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Evaluation of allergen-specific IgE antibodies by MAST for the diagnosis of nasal allergy*

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SUMMARY

Multiple-antigen simultaneous test (MAST) is a new system for detecting allergen-specific IgE antibodies. Multiple antigens can be examined simultaneously in a short period of time by this method. The purpose of this study is to evaluate the usefulness of this method and to compare the results of MAST with those of RAST and intradermal skin test using 133 serum samples obtained from patients with nasal allergy. The positive rates of the main allergens detected by the MAST system are 56% for Japanese cedar, 31 for Dermatophagoides farinae *(DF), 30% for* Dermatophagoides pteronyssinus *(DP), 27% for house dust (HD), and 27% for timothy grass. The positive rates of food allergens are very low. An average of 3.5 different allergens can be simultaneously detected in one serum. We have compared MAST and RAST with respect to nine allergens: HD, DF, cat, Japanese cedar, timothy, bahia, sweet vernal, velvet, and ragweed. There are statistically significant correlations between MAST and RAST for all allergens except ragweed, the correlation coefficients in the eight allergens are greater than r=0.60, and total agreements exceed over 70%. Similarly, there also is a good correlation between MAST and skin test for the allergens: HD, cat, Japanese cedar, timothy grass, and ragweed. These results indicate the clinical usefulness of the MAST system for detecting specific IgE antibodies in patients with nasal allergy.*

Key words : MAST, RAST, skin test, nasal allergy

INTRODUCTION

The first step in the treatment of allergic diseases, such as nasal allergy and asthma, is the determination of the allergens. For this purpose many procedures may be used: skin tests, provocation tests, radio-allergosorbent test (RAST). Although RAST is very useful for determining the allergens, it has some disadvantages, i.e. the use of radioactive agents, being expensive, and requiring a relatively long time before results are obtained.

Multiple-antigen simultaneous test (MAST) is a new system used for detecting allergen-specific IgE antibodies (Miller et al., 1984; Brown et al., 1985). This system is simple, does not use radioactive agents, and enables the simultaneous examination of multiple allergens. In this study, MAST has been evaluated using 133 serum samples obtained from patients with nasal allergy. In addition, we have examined the correlation between MAST, RAST, and skin test to evaluate the clinical efficacy.

MATERIALS AND METHODS

Subjects

Serum samples were collected from patients (n=133; 52 male and 81 female) diagnosed as having nasal allergy who were treated at the Department of Otolaryngology of Osaka University Medical School, from November 1989 to April 1991. The mean age of the subjects was 42.5 years, and ranged from 12 to 62 years.

A 10-ml sample of venous blood was obtained and allowed to clot at room temperature for 2 h. Serum for the MAST and RAST was obtained by centrifugation at 2,000 rpm for 15 min at room temperature and stored at −20°C until measurement.

Multiple-antigen simultaneous test

Figure 1 shows the principle of MAST. This test is an *in vitro* system using an enzyme-linked anti-human IgE and chemiluminescent assay.

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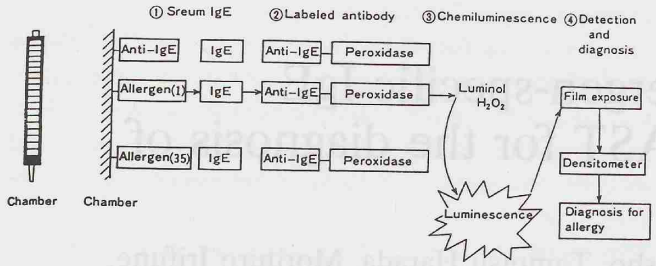

Figure 1. The principle of multiple-allergen simultaneous test (MAST).

The MAST-pette test chamber contains 35 allergens, and positive and negative controls (Hitachi Chemical Co. Ltd., Japan; MAST Immunosystems Inc., USA). The methodology has been fully described elsewhere (Miller et al., 1984; Brown et al., 1985). Briefly, serum is drawn into the MAST-pette and incubated at room temperature for 16–24 h. The MAST-pette is washed three times with 10 ml of buffer after each measurement. Diluted antibody (100 μl neat antibody added to 4.9 ml of buffer) is drawn into the MAST-pette until the top thread is covered. The antibody is incubated in the MAST-pette for 4 h. The MAST-pette is washed three times with 10 ml of buffer. Each MAST-pette is then filled with a photoreagent solution. The photoreagent solution is a mixture of the reagents A, B, C, and D (A: luminescent reagent; B: 0.05 M borate buffer, pH 9.4; C: dye reagent; D: hydrogen peroxide reagent). The MAST-pette filled with the photoreagent solution is inserted into the photocassette which is also loaded with a high-speed Polaroid film. After exposing the film for 10 min, the film is developed for 60 s. The film is then read in the densitometer using the reader card.

