

Crossreactivity of IgE antibody against *Dermatophagoides farinae* with *Limulus polyphemus* agglutinin

M. SHIBASAKI, S. ISOYAMA, R. SUMAZAKI and H. TAKITA

Department of Pediatrics, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Japan

Summary

Crossreactivity of IgE antibody against *Dermatophagoides farinae* (*Der f*) with *Limulus polyphemus* agglutinin (LPA) was examined using RAST and immunoblot analysis. Of 40 *Der f*-sensitive asthmatic patients, 28 revealed a positive RAST reaction to LPA, while none of 20 *Der f*-insensitive hay fever patients showed this reaction. LPA-specific RAST levels of the 40 asthmatic patients correlated with their *Der f*-specific levels. The RAST reactivity to LPA was competitively inhibited by the addition of either soluble *Der f* or LPA, but not by the specific inhibitory sugar of sialic acid. LPA could also induce histamine release from leucocytes of *Der f*-sensitive asthmatic patients. IgE immunoblot analyses showed that the positive RAST sera for LPA had a strong IgE binding activity to the 30 kDa and 80 kDa components of *Der f* body extract, whereas gel filtration studies showed that the high molecular weight fractions above 150 kDa retained antigenic constituents associated with IgE reactivity to LPA. These results suggest that the antigenic materials of *Dermatophagoides* mites share some determinants with the haemagglutinin of horseshoe crabs.

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Introduction

Because of its unique property of binding sialic acid, *Limulus polyphemus* agglutinin (LPA) is now widely used as a probe for structural analysis of the carbohydrate moiety of various glycoproteins [1–3]. We have recently examined the variation and functional role of the oligosaccharide components of the IgE proteins of allergic patients by the use of various lectins, including LPA [4]. During these attempts, we found that IgE antibody against LPA was frequently demonstrated in mite-sensitive asthmatic patients. The IgE-binding reaction to LPA was competitively inhibited by the addition of mite antigen or LPA, but not by sialic acid. The present paper describes the evidence that the antigenic materials of house dust mites share some determinants with the haemagglutinin of the horseshoe crab.

Materials and methods

Subjects

The mite-sensitive sera were obtained from 40 asthmatic children aged between 3 and 15 years, who had positive RAST values for *Dermatophagoides farinae* (Df) as determined using the Phadebas RAST® (Pharmacia, Uppsala, Sweden) system. Their average IgE value was 530 ± 318 IU/ml. The mite-insensitive sera were obtained from 20 hay fever patients aged 10–20 years, who had negative RAST values for Df, but positive RAST values for orchard grass (nine cases) or Japanese cedar (pollens) (11 cases). Their mean IgE value was 420 ± 211 IU/ml.

The sera for IgE immunoblot analysis were separately obtained from 20 Df-sensitive asthmatic patients, including 10 RAST-positive and 10 RAST-negative sera for LPA.

Blood was drawn with the patient's or parents' permission to be included in this study.

Antigens

LPA preparation purified by affinity chromatography

Correspondence: Dr M. Shibasaki, Department of Pediatrics, Institute of Clinical Medicine, University of Tsukuba, Tsukuba, Ibaraki-ken 305, Japan.

was purchased from E.Y. Laboratories, Inc. (San Mateo, CA, USA), hemocyanin and *Limulus polyphemus* plasma powder from Sigma Chemical Co. (USA). Mite body extract prepared from isolated mite bodies of Df [5] was kindly supplied by Torii Co., Ltd (Tokyo, Japan), and was used as Df antigen.

Fractionation of Df antigen

The Df antigen (5 mg) dissolved in 0.5 ml of 0.05 M Tris-HCl buffer (pH 8.0) containing 0.5 M NaCl was applied on a 0.9 × 70 cm Sepharose 6 B column (Pharmacia) equilibrated beforehand with the same buffer, and 1 ml fractions were collected at a flow rate of 4 ml/h. Molecular weight determinations of the fractions were performed by the use of gel filtration calibration kits (Pharmacia), containing thyroglobulin 669 kDa ferritin 440 kDa, catalase 232 kDa and aldolase 158 kDa.

Sepharose-RAST

The Sepharose-RAST method was described in detail in a previous article [6]. Briefly, 1 mg of LPA, Df antigen, or Df gel filtration fractions were coupled to 300 mg (dry weight) of CNBr-activated Sepharose 4 B (Pharmacia), according to the method described by Wide *et al.* [7]. The antigen-coupled particles (ACP) were suspended in the RAST buffer (0.1 M KH₂PO₄-K₂HPO₄, pH 7.4, 1% (w/v) Tween 20) at a concentration of 10 mg/ml. Fifty microliters of ACP suspension was incubated with 50 µl of test serum for 2 h at room temperature. After three washings with 4 ml of RAST buffer, ACP were further incubated with 50 µl of ¹²⁵I-labelled anti-IgE, obtained from Phadebas RAST kits, for 16 h at room temperature. Excess of labelled anti-IgE was washed away with 0.15 mol/l of NaCl solution containing 1% Tween 20. The radioactivity bound to the ACP was measured by a gamma counter. RAST determinations were done in duplicate, and the mean percentage of binding was calculated by dividing the counts bound to ACP by total counts times 100.

