

## [ORIGINAL]

DIFFERENCES IN RESPONSE OF PATIENT'S BLOOD CELLS WITH BRONCHIAL  
ASTHMA TO DIVERSE HOUSE DUST MITE ALLERGENS  
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A HPLC method was developed for the determination of histamine released from washed blood cells of allergic patients after exposure to allergens in the presence or absence of plasma. The blood cells used were preserved in a vital state at 4°C during at least 6 hours after blood-collecting. The responses of blood cells to an appropriately diluted series of a mite feces extract (Dff) solution were evaluated in three patients' groups (68 cases of hyposensitized, 59 cases of newly visited and 44 cases of the others) with bronchial asthma. Washed blood cells from the hyposensitized groups had a tendency to release histamine easily even with lower concentration of the Dff solution. Both the sensitivities of washed blood cells to Dff or mite body extract (Dfb) and the responses of the cells to diverse antigens in either extract differed largely in individual cases. The titer of blocking antibody could be expressed as the amount proportional to the concentration of Dff or Dfb.

**Key words:** bronchial asthma — *Dermatophagoides farinae* — histamine release assay — mite — mite allergen

## INTRODUCTION

Allergens from two predominant and closely related mite species, *Dermatophagoides farinae* and *D. pteronyssinus*, in house dust are major triggers associated with allergic diseases such as asthma, rhinitis and dermatitis<sup>1)–3)</sup>.

Mite allergens appear to be complicated mixture of intact or decomposed components of the mite body and feces. Of the 30 or more IgE-binding and heterogeneous components in a whole mite culture extract detected by crossed radioimmuno-electrophoresis or immunoblotting analysis using allergic sera, at least six was reported to be possibly important allergens on the basis of their frequency and intensity of IgE-binding on

blots<sup>4)–6)</sup>. However, only the major allergens of *Der* I and *Der* II groups, which react with IgE in 60 to 90% of allergic sera, in the small quantities of extract available have been extensively studied<sup>7)–15)</sup>. Other important allergens remain to be characterized.

Many reports on histamine release assay were available as a method for determination of in vitro allergenic activity. Together with RAST and skin test, histamine release assay was carried out only for a few cases using partially purified allergen<sup>13)16)–24)</sup>. However, few report was found that examined in detail a relation between histamine release assay and skin test or hyposensitization therapy.

In this report, we develop a HPLC system for histamine release assay. Using the system, 171 cases with bronchial asthma were examined for the sensitivities of their blood cells to Dff and Dfb, the responses of their blood cells to diverse antigens in either extract, and the titers of their

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**Abbreviations:** Dfb mite body extract; Dff mite feces extract; H house dust extract; HPLC high performance liquid chromatography; M mite extract

blocking antibody to either extract.

## MATERIALS AND METHODS

### Materials

A growth spent medium, in which *Derмато-phagoides farinae* was bred at 25°C and 75% humidity for 30 days, was supplied by Fumakilla Ltd. (Hiroshima, Japan). Ultrafiltration membrane (UF-20CS-10PS, 10<sup>4</sup> Mr-cutoff) was purchased from Tosoh Co. (Tokyo, Japan). House dust extract (H) and mite extract (M) (Torii & Co., Tokyo, Japan) were used for in vivo skin test and H was also used for hyposensitization. Ultrogel AcA 54 (IBF, France) was used for gel filtration chromatography. Blue Dextran 2000 (2,000,000) (Pharmacia LKB Biotechnology, Uppsala, Sweden), bovine serum albumin (67,000), ovalbumin (194) (45,000) (Katayama Chemical, Osaka, Japan) chymotrypsinogen A (25,000) (Boehringer Mannheim, Mannheim, Germany), cytochrome c (12,500) (Sigma, St. Louis, MO) and K<sub>2</sub>CrO<sub>4</sub> (Katayama Chemical) were used for molecular weight calibration markers.

### Subjects

Blood samples from 171 patients with bronchial asthma, who came to either the Third Department of Internal Medicine, Hiroshima Prefectural Hospital, or Otsuka Clinic (Hiroshima) as regular out-patients, were used in this study (Table 1). Patients with bronchial asthma were classified into three groups ((1) hyposensitized cases receiving a subcutaneous injection of a ten- to hundred-fold diluted house dust extract solution (Torii & Co.) for more than 1 year, (2) newly visited cases, and (3) the other cases receiving symptomatic therapy) on the basis of clinical history. Furthermore, the three groups were sorted according to the results obtained by skin test and histamine release assay. Differences among the groups or the subgroups were statistically evaluated with chi-square test.

