.6	17	10.6	15	13.4	6	62.9
3	++	++	0.0068	79	0.018	89	0.17	83	nd		nd		24.8
4	+	—	0.04	70	0.068	79	0.32	65	nd		nd		26.4
5	++	—	nd		nd		0.34	69	1.4	84	1.0	60	24.1
6	+	++	nd		nd		0.9	96	1.5	100	1.2	72	11.4

a, yields from a whole mite culture (1kg).

b, ng/ml.

1, Sensitivity (the sensitivity (μ g of allergen/ml of blood) of patient to an allergen was defined as the allergen concentration corresponding to 50% of the maximum histamine release amount).

2, Maximum histamine release.

nd, not determined.

LM fraction was completed. Fractionation of LM on the column resulted in two fractions having an ability to elicit histamine release (Fig. 1A), and these fractions were referred to as LM1 (842 mg) and LM2 (5.07 g), in their order of elution. Neither fraction reacted with IgE in sera from healthy donors or with IgE from sera with a high IgE titer which is not mite-specific. On the other hand, binding activities to an anti-Dff serum and were largely distributed over the positions eluted faster than LM1 fraction when the anti-Dff serum was used a probe, but no activity to release histamine was detected at all in fraction numbers 18–35.

To remove low molecular impurities, LM1 fraction (50 mg/3 ml of 0.9% NaCl) was chromatographed seventeen times on a column of Sepadex G-50 (1.5×100 cm) with 0.9% NaCl at a flow rate of 30 ml/hour into 5-ml fractions. The allergenic fraction (252 mg) was eluted around the position of cytochrome c (data not shown) when monitored by histamine release assay on three patients allergic to mite.

The allergenic fraction (10 mg/100 μ l of 20 mM Tris-HCl, pH 8.0) was put twenty-five times on a column of GM-DEAE (0.78×10 cm) with 20 mM Tris-HCl, pH 8.0, at a flow rate of 1 ml/min into 2-ml fractions and eluted with increasing concentrations of NaCl (0–500 mM) in the same buffer, and the fractions were assayed for histamine-releasing activity (data not shown). Although different intensities of response were observed among the three patients, fraction numbers 7–10,

in which activity was detected in at least one case, were pooled and lyophilized (50.4 mg).

The allergenic fraction (10 mg) was chromatographed on a column of TSKgel ODS-120T (Fig. 1B). After a fifth repetition of column chromatography, a few peaks of allergenic activity were identified by histamine release assay on 6 patients allergic to mite. Fraction numbers 15, 17, and 22–25 were pooled, in which relatively high frequencies (2/6, 3/6, and 6/6, respectively) of response were observed with different intensities among the 6 patients. They were referred to as LM1a, LM1b, and LM1c in their order of elution. Purified LM1a (2.2 mg), LM1b (2.6 mg), and LM1c (2.5 mg) were finally obtained from an extract of a whole mite culture (1 kg).

Physicochemical properties of the purified LM1a, LM1b, and LM1c

The molecular weights of the purified LM1s (LM1a, LM1b, and LM1c) were all 12.5 kD on a Sephadex G-25 column. Electrophoretic analyses of LM1a, LM1b, and LM1c showed them all to be all heterogeneous glycoproteins having isoelectric points at pH 4–5 on an Ampholine PAG plate (data not shown), containing 185, 220, 258 mg of saccharide/g, respectively, and consisting of a single polypeptide chain, by SDS-PAGE, with molecular weights of 8 kD (Fig. 2). The inconsistency of the molecular weight might reflect some anomalous electrophoretic and hydrodynamic behavior of the purified LM1s as acidic glycoproteins.