The MAST densitometer measures the transmittance level (in V) of the white band corresponding to each thread. The classification of the allergen-specific IgE response (MAST class) is based on the following ranges. MAST class is from 0 to 3, and readings above 1 represent means positive values.

Radio-allergosorbent test (RAST)

RAST was measured using ordinary Phadebus RAST test. RAST is classified from 0 to 4.

Intradermal skin test

Skin test was performed on the same day after collecting the serum for MAST and RAST in most of the cases. None of the subjects had received oral antihistamines and corticosteroids for 4 days prior to the testing.

Skin tests were performed using five allergens: house dust (HD), ragweed (RW), Japanese cedar, timothy grass and cat dander (Torii, Japan). All allergens were commercially available and were diluted 1:1000 (w/v); 0.02 ml of allergen extract was injected intradermally, and the diameter of the flare occurring 15 min after injection was measured and diagnosed as follows: (–) <20 mm; (+) 20–30 mm; (++) 30–40 mm; (+++) >40 mm.

Data analysis

The diagnostic sensitivity, specificity, and efficiency were calculated using standard methods (Galen et al., 1975). Briefly, *sensitivity* is the percentage of patients with positive reaction who actually have the disease. *Specificity* is the percentage of patients with negative test reactions who do not have the disease. The *efficiency* of the test is the percentage of patients correctly classified as having or not having the disease according to test, among all the patients tested.

The r values were determined using Spearmans' correlation coefficient. Obtained p-values of <0.05 were considered to indicate statistical significance.

RESULTS

Positive rate of allergen-specific IgE antibodies detected by MAST

The positive reaction ratio for each specific IgE detected by MAST is shown in Figure 2. Japanese cedar showed the highest positive ratio (56%) among all allergens. The positive rates of other main inhalant allergens were 30% for *Dermatophagoides pteronyssinus* (DP), 31% for *Dermatophagoides farinae* (DF), 27% for house dust (HD), and 27% for timothy grass. Cat dander showed in 11% a positive reaction. The positive rates of food allergens were low, and all of them were below 6%.

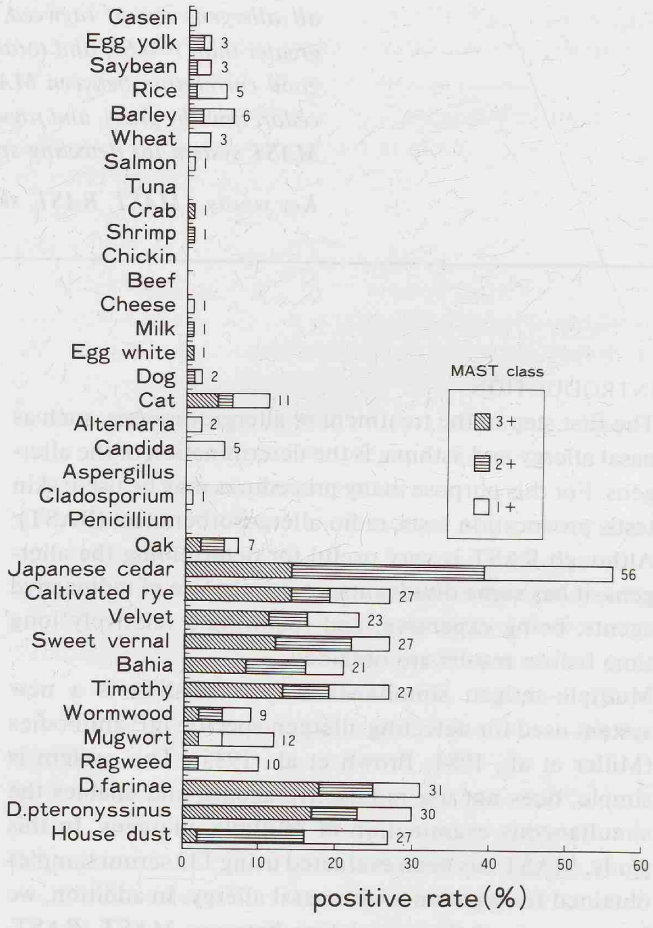

Figure 2. Positive ratio of allergen-specific IgE antibodies detected by MAST.