### Preparation and fractionation of mite body and mite feces extracts

Mites and feces were separated according to essentially the same method of Sasa et al.<sup>25)</sup>

Table 1 Histamine release assay among patients with bronchial asthma classified by clinical history and score of skin test

| Skin test (score)               | Group                    |                          |                          | Total cases       |
|---------------------------------|--------------------------|--------------------------|--------------------------|-------------------|
|                                 | Hyposensitized           | Newly visited            | Others                   |                   |
| H or M ≥ 2                      | 39/ 1*<br>(29/36)        | 21/ 6***<br>( 7/20)      | 16/ 7*<br>( 6/13)        | 76/14<br>(39/69)  |
| H = 1 & M ≤ 1,<br>M = 1 & H ≤ 1 | 20/ 5<br>(16/20)         | 2/14**<br>( 1/ 2)        | 0/ 3                     | 22/22<br>(17/22)  |
| H < 1 & M < 1                   | 2/ 1<br>( 1/ 2)          | 1/15**<br>( 0/ 1)        | 4/14<br>( 3/ 3)          | 7/30<br>( 4/ 6)   |
| Total cases                     | 61/ 7****<br>(43/58)**** | 24/35****<br>( 8/23)**** | 20/24****<br>( 9/16)**** | 105/66<br>(60/97) |

\*,  $p < 0.01$ . \*\*,  $p < 0.001$ . \*\*\*,  $p < 0.001$ . \*\*\*\*,  $p < 0.01$ .

Figure (XX/YY), XX=the number of histamine release positive cases and YY=the number of histamine release negative cases; figure in parentheses ((xx/yy)), xx=the number of high responders and yy=the number of total cases capable of obtaining histamine release curve; H, house dust extract; M, mite extract.

Case of less than 5% and nearly 0% histamine release when blood cells were stimulated with Dff solutions (200 µg/ml of blood and 100 µg/ml of blood, respectively) was defined as histamine release negative case.

In brief, a whole mite culture (1 kg) was suspended in 10 l of a saturated sodium chloride solution and centrifuged at 1400 × g for 30 min. Floating mites were collected by filtration and the filtrate was pooled. The sediment was then suspended in 10 l of 10 mM phosphate buffered saline (pH 7.6) and centrifuged. The supernatant was added into the filtrate. After the same procedure was repeated, the collected mites were ground with quartz in 10 mM phosphate buffered saline, followed by centrifugation. The supernatant and the pooled filtrate were dialyzed and lyophilized. The freeze-dried extracts were referred to as Dfb (lot 1) and Dff (lot 1), respectively.

Dfb or Dff (30 mg) was chromatographed on a column of Ultrogel AcA 54 (2 × 80 cm) with 0.9% NaCl at a flow rate of 0.5 ml/min into 20-ml fractions under monitoring absorbance at 280 nm.

### Histamine assay

A HPLC system consisted of an LC-6A liquid chromatograph (Shimadzu, Kyoto) equipped with a Shimadzu RF-535 fluorescence monitor fitted with a flow-cell unit (cell volume, 10 µl) operating at an emission wavelength of 440 nm and an excitation wavelength of 360 nm. CM-Toyopearl

650S and Shim-pack PAG-SP were used as preparative columns ( $8 \times 10$  mm) to retard histamine. Shim-pack ISC-05 was used as an interfacial ( $4 \times 10$  mm) and an analytical ( $4.6 \times 38$  mm) columns for capture and analysis of histamine, respectively. These two shim-pack ISC-05 columns and reaction coil were located in chemical reaction chamber maintained at  $50^\circ\text{C}$ .