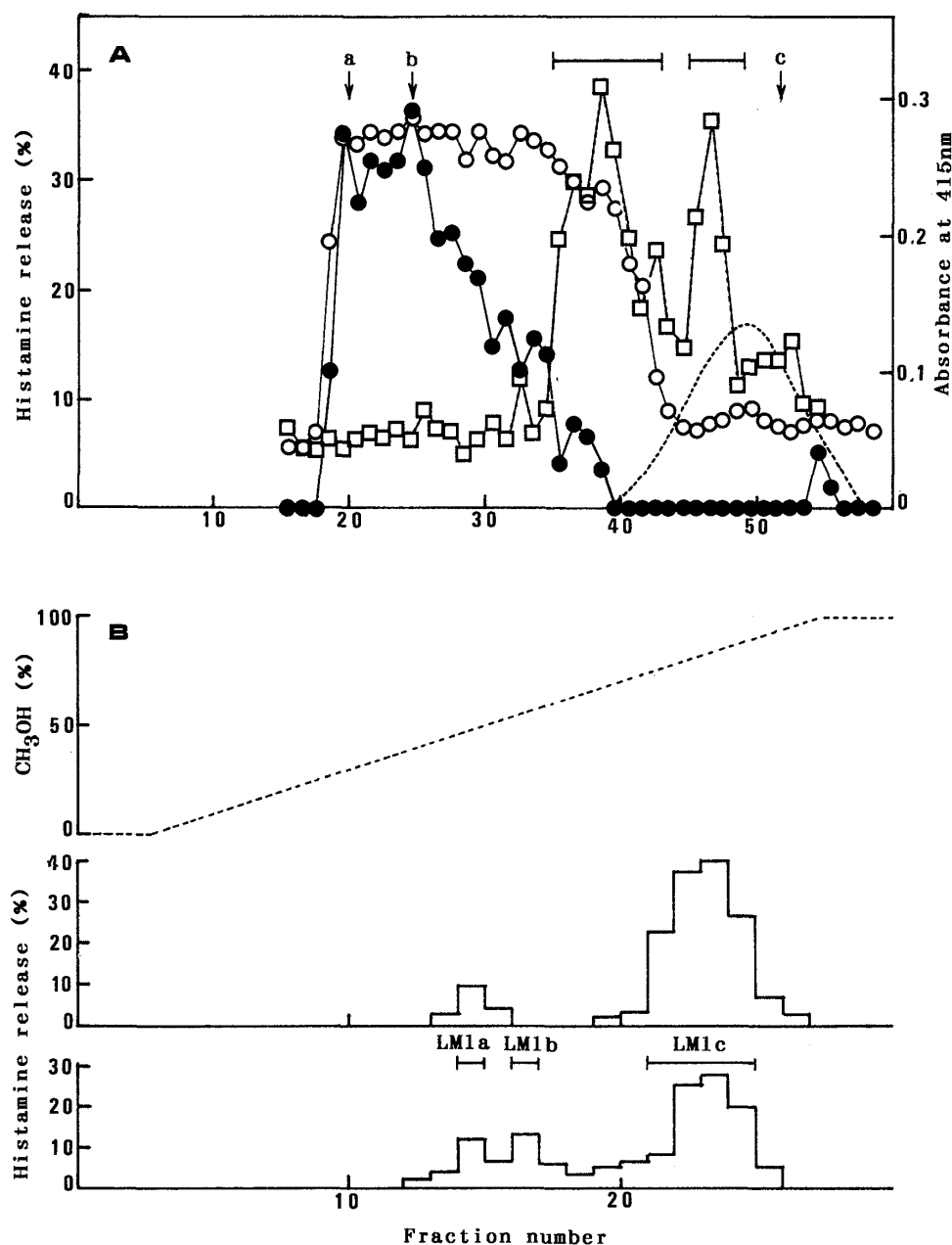


Fig. 1. Typical chromatographic fractionation of LM and LM1. (A) LM (500 mg/30 ml of 0.9% NaCl) was chromatographed on a column of Ultrogel AcA 54 (4.4×100 cm) with 0.9% NaCl at a flow rate of 120 ml/hour into 32-ml fractions. Fractions indicated with bar were pooled as LM1 and LM2 fractions having *in vitro* allergenic activity. a, b, and c with arrow indicated elution positions of molecular calibration markers (Blue Dextran 2000 (2,000,000), bovine serum albumin (67,000) and K_2CrO_4 (194)), respectively. (○) reactivity to anti-Dff serum: (●) reactivity to anti-Dfb serum: (□), histamine release assay: (---), absorbance at 280 nm. (B) LM1 (10 mg/200 ml of 0.1% TFA) was chromatographed on a column of TSKgel ODS-120T (0.46×25 cm) with 0.1% TFA at a flow rate of 1 ml/min into 4-ml fractions and eluted with increasing concentrations of methanol (0–100%) containing 0.1% TFA. *In vitro* allergenic activity was examined by measuring histamine release assay.

The amino acid composition of the purified LM1s is given in Table 3. None of the purified LM1s contained amino saccharides.