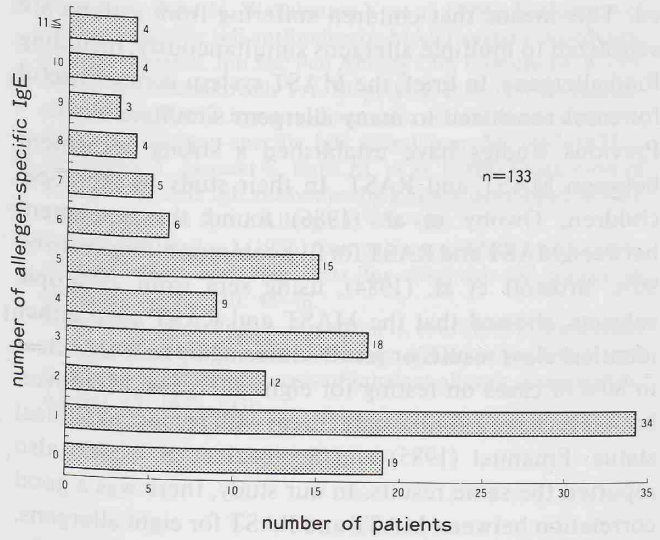

Figure 3. Number of allergen-specific IgE antibodies by MAST simultaneously. Among 35 allergens, an average of 3.5 different allergens was simultaneously detected in one serum sample.

Number of allergen-specific IgE antibodies detected by MAST simultaneously

Thirty-four sera were positive against one allergen, but eight sera were positive against more than 10 allergens. Of 35 allergens, an average of 3.5 different allergens were simultaneously detected in one serum sample (Figure 3).

Relationship between MAST and RAST

RAST was applied for nine allergens: HD, DF, cat, Japanese cedar, timothy, bahia, sweet vernal, velvet, and RW. We calculated the correlation coefficient between MAST and RAST, together with their sensitivity, specificity, and the efficiency of MAST and RAST for each allergens. For HD, the r-value was 0.64 ($p<0.01$), and the other r-values were as follows: for DF $r=0.90$ ($p<0.01$); for cat $r=0.88$ ($p<0.01$); for Japanese cedar $r=0.80$ ($p<0.01$); for timothy grass $r=0.88$ ($p<0.01$); for bahia $r=0.69$ ($p<0.01$); for sweet vernal $r=0.84$ ($p<0.01$); and for velvet $r=0.79$ ($p<0.01$). However, the correlation coefficient for RW was low and not significant ($r=0.24$).

The sensitivity, specificity, and efficiencies are shown in Table 1. A high value of sensitivity was obtained for all allergens except RW. However, high values of specificity and efficiency were exhibited for all allergens including RW.

Relationship between MAST and skin test

The correlation coefficients between MAST and skin test were significant for four allergens (HD, cat, Japanese cedar, timothy grass), but for RW the r-value was low (Table 2).

Specificity was high for all five allergens. Although the positive rate of each allergen by skin test was higher com-

Table 1. Relationship between MAST and RAST.

allergen	case	r	sensitivity	specificity	efficiency
house dust	81	0.64**	85.7	82.1	82.7
DF	46	0.90**	92.3	85.7	91.3
cat	43	0.88**	90.9	93.8	93.0
Japanese cedar	126	0.80**	76.1	88.2	79.4
timothy	78	0.88**	80.0	94.7	87.2
bahia	40	0.69**	80.8	64.3	75.0
sweet vernal	41	0.84**	96.7	90.9	95.1
velvet	38	0.79**	84.0	76.9	81.6
ragweed	70	0.24	25.0	80.6	74.3

DF: *Dermatophagoides farinae*; **: $p<0.01$

Table 2. Relationship between MAST and skin test.

allergen	case	r	sensitivity	specificity	efficiency
house dust	121	0.48*	40.7	95.0	56.7
cat	94	0.74**	66.7	96.3	92.6
Japanese cedar	121	0.60**	69.7	88.0	73.6
timothy	90	0.53**	72.7	86.8	83.3
ragweed	118	0.28	25.0	97.3	70.3

*: $p<0.01$; **: $p<0.05$

Table 3. Relationship between RAST and skin test.

allergen	case	r	sensitivity	specificity	efficiency
house dust	82	0.23	20.0	85.7	27.4
cat	38	0.67**	62.5	93.3	86.8
Japanese cedar	114	0.56*	85.6	70.3	82.5
timothy	62	0.53*	81.8	72.5	75.8
ragweed	62	0.02	5.9	92.9	45.2

*: $p<0.01$; **: $p<0.05$

pared with MAST, sensitivity was relatively low compared with the correlation between MAST and RAST, especially for HD and RW.