Histamine in a sample solution ( $100\ \mu\text{l}$ ) injected by autosampler was delivered at a flow rate of  $0.6\ \text{ml/min}$  with one mobile phase ( $0.1\ \text{M}$  EDTA buffer,  $\text{pH}\ 7.0$ ) for loading and washing by pump A and adsorbed on an interfacial Shim-pack ISC-05 column after retardation on successive columns of CM-Toyopearl and Shim-pack PAG-SP. The flow changeover valve was changed at 8 min to release histamine trapped on the Shim-pack ISC-05 column at a flow rate of  $0.6\ \text{ml/min}$  (pump B) by the other mobile phase ( $0.1\ \text{M}$  EDTA buffer containing  $1\ \text{M}$   $\text{NaClO}_4$ ,  $\text{pH}\ 8.5$ ) for elution. Released histamine was concentrated on an adjoining column of Shim-pack ISC-05, labeled at  $50^\circ\text{C}$  with o-phthalaldehyde reagent supplied consecutively at flow rate  $0.3\ \text{ml/min}$  by pump C, which was prepared by mixing o-phthalaldehyde  $0.5\ \text{g}$ ,  $\text{CH}_3\text{CN}$   $500\ \text{ml}$ ,  $\text{HClO}_4$   $5.5\ \text{ml}$ , and  $\text{H}_2\text{O}$   $500\ \text{ml}$ , and then measured by a fluorescence monitor. The valve was changed again at 20 min to get ready for the coming analysis. The assay was completed at 23 min. Simultaneously, next sample was injected.

#### *Histamine release assay*

About  $10\ \text{ml}$  of peripheral blood sample from allergic or healthy subject was separated into blood cells and plasma by centrifugation at  $370 \times g$  for 10 min. The blood cells were washed thrice with about 10-fold volumes of Hanks' buffer. After readjustment to the original volume by the supplement of the same buffer,  $200\ \mu\text{l}$  of the washed cell suspension was incubated at  $37^\circ\text{C}$  for 30 min with that of either Hanks' buffer or the plasma containing an appropriate diluted series of an antigen solution and centrifuged at  $370 \times g$  for 10 min. The supernatant was deproteinized by vortexing with one-tenth volumes of 60% perchloric acid and centrifuged at  $8200 \times g$  for 10 min. Total histamine was determined by perchloric acid lysis of cell aliquots (complete). Spontaneous release

(blank) was determined for cells incubated in the buffer. The histamine concentration in the supernatants was assayed using HPLC. Results are expressed as the mean percentage of histamine released for duplicated or triplicated assays where percentage histamine release =  $(\text{sample-blank}) / (\text{complete-blank}) \times 100$ .

#### *Intracutaneous test*

The allergenic activity was evaluated with *in vivo* skin test against allergic patients by injecting intracutaneously  $20\ \mu\text{l}$  of 0.9% NaCl and 0.5% phenol solution containing 0.005% H solution or 0.1% M solution. When erythema, appearing after 15 min, was 0 to 9, 10 to 19, 20 to 29, 30 to 39 or  $>40\ \text{mm}$  in mean diameter, score of 0, 0.5, 1, 2 or 3 was assigned, respectively.

## RESULTS

#### *Optimum condition for histamine release assay using HPLC*

As described under "MATERIALS AND-METHODS", the optimum condition for histamine assay was determined using a standard solution (histamine  $50\ \text{ng/ml}$ ) by examining valve changeover timings, times for initialization of the analytical column and flow rates, concentrations and pH values of the mobile phases for loading and elution. A linearity of relative fluorescence intensity for increasing amounts of histamine was obtained using the analytical condition in the range of histamine concentration from 1 to  $400\ \text{ng/ml}$  ( $r=0.9999$ ). Free amino acids in the same concentration as those in pooled plasma did not affect the determination of histamine.

#### *Stability of blood cells after blood-collecting*

Heparinized blood samples from mite-allergic patients and healthy donor were left at  $4^\circ\text{C}$  for desired times and then released histamine was determined by the HPLC method after washed blood cells were incubated with 2-fold dilution series of a Dff solution ( $100\ \mu\text{g/ml}$ ) (Fig. 1). When the data was plotted with percent histamine release as ordinate and antigen concentration ( $\mu\text{g/ml}$  of blood) as abscissa, no change was observed in histamine release curve of blood