Immunochemical properties of the purified LM1s

The *in vivo* and *in vitro* allergenic activities of the purified LM1s were examined by skin test

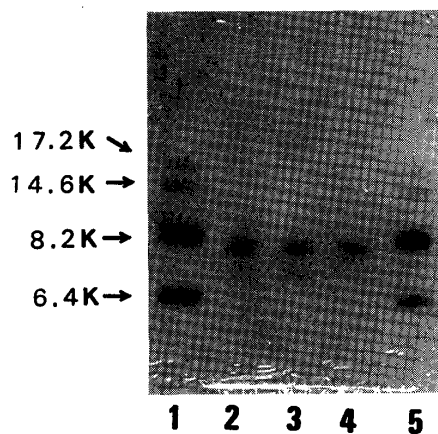


Fig. 2. SDS-PAGE analysis of the purified LM1s. The electrophoresis was performed on 15% polyacrylamide gel. The gel was visualized by staining with Commassie brilliant blue R-250. Lanes 1 and 5, molecular weight (MW) markers containing myoglobin and its peptide fragments (Pharmacia LKB Biotechnology), lane 2, LM1a (5 μ g); lane 3, LM1b (5 μ g); lane 4, LM1c (5 μ g).

Table 3 Chemical composition of purified LM1s

Amino acid	LM1a	LM1b	LM1c
Asp	213	314	278
Thr	102	131	85
Ser	161	207	195
Glu	603	836	307
Pro	355	384	225
Gly	581	684	430
Ala	153	178	229
Cys	9	23	41
Val	106	149	151
Met	20	25	12
Ile	74	110	134
Leu	101	162	192
Tyr	42	67	83
Phe	85	97	113
His	70	118	93
Lys	57	79	70
Arg	111	170	155

unit: nmol/mg

(Table 4) and histamine release assay (Table 5) on mite-allergic patients and patients allergic to other allergens with high IgE titers. In skin tests, the purified LM1s elicited erythema in the asthmatic patients allergic to mite. But these LM1s were not capable of inducing positive reactions for patients allergic to other allergens. In histamine release

Table 4 *In vivo* allergenicity of the purified LM1s

Case	Skin test				
	HD ^a	M ^b	LM1a ^c	LM1b ^c	LM1c ^c
7	++	nd	++	++	+
8	++	nd	++	++	+
9	++	++	+	+	+
10	++	++	+	+	+
11	++	nd	+	+	+
12	++	nd	+	+	+
13	++	—	—	—	—
14	+	nd	++	++	++
15	+	nd	+	+	+
16	+	nd	+	—	++
17	+	nd	—	—	+
18	+	nd	—	—	—
19	—	++	—	—	—
20	—	++	—	—	—
21	—	+	—	—	—
22	—	—	—	—	—
23	—	—	—	—	—

a, 1 : 1000 b, 1 : 10000 c, 5mg/ml nd, not determined

assay, the same results were obtained.

LM1a were cross-reactive with anti-*Der f* II but not with anti-*Der f* I. However, LM1s did not form any precipitate when tested by the double diffusion method (data not shown). To clarify the interrelationship of the purified LM1s, the reactivity of anti-*Der f* II serum to each LM1s was evaluated by immunoinhibition (Fig. 3). The reaction of LM1c to anti-*Der f* II serum was completely inhibited with the other antigens, while the reaction of LM1a to the serum were partially inhibited with LM1c or the other antigens, respectively.

DISCUSSION

When we attempt to isolate antigens in a mite-free spent culture, we have to determine whether the antigens are derived from mites or not. Statistically, LM fractions contain antigens derived from mites and feces. Purified LM1s have also been shown to be derived from mites and feces, since the antigens cross-react with anti-*Der f* II serum and have the ability to release histamine specifically from washed blood cells in mite-allergic patients and to elicit skin reactions only in mite-allergic patients.

Table 5 *In vitro* allergenicity of the purified LM1s

Case	Skin test		LM1a		LM1b		LM1c		Total histamine (ng/ml)	Serum IgE U/ml
	HD	M	1	2	1	2	1	2		
24	+	+	90.0	7.8	66.0	10.5	14.0	37.5	46.2	310
25	+	+	32.0	21.2	10.0	13.9	2.2	27.5	65.7	nd
26	++	++	NS	NS	NS	NS	3.4	10.5	100.7	310
27	+	nd	28.0	42.1	68.0	46.0	0.21	40.5	76.2	1000
28	+	++	NS	NS	NS	NS	14.0	22.5	75.2	690
29	+	++	26.0	38.4	10.0	16.3	2.0	45.8	55.2	nd
30	—	—	NS	NS	NS	NS	NS	NS	36.6	230
31	—	—	NS	NS	NS	NS	NS	NS	68.6	400
32	—	nd	NS	NS	NS	NS	NS	NS	48.2	570
33	—	nd	NS	NS	NS	NS	NS	NS	82.6	1133.8
34	—	nd	NS	NS	NS	NS	NS	NS	64.8	739.3
35	—	—	NS	NS	NS	NS	NS	NS	74.2	660
36	—	—	NS	NS	NS	NS	NS	NS	64.2	920-1100