The cut-off point between RAST positivity and negativity was defined as 300% of the background binding produced by 2% (w/v) human serum albumin (HSA). The results were regarded as positive when the percentage binding of the sample serum was higher than the cut-off value.

RAST inhibition test

RAST inhibition tests were performed with a serum pool from five asthmatic patients who have a high RAST value for LPA. Serial 10-fold dilutions of LPA or Df antigen

(2 mg/ml) in 2% (w/v) HSA were prepared and 100 µl of each was incubated in eightfold diluted serum aliquots on a horizontal shaker for 2 h. The last tube contained diluent alone. The serum-inhibitor mixture was then subjected to the RAST procedure. To investigate the effect of sugar on IgE binding to LPA, sera were incubated with serial twofold dilutions of sialic acid (2 mg/ml), instead of antigen, and then subjected to RAST assay for LPA. As a background binding, 2% (w/v) HSA RAST was similarly run. Percentage inhibition was calculated using the following formula:

$$\left[1 - \frac{\% \text{ binding with inhibitor} - \text{background binding}}{\% \text{ binding without inhibitor} - \text{background binding}} \right] \times 100$$

Histamine-releasing experiments

Leucocyte histamine release (HR) was examined as previously described [4]. Peripheral blood, anticoagulated with EDTA-2Na, was separated by Ficoll-Hypaque density gradient centrifugation [8]. The mononuclear cells including basophils were removed and washed twice with HEPES buffer (125 mM NaCl, 5 mM KCl, 20 mM HEPES, 0.009% glucose, pH 7.4) at 4°C and suspended in HEPES-CM buffer containing Ca₂₊ 1 mM and Mg₂₊ 1 mM. The leucocyte suspension (5 × 10⁶/ml) was added to 1.0 by 7.5 cm plastic tubes in 0.2 ml triplicate samples. After pre-incubation of the cells at 37°C for 30 min, freshly prepared LPA (0.02 ml) was added to the tubes, and the cells further incubated for 45 min at the same temperature, with occasional agitation. At the end of the incubation, the tubes containing cells were transferred to an ice bath and rapidly centrifuged at 450 g for 5 min at 4°C, and 0.1 ml of the supernatants was removed for histamine assay. The spontaneous release of histamine was determined by incubating cells in buffer alone. Total leucocyte histamine content was assayed in a cell suspension treated with 3% perchloric acid.

Histamine was assayed by 'Histamine' radioimmunoassay kit (Immunotech, Marseille, France).

The percentage of basophil HR was calculated as follows:

$$\% \text{ HR} = \left[\frac{\text{experimental HR} - \text{spontaneous HR}}{\text{total histamine} - \text{spontaneous HR}} \right] \times 100$$

IgE immunoblot analysis

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) was performed as described previously [9], with 12% homogeneous gel. The 9.0 × 4.0 cm mini-gel was used, and the sample gel was divided into two lanes by the use of a special comb; one 0.35 cm wide

for molecular weight marker standards (Pharmacia) and another 8.0 cm wide for a sample application.

The gels were loaded with 200 µg of protein and electrophoresed at 30 mA per gel under unreduced condition. After electrophoresis, gels were electroblotted onto a polyvinylidene difluoride membrane (Immobilon®, Millipore, Bedford, Mass, USA) in a Tris-glycine-SDS-methanol buffer at 0.8 mA/cm. After the transfer, the lane containing the molecular marker standards was removed and sequentially stained with amido black. The blotted samples were blocked for 3 h in PBS buffer containing 0.01% (w/v) Tween 20 and 3% (w/v) bovine serum albumin. The blots were then washed five times in PBS-Tween, and then cut into 22 strips, each being 0.35 cm wide. The strips were put into special strip-incubation tubes, incubated overnight with 1 ml of various RAST-positive sera diluted 1:10 in PBS-Tween, washed again, and incubated for 4 h with 0.2 µCi of ¹²⁵I-labelled anti-human IgE obtained from Phadebas RAST kits (Pharmacia). The membrane was washed again, dried, mounted and autoradiographed with X-OMAT AR film (Eastman Co., Rochester, NY, USA).

Immunoblotting inhibition experiments

One millilitre of each fivefold diluted serum was mixed with an equal volume of PBS-Tween solution, with or without soluble LPA inhibitor (20 µg/ml) or LPA-coupled Sepharose 4B (50 mg/ml), and the mixtures were incubated for 3 h at room temperature. The paired sera with and without inhibitor were applied for IgE immunoblot analysis with Df antigen, and their IgE binding patterns to Df components were compared by autoradiography.

Statistics

Data were analysed by a computer programme, Statistical Analysis System (SAS), using Student's *t*-test for paired and unpaired samples, and Pearson's correlation analysis.