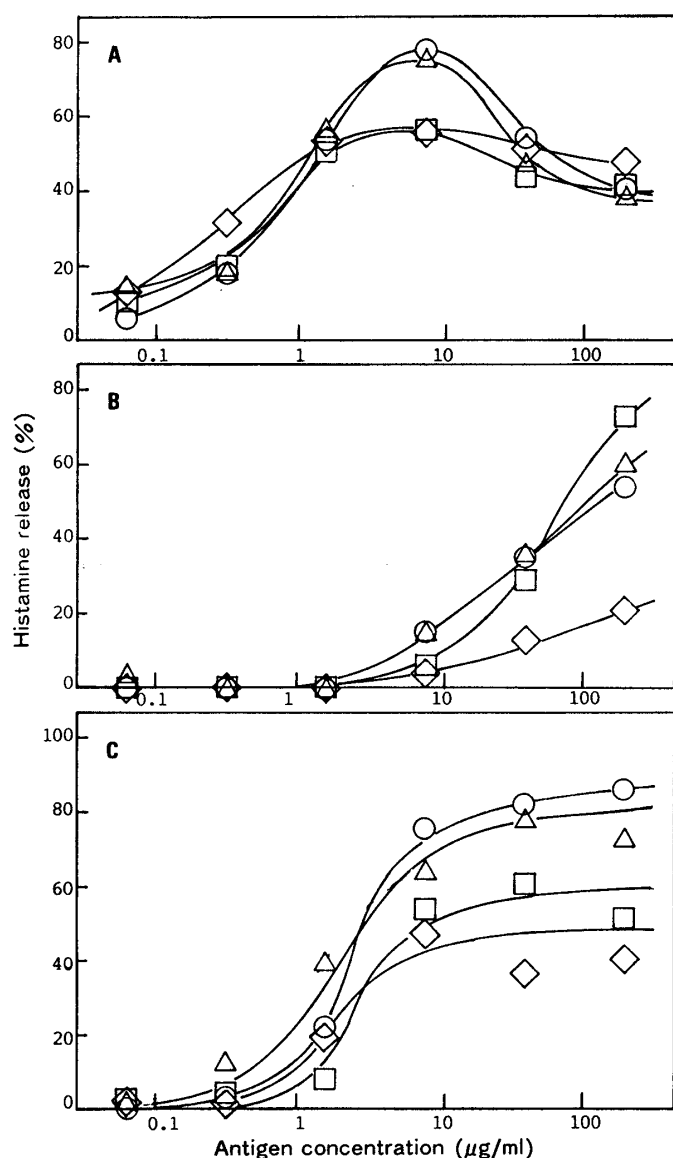


Fig. 1. Stability of leucocytes after blood-collecting. Heparinized blood samples (about 10 ml) from allergic (A, scores of skin test; M 2 and H 3), allergic (B, M 0 and H 0.5) and healthy (C, M 3 and H 3) subjects were allowed to stand at 4°C for 0 hours (○), 6 hours (△), 12 hours (□) and 24 hours (◇). After the indicated times, washed blood cells suspension (200  $\mu$ l) prepared from the aliquots (2 ml) was incubated at 37°C for 30 min with that of Hanks' buffer containing 2-fold diluted series of a Dff solution (100  $\mu$ g/ml) and then centrifuged. The supernatant was deproteinized with perchloric acid, followed by centrifugation. Total histamine was determined by perchloric acid lysis of cell aliquots (complete). Spontaneous release (blank) was determined for cells incubated in the buffer. The histamine concentration in the supernatants was assayed using HPLC.

samples left for at least 6 hours. This indicated that blood sample should be used within 6 hours after blood-collecting.

#### Correlation between histamine release assay and skin test

Blood samples (1) hyposensitized, (2) newly visited and (3) the other groups of patients with bronchial asthma were examined on histamine release assay (Table 1). The results of histamine release assay were not necessarily coincident with those of skin test. Highly significant differences between (1) and (2) or (3) were found in regard to histamine release (for total cases, (1) 61/7 and (2) 24/35 or (3) 20/24, \*\*\*,  $p < 0.001$ ; for intensely positive (M and/or H  $\geq 2$ ) subgroups, (1) 39/1 and