1, Sensitivity (the sensitivity (μg of allergen/ml of blood) of patient to an allergen was defined as the allergen concentration at corresponding to 50% of the maximum histamine release amount).

2, Maximum histamine release (%).

NS, Case of less than 2% histamine release when blood cells were stimulated with appropriately diluted antigen solutions ($13\text{ng}\sim 200\mu\text{g}/\text{ml}$ of blood) was defined as insignificant one for histamine release; nd, not determined.

Although the histamine release curve shifts according to the purity of the allergen in an antigen extract, the sensitivity of patients represents the allergen concentration corresponding to 50% of the maximum histamine release amount so long as the same preparation can be used among different cases²²⁾. We have been routinely using Dff (lot 1) and Dfb (lot 1) preparations as standard allergens. As shown in Table 2, the differences in the sensitivities to various preparations among patients and percentage of histamine release seems to reflect the differences in the sensitizing antigens.

To estimate roughly which preparations contain higher concentrations of allergen, the total allergen content may be expressed by multiplying the reciprocal of the sensitivity by the yield of a preparation, because the value of the sensitivity should be in inverse proportion to the amount of allergen (purity of allergen) in the preparation. The Dfb preparation contains the major allergen, *Der f* II. Total allergen content in the LM fraction was higher than that in the Dfb preparation, except in case 2 (Table 2). These results suggest that a significant amount of allergen is present

even in the LM fraction, although the specific activity (allergenic activity/dry weight) of the fractions becomes lower through the process of purification (Tables 2 and 5). This tendency is similarly observed in the purification process of sea squirt allergens²¹⁾²⁴⁾, *Der f* I¹²⁾, and *Der f* II¹²⁾.

ELISA test results indicate that LM1s cross-react with anti-*Der f* II. But LM1s contain saccharides, in spite of having a lower molecular weight than *Der f* II. These points strongly suggested that LM1s are not decomposed products of *Der f* II.

No increase in titer of antibody to LM was observed although LM had been injected into rabbits according to the usual procedure. Nevertheless, LM1s cross-reacted with rabbit anti-*Der f* II in ELISA test and reacted with mite-allergic patients' IgE in histamine release and skin test. This implied the existence of at least a sensitized antigen with high molecular weight. Few reports have been reported on allergens possessing haptenic properties. Krilis et al.¹⁶⁾ reported two dialysable fractions whose molecular weight are $500 < \text{mw} < 5,000$ and $5,000 < \text{mw}$ have significant allergenic activities when examined by skin prick