Results

RAST reactivity to LPA

RAST reactivity to LPA was compared between Df-sensitive asthmatic patients and Df-insensitive hay fever patients. As shown in Fig. 1, 28 of the 40 Df-sensitive asthmatic patients showed positive RAST reactivities to LPA, but none of the Df-insensitive hay fever patients responded definitely to LPA.

In the 40 asthmatic patients, RAST levels for LPA were compared with those for Df (Fig. 2). As can be seen, there was a weak but significant correlation ($r=0.33$, $P<0.05$) between the two.

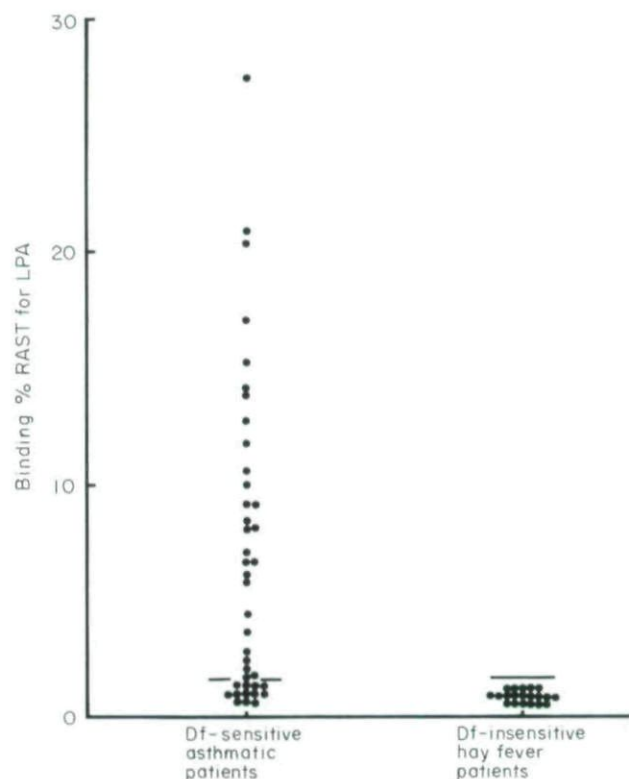


Fig. 1. Comparison of RAST levels for LPA between Df-sensitive asthmatic patients ($n=40$) and Df-insensitive hay fever patients ($n=20$). Horizontal bar in the graph represents the cut-off level between RAST positivity and negativity.

RAST inhibition experiments

As shown in Fig. 3a, both soluble LPA and Df inhibited LPA-RAST by 50% at the concentrations of 10 and 2 µg/ml, respectively. As shown in Fig. 3b, the soluble Df inhibited Df-RAST by 50% at a concentration of 8 µg/ml, whereas LPA did not, even at higher concentrations.

To examine the possibility that LPA directly binds to oligosaccharide components of IgE, sera were mixed with serial twofold dilutions of sialic acid, which was a specific inhibitory sugar against LPA, and then subjected to RAST assay for LPA. However, RAST reactivities to LPA were not significantly inhibited by the addition of the high concentration of sialic acid (1 mg/ml).

Leucocyte HR with LPA

As shown in Table 1, the leucocytes from Df-sensitive asthmatic patients released histamine significantly upon stimulation with LPA, while the cells from Df-insensitive hay fever patients did not respond to LPA.

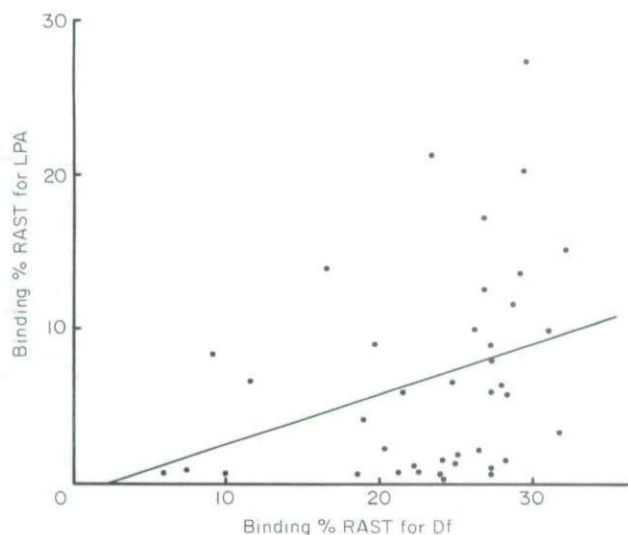


Fig. 2. Comparison of RAST levels for LPA and Df for 40 asthmatic patients. $R=0.33$. $P<0.05$.