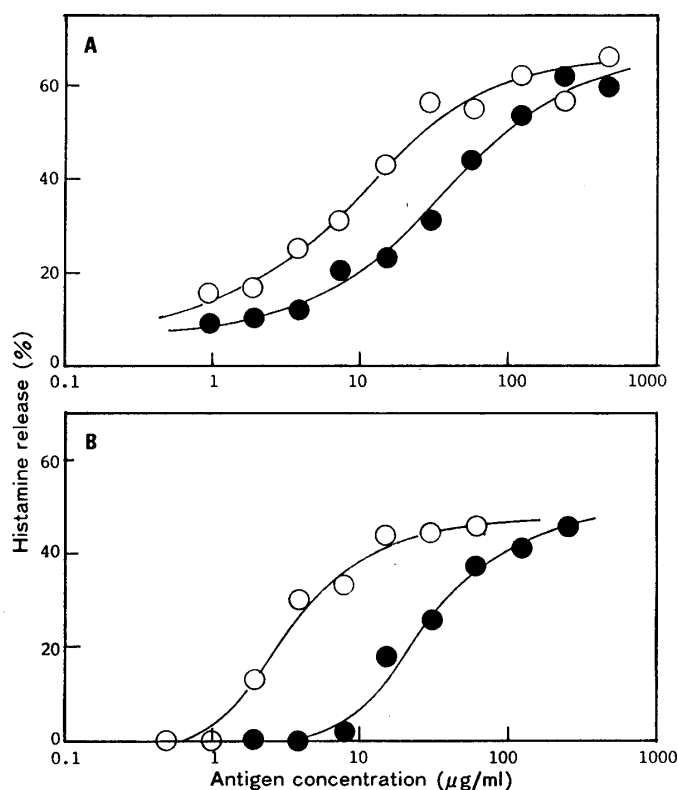


Fig. 2. Typical profiles of histamine release curve. About 10 ml of blood samples from hyposensitized subjects was centrifuged to prepare washed blood cells suspension according to the ordinate procedure. Two hundred  $\mu$ l of the washed cell suspension was incubated with that of Hanks' buffer (○) or the plasma (●) containing 2-fold diluted series of Dff (A, 0.98–500  $\mu$ g/ml of blood) or Dfb (B, 0.49–250  $\mu$ g/ml of blood) solution, followed by centrifugation. Histamine content in the supernatant was determined according to the protocol in the legend in Fig. 1.

Table 2 Differences in sensitivities of hyposensitized patients to Dff and Dfb

| Case | Skin test |   | Dff                                 |                                 | Dfb                                 |                                 | Total histamine <sup>c</sup><br>(ng/ml) |
|------|-----------|---|-------------------------------------|---------------------------------|-------------------------------------|---------------------------------|-----------------------------------------|
|      | H         | M | Sens. <sup>a</sup><br>( $\mu$ g/ml) | Max. HR <sup>b</sup><br>(ng/ml) | Sens. <sup>a</sup><br>( $\mu$ g/ml) | Max. HR <sup>b</sup><br>(ng/ml) |                                         |
| NG   | 3         | 2 | nd                                  | 32.1                            | 0.07                                | 31.4                            | 68.7                                    |
| YF   | 1         |   | 1.0                                 | 7.3                             | 0.8                                 | 9.5                             | 20.4                                    |
| SB   | 3         | 2 | 0.032                               | 2.8                             | 0.086                               | 3.8                             | 6.8                                     |
| EI   | 2         | 3 | 1.5                                 | 60.2                            | 0.66                                | 50.5                            | 64.5                                    |
| YW   | 2         | 1 | 0.26                                | 8.2                             | 0.74                                | 10.1                            | 19.2                                    |
| TI   | 3         | 2 | 1.6                                 | 60.8                            | 1.8                                 | 77.6                            | 81.1                                    |

nd; not determined.

a; sensitivity ( $\mu$ g/ml of blood) = an allergen concentration at inflection point corresponding to 50% of the maximum histamine release amount.

b; a maximum value of histamine release amount (ng/ml of blood).

c; unit (ng/ml of blood).

(2) 21/6 or (3) 16/7, \*,  $p < 0.01$ ). In (2), blood cells from intensely positive subgroup were capable of measuring histamine release with statistical significance (1) 21/6 and (2) 2/14 or (3) 1/15, \*\*,  $p < 0.001$ ).

Histamine release curves for patients allergic to Dff and/or Dfb were obtained with sigmoid shape (Fig. 2), or occasionally bell form with increasing concentration of Dff or Dfb. As shown in the parentheses of Table 1, proportion of high responders to Dff in (1) who took a maximum value of percent histamine release within the range of the concentration of the antigen used was much higher than that in the other groups ((1) 43/58 and (2) 8/23 or (3) 9/16, \*\*\*\*,  $p < 0.01$ ).

#### *Histamine release curves obtained after exposing washed blood cells from patients with bronchial asthma to Dff and Dfb*

Washed blood cells from hyposensitized patients allergic to mite were stimulated with varied concentrations of Dff or Dfb. Typical profiles of histamine release curve were shown in Fig. 2.

A maximum value of histamine released by the stimulation was obtained from the curve. Sensitivity of patient to the antigen (Dff or Dfb) was defined as an antigen concentration in ml of blood at inflection point corresponding to 50% of the maximum histamine release amount (Table 2).