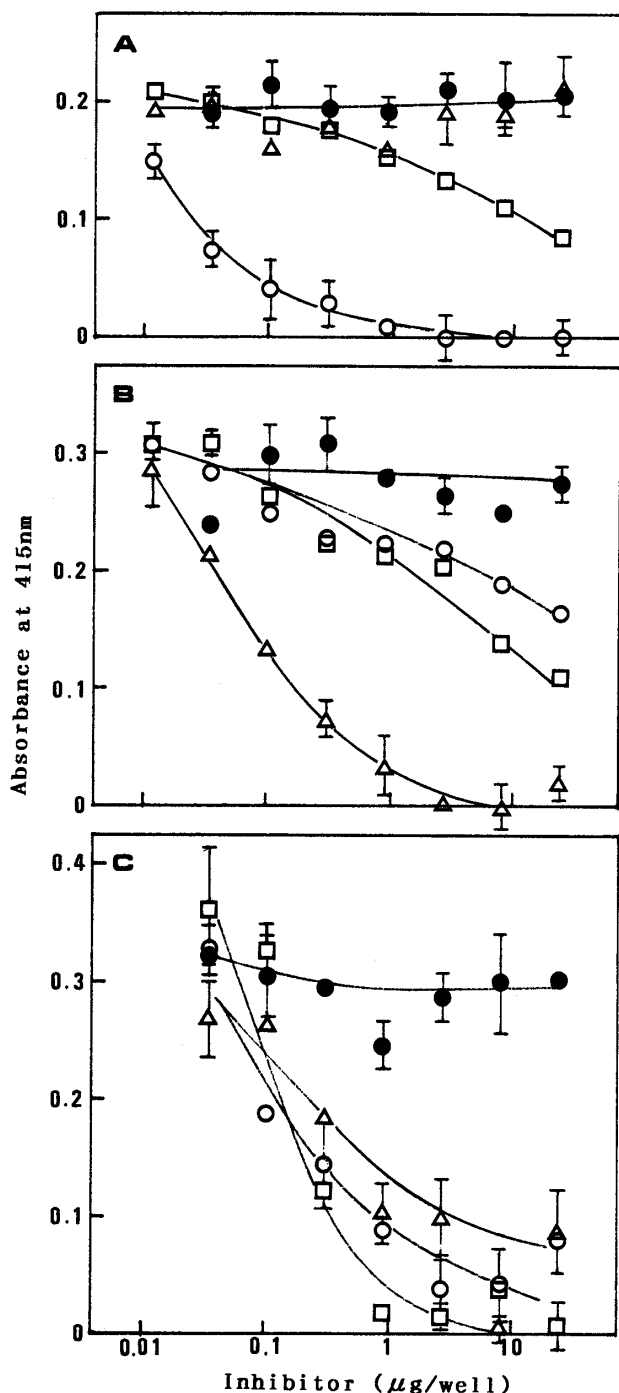


Fig. 3. ELISA inhibition test of the purified LMIs. Each well was coated with LM1a (A), LM1b (B) and LM1c (C). Before dispensing anti-*Der f* II serum to the antigen-coated wells, the serum was incubated with a series of properly diluted LM1a (○), LM1b (Δ), LM1c (□), and cytochrome c (●) solutions ranging from 34 or 102 ng to 25 μg/well. Inhibitory activities of the purified LMIs were measured at 415 nm according to ordinary ELISA method.

test, histamine release and RAST. Koda et al.¹⁷⁾ reported mite dialyzate suppressed homologous passive cutaneous anaphylaxis (PCA) in rats caused by a non-dialyzable fraction. Tamura et al.¹⁾ reported mite dialyzates were purified as 5 components which were basic glycoproteins and allergic in RAST and ELISA inhibition tests. LMIs are apparently different from these components in respect of non-dialyzable antigen. A polysaccharide-rich fraction (GP₈), whose molecular weight is 8 kD on SDS-PAGE, was reported by Le Mao et al.⁶⁾. But their detailed properties remain unknown. Similar studies of haptenic substances have been investigated using timothy pollen¹⁹⁾, ragweed pollen²⁰⁾, gree pea²⁵⁾ and Kentucky blue grass pollen²⁶⁾. Especially, antigen D of low molecular fraction from timothy pollen was shown to be more effective than crude antigen for immunotherapy against patients with timothy allergy²⁷⁾. LMIs may also be useful for immunotherapy against patients with mite allergy.

We are currently investigating the role of LMIs in immune responses in humans with mite allergy.

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コナヒョウヒダニ中の低分子アレルゲン

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コナヒョウヒダニの排泄物抽出物を分画分子量10kDの限外濾過膜で分離した低分子画分(LM)は、ダニアレルギー患者に特異的な強い皮内反応とヒスタミン遊離活性を有していた。この低分子画分をUltrogel AcA 54によるゲル濾過とヒスタミン遊離試験で分画し、2つのアレルゲン活性画分(LM1, LM2)を得た。高分子量のLM1画分を、更にイオン交換(DEAE)と逆相HPLC(ODS 120T)で分画して、3つの精製アレルゲン(LM1a, LM1b, LM1c)を得た。この3つの成分の分子量は、いずれもゲル濾過では13kD, SDS-PAGEでは8kDであり、等電点は4-5であった。この3つのアレルゲンは約20%の中性糖を含む糖タンパク質であり、いずれもウサギ抗*Der f II*抗体に反応したが抗*Der f I*抗体とは反応しなかったことから、抗*Der f II*と共通の抗原決定基を含むが、抗*Der f II*とは異なる低分子アレルゲンであることが示唆された。LM1cと抗*Der f II*抗体との反応はLM1aとLM1bによって完全に阻害されたが、LM1並びにLM1bと抗*Der f II*との反応はLM1cでは部分的にしか阻害されなかった。