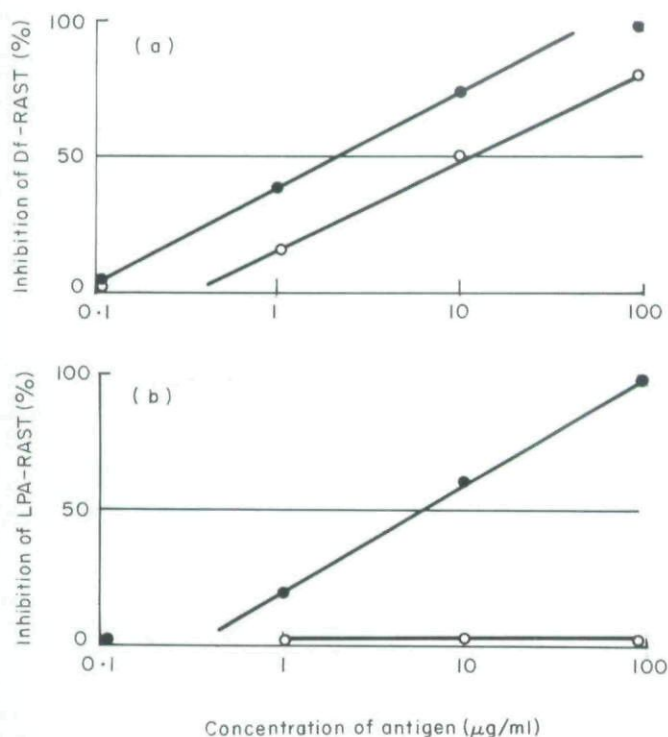


Fig. 3. RAST inhibition tests. (a). Inhibition of LPA-RAST by Df (●) or LPA (○). (b). Inhibition of Df-RAST by Df (●) or LPA (○). The sera from five asthmatic patients with high RAST values for LPA were pooled, diluted eightfold and applied to the RAST inhibition experiments.

Table 1. Comparison of LPA-induced histamine release between Df-sensitive and -insensitive subjects

Cell source	% HR
Df-sensitive asthmatic patients ($n=5$)	21.5 ± 8.2
Df-insensitive hay fever patients ($n=5$)	0.2 ± 0.2

Leucocytes were incubated with LPA at a concentration of $1 \mu\text{g}/\text{ml}$ for 45 min.

IgE immunoblot analysis for Df

IgE reactivities of positive and negative RAST sera for LPA to Df antigen were compared by immunoblotting. As shown in Fig. 4, the positive RAST sera for LPA bound preferentially to relatively high MW component, especially to 30 kDa, 59 kDa and 80 kDa (lanes A to J), while none of the negative RAST sera for LPA exhibited an IgE binding reaction to these components (lanes K to T). On the other hand, both positive and negative RAST sera for LPA showed similarly high IgE binding to 15 kDa and 25 kDa components.

Immunoblotting inhibition experiment

To determine which components of Df are crossreactive with LPA, immunoblotting inhibition experiments were performed using 10 positive RAST sera for LPA. As shown in Fig. 5, when IgE reactivity to Df was compared between soluble LPA-absorbed and unabsorbed sera, IgE-bindings to 30 kDa, 59 kDa and 80 kDa components were completely inhibited by preincubation with LPA (lanes A' to J'), but that to 15 kDa, 25 kDa, and 110 kDa components remained unchanged. When the sera were pre-absorbed with LPA-Sepharose 4B, IgE-bindings to high molecular weight components above 30 kDa were similarly inhibited.

Gel filtration analyses of Df antigen

The Sepharose 6 B gel filtration profile of the Df antigen was shown in Fig. 6. The eight fractions were pooled, and designated F1 through to F8. As shown in Table 2, when RAST reactivities to these fractions were compared between positive and negative RAST sera for LPA, both sera showed similarly high RAST reactivity to lower molecular weight fractions (F5, F6, F7 and F8), while only positive RAST sera LPA showed a considerable RAST reactivity to higher molecular weight fractions (F1, F2, F3 and F4). As shown in Table 3, when the

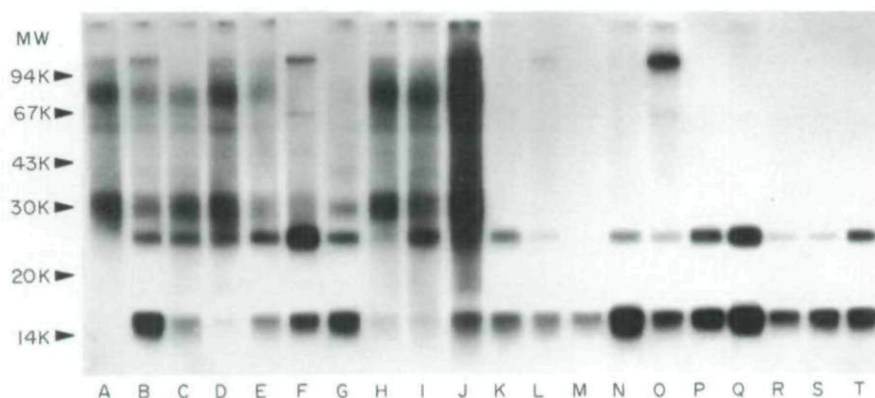


Fig. 4. Comparison of IgE reactivities to Df between positive and negative RAST sera for LPA. Lanes A to J, positive RAST sera for LPA; lanes K to T, negative RAST sera for LPA. The Df body extract was separated by 12% SDS-PAGE under non-reducing conditions, and electroblotted onto an Immobilon® membrane. The membrane was cut into strips, and each strip was incubated with mite-sensitive sera which were diluted to 1:10 with PBS.