Relationship between RAST and skin test

The correlation coefficient was significant only for cat, Japanese cedar and timothy. The sensitivity was poor for HD and RW. However, the specificity was relatively high for all allergens (Table 3).

DISCUSSION

The first step in the treatment of allergic diseases, such as nasal allergy and asthma, is the detection of the allergens. For this purpose, skin tests and RAST are commonly used. Skin testing is a very useful diagnostic tool for allergies, but it has some limitations (Tsay et al., 1984). When the patients take some medications such as antihistamines or have skin disorders (eczema, dermatographia, etc.), it is impossible to apply this test. It is very rare but it may lead to a risk of systemic response. RAST is very useful, but it also has some disadvantages which is reflected by the use of radioactive agent, its high cost, and its inability of being carried out quickly.

MAST is a new *in vitro* system used for detection allergen-specific IgE antibodies using an enzyme-linked anti-human IgE and chemiluminescent assay. The MAST system permits simultaneous detection of 35 different allergens and saves time, about half a day, compared with RAST.

The MAST system has shown a sensitivity, specificity, and precision which equal those of the RAST system. In addition, MAST has exhibited good correlation not only with RAST but also with skin prick test (Finnery et al., 1989). But up to now there have been only few reports giving comparisons between MAST, RAST and intradermal skin test in nasal allergy, and using more than 100 patients.

In this study, the highest positive ratio among the 35 allergens was Japanese cedar (56%) followed by DF, DP, HD and timothy grass while food showed a very low positive ratio. In Japan, HD mite is the most important allergen, and Nakagawa et al. (1989) and Iwasaki et al. (1990) reported that the positive ratio of DP and DF was highest among the 35 allergens using the MAST system. This discrepancy could not be verified, but it may be due to the fact that our subjects were affected by nasal allergy and most of them were adults whereas the subjects of Nakagawa et al. (1989) and Iwasaki et al. (1990) suffered from asthma, and most of them were children. Additionally, we collected the serum mainly during the pollination season.

An evaluation was also made of how many allergen-specific IgE antibodies could be detected in one sample. An average of 3.5 different allergens was simultaneously detected in one serum sample. Additionally, eight patients were found to be affected by more than 10 allergens simultaneously. Iwasaki et al. (1990) reported that an average of 5.5 different allergens in children with asthma was detected. This means that children suffering from asthma are sensitized to multiple allergens simultaneously, including food allergens. In brief, the MAST system is more useful for cases sensitized to many allergens simultaneously.

Previous studies have established a strong agreement between MAST and RAST. In their study of 20 atopic children, Ownby et al. (1986) found the agreement between MAST and RAST for five common allergens to be 90%. Brostoff et al. (1984), using sera from 75 atopic subjects, showed that the MAST and RAST gave either identical class results or results differing by only one class in 80% of cases on testing for eight allergens. Moreover, both tests have a similar agreement with respect to clinical status. Emanuel (1985) and Scolozzi et al. (1989) also reported the same results. In our study, there was a good correlation between MAST and RAST for eight allergens. Total agreements for different allergens were 82.7% for HD, 91.3% for DF, 79.4% for Japanese cedar, and 93.0% for cat. However, for RW poor efficiency was observed, especially in sensitivity. The reason for this discrepancy is obscure.

Our studies also indicated a good correlation between MAST and skin test for HD, Japanese cedar, timothy grass, and cat. But the correlation coefficient for RW was poor. In general, the total agreement and correlation coefficient in the relationship between MAST and RAST were lower compared with the relationship between MAST and skin test. Similar results were reported by Ownby et al. (1986); but several reports do demonstrate a good correlation between MAST and skin test. The reason of this may be due to a difference in allergenicity, because most MAST allergens are made in USA and Europe as compared to the allergens for skin tests which are made in Japan. In addition, most previous reports were made based on skin prick test, but in our study intradermal skin test was used.

In conclusion, the MAST system is a non-radioactive immunoassay and a practical approach to *in vitro* measurement for the diagnosis of allergic diseases such as nasal allergy. Also, the MAST system provides the same information as the RAST and skin test. We believe that MAST is useful for the diagnosis of specific allergens in patients suffering from allergic diseases.

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