#### *Titer of blocking antibody*

Histamine release curve obtained with reconstituted blood cells suspension (closed circles in

Fig. 2) was usually shifted to higher concentration than that obtained with washed cell suspension (open circles in Fig. 2). The difference in antigen concentrations at inflection points on both histamine release curves seemed to indicate the occurrence of IgG antibody (blocking antibody) capable of neutralizing the allergen in the plasma. The titers of blocking antibody for patients with bronchial asthma were listed in Table 3.

#### *Distribution of allergen species in Dff and Dfb*

Dff and Dfb were chromatographed on a column of Ultrogel AcA 54 into 10 fractions. Allergic activity was examined by incubating the washed blood cells suspension with dilution series of each fraction (Fig. 3). Many instances happened that histamine release curve was transformed into bell form in compliance with either molecular species of allergen separated in the fractions or allergic patient. This result suggested that molecular species of antigen eliciting histamine release varied greatly in individual differences.

## DISCUSSION

The reason we used EDTA buffer as mobile phase solutions in this assay is due to suppression of chelating action of histamine. Basically, optimum condition for histamine release assay was determined under the condition that peak area of standard histamine and its retention time became as larger and shorter, respectively, as possible.

However, the shorter became retention time with increasing flow rate in the range of 0.6–0.8 ml/min, the larger did coefficient of variation. Thus, fundamental concept was modified in part by taking coefficient of variation into consideration and optimum condition for flow rate of the mobile phases was decided.

No significant differences among (1) hyposensitized, (2) newly visited and (3) the other groups of patients with bronchial asthma were observed in the amount of total histamine ((1)  $\mu=55.4$  ng/ml, SD=33.5, n=58; (2)  $\mu=52.9$  ng/ml, SD=19.2,

n=24; (3)  $\mu=42.0$  ng/ml, SD=27.6, n=16) and percent histamine release ((1)  $\mu=51.2\%$ , SD=21.1, n=41; (2)  $\mu=46.5\%$ , SD=11.1, n=8; (3)  $\mu=52.9\%$ , SD=28.9, n=7). We could not recognize the difference (significant level 5%) in mean amount of total histamine between histamine release measurable cases ( $\mu=52.6$  ng/ml, SD=29.8, n=98) or the three groups and healthy donors ( $\mu=49.0$  ng/ml, SD=9.0, n=65)<sup>26)</sup> when these data were evaluated by Welch's test as variances of the patients was significantly larger than that of healthy donors. Consequently, we should consider that the whole

Table 3 Estimation of titer of blocking antibody for Dff or Dfb

| Case | Skin test |   | Sensitivity(Dff)            |                             | Blocking antibody<br>( $\mu\text{g/ml}$ ) | Hyposensitization<br>(+)<br>or<br>(-) therapeutic effect |
|------|-----------|---|-----------------------------|-----------------------------|-------------------------------------------|----------------------------------------------------------|
|      | H         | M | plasma                      |                             |                                           |                                                          |
|      |           |   | (+)<br>( $\mu\text{g/ml}$ ) | (-)<br>( $\mu\text{g/ml}$ ) |                                           |                                                          |
| HW   | 1         |   | 472.0                       | 12.0                        | 460.0                                     | + good                                                   |
| HT   | 1         | 1 | 72.5                        | 43.0                        | 29.5                                      | + good                                                   |
| SK   | 2         |   | 8.0                         | 2.3                         | 5.7                                       | + poor                                                   |
| MT   | 1         | 1 | 170.0                       | 8.5                         | 161.5                                     | + poor                                                   |
| TH   | 2         | 1 | 8.0                         | 2.3                         | 5.7                                       | + good                                                   |
| EI   | 1         | 1 | 20.0                        | 3.4                         | 16.6                                      | + excellent                                              |
| SM   | 0         | 3 | 1.3                         | 0.4                         | 0.9                                       | + good                                                   |
| KS   | 3         | 3 | 22.0                        | 12.5                        | 9.5                                       | + excellent                                              |
| TN   | 1         | 2 | 25.1                        | 2.3                         | 22.8                                      | + good                                                   |
| SD   | 3         | 3 | 545.0                       | 45.0                        | 500.0                                     | -                                                        |
| EM   | 0         | 0 | 0.0                         | 0.0                         | 0.0                                       | -                                                        |
| IT   | 0         | 0 | 0.0                         | 0.0                         | 0.0                                       | -                                                        |