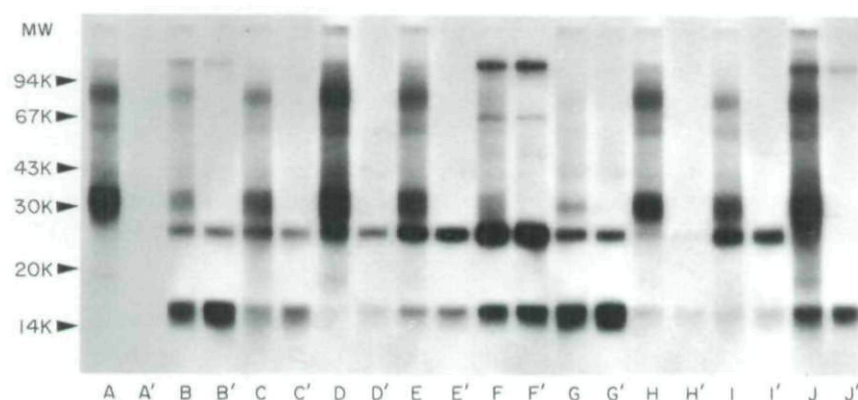


Fig. 5. Immunoblotting inhibition experiments. The positive RAST sera for LPA, corresponding to A to J in Fig. 4, were preincubated with either LPA or buffer alone and applied for IgE immunoblot analyses with Df antigen. Lanes A' to J' and lane A to J indicate IgE immunoblot for individual sera with and without LPA-absorption, respectively.

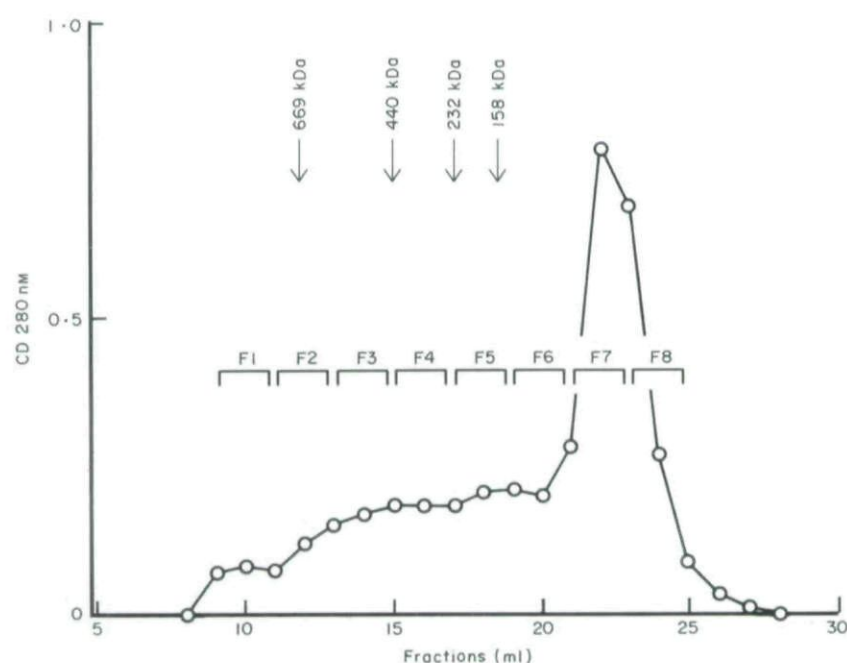


Fig. 6. Gel filtration of Df antigen on a Sepharose 6 B column equilibrated with 0.05 M Tris-HCl buffer (pH 8.0) containing 0.5 M NaCl. Df body extract (5 mg) was applied to the top of the column (0.9 × 70 cm), and 1 ml fractions were collected at a flow rate of 4 ml/h. Fractions as indicated (F1 to F8) were pooled, and used for RAST and immunoblot analysis.

Table 2. Comparison of RAST reactivity to Df fractions between positive and negative RAST sera for LPA

Df fractions	RAST binding to Df fractions	
	Positive RAST sera for LPA	Negative RAST sera for LPA
F1	6.1 ± 0.9	1.4 ± 0.1*
F2	9.2 ± 1.3	2.2 ± 0.1*
F3	9.6 ± 1.9	2.1 ± 0.2*
F4	7.3 ± 1.1	3.4 ± 0.4
F5	10.9 ± 1.5	8.6 ± 1.6
F6	16.2 ± 0.9	16.6 ± 0.5
F7	17.6 ± 0.7	17.2 ± 0.6
F8	11.1 ± 1.2	10.8 ± 1.3

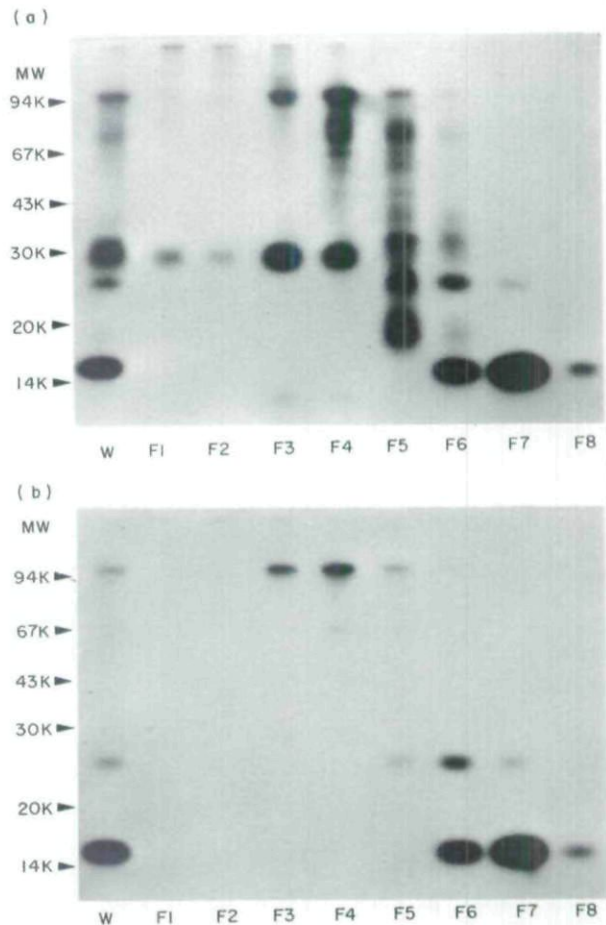
RAST determinations were done in triplicate, and the results were expressed as a mean % binding ± SD. *The mean RAST bindings were significantly lower than the cut-off level between RAST positivity and negativity for each fraction. Five positive RAST and five negative RAST sera for LPA were each pooled, and used for RAST assay.

Table 3. RAST Inhibition for Df fractions by LPA

Df fractions	Inhibition* (%)
F1	96
F2	88
F3	90
F4	72
F5	34
F6	0
F7	0
F8	0

* The value between -5% and +5% was expressed as 0. Five positive RAST sera for LPA were pooled and used for the inhibition experiments. The eightfold diluted pooled serum was mixed with an equal volume of LPA solution (20 µg/ml), incubated for 2 h, and then subjected to the RAST study for Df fractions. The % inhibition was calculated according to the equation described in Materials and methods.

antigenic crossreactivity between each of Df fractions and LPA was examined using a LPA-reactive pooled serum, the RAST bindings for higher MW fractions (F1 to F4) were suppressed markedly by the preincubation of serum with LPA and the binding to F5 moderately, but those to lower molecular weight fractions (F6 to F8) remained unchanged by such a treatment.

**Fig. 7.** Immunoblot analyses of the Df gel filtration fractions. IgE immunoblot for pooled serum without LPA-absorption (a) and with LPA-absorption (b). Lane W indicates Df whole body extract.

IgE reactivities to eight Df fractions were examined by the immunoblot analysis. As shown in Fig. 7a, the strongly stained 30 kDa band was found in F3 and F4 eluted at the position of 250 kDa–500 kDa, and the 80 kDa band was detected in F4 and F5 eluted at the position of 150 kDa–400 kDa. As shown in Fig. 7b, when the serum was pre-absorbed with LPA, IgE binding bands detected in the higher molecular weight fractions were almost completely suppressed.

Comparison of IgE binding activity between Limulus whole plasma, LPA and hemocyanin

Limulus whole plasma, LPA and hemocyanin were each compared for their protein profiles and capacity to bind IgE by SDS-PAGE and immunoblot analysis, respectively. On SDS-PAGE, *Limulus* whole plasma and hemocyanin preparations showed similar dark-stained protein