| Case | Skin test |   | Sensitivity(Dfb)            |                             | Blocking antibody<br>( $\mu\text{g/ml}$ ) | Hyposensitization<br>(+)<br>or<br>(-) therapeutic effect |
|------|-----------|---|-----------------------------|-----------------------------|-------------------------------------------|----------------------------------------------------------|
|      | H         | M | plasma                      |                             |                                           |                                                          |
|      |           |   | (+)<br>( $\mu\text{g/ml}$ ) | (-)<br>( $\mu\text{g/ml}$ ) |                                           |                                                          |
| KI   | 2         | 2 | 346.0                       | 45.0                        | 301.0                                     | + good                                                   |
| HH   | 2         |   | 1386.0                      | 86.0                        | 1300.0                                    | + good                                                   |
| ST   | 3         | 3 | 136.0                       | 136.0                       | 0.0                                       | + good                                                   |
| SO   | 3         | 3 | 45.3                        | 4.3                         | 41.0                                      | + poor                                                   |
| SK   | 3         | 3 | 253.0                       | 38.0                        | 215.0                                     | + excellent                                              |
| HI   | 1         | 2 | 203.0                       | 13.0                        | 190.0                                     | -                                                        |
| YT   | 3         | 2 | 100.0                       | 32.0                        | 68.0                                      | -                                                        |
| YM   | 0         | 0 | 291.0                       | 122.0                       | 169.0                                     | -                                                        |
| NH   | 0         | 0 | 262.0                       | 185.0                       | 77.0                                      | -                                                        |
| KU   | 0         | 0 | 0.0                         | 0.0                         | 0.0                                       | -                                                        |

The extent of shift from inflection point on histamine release curve obtained with reconstituted blood cells suspension to that obtained with washed cells suspension was defined as the titer of blocking antibody.

A criterion for judging of therapeutic effect is described as follows. excellent, successfully treated cases required neither suppressive drugs for histamine release nor symptomatic drugs; good, successfully treated cases required only a small amount of these drugs; poor, unsuccessfully treated cases required these drugs.

patients as well as their groups were not constructed with deviation of the population.

As listed in the parentheses of Table 1 (bottom), proportion of allergic cases in hyposensitized group had significantly higher sensitivity to Dff than those in the other groups. The reason remained still unsolved. But a possibility could be given that high responders to Dff might be chosen as the object of hyposensitization therapy. If the possibility held true, a new feasibility would have been hit upon that might have better conduct hyposensitization therapy for such selected cases in order to raise titers of blocking antibody and/or lower the sensitivity to allergens. In fact, allergen-specific IgG has been shown to increase dramatically with immunotherapy<sup>27/28)</sup>. However, remedial effect was not necessarily parallel with

increment of blocking antibody as shown in Table 3, although the total number of allergic cases is not sufficient for clinical assessment. This point would be a problem to be solved.

We have been using Dff (lot 1) and Dfb (lot 1) as standard allergen. The concentrations of these preparations (Dff and Dfb, 0.0005%) using skin test were much lower than those of the corresponding preparations (H, 0.005%; M, 0.1%) from Torii. Inflection point of histamine release curve did not represent sensitivity of patient to allergen in a strict sense because the curve was shifted by purity of allergen in Dff or Dfb. However, the sensitivity would be evaluated as the amount proportional to genuine sensitivity as far as the same preparation could be used among cases. To estimate roughly which preparations contained