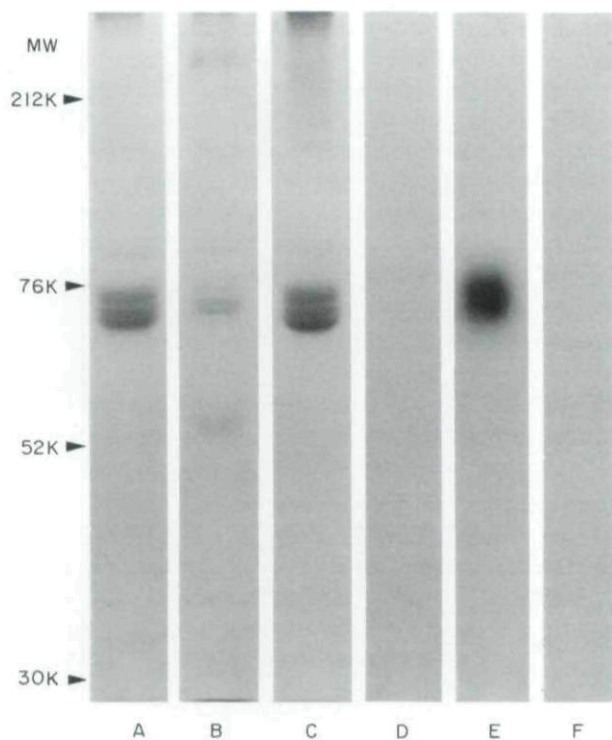


Fig. 8. SDS-PAGE and IgE Immunoblot analyses with *Limulus* plasma proteins. *Limulus* whole plasma, LPA and hemocyanin were each analysed for protein profiles (lanes A, B and C) by SDS-PAGE and for capacity to bind IgE by immunoblotting (lanes D, E and F). The gel was 7.5% and run under non-reducing conditions. Pooled serum from 10 asthmatic patients with positive RAST for LPA was used for IgE immunoblot analyses.

bands at the molecular weight region of around 70 kDa, corresponding to the hemocyanin sub-units (Fig. 8A and C). LPA preparation showed weakly stained protein band at around 70 kDa, corresponding to the LPA sub-units (Fig. 8B). In contrast, IgE binding activity was found only in the LPA preparation, and IgE bound preferentially to the 70 kDa–74 kDa components of LPA (Fig. 8 D–F).

Discussion

IgE antibody against LPA was frequently demonstrated in the sera from Df-sensitive asthmatic patients by RAST. The IgE reactivity to LPA was competitively inhibited by the addition of either soluble Df or LPA itself. These findings suggest that the antigenic materials of *Dermatophagoides* mites share some determinants with haemagglutinin of horseshoe crabs.

Since LPA is a sialic acid-binding lectin [1,2], it is theoretically possible that LPA binds directly to human IgE protein through its oligosaccharide units containing

sialic acid at the Fc-portion [10]. However, the sialic acid was not inhibitory for IgE binding to LPA in this study. Moreover, our RAST results have shown that the sera from Df-insensitive hay fever patients does not definitely react with LPA protein. It is also possible that LPA bind directly to certain Df components, and thus inhibit specific IgE binding. However, our inhibition experiments have shown that solid phase-LPA can also inhibit IgE binding to Df components on immunoblot. These findings indicate that IgE binding reaction to LPA is due to an immunologic reaction.

Since horseshoe crabs are a precious form of natural life, and designated as a protected species in Japan, the chances of our subjects becoming sensitized to LPA must be extremely limited. In contrast, Df is a universal sensitizer in house dust in this country. It is therefore likely that IgE antibody produced by specific antigenic stimuli of house dust mites may crossreact with certain epitopes of LPA.

The mediator release from basophils or mast cells requires at least a dimerization of the membrane-bound IgE or IgE receptors [11]. We found that LPA was capable of inducing *in vitro* HR from leucocytes of mite-sensitive asthmatic patients, suggesting that the LPA protein is a polyvalent antigen having two or more antigenic determinants on its molecule.

Our immunoblot analyses showed that both positive and negative RAST sera for LPA exhibited similarly high IgE binding activity to 15 kDa and 25 kDa components of Df, while positive RAST sera for LPA bound preferentially to 30 kDa and 80 kDa components. Furthermore, the IgE binding reactions to the latter components were competitively inhibited by pre-incubation of the sera with LPA. It is therefore suggested that the 30 kDa and 80 kDa components of Df are the major antigenic materials crossreactive to LPA.

Gel filtration studies combined with RAST assay have shown that the native antigenic constituents crossreactive with LPA have high molecular weight above 150 kDa. In addition, IgE immunoblot analyses with fractionated Df have shown that the 30 kDa and 80 kDa bands are associated with antigenic materials with apparent molecular weight 250 kDa–500 kDa, and those with 150 kDa–400 kDa, respectively. It is therefore likely that both 30 kDa and 80 kDa components may be dissociated sub-units of the two different high molecular weight proteins.

Two groups of allergens, *Der I* (24–15 kDa) and *Der II* (15 kDa) have been purified from *Dermatophagoides* mites, and generally recognized as the major mite allergens [12–15]. In this study, 25 kDa and 15 kDa IgE binding bands were demonstrated in most mite-allergic patients by immunoblotting with Df. The lack of the inhibition of Df-RAST by LPA in this study may be

associated with that RAST binding to Df is mainly directed toward *Der I* and *Der II*.

In addition, the third group of allergen (*Der III*) has been isolated from house-dust of mites [16–19]. The allergen is shown to be trypsin-like enzymes with an apparent molecular weight of approximately 30 kDa [17–19]. Stewart *et al.* [19] have shown that the trypsin-like enzymes are demonstrated in the fraction eluted at 28 kDa position on Sephadex G-75 gel filtration, whereas our results showed that the 30 kDa IgE binding component was detected in the Sepharose 6 B fraction eluted at 250 kDa–500 kDa position. Therefore, the 30 kDa component crossreactive with LPA may be different from the *Der f III* allergen.

The LPA preparation used for the present study still contains heterogeneous protein fractions. Our preliminary studies have shown that when LPA, hemocyanin and *Limulus* whole plasma protein preparations were each compared their capacity to bind IgE by RAST, LPA showed much higher RAST reactivity than the other two preparations (Data not shown). The present immunoblot study indicates that the IgE binding activity associated with Df-sensitivity is concentrated in the LPA.

The horseshoe crab *Limulus polyphemus* is the sole survivor of an extinct group of arthropods intermediate between the trilobites and arachnids [20]. The lack of the inhibition of Df-RAST by LPA in this study may be associated with that RAST binding to Df is mainly directed toward *Der I* and *Der II*. *Dermatophagoides* mites are also a member of the arthropod family, belonging to the order Acari, of the class Arachnida [21]. The two are known to have several morphological and phylogenetic similarities [20,21]. Although it is not clear from the present study what antigenic determinants are shared by LPA and Df, it seems likely that a chemically similar structure may constitute the epitope.

References

- Cohen E, Rose AW, Wissler FC. Heteroagglutinins of the horseshoe crab *Limulus polyphemus*. *Life Sci* 1965; 4: 2009–16.
- Pardoe GI, Uhlenbruck G, Bird GW. Studies on some heterophile receptors of the Burkitt EB2 lymphoma cell. *Immunology* 1970; 18:73–83.
- Nowak TP, Barondes SH. Agglutinin from *Limulus polyphemus*: Purification with formalized erythrocytes as the affinity adsorbent. *Biochim Biophys Acta* 1975; 393:115–23.
- Shibasaki M, Sumazaki R, Isoyama S, Takita H. Interaction of lectins with human IgE: IgE binding property and histamine releasing activity of 12 lectins. *Int Arch Allergy Immunol* 1992; 98:18–25.
- Haida M, Okudaira H, Ogata T, Ito K, Miyamoto T, Nakajima T, Hongo O. Allergens of the house dust mite *Dermatophagoides farinae*: Immunochemical studies of four allergenic fractions. *J Allergy Clin Immunol* 1985; 75:686–92.
- Shibasaki M, Tajima K, Morikawa A, Mitsunashi M, Sumazaki R, Tokuyama K. Relation between frequency of asthma and IgE antibody levels against *Dermatophagoides farinae* and total serum IgE levels in schoolchildren. *J Allergy Clin Immunol* 1988; 82:86–94.
- Wide L, Bennich H, Johansson SGO. Diagnosis of allergy by an in-vitro test for allergen antibodies. *Lancet* 1967; 2:1105–7.
- Böyum A. Isolation of mononuclear cells and granulocytes from human blood. *Scand J Clin Lab Invest* 1968; 21 (suppl 97):77–89.
- Laemmli UK. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 1970; 227:680–5.
- Baenziger J, Kornfeld S, Kochwa S. Structure of the carbohydrate units of IgE immunoglobulin. II. Sequence of the sialic acid-containing glycopeptides. *J Biol Chem* 1974; 249:1897–903.
- Ishizaka T, Ishizaka K, Conrad DH, Froese A. A new concept of triggering mechanisms of IgE-mediated histamine release. *J Allergy Clin Immunol* 1978; 61:320–30.
- Chapman MD, Platt-Mills TAE. Purification and characterization of the major allergen from *Dermatophagoides pteronyssinus*-antigen P1. *J Immunol* 1980; 125:587–92.
- Stewart GA. Isolation and characterization of the allergen Dpt 12 from *Dermatophagoides pteronyssinus* by chromatofocusing. *Int Arch Allergy Appl Immunol* 1982; 69: 224–30.
- Lind P. Purification and partial characterization of two major allergens from the house dust mite *Dermatophagoides pteronyssinus*. *J Allergy Clin Immunol* 1985; 76: 753–61.
- Yasueda H, Mita H, Yui Y, Shida T. Isolation and characterization of two allergens from *Dermatophagoides farinae*. *Int Arch Allergy Appl Immunol* 1986; 81:214–23.
- Heymann PW, Chapman MD, Aalberse RC, Fox JW, Platts-Mills TAE. Antigenic and structural analysis of group II allergens (*Der f II* and *Der p II*) from house dust mites (*Dermatophagoides* spp). *J Allergy Clin Immunol* 1989; 83:1055–67.
- Stewart GA, Thompson PJ, Simpson RJ. Protease antigens from house dust mite. *Lancet* 1989; 2:154–5.
- Takahashi K, Aoki T, Kohmoto S *et al.* Activation of Kallikrein system in human plasma with purified serine protease from *Dermatophagoides farinae*. *Int Arch Allergy Appl Immunol* 1990; 91:80–5.
- Stewart GA, Ward LD, Simpson RJ, Thompson RJ. The group III allergen from the house dust mite *Dermatophagoides pteronyssinus* is a trypsin-like enzyme. *Immunology* 1992; 75:29–35.
- Barnes RD. Invertebrate zoology: The Chelicerates. Philadelphia: Saunders Co. 1974:452–509.
- Tiegs OW, Manton SM. The evolution of the Arthropoda. *Biol Rev* 1958; 33:255–337.

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