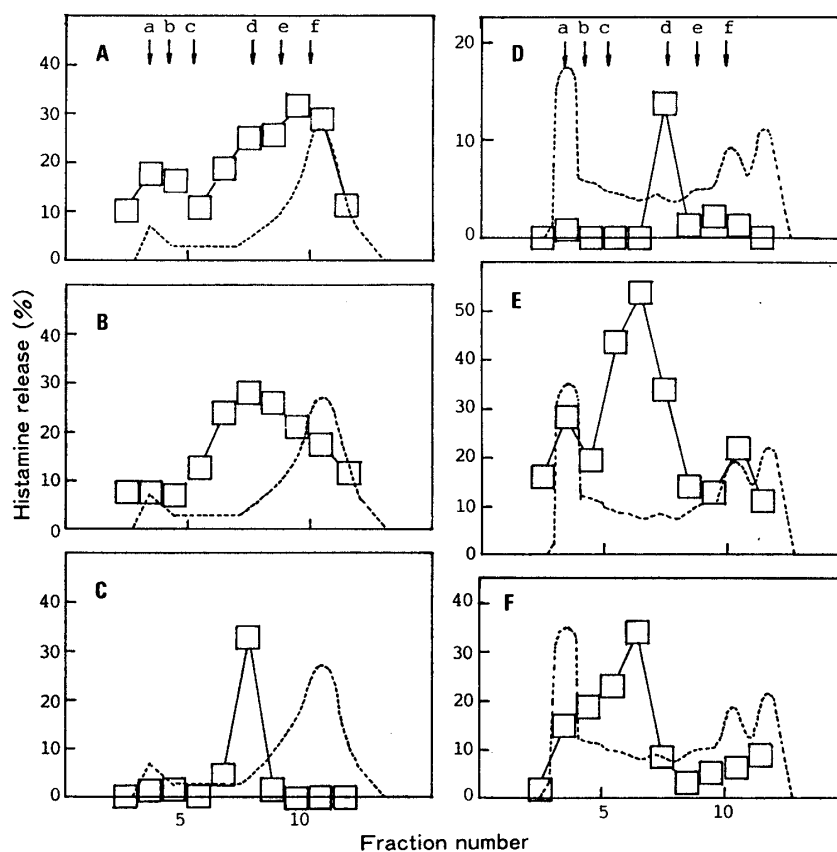


Fig. 3. Activity of diverse allergen species in Dff and Dfb to induce histamine release. Dff (A-C) or Dfb (D-F, 30 mg-each) was chromatographed on a column of Ultrogel AcA 54 (2 × 80 cm) with 0.9% NaCl at a flow rate of 0.5 ml/min into 20-ml fractions under monitoring absorbance at 280 nm. Allergenic activity was examined by measuring released histamine after incubating the washed blood cell suspension with dilution series of each fraction. a, b, c, d, e and f with arrow indicated elution positions of molecular weight calibration markers (Blue Dextran 2000 (2,000,000), bovine serum albumin (67,000), ovalbumin (45,000), chymotrypsinogen A (25,000), cytochrome c (12,500) and  $K_2CrO_4$  (194)), respectively.

higher concentration of allergen, total allergen content might be expressed by multiplying the reciprocal of the sensitivity by the yield of Dff or Dfb as the value of the sensitivity should be in inverse proportion to the amount of allergens (purity of allergen) in Dff or Dfb. Accordingly, total allergen content in Dff might be much higher than that in Dfb.

Similar results were obtained even when the data was plotted with percent histamine release as ordinate and protein concentration in fraction as abscissa after Dff and Dfb were fractionated by gel filtration (Fig. 3). A possibility is now under investigation that allergen eliciting histamine release in the lowest concentration might be the most important antigen for the allergic case.

In our studies on sea squirt allergy, we found that 1) increase in therapeutic effect by hyposensitization therapy was accompanied by remarkable decrease in histamine release induced by stimuli of patients' basophils with antigen, 2) histamine release induced by stimulus of patient's basophils with antigen was suppressed by preincubation of plasma with antigen, and 3) the suppression was caused by increase in IgG titer specific to sea squirt antigen in the plasma<sup>29)-31)</sup>. In histamine release test, we could consider the shift of histamine release curve as the increase in specific IgG antibody (blocking antibody) in plasma because the same phenomenon (decrease in histamine release by addition of plasma) was took place in this case.

Histamine release assay seems to provide a convenient and reliable method for the sensitivity of allergic patient to allergen, the identification of important allergen, and the titer of blocking antibody.

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## 種々のダニアレルゲンに対する気管支喘息患者の多様な応答性

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アレルギー患者の洗浄血球を血漿存在下及び非存在下で抗原刺激し、遊離するヒスタミンを、ポストラベル化法で定量する新しいヒスタミン分析 HPLC システムを開発した。この分析システムを用いて、減感作治療例、新患例、及びその他の治療例の 3 群に分けた気管支喘息患者の洗浄血球を、ダニ排泄物抗原 (Dff) で刺激して遊離するヒスタミンを測定した。その結果、ヒスタミン遊離が観察される症例は、減感作治療群と皮内反応強陽性群に多く、特に減感作治療群では抗原に対する高い感受性を示し、低濃度の抗原刺激でヒスタミンを遊離する傾向があった。また、この反応液に患者血漿を添加することにより、見かけ上の感受性の低下が認められた。この感受性のシフトから、血漿中のダニ抗原に対する特異 IgG、即ち、遮断抗体価を見積ることができた。Dff とダニ虫体抗原 (Dfb) のゲル濾過で分画した各成分に対する応答は、患者間でかなり異なっていたため、現在報告されている主要ダニアレルゲン以外にも、その患者にとって重要なアレルゲンの存在が示唆された。

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